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Comment

***Interactive comment on* “Temporal variations in abundance and composition of intact polar lipids in North Sea coastal marine water” by J. Brandsma et al.**

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We thank both the Anonymous Referee and Dr Van Mooy for the positive assessments of our manuscript and their constructive comments. As the reviewers' main comments are similar, we will address them simultaneously.

R1) reviewer 1 comment (Dr van Mooy)

R2) reviewer 2 comment (Anonymous Referee)

AC) author comment

R1) Is there a way to take greater advantage of the detailed data on algal community

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Discussion Paper



composition? It is not clear if the data in Fig 3 were integrated into a PCA test. Just perusing the IP-DAG data in Table S2, it looks like some of the molecules might be more abundant during the *Phaeocystis* blooms.

R2) As already mentioned by B. Van Mooy in his comments I also wondered if the algal community data and those of the IP-DAG data have also been included in the PCA test. At least in the major bloom period in april some IP-DAGs seem to be more prominent than before and after the major bloom in april. [...] It may be true that the overall IPL distribution does not allow an assignment to any organism at high diversity, but in the major bloom period an assignment of few IPLs to specific microalgae may be possible. The authors should check their data once again and verify if there are any specific IP-DAGs at least in the april bloom.

AC) The detailed algal community data (Fig. 3) were not included in the final PCA discussed in the paper and presented in Figure 5. As requested by the reviewers, a separate PCA was performed which included all the major IPL species, as well as the algal community data and cyanobacterial and bacterial abundances. This resulted in two major principal components, which together explained 61% of the variance. The additional PCA showed that the microbial taxa/groups *Phaeocystis* (single cell), *Pseudonitzschia* and unidentified flagellates were positively loaded on the first axis, while the remaining algal taxa, cyanobacteria and bacteria all showed minor loading factors (between 0.24 and -0.10). On the second axis, *Phaeocystis* (both types), *Prymnesiales* and unidentified flagellates were negatively loaded (between -0.64 and -0.44), while *Plagioselmis*, *Hemiselmis*, cyanobacteria and bacteria were positively loaded (between 0.35 and 0.70). The remaining taxa showed only minor loading factors between -0.28 and 0.04. The IPL species were all positively loaded on the first axis, with only 8 out of 130 species having a factor loading <0.40. On the second axis, the factor loadings of the IPL species were fairly uniformly spread between values of -0.64 and 0.76. This PCA thus shows that the IPL pool has a mixed origin, and no IPL species can be exclusively assigned to one particular algal taxon or (cyano)bacteria, something we concluded ear-

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lier on the basis of PCA analysis of the total IPL classes. What can be inferred more is that *Phaeocystis* (single cell), *Pseudonitzschia* and other (unidentified) flagellates are the dominant source of IPLs in the Marsdiep, as almost all IPL species are quite strongly positively loaded on axis 1. However, the same IPL species are also loaded on the second axis, indicating that they are also partly derived from *Phaeocystis* (both types), *Prymnesiales* and other (unidentified) flagellates (negatively loaded species), and partly from *Plagioselmis*, *Hemiselmis*, cyanobacteria and bacteria as well (positively loaded species). Finally, the few IPL species that are weakly loaded on both axes are likely derived from *Chaetoceros*, *Thalassiosira* or *Skeletonema* diatoms. Cross-referencing with the relative abundances in Table S2 and the correlations in Table S3 generally confirms these relationships between IPL species and microbial taxa/groups. The results of this additional PCA analysis thus add some additional chemotaxonomic resolution, but at the same time confirm our conclusion that the 130 main IPL species have mixed sources and thereby limited chemotaxonomic potential.

We will add this additional PCA analysis to the paper as a table containing the factor loadings, on both axes for each of the variables, but not the plot itself, as it contains too many data points. As a consequence of this addition, we will also make the following changes to the manuscript: 1) A description of the PCA analysis will be added to the Materials and Methods section. 2) The results as described above will be added to the Results section. 3) The relevant Discussion section will be amended and expanded to include the findings from this analysis. 4) The conclusion regarding the limited chemotaxonomic value of the IPLs will be phrased more strongly. 5) The PCA factor loadings will be added as a table in the Supplementary Material (Table S4).

R1) The conclusion that IP-DAGs have limited chemotaxonomic potential is very solid, and could be much more prominent in the paper. Perhaps this conclusion should be included in the title?

R2) I agree principally that IP-DAGs do indeed have a limited chemotaxonomic potential, but the authors should check once again if some of the IP-DAGs may be more

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useful as source-specific biomarkers (see comment above). If this is not the case I suggest to stress this finding already in the title.

AC) The title has been amended to reflect our conclusions more strongly, and now reads: “Low temporal variation in the intact polar lipid composition of North Sea coastal marine water reveals limited chemotaxonomic value”.

R1) The authors note that there was no correlation between the non-phosphorus lipids and phosphate concentrations, which is important to point out (they would be remiss if they did not). They also suggest that this means that non-phosphorus lipid substitution was not occurring in response to phosphate scarcity. However the nutrient data they present is really insufficient to conclude that phosphate was scarce from a physiological standpoint. So, doesn't the observed lack of a correlation between non-phosphorus lipids and phosphate concentrations suggest instead that phytoplankton were simply not stressed by phosphorus scarcity? It seems to me that this latter conclusion is more strongly supported by their data. Perhaps the authors should consider some batch culture experiments with *Phaeocystis*: these are the algae that dominated when phosphate concentrations were lowest. If they found that these algae do not substitute, then they would have a much stronger case (and an even more significant paper on their hands).

AC) Van Mooy and Fredricks (GCA, 2010) concluded that phosphorous to non-phosphorous IPL ratios are only influenced at dissolved P concentrations of less than 30 nmol L⁻¹. In our time series in the Marsdiep the lowest value was 70 nmol L⁻¹ at the height of the spring bloom, which is well above the threshold inferred by Van Mooy and Fredricks. We therefore agree with the reviewer that the phytoplankton community was probably not phosphate limited enough to induce any significant IPL remodelling, despite the high N:P ratios measured. We will rewrite the second half of the relevant Discussion paragraph (page 8909; lines 13-25) to reflect this.

Regarding the specific response of *Phaeocystis globosa* (the main bloom-forming al-

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gae in the Marsdiep): there are unfortunately no batch culture data available at present, but it would be valuable to study any nutrient limitation-driven IPL responses in this widespread and ecologically important taxon. However, this is beyond the scope of the present study.

R2) Page 8908, lines 14/15 the expression “microbial” is used for microalgae and bacteria, but I would prefer the expression microalgal community. The role of bacteria is only poorly discussed and not the major focus of the paper.

AC) While we agree with the reviewer that there is a strong focus on (micro)algae in the paper, we feel that to substitute “microbial” for “microalgal” would be a step to far, and in some respects even incorrect. While we did not differentiate between different cyanobacterial and bacterial taxa (as we did for the eukaryotic algae), the total numbers of these two microbial groups were included in all (statistical) analyses, and indeed our results show that neither cyanobacteria nor bacteria are major sources of IPLs in the Marsdiep. Thus, it is important that they are included in the discussion (even if we did not identify them to the same taxonomic level as the eukaryotic algae), and we therefore choose to maintain the term “microbial” throughout the manuscript.

R2) Technical comment Table S1/S2: The 35:5 PC and 37:6 PC are missing in Table S2, as well as the 35:0 PG. The authors should include these data or omit them from Table S1.

R2) A technical comment to table S2. This table is quite hard to read. Only on the first page the days can be found where the samples were taken. Please provide the date for each page separately.

AC) We apologise for these omissions and will amend the relevant Supplementary Tables to include the three missing IPL species, as well as the sampling dates on each separate page.

Please also note the supplement to this comment:

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<http://www.biogeosciences-discuss.net/8/C5506/2012/bgd-8-C5506-2012-supplement.pdf>

Interactive comment on Biogeosciences Discuss., 8, 8895, 2011.

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