

## **Referee #1:**

We would like to thank anonymous referee #1 for the constructive comments and opinions on the manuscript.

With respect to the novelty of the manuscript, there are two points we would like to highlight. Our study provides new knowledge on microbial production and transformation of biomolecules in the Ocean. First, we show that microbes grown from glucose release neutral sugars with a certain signature (mol% glucose, galactose etc.). This signature does not depend on initial DOM source (DOM and glucose or just glucose) or initial microbial community (Arctic or Atlantic). This result has never been reported previously in the literature. Ogawa et al. (2001) also incubated artificial seawater spiked with glucose and investigated the change in biomolecules, but they did not include different treatments as we did. Also, they did not report the molecular composition of neutral sugars released by the microbial community. They only reported the change in neutral sugar yield over time. Hence, our study is the first to present and discuss the molecular composition of neutral sugars released by microbes grown from glucose.

Second, we show that the molecular composition of neutral sugars and amino acids approach a common endpoint after 32 days. This result is new and remarkable taking the different starting conditions into account (natural or artificial seawater, glucose spiked or not, Arctic or Atlantic microbial community). The common endpoint indicates that microbes leave a similar microbial fingerprint of semi-labile or refractory biomolecules irrespective of starting substrate (semi-labile and refractory compounds present in the natural samples when collected or glucose in the artificial samples).

Neutral sugars and amino acids are found in very low concentrations in seawater which makes them very difficult to measure, and reliable measurements are therefore scarce. Therefore, only few incubation studies investigating the microbial production and/or transformation of biomolecules exist. We have rewritten the introduction to better highlight the current knowledge and gaps. This should hopefully improve the readability of the paper and make it more clear what the novelty of our study is.

Reply to specific comments:

Title: We agree that the term ‘bacterial production’ is likely to be misunderstood and have changed the title to ‘Production and transformation of dissolved neutral sugars and amino acids by bacteria in seawater’. The term ‘seawater’ (or the like) is necessary to point out that the paper deals with the marine environment. We have not been able to come up with a better term, and ‘seawater’ cannot be replaced with ‘ocean’, since that would imply an in situ study rather than an incubation study.

P6152: It is now stated in the abstract that the incubated seawater includes artificial, Arctic and North Atlantic seawater.

P6152, L13: It has been clarified in the abstract that the paper investigates combined neutral sugars and amino acids rather than just free biomolecules.

P6152, L24: As mentioned in the abstract, the D/L ratios generally increased during the incubations (although, from very different starting values). The important point here was not the exact quantitative change but rather the fact that all treatments (NSW, NSW<sub>glu</sub> and ASW<sub>glu</sub>) increase and end up having almost similar D/L ratios of the four individual amino acids. The numerical values are stated in the abstract to keep the abstract consistent, since neutral sugar values are stated as well.

P6152, L22: We have rewritten the sentence so that it does not include a reference to the microbial carbon pump.

P6152, L22 + L23: By 'long' we do not mean 32 days but rather years, hundreds of years or maybe even thousands of years. The point is we find that semi-labile and refractory biomolecules (those that are left in the natural seawater samples after 32 days) are almost similar to bacterially produced and altered biomolecules (those that are left in the artificial seawater after 32 days). The natural seawater samples on day 32 represent the leftover semi-labile and refractory biomolecules (which were probably present in the samples when collected in the ocean) and the artificial samples on day 32 represent bacterially-produced and degraded biomolecules. The fact that there is a close resemblance between these two types of treatments suggests that a fraction of bacterially-produced biomolecules are semi-labile or refractory – at least, they appear alike. We have tried to clarify this in the abstract by pointing out that the natural samples represent 'semi-labile and refractory DOM' and artificial samples represent 'bacterially-produced DOM'.

P5164, L25: Bacteria are well known producers and consumers of D-amino acids in the ocean. We have clarified in the text, that the balance between sources and sinks is an important factor in shaping the D/L ratio observed in the ocean.

P5164, L27: Instead of referring to the samples as Arctic and Atlantic in the introduction, they are now described as 'sampled from water masses originating from the surface water of the Arctic Ocean and the Atlantic Ocean'. We have kept the names 'Arctic' and 'Atlantic' throughout the remaining of the paper, but it is now clarified in the Methods section that these are just names that do not represent the entire Arctic or Atlantic Ocean.

P6155, L25: Subsection 2.1 in the Methods section is now organized in a way that it first describes the sampling sites, then the six different treatments and finally the preparation and setup of the incubations.

Table 1 and 2: We are aware that Tables 1 and 2 are rather boring but they contain data that are scarce and the compilation here will be of use for the scientific community. We agree that data on neutral sugars produced by bacteria and neutral sugars and amino acids left after 32 days are the most interesting in Tables 1 and 2. It is certainly possible to move these data into two new tables, but that would leave even more tables for the reader to keep track of and we believe that this will decrease the simplicity and readability of the paper. Instead, we suggest highlighting these numbers in Tables 1 and 2, e.g. by writing them in a bold font or by boxing the numbers.

#### References:

Ogawa, H., Y. Amagai, I. Koike, K. Kaiser, R. Benner. 2001. Production of refractory dissolved organic matter by bacteria. *Science*, 292: 917-920.

## Referee #2:

We would like to thank anonymous referee #2 for detailed comments on the manuscript.

Our study provides insight to microbial production and transformation of biomolecules in the Ocean. Referee #1 also questioned the novelty of the paper, and a detailed description of the new findings of our study is written in the reply to referee #1.

Reply to specific comments:

P6152, L9: The term 'long-term' has been changed to '32 days'.

P6152, L10: It is now stated in the abstract that the incubated seawater originates from the Arctic Ocean and the North Atlantic Ocean.

P6152, L21: Although the microbial carbon pump (MCP) needs to be studied in more detail to better understand it mechanistically, many studies support the existence of the MCP, and we therefore think it is reasonable, in fact important, to discuss our results in light of the MCP. The time it takes for microbes to generate semi-labile or refractory DOM via the MCP is unknown, and we agree that the setup of our experiment may be too simple to make 'strong statements' about the MCP. We have therefore tried to keep the conclusions to 'indications' and 'suggestions' which we do not think imply 'strong statements'. See also our reply to comment 'P6152, L22 + L23' from referee #1.

P6155, L7: The term 'long-term incubation' has been changed to '32 day incubations' in the Methods section and the figure legend for Figure 1.

P6156, L1: The word 'amble' has been changed to 'ample'.

P6158, L7: This is a good point. Making the calculation from  $ASW_{glu}$  alone reveals almost the same result, though. We have now addressed the uncertainty associated with calculating the bacterially-produced neutral sugars from the  $NSW_{glu}$  data in subsection 4.2 of the Discussion section.

P6164, L13-15: We do not claim that the molecular compositions of semi-labile/refractory biomolecules and microbially produced biomolecules are identical. We point out, that they are approaching each other during our 32-days experiment and use this fact to provide a possible explanation for production of semi-labile/refractory DOM. Based on the available data, we have presented this as a 'hypothesis' which we believe is reasonable.

P6167, L26-27: The MCP prolongs the residence time of DOM in the ocean (Jiao et al. 2010), but the operation time of the MCP is not necessarily on the same long time scale. At least, we do not

know of any studies that state that microbial production of semi-labile or refractory DOM only happens on year-long (or even longer) time scales. In fact, studies of fluorescent DOM indicates that microbial production of (what is believed to be) a refractory DOM component happens continuously over time (Jørgensen et al. 2014). Although our experiment follows a very simple design, we believe it provides preliminary insights into the microbial production of semi-labile and refractory biomolecules. The fact that there is a clear resemblance between semi-labile/refractory biomolecules (left in NSW samples after 32 days) and bacterially-produced biomolecules (left in ASW<sub>glu</sub> after 32 days) indicates that microbes can be a source of semi-labile or refractory biomolecules. See also our reply to comment ‘P6152, L22 + L23’ from referee #1.

Table 1: We will discuss this point with the copy-editors at Biogeosciences. They probably prefer having the units only at the top of the table, but we do not mind having the units further down in the table as well.

#### References:

Jiao, N., G.J. Herndl, D.A. Hansell, R. Benner, G. Kattner, S.W. Wilhelm, D.L. Kirchman, M.G. Weinbauer, T. Luo, F. Chen, F. Azam. 2010. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. *Nature Reviews Microbiology*, 8: 593-599.

Jørgensen, L., C.A. Stedmon, M.A. Granskog, M. Middelboe. 2014. Tracing the long-term microbial production of recalcitrant fluorescent dissolved organic matter in seawater. *Geophysical Research Letters*, 41 (7): 2481-2488.