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Interactive comment on "Enhanced rates of particulate organic matter remineralization by microzooplankton are diminished by added ballast minerals" *by* F. A. C. Le Moigne et al.

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MS BGD-10,3597-3625,2013-04-17 We have carefully considered these reviews and believe that we have been able to respond satisfactorily to each point raised by the referees. We are grateful to both referees for their useful comments on our manuscript.

Anonymous Referee #1 Received and published: 7 March 2013

Reviewer comment: 1) BiSi is not regenerated during organic matter decomposition. As the authors point out several times, it is chemically dissolved. The authors sometimes treat Si in the same way as N and P, and seem to be surprised when it behaves differently. One example: "In this case, the presence of rotifers resulted in enhanced

remineralization [production is meant here] of NH4+ and phosphate, but not of DSi, during the 8 days of the experiment." (p. 3611, line 7-8).

Our response: We will rearrange this sentence in the conclusion as "In this case, the presence of rotifers resulted in enhanced remineralization of NH4+ and phosphate during the 8 days of the experiment. The dissolution of BSi into DSi was not impacted by the presence of either microzooplankton or the presence of calcite". Also, we will rearrange the first sentence of part 4.1.

Reviewer comment: 2) The authors point out that their experiments are simple (I hope not simplistic as they say), but they should still be related to the real world. How do the rotifer and calcite concentrations added compare to what might be seen in the ocean? There was an analysis of rotifer nutrient regeneration rates on p. 3607. How do these compare with natural waters?

Our response: The only report of natural abundances of rotifers we found is given in a tropical coastal lagoon [Maioli Castilho and Sofia Arcifa, 2000]. They ranged from 0 to 8000 ind ml-1 which matches quite well with our number. However, this number has a limited interest as it does not reflect any rotifer:POM ratios. We unfortunately did not find any studies reporting rotifers abundance along with POM, POC or even Chl a concentrations. More interestingly, the maximum theoretical ammonium excretion rate of Brachionus at 15°C is 0.11 μg N mg-1 h-1 [Ejsmont-Karabin, 1983] and compare well with our estimation. This supports that there having been a significant feeding by the rotifers during the experiment

Reviewer comment: Would the concentrations of calcite added be similar to a coccolithophorid bloom? The experiments are very straightforward, and the results are clear, but what they possibly mean needs to be discussed further.

Our response: In the Bay of Biscay during intense coccolithophore blooms, calcite concentration ranges from 0.1 to 0.2 mg l-1 [Harlay et al., 2011]. In our experiment, calcite concentration was 1.3 mg l-1, one order of magnitude more. However, POC inoculated at d0 in our experiment ranged from 130 to 150 μM (1.560-1800 mg l-1) giving a PIC:POC ratio ranging from 0.72 to 0.83 g. g-1. [Harlay et al., 2011] report PIC:POC ratio ranging from 0.28 to 0.72 in the mixed layer. Our experiment is thus quite close of natural conditions in terms of calcite added per amount of POC in the tanks. We will add a paragraph at the end of the revised manuscript to put more emphasis on the implications of our experiment in "the real world". This paragraph includes specifications given above.

Minor editorial things: page 3598, line 19 Delete "as they were". I think "as they are" was meant, but it is not needed in any case. p. 3600, l. 4 Add r to Experimental p. 3603, l. 4 "as described in Poulton et al. (2006). p. 3604, l. 2 no differences sounds better than not differences p. 3611, l. 4 simple, not simplistic l. 14 : : :increased the initial rate, not the kinetics of aggregation: : :

Our response: All the "minor editorial things" will be corrected in the revised version of the manuscript.

Anonymous Referee #2 Received and published: 16 April 2013

Reviewer comment: The manuscript is well written, however, because results of each treatment are presented in different panels it is difficult to compare them. Looking at figures 1 to 4 one can hardly see any difference between treatments. Also, in figure 5 (possibly the most important one) one can hardly distinguish the lines corresponding to the different treatments.

Our response: When we revise the manuscript, we will carefully consider the issue regarding the figures and improve them as necessary for clarity.

Reviewer comment: Overall the only differences in the treatments seem to be the evolution of phosphate and ammonium concentrations, which increase in the presence of grazers. In the case of ammonium the increase is larger in the treatment with grazers alone, while for phosphate the increase is larger in the treatment with grazers and

minerals. Hence the argument for the impact of minerals could go both ways.

Our response: We disagree with referee#2. Phosphorus concentrations were not homogenous at d0. We regret that this is not obvious from Figure 8 alone but the increase in phosphate concentration by the end of the experiment is 2 times greater in the two rotifer-containing treatments (0.88 $\pm$ 0.10 μM) than in the two treatments lacking rotifers (0.46 $\pm$ 0.07 μM). More specifically, from d0 to d8, more phosphate were produced in PZ relative to PZM (0.95 uM in PZ, 0.81 uM in PZM). The increase of phosphate in treatment with grazers alone (PZ) is thus LARGER than in the treatment with grazers and minerals (PZM) further suggesting that the added calcite inhibited rotifer feeding. The argument for the impact of calcite is therefore consistent between those two variables (ammonium and phosphate production). We will add text during manuscript revision to make this point clearer.

Reviewer comment: Given the apparent lack of difference in the evolution of particulate matter between treatments one might question the fact that grazing was important at all as compared to treatments without grazers.

Our response: Because it is far more difficult to quantitatively sample the particulate matter in the experiment, in particular the non-suspended particulate matter smaller than 1 mm, and because measurements of particulate matter separated only into "aggregate > 1mm" and "suspended" pools don't distinguish between particulate matter which is still phytoplankton versus that which has been assimilated into bacteria or zooplankton during the experiment, it is better to draw conclusions about the effect of minerals and zooplankton on remineralization from the net change in concentration of inorganic solutes (i.e. nutrients) during the experiment. As noted in the paper, while there was no particular impact on the net regeneration of dissolved silicon (which could have been related to the removal of protective organic coatings from the surfaces of the biogenic silica), there were clear differences in the net regeneration of phosphate and ammonium between treatments (even without taking into consideration that the true extent of remineralization was slightly masked by bacterial uptake of these nutrients).

Therefore it is not correct of the reviewer to conclude that grazing had no impact.

Reviewer comment: Further, the decrease in particulate matter without concomitant increase in dissolved matter, in the treatment with phytoplankton only, is left unexplained. Does that mean that the methods used do not allow to follow real evolution of particulate and dissolved elements in the tanks? For the reasons mentioned above I find the evidence for protection of organic matter in the system studied poorly convincing. Results should be analyzed and discussed more thoroughly before publication.

Our response: As noted above, part of the problem is with the reviewer's expectation that there will be large differences between treatments in the quantity of particulate organic matter over the duration of the experiment. But that is not what will happen. Grazing does not destroy particulate organic matter, it merely shifts it from one form (i.e. phytoplankton) to another (zooplankton and bacteria). What does reduce the amount of particulate organic matter is respiration and the excretion of ammonium and phosphate. Unless there was a significantly larger living biomass in the tanks within which there were rotifers (and there was not), we would not expect notably greater respiration rates and therefore we would not expect a greater loss of POC from these tanks, unless there was a significantly greater production of DOC (which there was not). At the same time, losses of particulate organic phosphate to oxidation were slightly compensated for by assimilation of inorganic phosphate by the bacteria in the experiment, something which they are certainly known to do.

As was pointed out above, in terms of the ammonium and phosphate, the results are clear: there was significantly greater remineralization of nitrogen and phosphate in the rotifer containing tanks that did not contain minerals, and therefore higher grazing rates in these tanks thank in the ones that contained extra coccoliths. Certainly experiments should now be done to specifically investigate the impact of the coccoliths on grazing rates of small zooplankton. It is amazing to us that, aside from a talk given at ASLO this February on the impact of diatom silica on grazing by copepods, basically no work has been done on this truism that biominerals inhibit zooplankton grazing.

Reviewer comment: Additional comment: The rotifer species used in this study is referred to as microzooplankton (20_m-200_m; see Sieburth et al. 1978, L&O, 23, 1256-1263) in the text. Aren't they much larger than microzooplankton?

Our response: It is difficult to classified zooplankton according to their size. The microzooplankton size class includes species with body size ranging from 20 to 200μm. The next size category of zooplankton, mesozooplankton, ranges from 0.2 to 20mm. We used in our study a mix of Brachionus species. An investigation of body size variability among the rotifer Brachionus sp indicate body lengths ranging from 123 to 299 μm [Ciros-Perez et al., 2001; Snell and Carillo, 1984]. We therefore believe that classifying Brachionus sp. as microzooplankton is more accurate than putting them in the next larger (and really much larger) size class (mesozooplankton).

Anonymous Referee #3 Received and published: 24 April 2013

Reviewer comment: Detailed comments Abstract: Page 3598, line 5-7: This sentence could be written more clearly so that it is easier to follow.

Our response: Corrected.

Reviewer comment: Introduction: Page 3599, line 26: Change "effects" to "affects".

Our response: Corrected.

Reviewer comment: Results: Page 3604, line 23: Change "is" to "was".

Our response: Corrected.

Reviewer comment: Discussion: Page 3606, line 10-12: Strictly speaking it is not a balance. Erase "the balance between".

Our response: Corrected.

Reviewer comment: Page 3607, line 11-16: The abundance of rotifers used in equation 1, is that the amount added on day 0, or the amount counted from the sampling on day

8? Please make that clear in the text.

Our response: It is the amount added at day 0. We will clarify this point in section 4.1 of the revised version of the manuscript.

Reviewer comment: Page 3608, line 16-18: The physically interfering with grazing is then simply due to the rotifers filling their stomach with inorganic calcite resulting in lower regeneration of ammonium and phosphate. Would the organic-specific rates of regeneration therefore be the same if normalizing to the ingested amount of organic matter? This was not measured, and difficult to estimate, however, this could indicate that the lower remineralization rates in the presence of ballast minerals is not due to the inhibiting effect directly, but simply due to the "ballasting effect" of filling their stomach with non-organics. The end result is of course the same, but this is also interesting for areas with his silt in the water, i.e. river inputs, glacier melting etc. A small paragraph elaborating on this would be interesting.

Our response: This is an interesting point. We had only considered that the calcite particles were getting in the way of grazing, that is to say, making it more difficult for the rotifers to access, obtain, and ingest the particulate organic matter. But the reviewer is absolutely correct that if the rotifers are grazing non-selectively, then the rotifers in the calcite-containing tanks would grazing less efficiently in terms of taking in less organic matter per total amount of mass ingested. The reviewer is also correct, that the end result is the same. We will add some discussion of this to the manuscript because it is a very worthwhile point at the end of section 4.1.

Reviewer comment: Page 3608, line 20 onwards: It is interesting that the presence of rotifers lead to higher aggregate formation (comparing P and PZ). Did the rotifers themselves form aggregates together with the diatoms? For the ballast minerals (the calcite) the increased concentration of suspended particles in the water could be the main reason for the rapid aggregate formation. Can you include a small part of the P versus PZ treatment in the discussion, in addition to the little mentioning in the results?

Our response: The rotifers did form aggregates together with diatoms. However, we unfortunately did not count rotifers abundance in either background water, suspended or aggregated POM. It is therefore difficult to tell in which proportion they were associated with aggregates in PZ tanks. On day 2, TEP concentrations were higher in the PZ tanks relative to P tanks (Laurenceau pers com 2013). More TEP could have been produced by the phytoplankton as a result of the stress induced by the rotifers. This has already been observed in presence of larger grazers [Prieto et al., 2001]. A greater concentration of TEP and the presence of rotifer acting as nuclei for aggregation could potentially explain why we observed a faster aggregation in PZ relative to P treatment. We will add paragraph to clarify this point in line at the beginning of section 4.2.

Reviewer comment: Page 3609, line 4-13: As the authors state, the rapid formation of ballasted aggregates would potentially lead to elevated export. Is it likely that there would not be any differences after 8 days between a mineral ballasted and non-ballasted situation. As the authors state, the aggregates would rapidly sink out. Therefore, by keeping the aggregates in suspension within the same water volume over 8 days in the roller tanks allows the ballasted aggregates to become looser due to degradation and continuously disaggregation and re-aggregation while the non-ballasted aggregates potentially could become more compact due to degradation and scavenging. So despite no obvious differences in amounts of aggregated organic matter at the end of the incubation, it might be worth to consider these observations in relation to a more natural in situ situation. Meaning that the production of DOC from POC would likely occur at depths for the ballasted aggregates, while the non-ballasted aggregates could still have high DOC production within the mixed layer of the upper ocean. Maybe add a sentence or two after like 20 on page 3609?

Our response: We will add a paragraph at the end of the revised manuscript to put more emphasis on the implications of our experiment in "the real world" as also asked by ref#1.

Reviewer comment: Figures: Figure 5 is a bit unclear in the figure legend. It would make it clearer if you point out that the suspended and aggregated particulate compounds is there to show how the total particulate part is distributed.

Our response: We have now done this.

References used in our response to the reviewers:

Ciros-Perez, J., A. Gomez, and M. Serra (2001), On the taxonomy of three sympatric sibling species of the Brachionus plicatilis (Rotifera) complex from Spain, with the description of B. ibericus n. Sp, Journal of Plankton Research, 23(12), 1311-1328. Ejsmont-Karabin, J. (1983), Ecological characteristics of lakes in North-eastern Poland versus their trophic gradient. VIII. Role of nutrient regeneration by planktonic rotifers and crustaceans in 42 lakes., Ekol Pol, 31, 411-427. Gaul, W., A. N. Antia, and W. Koeve (1999), Microzooplankton grazing and nitrogen suplly of phytoplankton growth in the temperate and subtropical northeast Atlantic, Marine Ecology Progress Series, 189, 93-104. Harlay, J., et al. (2011), Biogeochemistry and carbon mass balance of a coccolithophore bloom in the nothern bay Bay of Biscay (June 2006), Deep Sea Research I, 58, 111-127. Li, Q. P., P. J. S. Franks, M. R. Landry, R. Goericke, and A. G. Taylor (2010), Modeling phytoplankton growth rates and chlorophyll to carbon ratios in California coastal and pelagic ecosystems, Journal of Geophysical Research, 115, 1-12. Maioli Castilho, M. S., and M. Sofia Arcifa (2000), Production of the rotifer Brachionus plicatilis (Ploimida: Brachionidae) in a Brazilian coastal lagoon, Revista de Biología Tropical, 48, 859-865. Martin, J. H., G. A. Knauer, D. M. Karl, and W. W. Broenkow (1987), Vertex - Carbon Cycling in the Northeast Pacific, Deep-Sea Research Part A, 34(2), 267-285. Prieto, L., F. Sommer, H. Stibor, and W. Koeve (2001), Effects of planktonic copepods on transparent exopolymeric particles (TEP) abundance and size spectra., Journal of Plankton Research, 23, 515-525. Snell, T. W., and K. Carillo (1984), Body size variation among strains of the rotifer Brachionus plicatilis., Aquaculture, 37(4), 359-367. Verity, P., S. C. Williams, and Y. Hong (2000), Formation, degradation and mass:volume ratios of detritus derived from decaying phytplankton, Marine Ecology Progress Series, 207, 53-68.

Interactive comment on Biogeosciences Discuss., 10, 3597, 2013.