

## ***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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We thank the referee for the extensive review which will undoubtedly improve the quality of our manuscript. We regret that the referee does not really appreciate the advances that are reported in this manuscript, where we, for the first time, report a Holocene high resolution HG record based on >250 samples. The referee also makes an unrealistic request for additional data. In summary the referee requests:

1) Reextraction of all 250 samples with the more “gentle” Bligh-Dyer method

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2) Reanalysis of the whole dataset of 250 samples with a method able to detect the “full range” of HGs

3) Reanalysis of the whole dataset in a quantitative fashion using an internal standard instead of the semiquantitative method used here

4) Generation of an independent temperature record based on biomarkers

5) Examination in all 250 samples of the distribution of branched hydrocarbons

6) Examination of HG distributions of heterocystous cyanobacteria isolated from the Baltic Sea

7) Generation of  $\delta^{15}\text{N}$  data for the multicore (100 samples)

This “shopping list” of the referee, which probably would require at least one full year work in the lab, is highly unrealistic and strongly contrasts the assessments of referee#2, who says that “the amount of analyses is staggering”. In the following, we will respond to the comments made by the referee by first repeating the statement of the referee (R), followed by our reply. We apologize to any reader for the length of this exchange of opinions, which actually contains more words than the main text of our manuscript.

(R) 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 cyanobac-

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teria 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.

The referee is correct that the method used targets the six HGs specified. We will more clearly state this in the manuscript. This choice is based on earlier analyses of Baltic Sea surface sediments (Bauersachs et al., 2010, PNAS), published work on the composition of HGs in heterocystous cyanobacteria, and on microbiological studies of the occurrence of cyanobacteria in the Baltic Sea. A recent extensive metagenomics study revealed that in the Baltic proper (the predominant area for cyanoHABs) 69% of the heterocystous cyanobacteria belong *Aphanizomenon*, 23% to *Dolichospermum* (formerly *Anabaena*), and 8% to *Nodularia*. Bauersachs et al. (2017, PlosOne 12, e0186360) have recently analysed HGs of eight representative heterocystous cyanobacterial strains isolated from the Baltic Sea and the six HGs analysed in our study form by far the majority (i.e. 97.7-100%) of the HGs. HGs with longer alkyl chains were not detected. The study concludes (last sentence of the abstract) "As heterocystous cyanobacteria of the genera *Aphanizomenon*, *Dolichospermum* and *Nodularia* are generally known to form massive blooms in many brackish as well as lacustrine systems worldwide, the chemotaxonomic markers introduced in this study may allow

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investigating cyanoHABs in a great variety of contemporary environments from polar to tropical latitudes". So our choice of HGs is very much in line with this independent study. Furthermore, we have analyzed a selected sample set from the brackish period with full scan HPLC-orbitrap-MS, allowing to make an inventory of all the HGs present, and were not able to find C28+ HGs. These results will be added to the manuscript. The referee is right that perhaps the HG distribution during the *Ancylus* lake phase may have comprised HGs outside our analytical window (i.e. the six HGs) because conditions were quite different from those today. We will mention this in a revised manuscript and adjust the discussion to some extent. However, we still feel that our data clearly demonstrates that a substantial change in the heterocystous cyanobacterial community must have occurred. This is carefully formulated in the abstract of the manuscript (p. 1, l. 26-28); "During the earlier freshwater phase of the Baltic (i.e. the *Ancylus* Lake phase) the distribution of the HGs varied much more than in the subsequent brackish phase and the absolute abundance of HGs was much lower than during the brackish phase." The referee suggests that all 250 samples should be reanalyzed for HGs before this study can be accepted. This is not possible since these analyses have been performed almost four years ago and we have noted that after filtration of extracts degradation of HGs may occur. Furthermore, for proper quantification, samples have been reanalyzed several times in that period and, especially for the samples with much lower amounts of HGs (i.e. the *Ancylus* lake phase sediments) there is certainly no material available anymore. We strongly feel that this suggestion for reanalysis is unjustified. Although our study has some limitations (as probably does every scientific study), the results provide novel insight into the use of HGs as potential markers for past blooms of cyanoHABs and is very timely.

(R) 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

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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.

This repeats much of what has been said before. We will extend Table 1 with the HG  
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data of Baltic Sea cyanobacterial strains (Bauersachs et al., 2017), check some of the issues mentioned by the referee, and more clearly indicate that this table lists the occurrence of the six targeted HGs in cultures.

(R) 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.

This comment is no longer relevant now that Bauersachs et al. (2017, PlosOne 12, e0186360) have published their work on the HG distribution of eight strains isolated from the Baltic Sea. We also note that some of the older HG distributional studies are based on some strains isolated from the water column of the Baltic Sea (i.e. *Nodularia* sp., CCY9414 and CCY9416; *Aphanizomenon* Åsp. CCY9905). We are surprised to learn that this referee doubts the usefulness of culture studies for biomarker studies since this basically forms the basis for the interpretation of the fossil biomarker record.

(R) 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 prove 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 referee has seen already that we have tested three extraction methods for HG determination in an earlier study (Bauersachs et al., 2010, PNAS), which showed that slightly higher yields were obtained by the ASE extraction used in this study. This study also clearly showed that the three extraction methods yielded almost identical HG distributions. The recently published method which uses an internal standard (see Bale et al. 2016; OG) became only available two years after all analyses were completed. As explained it is not possible to reliably reanalyze these samples. Furthermore, we have taken great care in the semiquantitative analysis of the HGs and have duplicated all (i.e. 250 samples) our analyses, which is commonly not done in such studies. The request for “proving beyond doubt that the extraction method did not flaw the generated HG profiles and that these profiles are indeed representatives for the sedimentary signal” can basically be made for every study working on organic components in sediments and follows the general strategy of this referee to ask for more work to be done wherever possible. Doubt is a highly personal and non-scientific expression in this sense.

(R) The issue of how degradation may affect HGs is also only little addressed but es-

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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 N<sub>2</sub> 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.

This referee clearly studied the other reviews of our manuscript in quite some detail. We responded to these concerns in our responses (both on the degradation issue and the use of carotenoid pigments) and do not repeat these arguments here. This referee also suggests that we should check our large sample set (>250 samples) for other biomarkers, i.e. branched alkanes. Branched alkanes are certainly not specific enough as biomarkers for heterocystous cyanobacteria as there are also reports of these components in other non-heterocystous cyanobacteria and algae. We also would like to remind the referee that we concluded from our work that “the abundance of HGs dropped substantially with depth and this may be caused by either a decrease of the cyanobacterial blooms or diagenesis, resulting in partial destruction of the HGs” (p. 1,

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l. 22-23) so we do not directly suggest that cyanobacterial HABs played a minor role in the past Baltic Sea”.

(R) 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 discussion 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.

Temperatures are not reported in the results because they are NOT a result but an interpretation of the data and, therefore, belong in the discussion. We feel it is interesting to see if the HG distribution, as seen in lakes, may be an indicator of water temperature and we feel we should spend a few lines on this topic. Clearly, this referee wants to have some more details and we will expand this section a bit and will also refer to a TEX86 record from this core (Warden et al., Sci. Rep., in press).

(R) Specific Corrections

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We appreciate that the referee has taken so much time to go in detail through the manuscript. The term “correction”, however, seems to indicate that all items listed below deal with errors in our manuscript, which we feel is unsubstantiated.

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

This will be added.

p. 2, l. 7. Adams was certainly not the first one to describe the role of the heterocyst in the process of N<sub>2</sub> 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.

We appreciate that the referee is concerned with the fact that original literature generally should be cited. However, we feel that this does not apply to review papers which provide a current overview on a topic. But it can't harm to add an older overview too, so we will add it.

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.

We will rephrase this.

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

We will change this.

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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?

Point taken; we will refer to the recently published detailed meta-omic analyses of Celepi et al. (*Environm. Microbiol.* 19, 673-686, 2017).

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.

This has been extensively discussed earlier and we refer to our response.

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.

We are not claiming that HGs are the best proxies for tracing anoxic events. In this paper we are evaluating the use of HGs as potential proxies for CyanoHABs in the past since in the present day Baltic Sea these HABs are of great importance in the

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development of anoxia. We do not exclude the possibility that other circumstances may also lead to anoxia. We agree that this sentence needs slight rephrasing.

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?

Well spotted. The section 0-240 cm was sampled as 1 cm slices. We will correct this.

p. 4, l. 17-26. Some of the descriptions of how the bulk-geochemical 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.

We will adjust this and refer to another paper for detailed information.

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

So do we; it may relate to the overall lower HG concentrations in this section.

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?

We will expand this description. As described and discussed in the Results and Discussion section these have been established for lakes. Our dataset just allows to test if they are applicable in the Baltic.

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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.

As described above, we will expand this description to make this clear and we will add the full-scan HPLC-orbitrap-LC experiments.

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?

We have discussed this in the manuscript and there is nothing we can add at the moment.

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'

We will check the manuscript for this terminology.

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.

How does this referee define "the full set of HGs"? When analyzing sediments one never can be sure that we look for all HGs that were once biosynthesized by heterocystous cyanobacteria residing the basin at that time. We have explained why the analysis was targeted on the six HGs analyzed in all the samples and we pointed out

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that in a suite of selected samples analyzed for all HGs these were by far the dominant HGs. Figure 4 clearly reveals that monitoring these 6 HGs allows to see changes in the composition of the heterocystous cyanobacteria over time and the relatively stable HG distribution does, in our view, allow us to say : "which suggests that the cyanobacterial community of the Baltic did not undergo major changes from the AL-LS transition to the MoWP and remained dominated by cyanobacteria belonging to the family Nostocaceae" (p. 8, l. 31-32). We, as the authors of this manuscript, take the responsibility for this statement. It is an interpretation of the data set and the referee is free to disagree on this but cannot prescribe what we should do and should have done.

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.

We have clearly phrased this as a suggestion. If we would have other evidence to back it up we would have done so but we will check this again with available inorganic geochemical data.

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?

We will fix this.

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.

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This issue was brought up earlier by the referee and we refer to our earlier response.

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.

We will refer to a recent paper by Warden et al. (Sci. Rep., in press) where these data are published.

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.

This is a repetition of a concern raised earlier by this referee and by Dan Conley and

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we refer to our earlier responses.

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?

There have not been any studies on the effect of partial oxidation on HG distribution. Microbial oxidation is likely to start with hydrolysis of the glycosidic bond of the HGs. If so, this would mean that this has no substantial effect on the HG distribution since all HGs contain glycosidic bonds but this remains to be tested.

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 N<sub>2</sub> 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.

The referee has much faith in  $\delta^{15}\text{N}$  as indicator of past nitrogen fixation. We did obtain  $\delta^{15}\text{N}$  data also for the MUC and we will compare the data with the HG concentrations to see if this fit and describe this in a modified manuscript.

(R) Technical Corrections

We will take all minor technical "corrections" into considerations.

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

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