

## Reply to review of Jack J Middelburg

**Reviewer's comment:** Line 37: 2.7-2.8 for clarity

**Reply:** Nöthig et al. (2015) report 2.7-8 °C, not 2.7-2.8°C, so we leave the temperature range as originally stated.

**Reviewer's comment:** Line 45: years phytoplankton blooms became more mixed

**Reply:** Agreed, changed accordingly.

**Reviewer's comment:** Line 59: delete [OM] because you introduce abbreviation again one line lower.

**Reply:** We thank the reviewer for noticing. Corrected accordingly.

**Reviewer's comment:** Line 83-89: The authors attribute potential differences in response to diatoms vs. coccolith phytodetritus almost entirely to differences in their skeletons (Si vs CaCO<sub>3</sub>) ignoring biochemical composition aspects.

**Reply:** The reviewer is correct. We added the biochemical composition aspects to lines 83-84 and 89-96:

“One mechanism would be the impediment of food source utilization by the physical protection of the cells.”

[...]

“The coccolithophore *E. huxleyi* for example, contains comparatively high levels of n-3 polyunsaturated fatty acids, essential for growth and reproduction of eukaryotic consumers (Pond and Harris, 1996). This high nutritional value has been used to explain the higher survival rate of planktonic foraminifera (Anderson et al., 1979) and egg production by calanoid copepods (Neystgaard et al., 1997) as compared to when these organisms were fed a diatom diet.”

**Reply (cont.)** In the discussion about the high foraminiferal preference for *Emiliana*, we also touched upon the biochemical composition again (lines 603-605):

“This agrees with the higher survival rate of planktonic foraminifera in feeding experiments with *Emiliana* than with diatoms (Anderson et al., 1979), which was later related to the higher nutritional value of *Emiliana* (Pond and Harris, 1996), ...”

**Reviewer's comment:** Line 118: The TOC of algae can never be 78 or 95%, because 100 % organic matter corresponds to 40-50% C depending on biochemical make up. Please correct.

**Reply:** The reviewer is right. We corrected the sentence to:

“The corresponding TOC of the algae was 78 % of TC (*Emiliana*) and 95 % of TC (*Thalassiosira*).”

**Reviewer's comment:** Line 122 & 125: TDN: is this indeed total dissolved nitrogen. But was the inorganic nitrogen not removed by washing three times, so that TDN is more or less DON?

**Reply:** The algae were only washed after thawing – not upon harvesting. Hence, inorganic nitrogen could have been on the cell surfaces at the binning of the washings steps. Since freezing and thawing can induce cell leakage, organic nitrogen from the inner part of the cells could have been released. We agree that this was not sufficiently explained and now changed to:

“These washing steps most likely also entailed a loss of DOM including dissolved organic nitrogen (TON). DOC and TDN (i.e., DIN from remains of culturing medium and DON from cell leakage upon thawing) were measured in the supernatant from the 3 washes...”

**Reviewer's comment:** Line 186: recovered or added labelled phytodetritus?

**Reply:** We added the necessary specifications:

“As will be discussed further, this missing representation of the subsurface sediments in the 4 d *Emiliania* chamber results in an underestimation of the labelled phytodetritus with < 10 % recovered as total processed carbon and uncharacterized OM”

**Reviewer's comment:** Line 267: Freeze-dried algae were measured for total  $^{13}\text{C}$  and  $^{15}\text{N}$ , thus for coccolith inorganic and organic carbon were combined. Both types of carbon will very likely be similarly enriched given the identical carbon sources. What is unclear though is how much of the  $^{13}\text{C}$ -DIC attributed to respiration (and recovered in overlying water and pore-water) is from dissolution of the carbonate. Combining  $^{15}\text{N}$ -DIN and  $^{13}\text{C}$ -DIC release might be give some hints whether  $^{13}\text{C}$  from carbonate dissolution matters or not.

**Reply:** This is indeed correct. Carbonate dissolution can contribute to the  $^{13}\text{C}$ -DIC. We therefore added the following paragraph:

“It seems that *Emiliania* OM was initially (4 d and start of 14 d experiment) more respired than *Thalassiosira* (in 4 d experiment: 4 % of the added *Emiliania* OM, of which 3.6 % by DIC release, as opposed to 2 % of the added *Thalassiosira* OM), but this could as well be ascribed to dissolution of the inorganic coccoliths. There was no observable  $\text{NH}_4^+$  or  $\text{NO}_x^-$  release as should co-occur with OM mineralization, which would agree with a significant contribution of coccolithophorid dissolution to the observed DIC release.”

We also integrated this insight in the paragraph on C:N in the discussion:

Line 619-622: In contrast, the preferred use of nitrogen in the *Emiliania* 4 d experiment might be masked by the dissolution of the carbonates from the coccoliths, leading to a higher C:N ratio in overlying and pore water as compared to the C:N in the biomass.

**Reviewer's comment:** Line 290-296: I do not see the use of these equations. You present all your data in excess  $^{13}\text{C}$  atom fractions. Why then are equation 4, 5, etc needed (these are copy-pasted from prior work in which del values were reported). Line 289-296 can be deleted without loss of information.

**Reply:** Equations 4-6 are needed for the calculations of bacterial enrichment, since the GC-IRMS results of the FAMES are classically given in delta values, not in atom fractions.

**Reviewer's comment:** All through try to avoid using on the other hand if there is no on the one hand (I have counted it 3 or 4 times).

**Reply:** Thanks for noticing. "On the other hand" has been replaced by synonyms throughout the manuscript.

**Reviewer's comment:** Line 364... after which the increase levelled off..... The increase of  $^{13}\text{C}$ -DIC...was higher and steady.

**Reply:** We agree that the wording could be improved. The sentence was corrected to:

"In the first three days of both *Emiliania* incubations,  $^{13}\text{C}$ -DIC concentrations quickly accumulated in the overlying water, after which the increase levelled off (Fig. 2 A). The increase of  $^{13}\text{C}$ -DIC in the *Thalassiosira* chambers was higher and steady throughout the 14 d incubation."

**Reviewer's comment:** Line 433: was respired (and recovered in overlying water and pore water).....

**Reply:** We agree and added "(and recovered in overlying water and pore water)" to clarify 'respiration'.

**Reviewer's comment:** Section 4.1: Cold, deep-sea systems sometimes show a delayed response, i.e. low activities during the first two-three days (e.g. Andersson et al 2008 in Arabian Sea). This is one of the reason why your experimental design (two incubation durations) makes sense. Culturing phytoplankton in the lab followed by freeze-drying before additions might perhaps have resulted in the addition of DOC to your experiment at the seafloor. This really depends on the very details of your phytodetritus preparation. Differences in response among studies in the literature can be partly explained by this. Resolution requires  $^{13}\text{C}$  measurement of the DOC pool and that is a daunting task.

**Reply:** We included a reference to the work of Andersson et al. (2008) in the Arabian Sea and included the possible effect of a potentially combined POC-DOC addition (lines 499-506) :

"... A delayed response with low activities for a few days was expected as this was also observed in other cold deep-sea ecosystems (e.g., Andersson et al., 2008 in the Arabian Sea) and resulted in the design of the experiment with a shorter and a longer incubation. In case the thawing of the cells resulted in continuous leaking after addition, the relatively slow response may be in part also be explained by the reduced availability of labelled DOM dispersed in the overlying water as opposed to POC at the sediment surface. The share of organic matter provided as DOM and its utilization would have required measurements of the  $^{13}\text{C}$ -labelled DOC pool in the water samples and pore-waters and could not be carried out as part of this study."

**Reviewer's comment:** Line 525: unclear, too cryptic, I understand but will all readers?

**Reply:** We further explained this sentence:

"As the sediment in our study area is well oxygenated in the upper centimeters, settling OM is most likely aerobically mineralized (Donis et al., 2016). Therefore, the probability that obligate anaerobic processes like denitrification take place, is very low. Hence we assume that denitrification of nitrate does not occur in the oxidized sediment layer of our experiments and that the observed accumulation of nitrate in the overlying water is caused solely by nitrification."

**Reviewer's comment:** Line 536: 0.4-1.5% of total nitrification is similar to 0.3-0.4 contribution to sediment ON pool.

**Reply:** We thank the reviewer for pointing this out and added the additional insight to this paragraph:

"Altogether, only 0.4-1.5 % of the total nitrification would then be attributable to nitrification of the ammonium released by the algal detritus, which corresponds to the original addition of algal nitrogen of 0.3-0.4 % to the sediment ON pool."

**Reviewer's comment:** Line 570-572: an additional/alternative explanation. OM delivered to the sediment surface is far away from microbes in the subsurface. Animals, through their bioturbation, mix OM down and deliver OM to bacteria that do not move. This is another component of the (inverted) sediment microbial loop.

**Reply:** We agree with the reviewer. We added the following lines to the discussion (lines 591-594):

"Alternatively, sediment reworking (bioturbation) by mobile fauna redistributes fresh organic matter deposited at the surface to deeper sediment layers, where subsurface bacteria can also access it. This redistribution of fresh carbon was indeed observed in the increase in subsurface algal-derived OM after 14 days (Figure 1A) and higher bacterial assimilation in the sediment subsurface (Figure 3)."

**Reviewer's comment:** Line 588: sediment OM

**Reply:** Agreed, we corrected "POC" to "OM".

**Reviewer's comment:** Line 593: the OM leftovers (POC cannot have a C:N ratio).

**Reply:** Agreed, we corrected "POC" to "the OM leftovers"

**Reviewer's comment:** Line 610-618: another reason for non-closure is that  $^{15}\text{N}$  in bacteria and  $^{13}\text{C}/^{15}\text{N}$  in archaea have not been measured (although the latter might contribute just a minor amount).

**Reply:** This is indeed an interesting additional aspect to cover. We added the following paragraphs (lines 644-654):

"(d) bacterial assimilation of phytodetrital N was not quantified. However, assuming a bacterial C:N ratio of 5 (Goldman and Dennett, 2000) and taking into account that growth of Arctic deep sea bacteria is N-limited (Boetius and Lochte, 1996), it can be expected that bacterial N assimilation was up to 5 times lower than carbon assimilation. This would have doubled the processed share of *Emiliania* detrital N after 14 days (from 6 to 12% of the added N) and almost tripled the processed

share of *Thalassiosira* detrital N after 14 days (from 16 to 42% of the added N). (e) Archaea were not considered in our study, but the experimental duration would probably have been too short, as shown for Thaumarcheota in shallow Icelandic shelf sediments (Lengger et al., 2014). Although sequence data suggest that Archaea contribute only 2-5% to the active members of the benthic prokaryotic community at the study site (Rapp 2018), deep-sea Archaea seem to be involved in protein degradation and carbohydrate metabolism (Li et al., 2015) and especially deep-sea Archaea from high latitudes have been shown to be especially sensitive to changes in food supply (Danovaro et al., 2016). “

**Reviewer's comment:** Line 641: delete p. 201

**Reply:** We thank the reviewer for noticing and corrected accordingly.

**Reviewer's comment:** Line 656: We thank.... ... We further thank Anja...

**Reply:** Agreed, corrected.

**Reviewer's comment:** References: balanced coverage, but I missed the Boetius et al. 2012 note in Science.

**Reply:** Thanks for the indeed necessary reference. Boetius et al. (2013) has now been referred to in the introduction in line 57:

“The deposition of phytodetritus from surface water primary production is of crucial importance for the deep-sea benthos (Boetius et al., 2013; Graf, 1989) ...”