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Interactive comment on "Seasonal changes in the D/H ratio of fatty acids of pelagic microorganisms in the coastal North Sea" *by* Sandra Mariam Heinzelmann et al.

Anonymous Referee #1

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Review of Heinzelmann et al. "Seasonal changes in the D/H of fatty acids of pelagic microorganisms in the coastal North Sea" for Biogeosciences

This manuscript provides an interesting environmental test to the hypothesis that changes in the net metabolism of microbial ecosystems are reflected in the hydrogen isotope composition of constituent lipid biomarkers. The authors collected a time series of lipid, DNA, and cell count samples from the North Sea over the course of an annual cycle. The authors do observe changes in both average and compound-specific fractionation and attribute those changes to varying abundances of heterotrophic vs. autotrophic microorganisms. While the dominant photoautotrophs are quantified, no data is presented on the abundance of heterotrophic bacteria. The sequencing data

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presented does suggest that heterotrophs are present and their relative abundance varies, but the reader is left to speculate on the true balance of autotrophy and heterotrophy in the system through time. As this is the proposed driver of isotopic variability, this is a problematic omission. In addition, there are substantial problems with the quantification and presentation of errors which appear to significantly underestimate the final uncertainty on isotope values. I suggest revision prior to publication of this manuscript.

Substantial comments: 85: What does 'general' metabolism mean? Perhaps 'Integrated' or 'Net' this is a problem throughout the manuscript. Please define exactly what you mean, then stick to a single term.

300: Is this relative to total fatty acid? I would much rather see these written per liter of sample. With the way it is presented it is impossible to tell if 18:0 went down because 20:5 went up, or because it actually went down. You could show both in a split figure if necessary.

324-327: This is not that strong a correlation and I am suspect you are underestimating the error associated with your water isotope estimates. Just looking that the plot a salinity around 27 could yield dDwater of anywhere from -5 to -10. Please justify the 1.5 permil number and revise as necessary. Also there are no errors shown on the water isotope measurements themselves, but all of the errors need to be propagated to the water isotope estimates. What do the errors on dD of fatty acid measurements mean? Does it include error on internal and external standards? 1 permil would be an exceptionally low error for compound specific isotope measurements... in fact lower than those for individual compounds in the Arndt B standard mix. Both water isotope errors and dD lipid errors need to be propagated into your epsilon values.

346-353: This is an interesting suggestion, but needs better support. What data exists on the use of storage products among algae? How long does and individual alga live (and thus is a seasonal physiological pattern reasonable)? I have heard of dark

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reactions becoming a dominant metabolic process on the diurnal, but not seasonal, timescales. What is the resonance time of a 20:5 in a diatom? Its not clear from your reference list which Zhang 2009 is 2009a, but Xinning Zhang's paper is not a good reference here as it does not include new data for phototrophs, metabolic switching, or concurrent metabolisms.

365-368: What do they make instead? Table 6 says that Rhodobacteria make mainly 18:1, 16:0, 16:1, and 18:0, so this statement seems to be both factually and logically inconsistent. If Flavobacteria dominate reads and make all 15:0 and branched 15, why don't you see these lipids? Either the lipid quantification or attribution must be wrong.

388-391: I don't agree with excluding 20:5 from the average. Algae are also contributing to the other pools. Also I am not sure that I agree with the average following the same trends as compound specific measurements. Perhaps plotting the average behind the C-S data would strengthen this statement.

408: This discussion needs to be more quantitative as you have most of the necessary data. The biggest hang up is a lack of data on the true bacterial abundance. I am not sure that you can really speak to the relative importance of autotrophy vs. heterotrophy at a given moment without at least an estimate. The presence of an algal bloom is not enough as we know that heterotrophic populations increase in response.

You do have estimates of relative microbial abundances and estimates of which fatty acids they makeâĂŤIf you estimate the expected FA abundances based on these data, do they match the observed FA pattern? If not does that provide insight into the abundance of heterotrophs? Then since you know the metabolism of each organism (broadly) and therefor have an expectation of fractionation, can you predict what the fractionation should be based on community and evaluate match to the measurements?

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17-18: The running title is just as long as the original title.

46: references are needed after ‘effect’

47-53: Be clear through this discussion if you are talking about all FAs, just C16, or an average fractionation.

57-60: This is a little misleading. ‘Main’ implies the majority whereas NAPDH donates ∼50% of H to fatty acid . H2O is the ultimate source of H to autotrophs.

63: I am not sure what ‘core’ metabolism is in the context.

65: Dawson et al. (2015) recently tested the effect of microbial interaction on D/H.

66-68: Specify that these were targeting marine samples

71-81: The discussion of Osburn et al. 2011 could be more concise. Also, the bigger issue related to the extrapolation of these results more broadly is not that it was a low diversity environment, but rather that is one full of highly unusual (hyper)thermophilic organisms. The Aquificales are not exactly cosmopolitan microbes.

95: Your data do not show an autumn bloom at all.

95-96: Is there data on bacterial abundance from this site? This is necessary complementary information to your phytoplankton counts.

112: What was the sampling depth? Was it consistent between samples? Is turbulence of the water a concern? Some of the changes in bacterial abundance that you note could be influenced by incorporation of sediment into the sample.

117: 0.7 is not very small compared to many marine heterotrophic bacteria. The clogging noted in 135-138 is also troubling. Was any microscopy performed to confirm what was actually analyzed? How can you be sure the bacterial population was captured?

186: Strange formatting on your instrumentation abbreviation protocol throughout 2.3. ‘elemental analysis/TC/irMS (EA/TC/irMS)’ is rather repetitive

189-195: Cyanobacteria are not usually considered algae. I find it strange that you counted the cyanobacteria, but did not do a total cell count.

242-244: This belongs in the methods.

247: 4 is not particularly low given the range you present. What is the error on these measurements? The graph seems to show a trend during this time period rather than a constant low.

258: Microbial Diversity would include that of the Archaea. You discuss only bacterial diversity.

289: The 'Other' group makes up a large percentage of your samples, You should add a section documenting any taxa that demonstrate seasonable variability.

335: Misleading. At times this is among the most D-enriched compound.

337-339: It becomes unclear at times if you are discussing your own data or that from the literature. I believe this is the literature and therefor requires references. Try to clarify this throughout the discussion.

342-344: But why would Phaeocystis produced enriched lipids? Any why does it matter of 20:5 is a trace or major constituent? To my knowledge there is no published information relating the relative abundance of a lipid to its relative D-enrichment or D-depletion.

354: I think on average it is the most D-depleted

372-376: The table needs to be reformatted in order to make these more general conclusions.

386: This is not evident for 18:0

392-394: Can the correlation of trends be shown statistically?

418-419: This could be said for fatty acids from almost all environments.

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422: Except that 20:5 also becomes very enriched and is derived from autotrophs.

Figure 1: Error bars on epsilon measurements (propagated as discussed above). If you come up with a measure of heterotrophic abundance, I would like to see it plotted here as well.

Figure 3: The 'Other' category is way to big. The usual standard is to lump phyla that comprise <1% of the total. This could be pushed to maybe 3 or 5% here since the trace constituents probably are not important. I would be particularly interested in any phyla with transient high abundances.

Figure 4 and 5: It would be helpful to have some shaded bars delineating seasons to guide the eye between these subplots. Table S4: Please report concentrations instead of relative abundance. Table S6: This would be far more useful reformatted in either tabular or heatmap form. It should be possible to scan down a column for a single FA and identify those that make it and those that don't. For the abundances that you discuss in the text, the original reference should be cited rather than the table itself. Figures S1-S3: I am not sure these trees are necessary. Figure S4: Error bars on each input measurement. As noted above you need to include more information on the error associate with the extrapolated water isotope values. Perhaps confidence intervals on this figure would be useful in that context. It would be also useful to color code these points by date to illustrate seasonal trends in salinity and water isotopes that might be underlying the organic isotope trends.

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