Response to reviewer 1

Brown and co-authors collected seawater samples from different depths of North Atlantic Ocean and investigated the response of microbial communities to the addition of diatom derived organic matter. I think this is an important study to understand the metabolic capabilities and activities of heterotrophic microorganisms in meso- and bathypelagic oceanic zones and illuminate the dynamics of carbon cycling in "dark" ocean. The experimental set up and the quality of the data shown in this study is very good; however, some aspects need to be considered before publication.

In the current flow of the manuscript, it is difficult understand the context of presented data when discussion points are provided in another section. If possible, I would suggest writing a combined Results & Discussion section to improve the readability of the manuscript. Another alternative would be to add some "bridge" sentences in Results section to guide readers to the points that will be discussed in the next section. This would yield a smoother read of the manuscript and better presentation of provided data. Moreover, I would suggest adding some extra information and modify some paragraphs in introduction. It is essential to mention the importance of proteins and polysaccharides in marine carbon cycling as they mostly focus on polysaccharide hydrolase and peptidases activities in the manuscript. It is also needed to innovative aspects of their work. They did not explicitly point out how the data presented in this study differs from Balmonte et al. 2019. Lastly, it would be useful to discuss the results with an ecological context. I suggested some reference studies below.

We added some "bridge" sentences to the results section (lines 290 and 301) as the reviewer suggested in order to help readers focus on the main points of the discussion. We also included more information about the importance of polysaccharides and peptides in the oceanic carbon cycle in the Introduction section (L63-66), as well as details on how this study differs from the one discussed in Balmonte et al. (2019) (L104-112). In the Methods, we included more information on the polysaccharides we used in the study (L63-66) and referenced some of this information in the Discussion section (L627-641) in order to provide some ecological context to the results of this study.

L25: The first sentence is of the abstract is too long. Diving into two sentences would help.

This sentence was divided into two (L25).

L26-27: Please define the depth of mesopelagic and bathypelagic zones in the abstract.

We added the meso- and bathypelagic depths listed in the Introduction into the Abstract (L30-31).

L35-39: Please be more specific and add some points to discuss the provided results.

Additional points from the results section were added to L36-41.

L74: Please mention the importance of polysaccharides and proteins in marine carbon cycling. This paper would also help to add some ecological context (https://www.biorxiv.org/content/10.1101/2022.08.04.502823v1)

We added information and citations on the importance of polysaccharides and proteins in the marine carbon cycle in the introduction (L63-66).

L97: Please provide more information for "the nature of that enzymatic response differed in some key respects". That will also help to define the motivation of the study.

We added sentences (L99-103) to this section to explain the key respects that we refer to.

L102: What does "moderate quantities" mean? Please be more specific.

Moderate quantities is defined as 658 uM HMW C as particulate organic carbon + dissolved organic carbon in the methods section; we included this information in the introduction (L112).

L231-236: Is there any particular reason to get samples from these stations? Adding some oceanographic key data would help.

We added information to the Results section (L266-270) to provide clarification on why we chose these specific stations.

L247: Please clearly define "endopeptidases". There are some substrates listed in the supplementary figure and it is not clear which ones are endopeptidases.

Endopeptidases cleave peptides/proteins mid-chain; in this manuscript, the term refers specifically to trypsin (measured with QAR and FSR) and chymotrypsin (measured with AAF and AAPF) activities. We modified this sentence (L281-282) to indicate that we are referring specifically to trypsin and chymotrypsin activities when we mention endopeptidases.

Figure 1: Please provide the full names of substrates in the figure or in the legend. Also, using a different scale for amended and unamended could be misleading. Maybe using broken axis or another solution would help?

Given the significant difference between that hydrolysis rates in amended and unamended mesocosms, we have found that plotting them on different axes is the best way to visualize them; plotting them on the same axis tends to make it difficult to see the lower unamended hydrolysis rates. However, we edited the figure caption to make it clear that the axes are quite different between the amended and unamended samples, and we added the full names of the substrates in the figure caption as well (L314-316).

L266: Please define alpha and beta-glucosidase activities. What do they use for? What is the difference between them?

α- and -β-glucosidase are both exoenzymes that hydrolyze glycosidic bonds (α- and -β glycosidic bonds, respectively), which are oriented differently. Cleaving these glycosidic bonds in oligosaccharides or polysaccharides frees a terminal glucose. Here, we measure α- and -β-glucosidase activities using 4-Methylumbelliferyl- α-D-glucoside and 4-Methylumbelliferyl- β-D-glucopyranoside. We added this information to the Methods section (L166-169).

L278: For this section, please introduce the polysaccharides used in this study. Short biogeochemical and ecological information would help. What are the sources of these polysaccharides? Why they are important? Why did you select these substrates?

We included additional information on the sources of these polysaccharides, their abundance and complexity, and our reasoning for their use, in the Methods section (L181-198), with relevant literature citations.

L316: Please explain why you measure bacterial protein production rates.

We measured bacterial productivity using leucine incorporation in order to measure bacterial protein-based growth rates. Sequencing samples provides a measure of the composition of the community; measuring protein production provides a measure of community activity. Although not all bacteria take up leucine, this method is widely used and is standard in the field of marine microbiology.

We added an additional paragraph to the Discussion section about bacterial production rates (L587-597).

Figure 3: Please explain how you classify ambiguous taxa in the legend. Also add the information in the methods section.

We defined the ambiguous taxa category in the Fig. 3 legend (L404-405).

Figure 4: Too much information is embedded in MNDS plot. Is it possible to divide this figure into different panels to show the differences between treatments, depth, and time.

Yes, we added an additional NMDS plot to the Supplemental Information section that divides station, depth, treatment, and timepoint into separate NMDS plots (Fig. S8). We additionally added a caption that better explains the significance of the NMDS plot in Fig. 4.

L475-490: I really like the discussion provided in this paragraph! It would be a very good example for the rest of discussion.

Thank you! We will try to revise the rest of the discussion accordingly.

Figure 5: Very nice summary! Yet, it is difficult to read the next and see the colours within dark background. Please make the background lighter.

Thank you! We edited the figure so that there was more contrast between the background and the text and enzyme colors.

L530: There is an elevated chondroitin hydrolase activity in bathypelagic. Why don't you discuss it here?

The bathypelagic chondroitin rates aren't elevated relative to the epi- or mesopelagic rates; the caption for Fig. 2 was edited to make this clear to the reader.

L569: For to discuss fucoidan, please also refer this paper: https://www.nature.com/articles/s41467-021-21009-6

We added this reference to the Discussion section (L629).

L584: Please provide a more relevant sentence to finalize the manuscript. I cannot see any direct link between your data and the "changing ocean conditions".

We edited the concluding paragraph so that the final sentence was more relevant to the data we presented in this manuscript.

Supplementary information: Please provide the full names of used substrates in Supp Fig. 3, 4 and 5

We edited the figure captions for Figs. S3, S4, and S5 so that they included the full names of each substrate.

Response to reviewer 2

Brown et al. present microbial enzymatic activities and community compositions in response to the addition of diatom-derived organic matter to water collected from the surface, mesopelagic, and bathypelagic depths in the North Atlantic. The manuscript is well written, and it is easy to follow the experimental setup and results comparing amended and unamended controls. I would recommend that this is published with only a few minor revisions to aid in the context of the study and its results. The study is very similar to the one in Balmonte et al., 2019, so some distinguishing characteristics should be included and/or more discussion about how the two studies compare and contrast. I believe more information is needed about the enzymes and their substrates — why were these enzymes chosen? What are the differences in these specific polysaccharides? What are their distributions in the marine environment? Do these particular hydrolases have any physiological significance for the microbes, e.g., are some more energetically expensive to produce than others? Just some things to consider…

We added additional information to the Introduction section (L104-112) to differentiate this study from that of Balmonte et al. (2019), and added details on the sources, abundance, complexity, and distribution of the polysaccharides we used to the Methods section (L181-198).

Some more oceanographic context about the stations selected would be welcomed as there is not much beyond just stating where the water was collected. Are DOC concentrations available for the in situ water?

Unfortunately, we do not have DOC concentrations. However, we included additional information on the stations we chose in the Results section (L266-270).

Please include full names of abbreviated enzymes in Figure 1 caption (line 273) as was done in Figure 2 caption. Full names are also needed in the supplemental figures 3, 4, and 5.

We included the full names of the substrates in the captions for Figs. 1, S3, S4, and S5.

Bacterial protein production is generally absent from the discussion: why was this measured? could these data be used to normalize the response in enzymatic activities in some way?

We included additional detail on bacterial productivity in the discussion section (L587-597). We measured bacterial productivity in order to examine the growth rates and activity of bacterial communities using a standard method. However, normalizing the responses of enzymatic activities using this data would not be meaningful, given that bacterial protein production provides information on protein production in general, not enzyme production specifically (we do not have the means to determine how much of the protein synthesized consists of the enzymes whose activities we measure).

The last sentence (line 584) about changing ocean conditions does not really tie into the prior discussion — if kept as is, please indicate earlier the analogs of the experimental setup to changing ocean conditions.

We edited the final paragraph of the discussion (L645-652) so that we had a more appropriate concluding sentence.