Dear Editor,

we thank the reviewers fort their thorough reviews of our manuscript "Biomarkers in the stratified water column of the Landsort Deep (Baltic Sea)". Please find below the individual responses to the points made by the reviewers as well as our revised manuscript.
As you will see, we followed almost all recommendations and added PCA as support for our original interpretations.

Kind regards,
Christine Berndmeyer (on behalf of all co-authors)

Reviewer #1, Solveig Bühring
General Comments
Reviewer: I find the presented work interesting and the conclusions reasonable and worth publishing. I have one main concern. For me it is not always feasible on which basis the authors choose the biomarkers for display. Why did they choose ai 15:0 and 10Me 16:0 to show sulfate reducers, but not i17:1 (e.g., Boschker & Middelburg, 2002)? From my point of view, it would be favorable to see all identified lipids. One way to show them would be in the appendix. But general trends in a huge data set are not so easily to see when displayed in a table. Ideally the authors would deliver a proof that they actually choose these compounds, because they are representative of the different groups of compounds that all show the same trends (groups 1 -4). I would suggest a correlation analyses, maybe displayed as a heat map or a principal component analyses (see example below), where lipids that show the same behavior along the transect could be easily identified. In this case an appendix with a table showing all lipid data would not be necessary any more. Using an approach like that is not only a much more transparent way, it also may allow implications for new biomarker assignments that cannot be seen by simply using the biomarkers that have been described before for a certain group of organisms or a process.
Answer: A principle component analysis was performed, and the results in the form of text and a figure were added in the revision. Our original interpretation was supported by PCA, but minor adaptations were necessary and biomarker groups were changed accordingly.

Reviewer: Furthermore, the biomarker assignments are sometimes too general, which needs attention. Tetrahymanol for example is not only produced by ciliates, but also known from ferns, fungi, andphotosynthetic bacteria (Zander et al., 1969; Kemp et al., 1984; Kleemann et al., 1990; Eickhoff et al.2003). Wouldn't a purple non-sulfur bacterium like Rhodopseudomonas also be a possible source forthis compound in the suboxic zone?
Answer: The different sources of tetrahymanol were added into the manuscript and discussed. Additional references were given. Our original interpretation, however, remained unchanged as DNA and other data argue against purple non-sulfur as source for tetrahymanol in the oxic-suboxic transition zone.

Reviewer: When the authors discuss higher abundances of certain compounds at the bottom of the suboxic zone they should also take into account that this layer is also a density layer. Cells (also dying or dead ones) might be caught in that layer, where, due to the low oxygen concentration, the degradation might be slowed down. This is a possibility that needs to be discussed especially for compounds that have a surface and a suboxic maximum.

Answer: Indeed, particles sinking through stratified water columns were observed to accumulate at density discontinuities, where the change of density with depth is largest (MacIntyre et al., 1995). The density discontinuity in the deeper Landsort Deep profile is located between ~60 and ~80 m with largest changes around ~70 m. The suboxic zone lies below this density discontinuity between 80 and 90 m. An accumulation of particles is likely for the depth between 60 and 70 m, but not that likely for the the lower boundary of the suboxic zone. High suboxic zone concentrations of compounds such as tetrahymanol and dinosterol are thus rather a signal of living organisms than an accumulated signal. The potential effects of density layers on the observed biomarker distributions were added in the discussion.

Reviewer: A submitted publication needs to be cited as unpublished (see Jakobs et al. 2014) and the publication Grasshoff et al. 1983 is missing in the publication list.
Answer: The paper from Jakobs et al. (2014) is now correctly cited in the references. The publication Grasshoff et al. (1983) was added in the references.

Reviewer: Maybe a concluding figure would be nice, which displays the vertical distribution of organisms also indicating the biomarkers that gave evidence for their identification.
Answer: We decided not to add a respective simplifying figure. From our point of view there are still too many discussable sources, which cannot be visualized in a respective figure.

Detailed comments
Material and methods
Page 9856, line 11: add "Grasshoff et al., 1983" to the reference list
Considered, see above.

Page 9865, line 11: change "l" to "L"
Considered.

Page 9857, line 11: explain LC-MS on first mentioning
LC-MS was explained.

Results
Give reference to Figure 3 in the sections 3.2.1., 3.2.2., 3.2.3. and 3.2.4. (see also comment on Figure 3).
References for now Figure 4 were given in the Results chapters 3.2.1 to 3.2.6.

Discussion
Page 9865, line 2: the sentence "As expected, in situ biomarkers for phototrophic organisms showed a clear preference for the surface layer." sounds like the biomarkers have a choice.... I suggest to change to: "As expected, in situ biomarkers for phototrophic organisms were most abundant in the surface layer."
Considered, the sentence was changed according to the suggestion of the reviewer.

Page 9867, line 24: even though production of 20:5ω3 from shorter PUFA has been described for some harpactoid copepods, I would not agree with the authors that this fatty acid is a generally produced by copepods. Much more likely is a production by phototrophs and a

subsequent incorporation by copepods, which are known to incorporate these fatty acids largely unchanged into their membrane (e.g., Kattner & Krause, 1989).
Considered. The source for 20:5ω3 PLFA is explained to be derived from phototrophic organisms. Additional references were added.

Page 9868, lines 6-9: "the 20:5ω3 FA shows high concentrations in the cold winter water layer, but it is also abundant in the surface and at the suboxic–anoxic interface (Fig. 3), suggesting multiple biological origins for this compound" I don't agree with this statement. I would suggest that it is always produced by phototrophs and the occurrence in the deeper layer can be explained by copepods that feed on them and are either migrating in the water column or it might be that they are caught in a density layer.
Considered. The discussion was changed according to the suggestions of the reviewer.

Figures
Figure 3: I suggest grouping the compounds according to their behaviour in the water column. In the first row you could write "Group 1" and show the profiles from Cholesterol, 7-methylheptadecane, and β-sitosterol, in the second row write "Group 2" and show 20:4, tetrahymanol, and dinosterol, in the third row "Group 3" and 16:1ω7, total BHPs, diploptene, and ai15:0, in the fourth row "Group 4" with 10-me-16:0, PMI and archaeol and in the last row you can display the "Others".
Considered. Figure (now) 4 was changed according to the groups resulting from the PCA and according to the reviewer's suggestion.

Reviewer #2
General comments
Reviewer: This manuscript describes the distribution of a number of biomarkers in 6 SPM samples of the Baltic Sea, particularly focused on the suboxic zone. In addition, the BHP composition of a surface SPM sample from a cyanobacterial bloom is reported. The biomarker distributions are complemented by selected isotope measurements and their sources are interpreted based on their distribution over the water column. This is a nice, solid contribution on biomarker assemblages in the water column of the Baltic Sea where few studies have yet been done. The interpretations are fine and consistent with previous studies. The manuscript does seem to be somewhat limited in scope, especially compared to the comprehensive studies done in the Black Sea (Wakeham et al. 2007 being the prime example), to which this system can be roughly compared. It creates, perhaps unintentionally, the impression of a data report rather than a focused study (such as with their previous work in the Baltic, focused on the methane cycle). There is nothing wrong with this, it can provide the foundation of future work, but I am not sure if this contribution provides further insights into 'the distribution of relevant biota' or 'biogeochemical processes' (end of introduction) or 'POM sources (middle of introduction). I think the metagenome study of Thureborn 2013 does a much more comprehensive job than this biomarker study (a more detailed comparison with this study might be useful, by the way). Rather, I think the aim of this study might be more to see which biomarker lipids in the Baltic Sea are promising to trace certain biogeochemically relevant microbes.
Answer: The reviewer states that our study is not providing further insights into "the distribution of relevant biota" or "biogeochemical processes" and thus, the aim of our study needs to be clarified. To avoid misunderstandings, we changed the aim to the analysis of biomarkers being relevant for the reconstruction of stratified water columns in the geological record (which is in important advantage of lipid biomarkers in

comparison to DNA). As the reviewer mentioned, the identification and distribution of biota and biogeochemical processes in the Landsort Deep water column today was already done comprehensively in the metagenome study by Thureborn et al. (2013). This study, in our opinion, is, however, not comprehensive. First, the metagenome study was restricted to prokaryotes only, whereas our biomarker study is taking eukaryotes into account. Second, only three water samples were analyzed in the metagenome study. Water bodies such as the cold winter water layer and the suboxic zone were not considered. Relevant processes such as the aerobic oxidation of methane and the related bacteria were overseen. Our biomarker study therefore complements the respective metagenome study and provides knowledge to translate recent DNA analysis into the geological record. A respective part was added in the discussion.

Reviewer: Although the present study is fairly comprehensive method-wise, I could not help thinking that some important biomarkers were not looked at : GDGTs, IPLs, ladderane lipids, carotenoids. For example, Bauersachs et al (2010, PNAS) reported the presence of cyanobacterial glycolipids in Baltic Sea sediments and it would have been great if the authors could have confirmed this in their samples as a good tracer for N_2 fixing cyano's. Despite this, I do think the data are worthwhile to be published in Biogeosciences provided the abstract and introduction more clearly state what the aim(s) is (are) of this study and some minor comments in the discussion are addressed.
Answer: The analysis of ladderane lipids was not possible with the extraction method we chose for our samples (Sinninghe Damsté, personal communication) and the devices available in our lab. Other biomarkers like GDGTs, IPLs, carotenoids, etc. were also not used to avoid overloading of the focused study. For this reason, our study was targeted to the selected representative GC- and LC-MS amenable components. The aims were adapted in the abstract and introduction.

Detailed comments
Page 9858, line 14: Indicate, what standards were used.
The standards squalane and n-eicosane-D42 were added.

Page 9855, lines 14-25: This part of the introduction is confusing. I do not see the need to mention in situ pumping here. It also jumps back and forth between Baltic Sea and Black Sea. Perhaps this can be rewritten by first saying that comprehensive biomarker water column studies such as those in the Black Sea can tell a lot about biomarker sources followed by a review of biomarker water column studies done in the Baltic Sea.
Considered. The introduction was restructured as suggested.

Page 9860, line 25: Fig. 3. I note that cholesterol and dinosterol are also present in the deepest point at 400 m where it is anoxic. Since the synthesis of sterols requires molecular oxygen (Summons et al, 2006 Phil. Trans.) this must mean that it is not produced in situ and is derived from fossil sinking material. I think you should make a remark on this as a potential complication of interpreting your depth profiles. This also comes back to the point of S. Burhing about density layers containing dead material.
The potential transport of dead material into this water depth was added in the discussion.

Page 9864, line 9: I would make it more clear, i.e. phytoplankton and zooplankton.
Considered.

Page 9866, lines 23-25: Mention Sinninghe Damste et al., 1995, GCA here. I agree with S. Buhring that purple sulfur bacteria might also be a good source for tetrahymanol. Were these bacteria detected in the metagenome study of Thureborn et al., 2013?
The reference was added and the presence of purple non-sulfur bacteria is discussed in the revised manuscript (see reviewer #1).

Page 9869, line 18: This is an interesting observation. The maxima of BHT-II would be in agreement with anammox but as stated here, the metagenomic study of Thureborn et al., 2013 does not find hydrazine genes in this zone. A note of caution of course is that this study was done at different time interval. What is noteworthy is that the 10-methyl C16 fatty acid also maximizes at this depth. This PLFA is discussed on the next page but what is not mentioned there is that it is also an important fatty acid in anammox bacteria (eg Sinninghe Damste et al., 2005; FEBS Journal) and has sometimes be used as a tracer for anammox (Schubert et al., 2006, Env Micro). It would be nice if the authors could check for the presence of ladderane fatty acids in their PLFA fractions just to make sure that anammox was really not there. Alternatively, and as also suggested by Rush et al., 2014, the BHT II could be occurring generally in planctomycetes, not only in anammox. Indeed, in the metagenome of Thureborn et al. 2013 they do find sequences belonging to planctomycetes. Could this be the source of BHT II? Some discussion on this option would be useful.
Considered. The discussion was expanded.

Page 9871 and 9872: The conclusion section is perhaps a bit on the long side. More importantly, I am not sure if I agree with the statements in the last few lines. I think several DNA studies have shed light on biogeochemical processes and the importance of certain microbes in the Baltic Sea. Nuance this statement. Perhaps a more important conclusion would be to further expand the biomarker tool box to see if more tracers are available to track important microbes and biogeochemical processes in the Baltic Sea.
The conclusion was slightly adapted and slightly shortened.