

## ***Interactive comment on “Molecular insights into the microbial formation of marine dissolved organic matter: recalcitrant or labile?” by B. P. Koch et al.***

**Anonymous Referee #2**

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The manuscript titled “Molecular insights into the microbial formation of marine dissolved organic matter: recalcitrant or labile?” by Koch et al. clearly showed microbial transformation of DOM, in mimicked Antarctic surface seawater. The experimental designs are ambiguous, such as the determination of element compositions of  $^{13}\text{C}$ -labeled non-labile DOM in the  $^{13}\text{C}$ -labeled glucose incubation experiment; Ultrahigh resolution mass spectrometry (FT-ICR-MS) analysis; The long experimental time duration (2 years). The results are useful and the data are valuable for inferring the functions and mechanisms of microbial transformation of marine DOM and carbon sequestration, supporting the newly proposed “Microbial Carbon Pump” conceptual framework. One of the most impressive findings is that “After 2 years, the molecular patterns of DOM

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in glucose incubations were more similar to deep ocean DOM whereas the degraded exudate was still different”. Overall this work is an important contribution to better understanding how microbes work on different organic matter toward different outputs and its implications in carbon cycling and sequestration in the ocean.

Major concerns: 1. The author noticed the cell size diverged, but they ignored the shift of community during the long term incubations. The natural community structure could collapse and reform more than one time. It could also be possible for specific populations to go extreme in the single carbon source incubation. Furthermore the acclimated populations in a sealed system may lose many metabolic ability for DOC compounds. Such issues should be discussed and considered for concluding the experimental outputs and implications. For example, the important conclusion “the molecular patterns of DOM in glucose incubations were more similar to deep ocean DOM whereas the degraded exudate was still different” lacks in depth interpretation. It would be much more convincing if the authors provided community structure information before and after the long term incubation with different carbon sources. It is not difficult to do phylogenetic and even metagenetic analysis anyway.

2. It would be nice to examine the chemical composition of the algal exudates. Compared to glucose, Exud may be more likely to be structural materials for bacteria. (The analyses based on the saturated and reduced states also proved that). Glucose is the main or core material in TCA cycle, it could be either energy source or sub-material for synthesis of many other compounds that are essential for bacterial growth and metabolism. In addition, extra nutrient was introduced in the Exud incubation, the lower C/N ratio might influence the microbial activities including carbon uptake. The steady nutrient concentration (especially ammonium concentration) in the Exud incubation also gave some clues.

3. The conclusion that “higher substrate levels result in a higher level of non-labile DOC which is an important prerequisite for carbon sequestration in the ocean” should be carefully derived from the specific experiment in the present work and through dis-

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cussion based on the literature.

4. The title "Molecular insights into the microbial formation of marine dissolved organic matter: recalcitrant or labile?" seems to focus on "lability" of microbial-formed DOM, which doesn't fit the contents and conclusion very well.

Specific comments: 1. Page 3067, Line 27 to Page 3068, Line 8: The ability or inability of the in situ microbial community to express membrane transporters for DOM uptake may also contribute to DOM degradation. 2. Page 3069, Line 18: There may be a need to give some explanation about the contribution of nitrogen to the refractory nature of DOM in ocean or some reasoning about the need to know the incorporation of nitrogen into DOM. 3. Page 3070, 2.2 Preparation of experiment: Did artificial seawater include some essential trace elements? Some microbial enzymes need certain trace elements to be functional. 4. Page 3071, Line 5: Is *Isochrysis galbana* a dominant microalgae in the Antarctic surface seawater. How close is its secreted DOM to the in situ algae-DOM composition? Does the source of algal DOM have any influence on the DOM degradability? 5. Page 3071, Lines 16-17: The inoculated seawater has already been stored for 5 months. Would the storage influence the microbial community and physiology, and thus influence the microbial DOM transformation performance? 6. Page 3078, Lines 6-7: please explain the cause of "Nitrate, nitrite and phosphate remained almost constant in all treatments". 7. Page 3080, Lines 4-5: "BGE was comparable in the treatments which contained glucose (0.1) and substantially higher in the [exud] treatments (0.6)". Does this mean labile DOC stimulates microbial respiration more strongly? This is actually consistent with some previous observations. You may also see a recent review on the relationship between DOC and microbial respiration (Dang et al, 2014. *Biogeosciences Discuss* 11:1479-1533). It seems necessary to discuss the points in this paper. 8. Page 3085, Lines 10-12: Was *I. galbana* cultivated in axenic condition? 9. Page 3086, Lines 25-28: Morphology is not good enough to distinguish microbial composition. Actually there is a need to characterize the microbial composition by molecular or even metagenomic method, at least in future investigations. 10.

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Page 3087, Lines 12-20: Maybe the added labile DOM lacks proper N content? Will adding nitrogen-rich DOM show priming effect? 11. Page 3087, Lines 21-22: It seems that the C:N ratio of the DOM may be important for TEM production.

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