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Discussions

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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) 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 labilities 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 incremetally 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.

AC: Thank you very much for taking the time to review our manuscript and for the insightful comments. We have addressed your comments below which we think has greatly improved the manuscript. Please see our detailed response and attached marked up manuscript showing all amendments.

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.

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.

AC: We agree that our work supports and builds upon and a number of recent studies and have tweaked the manuscript to ensure that we are not overstating the novelty of our work. For example in the introduction (review document page 33, line 31);

"To the best of our knowledge only two studies have combined direct measures of respiration on aggregates collected at depth with measurements of POC flux (Collins et al., 2015; McDonnell et al., 2015), both of which lack sufficient vertical resolution in the upper mesopelagic to capture the region of most rapid change. Collins et al. (2015) measure rates of substrate-specific microbial respiration of 0.007 ± 0.003 d^{-1} to 0.173 ± 0.105 d^{-1} in the North Atlantic, with rates of 0.01 ± 0.02 d^{-1} and 0.4 ± 0.1 d^{-1} measured by McDonnell et al. (2015) at the Western Antarctic Peninsula and Bermuda Atlantic Time Series respectively. Previous studies are therefore inconclusive as to the importance of particle associated microbes on the attenuation of POC, with some studies suggesting they play a minor role (Alldredge and Youngbluth, 1985; Collins et al., 2015; Ducklow et al., 1982; Karl et al., 1988) and others suggesting a larger contribution (Iversen and Ploug, 2013; Ploug et al., 1999; Turley and Stutt, 2000). Also on page 34 line 11:

"To build upon these previous studies we assess the role of particle associated microbial respiration in POC flux attenuation, presenting a vertical profile of particle associated respiration rates measured on individual marine snow particles collected at depth."

We also now note that we are building upon previous studies elsewhere in the discussion, such as in section 4.3 (review document page 45, line 4) and 4.4 (review document page 46, line 6):

"Despite the uncertainties in the mechanisms governing rates of particle associated microbial respiration, we are still able to assess the importance of particle associated microbial respiration on the attenuation of POC in the mesopelagic and compare our results to the small number of other recent studies (Collins et al., 2015; McDonnell et al., 2015)."

"Recent assessments of the mesopelagic carbon budget at the PAP site, although balanced over 50-1000 m, revealed an imbalance when the upper and lower mesopelagic were examined separately (Giering et al., 2014). Particle associated respiration was not directly measured in the aforementioned study, and hence we assess whether this term could help to explain observed imbalances. In this way we test whether the low respiration rates (0.001-0.173 d^{-1}) measured by Collins et al. (2015) are also applicable to our study site or whether the higher rates, such as observed in the western subtropical North Atlantic gyre (0.4 d^{-1}) by McDonnell et al. (2015) are more appropriate."

(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).

AC: We have changed our terminology from in situ aggregates to natural aggregates. We have addressed these concerns about particle manipulation in your specific comment below. Briefly, the use of a wide bore pipette avoids fragmentation of the particles and as carbon-specific respiration rates are size independent, if any size fractionation occurred then this would not affect our conclusions based on carbon-specific respiration rates.

(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.

AC: We have added text into the discussion to highlight the differences we have found between artificial and natural aggregates. Unfortunately we do not have a chemical measure of lability, or a measure of microbial abundance on the different aggregate types and so can only speculate as to the reasons for the differences between them. Artificial aggregates were formed from waters collected at a shallower depth than collected natural aggregates, and hence we cannot determine if the differences in respiration rates between the two aggregates were a result of the roller tank

formation or simply due to their different depth of origin. For this reason we exercise caution when describing the potential importance of these conclusions. We suggest in the second paragraph of section 4.2 that this as an important area for future research, and recommend comparing the lability and microbial abundances on natural aggregates with artificial aggregates formed from plankton collected from the same depth as the natural aggregates.

(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.

AC: In the previous version of the manuscript, when calculating average respiration rates (C_{spec}) for each depth horizon (which are used in subsequent calculations in the manuscript) we calculated upper and lower bounds based on the range of respiration rates measured in PA. Based on your recommendations we have now conducted a more thorough assessment of our uncertainties using a Monte Carlo approach (bootstrap analysis with 10,000 simulations). We have added details of this in section 2.6.

The calculated uncertainties are presented as error bars in Figure 9A. We believe, that despite uncertainties, our measurements make the most of available data (both our own and in the literature) to provide further insights into the role of particle associated microbial respiration on the attenuation of POC flux. We calculate that particle associated microbial respiration can account for only 1-14% of POC loss in the upper mesopelagic and hence the further error propagation we have carried out foes not change our conclusions. We have added the following to the manuscript (review document page 38, line 3):

"Time and methodological constraints of measuring very small particles, prohibited us from measuring the respiration rate of every particle collected in the MSC. Hence, before using these measurements to assess the contribution of particle associated microbial respiration to the mesopelagic carbon budget, we must first define upper and lower bounds to our estimates based on our uncertainties. We conduct a Monte Carlo analysis (with 10,000 iterations) of the individual parameters used in the calculations of carbon-specific respiration and remineralization length scale. We randomly sample (with replacement) our measured volumetric oxygen respiration rates at each depth. For each of these randomly selected particles we use the corresponding sinking velocity and ESD in subsequent calculations of carbon-specific respiration and remineralization length scale. For the RQ, used to convert O_2 to CO_2, we define a uniform distribution of possible values over the range of RQ values typically applied in the literature (0.7-1.2, Berggren et al., (2012)). PA were pooled into size classes and could only be measured once for POC content. For each depth, we create a normal distribution of possible POC:volume ratios for each size class, with our measured value as the mean, and standard deviation calculated from the standard deviation of the individual aggregate volumes within a size class. Based on the 10,000 iterations for each of the aforementioned parameters we obtain a range of estimates for the remineralization length scale (via particle associated microbial respiration) at each depth. We then use the mean of these distributions ±standard deviation to put error bounds on our estimates of the POC loss via particle associated microbial respiration."

Specific comments:

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

AC: The most widely relevant part of our work is the low measured rates of particle associated microbial respiration and hence we have written the abstract to match this. The comparison with roller tank and in situ collected aggregates is not the main focus of our study here, partly because they were not conducted with water from the same depth (see comment above). The roller tank aggregates provide additional respiration rate estimates at a shallower depth than was sampled from the marine snow catcher, but it is difficult to make direct comparisons to the in situ collected particles because of these depth differences. We therefore do not stress these results in the abstract. See also response to general comment above.

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."

AC: Restructured sentence as suggested.

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.

AC: We have altered the abstract to clarify that we are addressing the particulate phase only (review document page 32, line 23).
"We test the hypothesis that particle-attached microbes contribute significantly to community respiration in the mesopelagic, measuring particle associated microbial respiration of POC, through shipboard measurements on individual marine snow aggregates collected at depth. We find very low rates of both absolute and carbon-specific particle associated microbial respiration ($<3\%$ d^{-1}), suggesting that this term cannot solve imbalances in the upper mesopelagic POC budget."

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)

AC: Thank you for the good suggestion, the sentence has been altered.

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 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.

AC: We agree with your judgement and have replaced in situ with natural aggregates collected at depth (PA_n), and use this throughout the manuscript.

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

AC: The shift could indeed be to DOC also, but we attempt to account for losses to DOC via solubilization, and these alone do not seem to be able to address the mismatch. Previous studies including DOC still find imbalances and so although this may indeed be an ultimate fate, we focus here on processes that could account directly for the loss of 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-z0 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.

AC: Thank you for highlighting our mistake; we have removed the reference to Martin's b here.

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

sinking POC, this would still not be able to explain the high loss rates of POC that we observed in the upper mesopelagic. We have added the following additional text in section 4.3 (review document page 45, line 19) to state the limitations with particle type.

"Our measurements are based on a sub sample of the total assemblage of particles found in the water column, in particular only PA. If rates of microbial respiration are vastly different on other particle types then this would affect our calculations of POC removal by particle associated microbes. However, considering the dominance of PA in our samples (Fig. 2), we believe our calculations reflect the bulk of the sinking material at the time of sampling. We were not able to measure respiration rates on FP due to their low numbers and small size which adds uncertainty to our estimate of the contribution of particle associated microbial respiration to POC loss. However, even if FP were respired completely, they account for less than 10% of the flux between 36-128 m and thus could not resolve the large imbalances between POC supply and respiration that we observe in the upper mesopelagic."

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

AC: Edited sentence (review document page 37, line 12) to clarify:

"This enabled the carbon content per unit volume (mg C mm^{-3}) for each size class at each depth range to be calculated and hence POC content of individual PA to be estimated."

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-1. Perhaps the authors can clarify?

AC: Thank you for drawing our attention to this typo. We have amended the quoted units for Cresp, units are mg C mm^{-3}, which results in Cspec with units of d^{-1}.

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

AC: Added text (review document page 37, line 28) to clarify form:

"…compared to an exponential fit (R^2=0.30, p=0.128, n=9) (of form, $F_z = F_0 \exp(z-z_0/z^*)$, as in Buesseler and Boyd (2009))."

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

AC: Added 'based on discrete measurements' to clarify.

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.

AC: Yes, the timings are such that increased FP could be due to zooplankton migrating to depths >100m at sunrise, and these FP sinking further through the water column. We have added zooplankton diel vertical migration as a possible explanatory factor in this sentence (review document page 39, line 29).

"The increase in FP numbers below 100 m could be due to an increase in zooplankton populations with depth, zooplankton diel vertical migration, and/or increased FP loss in the upper mesopelagic due to processes such as fragmentation and coprophagy."

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.

AC: We have reworded these sentences to explain our meaning more clearly (review document page 42, line 17):

"Despite the consistency of average values of C_{spec} at each depth horizon, there was large variability in C_{spec} for individual aggregates within each depth horizon (full dataset range: 0.002-0.031 d^{-1}, Fig, 7). The calculated standard error was consistent across all depths (0.002—0.003) which implies that the factors driving the variability in C_{spec} are unchanging with depth, suggesting controls on degradation are already determined at shallow depths"

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

AC: Corrected publication date

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.

AC: Shipboard considerations meant it was only possible to incubate at one temperature, and 10 °C was chosen as the minimum temperature likely to be encountered in the upper 500 m of the water column. We have stated this in section 2.4 as recommended.

"Only one incubation temperature was possible due to laboratory and space limitations."

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

AC: Thank you for your enthusiasm regarding these findings. We believe the most important aspect of our study to be the low rates of particle associated microbial respiration (see response to specific comment 1); nether the less the differences we find between natural and artificially made aggregates are indeed interesting. Due to several uncertainties, we are only able to make speculations about the possible differences in microbial abundance in these aggregate types but believe that this should be considered a priority for future research. Following your advice, we have added text in the manuscript, particularly at the end of section 4.2 (review document page 44, line 24) to increase the prominence of this finding as suggested but also explain our uncertainties in making strong comparisons.
"Over the past couple of decades there have been numerous studies utilizing roller tanks to create artificial aggregates from natural phytoplankton assemblages in an attempt to replicate natural sinking particulates, investigating processes such as aggregation, sinking velocity, ballasting and degradation (e.g. Iversen and Robert, 2015; Iversen et al., 2010; Laurenceau-Cornec et al., 2015; Shanks and Edmondson, 1989). In the present study, we sampled the natural phytoplankton assemblage from waters combining the highest POC concentration and chlorophyll fluorescence in an attempt to assess the aggregation potential in the most productive water strata. As PA_r were formed from material at shallower depths than PA_n were collected, we cannot be certain that the observed differences are not depth related. Further, we left the roller tanks to rotate for a period of 9 days in the dark and cold to simulate POC aggregation in the water column, accelerating plankton death and vulnerability to microbial degradation. These conditions deviate from natural conditions in which the plankton sinking from 12 m depth would have experienced progressive temperature and light decreases as well as changes in grazing pressure. Therefore, it is difficult to ascertain the cause of differences between roller tank formed and in situ collected aggregates, and rather we use the roller tank formed aggregates to get an estimate of the carbon-specific respiration rate of the aggregates at shallow depths".

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.

AC: We agree that sinking particles are typically hotspots of activity in comparison to the surrounding water column, but suggest that zooplankton grazing and fragmentation leads to more rapid loss of large sinking particles. Fragmented material is likely ultimately respired by microbes, both particle associated and free-living, but we refer here to the direct loss of large sinking POC. We have amended the text as follows (review document page 45, line 30):

"Despite being hotspots for microbial activity compared to the water column (Thiele et al., 2015), particle associated microbial respiration may still be a minor contributor to the reduction in POC flux when compared to rapid loss via processes such as zooplankton grazing and fragmentation (Dilling and Alldredge, 2000; Stemmann et al., 2000; Svensen et al., 2014)."

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

AC: Following our additional uncertainty analysis we have recalculated our error bounds and quote them in the text (review document page 46, line 26):

"…excess POC supply of 118 mg C m^2 d^{-1} (60-177 mg C m^{-2} d^{-1} considering our calculated error bounds on rates of particle

associated microbial respiration and POC flux measurements) over the upper 36-200 m."

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

AC: Thank you for drawing this oversight to our attention. We have added the following paragraph in section 4.5 (review document page 48, line 18) where we address the possible missing parts of the budget:

"Additionally we are not able to measure mechanical disaggregation via processes such as fluid shear which could provide additional losses of large sinking POC. The forces required to break apart large marine snow aggregates have been shown to be higher than typical estimates of energy dissipation in the ocean, suggesting that this would not be a major loss process (Alldredge et al., 1990). Physical disaggregation could be more important in surface waters where dissipation rates can exceed the forces required to break marine snow aggregates (Alldredge et al., 1990; Burd and Jackson, 2009). However, we suspect that only a small fraction of sinking POC would be fragmented by abiotic processes to particles <0.15mm and hence would not explain the large loss of fast sinking POC measured in this study."

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.

AC: Thank you for drawing our attention to this interesting paper. We have added a reference to it to support our statement (review document page 48, line 25).

"In order to address the imbalances in the sources and sinks of fast sinking POC to the upper mesopelagic we require an additional loss process of POC. One key term missing from the budget is that of free-living protozoans which would not be collected in zooplankton nets and can make up a substantial part of marine planktonic ecosystems (Biard et al., 2016)".

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.

AC: Changed to 'at depth'.

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.

AC: Added points of chlorophyll from discrete measurements from CTD as recommended to figure 1C and also added profile of chlorophyll in figure 1B.

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.

AC: We only measure respiration rates in PA due to the low numbers and small size of collected FP. We acknowledge that this increases the uncertainty in our predicted POC loss via particle associated microbes, particularly at depth where FP are more important. We have added text to section 4.3 (review document page 45, line 23 of the manuscript to acknowledge this limitation.

"We were not able to measure respiration rates on FP due to their low numbers and small size which adds uncertainty to our estimate of the contribution of particle associated microbial respiration to POC loss. However, even if FP were respired at significantly different rates, they account for less than 10% of the flux between 36-128 m and thus could not resolve the large imbalances between POC supply and respiration that we observe in the upper mesopelagic."

Figure 3: An excellent figure. Very clearly and compelling presents the most interesting conclusion of the authors' manuscript. However, could the authors provide the R^2 for these fits in addition to the p-values?

AC: We have added in R^2 values to the plot

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

AC: To improve our error assessment of the b value obtained during this curve fit we have applied a bootstrap analysis with 100,000 simulations and quote the mean b+/- the standard deviation. This is stated in section 3.4 (review document page 41, line 2):

"To assess the uncertainty surrounding our calculated *b* value, we have applied a bootstrap analysis with 100,000 simulations giving a mean *b* of 0.67 ± 0.34 (standard deviation)."

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

AC: We have amended the figures to say "natural" rather than in situ.

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?

AC: The POC flux shown in Figure 9 includes all types of fast sinking POC collected by the marine snow catcher and we have amended to legend to make sure it matches this. Please see our earlier comment above where we justify the application of our respiration rates measured on PA to the whole flux based on their dominance in our samples. We have amended the text within the figure caption to better explain and refer to the methods employed for error bar calculation. As was previously in the figure caption, these did not include an error estimate of solubilization due to our inability to constrain it. We have now removed the error bars from the POC sinks in figure 9b) to avoid misleading the reader as to the accuracy of these calculations.

"Error bars in figure (a) relate to the range in POC flux measured at each depth and the range in respiration rates calculated (see section 2.6). We do not display error bars on our assessments of POC sinks due to the potentially large and unconstrained errors on solubilization."

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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 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.

AC: Dear reviewer, thank you very much for the thorough review. We are pleased that you generally liked our manuscript and hope that the changes in the revised manuscript now reconcile the hypothesis with the

measurements.

In particular,

(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.

AC: We agree with your concerns about the use of past data and have been able to source zooplankton net data taken during the 2015 research cruise through further collaborations with other scientists on board the ship. We now estimate zooplankton respiration from net tows carried out from 0-200 m during the cruise. Although we lose resolution by using these data, we believe that these data provide a more reliable assessment of POC loss processes at the time of our study. The zooplankton abundance and respiration estimates from this study (daytime: 0.3 g DW m^{-2}; night-time: 0.4-0.9 g DW m^{-2}; respiration: 5-10 mg C m^{-2} d^{-1} between 0-200 m) are in good agreement with the data collected 6 years ago (daytime: 0.2-0.4 g DW m^{-2}; night-time: 0.6-1.3 g DW m^{-2}; respiration: 10-18 mg C m^{-2} d^{-1} between 0-200 m). Respiration rates 6 years ago were slightly higher during one of their stations due to high abundance of amphipods and euphausiids. Nevertheless, the overall conclusion in our original submission has not been impacted, as zooplankton respiration is still only a small fraction of the POC loss over that depth horizon (~220 mg C m^{-2} d^{-1} between 0-200 m).

(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).

AC: Although indeed free-living bacteria can make up a large proportion of total bacterial respiration, this requires conversion of large sinking particles to dissolved organic matter (Cho and Azam, 1988; Karl et al., 1988). In contrast to Giering et al. (2014), we focus only on the direct loss of POC, investigating the balances between sources and sinks in this carbon pool, and hence we do not include free-living bacterial respiration. Free-living respiration is typically measured via leucine incorporation, and requires two conversion factors for which there is a large range of literature estimates (see methods of Giering et al., 2014). Leucine conversion factors and prokaryotic growth efficiencies range by 2 orders of magnitude and hence are, we believe, associated with too high a degree of uncertainty to provide a useful comparison. These large uncertainties motivated our focus on only the POC pool of the carbon budget and we assess the direct loss via particle associated microbial respiration only.

(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.

AC: We agree that information on bacterial abundance, and diversity would indeed be interesting and may help explain observed variability in our measured respiration rates. However, to obtain carbon-specific respiration rates it was necessary to combust the particles to obtain a POC measurement and therefore it was not possible to also obtain prokaryotic abundance for these aggregates. Moreover, in order to obtain the most accurate estimate of carbon flux from fast sinking particles, we chose to dedicate the rest of the particles to carbon flux measurement. However, during the cruise a microbiology team performed parallel MSC deployments devoted to linking aggregate carbon content with microbial abundance and activity. The analysis for these data is ongoing and will be the subject of a separate future paper by this team.

(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.

AC: See response to specific comment (p. 4 line 1) below where we state that slow sinking fluxes were <10% of the total flux and therefore were only a small component of the total POC flux. In addition rates of respiration on slow sinking particles have been shown to be different to those on fast sinking particles (Cavan et al. in review), and hence it would not be appropriate to apply our measured respiration rates to the slow sinking POC pool also.

(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.

AC: As note above, we focus only on the fast sinking POC part of the budget to avoid the introduction of additional uncertainties. We do not attempt to balance the whole carbon budget and have made modifications throughout the manuscript to make this clear and avoid confusion as to our intention. Assessing the balance of the whole carbon budget would indeed require measurements of DOC, however the source and sink terms of POC must also balance, enabling us to assess whether particle associated microbial respiration can help close this part of the full carbon budget. We believe that this is a useful step that will contribute to the wider scientific community and that the large number of carbon pools and interacting processes necessitate this step by step approach to make advances in the field. We must first balance the sources and sinks of POC before we can properly assess the transfer to DOC and subsequent sinks.

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 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.

AC: We thank the reviewer for taking the time to thoroughly review our manuscript and for the very useful insights and suggestions. We have amended the manuscript to clarify our intentions to assess the contribution of particle associated microbes to the loss of POC from the manuscript. See below our specific responses to the comments raised.

Following the suggestions of reviewer number 1, we have carried out more in depth uncertainty analysis, the results of which do not affect our conclusions. We are therefore confident that the addition of particle associated microbial respiration to the mesopelagic carbon budget, does not improve the balance. The purpose of out paper is not to try and create a mesopelagic carbon budget, but to assess an additional loss term and determine if this could explain some of the depth resolved mismatches in POC sources and sinks. We have changed the title of section 4.5 to "The missing piece of the mesopelagic carbon budget?", to better reflect that we are exploring the possible solutions to the current imbalances, rather than making a definitive budget.

We have edited the final paragraph of 4.4 (review document page 47, line 17) to make it clear that we are not suggesting

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that we are attempting to balance the budget in this study.

"POC loss via zooplankton respiration, particle associated microbial respiration and solubilization, as typically invoked in model studies (e.g. Anderson and Tang, 2010) can therefore not account for observed losses of POC in the upper mesopelagic, suggesting our knowledge of the mesopelagic carbon budget is still poorly constrained and/or incomplete."

SPECIFIC COMMENTS

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

AC: We have restructured the abstract and removed this confusing sentence.

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

AC: Thank you for highlighting our mistake; we have removed the reference to Martin's b here.

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

AC: Text amended to clarify we are comparing sources and sinks (review document page 33, line 16):

"However, this study contained large and compensating imbalances between sources and sinks in upper and lower mesopelagic layers, with an excess of POC supply to the upper mesopelagic (50-150 m depth), and an excess of respiration in the lower mesopelagic (150-1000 m depth)."

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.

AC: We have amended the text here to make it clear that we are not trying to close the mesopelagic carbon budget. Instead we focus on the balance between sources and sinks of fast sinking POC, and assess whether our new measurements of particle associated microbial respiration can help improve this balance (review document page 34, line 13):

"In an attempt to assess whether this term can improve our ability to balance the fast sinking POC budget in the upper mesopelagic…"

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.

AC: Following your advice we have calculated the mixed layer depth (MLD) based on a range of density criterion 0.02-0.125 σ_θ and find that the criterion of 0.125 (Levitus, 1982) best captures the pycnocline depths observed in our study. The depths obtained are within ±5 m of the depths we calculate based on a temperature criterion and so we stick with our original definition of MLD.

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.

AC: Thank you for highlighting our error in terminology here, we have corrected the sentence to better explain our intended use of the satellite data (review document page 35, line 3).

"We examine changes in surface chlorophyll prior to and post sampling by averaging chlorophyll data over the study region (48.5-49.5 °N, 16.0-17.0 °W)."

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 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.

AC: We made measurements of the slow sinking pool and found in contrast to Riley et al., (2012) that it only represented a small part of the total carbon flux (on average <10%). We therefore focus our study on only the loss of fast sinking POC as it is these particles that we measure respiration rates on. Recent work by Cavan et al., (in review) found significantly different rates of microbial respiration in fast and slow sinking carbon pools and therefore it would not be valid to apply our measured respiration rates to the slow sinking carbon pool. We have added the following additional text to the methods section (review document page 35, line 19):

"Slow sinking particles were also collected as of Riley et al., (2012), with slow sinking particle velocities calculated using the SETCOL method (Bienfang, 1981). Slow sinking fluxes were only a small part of the total sinking flux (on average <10%) and due to their slower sinking rate these particles do not penetrate as deeply in the mesopelagic. Hence we focus our study on fast sinking particles only."

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.

AC: Thank you for drawing our attention to this, we have replaced 'composition' with 'type' to clarify that we classified the particles into types as described in the text but did not assess composition (review document page 36, line 4).

"The type of fast sinking particles at each depth was assessed under a microscope and photographs taken using a Leica DM-IRB inverted microscope and Canon EOS 1100D camera. Particles were classified into phytodetrital aggregates (aggregations >0.15 mm equivalent spherical diameter (ESD) containing phytoplankton cells and other phytodetrital material, herein referred to as PA), faecal pellets (FP), and unidentified phytodetritus."

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)?

AC: Classifications were done manually by A.Belcher based on the particle appearance. The morphologies were distinct allowing confident classification.

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).

AC: Yes you are correct in that the uncertainty in carbon content of FP would affect our estimates of the % contribution of each particle type to the total POC (Figure 2). We only use area derived POC from photos and conversions to get an estimate of the contribution of each particle type to the total mass of sinking material. These conversions are not used for the calculation of total POC flux as presented in Figure 4. The POC flux is based on direct measurement of POC (a split of the particles were placed on GF/F filters, see section 2.3). The increase in POC at 500 m cannot therefore be explained by uncertainties in literature values as no literature values were used in calculation of these fluxes.

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.

AC: Sentence amended to:

"…before pooling PA into size classes based on ESD and placing onto pre-combusted…"

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

AC: Clarified as follows (review document page 37, line 14):

"We measured POC to volume ratios of two sizes classes (typically <0.6 mm ESD, >0.6 mm ESD) at each depth horizon (with the exception of samples at 128, 200 and 500m where all measured particles were <0.6 mm ESD so only one size class was used)…"

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

AC: Amended

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

AC: Amended

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 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.

AC: Thank you for these detailed and insightful comments. Indeed if the tank rotation speed is too fast then there is little time for the particles to settle which would reduce the rate of aggregation. We cannot be sure if the rotation speed had some inhibitory effect on the speed of aggregation but we observed particle formation after two days and aggregates increased in size during the incubation period. Additionally, a large number of the aggregates formed in the study of Iversen and Ploug (2010) fall in the range of sinking velocities that we measure on our roller tank formed aggregates (50-150 m d^{-1}), suggesting that aggregation processes are not inhibited at this speed. However, the long incubation time does increase the likelihood of "bottle-effects", and it could be that bacterial abundances were increased artificially high over the incubation period. However, long incubations (380 hr) were carried out by Iversen and Robert (2015) and no significant changes in carbon-specific respiration rates were observed in this time. Similarly Iversen and Ploug, (2013) carried out roller tank incubations at 4 and 15 °C over a period of a few weeks and did not observe any significant trend in carbon-specific respiration rates. These previous studies suggest that our incubation time of 9 days should not have influenced carbon-specific respiration rates. We have added the following sentence to section 4.2 (review document page 44, line 1):

"Although the length of the roller tank incubation may have allowed for a decrease in PA POC via microbial remineralisation, previous studies carrying out longer incubations (Iversen and Ploug, 2013; Iversen and Robert, 2015) do not measure significant changes of C$_{spec}$ over time suggesting that the long incubation would not bias rates of C$_{spec}$".

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.

AC: We have added new zooplankton data from vertical net tows carried out during our research cruise in order to remove this potential bias (see sections 2.7, and 3.5). As these net data are integrated over 0-200 m we are only able to assess their

role in the loss of POC over this region. The zooplankton abundance and respiration estimates from this study (daytime: 0.3 g DW m^{-2}; night-time: 0.4-0.9 g DW m^{-2}; respiration: 5-10 mg C m^{-2} d^{-1} between 0-200 m) are in good agreement with the data collected 6 years ago (daytime: 0.2-0.4 g DW m^{-2}; night-time: 0.6-1.3 g DW m^{-2}; respiration: 10-18 mg C m^{-2} d^{-1} between 0-200 m). Respiration rates 6 years ago were slightly higher during one of their stations due to high abundance of amphipods and euphausiids. Nevertheless, the overall conclusion in our original submission has not been impacted, as zooplankton respiration is still only a small fraction of the POC loss over that depth horizon (~220 mg C m^{-2} d^{-1} between 0-200 m).

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

AC: Thank you for spotting our mistake. This has been corrected.

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.

AC: The statistics quoted here show that there is a significant relationship between sinking velocity and ESD with the low R^2 describing that there is large variability surrounding this relationship. To make this variability clear we have added the following sentence (review document page 40, line 10):

"The low R^2 shows that there is variability around this relationship, suggesting some heterogeneity in PA composition."

We have also added the outliers to the relationship to Figure 3.

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

AC: Corrected

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.
AC: Thank you for picking up on this, we intended to only compare sinking velocities and have restructured the sentence. Although particle sinking velocities are also affected by many factors, are best compared for particles of similar sizes which is why we compare to only a subset of particles in the aforementioned study and quote the size range of this subset. We have restructured the sentence to make this clearer (review document page 40, line 15).
"A study in the Southern Ocean by Laurenceau-Cornec et al. (2015) also found size-specific (1.3-3.1 mm ESD) sinking velocities similar to those measured here (50-149 m d^{-1})."
p. 8, lines 3-9: this paragraph seems to belong to the Discussion section rather than the Results.
AC: Although we do attempt to explain results here in the manner of a discussion, we have chosen to do this within the results section to keep the manuscript as concise as possible. We briefly explain the possible scenarios leading to an increase in flux with depth, but quickly move on to the main focus of our paper, the role of particle associated microbial respiration. An additional section would be needed in the discussion to discuss these results which we believe would unnecessarily increase the length of the article and break up the flow of the article.
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.
AC: We have altered the sentence for clarity and have referred the reader to section 2.4 where the separation into two size classes (<0.6, >0.6 mm ESD) is described (review document page 41, line 12).
"For each depth horizon we measure the POC contents of the PA used in respiration experiments (see section 2.4). We find POC contents of PA$_n$ ranging…"

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].

AC: Although calculation of the fractal dimension would indeed provide another parameter to compare the roller tank aggregates with the in situ collected aggregates, as we do not have stacked images of each particle we think that the uncertainties in calculating the exact particle perimeter (as required for the Kilps et al. (1994)) are too high. Logan and Wilkinson (1990) derive a method using settling velocity versus size to estimate fractal dimension, however this too relies on a number of assumptions which we believe would increase uncertainty in our analysis and make the comparison less useful.

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

AC: See response to general comment

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

AC: Changed

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?

AC: We have amended this sentence to clarify our meaning here. Although a number of studies have noted some of the issues with roller tank studies, many studies still utilize roller tanks to collect particles, We therefore thought that we could make additional useful comparisons of respiration rates. We use the roller tank formed aggregates to provide data in the fluorescence maximum where we did not have a MSC deployment. The following text has been added in section 4.2 (review document page 44, line 24).

"Over the past couple of decades there have been numerous studies utilizing roller tanks to create artificial aggregates from natural phytoplankton assemblages in an attempt to replicate natural sinking particulates, investigating processes such as aggregation, sinking velocity, ballasting and degradation (e.g. Iversen and Robert, 2015; Iversen et al., 2010; Laurenceau-Cornec et al., 2015; Shanks and Edmondson, 1989). In the present study, we sampled the natural phytoplankton assemblage from waters combining the highest POC concentration and chlorophyll fluorescence in an attempt to assess the aggregation potential in the most productive water strata."

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.

AC: We have amended the sentence to clarify that only visual assessment of taxonomic composition was carried out and as already stated in the manuscript, we note that further chemical analyses would be needed to allow quantification of any differences between aggregates (review document page 44, line 8).

"However, we could not visually distinguish any clear differences in taxonomic composition of PA_n collected at depth and roller tank PA based on SEM or light microscope imagery (supplementary Fig. S1)."

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.

AC: We have amended the sentence to say that we were unable to measure bacterial abundance, rather than it being beyond the scope of the study. We recommend that future studies should measure bacterial abundance and if possible composition to better constrain the mesopelagic food web. Please see our response to general comment (3) above.

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

AC: We provide some possible explanations for this high value in section 3.4, and as noted in a previous comment, choose to do so in this section rather than break the flow of the discussion here which assesses the role of particle associated microbes on POC flux attenuation (review document page 40, line 28)

"Considering the decrease in resident zooplankton populations with depth (Giering et al., 2014), it seems unlikely that FP

production was higher at this depth unless there is a large contribution by diel vertical migrators, and may instead reflect reduced FP loss. However, this scenario could also be due to non steady state conditions with high FP at 500 m reflecting high abundances of FP sinking out from shallower in the water column at a time of previously increased FP production..”

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.

AC: We have amended these sentences to explain more clearly our justification and to correct our terminology (review document page 47, line 5).

“The direct hydrolysis by attached microbes likely supplies free-living communities with DOC (Cho and Azam, 1988; Karl et al., 1988; Kiørboe and Jackson, 2001). However by definition free-living microbes are not associated with particles and hence do not contribute directly to the loss of large fast sinking POC measured here, and as such we do not consider this loss process. The definition of dissolved and particulate is operational based on the pore size of a GF/F filter, and therefore microbes defined as 'free-living' may in fact be able to utilize colloids (Arístegui et al., 2009). However as we only measure the loss of large, fast sinking POC, we exclude free-living bacteria from our analysis of fast sinking POC loss processes. Free-living prokaryotic respiration may account for the ultimate loss of organic carbon from the organic carbon pool but we believe this is reliant on mechanical breakdown of large, fast sinking POC by zooplankton and protozoa (Lampitt et al., 1990; Poulsen and Iversen, 2008; Poulsen et al., 2011) and enzymatic hydrolysis (Smith et al., 1992).”

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?

AC: We intend this figure to convey the location of the PAP site and hence have chosen a scale that enables the incorporation of identifiable land masses. We choose to plot satellite chlorophyll for 18/06/2015-25/06/2015 (first MSC sample taken on 24/06/2015), in part because particles measured at depth over the first part of the cruise would have originated at the surface during this period. In addition, the cloud cover in the following 8 day satellite image encompasses the PAP study site so is a less useful comparison.

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.

AC: Following your suggestion we have re-plotted the y-axis of figure 3 on a log scale, which also allows us to plot all the outliers. We have investigated relationships of size and sinking velocity for each depth but do not believe we have enough data points within each depth to draw conclusions with certainty. Adding separate fits would, we believe, not be valid and would over complicate the figure.

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Depth-resolved particle associated microbial respiration in the northeast Atlantic

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Abstract. ~~The depth at which sinking particulate organic carbon (POC) is remineralized in the ocean is tightly linked to a~~Atmospheric levels of carbon dioxide are tightly linked to the depth at which sinking particulate organic carbon (POC) is remineralized in the ocean. Rapid attenuation of downward POC flux typically occurs in the upper mesopelagic (top few hundred meters of the water column), with much slower loss rates deeper in the ocean. Currently we lack understanding of the processes that drive POC attenuation, resulting in large uncertainties in the mesopelagic carbon budget. Attempts to balance the POC supply to depth discrete layers of the mesopelagic ~~in discrete depth layers~~ with respiration by zooplankton and microbes in those layers rarely succeed. ~~, with respiration sometimes being 50% lower than apparent carbon loss in the upper mesopelagic. One term that is often poorly quantified in such budgets is particle associated bacterial respiration, which we hypothesize could serve as the 'missing sink' for carbon in the upper mesopelagic. Here we test this hypothesis~~ In particular, it has been suggested that organic-carbon supply exceeds respiration by free-living microbes and zooplankton in the upper mesopelagic. We test the hypothesis that particle-attached microbes contribute significantly to community respiration in the mesopelagic, measuring particle associated microbial respiration of POC, through shipboard measurements on individual marine snow aggregates collected at depth. We find very low rates of both absolute and carbon-specific particle associated microbial respiration (<3% d[-1]), suggesting that this term cannot ~~close~~ solve imbalances in the upper mesopelagic ~~carbon~~ POC budget. The relative importance of particle associated microbial respiration increases with depth, accounting for up to 33% of POC loss in the mid mesopelagic (128-500 m). We suggest that POC attenuation in the upper mesopelagic is driven by the transformation of large, fast sinking particles to smaller, slowly sinking and suspended particles via processes such as zooplankton fragmentation, and that this shift to non-sinking POC may help to explain imbalances in the mesopelagic carbon budget.

1 Introduction

The biological carbon pump plays a key role in regulating the partitioning of carbon dioxide (CO_2) between the ocean and atmosphere, and without it atmospheric CO_2 would likely be 200 ppm higher than it is today (Parekh et al., 2006). Key to determining its effectiveness is the efficiency with which organic carbon sinks through the ocean interior (quantified as the transfer efficiency), and thus the depth at which material is remineralized ~~(quantified as the b parameter, Martin et al., 1987)~~ (Kwon et al., 2009). However, despite its importance, the processes governing the loss of organic carbon within the mesopelagic are poorly understood (Burd et al., 2010).

POC sinking out of the euphotic zone can be transformed within the mesopelagic in many ways including, zooplankton feeding, fragmentation via sloppy feeding, microbial solubilization to dissolved organic carbon (DOC), and physically driven aggregation and disaggregation processes (e.g. Azam and Malfatti, 2007; Burd and Jackson, 2009; Belcher et al., 2016). Ultimately carbon is lost from the organic carbon pool as CO_2 via respiration, and hence, in theory at steady state, the loss (attenuation) of POC should be balanced by community respiration (Buesseler and Boyd, 2009). However, settling organic matter is often found to be insufficient to meet the energy demands of microbes in the dark ocean, thus leading to an imbalanced mesopelagic carbon budget (Herndl and Reinthaler, 2013; Steinberg et al., 2008).

A recent study managed to close the mesopelagic carbon budget between 50-1000 m in the North Atlantic (Giering et al., 2014). However, this study contained large and compensating imbalances <u>between sources and sinks</u> in upper and lower mesopelagic layers, with an excess of POC supply to the upper mesopelagic (50-150 m depth), and an excess of respiration in the lower mesopelagic (150-1000 m depth). Prokaryotes were found to be responsible for most of the respiration (92%) across both depth ranges, however the sampling techniques used may have underestimated the respiratory loss due to particle associated prokaryotes which have typically not been included in mesopelagic carbon budget studies (Giering et al., 2014; Steinberg et al., 2008). Data from the subtropical North Atlantic and west Antarctic Peninsula show that particle associated microbial respiration can contribute 32-93% of the total respiration measured in situ (McDonnell et al., 2015), suggesting that particle associated microbes could play an important role in the loss of POC in the mesopelagic. Alternatively, Iversen et al., (2010) found that rapid flux attenuation was best explained by flux feeding by zooplankton off Cape Blanc (Mauritania). We hypothesize that POC losses via particle associated respiration (a term not directly measured by Giering et al., 2014 or Steinberg et al., 2008) may help to ~~close the~~<u>address imbalances in the</u> upper mesopelagic carbon budget.

Marine snow particles (aggregates of detritus, living organisms and inorganic matter larger than 0.5 mm in diameter, Alldredge and Silver, 1988) can make up a large fraction of the sinking POC in the ocean and host microbial abundances 2-5 orders of magnitude higher than those found free-living in the surrounding water column (Silver and Alldredge, 1981; Thiele et al., 2015). The fragile nature of marine snow particles makes sampling and measurement difficult; many previous measures of particle associated respiration have been carried out on roller tank formed marine snow aggregates, either from lab cultures of phytoplankton or natural sea water samples (Grossart and Ploug, 2001; Iversen and Ploug, 2010, 2013; Iversen et al., 2010). A few experiments have utilized SCUBA or submersibles to collect in situ aggregates and estimated

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heterotrophic bacterial production by measuring leucine uptake (Alldredge and Youngbluth, 1985; Smith et al., 1992) with few measuring respiration directly on individual aggregates (Ploug et al., 1999). To the best of our knowledge only two studies have combined direct measures of respiration on aggregates collected at depth in situ with measurements of POC flux (Collins et al., 2015; McDonnell et al., 2015), both of which lack sufficient vertical resolution in the upper mesopelagic to capture the region of most rapid change. Collins et al. (2015) measure rates of substrate-specific microbial respiration of 0.007 ± 0.003 d^{-1} to 0.173 ± 0.105 d^{-1} in the North Atlantic, with rates of 0.01 ± 0.02 d^{-1} and 0.4 ± 0.1 d^{-1} measured by McDonnell et al. (2015) at the Western Antarctic Peninsula and Bermuda Atlantic Time Series respectively. Previous studies are therefore inconclusive as to the importance of particle associated microbes on the attenuation of POC, with some studies suggesting they play a minor role (Alldredge and Youngbluth, 1985; Collins et al., 2015; Ducklow et al., 1982; Karl et al., 1988) and others suggesting a larger contribution (Iversen and Ploug, 2013; Ploug et al., 1999; Turley and Stutt, 2000).

To build upon these previous studiesHere we assess the role of particle associated microbial respiration in POC flux attenuation, presenting a the first vertical profile of particle associated respiration rates measured on individual marine snow particles collected at depthin situ. In an attempt to assess whether this term can improve our ability to balance the fast sinking POC budget in account for the 'missing carbon sink' in the upper mesopelagic, we make these measurements in the northeast Atlantic at the site of Giering et al., (2014) where we have the most complete knowledge of the mesopelagic carbon budget. In addition, we compare the natural particle sinking velocities and respiration rates with those of aggregates produced in roller tanks. We focus on the upper region of the mesopelagic (mixed layer depth-500 m) where the most rapid attenuation occurs, a region that is poorly understood and poorly represented in model studies (Henderson and Marchal, 2015).

2 Methods

2.1 Study site

Measurements were made during research cruise DY032 (20th June – 8th July 2015) to the Porcupine Abyssal Plain (PAP) observatory site (49 °N, 16.5 °W) in the northeast Atlantic aboard RRS *Discovery*. Vertical profiles of the water column at each site were made using a Conductivity-Temperature-Depth (CTD) unit (Seabird 9Plus with SBE32 carousel). The mixed layer depth (MLD) was determined as the depth where temperature was 0.5°C lower than surface temperature (Monterey and Levitus, 1997).

2.2 Chlorophyll-a

Depth profiles of chlorophyll-a were measured at a number of points during the cruise using water samples (200 mL) collected with the CTD rosette. Samples were filtered onto 0.8 μm MPF300 glass fibre filters, and frozen at -20 °C. Pigments were extracted in 90% acetone for 22-24 hours at 4 °C and fluorescence measured on a Trilogy Turner Designs 7200 lab fluorometer calibrated with a pure chlorophyll-a standard (Sigma, UK).

Aqua MODIS 9 km, 8 day satellite chlorophyll-a data (downloaded from the NASA Ocean Biology website; http://oceancolor.gsfc.nasa.gov/cms/) were used to assess mesoscale variability (e.g. passage of eddies) during the sampling period. ~~The~~ We examine changes in surface chlorophyll prior to and post sampling ~~stage of the bloom was inferred~~ by averaging chlorophyll data over the study region (48.5-49.5 °N, 16.0-17.0 °W) ~~and examining temporal changes prior to and post sampling~~.

2.3 Particle flux and composition

Particle flux and composition were measured using Marine Snow Catchers (MSC), large (95 L) PVC closing water bottles designed to minimize turbulence (Riley et al., 2012). MSCs were deployed between 36-500 m during the course of the cruise, at which depth the bottles were closed, retrieved on deck and left for particles to settle. Deployment depths were chosen based on MLD ~~defined~~ determined as the depth with steepest gradient in temperature from the most recent CTD profile. MSC deployments were carried out during the day with the exception of the two samples at 36 m and 128 m, which were deployed at night due to logistical limitations. Particles were allowed to settle onto a particle collector tray at the base of the MSC for two hours (we deem these 'fast sinking' as in Riley et al., (2012)), after which those visible by eye (>0.15 mm diameter) were picked from three quadrants using a wide bore pipette, filtered onto pre-combusted (450 °C, 24 h) glass fibre filters (25 mm diameter GF/F, Whatman), and oven dried at 50 °C for replicate analysis of POC. Filters were subsequently fumed with 37% HCl in a vacuum desiccator for 24 hours, and dried for 24 hours at 50 °C. Filters and filter blanks were placed in pre-combusted (450 °C, 24 h) tin capsules as in Hilton et al., (1986), and POC measured by a CE-440 Elemental analyser (Exeter Analytical.285 Inc). Particles in the remaining quadrant were transferred to a temperature controlled laboratory (10 °C) and used for measurements of sinking and respiration rates (section 2.4). Slow sinking particles were also collected as of (Riley et al., (2012), with slow sinking particle velocities calculated using the SETCOL method (Bienfang, 1981). Slow sinking fluxes were only a small part of the total sinking flux (on average <10%) and due to their slower sinking rate these particles do not penetrate as deeply in the mesopelagic. Hence we focus our study on fast sinking particles only.

The flux of POC (F in mg C m^{-2} d^{-1}) associated with fast sinking particles was calculated as follows:

$$F = \frac{m}{A} \times \frac{w}{h}, \tag{1}$$

where m refers to the total mass (mg) of fast sinking POC collected from the MSC, A the area (m^2) of the MSC based on inner MSC diameter, w the measured sinking velocity (m d^{-1}) from laboratory measurements, and h the height of the snow catcher (1.53 m). Sinking velocities of marine aggregates were measured in a flow chamber (section 2.4), and the median value for each depth horizon used to avoid bias by rare fast sinking aggregates. The rate of particle flux attenuation was assessed by fitting a power-law function (Martin et al., 1987) to the flux data,

$$F_z = F_0 \times (z/z_0)^{-b}, \tag{2}$$

where z is the depth of the flux, and F_0 is flux at the reference depth (in this case 26 m, i.e. the mixed layer depth). A high value of b corresponds to high attenuation (shallow remineralization) and vice versa. We note that as in situ particle production at depth is not considered, this represents a lower bound estimate of flux attenuation.

The ~~composition~~ type of fast sinking particles at each depth was assessed under a microscope and photographs taken using a Leica DM-IRB inverted microscope and Canon EOS 1100D camera. Particles were classified into phytodetrital aggregates (aggregations >0.15 mm equivalent spherical diameter (ESD) containing phytoplankton cells and other phytodetrital material, herein referred to as PA), faecal pellets (FP), and unidentified phytodetritus. Individual particle dimensions were measured using ImageJ (version 1.49p) and volumes calculated using formulae for a sphere, prolate ellipsoid or cylinder depending on particle shape. Conversions from PA volume were based on measurements of POC content of ~~in situ~~ marine aggregates collected at depth (section 2.4), and a carbon to volume ratio of 0.08 mg mm^{-3} used for FP based on literature estimates (range 0.01-0.15 mg mm^{-3}) (Wilson et al., 2008). FP carbon content can vary greatly even within species depending on factors such as food type and concentration (Urban-Rich, 2001), which introduces uncertainty into our estimates of their contribution to the total POC flux.

2.4 Oxygen gradients in marine snow aggregates

The rates at which sinking particles were degraded due to the respiration of particle associated microbes were calculated from direct measurements of oxygen gradients within PA. PA were transferred into a temperature controlled flow chamber system (Ploug and Jorgensen, 1999) containing filtered sea water (0.22 μm), taken from the MSC deployed at 36 m and maintained at 10 °C (at the low end of temperatures measured during the study, Fig. 1). Only one incubation temperature was possible due to laboratory and space limitations. The salinity in the flow chamber was 35.5 PSU which, considering the low variation in salinity profiles (standard deviation of 0.008 PSU at 36 m depth) should represent conditions at all depths sampled. Within 24 hours of collection, PA were placed carefully in the flow chamber using a wide bore pipette. The wide bore pipette lifts the particles with the surrounding water so that the particles remain suspended in water during the handling and minimal physical stress is exerted on the particles. The microbial communities associated with the aggregates are not removed by this method (Kiørboe et al., 2002). The~~ir~~ x, y, and z dimensions of PA were measured using a horizontal dissection microscope with a calibrated ocular, and three measurements of the sinking velocity made for each PA by suspending the PA with an upward flow (Ploug et al., 2010). PA volumes were calculated from x, y, z dimensions based on an ellipsoid, and equivalent spherical diameters (ESD) calculated.

A profile of oxygen was measured from the ambient water, through the diffusive boundary layer (DBL) and into the PA using a Clark-type oxygen microelectrode and guard cathode (Revsbech, 1989) mounted in a micromanipulator. Measurements were made in increments of (50-200 μm) on the downstream side of the particle and oxygen fluxes calculated using a diffusion-reaction model based on Fick's first law of diffusion (diffusion coefficients of 1.4691 x10^{-5} cm^2 s^{-1} for 10 °C and salinity 35 PSU, Broecker and Peng, 1974). Two to three replicate profiles were taken for each PA where possible. We used a solver routine to find the optimum solution minimizing the sum of the squares between measured and modelled

oxygen concentrations (see Ploug et al., (1997) for full details). Total oxygen consumption within the PA was calculated using the equation for the surface area of an ellipsoid assuming that net oxygen fluxes do not vary significantly on the upstream and downstream sides (Ploug and Jorgensen, 1999). As oxygen consumption in the DBL is a measure of the respiration rate of the microbial community associated with the PA due to net exchange of oxygen via molecular diffusion, the carbon respiration (C_{resp} in mg C $\underline{mm^{-3}}\cancel{m^{-2}}$ d^{-1}) can be calculated based on a respiratory quotient (RQ) of 1 mol O_2 to 1 mol CO_2 (Ploug and Grossart, 2000; Ploug et al., 1997). This was chosen as a conservative value in the range of literature values typically applied (0.7-1.2 mol:mol) for respiration of carbohydrates and lipids (Berggren et al., 2012), but adds uncertainty to our estimates that cannot be better constrained without knowledge of the form of carbon within the PA utilized for microbial respiration.

Following respiration measurements, PA were stored in 1.5 mL Eppendorf tubes before pooling PA into size classes based on ~~of known and similar~~ ESD and placing onto pre-combusted (450 °C, 24 h) glass fibre filters (25 mm diameter GF/F, Whatman) for measurement of POC as described in section 2.3. This enabled the carbon content per unit volume (mg C mm^{-3}) ~~carbon to volume relationships~~ for each size class at each depth range to be calculated and hence POC content of individual PA to be estimated. ~~Where possible W~~We measured POC to volume ratios of two sizes classes (typically <0.6 mm ESD, >0.6 mm ESD) at each depth horizon (with the exception of samples at 128, 200 and 500m where all measured particles were <0.6 mm ESD so only one size class was used), to take into account the fractal ~~shape~~ geometry of aggregates and non-linear volume to POC ratio (Alldredge, 1998). ~~(Ploug and Grossart, 2000; Ploug et al., 1999).~~ These PA POC contents ([POC] in mg C mm^{-3}) were then used to calculate carbon-specific respiration rates (C_{spec} in d^{-1}) as follows:

$$C_{spec} = C_{resp}/[POC]. \hspace{4cm} (3)$$

2.5 Roller tank experiments

Water was sampled on 24th June from 12 m depth (within the surface peak in chlorophyll) from the CTD rosette and transferred to 2 L acid-cleaned Nalgene polycarbonate bottles. The bottles (141 mm diameter, 249 mm height) were rotated on a 120V Benchtop Roller Culture Apparatus (Wheaton) at 3 rotations per minute (rpm, Iversen and Ploug, 2010) at 10 °C in the dark. The tanks were left to incubate in the dark and form aggregates for a period of 9 days before carefully removing aggregates and measuring respiration rates as described above.

2.6 Statistics and error analysis

Attenuation of fast sinking POC flux with depth was best described by a power-law relationship fit (R^2=0.42, p=0.06, n=9) (Martin et al., 1987) compared to an exponential fit (R^2=0.30, p=0.128, n=9) (of form, $F_z = F_0 \exp(z-z_0/z^*)$, as in Buesseler and Boyd (2009)). We also tested for any statistical relationship between carbon-specific respiration rates and depth. All statistics were carried out in RStudio (version 0.98.1091; R development core team, 2014). We calculate a relation between particle ESD and sinking velocity by applying a power law fit to the data using the NLS function in RStudio. The

choice of a power law relationship is based on the findings of previous studies (e.g. (Iversen and Ploug, (2010)) and the divergence of marine snow aggregates from Stoke's Law due to their fractal rather than spherical geometry.

Time and methodological constraints of measuring very small particles, prohibited us from measuring the respiration rate of every particle collected in the MSC. Hence, before using these measurements to assess the contribution of particle associated microbial respiration to the mesopelagic carbon budget, we must first define upper and lower bounds to our estimates based on our uncertainties. We conduct a Monte Carlo analysis (with 10,000 iterations) of the individual parameters used in the calculations of carbon-specific respiration and remineralization length scale. We randomly sample (with replacement) our measured volumetric oxygen respiration rates at each depth. For each of these randomly selected particles we use the corresponding sinking velocity and ESD in subsequent calculations of carbon-specific respiration and remineralization length scale. For the RQ, used to convert O_2 to CO_2, we define a uniform distribution of possible values over the range of RQ values typically applied in the literature (0.7-1.2, Berggren et al., (2012)). PA were pooled into size classes and could only be measured once for POC content. For each depth, we create a normal distribution of possible POC:volume ratios for each size class, with our measured value as the mean, and standard deviation calculated from the standard deviation of the individual aggregate volumes within a size class. Based on the 10,000 iterations for each of the aforementioned parameters we obtain a range of estimates for the remineralization length scale (via particle associated microbial respiration) at each depth. We then use the mean of these distributions ±standard deviation to put error bounds on our estimates of the POC loss via particle associated microbial respiration.

2.7 ~~Ancillary data~~Zooplankton respiration

Zooplankton were sampled in vertical net hauls (0-200 m) at 1 m s^{-1} speed using a 200 µm mesh size WP2 Net with a 57 cm frame diameter, fitted with filtering cod-ends. Collected organisms were fixed directly after collection with formalin at 5% final concentration for further analyses. In the laboratory, fixed samples were digitized with the ZooScan digital imaging system (Gorsky et al., 2010) to determine the size structure of the community. Each sample was divided into two fractions (<1000 and > 1000 µm) for accurate estimation of rare large organisms in the scanned subsample (Vandromme et al., 2012). Fractions were split using a Motoda splitting box until containing approximately 1000 objects. The resulting samples were poured onto the scanning cell and individual zooplankton were manually separated with a wooden spine, in order to avoid overlapping organisms. Each scanned image was later processed using ZooProcess (Gorsky et al., 2010). Each object in the image was automatically classified into five zooplankton categories (copepods, chaetognatha, appendicularia, other crustaceans, other zooplankton) and three non-living categories (detritus, fibers and out of focus) using Plankton Identifier (http://www.obs-vlfr.fr/~gaspari/Plankton_Identifier/index.php). These automatic results were manually validated. Finally, dry weight (DW) of each zooplankton object was estimated from its area using Lehette and Hernández-León's, (2009) allometric relationships corresponding to the five zooplankton categories. Respiration per individual (µC individual^{-1} h^{-1}).was computed from DW using relationship from Ikeda et al., (2001) for copepods and (Ikeda, 1985) for other groups:

In order to assess the importance of particle associated microbial respiration we compare our measured rates to other previously measured mesopelagic sinks of fast sinking POC at the PAP site. We calculated non migratory zooplankton respiration rates (mg m⁻² d⁻¹) over the depth horizons of interest using data from Giering et al. (2014) from the PAP site in August 2009. Non-migratory zooplankton respiration is based on measured abundances during two sets of day/night net deployments and the calculated respiration per individual (µC individual⁻¹ h⁻¹).

$$Zooplankton\ Respiration = exp(a_1 + a_2 \ln(DW) + a_3 T) \times RQ \times 12/22.4 \tag{4}$$

Here DW is dry weight (mg C individual^{-1}), RQ is the respiratory quotient (0.8 mol C/mol O$_2$), T is the average temperature over top 200 m (12.5 °C), 12/22.4 is the molar conversion factor and parameters a_1, a_2, and a_3 were dependent on the type of zooplankton. (see Giering et al. (2014) and references within for full details). Total zooplankton respiration (0-200 m) was calculated by summing the respiration values for each individual. Day and night respirations were calculated for 16 and 8 hours respectively based on day length at the study site. For each depth we took the average non migratory zooplankton respiration rate of the two deployments, before integrating over the required depth horizon for carbon budget comparisons.

3 Results

3.1 Hydrography and surface chlorophyll-a

The consistency of vertical temperature profiles suggests little variation in water mass structure during the cruise (Fig. 1b). Temperatures ranged from 15.2 °C at the surface to 10.9 °C at 500 m, with salinity remaining relatively constant with depth (average 35.34-35.56, Fig. 1b). The mixed layer shallowed from 32 m to 26 m (Fig. 1b), with peak chlorophyll just above the MLD at 15-25 m, and decreasing from 1.9 to 1.4 mg m^{-3} during the course of the cruise based on discrete measurements (Fig. 1c). The MLD was typically within ±5 m of than the 1% photosynthetically active radiation (PAR) level. Satellite chlorophyll data is consistent with in situ data, declining from 1.8 to 1.2 mg m^{-3} in the PAP region, suggesting sampling was carried out in the 'post peak' phase (Fig.1c).

3.2 Particle composition

A total of 10 MSC deployments were made over an 11 day period with particle composition and respiration measurements carried out for 7 deployments (Table 1).

The dominant component of fast sinking particles was PA at all depths sampled (one MSC sample per depth, Fig. 2), accounting for 96 % of sinking POC at 36 m and decreasing to 66 % at 500 m associated with an increasing abundance of FP with depth. The lack of FP observed in our sample at 113 m may be due to the heterogeneous distribution of FP at a particular depth associated with patchy zooplankton distributions. The increase in FP numbers below 100 m could be due to an increase in zooplankton populations with depth, zooplankton diel vertical migration, and/or increased FP loss in the upper mesopelagic due to processes such as fragmentation and grazing coprophagy. Qualitative assessment of FP morphology

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shows that FP were longer, thinner and darker deeper in the water column, implying a change in zooplankton community composition with depth.

3.3 Particle sinking velocities

Sinking velocities of ~~in situ~~ natural PA collected at depth (PA$_n$) ranged from 4-255 m d^{-1} (Fig. 3), reflecting both the range in size of PA measured (0.14-1.09 mm ESD) and the heterogeneous composition of PA. Median sinking velocities showed less variability ranging from 11-34 m d^{-1} (10-32 m d^{-1} and 21-62 m d^{-1} for ~~in situ~~ aggregates <0.6 mm (n=74) and >0.6 mm (n=24) ESD respectively), showing consistency in the composition of the bulk of sinking PA$_n$. There was no significant correlation between PA$_n$ sinking velocity and depth for either size class (R^2=0.004, p>0.1, n=98). PA$_n$ sinking velocity was significantly (R^2=0.17, p<0.0001, n=98) correlated with ESD (6 outliers, defined as being outside 2 standard deviations from the mean, were excluded in this relationship). The low R^2 shows that there is variability around this relationship, suggesting some heterogeneity in PA composition. PA formed in roller tanks, defined here as PA$_r$, were much larger in size (0.54-3.2 mm ESD), but had lower sinking rates for their size (6-173 m d^{-1}) as illustrated by the different power-law fits in Fig 3. We note here that sinking velocity measurements were limited to those particles visible by eye (ESD > 0.15 mm). However, measured velocities do agree with previous observations for the North Atlantic ocean which range from 0.2-181 m d^{-1} (supplementary table S1 in Collins et al. (2015)). A study in the Southern Ocean by Laurenceau-Cornec et al. (2015) also found ~~natural aggregates to be smaller than roller tank formed aggregates, and~~ size-specific (1.3-3.1 mm ESD) sinking ~~rate~~velocities ~~were~~ similar to those measured here (50-149 m d^{-1}) ~~considering roller tank aggregates of comparable sizes (1.3-3.1 mm ESD)~~.

3.4 Particle flux

Consistent with other studies we see a sharp decline in fast sinking POC concentration (not shown) and fast sinking POC flux with depth (Fig. 4). Rapid attenuation in the upper 128 m was followed by a slower decrease and possibly even an increase in POC flux below 128 m, suggesting that different processes may be controlling POC attenuation in the upper and lower mesopelagic, or that the rate of processes varies with depth. Interestingly we see an increase in flux between 203 and 500 m, which may reflect higher surface production in the days prior to sampling (Fig. 1c) and the time taken for material to reach this depth from the surface. Based on a median sinking rate of 34 m d^{-1} measured at 500 m, material at this depth would have originated at the surface on Julian day 164, 15 days prior to sampling, which corresponds to the peak in surface chlorophyll concentrations (Fig. 1c). This increase in flux is associated with twice as much FP POC at 500 m compared to 203 m, and a 29% increase in aggregate POC. Considering the decrease in resident zooplankton populations with depth (Giering et al., 2014), it seems unlikely that FP production was higher at this depth unless there is a large contribution by diel vertical migrators, and may instead reflect reduced FP loss. However, this scenario could also be due to non steady state conditions with high FP at 500 m reflecting high abundances of FP sinking out from shallower in the water column at a time of previously increased FP production. Excluding this potentially non steady state value at 500 m, we calculate a Martin's *b*

value of 0.71 which is in line with previous studies at this study site (Giering et al., 2014; Riley et al., 2012), but note that this fit is just outside the 5% significance level (R^2=0.42, p=0.06, n=9). To assess the uncertainty surrounding our calculated b value, we have applied a bootstrap analysis with 100,000 simulations giving a mean b of 0.67 ± 0.34 (standard deviation).

3.5 Microbial respiration in phytodetrital aggregates

Using the microelectrode approach we found that oxygen concentrations decreased from the ambient water towards the PA surface, reaching a minimum at the centre of the PA (but remaining well above anoxic conditions in all PA measured) (Fig. 5). Average oxygen fluxes to ~~in situ~~ PA_n did not vary significantly with the depth at which particles were collected, ranging from 11.7-19.1 nmol O_2 mm^{-3} d^{-1} (Fig. 6), but variability between ~~samples~~ PA collected at each depth was large (3.1-43.8 nmol O_2 mm^{-3} d^{-1} over the depth range measured, Table 2). Volumetric respiration rates on roller tank formed aggregates (PA_r) were smaller (2.5-7.7 nmol O_2 mm^{-3} d^{-1}) than those of ~~in situ~~ PA_n, although the large range in rates for ~~in situ~~ PA_n (4.7-37.7 nmol O_2 mm^{-3} d^{-1} for PA >0.6mm ESD) makes direct comparison difficult.

For each depth horizon we measure the POC contents of the PA used in respiration experiments (see section 2.4). ~~Based on pooled measurements of in situ collected PA_n at each depth horizon~~ Wwe find POC contents of PA_n ranging from 11.7-30.6 μg mm^{-3} (average 15.4 μg mm^{-3}) for PA <0.6 mm ESD, and 6.7-11 μg mm^{-3} (average 9.0 μg mm^{-3}) for PA >0.6 mm ESD. The POC content of both size classes peaked at 46 m depth, showing a general decline below this (supplementary material, table S1). PA_r had lower POC to volume ratios, 2.4 μg mm^{-3} for PA_r <1 mm and 1-2 mm ESD. POC to volume ratios for PA_r are more comparable to phytoplankton culture aggregates formed in roller tanks, which were also similarly sized (Iversen and Ploug, 2010). Our POC measurements are based on filters containing a relatively low number of aggregates; 9-13 aggregates and 4-6 aggregates for aggregates <0.6mm ESD and >0.6mm ESD, respectively. However, despite the low concentrations of carbon measured, sample POC was significantly higher than POC filter blanks (Welch's t-test, p<0.001).

3.5 Zooplankton respiration

Four zooplankton net tows were carried out during the cruise alongside MSC deployments, one during the day and three at night. Daytime zooplankton DW over the upper 200 m was 313.4 mg DW m^{-2}, with night values ranging from 419.1 to 942.7 mg DW m^{-2}. Calculated zooplankton respiration rates ranged from 5.1 to 10.1 mg C m^{-2} d^{-1}.

4 Discussion

4.1 Rate of particle associated microbial respiration

Although rates of respiration per PA volume were found to be relatively uniform with depth, we observed variability within each depth range. This may reflect the heterogeneity of aggregate composition in terms of the availability of labile

carbon and/or variation in microbial abundance, composition or activity. It may also simply be a result of the range in aggregate sizes at each depth, with higher respiration per volume in smaller aggregates that have higher POC:volume ratios (due to their fractal nature, Logan and Wilkinson, 1990). Our measured 0.14-1.09 mm ESD natural aggregate POC contents are 1.2-10.1 times higher than defined by the size relationship of Alldredge (1998) based on in situ collected 1-5 mm ESD

5 marine snow of mixed composition, and are at the high end of the range of values measured on roller tank formed phytoplankton culture aggregates (0.9-4.6 mm ESD) by Iversen and Ploug (2010). The POC contents of our >0.6mm ESD PA (6.7-11 µg mm^{-3}) and 1-2 mm ESD PA$_r$ (2.4 µg mm^{-3}) do however compare well with the study of Laurenceau-Cornec et al. (2015) on aggregates formed in roller tanks from in situ collected phytoplankton assemblages; we calculate aggregate POC of 7.4 µg mm^{-3} and 2.7 µg mm^{-3} for aggregates of 0.6 mm and 1.0 mm ESD respectively based on their regression

10 between aggregate volume and POC content (POC=0.58 · Volume$^{0.35}$). This indicates that the power-law defined by Laurenceau-Cornec et al. (2015) can be applied to our study site.

In order to assess whether size related changes in carbon content of PA is the main cause of variability in volume specific respiration rates, we have calculated the carbon-specific respiration rate (C$_{spec}$) (Fig. 7) based on the POC content of individual aggregates. There is a relatively small range in average C$_{spec}$ (0.011-0.014 d^{-1}) for ~~in situ collected~~ PA$_n$ for each

15 depth horizon. Iversen and Ploug, (2010) measured higher rates of C$_{spec}$ (0.13 d^{-1}) in roller tank formed phytoplankton culture aggregates of lower POC contents, suggesting that POC content was not the limiting factor for respiration in our study. ~~As suggested by standard error bars (Fig. 7),~~ Despite the consistency of average values of C$_{spec}$ at each depth horizon, there ~~was is still~~ large variability in C$_{spec}$ for individual aggregates within each depth horizon (full dataset range: 0.002-0.031 d^{-1},)Fig. 7). The calculated standard error was consistent across all depths (0.002—0.003) which ~~The similarity in the range of values~~

20 ~~at each depth~~ implies that the factors driving the~~is~~ variability in C$_{spec}$ are unchanging with depth, suggesting controls on degradation are already determined at shallow depths. Our study does not however account for any changes in respiration that may occur as a result of pressure changes with depth (see section 4.5). If microbes largely attach to particles in the surface ocean (Thiele et al., 2015), the starting abundance of microbes will be in part limited by the residence time of the particle in the surface ocean as dictated by sinking rate. The highest volume-specific abundances of microbes have been

25 measured on the smallest aggregates (Grossart et al., 2003) which we would expect to have lower sinking velocities. Variable microbial densities, driven by differences in sinking velocity and colonization time, may therefore account for some of the variability in the rate of respiration per aggregate volume or POC content. However, large aggregates could also have high microbial densities following the aggregation of smaller aggregates. There are a number of factors which influence colonization, and grazing has been modelled to have a higher impact than sinking rate (Kiørboe, 2003).

30 We must consider that all respiration measurements in this study were carried out at 10 °C (which is just below the temperature measured at 500 m depth), and therefore may not reveal the true vertical structure of particle associated microbial respiration due to the influence of temperature on metabolic rates. To account for this we have applied a Q10 factor of 3.5 from a study on PA (Iversen and Ploug, 2013) and adjusted each C$_{spec}$ to the in situ temperature (dashed line Fig. 7) with roller tank aggregates being adjusted to the temperature at 12 m (the depth that water was collected from for

their formation). In this way we calculate the rate we would expect to be occurring at in situ temperature. This gives higher rates in the upper ocean where temperature changes ~~s~~ are higher, but the range with depth is still relatively narrow (average 0.013-0.023 d^{-1}, full range 0.002-0.037 d^{-1}) and we observe no relationship between PA size and C_{spec} (Fig. 8). In comparison Ploug and Grossart (2000) measured C_{spec} of 0.083 +/- 0.034 d^{-1} on aggregates formed from phytoplankton cultures at 16 °C which is more comparable to rates of 0.055 +/- 0.006 d^{-1} measured on our roller tank formed aggregates. Iversen and Ploug (2010) measured average C_{spec} of 0.13 d^{-1} at 15 °C but a range of 0.005-0.422 d^{-1} for lab formed aggregates of three different phytoplankton cultures. Similarly, rates of 0.13 d^{-1} (range 0.02-0.36 d^{-1}) were measured at 18 °C in aggregates formed in roller tanks from peak fluorescence waters off Cape Blanc, Africa (Iversen et al., 2010). These studies find a lack of size dependency in C_{spec}, consistent with our observations. Our measurements are towards the low end of these measurements which we cannot explain by differences in temperature alone based on a Q10 factor of 3.5 (Iversen and Ploug, 2013). Recalculating the average C_{spec} at each depth based on the upper bound of respiratory quotients that are typically applied in the literature (0.7-1.2, (Berggren et al., (2012)), increases our values of 0.019-0.033 d^{-1} which is still lower than the aforementioned studies.

There have been limited measurements made on natural aggregates formed in situ. McDonnell et al. (2015) utilized in situ incubators to measure C_{spec} of 0.4 d^{-1} at the Bermuda Atlantic Time Series (BATS) station, and 0.01 d^{-1} off the Western Antarctic Peninsula (WAP). Collins et al. (2015) carried out incubations with and without sinking particles collected in the North Atlantic, revealing C_{spec} of 0.007-0.084 d^{-1} with one higher value at 0.173 d^{-1}. These rates are more in line with those measured here, yet there are still considerable differences between studies. We turn to a comparison of ~~in situ~~ natural and roller tank formed aggregates in an attempt to explain some of this variability.

4.2 In situ versus roller tank formed aggregates

The difficulty of sampling intact PA due to their fragility has led many studies to ~~the~~ use ~~of~~ roller tanks to create artificial aggregates (e.g. Grossart and Ploug, 2001; Iversen and Ploug, 2010, 2013; Iversen et al., 2010). E~~However, e~~ven considering the variability in our respiration estimates for ~~in situ~~ PA$_n$, we find much lower respiration rates in PA$_r$ (Fig. 6). This may be in part due to the lower POC to volume ratios (2.4 μg mm^{-3} for PA$_r$ <1 mm and 1-2 mm ESD) which could be a result of POC loss via respiration during incubation in roller tanks. Considering a carbon respiration rate of ~1 μg C mm^{-3} d^{-1} (Fig. 6) and a starting POC content of 9 μg C mm^{-3} d^{-1} (based on average values for ~~in situ~~ PA$_n$), PA$_r$ POC contents could be reduced to 2 μg C mm^{-3} over 7 days (time incubated after first signs of aggregate formation). However, the fractal nature of aggregates means that we would also expect large aggregates to have lower POC to volume ratios (Alldredge, 1998; Logan and Wilkinson, 1990). POC to volume ratios for PA$_r$ are more comparable to those of Iversen and Ploug (2010) which were also similarly sized. To remove the influence of aggregate size, we now compare PA$_n$ ~~in situ~~ and ~~roller tank aggregates~~ PA$_r$ between 0.7-1.1 mm ESD as this is the size category encompassing both PA types. Volumetric respiration rates of PA$_r$ are 45% lower than ~~in situ~~ PA$_n$, but as POC:volume ratios are 72% lower, this results in PA$_r$ C_{spec} rates that are actually higher than those of ~~in situ~~ PA$_n$ (Fig. 7). This could be due to greater abundances of microbes on individual PA$_r$, and/or higher

quality POC that is more readily respired. Although the length of the roller tank incubation may have allowed for a decrease in PA POC via microbial remineralisation, previous studies carrying out longer incubations (Iversen and Ploug, 2013; Iversen and Robert, 2015) did not measure significant changes of C_{spec} over time, suggesting that the long incubation would not bias rates of C_{spec}.

5   The lability of the carbon may differ between naturally formed and roller tank formed aggregates, with ~~in situ collected~~ PA_n collected at depth containing more reworked material such as FP fragments. Higher rates of solubilization and respiration of labile POC in the euphotic zone would leave more refractory POC to be slowly respired through the mesopelagic and could explain the differences observed between PA_r and ~~in situ~~ PA_n. However, we could not visually distinguish any clear differences in taxonomic composition of ~~aggregate composition between in situ~~ PA_n collected at depth

10   and roller tank PA based on SEM or light microscope imagery (supplementary Fig. S1). Future studies incorporating analysis of pigments, amino acids and neutral aldoses to determine aggregate age, source material and lability (Cowie and Hedges, 1994; Goutx et al., 2007; Skoog et al., 2008; Tamburini et al., 2009) would help to quantify these differences between PA_r and ~~in situ~~ PA_n.

    If we consider the process of aggregate formation, microbial populations in roller tanks have a much longer time to

15   attach to and colonize particles due to the infinite residence time created by the rotating bottles. This would allow much higher densities of microbes to colonize the PA_r compared to ~~in situ formed~~ natural aggregates collected at depth, which, assuming microbial respiration is not limited by any other factor, would drive higher C_{spec}. This hypothesis is also consistent with reduced variability in C_{spec} of PA_r compared to ~~in situ aggregates~~ PA_n where microbial colonization would be influenced by heterogeneity in particle sinking speed and residence time in the surface ocean. We believe artificially high microbial

20   densities on roller tank formed PA is the most likely cause of differences between our respiration measurements on ~~in situ~~ natural PA collected at depth ~~PA~~ and ~~previous~~ measurements on roller tank formed PA. Unfortunately ~~it was beyond the scope of this~~ we were not able to ~~study~~ to measure bacterial abundance in this study ~~but we suggest comparison between in situ and lab formed aggregate bacterial abundance as a key area of future work~~.

Over the past couple of decades there have been numerous studies utilising roller tanks to create artificial aggregates from

25   natural phytoplankton assemblages in an attempt to replicate natural sinking particulates, investigating processes such as aggregation, sinking velocity, ballasting and degradation (e.g. Iversen and Robert, 2015; Iversen et al., 2010; Laurenceau-Cornec et al., 2015; Shanks and Edmondson, 1989). In the present study, we sampled the natural phytoplankton assemblage from waters combining the highest POC concentration and chlorophyll fluorescence in an attempt to assess the aggregation potential in the most productive water strata. As PA_r were formed from material at shallower depths than PA_n were collected,

30   we cannot be certain that the observed differences are not depth related. Further, we left the roller tanks to rotate for a period of 9 days in the dark and cold to simulate POC aggregation in the water column, accelerating plankton death and vulnerability to microbial degradation. These conditions deviate from natural conditions in which the plankton sinking from 12 m depth would have experienced progressive light and temperature decreases as well as changes in grazing pressure. Therefore, it is difficult to ascertain the cause of differences between roller tank formed and in situ collected aggregates, and

rather we use the roller tank formed aggregates to get an estimate of the carbon-specific respiration rate of the aggregates at shallow depths.

4.3 Role of particle associated microbes in mesopelagic POC flux attenuation

Despite the uncertainties in the mechanisms governing rates of particle associated microbial respiration, we are still able to assess the importance of particle associated microbial respiration on the attenuation of fast sinking POC in the mesopelagic and compare our results to the small number of other recent studies (Collins et al., 2015; McDonnell et al., 2015). We calculate the flux of fast sinking POC (F_z) at each depth (z) that would result if the only loss was via particle associated microbial respiration. Calculations were based on the relationship between the remineralization length scale (L in m^{-1}) (see Iversen and Ploug, 2013; Iversen et al., 2010), carbon-specific respiration rate (C_{spec} in d^{-1}) and sinking velocity (w in m d^{-1}).

$$L = \frac{C_{spec}}{w} = (-ln(F_z/F_0)/(z - z_0)) \tag{5}$$

We calculate upper and lower bounds on the remineralization length scale based on uncertainties in our measurements of C_{spec} and w, as described in section 2.6. We compare observed fast sinking POC flux attenuation and predicted losses via particle associated microbes over two discrete depth horizons; a region of rapid attenuation of 36-128 m and slow attenuation zone of 128-500 m. Note that we exclude the non steady state value of POC flux at 500 m and instead use the value predicted from our power-law fit (Fig. 4) with bounds based on the standard deviation of our b value estimated via bootstrap analysis (see section 2.7). Our data suggest that particle associated microbial respiration plays only a minor role in POC attenuation in the upper water column (8%; range: 1-~~13~~14%), but becomes more important below this (33%; range: 12~~30~~-~~6~~50%) as the rate of POC attenuation decreases (Fig. 9a). Our measurements are based on a sub sample of the total assemblage of particles found in the water column, in particular only PA. If rates of microbial respiration are vastly different on other particle types then this would affect our calculations of POC removal by particle associated microbes. However, considering the dominance of PA in our samples (Fig. 2), we believe our calculations reflect the bulk of the sinking material at the time of sampling. We were not able to measure respiration rates on FP due to their low numbers and small size which adds uncertainty to our estimate of the contribution of particle associated microbial respiration to POC loss. However, even if FP were respired ~~at significantly different rates~~completely, they account for less than 10% of the flux between 36-128 m and thus could not resolve the large imbalances between POC supply and respiration that we observe in the upper mesopelagic.

Low rates of respiration result in only a very small loss of POC with depth below the euphotic zone. Thus our data agree with a recent study (Collins et al., 2015), suggesting that only a small fraction of sinking POC is removed by particle associated bacteria. Despite being hotspots for microbial activity compared to the water column (Thiele et al., 2015), ~~P~~particle associated microbial respiration may still be a minor contributor to the reduction ~~represent a slow background~~ in POC flux when compared to rapid loss via~~process which only becomes the dominant control on POC flux attenuation when~~

~~other~~ processes such as zooplankton grazing and fragmentation (Dilling and Alldredge, 2000; Stemmann et al., 2000; Svensen et al., 2014) ~~become less important~~. This hypothesis is supported by measurements made in the mesopelagic of the Scotia Sea on faecal pellets (Belcher et al., 2016) and on PA off Cape Blanc, Africa (Iversen et al., 2010), as well as model studies (Gehlen et al., 2006; Stemmann et al., 2004).

4.4 Mesopelagic carbon budget

Recent assessments of the mesopelagic carbon budget at the PAP site, although balanced over 50-1000 m, revealed an imbalance when the upper and lower mesopelagic were examined separately (Giering et al., 2014). Particle associated respiration was not directly measured in the aforementioned study, and hence we assess whether this term could help to explain observed imbalances. In this way we test whether the low respiration rates (0.001-0.173 d^{-1}) measured by Collins et al. (2015) are also applicable to our study site or whether the higher rates, such as observed in the western subtropical North Atlantic gyre (0.4 d^{-1}) by McDonnell et al. (2015) are more appropriate. As our zooplankton net tows are integrated to 200 m, we compare sources and sinks of POC over the depth range of 36-200 m.

~~however~~ Although we measure low rates of both absolute and carbon-specific PA microbial respiration (<3% d^{-1}) ~~measured here reveal~~ suggesting that this term cannot ~~close~~ resolve imbalances in the upper mesopelagic carbon budget~~.~~ ~~However,~~ we may underestimate ~~,~~ the importance of particle associated microbes ~~may be underestimated~~ if solubilization of POC to DOC by ecto-enzymatic hydrolysis is significant (Alldredge, 2000; Grossart and Simon, 1998; Smith et al., 1992). This solubilization to DOC is likely to fuel the respiration of free-living microbes. Smith et al. (1992) estimated that 97% of the hydrolysates produced by bacteria in marine snow were released, with the remaining 3% being utilized by bacteria in the aggregate. However, this value was based on nitrogen-rich amino acids and hydrolysis for carbon is likely lower as it is lost more slowly than nitrogen from sinking particles. To calculate potential hydrolysis of carbon from particles, we conservatively assume a value of 75% (i.e. assuming our measured loss via respiration is 25% of the total POC loss via particle associated microbes), which sits between Smith et al.'s (1992) value and carbon solubilization losses of <30% measured in copepod faecal pellets which are much less porous (Møller et al., 2003). With additional loss of fast sinking POC ~~loss~~ via solubilization we find particle associated microbes can explain 49~~31~~% (2~~3~~9-5~~3~~7%) of POC losses in the upper ~~and lower~~ mesopelagic (36-200~~128~~ m~~, and 128-500 m~~) ~~respectively~~ (Fig. 9b). In spite of the limitations in our estimations of solubilization it is clear that a large discrepancy still remains in terms of an excess POC supply of 1184 mg C m^2 d^{-1} (608-1797 mg C m^{-2} d^{-1} considering our calculated error bounds on rates of particle associated microbial respiration and fast sinking POC flux measurements) over the upper 36-~~128~~200 m.

The other direct loss of POC in the mesopelagic is via zooplankton respiration and 'sloppy feeding' (cell breakage during feeding and subsequent release of DOC) (Jumars et al., 1989). Our measured zooplankton respiration rates are likely an overestimate of their contribution to POC losses in the upper mesopelagic (36-200 m) as they include both migratory and non-migratory individuals, as well as individuals above the mixed layer depth. Even with these overestimations, zooplankton respiration ~~Although we did not measure rates of zooplankton respiration here, data previously collected at the same site in~~

July/August 2009, (gives values of ~~11.3~~5.1-10.1~~13.7~~ mg C m^{-2} d^{-1} ~~o)~~~~ver our depth range (36-500 m) (Giering et al., 2014),~~ ~~implying that zooplankton respiration~~ accounts for~~is~~ only a small POC sink in the upper mesopelagic (Fig. 9b). We are not able to account for losses of POC to DOC or suspended POC via sloppy feeding.

~~We do not directly consider free-living microbial respiration as a loss process here as our analysis is focussed on the loss of large fast sinking POC~~The direct hydrolysis by attached microbes likely supplies free-living communities with DOC ~~only which is likely to be less accessible to free-living microbes~~(Cho and Azam, 1988; Karl et al., 1988; Kiørboe and Jackson, 2001). However by definition free-living microbes are not associated with particles and hence do not contribute directly to the loss of large fast sinking POC measured here, and as such we do not consider this loss process. The definition of dissolved and particulate is operational based on the pore size of a GF/F filter, and therefore microbes defined as 'free-living' may in fact be able to utilize colloids (Arístegui et al., 2009). However as we only measure the loss of large, fast sinking POC, we exclude free-living bacteria from our analysis of fast sinking POC loss processes. Free-living prokaryotic respiration may account for the ultimate loss of organic carbon from the organic carbon pool but we believe this is reliant on mechanical breakdown of large, fast sinking POC by zooplankton and protozoa (Lampitt et al., 1990; Poulsen and Iversen, 2008; Poulsen et al., 2011) and enzymatic hydrolysis (Smith et al., 1992). Previous measurements at the PAP site suggest that prokaryotic respiration results in loss rates of 42 mg C m^2 d^{-1} between 36-203 m which greatly exceed estimated DOC input to the upper 1000 m (15 mg C m^2 d^{-1}) (Giering et al., 2014), supporting this hypothesis.

POC loss via zooplankton respiration, particle associated microbial respiration and solubilization, as typically invoked in model studies (e.g. Anderson and Tang, 2010) can therefore not account for observed losses of fast sinking POC in the upper mesopelagic, suggesting our knowledge of the mesopelagic carbon budget is still poorly constrained and/or incomplete.

4.5 The missing piece of the mesopelagic carbon budget?

Before we begin to examine whether we are indeed ~~search for the~~ missing a piece of the upper mesopelagic carbon budget puzzle we must acknowledge the limitations of our estimates thus far which may in themselves ~~complete the puzzle~~rectify imbalances. ~~Our calculations rely on zooplankton abundances and respiration rates measured by Giering et al., (2014) in summer 2009 being representative of the conditions observed in our study. Although at a similar stage of the seasonal cycle, we measured POC fluxes that were 2-3 times higher than Giering et al. (2014) which could support higher losses if zooplankton respiration is limited by substrate concentration. Zooplankton populations can be patchy, resulting in different estimates of abundance even on short time scales. However, zooplankton respiration would need to be 265 times greater between 36-128 m to balance the budget, and is therefore unlikely to be able to close the carbon budget.~~

Large uncertainties surround our estimates of solubilization by particle associated microbes. We would require solubilization of 87~~9~~% ~~and 6% in the upper and lower mesopelagic respectively~~ to balance our budget which w~~. W~~e believe ~~9% solubilization~~ to be high considering estimates of 97% for nitrogen (Smith et al., 1992) which is preferentially remineralized over carbon. In addition, as particle associated microbial respiration is able to account for a greater proportion

of the fast sinking POC loss in the mid mesopelagic (200-500 m), solubilization losses would need to be lower over this depth region (66%) to maintain a balance. We are also not aware of any studies suggesting that rates of solubilization would vary with depth. Increased solubilization would present itself in the form of increased DOC and/or increased rates of microbial respiration, however these terms are included in the estimate by Giering et al. (2014) and a large imbalance in the upper mesopelagic is still apparent in their budget. We are not able to rule out increased solubilization in the upper mesopelagic as an additional sink term, but consider it unlikely to solve the imbalance.

Although our method of measuring particle associated microbial respiration attempts to avoid bottle effects and accurately simulate the environment of a sinking particle, we were not able to simulate pressure changes. Colonization of particles by pressure adapted microbial communities at depth may lead to an underestimation of in situ microbial activity using decompressed samples (Tamburini et al., 2013). Recent work suggests that the attached microbial community on sinking particles is 'inherited' from the fluorescence maximum (Thiele et al., 2015); these organisms are not adapted to changes in pressure (Tamburini et al., 2006, 2009) and temperature, and therefore exhibit lower prokaryotic growth efficiencies (PGE) and overall metabolic rates. Similarly, our experiments were carried out at constant temperatures whereas particles sinking through the water column will experience the range of water column temperatures, likely impacting all metabolic processes. We are also limited in our study by the lack of replicate MSC deployments at each depth. Although numerous aggregate respiration rates were measured from each sample, high patchiness in the type and source location of sinking particles could result in greater variability in respiration rates.

Additionally we are not able to measure mechanical disaggregation via processes such as fluid shear which could provide additional losses of large sinking POC. The forces required to break apart large marine snow aggregates have been shown to be higher than typical estimates of energy dissipation in the ocean, suggesting that this would not be a major loss process (Alldredge et al., 1990). However, Pphysical disaggregation could be more important in surface waters where dissipation rates can exceed the forces required to break marine snow aggregates (Alldredge et al., 1990; Burd and Jackson, 2009). However, we suspect that only a small fraction of sinking POC would be fragmented by abiotic processes to particles <0.15mm and hence would not explain the large loss of fast sinking POC measured in this study.

In order to address imbalances in the sources and sinks of fast sinking carbonPOC to the upper mesopelagic we require an additional loss process of POC. One key term missing from the budget is that of free-living protozoans which would not be collected in zooplankton nets and can make up a substantial part of marine planktonic ecosystems (Biard et al., 2016). Laboratory experiments on copepod FP reveals that dinoflagellates degraded FP over three times faster than bacteria (0.18 d^{-1} compared to 0.04 d^{-1}), and the combined effects of bacteria, dinoflagellates and copepods led to FP degradation rates of 1.12 d^{-1} (Svensen et al., 2014). Dinoflagellates and ciliates have been shown to feed on fecal pellets (Poulsen and Iversen, 2008; Poulsen et al., 2011) and PA (Tiselius and Kiørboe, 1998). Therefore POC loss via protozoan respiration may account for at least some of the additional POC loss we require to resolve im-balances in our upper mesopelagic carbon budgets.

Loss of sinking POC via fragmentation of large sinking particles into small (<0.15 mm ESD) and non-sinking particles by both abiotic and biotic means may also explain some of our observed imbalance in the upper mesopelagic as the POC fluxes measured here are for 'fast sinking' particles only (see methods). We hypothesize, in line with a growing number of other studies (Cavan et al., 2015; Collins et al., 2015), that zooplankton living in the upper mesopelagic may stimulate this loss in POC via fragmentation from sloppy feeding, swimming activities and/or microbial gardening (Iversen and Poulsen, 2007; Mayor et al., 2014). Fast sinking particles can reach the deep ocean with minimal degradation due to slow rates of particle associated respiration. Conversely, once fragmented, the increased residence times (in terms of their sinking rate) of slow and non-sinking POC could allow a sustained loss of POC over the season by microbial respiration. In theory, this seasonal balance should present itself in the form of an increase in slow and non-sinking POC following the seasonal peak in fast sinking POC and a more gradual decline over the season. This less rapid seasonal decline in slowly sinking material is apparent in the results of a biogeochemical model study for subpolar regions (Henson et al., 2015). Seasonal cycles in POC (0.8-200 µm via GF/F filtering) have been detected following analysis of long term time series data at station ALOHA in the Pacific (Hebel and Karl, 2001). They suggest that the build up and removal of standing stocks of POC do not require a large degree of decoupling between production and loss processes and can exist due to small but sustained differences. Considering the highly dynamic nature of the typical bloom-bust scenario of the North Atlantic it seems unlikely that a balance in source and sink processes would be found by 'snapshot' measurements such as made here.

Additional inputs/losses of organic carbon could be driven via physical processes such as advection or changes in mixed layer depth (e.g. Dall'Olmo and Mork, 2014). Although the mixed layer was relatively stable during our study period, the winter deepening in MLD to 250 m (Hartman et al., 2015) could provide a seasonal balance to the budget if concentrations of slow and non-sinking particles are sufficiently high (Bochdansky et al., 2016). Similarly, advective processes are an unaccounted for source/sink of carbon in this study and could result in closer agreement between sources and sinks.

5 Conclusions

We present here a unique vertical profile of particle associated microbial respiration measured directly on sinking marine aggregates collected at depth~~in situ~~. Rates of carbon-specific respiration were relatively constant with depth, and particle associated microbial respiration amounts to a small loss term in the mesopelagic carbon balance. We suggest that it may be possible to explain the loss of fast sinking particles (>0.15 mm ESD) in the upper mesopelagic through a combination of particle associated microbial respiration, solubilization, and the conversion into small (<0.15 mm ESD) and non-sinking POC via zooplankton and protozoan mediated processes.~~. In the lower mesopelagic (128-500 m depth), respiration and hydrolysis by particle associated microbes appear to explain ~100% of the POC loss, whereas in the upper mesopelagic, fragmentation process appear to dominate (69%).~~ Material lost through fragmentation would be retained in the upper mesopelagic allowing it to be slowly respired over time and enabling a balance of the mesopelagic carbon budget only

over seasonal timescales. However, detailed information about fragmentation processes are lacking and are needed to better constrain the upper mesopelagic carbon flows. Moreover, there is a need for seasonally resolved studies (of fast and slow sinking pools of carbon) to get a better appreciation of how changing primary production in a non-steady state system can influence seasonal fluxes of POC in the mesopelagic.

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Figures and Figure Legends
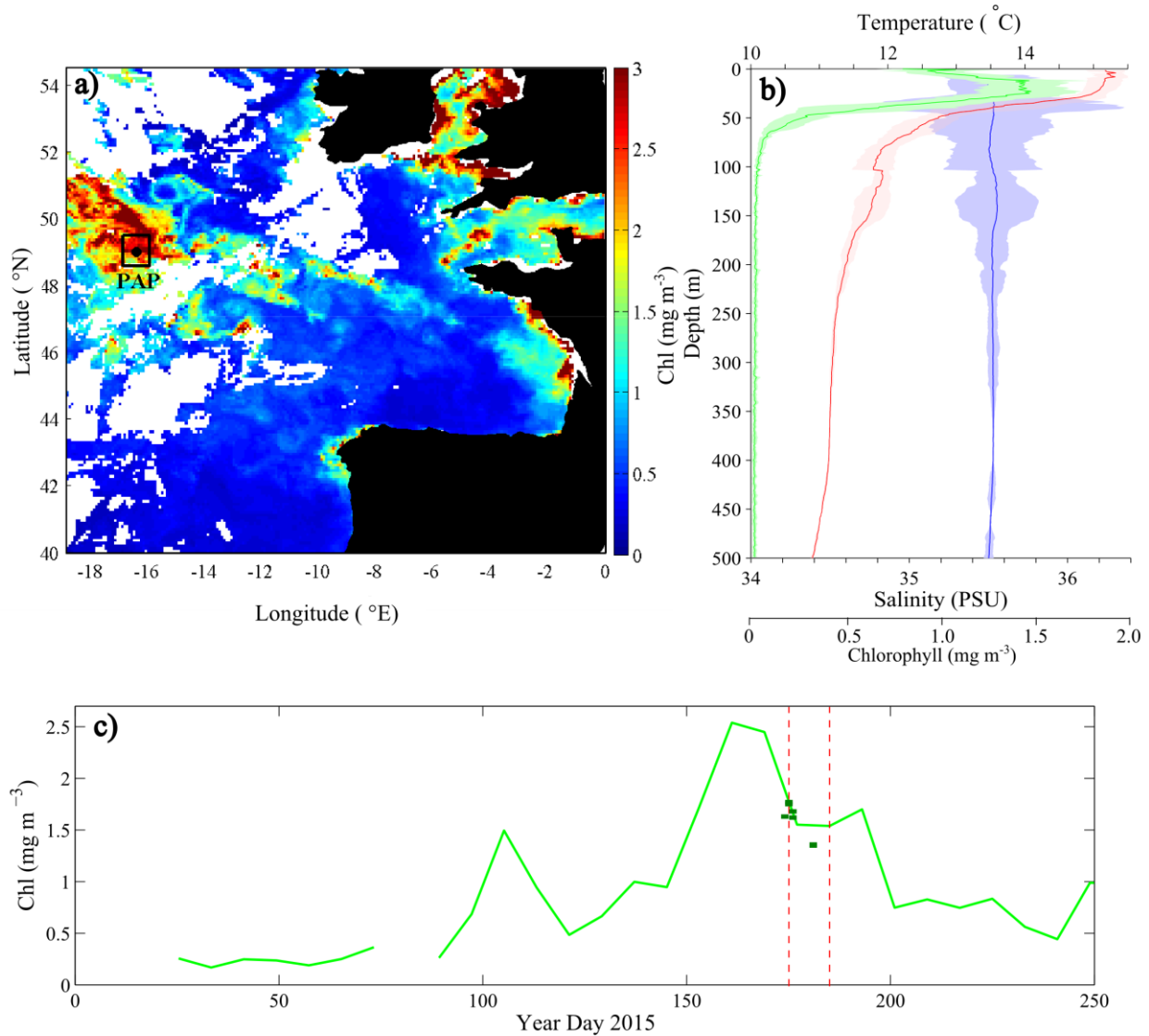
Figure 1: Surface chlorophyll concentration (mg m^{-3}) at the PAP site, (a) PAP study region (black box) overlain on 9 km Aqua MODIS satellite chlorophyll for 18/06/2015-25/06/2015 ~~(week prior to sampling)~~. (b) Average vertical temperature, ~~and~~ salinity and chlorophyll profiles measured at the PAP site (red, ~~and~~ blue and green lines respectively) and the standard deviation (light shading) of CTD deployments coinciding with MSC deployments. (c) Temporal change in surface chlorophyll (mg m^{-3}) over the PAP study region based on 8 day, 9 km Aqua MODIS satellite data. Gaps in data are due to cloud cover. Vertical red lines indicate start and end of sampling period, and dark green squares are discrete measurements made from the CTD at depths of 5-10 m.
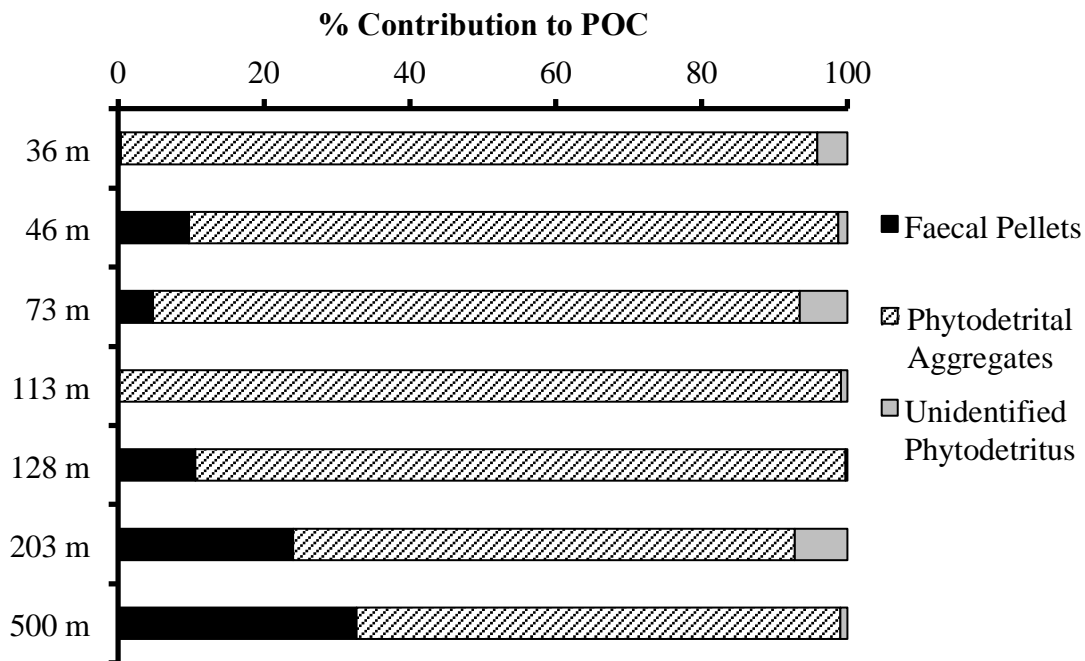
57

% Contribution to POC

Figure 2: Composition of fast sinking POC at each measured depth horizon. The percent (%) contribution of faecal pellets (black), phytodetrital aggregates (hatched), and unidentified phytodetritus (grey) to the total mass of fast sinking POC collected in Marine Snow Catchers at each depth horizon. See Table 1 for numbers of particles in each category.
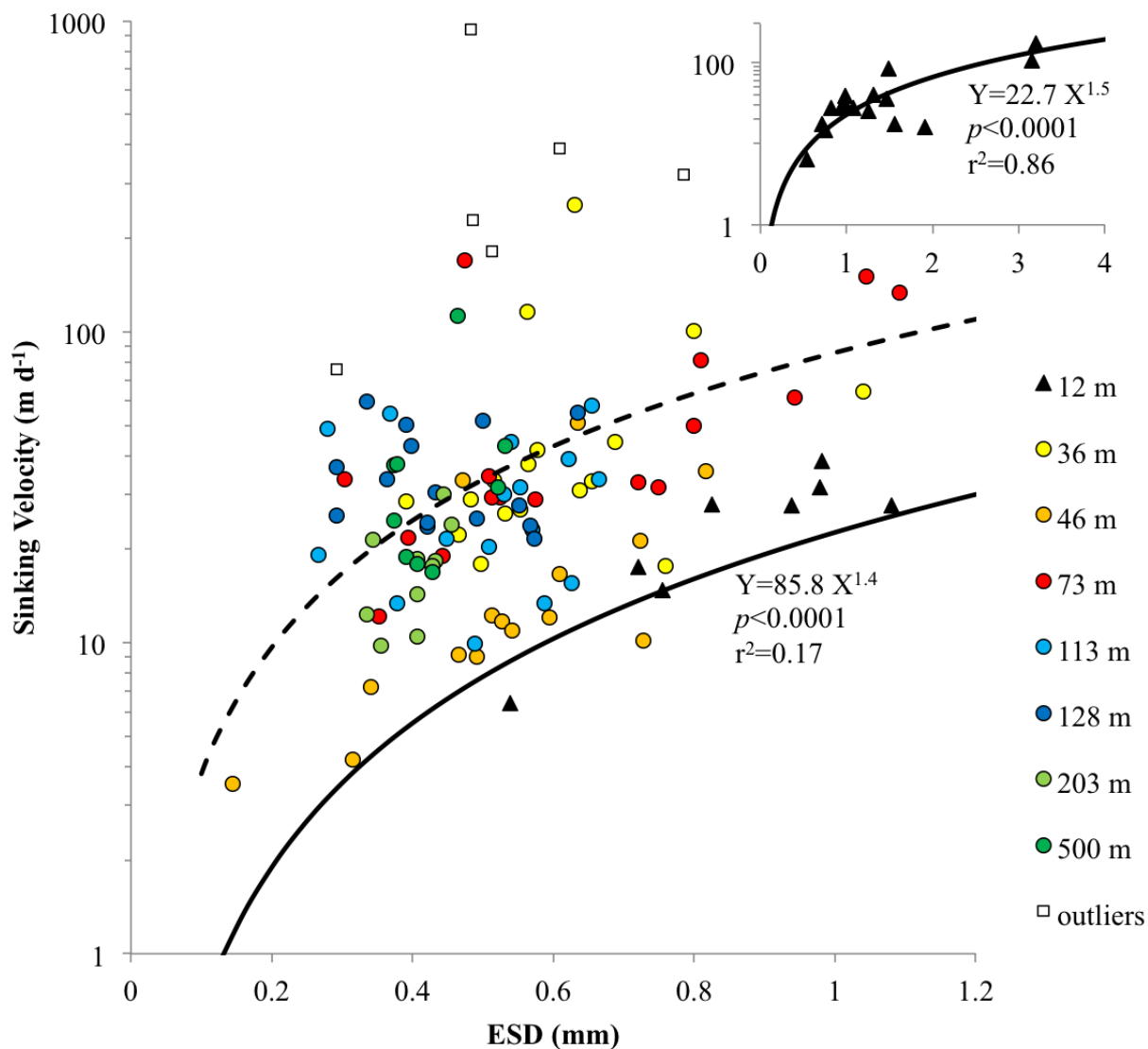
Figure 3: Relationship between sinking velocity (m d^{-1}) and equivalent spherical diameter (ESD, mm) of phytodetrital aggregates. Roller tank formed aggregates are shown by triangles (black) and ~~in situ~~ natural aggregates collected at depth by circles coloured by depth (36 m=yellow, 46 m=orange, 73 m=red, 113 m, light blue, 128 m=dark blue, 203 m=light green, 500 m=dark green). Note the log scale on the Y axis. We apply a power-law fit for roller tank (solid line Y = 22.7X$^{1.5}$) and ~~in situ~~ natural (dotted line Y = 85.8X$^{1.4}$) aggregates. Inset displays roller tank particle data only showing full size range. 6 outliers (black open squares), defined as being outside 2 standard deviations from the mean, were excluded in the relationship for natural aggregates.
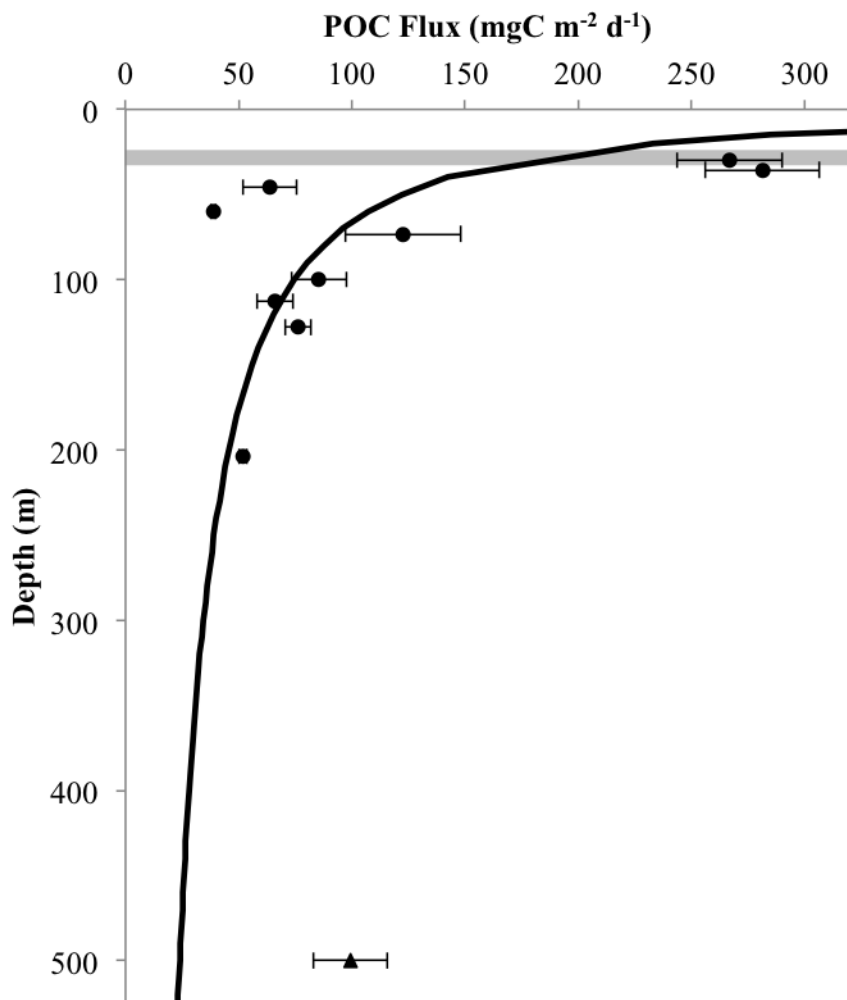
Figure 4: Flux of POC (mg C m^{-2} d^{-1}) with depth at the PAP site. POC fluxes of fast sinking particles measured in June 2015 at the PAP site via deployment of Marine Snow Catchers. Error bars relate to duplicate filters per sample. A power-law curve was fitted to the data (black line), Y=194.9 ·(X/MLD)$^{-0.71}$ (R^2= 0.42, p=0.060, n=9), excluding the point at 500 m (triangle) which is likely due to non steady state conditions. The grey shaded area indicates the mixed layer depth over the study period.
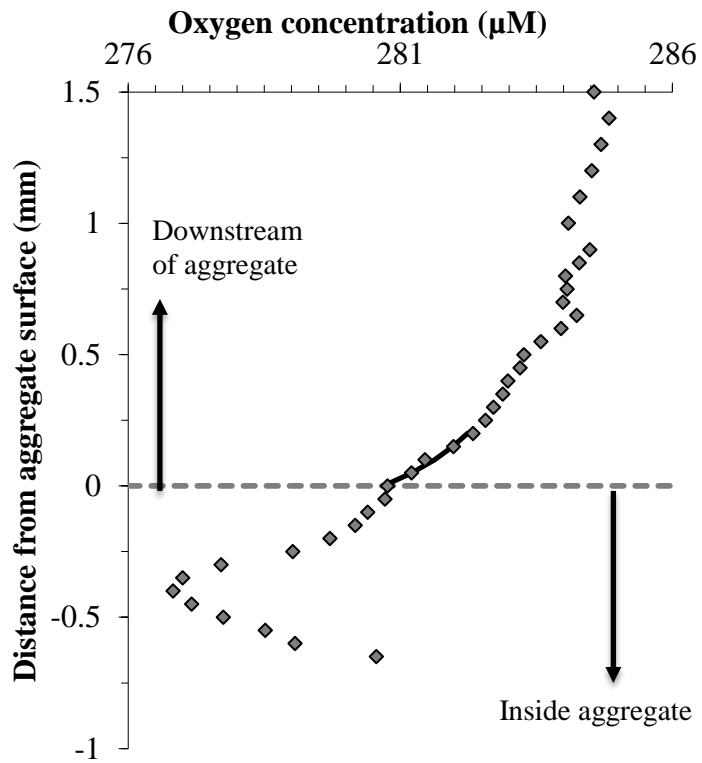
Figure 5: Example oxygen profile (μM) through a phytodetrital aggregate collected at 46 m depth. Measurements were made with microsensors in steps of 50-100 μm, with negative values reflecting the distance into the aggregate from the surface. The solid black line shows the model fit used to calculate the oxygen flux in the diffusive boundary layer.
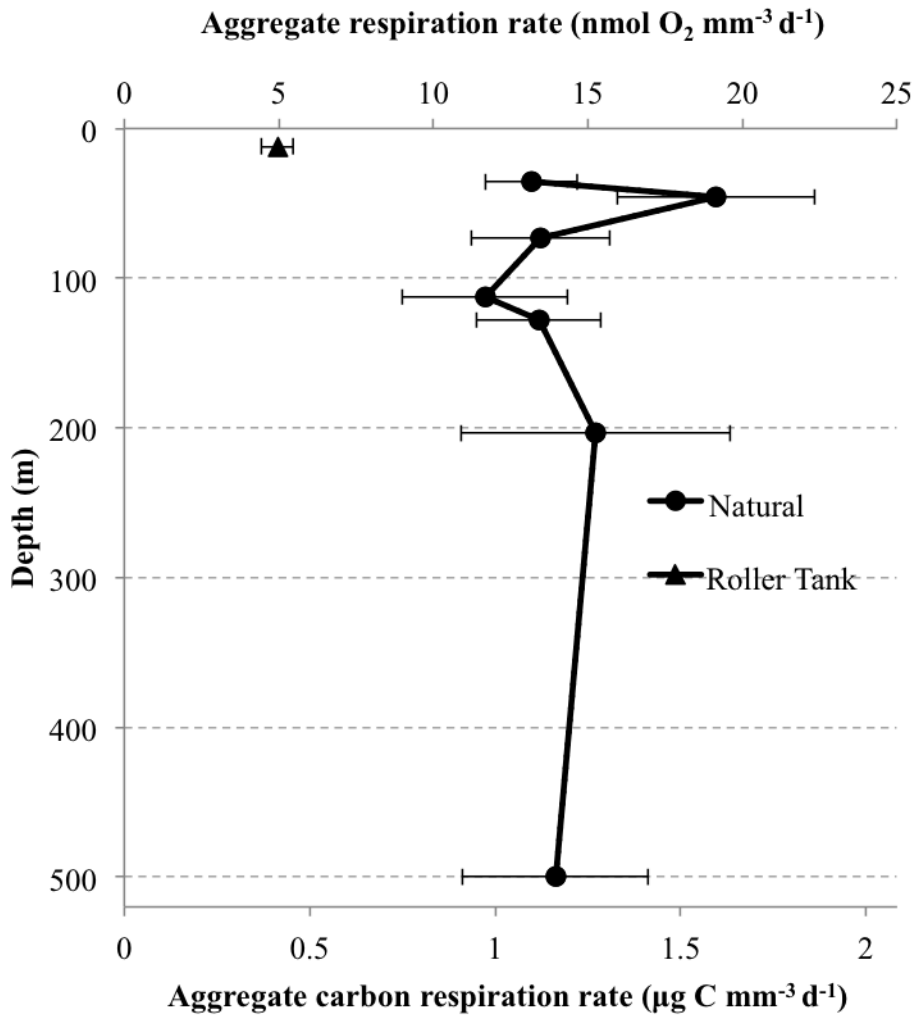
Figure 6: Respiration rates of phytodetrital aggregates with depth. Oxygen fluxes to aggregates (nmol O$_2$ mm^{-3} d^{-1}) for ~~in situ~~ natural~~collected~~ aggregates collected at depth (black circles, solid black line) and roller tank formed aggregates (black triangle). For reference aggregate respiration rates are also shown in terms of carbon per aggregate volume (µg C mm^{-3} d^{-1}). Data are for experiments carried out at 10 °C. Error bars represent +/- 1 standard error.
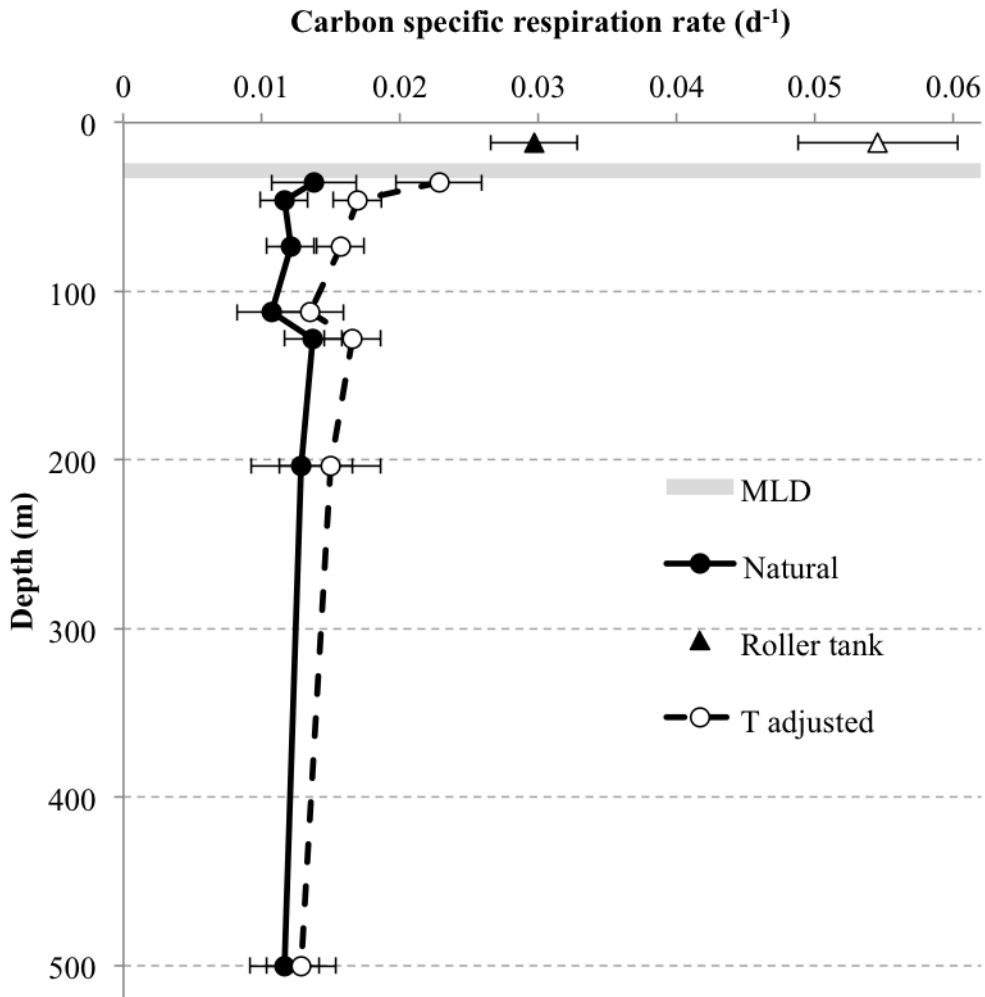
Figure 7: Carbon-specific respiration rates (d^{-1}) for ~~in situ collected~~ natural aggregates collected at depth (black circles, solid line) and roller tank (triangles) formed aggregates. Rates adjusted to the in situ temperature (T) are shown by the dashed black line (open circles and open triangle for roller tank). Grey shading shows the range in mixed layer depth over the study period, and error bars represent standard errors.
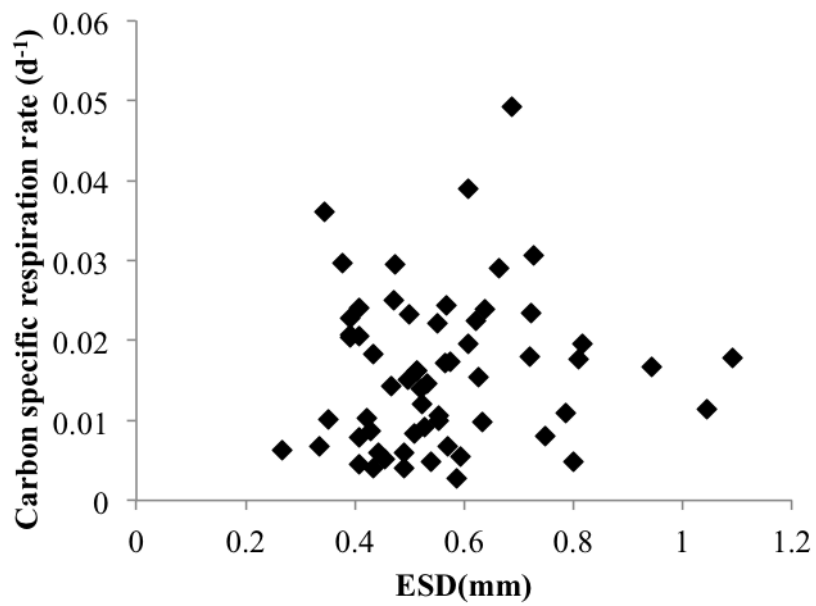
Figure 8: Carbon-specific respiration rates (d⁻¹) of ~~in situ~~ natural ~~collected~~ phytodetrital aggregates collected at depth. Data have been adjusted for in situ temperatures (see text).
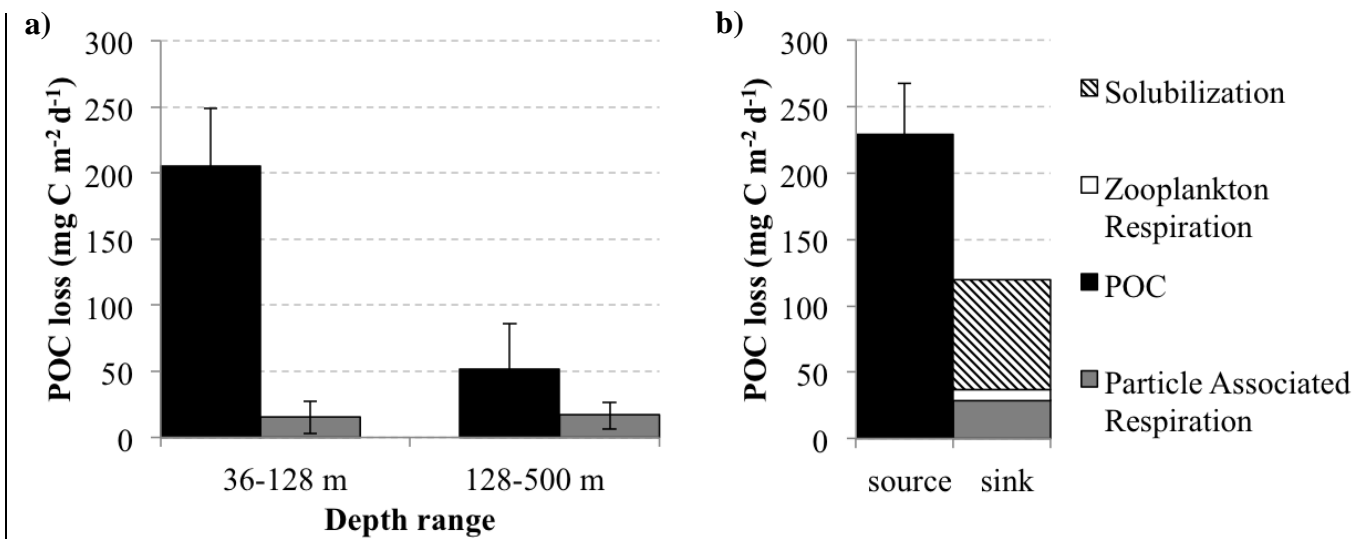
Figure 9: Balance of processes controlling POC flux attenuation. (a) Comparison of observed POC loss (black bars) and estimated POC loss based on particle associated microbial respiration only (grey bars) over two depth horizons (36-128 m, and 128-500 m). (b) Assessment of POC sources and sinks in upper 200m. Additional estimated losses via solubilization by particle associated microbes (calculated assuming respiration accounts for 25% of the total loss by particle associated microbes and solubilization the remaining 75%, see section 4.4), and zooplankton respiration based on integrated (0-200 m) net data. (from _Giering et al._, (2014)). Error bars in figure (a) relate to the range in POC flux measured at each depth and the range in respiration rates calculated (see section 2.6). We do not display error bars on our assessments of POC sinks due to the potentially large and unconstrained errors on solubilization.

Tables

Table 1: Deployment table for cruise DY032 to the PAP site.

Depth (m)	Date	Time (GMT)	POC Flux (mg C m^{-2} d^{-1})	# PA (in 95 L sample)**	# FP (in 95 L sample)**	Average PA ESD (mm)	Median PA sinking rate (m d^{-1})
36	24/06/2015	02:10	281.4	785	23	0.60	33.2
128	24/06/2015	02:35	76.2	259	61	0.53	11.7
73	26/06/2015	14:45	122.8	252	15	0.65	34.0
113	28/06/2015	11:50	66.0	198	0	0.49	30.1
500	28/06/2015	17:50	99.4	282	92	0.44	30.6
46	30/06/2015	18:45	63.7	702	61	0.41	18.3
204	02/07/2015	10:00	51.8	275	76	0.44	34.4
30	04/07/2015	13:00	266.8				
60	04/07/2015	13:20	38.7				
100	04/07/2015	13:30	85.5				
12*	24/06/2015	00:38				1.39	29.4

PA: Phytodetrital aggregate; FP: Faecal Pellet; ESD: Equivalent spherical diameter

* Roller tank data: Deployment date, time and depth refer to CTD cast from which water for roller tank experiments were obtained

**Refers to counts of fast sinking material collected from deployment of 95 L snow catcher bottle. Counts have been scaled up from smaller sample split (1/4).

Table 2: Rates of particle associated microbial respiration rates in phytodetrital aggregates. Averages are given for each depth with full range in brackets. Results are for experiments carried out at 10 °C.

Depth (m)	Total O_2 consumption (nmol O_2 agg^{-1} d^{-1})	Volumetric O_2 consumption (nmol O_2 mm^{-3} d^{-1})	C_{resp} (ng C mm^{-3} d^{-1})*	C_{spec} (d^{-1}) **	# aggregates measured
12 ^	12.62 (1.23-62.80)	4.96 (2.48-7.65)	0.059 (0.030-0.092)	0.030 (0.012 -0.054)	12
36	1.25 (0.56-2.81)	13.18 (8.03-17.77)	0.158 (0.096-0.213)	0.014 (0.006-0.030)	6
46	2.04 (0.65-3.89)	19.12 (9.49-43.76)	0.230 (0.114-0.525)	0.012 (0.004-0.021)	10
73	2.46 (0.22-6.92)	13.47 (4.69-37.67)	0.162 (0.056-0.452)	0.012 (0.004-0.030)	15
113	0.98 (0.07-2.80)	11.66 (3.10-32.39)	0.140 (0.037-0.390)	0.011 (0.002-0.024)	10
128	0.93 (0.20-1.88)	13.40 (3.22-19.73)	0.161 (0.039-0.237)	0.014 (0.003-0.020)	8
204	0.46 (0.14-0.87)	15.25 (4.05-36.77)	0.183 (0.049-0.441)	0.013 (0.003-0.031)	7
500	0.74 (0.17-1.24)	13.95 (4.86-24.71)	0.167 (0.058-0.297)	0.012 (0.004-0.021)	5

^ Roller tank

* Volume specific respiration rate (C_{resp})

5   **Carbon-specific respiration rate (C_{spec})