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Biogeosciences Discussions

Interactive comment on "A molecular perspective on the ageing of marine dissolved organic matter" *by* R. Flerus et al.

Anonymous Referee #2

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This paper presents the first large dataset on marine SPE-DOM FTICR-MS from the North Atlantic Ocean. It also attempts, for the first time, to examine those MS data in the context of bulk SPE-DOM radiocarbon signatures, total DOC concentration, total dissolved amino sugars etc.

These data are interesting and worthy of publication. However, in my opinion, the paper needs some revision. To be honest, I spent a lot of time on this review, trying to justify the interpretations in section 3, but I was unsuccessful in many cases. I raise a few key points below.

1. Molecular – the real novel contribution in this dataset is the large number of DOC samples that were analyzed by FTICR-MS. The samples also spanned a gradient in space and radiocarbon signature and so, presented the authors with the opportunity to

examine molecular signatures that changed with DOC age and other biogeochemical parameters. The authors discuss the samples in van Krevelen space but little effort is made to go beyond C/H and O/H ratios. Surprisingly, the largest dataset presented in this paper is the least explored one.

2. Normalization – The y-axis in Figure 3, the grey scale bar in Figure 7 and the x-axis in Figure 10 caused me quite a bit of frustration. The authors do a poor job of explaining how they arrived at these "scales." In particular, the discussion of normalizing peak areas to enable comparison of peak intensity with other independent analyses needs rewriting.

For example, the authors speak of a 100 ppm threshold – what exactly is this? If you can put a number on the threshold then you can certainly put a number on the y-axis in Figure 3. Without an axis, I have no way of evaluating the slope in Figure 3. What are the relative changes in signal intensity over this relatively large range in Δ14C signature. The slope is critical for determining whether these changes are real. To me this section is critical to the rest of the paper. Much of the subsequent discussion is built on this linear relationship and what it means.

My interpretation of the normalization that was performed for Figure 3 is a follows: the areas under every peak in the MS that increased with increasing Δ14C signature (i.e. decreasing "age") were summed together for any given sample, and this total area was normalized to the total MS area. This was necessary because samples were not all run at the same DOC concentration (factor of 3-9 difference across samples)? The quality of this relationship depends strongly on the variability in "normalized" magnitude for a particular peak in a particular sample. How conserved is this for a single sample over different days and instrument conditions? I think this concern is what drives other authors to focus on presence-absence rather than intensity. No information is provided on this important parameter– e.g., were duplicates run to constrain y-axis error bars in Figure 3? The rest of the paper cannot be appreciated without this information.

If the data were normalized as I imagine them to be (i.e., relative to the total MS) then aren't the peaks that increase in relative intensity just one component of a two component mass balance; the other component being peaks that decrease in relative intensity. In fact, peaks that were correlated with Δ14C accounted for 97% of the total intensity. So, if one component (e.g., POS) is related to Δ14C then the other component (e.g., NEG) should also be related to Δ14C, likely in a 1:1 ratio. I don't see how these are entirely independent relationships? I may be misinterpreting the process of normalization. If the slope is significant, then the observation that there is an intensity relationship with radiocarbon is indeed interesting (and perhaps not unexpected, given that DOC concentrations decrease and extraction efficiency remains the same). This is worth focusing on but the over interpretation that follows diminishes the value of the actual data.

p.11461 line 4: "we calculated "average" "relative magnitude" "ratios"" - I have no idea what this means (is "relative magnitude" equivalent to their previous normalized ratio?). What does the ratio of 1 represent? I apologize for my inability to understand this paragraph but I doubt I will be the only one to be confused by the language used. Basically, what I do understand is the following: the authors are trying to use their data to identify molecules that are removed with depth (100m versus >800 m) versus those that are not (i.e. same "relative" magnitude in 100 m and >800 m samples). I can't tell exactly how they used the peak areas reported in their mass spectra (i.e., what was actually measured) to make this observation. I assume they first normalized to total MS area and then averaged this value for all samples in the upper 100 m and separately, for all samples >800 m? 800 m values were divided by 100 m values? 1 or >1 means these are "preserved" but not produced? The latter is inferred (but really assumed) because total DOC and bulk age decreases with depth.

If I am even in the right ball-park with this interpretation then I recommend a simpler rewrite.

3. Mass balance - Perhaps the sections I found most frustrating were those "analyses"

that attempted to use MS peak intensities to conduct carbon-isotope mass balances. This analysis hinges on the dataset presented in Figure 3, which as I point out above, requires further scrutiny. Furthermore, it hinges on the assumption that changes in the intensity of particular FTICR peaks drive the observed changes in Δ14C of bulk SPE. This is not a valid use of FTICR peak areas. There is no way to quantify the fraction of total SPE-DOM that is being ionized and analyzed via this technique. No attempt is even made to do this – for example, how does C:N of the bulk SPE fraction compare to C:N calculated via FTICR-MS? The point here is that the peaks visible by FTICR do not necessarily represent molecules that drive the radiocarbon content of SPE-DOM. For example, all of these peaks could represent compounds with a modern radiocarbon signature throughout the ocean. Even in that case they could show an intensity relationship with radiocarbon. Of course, this would require all of these peaks to represent only a very small fraction of the PPL-resin, but there is no evidence to the contrary. In summary, the fact that certain peaks show a linear relationship in their intensity to bulk SPE-DOC Δ14C provides no information on the Δ14C signature of that compound.

There are some indications that the Δ14C signature of bulk SPE is not representative of compounds ionized by FTICR-MS. For example, the varying Δ14C signature of SPE-DOM with depth shows that modern compounds are added toward the surface ocean. (A two component mass balance would require at least 30% of the SPE DOC in surface waters to be enriched in Δ14C if the remaining mix was $\leq$ -450 per mil.) Yet, only a few MS peaks had relative magnitude ratios <0.6 (Figure 10). Also, given the large number of peaks that have Δ14C limits $\leq$ -600 per mil, one would expect a large number of peaks in the FTICR-MS to show no relationship between intensity and SPE-Δ14C (yet 97% of the MS intensity was correlated with radiocarbon).

These discussions are all geared toward showing some relationship to the reactivity continuum. However, FTICR data cannot be used to get at the distribution of ages in DOM because it provides no absolute quantitative information – something that is

required for an isotope-mass balance to be considered valid.

I don't understand the value of the Δ14C LIM exercise in the context of FTICR-MS data. I would have liked to see all the various relationships that went into generating Figure 10. How different were the slopes of peak intensity versus radiocarbon signature, and wouldn't the point be made more clearly if intensity was plotted against water mass age or depth? Would peak intensity versus density or salinity give you a linear relationship for many molecular ions? Using that relationship would it be correct to calculate a salinity/density value at which that compound was no longer present? For example, DOC is often linearly related to density or temperature, but extrapolating that relationship to a DOC concentration of 0 is meaningless.

Some specific comments.

p.11459. Neither blank information nor sample size information is provided for radiocarbon data (i.e. were they blank corrected for column bleed, combustion blanks, process blanks etc). Approximately 5L of seawater were extracted for each sample and assuming 40% C recovery one would expect surface samples to generate about 2 mg C and deep samples to generate on the order of 1 mg C. Given the C/H ratios etc the total mass could not have been much more than 5 mg for any sample. What fraction was processed for Δ14C measurements? The transfer appears to have been done in methanol since the solvent was removed under N2. A blank is typically processed for this kind of analysis. Yet, if radiocarbon samples were big enough (>200 ug C or so) then blanks may not be an issue. Thus, it is useful to provide information on the amount of carbon processed for radiocarbon measurements. How many samples from each depth range were measured? I could try to count them in Figure 2 but it is useful to provide an n=x term. p.11459. "Samples were adjusted to similar DOC concentrations" - How were dilution factors (3-9) considered when comparing intensities across spectra; was this achieved by normalizing to total MS area?

p.11460 a S/N ratio of > = to 3 was applied; a signal intensity threshold of 100 ppm

was used. Are these referring to the same thing?

p.11461. Observations described in the first paragraph of 3.1 are interesting. Lower molecular weight peak intensity increases with decreasing radiocarbon "age" – i.e., the same LMW compounds are more abundant in younger samples; compounds that ionized more efficiently and/or were more abundant increased in intensity with decreasing "age". The latter could be compounds with ES ionizable functionalities like carboxylic acids etc – so increasing oxidation state with increasing age? But this is not borne out in the VK diagrams – O/C ratios are quite similar among POS and NEG peaks, and changes in MW are driven primarily by H/C.

p.11462. Is a discussion on the size-reactivity continuum really necessary? The results will have more impact when the authors simply confine themselves to the data. I.e., within the mass range observed (200-500) and the extraction technique used, higher molecular weight peaks appear to actually increase in intensity with increasing radiocarbon age. The rest of this discussion is completely unnecessary and confusing. Hertkorn's results are not accessible to the reviewer so cannot be evaluated in this context. Which trend are they referring to? LMW DOM decreases with age or compounds more susceptible to ionization increase with radiocarbon age?

It is curious that most compounds that are POS related to radiocarbon have essentially the same H/C ratio (differing primarily in O content). This is somewhat true for the negatively correlated peaks as well. Are these compounds structurally related? Could some of them be fragments of larger molecules – this could explain the linear relationships with radiocarbon

Paragraph beginning on line 11. Some examples of compounds that fit into the VK space being discussed would be useful. The paper would also benefit from some simple conclusions (if they are accurate): Do compounds that dominate the deep ocean SPE extractable, ES ionizable DOM reservoir appear to contain a greater number of double bonds? This could be consistent with the conclusions of Lam et al (2007). Line

26. Provide examples of compounds that have an H/C of 1.27 versus 1.25. Are these values significantly different? This paper provides the largest “chemical dataset” for DOM but makes no attempt to discuss the types of molecules that are present – it is quite frustrating.

(Figure 7). Some notation should be added to the greyscale bar to show that high ratios correspond to samples that are considered to be “not or less degraded” and low ratios correspond to compounds that are considered to be “removed” with depth. The figure caption is not clear enough to meaningfully describe the trend

p.11463. Line 1 – what ratios are being referred to here (in Figure 7)? Elemental ratios? Greyscale ratios? What is meant by continuum of reactivity? Only two groups of “reactivity” were examined – “younger” and “older” right? Or are the authors referring to the “continuum” of H/C ratios? This discussion needs to be less abstract.

The point made in Line 5 is what I raised earlier. It is odd that no change in O/C is observed with age/depth.

Line 9-10. Hertkorn suggested increasing CRAM with depth and these authors observe a similar trend in VK space here as well . However, to make this more explicit it would make sense to change the greyscale caption to be more reflective of a ”process” (as noted before in my review). p. 11464 I don’t see the need for both IÂňDEGÂň and Δ14CCALC. Certainly both contour plots are not needed; and the parameters are related – both rely on the relative change in intensity of POS and NEG peaks. I haven’t quite figured out why the index has 100 and +1 in the denominator – seems more complicated than necessary. Also seems that it should be normalized to a particular depth.

It should be explicitly noted here that amino sugars were measured on bulk DOM and not SPE extracts (at least this is what the methods currently imply). How does the relationship in Figure 9a compare to the relationship of amino sugar concentrations (yield) to depth?

The paragraph starting on line 22 is not particularly illuminating. Bacterial activity is high in a region with high DOC concentration and enriched Δ14C signature – and this somehow provides evidence to indicate bacterial reprocessing of SPE-DOM? In fact, their calculated IDEG is low at this site, suggesting, in their interpretation, the presence of DOM that has not been degraded or altered by bacterial reprocessing. This again strikes me as a misplaced sentence.

p. 11465 section 3.3. Were keeling plots constructed using total DOC concentrations or the calculated concentration of SPE-DOC? The fresh endmember contribution should, by definition, have the same radiocarbon signature in all and any DOM fraction being examined. In Table 3, provide information on the quality of the fit to the data. Line 13-16 - The authors have lost me again here with regard to the relationship between keeling plots, reactivity continuum and relative magnitude ratios. Keeling plots typically assume the presence of only two radiocarbon endmembers.

Last paragraph. I get lost trying to understand how the authors are interpreting this linear relationship. For example, this relationship potentially tells you nothing about the Δ14C signature of the ion of interest. As mentioned above, mass balancing without quantitative information is impossible to justify.

p.11466. There are many assumptions in this last paragraph that are not necessarily supported by the data. Authors state that SPE-DOM represents a fraction of DOM for which most compounds are expected to persist on one or more cycles of ocean circulation – why is this assumed? The authors use the Keeling Plot analysis to demonstrate that a fresh, modern component is added to this SPE fraction to modify its radiocarbon signature. It is possible and likely that these compounds don’t appear in FTICR-MS, but they are nevertheless present in SPE-DOM. Old carbon recycling – I am not sure what this refers to but the Druffel, Williams, Bauer et al datasets on radiocarbon are the only ones to directly demonstrate that “old” DOM is mixed throughout the water column. “Continuous distribution of 14C ages” – as pointed out earlier, the results presented here cannot address the radiocarbon distribution in DOM. In my opinion, the

reactivity continuum is not well addressed by this dataset – I am not convinced that Figure 10 is meaningful.

Finally, this dataset has the potential to provide some interesting molecular-level information and it frustrates me to see that being abandoned in favor of over-interpreting the radiocarbon data.

p.11467. There is entirely too much speculation in section 3.5. High bacterial activities in the surface are not unexpected, and yes, refractory DOM may be produced at the surface, but the data presented in this paper cannot address this explicitly. The high abundance of masses with low reactivity could just be related to the fact that refractory compounds are well mixed throughout the ocean. There is no reason, based on the data presented here, to suspect that these compounds are actually being produced in the surface by bacteria. Were primary production rates measured? Chl a concentration? These parameters may also be related.

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