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Interactive comment on "Size-fractionated dissolved primary production and carbohydrate composition of the coccolithophore *Emiliania huxleyi*" *by* C. Borchard and A. Engel

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General comments

Referee: This BGD paper presents particulate and dissolved primary production, and a detailed description of the neutral sugar composition of 4 different size classes of the extracellular release of E. huxleyi grown in chemostat cultures. The paper is overall well written and the results presented in a clear manner. I would like to highlight two statements that both point to issues that, in my opinion, merit much more attention and revisions of the present version of the manuscript. The first statement, on p. 15295, line 10-11, concerns the two CO2 regimes (380atm and 750atm) under which

the chemostat cultures were run. I was surprised that this important aspect was not considered from the beginning. Due to the lack of differences in the parameters determined, the authors treat the results from the chemostats run under contrasting pCO2 as replicates.I strongly suggest to change this presentation of the results. I think the authors miss the opportunity to present their data in an ecologically highly relevant and timely context that is ocean acidification. In their actual presentation the results will not be read and viewed by the large scientific community working on the effect of increased pCO2 on biogeochemical processes. Further, these types of experiments are technically quite challenging to run, and they provide very valuable information. The finding that the processes investigated in the present study are not affected by increased pCO2 is important and it should be shared in that way. I therefore would also suggest to add this aspect in the title, similar to the recent publication by Engel et al. (2014). Presenting the results in this context would further render them original with respect to the many studies performed previously on the same topic.

Response: We appreciate that the referee wants to highlight our study. Chemostat experiments are indeed extensive and we surely would like to give this study the highest possible attention. However, we discussed the absence of a CO2 effect during this study in general and in the context of phytoplankton exudation in another publication (Engel, A., Cisternas Novoa, C., Wurst, M., Endres, S., Tang, T., Schartau, M. und Lee, C. (2014): No detectable effect of CO2 on elemental stoichiometry of Emiliania huxleyi in nutrient-limited, acclimated continuous cultures; Marine Ecology Progress Series, 507 . pp. 15-30) and we didn't want to be repetitive. We fully agree that we should refer earlier and with more detail to the Engel et al. publication, which we will do in the revised version. We will also show data on primary production and carbohydrate composition for the present day and high CO2 treatment individually to demonstrate the absence of a CO2 effect (See figures below). We can do so, because the data of this manuscript were not published before. However, we would like to keep the focus of this publication on exudation of primary production and the compositional differences of exudates. Our results give deeper insight to the progress of carbon release by

phytoplankton cell, a process that is still not well understood.

Referee: The second statement, on p. 15306, line 5-12, concerns the high bacterial abundance (106 cells ml-1) in the E. huxleyi cultures. Given these high abundances, I wonder how representative the production rates of dissolved organic carbon and neutral sugars are? I assume these values underestimate the actual release rates due to the concurrent uptake by heterotrophs. I strongly suggest the authors describe the non-axenic feature of the chemostat cultures in the first paragraph of the Material & Methods Section (Experimental Setup), so that the reader is aware of this fact for the interpretation of the results that follow. I also suggest the authors discuss their results (eg comparison with other studies and natural seawater) with more focus on the potential role of heterotrophic bacteria on the observed chemical signature.

Response: We discussed the potential role of bacteria in this study on pages 15306 and 15307 and estimated a maximum utilization of fresh DOC by bacteria in order of 20%. This means that exudation rates would be underestimated by 20% at most. We will specify this better in the revised version. We agree with the referee that it is necessary to mention the fact that cultures were not axenic earlier, i.e. in the M&M section, which we will do in the revised version. We will revise the discussion text dealing with the impact of bacterial activity on the composition on natural seawater for clarification (see comment below).

Specific comments: R#1: I suggest the authors provide a rationale for the many different size fractions that were examined.

Response: Dissolved organic matter covers a size continuum of substances that are not retained by a 0.7 μm GF/F (classical method discriminate between dissolved and particulate) or (as applied for data obtained here) by a 0.45 μm filter. In order to further resolve this size continuum of substances, and to better understand the impact of algal release on marine DOM, we used commercially available membranes (<1000, <100 and <10 kDa). We will implement this information in the methods section.

R#1: Are DOC concentrations available?

Response: For the present manuscript we focus on the freshly produced material. “Background-subtracted” (=freshly produced) data for combined carbohydrates (CCHO) are shown in relation to DO14C (=freshly produced) which in our opinion provides the relation of interest (fresh to fresh) for the present study.

R#1: 15290, line 10-11: How can particulate carbohydrates be part of the dissolved pool of extracellular release? Do the authors mean by particulate material the colloidal fraction of E. huxleyi release products? In that case, I suggest to reformulate this term, because many readers will associate with the term “particulate” the E. huxleyi cells and the associated material, in accordance with particulate primary production.

Response: We agree that the sentence was misleading, ER is referred to the high molecular weight (1 kDa, HMW) dissolved combined carbohydrates (dCCHO). We will reformulate it in the revised version.

R#1: Fig. 3 I suggest the authors change the heading “E. huxleyi” to “E.huxleyi exudate”, otherwise it might be interpreted as the E. huxleyi cellular material.

Response: In this figure we also show the particulate combined carbohydrates which actually are the E. huxleyi cellular material. Changing the heading to “E. huxleyi exudate” would thus be wrong. Obviously this fact is not shown clear enough and we will rework the figure to clarify the fact that dissolved and particulate carbohydrate fractions are shown here.

R#1: Table 3 and corresponding text (p. 15297). I suggest to explain the abbreviations tCCHO, pCCHO and dCCHO in each of the Table Headings and Figure Legends.

Response: We will add explanations of the abbreviations for the figure captions and table headings.

R#1: Can the authors describe more precisely in the text, what the term pCCHO stands for?

Response: Yes – we only defined pCCHO in the methods section and otherwise missed to introduce what it really stands for: the combined carbohydrates in the particulate fraction = pCCHO (>0.45 μm). We will add this information.

R#1: p. 15305, lines 15-18: This is an interesting observation. But how much is explained by bacterial heterotrophic activity on the release products? “Cellular pCCHO of E. huxleyi differed clearly not only from NSW but also from HMW-dCCHO (Fig. 3b, right panel). This is in accordance with previous studies showing differences between intracellular and extracellular CCHO compositions for various algae (Mague,1980; Aluwihare, 1999, 2002).”

Response: We discuss this issue in the 2 paragraphs following this statement. More specific on p. 15306, l. 8-12 (quantitatively) and on p. 15307, l. 14-16 (qualitatively). We will check the structure of these paragraphs for clarification. We will further change the sentence p. 15307, l. 13 which is indeed misleading concerning the non-axenic condition in our chemostats.

R#1: p. 15305, line 24: The authors should precise here that Biersmith and Benner (1998) determined the neutral sugar composition also in E. huxleyi cultures. So, why were the concentrations of Ara so different between the two studies?

Response: We will specify that Biersmith and Benner (1998) studied cultures of E. huxleyi comparable to our and the Aluwihare and Repeta (1999) study and different to the field study accomplished by Engel et al., (2012). The variations in proportions of Ara, which were determined between our study to Biersmith and Benner (1998), but not to Aluwihare (who also reported Ara as the major component in non-axenic E. huxleyi cultures) are discussed on p. 15305, l. 19 to p. 15306, l. 19: “Neutral sugars generally dominated the HMW-dCCHO composition with $\sim$83 mol %. These results are consistent with findings by Aluwihare (1999), who report on HMW exudates from E. huxleyi being mainly composed by neutral polysaccharides with Ara as the dominant monomer (30 Mol %). However, the fraction of Ara observed during this

study is considerably higher than reported for ultrafiltered DOM (>1 kDa) by Biersmith and Benner (1998), and for HMW-dCCHO sampled during a field study in the Bay of Biscay, when coccolithophores and presumably E. huxleyi was the dominating phytoplankton organism (Engel et al. 2012); both studies reported Ara of $\sim$3 % Mol. Apart from well documented species specific differences in CCHO composition (Aluwihare and Repeta, 1999, Myklestad, 1974, Myklestad et al., 1989), variations in the composition of algal extracellular carbohydrates may be related to physiological and ecological functions. Although freshly produced DOC is generally a primary substrate for heterotrophic uptake, E. huxleyi exudates were shown to exhibit recalcitrant features (Nanninga et al., 1996). Degradation experiments with the diatom Thalassiosira weissflogii revealed a special role of Ara in carbohydrate accessibility, as it escaped bacterial degradation over a period of two weeks (Aluwihare and Repeta, 1999). Bacterial cell numbers during the present experiment were relatively high, between 2 and 3 x 106 mL-1, contributing $\sim$2 % to particulate organic carbon (POC) and $\sim$3 % to DOC (Engel et al. 2014). Assuming a bacterial growth efficiency of 60 % (upper limit, Del Giorgio and Cole, 1998), the bacterial carbon demand could have been about 2 % of POC and 5 % of DOC. Relative to the freshly produced DO14C derived from rate measurements, however, a share of up to 20 % may have been channeled into heterotrophic turn-over. Thus, the HMW-CCHO was potentially subject to bacterial reworking and the high proportions of Ara could have been a result of the selective removal of other monomers. In accordance with the findings of Aluwihare (1999), concentration of Ara in dCCHO remained unchanged during a degradation experiment with the same E. huxleyi strain investigated here, while dCCHO were reduced by $\sim$60 % (Piontek et al. 2010; J. Piontek pers. comm.). However, we would expect that extensive microbial degradation of larger dCCHO would lead to an increase of Ara Mol %in the small size fraction. But this was not observed. Alternatively, high Mol % Ara and low Mol % Glc may indeed be a characteristic of larger carbohydrate molecules released by E. huxleyi that are recalcitrant to microbial decomposition. Assuming these components are bad substrates for microbial utilization, their controlled exudation, if physiologically neces-

sary, may be ecologically advantageous for algal cells that are competing with bacteria for nutrients such as phosphorus. This corroborates earlier findings of DOM produced at P-depletion being more resistant to bacterial degradation (Obernosterer and Herndl, 1995, Puddu, 2003). On the other hand bacteria recycle organic phosphorus and a certain degree of bacterial activity will be advantageous for regenerated productivity of algal cells. So far, little is known on how nutrient limitation affects the composition of algal release products. We suggest that nutrient availability may be one factor responsible for variability in carbohydrate composition observed during various studies (Giroldo et al. 2005, Goldberg et al. 2010, Engel et al. 2013).

Assuming a certain degree of microbial modification, another explanation for the difference of CCHO composition between culture studies, and those observed in natural seawater may be the highly specific linkage between algal release and bacterial community response, proposed by a series of recent studies (Teeling et al 2012, Taylor et al. 2014, Kabisch et al. 2014). These showed that the release of algal polysaccharides can induce a succession of bacterial communities inhabiting different abilities for enzymes expression related to specific carbohydrate degradation. Because the majority of marine bacteria cannot be kept in culture, bacteria present in this chemostat study, and likely in all culture experiments, represent only a small fraction of the natural diversity. Hence, even if bacteria were present in this study they may have left a different fingerprint on polysaccharide composition than natural communities. Short-term incubation studies with natural bacterial communities may be required to better understand the microbial fingerprint on DOM, specifically polysaccharide degradation. A better understanding of the microbial fingerprint on DOM could also allow for tracing microbial degradation activities in specific environments, such as the ocean's anoxic zones, or the extreme oligotrophic seas."

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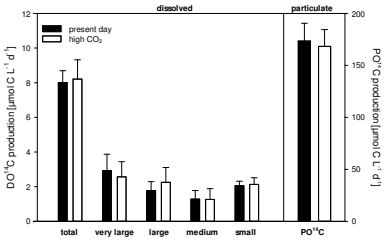

Fig. 1.

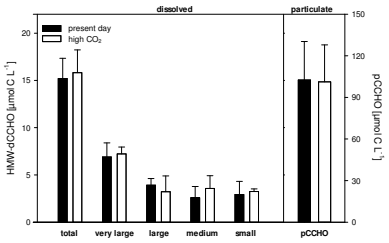
dissolved
particulate
present day
high CO_2
HMW-dCCHO [µmol C L^{-1}]
pCCHO [µmol C L^{-1}]
0
5
10
15
20
0
30
60
90
120
150
total
very large
large
medium
small
pCCHO

Fig. 2.