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Biogeosciences Discussions



# *Interactive comment on* "Bacteriohopanepolyols record stratification, nitrogen fixation and other biogeochemical perturbations in Holocene sediments of the Central Baltic Sea" *by* M. Blumenberg et al.

**M. Blumenberg et al.**

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This manuscript by Blumenberg et al., describes observations of the variation in bulk organic properties (organic carbon, C/N, and $^{13}C$ of organic carbon), n-$C_{29}$ alkane abundance, and bacteriohopanepolyol (BHP) abundance and structural diversity in Baltic Sea sediments spanning the Holocene. Distinct changes in the organic composition of these sediments occurs around 7 kyr during the transition from lake to brackish basin and the onset of upper water column stratification. These changes are interpreted as being consistent with an emerging contribution to organic carbon export from nitrogen

fixing cyanobacteria and microorganisms associated with the chemocline. A particularly striking result is the emergence of an isomer of BHT – a putative marker for water column suboxia that was previously shown to be associated with suboxic/anoxic marine environments – coinciding with the transition to a brakish basin and the onset of stratification. The observations in this study are novel and are of interest to the biogeochemical and organic geochemical communities. However, I am concerned by the absence of error estimates in the reported data, especially for BHPs. It is not possible to discern the significance of absolute variations in abundance without knowing the uncertainty associated with these measurements. If the authors can address this, then I recommend this paper be published.

**Reply: Quantitative biomarker studies include several potential errors resulting mostly from sample heterogeneities, the efficiency of the extraction, and the analyses. The importance of the first two, however, can be considered low (<5 percent) due to the use of homogenized samples and an efficient extraction technique that was applied in replicate. For biomarkers analysed with GC-MS the analytical error is also relatively low, but hardly quantifiable for individual compounds in this multiple proxy study. As described below, standard deviations for bacteriohopanepolyol (BHP) analyses are slightly higher. But, neither the deviation for gas chromatography-amenable hopanoids nor that for BHPs affects any of our interpretations. We therefore refrained from adding errors in our figures, but added an explaining statement in the methodological section (see also below).**

Comments: Microwave extraction was used. Is it known whether some compounds are degraded under these conditions?

**Reply: Microwave-assisted extraction is a widespread technique in organic geochemical studies and is frequently also used for studies of functionalised lipids such as intact polar lipids (IPLs, e.g. Rossel et al., 2008) and BHPs (e.g., Schmidt et al., 2010; Schmale et al., 2012; Berndmeyer et al., 2013). In previous studies,**



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**we also used a different extraction technique (ultrasonic-assisted) for the analysis of BHP in similar samples. Under the conditions used for our current study, transformation of BHPs occurred with neither of the methods used. Therefore, and because of the high extraction efficiency, we selected microwave extraction as the method of choice in our current study.**

Which BHP standards were used for quantification? Was an internal or external standard used? Some more description of the means of quantification would be helpful.

**Reply: As briefly described in the paper (and in detail in the referenced publication) we used external standards for quantification. BHPs are not commercially available. However, two standards with known concentrations, extracted and purified from bacterial cultures, were used for quantification, bacteriohopanetetrol and 35-aminobacteriohopanetriol. The first was used as a reference for non-amino group containing BHPs, and the second for amino-group containing BHPs, as both have severely different responses during APCI LC-MS. Standards gave linear responses and were analysed prior and after sample extracts. Replicate measurements resulted in a standard deviation of $\pm$20%. We added a respective sentence in the method section. New sentence: "Routine replicate analyses of the standard BHPs revealed an error in quantification of $\pm$20%.**

Page 7, lines 14-15: I have some concerns about inferring dates from a comparison of peak OM concentrations to existing cores that have been dated. How closely spaced are these locations? How can it be certain that peaks in OM are widespread and synchronous features of this basin? If the authors insist on using these dates, it would be useful for the reader to assess the robustness of this method by providing a supplementary figure showing the OM profiles from all cores considered and some graphical indication of how the variations in OM were compared/matched between cores.

**Reply: We are aware that the stratigraphy of the core is a crucial requirement of**



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**our study. The central part of the basin, where the core was taken, can be considered as stable and unaffected by sedimentological disturbances. The Gotland Deep basin is, concerning sedimentological and geological aspects, one of the best studied marine basins worldwide and is also one of the few permanent stations of the monitoring programme of the Leibniz Institute of Baltic Sea Research in Warnemünde. The co-author from this institute, the geologist Matthias Moros, has a long year experience in the lithostratigraphical classification of sediment cores from the Central Baltic Sea, and he also authored the paper describing the dated core used for comparison (Lougheed et al., 2012). The lithostratigraphy of the core used for our study was based on fine scale OM distributions. The correlation was further substantiated by visual inspections, as particularly the transition to laminated sediments (above the Ancylus Lake/Littorina Sea transition) is easily recognizable. To illustrate the excellent correlation, the attached figure compares LOI data of our core with those of the dated cores published in Lougheed et al. (2012). To avoid overloading of the manuscript, we decided not to add this figures to the revised MS.**

Page 8, lines 1-15: How do other terrestrial plant markers (e.g. long chain fatty acids) compare with n-C29 concentration profiles? What is the predominance of odd over even chains? This would provide some additional support to interpret this as a decrease in terrestrial plant input (and not a decrease in fossil hydrocarbon source?)

**Reply: Our interpretation of the decreasing n-C29 with decreasing depth is in line with other studies on changing contributions from terrestrial plants into the Gotland Deep (e.g., from triterpenoids; Nytoft et al. (2001)). However, varying influx of fossil hydrocarbons may alter the use of n-C29 as terrestrial marker. We therefore calculated the carbon preference index (CPI) for all samples and found them high and stable (>5) throughout the core (fossil, petroleum-derived hydrocarbons have a CPI of 1, while fresh terrestrial organic matter shows much higher values). A respective sentence will be included in the revised MS. This**



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**and the in fact also co-varying abundances of long and even-chained n-alcohols and n-fatty acids substantiates that the majority of n-C29 has a terrestrial source.**

**New sentence: "All core samples revealed a high carbon preference index (CPI) of >5, reflecting a strong odd-over-even carbon number predominance and thus, a mostly terrestrial origin of n-C29 and other long chain n-alkanes (Bray and Evans, 1961)."**

Page 8, lines 9-14: n-C29 is a fairly refractory compound compared with other components of bulk OM (sugars, amino acids, polar lipids etc). Why would variations in conditions that affect bulk OM preservation would affect alkanes the same?

**Reply: We agree with the reviewer and deleted this sentence. This does not affect any of our interpretations.**

Page 11, line 25: Adenosylhopane is not proven to be specific to bacteria living in soils, and is, in fact, thought to be an intermediate in the synthesis of BHP side chains (see Bradley, A. S., A. Pearson, J. P. Sáenz, and C. J. Marx. 2010. Adenosylhopane: The first intermediate in hopanoid side chain biosynthesis. Organic Geochemistry 41:1075-1081). So, in theory, all bacteria with BHPs should contain some adenosylhopane. It would be more accurate to say that adenosylhopane is generally enriched in soils, and has not been detected in marine bacteria or marine suspended particulate matter.

**Reply: We agree with the reviewer, and modified the respective sentence. New: "The consistently low amounts of adenosylhopane, a BHP abundant in soil bacteria (Talbot and Farrimond, 2007; Cooke et al., 2008; Fig. 5g), argues against variations in land-derived allochthonous BHP contributions as a major control on BHP patterns."**

Page 11, lines 25-27: "Exclude" is too strong of a word for this argument. The low abundance of adenosylhopane certainly suggests that terrestrial BHP input is relatively small compared with marine sources, but it does not exclude the possibility of an



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adenosylhopane-depleted source of terrigenous material. Compound specific stable isotopic measurements would provide a much more concrete measure of the relative contribution of marine and terrestrial sources to the sedimentary BHP inventory.

**Reply: Considered. See new sentence above.**

Page 12, line 6: The cited paper provides no information on the susceptibility of BHPs to microbial degradation. To my knowledge, the enzymatic pathways for BHP degradation have not been well characterized.

**Reply: The reviewer is correct. Indeed, the paper cited presents no specific information on BHPs, but on the general microbial transformation behavior of lipids versus carbohydrates and proteins. We therefore deleted the references, but kept the very general statement.**

Page 12, line 11: or could be input from an allocthonous source enriched in anhydro-BHT.

**Reply: We think that an allochthonous source of anhydroBHT is very unlikely as no indications for enhanced external (terrestrial) input were observed in the respective samples. We therefore refrain from changing the text in the modified MS.**

Page 12, line 20: I don't understand this argument. Needs some clarification. Do the authors mean the variations in abundances are less pronounced for anhydro-BHT? Are these variations statistically significant given the errors involved in extraction and analysis?

**Reply: Yes, we meant that variations in anhydroBHT are less pronounced and take that as additional support for mostly productivity-induced changes in sedimentary BHPs. However, as we can follow the criticism and agree that this paragraph may be confusing to the reader we deleted this part of the sentence.**

Section 5.2.3 (page 13): Some estimate of error needs to be provided to interpret the



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variations in BHP abundance within this period.

**Reply: See above. We added error bars for the analytical uncertainties in the modified Figure 4. All discussed changes are by far higher than the ±20%!**

Page 14, line 20: how is intensity of stratification quantified?

**Reply: This was a misleading formulation which has now been modified. New: "It appears, however, that the concentrations of this compound cannot directly be translated into the stability of the stratification,..."**

References used in this reply

Berndmeyer, C., Thiel, V., Schmale, O., and Blumenberg, M.: Biomarkers for aerobic methanotrophy in the water column of the stratified Gotland Deep (Baltic Sea) Org. Geochem., 55, 103-111, 2013.

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Lougheed, B. C., Snowball, I., Moros, M., Kabel, K., Muschelera, R., Virtasalo, J. J., and Wacker, L.: Using an independent geochronology based on palaeomagnetic secular variation (PSV) and atmospheric Pb deposition to date Baltic Sea sediments and infer 14C reservoir age, Quaternary Science Reviews, 42, 43-58, 2012.

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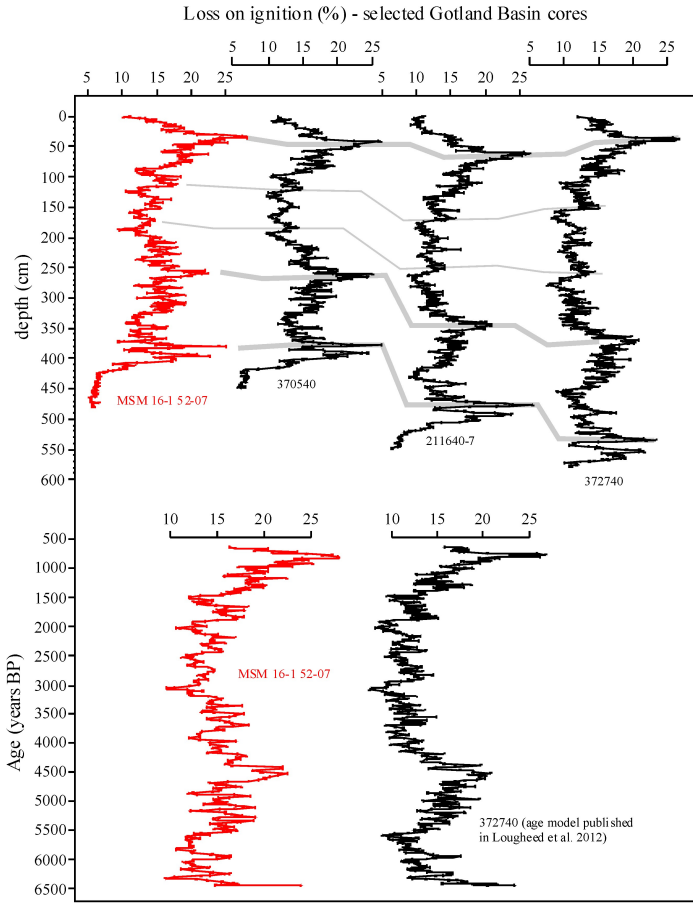


**Fig. 1.** Lithostratigraphic correlation of the core of our study (red) with the dated cores in Lougheed et al. (2012). Modified after Kabel et al. (2012).



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