Dear Gerhard,

Herewith, I would like to submit the revised version of the manuscript, 'Size-fractionated dissolved primary production and carbohydrate composition of the coccolithophore *Emiliania huxleyi* ' prepared by Corinna Borchard and myself.
We are thankful to the three referees, who thoroughly evaluated the manuscript and gave valuable suggestions for its improvement. We are confident to have satisfactorily addressed all major comments, and hope that the revision will meet with yours and the referee's approval.

Best regards,

Anja

Response to anonymous referee #1 (R#1)

General comments

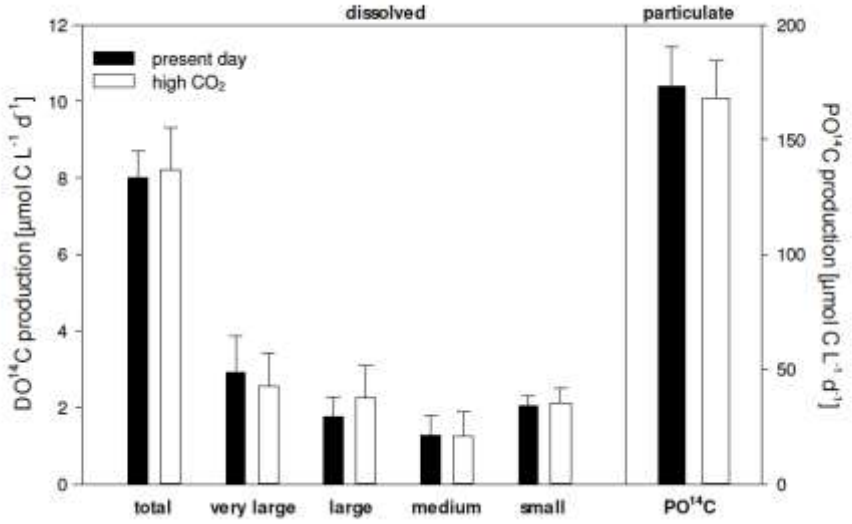
Referee:

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.

Feldfunktion geändert



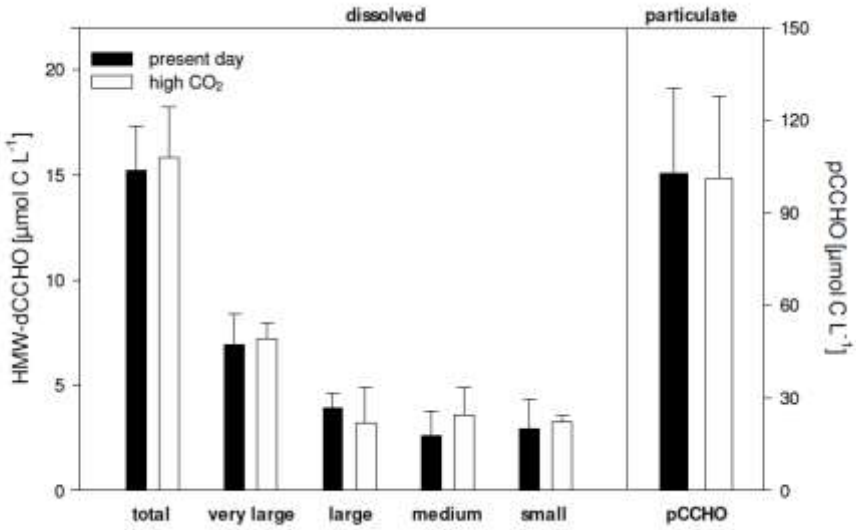


Figure 1

Dissolved ($DO^{14}C$, left) and particulate ($PO^{14}C$, right) primary production [µmol C L^{-1} d^{-1}] of *Emiliania huxleyi* at present day (filled bars) and high CO_2 (open bars) conditions. Daily rates are additionally given for each $DO^{14}C$ size fraction. Each bar corresponds to the average (± standard deviation) of replicate samplings (sampling 1-5, n=5) accomplished during the steady state period of the experiment.

Figure 2

Freshly produced high molecular weight (HMW > 1 kDa) dissolved combined carbohydrates (HMW-dCCHO, left), particulate CCHO (pCCHO, right) [µmol C L^{-1}] derived from *E. huxleyi* at present day (filled bars) and high CO_2 (open bars) conditions. Concentrations are additionally given for each size fraction of HMW-dCCHO. Each bar corresponds to the average (± standard deviation) of replicate samplings (samplings 3-5, n=3) accomplished during the steady state period of the experiment.

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 ~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 ~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 10^6 mL^{-1}, contributing ~2 % to particulate organic carbon (POC) and ~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 DO^{14}C 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 ~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 necessary, 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."

References:

Aluwihare, L.I., Repeta, D.J.: A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae, Mar. Ecol.- Prog. Ser., 186, 105-117, 1999.

Biersmith, A. and Benner, R.: Carbohydrates in phytoplankton and freshly produced dissolved organic matter, Mar. Chem., 63, 131-144, 1998.

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Nanninga, H.J., Ringenaldus, P., Westbroek, P.: Immunological quantitation of a polysaccharide formed by *Emiliania huxleyi*, J. Marine Syst., 9, 67-74, 1996.

Obernosterer, I., and Herndl, G. J.: Phytoplankton extracellular release and bacterial growth: dependence on inorganic N:P ratio, Mar. Ecol. Prog. Ser., 116, 247-257, 1995.

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Puddu, A., Zoppini, A., Fazi, S., Rosati, M., Amalfitano, S., Magaletti, E.: Bacterial uptake of DOM released from P-limited phytoplankton, FEMS Microbiol. Ecol., 46, 257-268, 2003.

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Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M.et al.: Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom, Science, 336, 608-611, 2012.

Feldfunktion geändert

Response to anonymous referee #2 (R#2)

General comments

Referee:

The manuscript presents particulate and dissolved organic production in Emiliania huxleyi chemostat culture with interest on the composition of particulate and dissolved carbohydrates for 4 different size classes. As a general comment, the manuscript is well written and the results present a clear scientific interest, as size fractionation and composition of carbohydrates are relatively weakly studied. However, this manuscript "merit much more attention and revisions of the present version", as well expressed by the other referee. Indeed, as mentioned by the other referee, while reading the M&M

we discover that this data set is part of a bigger data set on which the effect of different pCO2 treatments has been studied on several parameters (Engel et al., 2014). However, nothing is mentioned on this aspect on the introduction as the authors directly assumed that there is no effect. But, the results are interesting for: 1) knowledge's on PP, carbohydrates, size fraction, etc and 2) for the possible modification under future pCO2 levels. The title should therefore be modified to include the pCO2 aspect. The no OA effect should be clearly and honestly assumed by the authors

Apart this OA aspect, the interpretation of the results is, in my opinion, good and discussion related to heterotropic compartment is interesting. As mentioned by the other referee, the presence of bacteria in the chemostat should be clearly expressed earlier in the manuscript.

Response:

The response to this comment has been dealt with in the response to referee 1.See also Figures 1 and 2 in the response to referee1.

Referee:

Authors should reconsider some paragraphs of the discussion that are more related to results than discussion. Also, the author start their discussion with results from other studies on plankton communities then come to their results and other results on E. huxleyi culture. This should be reconsidered in the next version of the manuscript, because from their chemostats on single strain it is not realistic to compare for example PER obtained at community level.

Finally some paragraphs of the discussion do not finish on a clear take home message.

Response:

We will take these comments into consideration when revising the manuscript.

Specific comments:

R#2: ***The title should therefore be modified to include the pCO2 aspect.***

Response: As mentioned in the response to referee 1 we will include the CO2 aspect in the revised version. However, we will keep the focus on the process of exudation and composition of exudates and not include the absence of a CO2 effect in the title.

R#2: ***Also, why does the 180 atm chemostat not taken in consideration here?***

Response: For the present study we decided to focus on present day and future ocean conditions and did not sample the chemostat aerated with 180 µatm CO2 (glacial conditions). Due to practical reasons we decided to choose a present day and a future ocean treatment.

R#2: ***The no OA effect could be simply shown with one-two Figure(s)***

Response: We will show the no OA effect in revised versions of figure 1 and 2: Data obtained during the steady state period will be shown separately for "present day" and "high CO2".

R#2: ***While reading the M&M we imagine that axenic conditions were maintained, but we discover in the discussion it was not the case and non-axenic conditions have to be considered.***

Response: We will give information of the non-axenity in the chemostats in the methods section.

R#2: ***What CCHO composition of other NSW bring to the discussion? It merit to be related to the rest of the discussion.***

Response: We include the data of Aluwihare for comparison with our data and to show that the change in CCHO composition, particularly the increase in Ara has been observed for E. huxleyi before.

R#2: ***We don't need stats everywhere but there are results without stats (e.g., size fractionated DO14C production), is there any reason(s)?***

Response: We will check the results section and add stats wherever needed.

R#2: ***Does the ER expressed in % is the same as PER?***
Response: Yes.

R#2: ***P15291, l.21: "CO2" is not defined before***
Response: "CO2" will be defined as carbon dioxide (CO2) at first mention.

R#2: ***P15292, l. 23: "PP" is used but not defined before but after (same page, l.29).***
Response: "PP" will be defined as primary production (PP) at first mention.

R#2: ***P15299: There is no sentence confirming that the processes and parameters measured presented no temporal dynamic (chemostat culture) and therefore that the values expressed are average SD of the process or parameter during the X days of experiment. Therefore, if I understood, the results presented are the average for the X days of experiment and of the 2 pCO2 treatments? (except for NSW concentration and composition of carbohydrate in Figure 3).***
Response: We will add an explanation to the Data treatment section stating that average values and standard deviations were derived from all samplings including the replicate samplings over time during the steady state period in both the "present day" and the "highCO2" chemostat.
R#2: ***P15299, l.9-10: it is expressed that cell densities of E. hux. in cells mL-1 while in Engel et al. (2014) E. hux. cell densities are in cells L-1, for clarity it could be standardized?***
Response: We will adapt the presented cell numbers to per liter values in accordance to Engel et al. 2014.

R#2: ***Table 1 is cited but do not correspond to the actual Table 1 of the manuscript, as cell abundance data are not presented.***
Response: Referring to table 1 will be deleted at his point.

R#2: ***P15299, l.17: I would suggest to express the different pCO2 treatments different way than "337±94 (350) and 623±139 (750) atm" and use like in P15300, l. 18: value SD (present day) and value SD (high CO2).***
Response: We will adopt the referee's suggestion.

R#2: ***P15299, l.22: a sentence confirming that PP (PO14C, DO14C) productions were constant during the time of the sampling in stationary phase would be required (see above also).***
Response: We will add a sentence confirming the steady state in the chemostat cultures.

R#2: ***P15300, l.7: "Table 1" should be "Table 2"?***
Response: Reference to figures and tables will be thoroughly checked and corrected in the next version.

R#2: ***P15300, l. 14: "nutrient seawater (NSW)" should be modified to "natural seawater"?***
Response: Yes. We will change "nutrient" to "natural".

R#2: ***P15300, l. 16: Fig. 3a is cited before Fig. 2 has been cited.***
Response: Will be adapted.

R#2: ***P15301, l.12: "significant variation in monomeric" is used but no statistics are provided to confirm the "significant".***
Response: We will add the *p* value.

R#2: ***P15301, l.17: Fig. 4 is also the average of present and high CO2? Could be precised here as "(Table 3 and Figure 3 and 4)".***
Response: We will provide more specific information in the figure caption.

R#2: ***P15301, l.19 and 20: should be "Fig. 3b, right panel" instead of "left"?***
Response: In this case referring to the left panel is correct. However, references to figures and tables will be corrected and thoroughly checked in the next version.

R#2: ***P15301, l.20: it should be choose between Emiliania huxleyi or E. huxleyi; there is a "and" that should be removed.***
Response: We will use "Emiliania huxleyi" only once for the first time mentioned in each paragraph and use "E. huxleyi" afterwards.
The "and" will be deleted.

R#2: ***P15302, l.24: a new result is provided and there is no information on how the cell abundance were converted to carbon (conversion factor used?).***
Response: We wrote 'cell normalized production of PO14C' which implicates that the PO14C data were normalized to (divided by) the cell abundance. We will introduce the cell normalized values in the results section.

R#2: ***P15303, l.11: Marañón et al. (2005) have also shown relative constant PER over different ecosystem from eutrophic and oligotrophic with field samples.***
Response: True. We will add a sentence to specify this important finding by Marañón et al. (2005) and cite the respective paper at this point.

R#2: ***P15303, l.15: What do you mean by culture? Laboratory cultures? Because I don't read that Marañón et al. (2005) or López-Sandoval et al. (2010, 2011) or Engel et al. (2013) are related to culture but to natural samples samples in field or in mesocosm conditions.***
Response: We will change "cultures" to "natural phytoplankton communities".

R#2: ***P15304, l.10: what is "LMW-DOC"? (not defined before)***
Response: We will add the definition as "low molecular weight (<1 kDa, LMW)"

R#2: ***P15395, l.9: should be "(Fig. 3 b, left and right panels)"?***
Response: Reference to figures and tables will be corrected and thoroughly checked in the next version.

R#2: ***P15395, l.29: should it be "may be related to physiological and ecological functions"?***
Response: Yes. We will add a "to" to the sentence.

R#2: ***P15307, l.23: I don't see that large and small fractions have different contributions.***
Response: We wrote 'slightly higher (DO14C) proportions in the very large and small fraction'. Since it is more apparent for the very large fraction than for the small fraction we will revise the sentences to 'slightly higher (DO14C) proportions in the very large fraction'.

R#2: ***Figure 2: is this Figure 2 the same as Figure 3a (right panel) with the NSW concentration removed to have the freshly produced component? This figure is cited in the text but not very used, the values could be provided in the text and the Figure 2 could be replaced by a figure showing that there is no effect of increase pCO2 on some of the parameters and processes measured.***
Response: Showing the whole and the background corrected data for combined carbohydrates is on our opinion reasonable for an easier comparison with other studies. Therefore we rather change figure 1 and 2 by showing the data for present day and high CO2 individually to visualize the no CO2 effect and keep figure 3a (right panel) as is.

R#2: ***Figure 3: cited before Fig. 2 (see above). The a) and b) should be more visible.***
Response: Will be adapted and a) and b) will be shifted to a more visible position.

R#2: ***Figure 4: the text on the right y-axis should be oriented inside***
Response: We will change the text on the axis in accordance to the referee's suggestion.

R#2: ***like Figures 1 and 2. All: as mentioned by the other referee for each Figures and Table headings, it should be define tCCHO, pCCHO, as well as results represent average between the two pCO2 treatments over the experimental period. In the present version Figures and Tables are not self-sufficient (reading text is required).***
Response: We will change the figure captions and table headings in accordance to the referee's suggestion.

References

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

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Feldfunktion geändert

Response to anonymous referee #3 (R#3)

General comment:

Advancing the discussion of the passive versus active release findings and the meaning of the compositional differences in the freshly produced LMW DOM and HMW DOM fractions to their reactivities in the environment would enrich this study.

Nice discussion points. However, some additional attention could be given to the resolution of the active versus passive release question that is raised in the Abstract as well as the Introduction – but goes unaddressed in the Discussion in the current form. Discussions of the reactivity of size-fractionated DOM could also be advanced further – leading from experimental results to their environmental role.

Response:

We discussed these issues in 4.2.2, but we will revise and enhance this paragraph to explicitly state the hypotheses as well as implications.

Specific comments:

R#3: Please expand PP upon first mention in p. 1592, line 23.

Response: Done

R#3: Please expand fully TA upon first mention in p. 15295, line 8.

Response: Done

Size-fractionated dissolved primary production and carbohydrate composition of the coccolithophore *Emiliania huxleyi*

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Abstract

Extracellular release (ER) by phytoplankton is the major source of fresh dissolved organic carbon (DOC) in marine ecosystems and accompanies primary production during all growth phases. Little is known, so far, on size and composition of released molecules, and to which extent ER occurs passively, by leakage, or actively, by exudation. Here, we report on ER by the widespread and bloom-forming coccolithophore *Emiliania huxleyi* grown under steady state conditions in phosphorus controlled chemostats (N:P=29, growth rate of μ=0.2 d^{-1}~~),~~) at present day and high CO_2 concentrations. ^{14}C incubations were accomplished to determine primary production (PP), comprised by particulate ($PO^{14}C$) and dissolved organic carbon ($DO^{14}C$~~), and the concentration~~). Concentration and composition of particulate combined carbohydrates (pCCHO), and of high molecular weight (>1 kDa, HMW) dissolved combined carbohydrates (dCCHO) ~~as major components of ER.~~ were determined by ion chromatography. Information on size distribution of ER products was obtained by investigating distinct size classes (<0.40 µm~~,~~ ($DO^{14}C$), <0.45 µm (HMW-dCCHO), <1000 kDa, <100 kDa and <10 kDa) of $DO^{14}C$ and HMW-dCCHO. Our results revealed relatively low ER during steady state growth, corresponding to ~4.5% of primary production, and similar ER rates for all size classes. Acidic sugars had a significant share on freshly produced pCCHO as well as on HMW-dCCHO. While pCCHO and the smallest size fraction (<10 kDa)~~ fraction~~ of HMW-dCCHO exhibited a similar sugar composition, dominated by high percentages of glucose (74-80 Mol %), the composition of HMW-dCCHO size-classes >10 kDa was significantly different with higher Mol % of arabinose. Mol % of acidic sugars increased and Mol % glucose decreased with increasing size of HMW-dCCHO. ~~–~~We conclude that larger polysaccharides follow different production and release pathways than smaller molecules, potentially serving distinct ecological and biogeochemical functions.

Keywords: Exudation, carbohydrates, DOC, primary production, coccolithophores

1. Introduction

The global ocean inventory of dissolved organic carbon (DOC) is estimated to be in a range of 662 – 700 Gt (Hansell and Carlson 1998, Ogawa and Tanoue 2003). A common classification of marine DOC relies on its reactivity and discriminates between labile (LDOC), semi-labile (SLDOC), semi-refractory (SRDOC), refractory (RDOC) and ultra-refractory (URDOC) DOC with lifetimes of hours to days, weeks to months, month to years, centuries or even millennia (Kirchman 1993, Carlson & Ducklow 1995, Anderson & Williams 1999, Hansell, 2013). Only a small fraction of marine DOC is considered reactive; LDOC (< 0.2 Gt) and SLDOC (6 ± 2 Gt) (Hansell, 2013). In general, these compounds are freshly produced by plankton and represent the major nutritional resource for heterotrophic microorganisms (Cherrier et al., 1996; Amon & Benner 1996, Amon et al., 2001, Benner, 2002, Azam & Malfatti 2007, Davis et al., 2009). Especially during the summer season, SLDOC can accumulate in temperate waters, and becomes available for deep convective mixing, contributing to the biological carbon pump (Hopkinson and Vallino, 2005; Hansell et al., 2009). Microbial assimilation of DOC as well as the formation of gel particles, such as transparent exopolymer particles (TEP), lead to a repartitioning of DOC into the particulate organic carbon (POC) pool (Alldredge et al., 1993; Chin et al., 1998; Engel et al., 2004), the sinking of which represents another pathway for carbon export and storage in the ocean. In addition, microbial processing of fresh DOC may result in formation of recalcitrant compounds with longer residence time, also increasing the carbon dioxide (CO_2) storage potential in the ocean (Jiao and Zheng 2011). Thus, deeper insights to the origin and quality of DOC in the ocean can greatly abet our ability to quantify carbon and nutrient cycling in the ocean.

The ultimate source of organic carbon in the ocean is primary production, and extracellular release (ER, also referred to as dissolved primary production) of organic carbon is the primary source of fresh DOC, followed by cell lysis (Fuhrman 1999), grazing (Møller 2005), enzymatic particle solubilisation (Cho and Azam, 1988; Smith et al., 1992) and sloppy feeding (Copping and Lorenzen, 1980; Nagata, 2000). The major components of phytoplankton ER are high molecular weight (HMW, >1 kDa) dissolved combined carbohydrates (dCCHO), representing also the largest characterizable fraction of marine dissolved organic matter (DOM); 15-35 % DOC in the surface ocean, 5-10 % DOC in the deep ocean (Benner et al., 1992 Pakulski & Benner 1994, Biddanda and Benner, 1997, Ogawa and Tanoue, 2003). Composition of HMW-dCCHO in seawater is usually determined on the basis of monomeric sugars after hydrolysis of the polymer chains, and resembles

either phytoplankton biomass itself (Pakulski & Benner 1994, Børsheim et al. 1999) or extracellular CCHO from phytoplankton cultures (Biersmith & Benner 1998, Aluwihare & Repeta 1999, Aluwihare et al. 2002). The latter are usually comprised by neutral hexoses, pentoses and deoxysugars like glucose, galactose and mannose, by amino sugars like glucosamine and galactosamine and by uronic acids e.g. galacturonic acid and glucuronic acid (Aluwihare et al., 1997; Biersmith & Benner, 1998; Aluwihare & Repeta, 1999; Engel et al. 2010, Borchard & Engel, 2012).

ER is a normal function of healthy algae cells during all stages of growth (Fogg 1966, Mague et al. 1980, Bjørnsen 1988, Borchard and Engel 2012, Lopez-Sandoval et al. 2011) and can comprise up to 80 % of primary production (Sharp, 1977, Mague, 1980, Fogg, 1983, Bjørnsen, 1988). Two conceptual models have been proposed for phytoplankton ER: *i)* the *passive diffusion model* that describes the leakage of smaller molecules from inside the cell to its surrounding environment (Fogg, 1983, Bjørnsen, 1988), and *ii)* the *overflow model* that assumes an energy consuming exudation of HMW compounds (Fogg 1983, Nagata 2000, Schartau et al. 2007). According to the *passive diffusion model*, DOC crosses the cell membrane independently from primary production (PP) during day and night, and ER correlates to phytoplankton biomass and cell size. A higher relative contribution of ER to total PP would therefore be expected in communities dominated by small cells due to their higher surface to volume ratio (Bjørnsen 1988, Kiørboe & Hansen 1993, Marañón et al. 1996).

Central aspects of the *overflow model* are a dependence of ER on ~~primary production (PP)~~PP rates, the absence of ER at night and a high share of HMW substances (Williams 1990 and references therein, Nagata 2000). Fogg (1966) proposed that photosynthesis and build-up of organic carbon is primarily regulated by irradiance, while cell growth is controlled by the availability of inorganic nutrients. The discharge of photosynthesates, not utilized for cell growth, was suggested to be more energy-efficient than intracellular storage (Wangersky 1978, Wood & van Valen 1990). In accordance with the *overflow model*, data from coastal, marine and estuarine systems revealed a linear relationship between PP and ER, and factors influencing PP were suggested to also affect ER (Baines and Pace 1991). Such effects were shown for light (Zlotnik & Dubinsky 1989) and later suggested also for CO_2 (Engel 2002) and temperature (Moran et al. 2006). Under nutrient limitation, however, substantial ER was observed when PP was reduced, leading to higher percentages of extracellular release (PER) (Myklestad et al. 1989, Goldman et al. 1992, Obernosterer & Herndl 1995, Halewood et al. 2012). Under such conditions, decoupled from PP and biomass, ER becomes difficult to

estimate, both in terms of quantity and quality. Moreover, phytoplankton cells display a large physiological plasticity for nutrient requirements, i.e. the nutrient cell quota, which varies with environmental conditions or among different taxonomic groups (Geider and LaRoche, 2002).

Despite their role in marine carbon cycling, processes involved in the production, consumption and remineralisation of extracellular organic matter are little understood and have largely been neglected in biogeochemical models (Flynn et al., 2008, Repeta & Aluwihare, 2006; Hansell et al., 2009, Hansell, 2013). So far, it is not known if extracellular products are mainly released by leakage or by exudation processes, or how much leakage and exudation products differ. We also don't know if and how the physiological status of the cell influences the composition of extracellular products, and whether or not such differences in chemical signatures subsequently affect their microbial cycling, remineralisation rate, or affinity to form gel particles.

In order to improve our understanding on ER, we conducted a chemostat experiment with *E. huxleyi* under fully controlled nutrient supply and growth rate. *Emiliania huxleyi* is a bloom forming cosmopolitan coccolithophore species, and known to produce a methylated, acidic polysaccharide that plays a central role in coccolith formation and agglutination (Fichtinger-Schepmann, 1979, De Jong, 1979). ER by *E. huxleyi* cells was reported earlier (Aluwihare and Repeta 1999, Biddanda and Benner 1997, Borchard and Engel 2012) and carbohydrates were shown to provide a substantial fraction of freshly produced HMW-DOC (35-94 %) (Aluwihare and Repeta 1999, Biddanda and Benner 1997).

This study was part of a larger experiment investigating carbon and nutrient cycling under different pCO_2 conditions at steady state growth in *E. huxleyi*. No effect of the CO_2 treatment was observed for elemental stoichiometry of cells as well as for TEP production (Engel et al. 2014). This study focusses on primary production of POC and DOC by *E. huxleyi*, the carbohydrate composition of cells and for the first time on different size fractions of released compounds.

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With our study we wanted *(i)* to determine ER of DOC and carbohydrates by combining rate measurements for particulate and dissolved primary production with analyses of carbohydrate concentration, and *(ii)* to characterize monomeric carbohydrate composition in different size classes of DOC in order to elucidate mechanisms of ER. We chose the continuous culture approach, because here cells can be grown under nutrient limitation at steady state biomass.

Thus, in a chemostat the increase in extracellular organic matter can primarily be attributed to growing phytoplankton cells and not to cell lysis and decay, processes that co-occur with ER when batch cultures or natural populations become nutrient depleted.

2. Methods

2.1 Experimental setup

A calcifying strain of *E. huxleyi* (PML B92/11) was grown as continuous culture in two chemostats (~9.2 L each) at a constant dilution rate of D = 0.2 d^{-1}. A more detailed description of the chemostat principle and the experimental set-up are given by Borchard et al. (2011), Borchard and Engel (2012), and Engel et al. (2014), respectively. Temperature was set to 14.0±0.1°C. Irradiance was provided at a 16h:8h light:dark cycle with a photon flux density of 190 µmol photons m^{-2} s^{-1} (TL-D Delux Pro, Philips; QSL 100, Biospherical Instruments, Inc.). Nutrient medium was prepared from sterile-filtered (Sartobran P, 0.2 µm capsule, Sartorius) aged natural seawater (NSW) with a salinity of 33, total alkalinity (TA) of 2250 µmol kg^{-1} seawater and a pH of 8.24. The seawater was enriched with nutrients according to the f/2 recipe of Guillard and Ryther (Guillard & Ryther 1962) with final concentrations of 43 µmol L^{-1} NO_3^- and 1.5 µmol L^{-1} PO_4^{3-}. The nutrient medium was treated for 3 h with UV irradiation (Microfloat 1/0, a.c.k. aqua concept GmbH) for sterilisation before the addition of sterile-filtered (0.2 µm, Minisart, Sartorius) f/2-vitamins. Axenic conditions, however, could not be maintained in the 9.2 L chemostats over the long period of time.

Equilibration of the medium with CO_2 was obtained by constant aeration with 380 and 750 µatm CO_2, respectively. To minimize effects of calcification by *E. huxleyi* on carbonate chemistry in the incubators, TA in the reservoir tank was increased by addition of bicarbonate (LaRoche et al. 2010) resulting in 2460 µmol kg^{-1} seawater. *E. huxleyi* cells were pre-cultured for 30 d at prescribed CO_2 concentrations and temperature conditions in f/2 media in order to avoid short term stress effects on cell physiology. Each chemostat incubator was then inoculated to a final density of ~5000 cells ml^{-1}. Cultures were grown in batch mode for 5 d until the constant medium supply was applied at a dilution rate (D) of D=0.2 d^{-1}. Cells were kept in suspension by gentle mixing at 50 rotations min^{-1}. Here, we report data derived from samplings during steady state growth on experimental day 30, 34, 38, 42 and 44 for ^{14}C rate measurements and on day 38, 42 and 44 for carbohydrate analyses and size fractionations of those and ^{14}C exudation. All samples were taken 3 hours after lights on to avoid biases due to physiological variations during the day-night cycle.

2.2 Cell density and chemical analysis

***2.2.1* Cell density** was determined daily as the mean of three consecutive measurements of 500 µl by an electronic particle counter (Coulter Multisizer III, Beckman Coulter) equipped with a 100 µm aperture. 0.2 µm pre-filtered (Minisart 2000, Sartorius) NSW with a salinity

of 33 was used to dilute the samples 1:100. After microscopic inspection, particles with an equivalent spherical diameter in a range of 3.2 µm to 8.0 µm were identified as *E. huxleyi* cells.

2.2.2 **Nutrient samples** were filtered through 0.2 µm syringe filters (Minisart, Sartorius) and stored frozen at -20ºC until analysis. Measurements of NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} were made spectrophotometrically after Grasshof et al. (1999) using an Evolution 3 autoanalyzer (Alliance Instruments). Detection limits were 0.3 µmol L^{-1} for N and 0.01 µmol L^{-1} for P.

2.2.3 **Primary production and exudation** were measured by applying the ^{14}C incubation method according to Steemann Nielsen (Steemann Nielsen 1952) and Gargas (Gargas 1975). Triplicate samples (75 ml each) were taken from each chemostat, transferred into cell culture flasks (~~25cm²~~25 cm^2, Corning®) and spiked with approximately 5 µCi $NaHCO_3^-$ (Hartmann Analytics, specific activity 40-60 mCi/mmole). Each triplicate set was incubated for about 4 h at original experimental light and temperature settings, but without aeration. Simultaneously, dark uptake was measured in triplicate from 75 ml samples incubated in the dark. Added activity in the samples was determined by removing a 100 µl aliquot from three dark bottles prior to incubation and transferred to 6 ml liquid scintillation vials in which 200µl of 2N NaOH were placed. 4 ml liquid scintillation cocktail (Ultima Gold AB) were added before counting. Incubations were stopped by gentle filtration on 0.40 µm polycarbonate filters (Nucleopore) at low vacuum (<150 mbar) to avoid cell breakage. The filters ($PO^{14}C$) were covered with ~~250µl~~250 µl 1 M HCl in order to remove inorganic ^{14}C. After a few seconds they were rinsed with 10 ml filtered seawater. Filters were transferred to 6 ml scintillation vials, 4 ml liquid scintillation cocktail (Ultima Gold AB) were added and samples were stored overnight before being counted in a Packard Tri Carb Liquid Scintillation Counter. Carbon incorporation rates were calculated in accordance to Borchard and Engel (2012).

For the determination of released dissolved organic carbon ($DO^{14}C$), 4 ml of the filtrate were transferred into 20 ml scintillation vials and acidified to pH <2 by the addition of 100 µl 1 M HCl and left open under the fume hood for 24 hours. For size fractionation of $DO^{14}C$, triplicate sets of 10 ml sample were transferred into Macrosep® centrifugal devices with membrane cut off of <1000 kDa, <100 kDa and <10 kDa, respectively. After centrifugation (Heraeus, Megafuge® 1.0 R) for 15 min at 4000 rounds per minute, 4 ml sample were transferred into 20 ml liquid scintillation vials. In the following, samples were treated as the whole $DO^{14}C$ samples and after the outgassing of inorganic ^{14}C, 15 ml liquid scintillation cocktail were added. Counting and calculations were accomplished after Borchard and Engel (2012).

731 Primary Production (PP) was derived from the sum of $PO^{14}C$ and $DO^{14}C$. The percentage of
732 extracellular release (PER) was calculated as ($DO^{14}C$/PP)*100.
733
734 ***2.2.4.*** **Total combined carbohydrates** (tCCHO) and high molecular weight (HMW; >1 kDa)
735 **dissolved combined carbohydrates** (dCCHO) were determined by ion chromatography after
736 Engel and Händel (2011). Duplicate samples for HMW-dCCHO were filtered through 0.45
737 µm syringe-filters (GHP membrane, Acrodisk, Pall Corporation) and stored in combusted (8
738 h at 500°C) glass vials at -20°C. Samples for tCCHO remained unfiltered and were stored
739 identically.
740 For size fractionation of HMW-dCCHO, 10 ml sample were transferred into Macrosep®
741 centrifugal devices with a molecular weight cut-off (MWCO) of 1000 kDa, 100 kDa and 10
742 kDa, respectively. After centrifugation (Heraeus, Megafuge® 1.0 R) for 15 min at 4000
743 rounds per minute, samples were transferred into combusted (8 h at 500°C) glass vials and
744 stored at -20°C. Before usage, Macrosep® devices were rinsed twice by centrifugation with
745 ultrapure water to avoid any contamination with carbohydrate compounds in the membrane.
746 Concentrations of CCHO in these blanks were tested to be below the detection limit and did
747 therefore not affect analyses.
748 Prior to analysis, samples were desalinated by membrane dialysis (1 kDa MWCO, Spectra
749 Por) for 6 h at 0°C and thereafter hydrolyzed with HCl at a final concentration of 0.8 M for
750 20 h at 100°C to yield monomeric CHO. Samples were stored at -20°C over night and then
751 neutralized by acid evaporation (N_2) at 50°C. Dried samples were solubilised in ultra pure
752 water before determination of CHO monomers by high performance anion exchange
753 chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) on a Dionex
754 ICS 3000 (Engel & Händel 2011). A Dionex CarboPac PA10 guard column (2x50 mm)
755 coupled to a Dionex CarboPac PA10 analytical column (2x250 mm) was applied for
756 separation of fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactosamine (GalN),
757 glucoseamine (GlcN), galactose (Gal), glucose (Glc), mannose/xylose (Man/Xyl) (quantified
758 together due to co-elution), galacturonic acid (Gal-URA) and glucuronic acid (Glc-URA).
759 Detection limits are 10 nM. **Particulate CCHO** (pCCHO) were derived from subtraction of
760 HMW-dCCHO from tCCHO~~.~~ and thus represent carbohydrates in the size fraction > 0.45
761 µm. Concentrations of CHO are given as µmol carbon per volume of seawater (µmol C L^{-1})
762 and composition of CCHO is expressed as Mol % CCHO.
763 **Size fractions** of $DO^{14}C$ and HMW-dCCHO obtained by Macrosep® centrifugal devices
764 were subtracted from each other in order to present data for each size class. Definitions for
765 size classes are given in table 1.

2.2.5 For **total alkalinity** (TA), 25 ml of each sample were measured by titrating with 0.05 M HCl until the buffering capacity of the water samples was consumed and all bases of interest were protonated to zero level species. Analysis was accomplished with an automatic titrator (TitroLine® alpha *plus*, SI Analytics) equipped with a sample changer (TW *alpha plus*, SI Analytics) and a piston burette (Titronic®110 *plus*, SI Analytics). The pH was monitored by a two-point calibrated (buffer solution pH 4.006 and pH 6.865; Applichem, standardised according to DIN 19266) electrode (Schott® Instruments IoLine). The concentration of TA in µmol kg^{-1} seawater was calculated from linear regression of the absolute numbers of protons in solution and the total volume (sample plus HCl) in the range of pH 4 and 3. Determination of the seawater carbonate chemistry was conducted by using the program co2sys (Lewis & Wallace 1998) with pH (calibrated by the use of reference materials provided by A. Dickson) and TA being the input parameters.

2.3 Data treatment

All samplings were accomplished during the steady state period of the experiment when the growth rate (μ) was equal to the dilution rate (D). The samplings over time thus represent replicates of the same physiological state and values of the respective parameters are given as average ± standard deviation. Since CO_2 induced no differences between the *present day* and the *high* CO_2 chemostat, they were used as replicate treatments and values are given as mean values with single standard deviation if not stated otherwise.

In order to ~~directly~~ relate daily rates (µmol L^{-1} d^{-1}) ~~and~~directly to concentrations (µmol L^{-1}), data were converted into each other by applying a growth rate of 0.2 d^{-1}. For cell normalized carbon values concentrations and rates were divided by the cell number.

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Differences in carbohydrate composition for the different size fractions were tested by means of analysis of co-variance (two-way ANOVA). Differences as response to CO_2 conditions were tested by means of a *t*-test. Statistical significance was accepted for $p<0.05$. All calculations were performed using the software package Sigma Plot 10.01 (SysStat).

3. Results

3.1 Growth, nutrients and carbonate chemistry

Growth ~~of *E.*~~and biogeochemical composition of *Emiliania huxleyi* as well as carbonate and nutrient chemistry during this chemostat experiment are described in more detail in Engel et al. (2014). Briefly, ~~during~~on day 28 of the experiment, the steady state was reached with the dilution rate (D) being equal to the growth rate (μ) of *E. huxleyi.* Cell abundances and basic parameters such as particulate organic carbon (POC), nitrogen (PON), phosphorus (POP) and chlorophyll *a* (chl *a*) remained constant until the end of the experiment proving the constant physiological state of *E. huxleyi* (Engel et al., 2014).

During the steady state period, cell densities were similar in the ~~380~~*present day* and ~~750~~*high* CO_2 treatment and averaged 5.2*~~10^5~~10^8 ± 18.6 % cells ~~ml~~L^{-1} and 5.1*~~10^5~~10^8 ± 19.7 % cells ~~ml~~L^{-1}, respectively~~, over the whole sampling period (Table 1).~~. High variations resulted exclusively from intensive sampling between days 42 and 44. Until day 42 variations did not exceed 11.6 % and biomass production was accepted as balanced growth as a result of controlled nutrient supply. During steady state (days 30-44), both, NO_3^- and PO_4^{3-} concentrations were below the detection limit in both treatments. P-limitation was likely more severe than N-limitation, given a nutrient supply N:P ratio of ~29 and indicated also by PON:POP ratios ~~>16.~~clearly >16 (Engel et al., 2014). pCO_2 was calculated from pH and TA and yielded significantly different values between treatments of 337 ± 94 (~~380~~*present day*) and 623 ± 139 (~~750~~*high* CO_2) µatm. Time averaged values given here differ slightly from those given by Engel et al. (2014) as the latter used data from replicate chemostats per CO_2 treatment, while only one chemostat per treatment was sampled for the purpose of this study.

3.2 Primary production and exudation

As determined for cell densities, $PO^{14}C$ and $DO^{14}C$ production rates derived from replicate sampling during steady state growth varied <11 % confirming the physiological steady state of *E. huxleyi* grown in the chemostats. $PO^{14}C$ production of 173 ± 17 and 168 ± 16 µmol C L^{-1} d^{-1} and $DO^{14}C$ production of 8.0 ± 0.7 and 8.2 ± 1.1 µmol C L^{-1} d^{-1} were determined for the ~~380~~*present day* and ~~750~~*high* CO_2 µatm treatment, respectively (Fig. 1). Production rates of $PO^{14}C$ and $DO^{14}C$ were not significantly different between the CO_2 treatments (Mann-Whitney Rank sum tests and t-tests, n=5, p>0.69) and were thus averaged for both treatments: 171 ± 16 µmol C L^{-1} d^{-1} ($PO^{14}C$) and 8.1 ± 0.9 µmol C L^{-1} d^{-1} ($DO^{14}C$).

Cell normalized production of $PO^{14}C$ and $DO^{14}C$ during the steady state period were on average 0.33 ± 0.04 and 0.015 ± 0.002 pmol C $cell^{-1}$ d^{-1} for both treatments. Similar $PO^{14}C$ and $DO^{14}C$ production rates in both chemostats are reflected in comparable percentages of

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extracellular release (PER) of 4.42 ± 0.22 ~~and 4.70 ± 0.92 %, respectively. Because production rates of PP, $PO^{14}C$, $DO^{14}C$ and of associated size fractions were not significantly different between the CO_2 treatments (Mann Whitney Rank sum tests and t tests, n=5, p>0.69), data of both CO_2 treatments and repeated samplings are reported as average values ± one standard deviation (Figure 1, Table 2~~% (*present day*) and 4.70 ± 0.92 % (*high CO_2*) and also for the size classes of $DO^{14}C$ no CO_2 effect was determined (Fig. 1).

~~Size~~Averaged for both treatments, size fractionated (see table 1 for definition) $DO^{14}C$ production ranged between 1.27 ± 0.53 (*medium*) and 2.74 ± 0.88 (*very large*) µmol C L^{-1} d^{-1} ~~(Fig. 1, Table 1).~~ Relative contribution of different $DO^{14}C$ size classes to total $DO^{14}C$ was 33.6 ± 9.31 %, (*very large*), 24.6 ± 7.90 % (*large*), 15.9 ± 7.15 % (*medium*) and 25.8 ± 3.55 % (*small*). Thus, total $DO^{14}C$ was comprised by comparable shares of $DO^{14}C$ in these size classes with slightly higher proportions in the *very large* ~~and *small* fractions~~fraction.

3.3 Combined carbohydrates

Initial HMW-dCCHO concentrations of 7.02 ± 0.15 µmol C L^{-1} were determined in the ~~nutrient seawater (NSW) media. Size fractionation (see table 1 for definition) of~~natural seawater (NSW) media. Corrected for NSW values, carbohydrate concentration during steady state growth of *Emiliania huxleyi* was 103 ± 28 (*present day*) and 104 ± 31 µmol C L^{-1} (*high CO_2*) for pCCHO, and 15.2 ± 2.1 (*present day*) and 15.8 ± 2.4 (*high CO_2*) µmol C L^{-1} for fresh HMW-dCCHO, and hence very similar between the two CO_2 treatments (Fig. 2). Averaged for both treatments, 87 ± 3 % of tCCHO were present in the particulate fraction (pCCHO). *E. huxleyi* produced pCCHO in order of 104 ± 27 µmol C L^{-1} (0.20 ± 0.02 pmol C $cell^{-1}$) equivalent to 20.7 ± 5.3 µmol C L^{-1} d^{-1} at a growth rate of 0.2 d^{-1}, representing about 12.5 % of the daily produced $PO^{14}C$ (Table 2). Freshly produced HMW-dCCHO was 15 µmol C L^{-1} (0.043 ± 0.004 pmol C $cell^{-1}$), equivalent to about 40 % of freshly produced $DO^{14}C$. Fresh carbohydrate concentrations in various size classes (see table 1 for definition) also revealed a strong similarity between the *present day* and the *high CO_2* treatment (Fig. 2, t-tests, n=6, p>0.269) and are therefore given as average values in the following. In the different size classes, HMW-dCCHO comprised between 29.5 ± 9.3 % (*small*) and 59.7 ± 17.2 % (*Very large*) of $DO^{14}C$ (Table 2).

HMW-dCCHO yielded 7.07 ± 1.06 (*very large*), 3.57 ± 1.21 (*large*), 3.08 ± 1.26 (*medium*) and 3.09 ± 0.92 µmol C L^{-1} (*small*), suggesting that freshly released HMW-dCCHO were primarily comprised by *very large* HMW-dCCHO (46 ± 3% C). HMW-dCCHO in *large*, *medium*, and *small* contributed 23 ± 6, 20 ±4 and 20 ± 3 % C, respectively, to total HMW-dCCHO.

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Size fractionation of HMW-dCCHO in NSW yielded concentrations of 2.39 ± 0.21 (*very large*), 1.31 ± 0.09 (*large*), 1.28 ± 0.09 (*medium*) and 1.95 ± 0.10 *(small)* µmol C L^{-1} (Fig. 3a, left panel). During the experiment, ~~fresh HMW-dCCHO derived from~~extracellular release by *E. huxleyi* enriched the ~~natural seawater~~NSW to ~~steady state mean~~ HMW-dCCHO concentrations of ~~21.9 ± 2.2 (*present day*) and~~ 22.~~5 ± 2.4 (*high CO_2*)~~2 ± 2.1 µmol C L^{-1}~~; not significantly affected by elevated CO_2 (t test: n=6, p=0.985). The similarity between both treatments also holds for pCCHO and each~~ with size ~~fraction~~fractions of ~~HMW-dCCHO (t-tests, n=6, p>0.269), thus CCHO data are reported as average values for steady state conditions of the *present day* and *high CO_2* treatments in the following~~8.55 ± 1.08 (*very large*), 4.64 ± 1.33 (*large*), 4.09 ±1.21 (*medium*) and 5.18 ± 0.72 µmol C L^{-1} (*small*) (Fig. ~~2 and 3, Table 2~~3a, right panel).

~~Corrected for NSW values, carbohydrate concentration was 119 ± 28 µmol C L^{-1} for tCCHO, 16 ± 2.1 µmol C L^{-1} for fresh HMW-dCCHO and thus 103 ± 27 µmol C L^{-1} for pCCHO (Fig. 2). Hence, 87 ± 3 % of tCCHO were present in the particulate fraction (pCCHO). The size fractionation of HMW-dCCHO yielded 7.07 ± 1.06 (*very large*), 3.57 ± 1.21 (*large*), 3.08 ± 1.26 (*medium*) and 3.09 ± 0.92 µmol C L^{-1} (*small*) (Fig. 2, and daily rates given in table 2), suggesting that freshly produced HMW dCCHO were primarily comprised by *very large* HMW dCCHO (46 ± 3% C). HMW dCCHO in *large*, *medium*, and *small* contributed 23 ± 6, 20 ±4 and 20 ± 3 %C, respectively to HMW dCCHO.~~

3.4 Carbohydrate composition of exudates

Sugar monomers of three different types comprised the combined carbohydrates (CCHO) determined during the present experiment: Neutral sugars (Fuc, Rha, Ara, Gal, Glc and coeluting Man/Xyl), amino sugars (GalN and GlcN) and uronic acids (Gal-URA and Glc-URA). Various amounts of these monomers were detected in HMW-dCCHO of the initial NSW used for the present experiment (Fig. 3b, left panel). Size fractions of HMW-dCCHO in NSW did not show any significant variation in monomeric composition~~.~~ (p>0.462). However, relative to the other size fractions Man/Xyl was slightly enriched in *small*, while a smaller proportion of Fuc was detected in this fraction. No significant differences in monomeric composition of CCHO produced by ~~*E.*~~*Emiliania huxleyi* were determined between the *present day* and *high CO_2* treatment (p>0.~~05~~881). Therefore, average values ~~include~~ are given for replicate sampling during steady state growth and both treatments in the following (~~Table~~Fig. 3 and ~~Fig.~~Table 3).

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ER by *E. huxleyi* led to a clear change in HMW-dCCHO composition of the NSW (Fig. 3b~~, left panel~~); primarily caused by Ara being significantly higher (p<0.001) in all size classes, except for *small* (Fig. 3b~~, left panel~~). In HMW-dCCHO derived from ~~*Emiliania*~~*E. huxleyi*, Ara and Glc ~~and~~ were the dominant sugars with 46 and 21 Mol %, respectively followed by Gal-URA (11 Mol %), Man/Xyl (10 Mol %), Glc-URA (5.7 Mol %), Gal (4.6 Mol %) and Rha (2.4 Mol %) (Table 3~~).~~ and Fig. 3b, right panel). Proportions of other monomers comprised less than 0.5 mol % and declined in the following order: Fuc > GalN > GlcN (Table 3). In pCCHO, Glc was the most abundant sugar (74 ± 4 Mol %), followed by Rha (6.6 ± 0.8 ~~mol)~~Mol %) and Gal-URA (5.2 ± 0.7 ~~mol~~Mol %). Man/Xyl, Ara and Glc-URA ranged between 3.2 ±1.7 and 4.2 ± 1.0 ~~mol~~Mol % while Fuc and the amino sugars GalN and GlcN contributed only a minor fraction (<0.5 Mol %) to pCCHO (Table 3).

Hence, composition of pCCHO was substantially different from the composition determined for freshly produced HMW-dCCHO (p<0.002), except for the proportions of Gal and Glc-URA. This difference is mainly attributed to a smaller proportion of Glc in the dissolved fraction along with a more than 10 fold higher share of Ara and also higher proportions of Man/Xyl and Gal-URA (Table 3).

Carbohydrate composition of the investigated dCCHO size fractions was significantly different also~~.~~ (p<0.002). Ara was dominant in *very large*, *large*, and *medium* HMW-dCCHO, but not in *the small* fraction in which its contribution was significantly smaller than in all other size classes (p<0.01). Most interestingly, the proportion of Glc increased with decreasing size class, while the proportion of Gal-URA clearly decreased (Fig. 4). In *small,* Glc contribution was 80 ± 12 Mol % and significantly higher than in all other size classes of HMW-dCCHO (p<0.002) (Table 3). Contribution of Glc to *very large* dCCHO was negligible (<0.5 Mol %). In contrast, Gal-URA contributed 18 Mol % to *very large*, but only 1 Mol % to *small*. Proportions of Gal were also decreasing the smaller the HMW-dCCHO size classes, albeit not as clearly as for Gal-URA. Gal ranged from 6 (*very large*) to <0.5 Mol % (*small*). Contributions of Rha and Man/Xyl varied among size classes. Mol % of Fuc and both amino sugars, GalN and GlcN were negligible.

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4. Discussion

4.1 Particulate and dissolved primary production

Nutrient limitation and low growth rate did not hamper organic carbon production of *Emiliania huxleyi* during the present study. Cell normalized production of $PO^{14}C$ was on average ~0.33 pmol C $cell^{-1}$ d^{-1} and well within the range of published values (0.12 – 0.64 pmol C $cell^{-1}$ d^{-1}; Biddanda and Benner 1997, Borchard and Engel, 2012). The partitioning of organic carbon between dissolved and particulate pool was shown earlier to be highly influenced by environmental conditions such as light, temperature and nutrient supply (Myklestad and Haug, 1972, Zlotnik and Dubinsky 1989, Staats et al., 2000, Wetz and Wheeler, 2007). Nutrient depletion, however, seems to be the major factor leading to excess DOC excretion from algae cells to the surrounding environment and was reported from a variety of field and lab experiments (Fogg, 1983, Wood and VanValen 1990, Smith and Underwood, 2000, Lopez Sandoval 2010, 2011). ~~ER~~Extracellular release (ER) in the range from 0-80 % was reported over the past decades and only after a long lasting debate primarily concerning methodological constraints (Sharp, 1977, Mague, 1980, Fogg, 1983, Bjørnsen, 1988), it is nowadays accepted, that ER is a normal function of healthy algae cells occurring during all stages of growth. In exponentially growing cells in culture, ER typically ranges between 2 and 10 %, while in natural marine environments ER is generally higher by 10-20 % (see Nagata, 2000 and references therein). ~~Increased percentages of extracellular release (PER)~~A relatively constant percentage of extracellular release (PER) of 20 % was reported for field samples over different ecosystems covering oligotrophic and eutrophic regions (Marañón et al., 2005). Increased PER (up to 37 %) however, were observed for nutrient limited algae, during the transition period of exponential to stationary growth and during senescence of ~~cultures (Marañón, 2005,~~ natural phytoplankton communities (Lopez-Sandoval, 2010, 2011, Engel et al. 2013). In chemostats, despite the strict control of nutrient supply and growth rate, cells still grow exponentially. A decoupling of carbon to nutrient metabolism in continuous cultures can occur due to a change in growth rate (e.g. change the inflow of nutrient media) and results in changes in the partitioning between dissolved and particulate carbon pools, as shown with the same *E. huxleyi* strain (B 92/11) by Borchard and Engel (2012). In their study, down-regulation of the growth rate from μ=0.3 d^{-1} to μ=0.1 d^{-1} induced a slight increase in $DO^{14}C$ production, while the $PO^{14}C$ production was significantly minimized, resulting in higher PER. Cells then adapted to the steady state and high PER remained constant. During the present study, growth of *E. huxleyi* was also balanced to the nutrient supply but cells were not exposed to any stress due to nutritional changes. Thus, production of $DO^{14}C$ was not explicitly stimulated by changing experimental conditions, and,

albeit constantly P-limited, the cell normalized $DO^{14}C$ production of ~0.015 pmol C $cell^{-1}$ d^{-1} represented ~~a ER of ~ 4.5 %, well within the above mentioned range for non-stressed algae.~~ an ER of ~4.5 %, well within the above mentioned range for non-stressed algae. Full acclimation to environmental conditions during steady state growth may also explain the absence of a CO_2 effect on primary production and exudation during this study, and shows that *E. huxleyi* is in principle capable of acclimating to different CO_2 concentrations. Engel et al. (2014) suggested that exudation may be more sensitive to changes in pCO_2 during transient growth phase, such as towards the end of phytoplankton blooms, when cells become nutrient limited. Indeed, significant responses of ER to changes in pCO_2 have mainly been reported for phytoplankton blooms (Engel et al. 2013), batch and semi-continuous cultures (Thornton 2009, Barcelos e Ramos 2014), or when growing conditions changed during chemostat studies (Borchard and Engel 2012).

4.2 Combined ~~carbohydrates~~carbohydrate production

~~For cell growth, *E. huxleyi* produced particulate combined carbohydrates in order of 20.7±5.3 µmol C L^{-1} d^{-1} (equivalent to 103.6 ± 26.7 µmol C L^{-1} at a growth rate of 0.2 d^{-1}), representing about 12.5 % of the daily produced $PO^{14}C$ (Table 2). Cell normalized values for pCCHO were ~ 0.2 pmol C $cell^{-1}$ and close to values previously given for *E. huxleyi* (e.g. ~ 0.3 pmol C $cell^{-1}$, Biddanda and Benner 1997).~~

~~Carbon content of~~ HMW-dCCHO ~~in the NSW was ~ 7 µmol C L^{-1}. In the chemostats, HMW-dCCHO were enriched to an average value of ~ 22 µmol C L^{-1}. The~~ freshly produced ~~~15 µmol C L^{-1} suggest a HMW-dCCHO content of~~by *C. huxleyi* during steady state growth represented about 40 % of freshly produced $DO^{14}C$ (Table 2). This is a lower estimate because ~~LMW~~low molecular weight-DOC (<1 kDa, LMW) would be detected by the ^{14}C-incubation method (Steemann Nielsen, 1952) during the determination of $DO^{14}C$, but would escape the analysis of HMW-dCCHO due to the molecular cut off >1 kDa during desalinization of seawater samples (Engel & Händel 2011). In the surface ocean, HMW compounds of dissolved organic matter (DOM) were found to be more abundant (30-35 %) compared to deeper waters (20-25 %) and it was concluded that HMW-DOM inherits a higher reactivity and shorter lifetimes, while LMW-DOM is rather refractory (Amon and Benner, 1996, Ogawa and Tanoue, 2003). Major reaction processes of HMW compounds are heterotrophic degradation (Amon and Benner, 1996, Guo et al., 2002, Aluwihare and Repeta, 1999) and gel particle formation (Mari & Burd, 1998, Leppard, 1995, Passow, 2000, Passow 2002 and references therein). Thus, the HMW-DOM pool is directly linked to processes significant for organic carbon dynamics, nutrient cycling and oxygen consumption in the

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ocean. Assembly and coagulation of polymeric precursors has been proposed as mechanism leading to the formation of marine gel particles, such as TEP. Specifically, divalent cation bridging of acidic sugars, such as uronic acids is assumed to be involved in bonding between polysaccharide chains. The release of larger polysaccharides with relatively high Mol % Gal-URA as observed for *E. huxleyi* in this study may be an important first step for high TEP concentrations, observed previously (Engel et al. 2004, Harlay et al. 2009). However, absolute rates of ER were relatively low and apparently insufficient to induce TEP formation during this study. Engel et al. (2014) suggested that ~~acclimations~~responses to variations in environmental factors, specifically to changes in nutrient supply, are responsible for excess carbon accumulation inside the cell and for exudation of carbohydrates. Sampling during this study was conducted during the period of steady state growth. This may explain the observed relatively low rates of ER, including potential TEP precursors.

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4.2.1 Monomeric composition of CCHO

Natural seawater (NSW) used in the present study to prepare the nutrient media was collected from the North Sea and kept under dark and cool conditions for several months before usage. HMW-dCCHO monosaccharide composition of NSW was dominated by Glc (24 Mol %) and Man/Xyl (24 Mol %). Also high Mol % (~10) of Fuc, Gal, Gal-URA and GlcURA were determined, while other monomers were of minor importance (Fig. 3b, left panel). The composition of the aged NSW used here differs from those obtained from the Northwest Atlantic, the Sargasso Sea and the Gulf of Mexico (Aluwihare et al., 1997 and references therein), especially concerning comparably low proportions of Rha and Gal (Fig. 3b, left panel). Differences in carbohydrate composition of the seawater can be explained by seasonal or geographical divergences as well as by storage time of NSW.

Monomeric composition of HMW-dCCHO released by *E. hux*leyi during the present experiment was substantially different from the initial NSW composition (Fig ~~3a/b, right panels~~, 3b) and the compositional shift was primarily induced by a profound relative increase in Ara. The HMW-dCCHO and pCCHO derived from *E. huxleyi* during this experiment contained a similar composition as determined earlier for cellular and extracellular carbohydrates derived from this species (De Jong et al. 1979, Fichtinger Schepman et al. 1979, Nanninga et al. 1996, Bilan & Usov 2001). 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).

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Neutral sugars generally dominated the HMW-dCCHO composition with ~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~~),~~) who also investigated non-axenic *E. huxleyi* as batch culture, 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 ~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 10^6 mL^{-1}, contributing ~2 % to particulate organic carbon (POC) and ~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 DO^{14}C derived from rate measurements, however, a share of up to 20 % may have been channeled into heterotrophic turn-over. ~~Thus, the~~This means that e ER would be underestimated by 20% at most. The HMW-CCHO was ~~potentially~~thus to some extent subject to bacterial reworking and the high proportions of Ara ~~could have been~~may be 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 ~60 % (Piontek et al. 2010; J. Piontek pers. comm~~).~~., 2014). 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

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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 necessary, 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~~enzyme 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~~The 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.

4.2.2 Size fractionation of CCHO and DOC – Considerations on extracellular release

Quantitatively, each $DO^{14}C$ size fraction contributed similar amounts to total $DO^{14}C$ with slightly higher proportions in the *very large* ~~and *small*~~ fraction (Fig. 1 and Table 2). Release rates of HMW-dCCHO were similar for the different size fractions, but highest in the *very large* fraction (Fig. 2, Table 2). On a total basis, ~40 % of produced $DO^{14}C$ were characterized as freshly produced HMW-dCCHO (Table 2). Contribution of dCCHO to fresh DOC was lowest in the *small* size fraction (30 %) and highest in the *very large* (60 %)

1103 fraction (Table 2). Monomeric composition of different size classes of dCCHO enriched by
1104 *E. huxleyi* exudates was profoundly different from those of the aged NSW used as culture
1105 media (Fig. 3). In aged NSW, monomers were more evenly distributed among size fractions
1106 (Fig. 3b, left panel). In comparison, differences in monomeric composition of size classes in
1107 *E. huxleyi* exudates were largely due to changes in Ara, Glc, and Gal-URA. Most
1108 remarkably, Ara the dominant monomer in all larger dCCHO size classes, was of minor
1109 importance in the *small* dCCHO size fraction and lowest in the particulate fraction (Fig. 3,
1110 right panel). This is in accordance with the findings of Biersmith and Benner (1998), who
1111 also observed lower Mol % Ara for particulate components of an *E. huxleyi* culture as well as
1112 for the cell lysate. In contrast to Ara, Mol % Glc in our study was highest in the particulate
1113 and small fraction, relatively small in the *medium* to *large*, and negligible in the *very large*
1114 fraction. This also agrees well to earlier findings; Skoog et al. (2008) observed larger Mol %
1115 of Glc in LMW-CCHO than in HMW-CCHO, while reporting less Mol % Ara in LMW- than
1116 in HMW-CCHO. Thus, differences in size fractions of combined sugar molecules may be
1117 one factor responsible for differences in CCHO composition of DOC between study sites.
1118
1119 In general, carbohydrate composition in the smallest size class was similar to cellular
1120 *p*CCHO composition, while larger molecules were more distinct (Fig. 3, right panel). The ^{14}C
1121 method (Steemann Nielsen, 1952), applied here to measure primary production and ER of
1122 organic carbon does not allow distinguishing if DOC is released from the cell passively, i.e.
1123 by leakage, or actively by exudation. Leakage is hypothesised to be directly related to
1124 biomass and cell size, suggesting a constant value of passive PER. The composition of the
1125 *small* size class, and particularly the high share of Glc, resembled the cellular carbohydrate
1126 composition (Fig. 3b, right panel). This finding suggests a non-selective, i.e. passive, release
1127 of carbohydrates in the smallest size class determined here. Storage glucans in algae are
1128 comprised exclusively by Glc in D formation and have a molecular weight of 5 – 10 kDa. D-
1129 Glc was reported as major component of coccolith polysaccharide (CP) of *E. huxleyi*
1130 (Fichtinger Schepman, 1979). For chloroplasts in higher plants, porins are described that
1131 allow trans-membrane passage of hydrophilic molecules like sugars and amino acids up to a
1132 molecular weight of 10 kDa without the use of energy (Flügge and Benz, 1984; Mohr and
1133 Schopfer, 1992). The existence of porins in cell membranes of algae is likely but not
1134 explicitly reported. If DO^{14}C >1 and <10 kDa and associated carbohydrates leak from the cell
1135 in accordance to the passive diffusion model, this extracellular release is presumably linear
1136 correlated to biomass (*property tax* – Sharp 1977). For molecules >10 kDa, however,
1137 different mechanisms for the extracellular release are to be expected, since larger molecules

~~can not~~cannot pass the membrane by diffusion, and CCHO composition clearly differs from intracellular CCHO (Fig. 3b, right panel). If active release, i.e. exudation, follows the overflow model, biomass growth and dissolved primary production might be strongly decoupled (*income tax* – Sharp 1977). Moreover, exudation requires a series of physiological processes involved in the synthesis, transport and trans-membrane release of exudates. Hence, exudates likely vary in composition. Data obtained during the present study indicate, that components >10 kDa, rich in Ara and Gal-URA and poor in Glc, are transported actively through the cell membrane. ~~Assembly and coagulation of polymeric precursors has been proposed as mechanism leading to the formation of marine gel particles, such as TEP. Specifially, divalent cation bridging of acidic sugars, such as uronic acids is assumed to be involved in bonding between polysaccharide chains. The release of larger polysaccharides with relatively high Mol % Gal-URA as observed for *E. huxleyi* in this study may be an important first step for high TEP concentrations, observed previously (Engel et al. 2004, Harlay et al. 2009). However, absolute rates of ER were relatively low and apparently insufficient to induce TEP formation during this study. Engel et al. (2014) suggested that acclimations to variations in environmental factors, specifically to changes in nutrient supply, are responsible for excess carbon accumulation inside the cell and for exudation of carbohydrates. Sampling during this study was conducted during the period of steady state growth. This may explain the observed relatively low rates of ER, including potential TEP precursors.~~

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5. Conclusion

Carbohydrates of high molecular weight (>1 kDa) as a product of primary production are released from nutrient limited *E. huxleyi* during steady state growth. Compositional difference between size fractions of combined carbohydrate suggest that dCCHO >10 kDa are released by active exudation across the cell membrane whereas lower molecular weight carbohydrates (<10 kDa) can pass the membrane passively by leakage. The underlying mechanism of the release, however, needs to be further elucidated. If the presence of Ara is indeed an indicator for less degradable exudates as suggested by this study or, if Ara degradation requires activities of specific bacterial ~~strains~~assemblages, needs further exploration, i.e. by using axenic phytoplankton cultures ~~or~~combined with the addition of natural bacterioplankton communities. At present our understanding of how microbial processes shape the molecular composition of DOM, specifically of carbohydrates, is still at its infancy. This study suggests that dCCHO composition and size may be valuable indicators of processes related to autotrophy such as primary production and exudation, but may also keep the fingerprint of heterotrophic degradation. A better understanding of compositional changes in dCCHO, as major fraction of semi-labile DOC, may therefore help to unravel carbon cycling and ecosystem dynamics in the ocean.

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Table 1: Definition of size classes for fractionated high molecular weight (~~>~~(HMW, >1 kDa) dissolved combined carbohydrates (HMW-DCCHO) and dissolved organic carbon ($DO^{14}C$).

	HMW-dCCHO	***$DO^{14}C$***
Total	1kDa< HMW-dCCHO < 0.45 µm	$DO^{14}C$ < 0.40µm
Very Large	1000 kDa < HMW-dCCHO <0.45 µm	1000 kDa < $DO^{14}C$ < 0.40µm
Large	100 kDa < HMW-dCCHO <1000 kDa	100 kDa < $DO^{14}C$ <1000 kDa
medium	10 kDa < HMW-dCCHO <100 kDa	10 kDa < $DO^{14}C$ <100 kDa
Small	1kDa< HMW-dCCHO <10 kDa	$DO^{14}C$ <10 kDa

Table 2: Size class resolved production rates of ~~fresh organic carbon and of~~ high molecular weight (>1 kDa) carbohydrates (HMW-CCHO) and of fresh organic carbon (^{14}C) during the chemostat experiment, as well as contribution of carbon contained in HMW-CCHO ~~–~~to primary production (^{14}C) ~~of~~in particulate matter and ~~of~~in different size fractions of dissolved organic carbon ~~fractions.~~. Values represent averages ± standard deviation of replicate samplings and both treatments, n=6.

		HMW - CCHO [µmol C L^{-1} d^{-1}] avg. ~~± 1~~ ± *sd* ~~(n = 6)~~	*^{14}C [µmol C L^{-1} d^{-1}]* *avg.* ~~± 1~~ sd ~~(n = 10)~~	*HMW-CCHO : ^{14}C [%]* *avg.* ± sd
Particulate		20.7 ± 5.34	171 ± 15.9	12.5 ± 3.54
Dissolved	***Total***	3.10 ± 0.41	8.11 ± 0.88	40.0 ± 5.37
	Very Large	1.41 ± 0.21	2.74 ± 0.88	59.7 ± 17.2
	Large	0.71 ± 0.24	2.01 ± 0.72	44.6 ± 24.7
	Medium	0.62 ± 0.25	1.27 ± 0.53	52.9 ± 33.7
	Small	0.62 ± 0.18	2.09 ± 0.32	29.5 ± 9.30

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Table 3: Freshly produced combined carbohydrates (CCHO) in various size fractions. Average values (bold) and standard deviations (*italics*) in Mol % CCHO are given for replicate samplings ~~during steady state cell growth,~~and both treatments., n=6. Fuc, GalN and GlcN were always <0.5 Mol % and are not included.

CCHO [~~mol~~Mol %] Size fraction	Rha	Ara	Gal	Glc	Man/Xyl	Gal-URA	Glc-URA
pCCHO	**6.56** *0.84*	**3.69** *0.99*	**3.09** *1.45*	**74.0** *4.08*	**3.22** *1.65*	**5.18** *0.68*	**4.23** *1.01*
HMW-dCCHO (total)	**2.44** *0.70*	**46.0** *3.0*	**4.64** *1.95*	**20.5** *7.48*	**9.68** *2.37*	**11.0** *4.40*	**5.74** *2.99*
very large	**3.46** *1.88*	**54.2** *13.3*	**6.34** *4.11*	**<0.5** -	**9.62** *6.35*	**18.2** *5.35*	**8.17** *6.11*
large	**0.91** *0.85*	**41.0** *24.2*	**5.92** *5.10*	**18.9** *11.2*	**16.5** *18.9*	**8.45** *13.3*	**8.34** *13.41*
medium	**1.71** *1.03*	**48.8** *9.41*	**3.54** *3.41*	**34.9** *19.2*	**8.20** *9.88*	**2.96** *3.35*	**<0.5** -
small	**2.25** *1.54*	**9.70** *6.17*	**<0.5** -	**79.8** *11.9*	**4.03** *3.74*	**1.13** *3.35*	**2.64** *2.89*

pCCHO: particulate combined carbohydrates; HMW-dCCHO: high molecular weight (> 1 kDa, HMW) dissolved combined carbohydrates; Rha: rhamnose; Ara: arabinose; Gal: galactose; Glc: glucose; Man/Xyl: co-eluting mannose and xylose; Gal-URA: galacturonic acid; Glc-URA: glucuronic acid;

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Figure 1

Dissolved ($DO^{14}C$, left) and particulate ($PO^{14}C$, right) primary production [µmol C L^{-1} d^{-1}] of *Emiliania huxleyi*~~.~~ at present day (filled bars) and high CO_2 (open bars) conditions. Daily rates are additionally given for each $DO^{14}C$ size ~~fractions.~~fraction. Each bar corresponds to the average (± standard deviation) of replicate samplings (sampling 1-5, *n*=5) accomplished during the steady state period of the experiment.

Figure 2

Freshly produced high molecular weight (HMW > 1 kDa) dissolved combined carbohydrates (HMW-dCCHO, left), particulate CCHO (pCCHO, right) [µmol C L^{-1}] derived from *E. huxleyi*~~.~~ at present day (filled bars) and high CO_2 (open bars) conditions. Concentrations are additionally given for each size fraction of HMW-dCCHO. Each bar corresponds to the average (± standard deviation) of replicate samplings (samplings 3-5, *n*=3) accomplished during the steady state period of the experiment.

Figure 3

Concentration [µmol C L^{-1}] *(a)* and composition [Mol % CCHO] *(b)* of high molecular weight (>1 kDa) dissolved combined carbohydrates (HMW-dCCHO). Data are shown for natural seawater used to prepare the experimental culture media (left panels) and composition in natural seawater enriched with freshly produced HMW-dCCHO derived from *E. huxleyi* (rights panels) grown in chemostats. Due to the strong similarity between the present day and high CO_2 treatment, both were treated as replicates. Stacked bars show the average of replicate samplings (samplings 3-5, *n*=6) accomplished during the steady state period of the experiment.

*: Data for *HMW-dCCHO for Natural seawater* and *E. huxleyi* taken from Aluwihare (1999) for comparison. Here, only neutral carbohydrates are included, since amino- and acidic HMW-dCCHO were not analyzed.

Figure 4

~~Changes in molar composition~~Proportions of glucose (Glc) and galacturonic acid (Gal-URA) in ~~dCCHO with the~~high molecular weight (HMW > 1 kDa) dissolved combined carbohydrates (dCCHO) of ~~released dCCHO~~different molecular weight size classes as defined in table 1. Due to the strong similarity between the present day and high CO_2 culture, both were treated as replicates. Bars show the average ~~values; error bars ± 1~~(± standard

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deviation) of replicate samplings (sampling 3-5, n=6-) accomplished during the steady state period of the experiment.

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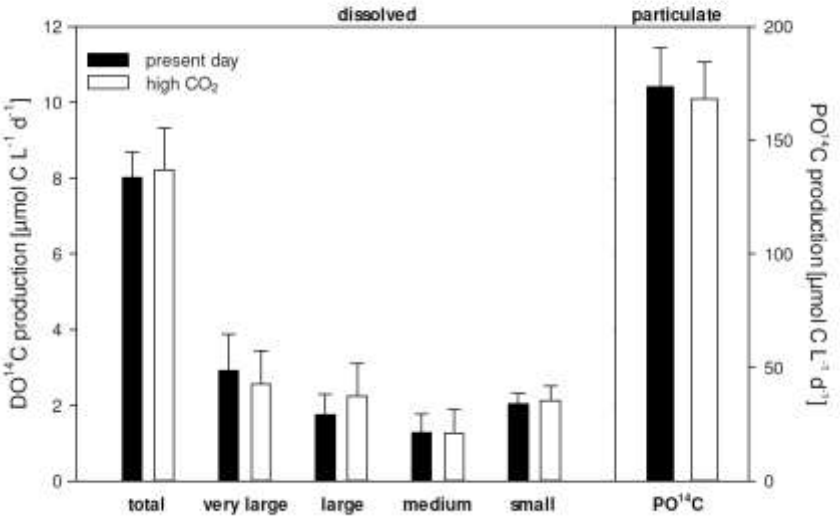
dissolved
particulate
present day
high CO_2
$DO^{14}C$ production [µmol C L^{-1} d^{-1}]
$PO^{14}C$ production [µmol C L^{-1} d^{-1}]
0
2
4
6
8
10
12
0
50
100
150
200
total
very large
large
medium
small
$PO^{14}C$

Figure 1

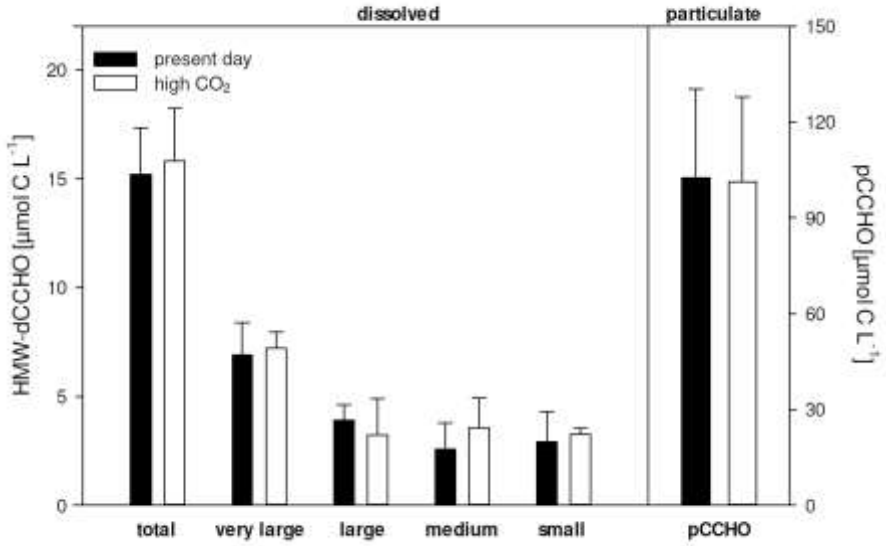
dissolved
particulate
present day
high CO_2
HMW-dCCHO [µmol C L^{-1}]
pCCHO [µmol C L^{-1}]
0
5
10
15
20
0
30
60
90
120
150
total
very large
large
medium
small
pCCHO

Figure 2

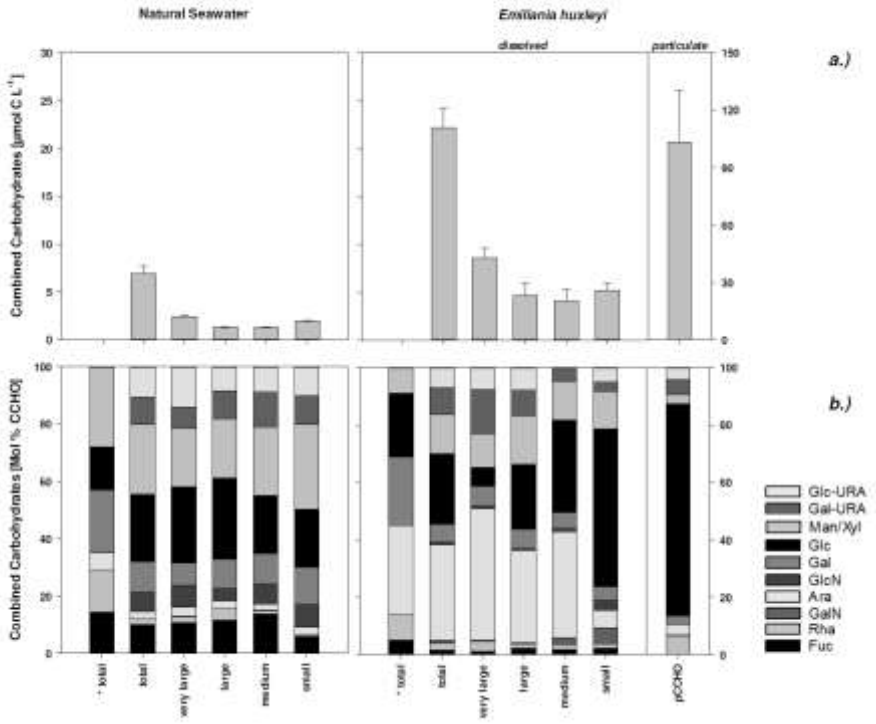
Natural Seawater
Emiliania huxleyi
dissolved
particulate
a.)
b.)
Combined Carbohydrates [µmol C L⁻¹]
Combined Carbohydrates [Mol % CCHO]
Glc-URA
Gal-URA
Man/Xyl
Glc
Gal
GlcN
Ara
GalN
Rha
Fuc
total
total
very large
large
medium
small
total
total
very large
large
medium
small
pCCHO

Figure 3

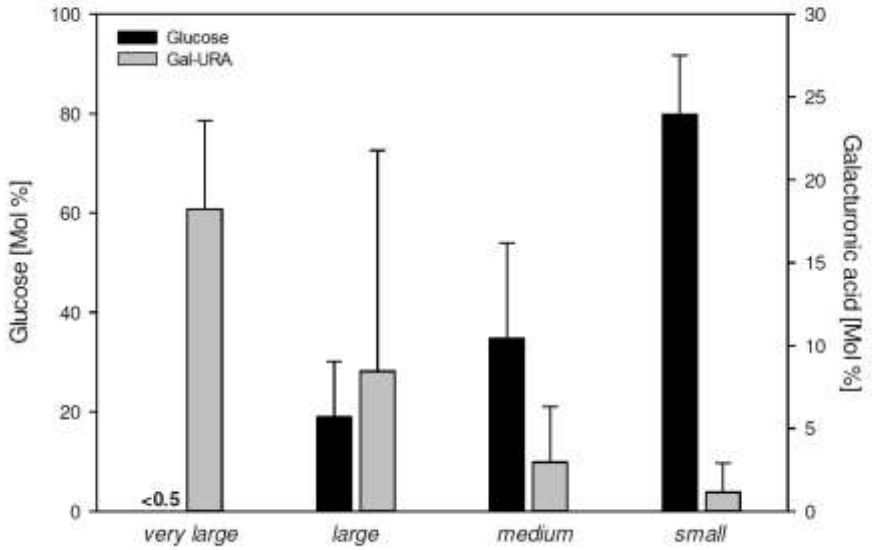
Glucose
Gal-URA
Glucose [Mol %]
Galacturonic acid [Mol %]
100
80
60
40
20
0
30
25
20
15
10
5
0
<0.5
very large
large
medium
small

Figure 4