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Interactive comment on “High field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter from the South Atlantic Ocean” by N. Hertkorn et al.

Anonymous Referee #2

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This manuscript represents an important advance in analytical organic geochemistry. It combines highly sophisticated ^1H and ^{13}C NMR techniques with the most powerful mass spectrometry method to probe subtle features in the composition of dissolved organic matter (DOM) from multiple depths at one site in the South Atlantic. While only one site was sampled, the depths from which the samples were obtained (near surface photic zone, fluorescence maximum, upper mesopelagic zone and near bottom at 5440 m) are likely of general interest to the marine geochemistry community. Given

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that the intensity of the analyses utilized was so significant and that broad temporal and/or spatial characterizations were thus precluded, I believe the authors have chosen their sampling strategy wisely.

As noted, the primary contribution of this manuscript is in the detailed investigation of “advanced” analytical NMR and mass spectrometry. The descriptions of the NMR experiments and the resulting data they provide will be particularly useful for future researchers in this field. The same cannot be said for the mass spectrometry analyses, as high-field FT-ICR MS is now a “maturing” technique and the authors do not provide any really new insights in its application to DOM. However, the combination of multiple ^1H and ^{13}C experiments with the formula data from FT-MS allow the reader to better understand the relative strengths and weaknesses of these complimentary advanced methods.

I can understand that some if not most readers of Biogeosciences may not fully appreciate this article in its entirety and may not see it as appropriate for this journal. Indeed, this reviewer, moderately versed in NMR methodology, had difficulty following the elaborate discussions of structural complexity that are based to a great extent on NMR theory and NOT on additional analytical chemical data. However, I do think this essentially methods paper is important and does belong in a journal such as Biogeosciences.

The manuscript is of generally high quality, well-written, and I can find little in the experimental methodology that I can argue with. The NMR experiments in particular are almost breathtaking in their scope (i.e. ^1H and ^{13}C multiple pulse sequences), resolution (e.g. high field 800 MHz used), and interpretation. I thus will not provide detailed comments to any significant degree on those experiments. My comments are more general and are intended to provide the reader with some context as to the significance of the data interpretations and conclusions.

1. Although the authors correctly note in the Introduction that depth profiles of DOM

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composition are a complex function of many variables, and not just simple downward mixing (e.g. downward flux and processing of particulate DOM, long-range horizontal transport) they do occasionally try to link surface, photic zone and deep samples in their discussions of processes that produce the differences they observe. They really should discuss the molecular characteristics they observe as being depth-specific and not as being site-specific. 2. DOM was isolated and concentrated from large volume sea water samples by solid phase extraction (SPE), and to the authors' credit they remind the reader at several junctures that they are describing SPE-DOM. However, some additional discussion of how representative such sampling is, particularly with regard to the ^1H NMR measurements which are "near quantitative", would be useful. "Likely discriminated against carbohydrates" is not sufficient. There are likely compounds which are selectively isolated as well. It should be noted that they report (probably accurately) $\sim 40\%$ recovery. 3. I find the discussion in section 3.10 to be quite speculative. While "black carbon" type molecules have certainly been observed by others, I do not think the authors can really identify such black carbon with the low S/N of peaks in the aromatic region. There is a large background of aromatic materials in these samples and to assume they are thermogenically derived without further evidence is a stretch. 4. The discussions in section 3.12 are fraught with potential problems, primarily because of the selective nature of electrospray ionization (ESI). While I appreciate the fact that they are attempting to make relative comparisons of oxygen content between the samples (i.e. trends) and not report absolute oxygen content, they still cannot ignore selective ionization and ionization suppression in ESI. For example, CHOS compounds will ionize much differently than simple CHO compounds, and variations in composition of these species can alone account for observed differences in H/C and O/C ratios. This is particularly true when using intensity-weighted peaks in the elemental composition calculations. 5. Again, I have doubts about average mass calculations from MS data. I assume these are also based on intensity-weighted peaks, which can be notoriously unreliable. Furthermore, it is highly unlikely that a 10 Da difference is significant. Indeed, the authors even admit this in section 3.12.1! 6. Finally, the authors argue that

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all other methods for characterizing DOM are unreliable because they “rely on transfer functions of mainly unknown shape and amplitude.” I agree with that statement, but disagree with their argument that NMR and FT-MS are without such transfer functions. The first such transfer function is the SPE sampling, which we know of and which the authors readily admit provides an initial and highly selective transfer function. Clearly, SPE is necessary and I am not suggesting that it invalidates such studies. Indeed, such advanced analytical characterizations as provided here would not be possible without such an elegant and highly-concentrating method. But it does impress upon all subsequent measurements a certain bias. In my own work we have noted high selectivity for aromatic, lignin-like compounds and low selectivity for carbohydrates with the same PPL cartridges used here.

The “transfer functions” imposed by ionization method in FT-MS are provide also problematical. Indeed, these authors have published on this and I am surprised they have ignored it here. For example, they know that many if not most of the dissolved organic nitrogen component of DOM is invisible in negative-mode ESI. The biasing in FT-MS can be greatly reduced with multiple ionization experiments (e.g. –ESI, +CI, +APPI).

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