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Interactive comment on "Depth-resolved particle associated microbial respiration in the northeast Atlantic" *by* A. Belcher et al.

Anonymous Referee #2

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SUMMARY

This manuscript tackles a major and current question in the understanding of biogeochemical cycles in the ocean regarding the processes which affect the remineralisation length scale in the twilight zone and influencing the efficiency of carbon sequestration in the deep ocean. More specifically, the authors address the question of the relative influence of particle-attached bacterial remineralisation to other processes responsible for Particulate Organic Carbon (POC) flux attenuation in the upper mesopelagic zone. A large range a methodologies are used and combined to test their main hypothesis that the loss of organic carbon due to bacterial communities attached to 'fast-sinking particles' is the missing term that may help to close the carbon budget in the mesopelagic. Most of the methods used are recent but not novel and proved to be robust in previous published studies. The choice of the study site located in the Porcupine Abyssal

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Plain (PAP) in the North Atlantic is motivated by an extensive and recent pre-existing literature in this area. The resulting dataset is certainly valuable and could contribute to the scientific community. Results of their measurements combined with numerous calculations – and assumptions where needed – suggest that particle-associated bacterial respiration is not sufficient to explain the missing loss of organic carbon in the upper mesopelagic. As an alternative hypothesis, the authors propose that organic carbon losses due to the fragmentation of large fast-sinking particles into smaller slow-sinking/suspended particles operated by zooplankton in the mesopelagic could be the main process to account for to explain the imbalances observed in the mesopelagic carbon budget. Apart from a few sections, the manuscript is well-written and easy to read. The figures are clear and well presented, but in some places modifications are needed to improve the messages intended.

GENERAL COMMENTS AND RECOMMENDATION

Overall, this manuscript leaves a good impression on the goals targeted and the amount of work achieved. However, there is a striking mismatch between the hypothesis tested, the type of measurements conducted and the conclusions developed. I acknowledge the large number of measurements and understand the challenge presented by onboard analyses and that compromises have always to be made in the choice of the parameters measured but the conclusions drawn in a study have to align with the data acquired and it is unfortunately not the case here. In order to properly test the relative importance of the respiration associated with particle-attached bacterial communities, a complete set of the other known processes responsible for organic carbon loss, along with a comprehensive inventory of carbon sources should have been measured. This work lacks measurements of major parameters essential to establish a valid carbon budget in the mesopelagic. As stated in the title of the last section of the Discussion ("The missing piece of the mesopelagic budget"), the authors make an attempt to find a missing piece of a puzzle which seems to already miss several other very important pieces. In particular,

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(1) the values of zooplankton abundance and respiration rates used in the calculations are inferred from another study carried out in the same area but 6 years before. To justify the validity of applying these external data to the present work, the authors state that sampling was conducted in both studies at the same stage of the seasonal cycle but no evidence are given to support it. For instance a measure of phytoplankton cell physiological state (Fv/Fm), phytoplankton community structure (often displaying the same successions each year), nutrient levels potentially indicating exhaustion, and zooplankton community structure could have provided some element of response.

(2) no estimation of the respiration due to free-living bacterias in the water column is made. Previous studies noted however that it may be the predominant term in total bacterial respiration (see Extended Data Figure 6, Giering et al., 2014).

(3) the measurements of bacteria abundance in aggregates is also missing although it would have bring essential information to conclude on the observed variations of POC content and calculated specific respiration rates in the marine snow aggregates.

(4) Only the 'fast-sinking' fraction of the particle flux is analysed. This is a major flaw in the study. While the 'slow-sinking' fraction might poorly contribute to the deep carbon export as noted by Riley et al. [2012], it is precisely because most of this fraction of the flux appears to be remineralised in the mesopelagic zone. It is very surprising to me that no total POC measurements from the MSC collection have been done to allow a comparison with POC contents measured in the aggregates selected for the analysis.

(5) no measurements of DOC was made even if it represents a non-negligible source of carbon to depth and surely needs to be accounted for in any carbon budget. Having this term would have informed – and possibly confirmed– some of the numerous hypotheses postulated in the Discussion section.

In a very honest approach, the authors detail all these missing terms in the last section of the Discussion and try to weigh their potential influence based on the literature. Unfortunately, no valid conclusion can be drawn based on so many assumptions and by

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using values which are themselves subjected to very large uncertainties and potentially not applicable in this system. As a result, the main conclusion – in fact an alternative hypothesis – is disappointing since it echoes the conclusion made by Giering et al. [2014], but without bringing any additional piece of evidence to confirm it. Despite all these caveats, I believe that this work is worthy of being published, mostly for its very valuable dataset. However, a complete restructuring of the manuscript is required. The formulation of new hypotheses more in-line with the measurements conducted should help in this difficult task. The objectives initially aimed in this work (i.e. to close the carbon budget in the mesopelagic zone) have to be downgraded, but it should not minimise the importance of the findings centred on the variations of particle-associated bacterial respiration as a function of depth. As already noted in the title, I suggest to present this work as a focus on this single parameter of importance: the depth-resolved particle-associated microbial respiration in the northeast Atlantic. I recommend to highlight the variations of respiration observed at each depth and in each set of aggregates from the same depth. A large fraction of the discussion is already framed around this aspect, but a thorough evaluation of the variability attributable to errors inherent to each measurement should be examined before trying to explain the potential real variability. It might appear that propagation of the uncertainties could alone explain the variability observed at a given depth, allowing then to fully explore the depth-related variability. Finally, I wonder if the roller tank aggregation experiment is a valuable addition to this work or at the opposite if it weakens the study by showing results that contradict those made in the water column. Roller tank-made aggregates have been excluded a long time ago as models to quantify processes in real particles [Jackson, 1994]. In addition, the choice of settings used in the roller tank experiment and the methods conducted are highly questionable (see specific comments), and any evidence based on these results should be taken very carefully.

SPECIFIC COMMENTS

Abstract, line 16: "...with respiration sometimes being 50% lower than apparent carbon

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loss in the upper mesopelagic." This sentence is confusing and possibly incorrect. Did the authors mean 50% "higher" rather than "lower"? Most of the studies presenting an imbalance in the mesopelagic carbon budget highlight that respiration exceeds the organic carbon supply (i.e. Burd et al., 2010).

p. 1, line 30: the b parameter in the Martin's curve is not the depth at which the material is remineralised.

p. 2, line 12: the "excess of POC supply" and "excess of respiration" compared to...?

p. 3, line 5: "missing carbon sink", again even if the reader understands what it is meant here, this is confusing as the missing term is an amount of organic matter needed to sustain observed respiration rates.

p. 3, line 15: why not calculating the mixed layer depth based on seawater density? Park et al. [1998] showed that the temperature alone might not be a reliable proxy for mixed layer calculations. Given the small variations of salinity with depth, I do not suspect any major change of the MLD calculations if based for instance on the 0.02 sigma-theta density difference criteria, but this could be checked easily.

p. 3, line 24: I doubt that bloom stage can be inferred that way. At most, Chl. a surface concentrations from satellite data can inform on biomass levels at a given time. Unless the bloom is considered as a whole, regardless of species successions and size classes – which should be avoided – the term 'bloom stage' denotes rather a moment in phytoplankton community successions and physiological state and can be estimated by sampling the plankton communities.

p. 4, line 1: considering that the height of the MSC is 1.53 m, it means that the particles collected are those which settled at approximately 18.4 m d-1 or faster. It seems that excluding all particles settling slower than this speed could have removed a non-negligible fraction of the flux, especially because of the findings of Riley et al. [2012] who deployed MSCs at the same site in summer 2009. They found that the

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POC flux from fast-sinking particles was 54 mg C m-2 d-1, while the POC flux carried by slow-sinking particles (not sampled here) was 92 mg C m-2 d-1, suggesting that slow-sinking particles might sustain most of the POC flux. As noted above, it would have been necessary to also estimate the total POC flux collected in the MSC to have some idea of the contribution of the fast-sinking POC to the total flux.

p. 4, line 20: what sort of composition is assessed here? If based on the images showed in the supplementary material, only a very subjective idea of particle composition, and very likely not quantitative, can be accessed this way.

p. 4, line 25: how the decision was made to apply a given formula to each particle? Did the authors used morphological data from Image J (e.g. aspect ratio threshold to apply formulae for a cylinder or a sphere)?

p. 4, line 27: if an uncertainty is related to carbon content in FP then it should increase with depth where FP become predominant. It should be taken into account when trying to explain the unexpected increase of the POC flux between 203 and 500 m (section 3.4).

p. 5, line 24: "similar ESD" needs to be rephrased as if I well understood aggregates have been sorted in size classes not similar ESDs.

p. 5, line 26: "Where possible we measured POC...". This needs to be clarified.

p. 5, line 27: please change "fractal shape" to "fractal geometry".

p. 5, line 28: the correct reference is Alldredge [1998].

p. 6, line 1: the rotation speed of 3 rpm seems incredibly fast, and the incubation time of 7 days (p. 10, line 21) needed to obtain the first signs of aggregation surprisingly long. The speed of 3 rpm chosen by Iversen and Ploug (2010) was adapted to aggregates sinking much faster (due to ballast effect), and thus needing a very high rotation speed in order to keep them in suspension and avoid collision with the walls of the tank. I suspect that the same rotation speed used here was too fast to allow the particles to

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settle at any point of their rotation around the centre of the tank, minimising aggregation by differential settling. It would explain why obtaining 'decent-size' aggregates have required a very long incubation of 9 days which is surely a factor that played in bacterial remineralisation. A very large fraction of the POC content in the aggregates may have been respired by the end of the incubation. Since no measure of POC, DOC and Dissolved Inorganic Carbon (DIC) was made at the beginning of the incubation, but only POC content measured at the end, no information is available on the solubilisation of the POC to DOC and its subsequent remineralisation to Dissolved Inorganic Carbon (DIC), rendering any conclusion impossible.

p. 6, line 13: this is another potential bias in the study since no evidence is given that zooplankton abundances and respiration rates measured in August 2009 by Giering et al. (2014) are representative of the system studied here. Maybe the authors can look for existing data of inter-annual variations of zooplankton abundances at the PAP site. Even if the importance of this term is minimised in the Section 4.5 and the authors estimate that it cannot close the carbon budget, it also needs to be calculated as accurately as possible (by direct measurements) as any biogeochemical budget needs a careful consideration of every term.

p. 7, line 9: please replace "grazing" by "coprophagy" as it is the correct term.

p. 7, lines 16-17: "PA sinking velocity was significantly ($R2 = 0.163$, $p<0.0001$, $n=98$) correlated with ESD". It seems to me that these statistics suggest precisely the opposite here. It needs clarification. Also, the 6 outliers excluded from the relationship should be marked in Figure 3.

p. 7, line 22: correct reference is Laurenceau-Cornec et al. (2015).

p. 7, line 23: the size of aggregates formed in roller tanks is controlled by the time of incubation, initial cell concentration, stickiness, etc. and can hardly be compared between studies.

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p. 8, lines 3-9: this paragraph seems to belong to the Discussion section rather than the Results.

p. 8, line 18: "Based on pool measurements...". This needs more details. In particular what were the size classes (and their width) used to pool the aggregates.

p. 8, line 21: this refers to the comment on p. 6, line 1, that a large fraction of the POC should have been respired during the prolonged incubation (as noted by the authors p. 10, lines 18-19). Also, the aggregation processes likely affected by tank rotation speed may have controlled the fractal dimension of the aggregates and influence their POC:Vol ratios. Maybe the authors can somehow estimate the fractal structure of the aggregates using their images following Kilps et al. [1994].

p. 8, line 31: this refers to the previous general comment that estimating bacteria abundance in the aggregates could have brought valuable insights here.

p. 8, line 33: please change "temperature are higher" to "temperature changes are higher".

p. 10, line 15: does it imply that the aggregates collected with the MSC have been subjected to fragmentation or aggregation subsequent to their sampling in the water column? If the data from the MSC are assumed to be valid then what was the motivation of using artificial roller tank aggregates known to represent poorly real particles?

p. 10, line 33: again, "aggregate composition" is a very vague description. A proper composition analysis should be chemical and/or taxonomic. More details on what was really assessed here are needed.

p. 11, line 11: I strongly disagree and think that a measure of bacterial abundance was in the scope of this study. The authors need to justify the absence of these measurements in other ways.

p. 11, line 22: why not trying to explain this unexpected (and thus interesting) value rather than excluding it from the study?

p. 12, line 27: "... which is likely to be less accessible to free-living microbes". This is a very important statement on which rely the justification that free-living bacterial respiration was not measured here. More details are needed on "less accessible". To what extent? Some additional quantitative informations supported by adequate references are needed.

COMMENTS ON FIGURES

Fig. 1. a: perhaps the authors could reduce the scale of this map and centre it on the PAP site as it is very difficult to see any mesoscale structure at this scale (if this is what is intended). Also, why not choose a satellite product which encompasses the sampling period of the study?

Fig. 3: A log scale on the Y-axis might improve the readability of the different sets of aggregates from each depth. Also, separate sinking velocity-size fits for each set of aggregates from distinct depth (one fit by color) could reveal interesting findings, especially if the structure and/or composition of the aggregates are assumed to vary with depth.

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