Biogeosciences

Discussions

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Interactive comment on "Whole-lake spatial variability of organic matter molecular composition and elemental inorganic properties in a small boreal Swedish lake" *by* Julie Tolu et al.

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We thank referee 2 for reviewing our manuscript. The response to the different comments and the intended modification in the manuscript are detailed thereafter.

1. "A spatial geochemical study of a lacustrine environments is original and represents a largely unexplored aspect of biogeochemistry. Most studies reach directly to the coring equipment and retrieve a core which then becomes the focus of the study assuming we understand all those spatial details. Although the findings are perhaps not that surprising this is a valuable contribution which shows how OM and inorganic geo- chemistry varies in a relatively small but complex lake. These findings will stimulate thinking about the processes involved in other lake systems, but the paper could

have had more impact if this concept would have been developed a bit more. Why not show a few sediment profiles from this lake in relation to these findings and show the implications. That would be really interesting and relatively easy to add. Now it remains just a hypothetical case."

We appreciate the positive view the reviewer has on our exploration of the geochemical and molecular composition of the sediments of a whole-lake basin. While we agree sediment profiles would be interesting, we disagree that it would be appropriate here for two main reasons. First, including an evaluation of sediment cores and all that such an evaluation would entail are outside the context of our study objective focused on spatial patterns in OM molecular composition and how these relate to geochemical variations and in-lake processes. We think there is a novelty already in this focus, with our combination of the pyrolysis-GC/MS characterization of OM molecular composition with elemental geochemistry. Second, including a temporal perspective by analyzing downcore changes would entail a fundamentally different discussion and would be a paper in its own right. We are uncertain why the reviewer considers this a hypothetical case. We believe the spatial context here is valuable for process-oriented studies on, e.g., carbon or trace elements cycling, which we think at this point is the main implication of our work. Indeed, our study clearly demonstrates that the spatial distribution of OM molecular composition is well explained by general/common factors and processes of lake ecosystems (i.e. sediment focusing, macrophytes, catchment input, mineralization). In consequence, our results provide insights into the locations where it would be relevant to determine and compare the fate of C, nutrients and trace elements within a single lake, especially regarding the reactions for which OM molecular composition play a critical role, e.g., OC mineralization, mercury methylation, phosphorus mobility. While the practical steps in analyzing sediment core samples themselves may not be a complicated task, presenting and evaluating downcore changes would require assessing diagenetic changes within each core (cf. our methods paper, which used varved sediments; Tolu et al. 2015), as well as including a full assessment of how the sediment record(s) reflect environmental changes over the represented timeframe of each

core. For Härsvatten, this would include the effects of acidification on lake biota (e.g., diatoms; Renberg et al. 1993), effects of acidification on carbon cycling (e.g., declines in TOC; Rosén et al. 2011), long-term changes in land use (e.g., cultural alkalization over past c. 1000 yrs until c. AD 1900; Renberg et al. 1993, Rosén et al. 2011), influence of 2000 yrs of atmospheric pollution deposition (e.g., Bindler et al. 2001), changes in catchment vegetation (e.g., spruce immigration), etc. These would all be interesting concepts to examine with lake sediment records and would all have to be addressed even with short surface cores because of the low sediment accumulation in this lake. A 30-cm surface core from the deeper basins would comprise >500 yrs of environmental changes, which would imprint on the OM quality. But as we first comment, all of this is outside the scope of our spatial assessment in OM quality, which we think is particularly relevant for process-oriented studies such as organic carbon mineralization, trace metals and nutrients sorption and transformations.

2. "Firstly, the title suggests that this study is characterising an entire lake. This is a bit misleading; I suggest to reword this to reflect more accurately the exact nature of this study."

We think that the referee 2 found our title to be misleading because we did not include the word "sediments", which is a keyword of our study as pointed out by the referee 1. The title of the manuscript will be replaced by "Spatial variability of organic matter molecular composition and elemental geochemistry of sediments in a small boreal Swedish lake".

3. "The opening line of the abstract states: "The composition of organic matter (OM) exerts a strong control on biogeochemical processes in lakes, such as for carbon, nutrients and trace metals". Carbon, nutrients and trace metals are not processes. . . I suggest to reword this."

To avoid such confusion, we will reword the sentence as follows: "The composition of sediment organic matter (OM) exerts a strong control on biogeochemical processes in

lakes, such as those involved in the fate of carbon, nutrients and trace metals."

4. "L8 no hyphen between pyrolysis and gas", "L24 Py-GC-MS = Py-GC/MS this is the accepted convention of abbreviated this technique.", "L15 no hyphen between source and pools" and "L45 delete: C being the main constituent of OM (I think that is obvious)."

These small technical/editing corrections will be made in the revised manuscript.

5. "L65 "little work has been done to detail the compositional variability of the OM matrix as a whole". Whilst I agree that in terms of spatial variation this is a virtually unknown territory, variation of the OM matrix in general is what many in the organic geochemistry community deal with. As such this is an overstatement and I believe the manuscript is doing an injustice to the many studies that have gone before. In Tolu et 2015 a more robust overview is given of previous Py-GC/MS studies. I would like to see more such acknowledgement and brief detail about previous achievements on OM matter composition studies."

In this part of the sentence line 65 "little work has been done to detail the compositional variability of the OM matrix as a whole", we are actually referring to a lack of data on the in-lake spatial variability of OM composition at the molecular level, for which the referee agrees that it is unexplored territory. Indeed, this sentence starts on discussing what has been done in term of in-lake spatial variability of OM quality, i.e. "Although there have been a few studies where the spatial complexity in OM quality within a whole-lake basin has been assessed using infrared spectroscopy, which yields qualitative information on variations in OM quality". However, to avoid such confusion and risking to make injustice to the high number of studies that have gone before on sediment OM molecular composition with different objectives, we would like to re-write this part of the sentence line 65 as follows "little work has been done to detail how the molecular composition of the sediment OM matrix as a whole varies spatially within a single lake". Given the large number of studies looking at variability of OM molecular composition

in sediments within a wide number of goals and contexts (such as OM mineralization and redox conditions, lake trophic status, urban pollution) and using methods that provide different levels of molecular information (i.e., Py-GC/MS, specific liquid extractions associated to LC/GC-MS or LC-FTICR-MS analyses), we do not see how we can give "brief details about previous achievements on OM composition" in sediments as asked by the referee 2. We believe that adding a paragraph on presenting previous achievements on OM composition in sediments would greatly extend the length of the introduction and would be out of the scope of the study focused on a comprehensive understanding of in-lake spatial variability and distribution of OM composition.

6. "Method section."

6.a. "Pyrolysis GCMS is traditionally carried out at 650 degrees or there about and 450 is significantly lower than the conventional methods. Since this deviation may have consequences, more detail should be given here. I read Tolu et al 2015 which is a very interesting paper. But I am not sure if for example it can be said that aliphatic signal in lacustrine sediments, that is often observed at 650 degrees can be dismissed as being an artefact of the pyrolysis process. Previous work from others has clearly shown that this is to be expected when algenan is present, there is no question about the fact that the aliphatic signal is an important constituent of lacustrine OM: : : The fact that this particular signal significantly lowers at 450 concerns me. As such, it may be that lowering the temperature to 450 may cause bias toward the terrestrial components."

Pyrolysis-GC/MS is indeed generally carried out in environmental sciences at 650°C where the sample mass used for the Py-GC/MS analysis is generally above 1 mg. We have demonstrated in our methodological development (Tolu et al., 2015), in which we compared specifically 450 and 650°C, that when using 200 μg of sediment, we lose many Py products of lignin oligomers and especially all Py products of syringyl lignin oligomers due to increase in secondary reactions with a pyrolysis at 650°C. We also lose Py products indicative of fresh, algal or higher plant and mosses OM, e.g., some levosugars that are Py products of fresh higher plant and moss polysaccharides

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or cellulose, and some 2,5 diketopiperazines that are Py products of proteins. As referee 2 pointed out, lower proportions of aliphatic compounds were obtained with a pyrolysis at 450°C, most probably due to less efficient volatilization of refractory organic molecules, which may include bio-macromolecules such as algaenan, cutin, suberin that are know to form aliphatic compounds during Py-GC/MS and especially n-alkenes/n-alkanes doublets with a number of C comprised between 16 and 24 carbons. However, we do not understand why "lowering the temperature to 450 may cause bias toward the terrestrial components as compared to a Py at 650 degree." We would like to first underline that not only bio-macromolecules from algae (such as algaenan), but also bio-macromolecules from higher plant and moss (such as cutin and suberin) are known to contribute strongly to the aliphatic signal in Py-GC/MS. Secondly, at 450°C, we are still identifying the series of n-alkenes/n-alkanes doublets characteristic of these bio-macromolecules and, actually, there is one principal component from the PCA analysis (i.e. PC2OM; Fig. 3a in the manuscript) which is associated with the n-alkenes/n-alkenes doublets with 17 to 22 carbon number and others organic compounds known for their recalcitrance. On the other hand, we believe that a pyrolysis at 650°C may cause a bias toward the refractory components of sediments OM (loss of many Py products from labile/fresh organic compounds such as polysaccharides and proteins), while this fraction of sediment OM is of much less relevance for process-oriented studies, which are one of the main implication of our study. We would prefer to not extend our manuscript by discussing details about the differences between pyrolysis at 450 and 650°C, given that Py at 650 °C was not carried out in this study and that this was already discussed in our paper presenting the methodological development where we compared results from the two treatments (Tolu et al., 2015).

6.b "Have you considered an alternative approach such as TMAH pyrolysis?"

We have considered, and even briefly tested, TMAH pyrolysis when we have performed our methodological development. The main differences between TMAH pyrolysis and regular pyrolysis is that it enables to identify more accurately acid protons containing

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compounds, i.e. carboxylic acids and alcohols. However, this method has major drawbacks: 1. TMAH pyrolysis has been shown to be associated with really poor data reproducibility because the methylation process is non-exhaustive owing to steric hindrance or too large quantity of the original compounds (Chiavari et al. 1994). During our test (non pubished) with TMAH pyrolysis, we could clearly see very low reproducibility in the peak areas of compounds of interest (i.e. carboxylic acids). 2. We cannot identify the compounds that elute during the first 3-5 min due to solvent/TMAH elution. This part of the chromatograms contained most of the (alkyl)furans and (alkyl)pyrroles/pyridines, which are used as indicator of degraded OM by comparison with the levosugars (Py products of plant polysaccharides and cellulose) or the 2,5 diketopiperazines (Py products of proteins). Hence, TMAH pyrolysis is a complementary analysis to regular pyrolysis for identifying the acid fraction of OM, but if one method has to be chosen, the regular pyrolysis is for us the most relevant one. We agree that providing data on the spatial variability of the abundance of the acid fraction of OM would be interesting. However, we believe they would not bring more insights on the factors and processes involved in the spatial distribution of OM composition than what we are discussing with our data from regular pyrolysis. Indeed, the acid fraction would most probably only show variations in the sources and degradation status of the sedimentary OM across Härsvatten, which are already clearly observed from the data obtained with regular pyrolysis. On the other hand, we would have to present the full dataset obtained by TMAH pyrolysis and the results of PCA and cluster analyses made on this specific dataset, which means it would significantly extend the length of our manuscript.

6.c. "Also it is not clear to me if the authors can distinguish between thermal desorption and pyrolysis. A lot of the compounds listed could also be explained as thermally extracted and may not be pyrolysis products at al (this might also explain the difference between 650 and 450)."

In Py-GC/MS, the sample reaches the Py oven which is already at 450°C and the volatilized fraction is injected in the GC/MS in a few seconds. This is technically

very different from thermodesorption-GC/MS analysis. May be, a part of the identified compounds that are volatilized during pyrolysis could also be released during thermodesorption analysis. However, at our knowledge, there is no way to identify simultaneously to Py-GC/MS what are those compounds, but it would require to run separately thermodesorption-GC/MS analyses. Moreover, we do not see how knowing whether the compound is directly released or is a "real" Py product will enable us to get more insights in our study because the most significant trends highlighted by the statistical analyses and by the comparison with the elemental geochemistry are about OM sources (autochthonous algal vs autochthonous higher plant and moss versus terrestrial OM) and degradation status, and are thus based on information about the structure, the origin and the reactivity of the identified organic compounds.

7. "L185 "Because these sediment samples also contained too little OM for Py-GC-MS analysis." Py-GCMS normally should be able to detect extremely low amounts of OM even with the small sample size that is described here, I find it hard to believe that nothing could be detected…give the %TOC of these samples in the discussion. Is it really nothing or were the results unexpected? This might also be a 450 vs 650 problem. I would imagine there is more complex OM than material that can thermally desorb."

Our explanation for the fact that we have no Py data for the sediments at sites M4 and S15 was not clear. We have no Py data for those samples because we could not analyze these samples, even after having grind them strongly (30 Hz for 3 min using a stainless steel Retsch ball mill). Indeed, these sediments are corresponding to coarse sand, and thus it was impossible to prepare a bowl of 200 μg with our capillary system which is used to weight accurately ($\pm$ 20 μg) and transfer our samples to the Py cup. Hence, we agree by clarifying this technical issue in our manuscript by replacing the sentence lines 185-186 by "Because these sediment samples are too coarse (predominantly sand) for Py-GC/MS analysis according to our method based on 200 $\pm$ 20 μg analyzed sample mass, they are excluded from the data analyses and discussion".

We could not have analyzed those two sediments with higher sediment mass in order to include the resulting data in our statistical analyses because we employed a data processing pipeline to automatically integrate the peaks and extract the corresponding mass spectra. For getting accurate and precise peak integration and extracted mass spectra from this data processing pipeline, the baseline of the Py chromatogram has to be similar between the different sample Py chromatogram. However, when injecting strongly different sample mass the baseline is strongly affected.

8. "Given the effort that has been put into analyzing the geochemical data I think it would have been better to determine the %TOC using elemental analysis as was done in Tolu et al 2015. LOI is not a very precise way to determine TOC. the pyrolysis results could be expressed per TOC."

Although it would have been great to determine TOC content, LOI is a very good indicator of OM content, which has been, and is still, widely used. Moreover, it does not make more sense to express the pyrolysis results per TOC than as relative abundance (%) based on the sum of peak areas of identified compounds, as done here and all previous studies using Py-GC/MS in environmental matrix. Indeed, in others of our studies where we look at OM composition by Py-GC/MS in long sediments records (for Holocene paleo-re construction) or varved sediments (to study OM diagenesis over 30 years), we could observe that the sums of peak areas of identified compounds are always significantly positively correlated to TOC content (Figure R1 in the supplement file). In this study on the spatial variability of OM composition in Härsvatten, there is also a significant correlation between the sum of peak areas of identified compounds and LOI (Figure R2 in the supplement file). Therefore, using the pyrolysis-GC/MS data expressed per TOC or as relative abundance will give the same trends and thus the same data interpretation and conclusions. However, it is much more convenient for a reader to compare and check the trends we discussed, in term of OM composition between a large number of compounds groups and sediment samples, when the data are presented as relative abundance than as peak areas normalized by TOC or LOI.

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9. "Table 2: Give references to the information that is underlying the grouping. This table looks a bit rushed. The compound classes are not always clear and confusingly grouped. I object against the terminology used to group the compounds and the way how the grouping is organised. The authors must decide how to group the compounds and stick to a very clear well explained system of doing so. What are "Big furans"? please improve. Maleimie = Maleimide Proteins as a class of pyrolysis compounds is not possible, I suspect protein derived is meant here. Lipids is the wrong term other compounds in the list are lipids too (eg hopanoids). Aliphatics is a better label. Steroids and hopanoids have different biological origins, for a statistical approach such as what is presented here it would make a lot of sense to keep these separated. This would help with statistical analysis. A term such as lignin has a clear origin associated with it, a term such as "lipid" or "high mass compound" caries no such value. The table as given in the supplement is much more useful. It is very important to see what contributes to these categories. Without it, the methodology becomes a black box. I suggest to combine table 2 and S1. This will avoid that the reader is wonders about what might be in a "big furan" category. I would suggest to rename some of the categories and rethink the groupings. With such a small change, our PCA results are not going to change significantly."

We agree with the referee that better homogeneity in the terminology used to present the compounds groups will improve our manuscript and will be useful for the readers. We will thus rename some of the categories in the revised manuscript, and will change Table 2. However, we would like to underline that our grouping is homogeneous because it is based on similarity in the molecular structure, and that the example given by referee 2 to discuss our grouping, i.e. "Steroids and hopanoids have different biological origins, for a statistical approach such as what is presented here it would make a lot of sense to keep these separated", is not fully correct. Indeed, while we present the "steroids" and "hopanoids" within the same groups in Table 2 of the manuscript, these two groups of compounds have been kept separated for the statistical analyses (cf. Fig. 3 in the manuscript, which presents the output of the PCA analysis). The

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only major change that could be done in our grouping would be to gather the "furans" with the "big-furans", this latter group being mentioned by the referee 2 for its unclear terminology. Therefore, our PCA results are not going to change.

10. "I would like to see Retention Index values for each of the listed compounds in the table. This is very important as all compounds have been identified using the NIST database (according to the text) and this can be very unreliable. RI values together with the mass spectra (as have correctly been given) will give extra confidence. The mass spectra given are only a rough summary and do not have much value for a reader but with RI values it becomes much more solid."

Adding the retention index (RI) for each of the identified peak (compounds listed in Table S1) will effectively provide extra confidence to our identification and could be useful for future users of Py-GC/MS. We will thus add in the revised supplementary information, when possible, the RI values of the identified compounds resulting from our analyses and the corresponding reference RI values, i.e. RI values determined by previous studies using similar GC operating conditions (non-polar column and temperature-ramp GC program) which are provided by the NIST in their "NIST Chemistry Webbook" website (http://webbook.nist.gov/chemistry/) and/or in the 'NIST/EPA/NIH 2011' library included in the software "NIST MS Search v.2.0" (cf. Table R1 in the supplement file). Because the RI of a certain chemical compound is its retention time normalized to the retention times of adjacently eluting n-alkanes, we unfortunately cannot provide the RI values for each of our identified compounds. Indeed, we only detected n-alkanes from the "nonane, C9:0" which elutes at 282.2 seconds. This makes impossible to calculate the RI for compounds eluting before 282.2 s, i.e. for 15 compounds belonging to carbohydrates, acetamino-sugars or N-compounds. On the contrary, there are $\sim$50 compounds for which we have determined RI values but could not find any corresponding reference values. For the remaining ones (i.e. $\sim$70 compounds), we obtain a very good match between our determined RI values and the reference ones (cf. Table R1 in the supplement file), showing that our peak identification is very reliable. The RI

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values give for sure extra confidence, but there is a large number of Py organic compounds that can be identified but for which there are no previously reported RI values. For those, we believe that a careful comparison between the experimental mass spectra and theoretical mass spectra (available from NIST MS Search v2.0 software and 'NIST/EPA/NIH 2011' libraries) or those given in published studies is the best way to identify peaks in a reliable way. As we have discussed in our methodology paper, a compound can be proposed by the software to match the experimental spectra with a R. match value above 750, or even 800, while the unknown and proposed theoretical spectra do not obviously match according (cf. Tolu et al., 2015 ACA, 880, 93-102).

11. "There are quite a few unknowns. I would like to challenge the authors: if they are not certain about the ID of a compound, how can you put a compound in a specific category?"

First we will rectify mistakes we have made for some compounds which have been labelled as unknown (which does not mean our ID is uncertain) while the molecular structure is actually known, i.e. for levoglucosenone, levomannosan, levogalactosan, levoglucosan, Stigmasta-3,5-dien-7-one. Thereafter, there will be only 14 compounds for which the molecular structure remains unclear (over >150 identified Py compounds), i.e. anhydrohexose, 3 acetamido-4-pyrone, Oxazoline structure, diketodipyrrole, six alkylamides, and four hopanoids (Trisnorhopane, Norhopene (triterpene C29), norhopane (C30/C31?), Norhopene (C30/C31?)). The "anhydro-hexose" has been assigned to a mass spectrum corresponding to a mass spectrum reported by Faix et al., 1991 that have specifically studied the pyrolytic products of woods polysaccharides. Therefore, although we do not know exactly the molecular structure of this compound, we know this compound is a pyrolytic product of carbohydrates/polysaccharides. Similarly, we assigned acetamido-4-pyrone, oxazoline structure, and diketodipyrrole based on mass spectra reported in previous studies aiming at identifying the pyrolytic nitrogen-containing products of chitin and/or proteins. For those compounds that are based on published mass spectra instead of the

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NIST library, a reference is given in Table S1 of the supplementary information. The alkylamides are molecules made of C, H, N and O atoms which present highly specific mass spectra in electron impact-MS; their mass spectra is made of two m/z with high abundance (i.e. m/z 59 and 72) and many m/z of very low abundance including the m/z corresponding to the molecular ion of the specific alkylamide, such as 227 for tetradecanamide (alkylamide C14) or 255 for hexadecanamide (alkylamide C16). We did observe peaks with such mass spectra but identifying the highest m/z corresponding to the molecular ions was too uncertain because their signal intensity was too close from the background. Hence, we have only assigned these peaks to "alkylamide" rather than to a certain alkylamide compound. Similarly, the hopanes and hopenes are well known to have one or two important and highly specific m/z in their mass spectra (i.e. 191 and 177), but the m/z corresponding to the molecular ions are of very low abundance and too close from the background as well.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-361/bg-2016-361-AC2-supplement.pdf

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-361, 2016.

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