**Author response to reviewer's comments**

We would like to thank the two anonymous reviewers and Dr Benner for the helpful, constructive suggestions and positive comments. In the revised version of our manuscript, the suggestions will be addressed as follows:

**Referee 1:**

*Abstract: the fact that priming was not observed should be noted in the abstract.*

In our revised manuscript, we will add a statement on the results of our priming experiment.

*3068/9-23. The term "TEP" is used, but the description in part appears to be that for microgels; certainly the reference to Verdugo papers suggests gels while the mention of aggregation and vertical flux suggests TEP. Is "TEP" being used for both true gels and standard TEP particles? Please revise for clarity.*

On a chemical level, the terms TEP and gels are not well defined. It is likely that TEP and microgels, at least in part, comprise the same subset of molecules. Our statement in the introduction will be clarified in the revised version of the paper.

*3069/6: the word "transformations" should be singular*

Corrected.

*3069/7-11: I do not understand "a better mechanistic understanding of kinetics". Kinetics deal with rates, not mechanisms. The use of the word "kinetics" in this section is confusing. How are "kinetics..crucial for …conservation.."?*

We tried to state that knowing the molecular transformation processes would allow a better understanding how easily a substrate can be transformed into refractory material. The degree of bioavailability inherently also affects bulk DOC kinetics/transformation rates. We agree that our statement was slightly misleading. In the manuscript, this will be primarily addressed by replacing the term "kinetics" by "processes".

*3069/14: only one goal is given yet "main goals" is written.*

Corrected.

*3078/25: "(Fig. 4)" should be Fig. 5, right?*

Corrected.

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**Referee 2:**

The manuscript titled "Molecular insights into the microbial formation of marine dissolved organic matter: recalcitrant or labile?" by Koch at 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 $^{13}C$-labeled non-labile DOM in the $^{13}C$-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 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 convincible 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.*

> We completely agree that microbial community succession likely influences molecular changes as demonstrated in many previous studies (e.g. Herlemann et al. 2014; PloS ONE). We tried to address the potential impact of community structure changes in our last statement (3092; l 14) and will put more emphasize on this issue in the discussion of our revised manuscript. However, samples for subsequent community structure analyses are, unfortunately, not available.

*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.*

> The molecular elemental composition of the original algal exudate is discernible from the sterile control ($^{sc}$[exud]). All molecular changes in [exud] treatments, presented in Figure 5, were displayed as relative changes compared to this control.

*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 discussion based on the literature.*

> We agree that our statement in the abstract was probably generalizing too much. It is clear that a multitude of parameters can influence the long-term decay of marine DOM (as discussed in chapter 4.4) and it is one of the great challenges to find a scientific approach to explore these potential changes. In our manuscript, we will tone down the statement in the abstract to match it with the discussion in chapter 4.4. We will add following statement: “For our experimental setup (and similar previous studies), […]”.

*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.*

> As the title includes the terms “recalcitrant” and “labile” - and both are key topics in the aims and discussion of the manuscript - we would prefer to stick to our original title.

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.

> We revised our original statement:
> ”The ability or inability of the in situ microbial community to express membrane transporters for DOM uptake may also control 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.*

> We rephrased the statement:
> “The relative contribution of nitrogen and sulphur heteroatoms in organic matter can determine bioavailability. Therefore we also investigated their incorporation into persistent 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.*

> The artificial seawater medium did not contain trace elements because the standard trace element (and vitamin) solutions would have introduced additional organic compounds (such as EDTA). However, the substrate of the [DOM] treatments might have contributed trace elements as part of the f/2 medium which was used to grow *Isochrysis galbana*. However, a molecular mass that matched EDTA was not found in our spectra.
> The priming experiment suggested that the microbial community was not generally trace element limited because microbial growth and degradation of glucose was still possible. However, we agree that trace element limitation might play a role in the degradation of the non-labile DOM in the experiment. Therefore, we will include additional statements on the role of trace elements in the introduction and discussion (chapter 4.1).

*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?*

> Isochrysis is not a dominant species in Antarctic surface seawater. We considered our exudate substrate to be freshly produced dissolved organic matter. It is possible that there are deviations from the in situ DOM composition in Antarctic surface water. This, however, could not be addressed within the scope of our experiment.

*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?*

> As we did not monitor microbial community changes in the inoculum, we cannot comment on this. However, in the discussion we tried to address that community changes are very likely to be important for the transformation quality and performance.

*6. Page 3078, Lines 6-7: please explain the cause of "Nitrate, nitrite and phosphate remained almost constant in all treatments".*

> We added a statement on the results of the inorganic nutrients in the discussion.

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.

On page 3087, we discussed the differences in bacterial growth efficiency (BGE) between the two treatments. In consistence with previous studies, we think that the differences in nitrogen content are probably most important for BGE within the first weeks of the experiment. Our BGE calculations were based on POC concentration which was below detection after the first phase of the experiment. Therefore, we could not accurately calculate BGE for the non-labile DOM produced in the experiment. However, the degradation rate of the non-labile DOC was faster for [exud] compared to [Glu] (see 3086). In our revised manuscript, we will discuss this observation in the context of the MCP.

*8. Page 3085, Lines 10-12: Was I. galbana cultivated in axenic condition?*

The original algal culture was supposed to be axenic. However, after filtration, the exudate substrate was stored cool for approximately 10 days until the start of the experiment. We think that the filtration was not completely sterile so that microbial degradation occurred within this period. For clarity, we will slightly revise our statement on page 3085:
"This suggests that the algae exudates which were added to the [exud] treatments were already degraded when the experiment started. Obviously the most labile fraction of the exudation products was utilized immediately upon their release or during storage, before our experiment."

*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.*

See comment above.

*10. Page 3087, Lines 12-20: Maybe the added labile DOM lacks proper N content? Will adding nitrogen-rich DOM show priming effect?*

This is of course possible. Many other factors could potentially yield different results - such as addition of trace elements, changes in temperature etc. To test this hypothesis additional experiments would be necessary. For our interpretation, we can only stick to the given experimental setup.

*11. Page 3087, Lines 21-22: It seems that the C:N ratio of the DOM may be important for TEP production.*

In our experiment, we did not follow the dynamics of the bulk or average molecular DOC/DON ratio. This would be a prerequisite to relate these parameters to the more detailed dataset of TEP dynamics. However, this would be an interesting aspect to follow in future studies.

**Ron Benner**

Long-term laboratory experiments were used to explore the production and decomposition of marine DOM. Changes in the concentrations of DOC, TEP and total amino acids were monitored along with measurements of the composition of the DOM using ultrahigh resolution mass spectrometry. The main conclusions of the study are that microbial production of DOM is dependent upon and proportional to the amount of labile DOM, TEP is rapidly degraded and does not accumulate, different substrates can lead to different forms of refractory DOM. It is interesting to note that the addition of a labile substrate, glucose, to incubations with refractory DOM did not enhance the degradation of the refractory DOM, indicating microbial utilization of refractory DOM was not limited by an energy and carbon source. These results do not support the hypothesis that refractory DOM can be degraded through cometabolic processes. They are consistent with the hypothesis that the composition of refractory DOM is the primary factor limiting its degradation.

The incubations received an inoculum (<3 um) that included a diverse assemblage of microoganisms (e.g. bacteria, protozoans, viruses). In section 2.5 it is stated that 2 samples were collected on day 28 and analyzed for the presence of flagellates. Both samples did not have detectable flagellates. This is surprising, so it is important to provide more details, such as the volumes filtered, vacuum applied, and how this procedure was specific for flagellates (i.e. not ciliates). Is it possible flagellates and ciliates passed through the 0.8 um pore-size filter? Are the authors stating there were no flagellates or ciliates in these incubations during the experiment? How do you know TEP was formed by bacteria and not by other microorganisms?

> We think that the dynamics of TEP formation and DAPI counts, especially in the [Glc] treatments, suggest that TEP was formed by bacteria. However, as we did not monitor other microbial species there is no ultimate evidence. We will expand on these important aspects in the discussion of the revised manuscript. Also, we will supply more details on the methodology for the flagellate test.

It would be informative to present the concentration and compositional data from the total amino acid analyses (e.g. Fig. 3).

> As most amino acids were near or below the limit of detection we decided to omit the compositional amino acid data in our original submission. The amino acid composition (on the three days we analysed AAs) was dominated by Gly, Glu, and Leu. In the revision, we will add a statement on these results.

*Figure 5 should come before Figs. 2, 3, 4.*

> This will be changed in our revised manuscript.

*Figure quality and text size should be improved (Fig. 2, 5).*

> Corrected.

*The abbreviation used for glucose (Glu) is commonly used for glutamic acid. A different abbreviation (Glc) should be used.*

> Corrected.

*The long lag phase (16 d) before glucose utilization is interesting (Fig. 5a). What was the duration of the lag phase after addition of glucose at 699 d?*

We assume that the long lag phase can mainly be attributed to the low incubation temperature (0°C). However, for an accurate description of the growth dynamics towards the end of the experiment the sampling frequency was too low.

Does TEP-C dynamics follow those of DOC? How significant is TEP-C/DOC?

TEP (in µg $X_{eq}$ $L^{-1}$) and DOC (in µM) were not correlated. For a previous version of our manuscript we tried to address the issue of TEP-C but omitted it eventually in the submitted version:

For the organic carbon budget, we tried to estimate the amount of TEP-derived carbon (TEP-C): The contribution of organic carbon in xanthan gum monomers ($C_{35}H_{49}O_{29}$) is 45% (w/w). The maximum TEP production, e.g. in the $[Glu]_{21}$ treatments (1,683 µg $X_{eq}$ $L^{-1}$) would therefore be equivalent to 63 µmol TEP-C $L^{-1}$. This value exceeded the POC concentration on the same day (30 µmol C $L^{-1}$). Similar results were obtained for the other treatments as well. It has been previously pointed out that the calibration with xanthan gum can only yield semi-quantitative carbon data (Passow 2002). Parts of this discrepancy might be explained by the differing filter pore sizes used for POC (0.2 µm) and TEP (0.4 µm). It is more likely, however, that the composition of TEP produced in this study differed from TEP which is produced by e.g. diatoms (Engel and Passow, 2001) leading to different absorption efficiencies at 787 nm.

The discussion of the reactivity of different components of DOM is somewhat weak and confusing. The terminology is awkward: labile, bioavailable, non-labile, and bioavailable non-labile, refractory. Fewer terms and clearer definitions would improve the manuscript.

We completely removed the term “bioavailable” as it was used as a synonym for “labile”. The expression “bioavailable non-labile” was a typo and changed to “labile”. The term “non-labile” was introduced to differentiate DOM generated in the experiment from “refractory” fractions, which are generated on time scales beyond those applied in our experiment. We will try to streamline the statements on reactivity in our revised manuscript.