

Interactive comment on “Molecular characterization of organic matter mobilized from Bangladeshi aquifer sediment: tracking carbon compositional change during microbial utilization” by Lara E. Pracht et al.

Anonymous Referee #2

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GENERAL COMMENTS

The present study builds on an earlier paper by the group which examined microbial utilization of sedimentary organic carbon upon disturbance (physical homogenization). Here they utilize high resolution mass spectrometry to determine the organic carbon chemistry during microbial processing of the carbon. There are many things to like about the study. Expanding the science of organic carbon in sediments and soils is of importance of understanding the processing (and inversely, storage) of carbon within the subsurface, how it impacts water quality (chemically and biologically), and potential

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impacts on atmospheric gas exchange. The mass spectral technique utilized within the study offer a means of examining organic carbon with unprecedented resolution, although there are many caveats that the authors should be careful of and be cautious with their conclusions, particularly without secondary supporting techniques. The physical displacement of the organic carbon provide great insight into what is essentially hiding in the sediments, unavailable for microbial decomposition. Following the displacement with incubation studies helps to confirm that these compounds are decomposable, but not in the physically unaltered sediments. This is an important insight that should be the highlight of the manuscript.

The downside of the manuscript, as it presently stands, is that the primary conclusion, and, in fact, the central theme, is that carbon displaced by physical homogenization will lead to its decomposition by microbial action. There is just no way for me to conceive how sediments 9-m below the ground surface could be homogenized, and making this a central theme of the manuscript is highly problematic. However, by reversing the concept and considering this an examination of the carbon stored in the sediments, the study not only moves to solid ground (no pun intended), but the displacement and subsequent microbial utilization of the sediment organic carbon becomes a highly clever means to illuminate the chemistry of the compounds. I would therefore strongly suggest that the authors consider inverting their view of the central theme and making the paper about the chemistry of organic carbon within the Holocene aquifer of Bangladesh.

One final general comment is that there seems to be a number of locations in the manuscript where microbial processing of organic carbon is not correctly portrayed. I would therefore also suggest the authors seek to ensure microbial and biochemical accuracy (possibly consulting with a microbiologist).

DETAILS

Page 1

Lines 17-18: Organic carbon is not fermented into methane. It's at two (to three) step

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process where first the organic carbon (that less than 600 Da) is bacterially fermented (sometimes with a secondary fermentation step), then the metabolites are respired by archaea to methane (methanogenesis).

Lines 19-20: Stick with abiotic sulfidization; as noted below, the methanogen pathway is not warranted here.

Line 22: Here and elsewhere, the term or inference of “recalcitrance” needs to be clarified. As noted in citation later in the manuscript (Schmidt et al, 2011; Lehmann and Kleber, 2015), “molecular recalcitrance” has become an antiquated notion and should be placed in the correct context here. In short, the new realization is that recalcitrance is an ecosystem specific feature. As summarized in Schmidt et al. (2011), lignin and other complex aromatic structures degrade at rates not dissimilar to many starches.

Page 2

Lines 10-11: Again, the inference to recalcitrance needs to be clarified (or avoided).

Lines 22-23: same comment as for lines 17-18 on page 1.

Line 30: Exoenzymes are indeed needed for depolymerization but not necessarily to monomers. The size cutoff is the critical factor and is largely considered in the 600 Da range (as noted in the manuscript).

Page 3

Line 7: The analysis provides insight into the chemistry of the DOC; it does not, however, translate to the bioavailability, which is a far more complex pathway that varies between specific organisms. Even bioaccessibility is not really tracked here—possibly the lack of bioaccessibility is obtained.

Lines 16-17: I think this statement of “aquifer sediments” is splitting hairs. There is an abundance of work done on marine sediments that should be noted, and which could help guide the authors to stronger conclusions, and an extensive body of literature

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on soils is also available. For marine sediment analogies, Hedges and co-workers, for example, have done extensive work that has been, in my opinion, groundbreaking and could help with the interpretation here. Also, two recent papers using FT-ICR-MS (Bailey et al., *Soil Biol Biochem.* 2017, 107: 133-; Boye et al., *Nature Geosci.* 2017, 10, 415-), in which one of the authors was involved, could also be helpful and describe organic carbon physically isolated in soils and thermodynamically protected in terrestrial sediments.

Lines 22-32: How long were the samples stored? And at what temperature?

Line 24: Homogenization of the sediments alters that physical accessibility massively. As noted in Bailey et al. (*Soil Biol Biochem.* 2017, 107: 133-), the chemistry of organic carbon changes with pore-size. Adding in the displacement of physically occluded organic carbon, and the system has been changed massively. This can be used to the authors advantage, but a realization that no disturbance can release this proportion of the OC needs to be made. Rather, it provides an ability to access what is not being processed by the microbiota.

Page 5

Lines 17-: What proportion of the mass spec data was successfully assigned? Or, in other words, what proportion of the mass spectral data were left unassigned. This is a really important aspect of the analysis when (semi-) quantification is being attempted.

Page 6

Line 25: This is already an outdated concept. The present manuscript notes this later in the references to Schmidt et al. (2011) and Lehmann and Kleber (2015), and the authors should hold to the updated theory of sediment/soil organic carbon.

Page 7

Line 22-23: The process described here for microbial metabolism is not correct. Catabolism is the transfer of compounds into useful energy. The authors are correct

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that for the organisms to utilize carbon compounds for catabolism or anabolism they must be less than ca. 600 Da. However, to decrease polymer size, extracellular enzymes are (typically) used, and this is independent of catabolism. In fact, catabolic energy is needed to synthesize the molecules, and thus would be an endergonic process.

Line 27-29: As noted in the M-M section, FT-ICR-MS is not quantitative, and thus providing the % differences should be done with caution.

Line 30: Once again, the concept of chemical recalcitrance is now antiquated and should be placed within the ecological context of the environment (see Schmidt et al., 2011, for example).

Page 8 Line 6: "thermodynamically accessible" is not an appropriate description. A reaction is either thermodynamically viable or it is not—there is no middle ground. And accessible is not a term that would equate to viability. Accessible leads one to think of physical access rather than biochemical viability.

Line 25: There is more than fermentation and methanogenesis happening here. Dissimilatory sulfate reduction would be taking place and other respiration may as well (DIRB, for example).

Lines 32-33: Are the number of compounds identified meaningful given the noted limitation of the FT-ICR-MS approach?

Page 9

Line 15-16: I don't understand this statement. If I look at Figure 2 and compare DOC levels for pond water (b) to rice (d) for the biotic incubation, they look exactly the same; for the abiotic incubations, the rice paddy water created great DOC than the pond water, which is the opposite of the text.

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Line 4: This is (at least) a two-step process: Fermentation and then methanogenesis.

Line 13: Consider the concepts in these referenced (Lehmann and Kleber, 2015; Schmidt et al., 2011) earlier in the manuscript, and place your findings within the context of current organic matter processing paradigms.

Lines 17-27: This is an excellent paragraph.

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Lines 9-10: I would recommend removing the implication of methanogenesis in sulfidization of organic matter. The sulfur enzymes would not be sufficient to provide this signal.

Line 18: Again, remove "fermented into methane". The organic carbon compounds are fermented, and then methanogenesis transpires.

Line 18: The posed "If this pool of SOC were destabilized in situ" is an important point. How would the organic matter be destabilized? In the Neumann et al. (2014) study, the sediments are homogenized, leading to the release of organic matter that is then subject to microbial degradation. It is interesting that the organic matter is rapidly consumed but not surprising. Presumably the OM is physically isolated, or partially mineral protected, and not available for microbial utilization. Residing 9 m below the surface, it is hard to imagine a process that would lead to destabilization. As such, I would recommend moving away from this position and instead focus on the interesting aspect of its chemistry—the metabolism of the OM advances our understanding of their composition and that a protection mechanism must be in play.

Lines 20-26: I don't see the present study supporting the conclusions drawn in this paragraph. The summary provided in lines 27-31 are reasonable and should remain the emphasis. Albeit that I support reasonable speculation, the extension of a homogenized sediment release well-protected organic carbon as an inference into field setting is not warranted. As noted in the comment above, the power of this study is

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in characterizing the OM that is protected, not in biogeochemical processes that follow unrealistic release upon physical homogenization.

Page 12

Line 17: The authors are correct to compare their finding to the soil science literature. It's important to note that there is really a fine line between subsurface soils and sediments. In fact, when soil scientist describe a C horizon (or horizons), they have effectively crossed the boundary into sediments. Top soils that undergo greater input and turnover are a different story, but the subsurface across depths is more similar.

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