

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

### **Anonymous Referee #1**

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Summary: The authors use a combination of emerging and established methods to address a major outstanding question in chemical oceanography; namely, the degree to which particle flux attenuation (i.e., the progressive remineralization of sinking marine particles with depth) is a result of respiration by attached bacterial communities, compared with various other biological and abiotic processes. The authors have a robust and very welcome dataset that has the potential to contribute significantly to a growing body of work in this area. Using shipboard measurements of respiration on particle material retrieved from depth, the authors conclude (in agreement with several other very recent studies) that direct respiration by particle-attached bacterial communities can explain only a fraction of the attenuation that is observed in the environment using sediment traps. (The relative importance of attached bacterial respiration appears to increase with depth compared with other processes.) The other major (and truly novel)

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finding of the paper – which is not presently addressed in the abstract, but should be – is the very apparent and mysterious difference in amenability to heterotrophic respiration between actual sinking marine particle material and artificial aggregates created in roller tanks. This is striking, because the latter have been invoked in dozens of experiments over the past 20 years as proxies for true sinking particle material. In the present study, the liabilities of the two types of particles appear to differ very significantly.

General impression and recommendation: This is scientifically interesting work that deserves publication in Biogeosciences. The conclusions advance the dialog incrementally in one avenue of research (the partitioning of particle flux attenuation between attached respiration and other processes) and present a very new and striking finding with regard to the roller tank particles versus "real" particles. The authors' central findings and conclusions are acceptable and meet the criteria for publication in Biogeosciences. However, I have some very significant concerns which pertain primarily to (1) the authors' reading, interpretation, and presentation of the existing literature and state of the art as regards the biological pump (several issues), (2) the interpretation of respiration rates obtained using the authors' method, and (3) the structure of their manuscript. The authors could address these concerns with some re-analysis (particularly a more diligent consideration and assimilation of uncertainties), a more careful (and nuanced) interpretation of the data, to include mention in the manuscript of some very relevant caveats, and some re-writing of the manuscript, in places. In addition, important details of calculations and mathematical methods are omitted.

The manuscript is generally well-written and the figures are generally clear. I have some suggestions for the authors on two of their figures; addressing these should be trivial.

General comments:

(1) In both the abstract and body of the manuscript, the authors generally overstate the novelty of their finding with regard to the importance of particle-attached respiration.

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As they note on pp. 11-12, a small number of other recent studies have also presented similar results. I would urge the authors' to revisit the manuscript and ensure its tone reflects the findings in these existing studies apart from the citations on pp. 11-12.

(2) I have some significant concerns about the degree of handling, size-fractionation, and manipulation involved in the collection and incubation of the particle material in this study. First, the authors invoke the term *in situ* for these measurements; this is neither correct nor appropriate. Second, I am concerned that the particle material used for the incubation studies was manipulated and size-fractionated to a degree that makes comparison of respiration rates measured on the material to the MSC sediment trap flux measurements very questionable (specific concerns below).

(3) The authors' findings regarding the difference in lability between the two types of particles is really the novel contribution of this paper; I would encourage them to revisit the manuscript with an eye toward elevating the importance of these conclusions.

(4) The authors do not describe at all their methods for error assimilation and propagation; in fact, it is unclear whether this was even considered. As the authors are aware, the business of extrapolating rates of respiration on single particles in a highly controlled environment to the carbon cycle of a marine water column involves a large number of calculations involving a number of conversion and scaling factors, each of which have their own uncertainties. I would like to see some sort of more robust error propagation/analysis in the manuscript.

Specific comments:

Abstract generally: Why no mention of the roller tank versus "real" particle respiration rate comparison?

p. 1, lines 11-12: Logic suggests the first sentence of the abstract should be restated in the converse, i.e., "Atmospheric levels of carbon dioxide are tightly linked to the depth at which sinking POC is remineralized in the ocean."

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p. 1, lines 17-18: I am confused by the authors' statement of their hypothesis here. Perhaps they mean something more specific, i.e., "... the missing sink for particulate carbon in the upper mesopelagic"? In the growing number of recent studies in which other oceanographers have been unable to close mesopelagic carbon budgets (those in which both particulate and dissolved/suspended water column phases have been considered together), the problem has been one of an apparent undersupply of total carbon to the system, not a problem of too little respiration. However, if the authors are only considering the particulate phase, then this supposition would make more sense since the studies they reference on pp. 11-12 have demonstrated the opposite is true when considering the sinking particulate phase exclusively.

p. 1, line 15: Confusing as currently stated. Try: "Attempts to balance POC supply to discrete depth layers of the mesopelagic..." (if that is in fact what the authors mean)

p. 1, line 19 ff: Use of *in situ* confusing and inappropriate. If the authors mean here that the particles were collected from depth, then that should be stated; anything collected from the ocean in the course of oceanographic research can be said to have been collected "*in situ*." The respiration measurements the authors make in this study are not *in situ* measurements; the only true *in situ* particle respiration measurements that I know of are those of McDonnell et al. (2015), obtained using the RESPIRE device. The measurements in the present study, as in Collins et al. (2015), are shipboard measurements made using material collected from depth.

p. 1, line 25: This shift could also be ultimately to DOC (not simply to suspended POC).

p. 1, line 30: This is an incorrect interpretation of Martin's "b"; b does not represent the depth at which material is remineralized. (In the length-scale-based parameterization the authors invoke on p. 11, the interval  $z-z_0$  represents the depth interval over which material is remineralized.) This is one of the limitations of the Martin formulation, since the exponent doesn't really have any explicit meaning.

p. 3, lines 3-4: I am not sure this is true; McDonnell et al. and Collins et al. both

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made profiles of particle-associated respiration rates using actual particle material. Is it the authors' contention that their use of "individual" particles makes this the first such study? Seems like a rather qualified claim to novelty that could be omitted from the paper without affecting its impact or conclusions.

p. 3, line 15: How did this measure of MLD compare to the 1% PAR level (traditionally invoked as the base of the euphotic zone, which is the reference depth for most other particle flux studies).

p. 4: I have some concerns about the representativeness of the respiration rates obtained from the authors' method, which appeared to require very extensive handling and manipulation in the selection and isolation of individual particles. Wouldn't this method of "plucking" by eye perhaps result in separation of the particles from the microbial communities with which they were likely associated in the environment? Further, how do the authors justify the assumption that the sum of the individual rates obtained on a few chosen particles can be applied to estimate rates of removal vis-a-vis the complete, heterogeneous collection of particles in the corresponding MSC fractions used for flux determination? Would it not have been better to measure rates on an assemblage of particles that was (a) subjected to less handling and (b) was more completely representative of the wider spectrum of shapes and sizes of the particles in the traps? I am not suggesting the errors introduced by this approach render it invalid (particularly given the enormous uncertainties involved in every other aspect of this sort of work), but a more complete discussion of the implications of their decision would be welcome.

p. 5, line 25: Conversions of PA volume to what?

p. 5, line 30: The way the authors have currently defined Cresp (line 18) and [POC] (line 29), it seems to me this equation yields a quantity Cspec with units of length (m) per unit time, not simply time<sup>-1</sup>. Perhaps the authors can clarify?

p. 6, line 6: What is meant by "exponential fit" (i.e., what is the form of the equation)?

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p. 6, line 25: Are the 1.9 and 1.4 mg m<sup>-3</sup> figures from discrete measurements or ocean color data?

p. 7, lines 7-10: Depending on the time of day at which the traps were collected, this increase in FP numbers could also be aliasing daily vertical migration.

p. 9, lines 16-17: I do not understand the authors' meaning here. In addition, what is meant by "similarity"; this is a nonspecific and vague term.

p. 9, lines 19-20: Version of record of this paper is a 2015 date of publication.

p. 9, last paragraph: Given the very significant influence of temperature on metabolism, why were the particles incubated at a static 10°C rather than at in situ temperature? If because only a limited number of incubators were available for large number of particles, this should be stated on p. 4.

p. 11, first full paragraph: This is the most intriguing and novel finding of the authors' study, and should be given more prominence.

p. 11, line 29: "Slow" compared to what? I would suggest the rates of respiration measured by others in water column samples are truly "slow" compared with particle-attached/associated rates.

p. 12, line 19: What are the error bounds on this 139.3 mg C m<sup>-2</sup> d<sup>-1</sup> figure?

p. 13, first paragraph: The authors do not mention the additional possible mechanical processes of shear or turbulent disaggregation.

p. 13, line 33: I concur wholeheartedly! The authors would do well to make a connection here to very recent and compelling work by Biard et al. (2016), showing the ocean may contain large numbers of these protists.

pp. 14-15: I commend the authors for their thoughtful and excellent discussion here.

p. 14, line 30: Use of in situ misleading and redundant.

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Comments on figures and tables:

Figure 1: Panel (c) might be improved by the superimposition of some chlorophyll concentrations measured concurrently in the water samples.

Figure 2: From inspection of the figure, it seems to me the authors' decision to use only PA-derived rates (and not also FP-derived rates) is valid only for depths < 113 m. Below this depth, FP constitute a very significant fraction of the POC.

Figure 3: An excellent figure. Very clearly and compelling presents the most interesting conclusion of the authors' manuscript. However, could the authors provide the R2 for these fits in addition to the p-values?

Figure 4 (and corresponding presentation of this curve fit in the manuscript): What are the error bounds on the fitted parameters?

Figures 6, 7: Use of "in situ" to characterize the rates presented in these figures is particularly misleading.

Figure 9: In line with my concern regarding the compatibility of the authors' respiration rates with their trap flux data: Is the "aggregate POC" represented by the black bars the same POC as that supplied in the incubations used to determine the respiration rates? Seems to me this is not the case. I am not sure these can be directly compared in such a way. I am not sure I understand the meaning of the error bars ("upper" and "lower" estimates; are these error bounds obtained from some sort of uncertainty analysis?). Specifically, how were the error bars on the solubilization rates obtained, since these were based on a very hypothetical assumption?

References cited in review:

Biard, T.; Stemann, L.; Picheral, M.; Mayot, N.; Vandromme, P.; Hauss, H.; Gorsky, G.; Guidi, L.; Kiko, R.; Not, F. 2016. In situ imaging reveals the biomass of giant protists in the global ocean. *Nature* 532, 504-507.

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Collins, J. R.; Edwards, B. R.; Thamtrakoln, K.; Ossolinski, J. E.; DiTullio, G. R.; Bidle, K. D.; Doney, S. C.; Van Mooy, B. A. S. 2015. The multiple fates of sinking particles in the North Atlantic Ocean. *Global Biogeochem. Cycles* 29, 1471-1494

McDonnell, A. M. P.; Boyd, P. W.; Buesseler, K. O. 2015. Effects of sinking velocities and microbial respiration rates on the attenuation of particulate carbon fluxes through the mesopelagic zone. *Global Biogeochem. Cycles* 29, 2014GB004935

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