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Organic matter exudation by *Emiliania huxleyi* under simulated future ocean conditions

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Abstract

Emiliania huxleyi (strain B 92/11) was exposed to different growth, CO_2 and temperature conditions in phosphorous controlled chemostats, to investigate effects on organic carbon exudation, and partitioning between the pools of particulate organic carbon (POC) and dissolved organic carbon (DOC). ^{14}C incubation measurements for primary production (PP) and for extracellular release (ER) were performed. Chemical analysis included amount and composition of high molecular weight dissolved combined carbohydrates (>1 kDa, HMW-dCCHO), particulate combined carbohydrates (pCCHO) and the carbon content of transparent exopolymer particles (TEP-C). Applied CO_2 and temperature conditions were 300, 550 and 900 µatm pCO_2 at 14 °C, and additionally 900 µatm pCO_2 at 18 °C simulating a greenhouse ocean scenario.

A reduction in growth rate from $\mu = 0.3\,d^{-1}$ to $\mu = 0.1\,d^{-1}$ induced the most profound effect on the performance of *E. huxleyi*, relative to the effect of elevated CO_2 and temperature. At $\mu = 0.3\,d^{-1}$, PP was significantly higher at elevated CO_2 and temperature. $DO^{14}C$ production correlated to $PO^{14}C$ production in all cultures, resulting in similar percentages of extracellular release ($DO^{14}C/PP \times 100$; PER) of averaged 3.74 ± 0.94 %. At $\mu = 0.1\,d^{-1}$, $PO^{14}C$ decreased significantly, while exudation of $DO^{14}C$ increased, thus leading to a stronger partitioning from the particulate to the dissolved pool. Maximum PER of 16.3 ± 2.3 % were observed at $\mu = 0.1\,d^{-1}$ at greenhouse conditions.

Concentrations of HMW-dCCHO and pCCHO were generally higher at $\mu = 0.1\,d^{-1}$ compared to $\mu = 0.3\,d^{-1}$. At $\mu = 0.3\,d^{-1}$, pCCHO concentration increased significantly along with elevated CO_2 and temperature. Despite of high PER, the percentage of HMW-dCCHO was smallest at greenhouse conditions. However, highest TEP-formation was observed under greenhouse conditions, together with a pronounced increase in pCCHO concentration, suggesting a stronger partitioning of PP from DOC to POC by coagulation of exudates.

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Our results imply that greenhouse condition will enhance exudation processes in *E. huxleyi* and may affect organic carbon partitioning in the ocean due to an enhanced transfer of HMW-dCCHO to TEP by aggregation processes.

1 Introduction

Primary production (PP) in the sunlit surface ocean is the driving force for the uptake of CO_2 and basis for its potential sequestration into the ocean's interior (Chisholm, 2000; Falkowski et al., 2000). The production of particulate organic carbon (POC) via primary production is accompanied by the extracellular release (ER) of various amounts of dissolved organic carbon (DOC). ER represents a significant fraction of PP (Myklestad, 1977; Mague et al., 1980; Baines and Pace, 1991) and was reported from a variety of phytoplankton species in several studies over the last decades (Fogg, 1966; Myklestad, 1995; Biddanda and Benner, 1997; Descy et al., 2002; Wetz and Wheeler, 2007; Lopez-Sandoval et al., 2011). Results from a modelling study with data from coastal, marine and estuarine systems revealed a linear relationship between PP and ER for non freshwater systems with a percentage of extracellular release (PER) in a range of 2–50 % (Baines and Pace, 1991). A correlation between PP and ER was also shown for light and temperature for marine (Verity et al., 1991) and freshwater algae (Zlotnik and Dubinsky, 1989) as long as applied conditions are in a range of optimum conditions of the tested algae. Data obtained under nutrient limitation frequently revealed PP and ER to be decoupled, generally leading to significantly higher PER (Myklestad, 1977; Obernosterer and Herndl, 1995; Lopez-Sandoval et al., 2011). Changes of the partitioning of POC and DOC have strong implications for carbon export fluxes, because DOC itself, unlike POC, does not sink. DOC can only be transported to deeper waters by mixing processes, e.g. downwelling and eddy diffusion (Kaehler et al., 1997; Ducklow et al., 2001; Karl et al., 2001). However, if not subject to bacterial degradation, freshly produced DOC accumulates in surface waters and may affect the transfer efficiency of dissolved compounds into the particulate size spectrum of POC

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(>0.7 µm) via coagulation processes: exudates provide precursors for transparent exopolymer particles (TEP) (Passow, 2000), which represent an abiotic linkage between DOC and POC in marine carbon cycling (Chin et al., 1998; Wells, 1998; Engel et al., 2004b; Verdugo et al., 2004, 2008; Gogou and Repeta, 2010). Phytoplankton exudates are by up to 80 % comprised by carbohydrates (Myklestad, 1977, 1989, 1995; Ittekkot et al., 1981; Lancelot, 1984). Exudation of TEP-precursors by microalgae was shown to be enhanced at nutrient stress (Staats et al., 2000; Passow, 2002a; Underwood et al., 2004), as a consequence of carbon overflow (Schartau et al., 2007).

Since preindustrial times, emissions of the greenhouse gas CO_2 into the atmosphere increased dramatically due to human activities (Caldeira and Wickett, 2003; Meehl et al., 2007). The sea surface is expected to be most affected by global change (Raven et al., 2005) and acidification and warming will most likely influence the photosynthetic fixation of CO_2 and therefore the ocean's organic carbon pump and the fate of organic matter in the ocean (Falkowski, 1994; Arrigo, 2007; Caldeira, 2007).

Carbon partitioning and carbon fluxes were hypothesised to be affected by CO_2 and temperature (Engel et al., 2004a; Leonardos and Geider, 2005; Wohlers et al., 2009). In accordance, TEP has been shown to be related to seawater CO_2- concentrations (Engel et al., 2004a; Moran et al., 2006) and its formation was reported to be facilitated at elevated temperature (Claquin et al., 2008). Factors that influence carbon overconsumption, exudation and TEP-formation will thus affect elemental ratios of particulate organic matter (POM) and may have strong implications for the organic carbon pump (Engel et al., 2004b, Riebesell et al., 2007). However, ER in referred studies was deduced from DOC accumulation rather than from direct rate measurements.

Emiliania huxleyi, a cosmopolitan species of coccolithophores, was subject of several studies in the context of global change (Riebesell et al., 2000; Zondervan et al., 2002; Sciandra et al., 2003; Delille et al., 2005; Leonardos and Geider, 2005; Feng et al., 2008; Langer et al., 2009). *E. huxleyi* primary production was shown to be stimulated by elevated partial pressure of CO_2 (pCO_2) (Riebesell et al., 2000, 2007; Egge et al., 2009) and elevated temperature (Langer et al., 2007; Feng et al., 2008).

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Studies with *E. huxleyi* reporting on effects of nutrient limitation in the context of global change are scarce, but a clear decoupling of nutrient assimilation from carbon assimilation at high CO_2 conditions has been shown for nitrogen (Leonardos and Geider, 2005) and phosphorous limitation (Borchard et al., 2011). The production of extracellular polysaccharides and ER of organic carbon was reported for *E. huxleyi* (Nanninga et al., 1996; Biddanda and Benner, 1997; Godoi et al., 2009). Furthermore, the presence (Westbroek et al., 1973) and structure of coccolith polysaccharides, as well as the carbohydrate composition in this species is well described (De Jong et al., 1979; Fichtinger Schepman et al., 1979; Kayano and Shiraiwa, 2009). Galacturonic acid, an acidic polysaccharide, was determined in *E. huxleyi* cultures (Nanninga et al., 1996), and is of great interest, since polysaccharides in general and acidic polysaccharides in particular, are known to facilitate TEP-formation (Mopper et al., 1995; Passow, 2000, 2002b).

According to future projections on climate change, a rise in CO_2 and temperature will occur simultaneously (IPCC, Solomon et al., 2007). We therefore applied both, conditions of warming and acidification, in the present study to test for combined effects, representative for a greenhouse ocean. The experimental design used here, mimics a situation of the future ocean that potentially favours carbon overconsumption and exudation by testing the effect of phosphorous limitation at two low growth rates. Results on the cell composition are presented in Borchard et al. (2011) and high C:N:P ratios clearly show a decoupling of carbon and nutrient assimilation. Here we give detailed information on dissolved and particulate primary production derived from the same experiment in order to assess information on the partitioning of DOC and POC derived from *E. huxleyi* as a function of growth, CO_2 and temperature. The main objectives during the present study were to determine (1) the partitioning of photosynthetically derived DOC and POC, (2) the amount and composition of high molecular weight (>1 kDa) dissolved combined carbohydrates (HMW-dCCHO) and particulate CCHO (pCCHO) and (3) to test for linkages to TEP-formation.

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2 Methods

2.1 Experimental setup

Data shown here were obtained during the chemostat experiment described in more detail by Borchard et al. (2011). Briefly, a calcifying strain of *Emiliania huxleyi* (PML B92/11) was grown in CO_2- and temperature-controlled chemostats with a culture volume of 9.2 l. Nutrient medium for the constant supply was prepared from sterile-filtered (Sartobran P, 0.2 µm capsule, Sartorius) natural seawater (SW) with a salinity of 32, pH of 7.97 and total alkalinity (TA) of 2281 µmol kg^{-1} SW. SW was enriched with nutrients to yield final concentrations of 29 µmol l^{-1} NO_3^- and 1.1 µmol l^{-1} PO_4^{3-}, and with a metal mix according to the f/2-recipe (Guillard and Ryther, 1962). Medium for each chemostat was stored in 100 l reservoirs. In order to sterilize the medium, it was treated with UV irradiation (Microfloat 1/0, a.c.k. Aqua Concept GmbH) for 3 h before the addition of sterile-filtered f/2-vitamins (0.2 µm, Minisart, Sartorius).

After filling 9.2 l nutrient medium into each chemostat incubator, CO_2 and temperature conditions of 300 and 550 µatm at 14 °C and 900 µatm at 14 and 18 °C were applied and cultures are hereafter referred to as 300-14, 550-14, 900-14 and 900-18. CO_2-concentrations in chemostats were achieved by constant aeration with gas obtained from a CO_2 regulation system based on the mixing of CO_2 free air and pure CO_2 using mass flow controllers (MFC, Type 1179 Mass Flo Controller; MKS Instruments, Germany) (Borchard et al., 2011). Briefly, premixed gases were channelled into each incubator via silicon and gas distribution tubes (ROBU; Type A, Por. 1) and allowed to equilibrate for 2 d before inoculation with cells. Each chemostat incubator was surrounded by a water jacket connected to a thermostat (Lauda, Ecoline Staredition, RE 104) setting temperatures to target values ± 0.1 °C. A 16h:8h light:dark cycle with a photon flux density of 300 µmol photons m^{-2} s^{-1} was applied (TL-D Delux Pro, Philips; QSL 100, Biospherical Instruments, Inc.).

All pipes and tubes connected to the incubator were rinsed first with 10% hydrochloric acid (HCl), subsequently with deionised water, and then sterilized by autoclaving at

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121 °C for 30 min before usage. The incubator and the reservoir vessels were cleaned with phosphate-free detergent, then soaked in 10 % HCl for 2 h and thoroughly rinsed with deionised water thereafter.

Cells were inoculated to each chemostat incubator to a final density of 3000 cells ml^{-1} and grown in batch mode for 3 d until the constant medium supply was applied for 12 d at a dilution rate (D) of $D = 0.3\,d^{-1}$ followed by 12 d at $D = 0.1\,d^{-1}$. Gentle mixing at 50 r.p.m by a mechanical stirrer inside the incubator kept cells in suspension. Three samplings were accomplished on days 10, 14 and 17 for $\mu = 0.3\,d^{-1}$ and days 22, 25 and 28 for $\mu = 0.1\,d^{-1}$ at 08:00 am (three hours after lights on).

Temperature and pH values in the cultures were measured with a proton sensitive combined pH- temperature electrode (Sentix 41; WTW standard DIN/NBS buffers PL 4, PL 7 and PL 9) and recorded hourly on a data logger (WTW; pH 340i) throughout the experiment. Accuracy of pH measurement was better than ± 0.01 pH units and ± 0.1 °C for temperature.

2.2 Analytical methods

Cell densities were determined daily as the mean of three consecutive measurements of 500 µl by a Beckman Coulter Counter (Coulter Multisizer III) equipped with a 100 µm aperture. Prior to measurements, samples were diluted 1:100 with 0.2 µm pre-filtered (Minisart, Sartorius) seawater with a salinity of 32. After microscopic evaluation, the equivalent spherical diameter (ESD) of *E. huxleyi* was determined to be within a particle size range of 3.27–7.96 µm ESD. A constant cell density over three consecutive days with a maximum variation of 10 % was accepted as criterion for steady state growth in continuous cultures after Leonardos and Geider (2005).

For nutrients, 50 ml sample were filtered through 0.2 µm (Minisart 2000, Sartorius) syringe filters and frozen at −20 °C until analysis. NO_3^- and PO_4^{3-} were determined spectrophotometrically after Grasshoff (Grasshoff et al., 1999) (Autoanalyzer Evolution 3, Alliance Instruments). Detection limits were 0.3 µmol l^{-1} for N and 0.1 µmol l^{-1} for P.

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$PO^{14}C$ was measured using the ^{14}C incubation method according to Steemann-Nielsen (Steemann Nielsen, 1952) and Gargas (Gargas, 1975). 50 ml sample were taken in triplicates from each chemostat, transferred into cell culture flasks (25 cm^2, Corning) and spiked with approximately 5 µCi $NaHCO_3^-$ (Hartmann Analytics, specific activity 40–60 mCi $mmole^{-1}$). Each triplicate set was incubated for 4 h at the same light and temperature conditions of the original chemostat culture, but without aeration. Simultaneously, dark uptake was determined in triplicate from 50 ml sample wrapped in aluminium foil and incubated in the dark for 4 h. 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. All incubations were stopped by gentle filtration on 0.4 µm polycarbonate filters (Nucleopore) at <150 mbar to avoid cell breakage. After rinsing with 10 ml sterile filtered seawater, filters were treated with fuming HCl in order to remove inorganic ^{14}C. 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 Gargas (Gargas, 1975).

Exuded dissolved organic carbon ($DO^{14}C$) was measured in the 0.4 µm-filtrate obtained during the $PO^{14}C$-filtration. 4 ml liquid sample were acidified with 100 µl 2 M HCl and left for 7 days in an desiccator with an NaOH-trap at ~600 mbar depression to allow for outgassing of inorganic ^{14}C. After the addition of 15 ml liquid scintillation cocktail (Ultima Gold AB), overnight storage, counting and calculations were accomplished as described above.

Primary Production (PP) was derived from the sum of $PO^{14}C$ and $DO^{14}C$. The percentage of extracellular release (PER) was calculated as ($DO^{14}C$/PP) × 100.

The analysis of high molecular weight (>1 kDa) dissolved combined carbohydrates (HMW-dCCHO) and of total combined carbohydrates >1 kDa, i.e. HMW-dCCHO and particulate combined carbohydrates (pCCHO), hereafter referred to as tCCHO, were conducted by ionchromatographie. Duplicate samples for HMW-dCCHO were filtered through 0.45 µm syringe-filters (GHP membrane, Acrodisk, Pall Corporation) and

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stored in combusted (8 h at 500 °C) glass vials at −20 °C, while the tCCHO- samples remained unfiltered and stored identically. Prior to analysis, samples had to be desalinated by membrane dialysis (1 kDa MWCO, Spectra Por) for 6 h at 0 °C. Thereafter, samples were hydrolyzed with HCl at a final concentration of 0.8 M for 20 h at 100 °C to yield monomeric CHO. Samples were stored at −20 °C over night and then neutralized by acid evaporation (N_2) at 50 °C. Dried samples were solubilised in ultra pure water before determination of CHO monomers by high performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) on a Dionex ICS 3000 following Engel and Händel (2011). Separation of fucose (Fuc), rhamnose (Rha), arabinose (Ara)/galactosamine (GalN) (quantified together due to co-elution), glucosamine (GlcN), galactose (Gal), glucose (Glc), mannose (Man)/xylose (Xyl) (quantified together due to co-elution), galacturonic acid (Gal-URA) and glucuronic acid (Glc-URA) was achieved by applying a Dionex CarboPac PA10 guard column (2 × 50 mm) coupled to a Dionex CarboPac PA10 analytical column (2 × 250 mm). Detection limits were 10 nM. Particulate CCHO (pCCHO) were determined by substracting HMW-dCCHO from tCCHO. Concentrations of CCHO are given as mol carbon.

Transparent exopolymer particles (TEP) were determined according to the colorimetric method by (Passow and Alldredge, 1995). Between 10 and 20 ml sample were filtered in triplicate onto 0.4 µm polycarbonate filters (Nuclepore), stained with Alcian Blue and rinsed with several ml of ultrapure water. Filters were stored in polypropylene tubes at −20 °C until analysis. TEP-concentrations are given in units of µg Xanthan-equivalents per liter ($\mu g\, X_{eq}\, l^{-1}$). Conversation to carbon-units (TEP-C) in $\mu mol\, C\ l^{-1}$ was done by applying a factor of $f_0 = 0.63$ as suggested by Engel et al. (2004a). Alcian Blue was shown to stain the surface of *E. huxleyi* coccoliths. Therefore, TEP-C concentrations were corrected for the amount of Alcian Blue per cell as derived from Engel (2004a).

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2.3 Data treatment

During steady state, the total production rates (γ) in a chemostat are given as:

$$\gamma[\mu\text{mol l}^{-1}\,\text{d}^{-1}] = \mu[\text{d}^{-1}] \cdot x[\mu\text{mol l}^{-1}] \quad (1)$$

with μ being the growth rate and x being the concentration of produced compounds.

Cell normalised production rates (γ_{cell}) were derived from

$$\gamma[\text{p mol cell}^{-1}\,\text{d}^{-1}] = \mu[\text{d}^{-1}] \cdot \frac{x[\mu\text{mol l}^{-1}]}{n[\text{l}^{-1}]} \quad (2)$$

with n being the cell density.

Concentrations and cell normalised values for $PO^{14}C$ and $DO^{14}C$ were derived from production rates by rearranging Eqs. (1) and (2).

Lowering the dilution rate from $D = 0.3\,\text{d}^{-1}$ to $D = 0.1\,\text{d}^{-1}$ on day 17 yielded a reduction of the media inflow by ~67 %. Thus, a lowering of production rates (mol $\text{l}^{-1}\,\text{d}^{-1}$) <67 % led to higher concentrations (mol l^{-1}), while changes in rates >67 % yielded lower concentrations (mol l^{-1}).

All cultures reached the steady state between days 10 and 12 of the experiment. Growth of *E. huxleyi* was stimulated by a rise in CO_2 and temperature during the initial nutrient replete batch phase and during the transition to phosphorous limitation (see Borchard et al., 2011 for details). Cell yield differed significantly (t-test, $n = 17$, $p < 0.001$) among cultures ranging from $3.1 \times 10^8 \pm 8.1\%$ cells l^{-1} in the 300-14 culture and maximum $4.2 \times 10^8 \pm 7.5\%$ cells l^{-1} in the 900-18 culture. Since cell densities within each culture remained constant at both dilution rates with a maximum variation of 10.6 % (550-14), growth rates adapted to dilution rates with $D = \mu = 0.3\,\text{d}^{-1}$ and $D = \mu = 0.1\,\text{d}^{-1}$.

Mean values ± standard deviations for each dilution rate were derived from sampling 1, 2 and 3 ($\mu = 0.3\,\text{d}^{-1}$) and 4, 5 and 6 ($\mu = 0.1\,\text{d}^{-1}$), respectively. Differences between cultures were tested by means of analysis of variance (two-way ANOVA), and by means

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of t-tests. Statistical treatment of data was performed by using the software package SigmaPlot 10.01 (SysStat).

3 Results

3.1 Primary production

Primary production (PP) was strongly affected by differences in growth, CO_2 and temperature conditions (Fig. 1, upper panel). PP was significantly higher at $\mu = 0.3\,d^{-1}$ than at $\mu = 0.1\,d^{-1}$ (two way ANOVA, $p < 0.001$). At $\mu = 0.3\,d^{-1}$, PP increased significantly (two way ANOVA, Holm-Sidak-Method, $p < 0.005$) with CO_2 and temperature from $153.6 \pm 8.6\,\mu mol\,C\,l^{-1}\,d^{-1}$ (300-14) to $230.3 \pm 12.9\,\mu mol\,C\,l^{-1}\,d^{-1}$ (900-18). At $\mu = 0.1\,d^{-1}$, PP ranged between $106.3 \pm 33.4\,\mu mol\,C\,l^{-1}\,d^{-1}$ (300-14) and $96.9 \pm 4.9\,\mu mol\,C\,l^{-1}\,d^{-1}$ (900-14) and did not differ between cultures (Fig. 1, upper panel).

Growth, CO_2 and temperature conditions also affected the PP partitioning into $DO^{14}C$ and $PO^{14}C$. At $\mu = 0.3\,d^{-1}$, exudation correlated in all cultures with $PO^{14}C$ production and ranged between $6.02 \pm 2.57\,\mu mol\,C\,l^{-1}\,d^{-1}$ (550-14) and $8.59 \pm 2.85\,\mu mol\,C\,l^{-1}\,d^{-1}$ (900-18) (Fig. 1, middle panel). This led to an average percentage of extracellular release (PER) of $3.74 \pm 0.94\,\%$ at $\mu = 0.3\,d^{-1}$ (Fig. 1, lower panel). Relative to $\mu = 0.3\,d^{-1}$, $PO^{14}C$ production at $\mu = 0.1\,d^{-1}$ was up to 61 % (900-18) lower (two way ANOVA, $p \leq 0.013$). $DO^{14}C$ production increased in all cultures at $\mu = 0.1\,d^{-1}$, ranging between $9.38 \pm 3.29\,\mu mol\,C\,l^{-1}\,d^{-1}$ (550-14) and $17.06 \pm 1.71\,\mu mol\,C\,l^{-1}\,d^{-1}$ (900-18) (Fig. 1, middle panel). This opposite effect on $DO^{14}C$ and $PO^{14}C$ production at $\mu = 0.1\,d^{-1}$ yielded an average PER of $11.00 \pm 4.64\,\%$, significantly higher than at $\mu = 0.3\,d^{-1}$ (two way ANOVA, $p < 0.001$). At $\mu = 0.1\,d^{-1}$, highest values for PER of $16.29 \pm 2.33\,\%$ were observed for 900-18, significantly different from the other cultures (two way ANOVA, Holm-Sidak-Method, $p = 0.004$) (Fig. 1, lower panel).

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3.2 Particulate and dissolved combined carbohydrates

Combined carbohydrate (CCHO) concentration varied significantly due to changed growth, CO_2 and temperature conditions (Two way ANOVA, Holm-Sidak-Method, $p < 0.001$) (Fig. 2, upper panel).

At $\mu = 0.3\,d^{-1}$, tCCHO were significantly affected by CO_2 and temperature conditions, yielding values between 40.7 ± 3.8 (300-14) and $73.0 \pm 8.3\,\mu mol\,C\,l^{-1}$ (900-18) (Fig. 2, upper panel). The CO_2 and temperature induced increase in tCCHO concentration was primarily due to pCCHO that showed a similar trend as tCCHO in a range of 32.4 ± 5.1 (300-14) and $61.6 \pm 6.8\,\mu mol\,C\,l^{-1}$ (900-18) (Table 1). Concentration of tCCHO at 900-18 were significantly higher than at 300-14 and 550-14 (Two way ANOVA, Holm-Sidak-Method, $p = 0.001$ and $p = 0.002$; Table 1). Accordingly, pCCHO in both high CO_2 cultures (900-14 and 900-18) were significantly higher than pCCHO derived at 300-14 and 550-14 (Two way ANOVA, Holm-Sidak-Method, $p \leq 0.009$).

Concentrations of pCCHO and HMW-dCCHO in all cultures were significantly higher at $\mu = 0.1\,d^{-1}$ compared to $\mu = 0.3\,d^{-1}$ (two way ANOVA, $p < 0.001$). Reduction of growth rate yielded the strongest relative increase of pCCHO concentration at 300-14. However, highest absolute concentrations of pCCHO at μ=0.1 d^{-1} were still observed at 900-14 and 900-18 (Table 1) Thus, tCCHO concentrations increased with CO_2 from 87.8 ± 6.1 (550-14) to $104.8 \pm 3.7\,\mu mol\,C\,l^{-1}$ (900-14) (Fig. 2, upper panel).

The higher concentrations ($\mu mol\,l^{-1}$) of pCCHO at $\mu = 0.1\,d^{-1}$ compared to $\mu = 0.3\,d^{-1}$ were most pronounced for Man/Xyl ($p < 0.001$), Fuc ($p < 0.001$), Rha ($p \leq 0.004$, except for 900-18 for which Rha decreased), Ara/GalN ($p < 0.001$), GlcN ($p \leq 0.001$), Gal ($p \leq 0.026$, except for 900-18), Glc ($p \leq 0.001$), and Gal-URA ($p \leq 0.001$). Glc-URA was unaffected by the change of growth rate (Table 2).

HMW-dCCHO at $\mu = 0.3\,d^{-1}$ ranged between 8.3 ± 1.4 (300-14) and $11.5 \pm 1.4\,\mu mol\,C\,l^{-1}$ (900-18) and concentrations increased with increasing CO_2 and temperature (Table 1). Compared to $\mu = 0.3\,d^{-1}$, HMW-dCCHO concentration ($\mu mol\,l^{-1}$) was significantly higher at $\mu = 0.1\,d^{-1}$ (two way ANOVA, $p < 0.001$) and

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now ranged between 25.8 ± 1.4 (300-14) and 19.9 ± 1.5 (900-14). The increase was significant for Fuc ($p < 0.001$), Rha ($p \leq 0.004$), Ara/GalN ($p < 0.001$), GlcN ($p \leq 0.021$, except for 900-18), Gal ($p \leq 0.001$), Glc ($p \leq 0.016$, except for 900-14 and 900-18), Man/Xyl ($p < 0.001$), Gal-URA ($p \leq 0.032$). Glc-URA was unaffected by the change of growth rate (Table 2).

Averaged for all cultures, HMW-dCCHO comprised 18.6 ± 3.4 % of tCCHO with lowest proportions of 15.8 ± 1.2 % for 900-18 (Fig. 2, lower panel). Partitioning of HMW-dCCHO and pCCHO at $\mu = 0.3\,d^{-1}$ clearly shifted stronger to the particulate pool with increasing CO_2 and temperature.

As a consequence of the steep rise in HMW-dCCHO, the ratio of HMW-dCCHO to tCCHO was significantly higher at $\mu = 0.1\,d^{-1}$ (two way ANOVA, $p = 0.005$) than at $\mu = 0.3\,d^{-1}$. Additionally, the ratio derived from 300-14 was significantly higher compared to the high CO_2 cultures (900-14 and 900-18, two way ANOVA, Holm-Sidak method, $p \leq 0.004$). While HMW-dCCHO:tCCHO ratios were clearly higher for 300-14 and 550-14 (27.1 ± 2.9 % and 25.1 ± 1.3 %), they were only slightly increasing for 900-14 (19.3 ± 1.6 %) and 900-18 (20.3 ± 3.4 %) (Fig. 2, lower panel) showing a similar partitioning between HMW-dCCHO and pCCHO at $\mu = 0.1\,d^{-1}$ as determined for $\mu = 0.3\,d^{-1}$.

Cell normalised pCCHO and HMW-dCCHO production was significantly slower at $\mu = 0.1\,d^{-1}$ compared to $\mu = 0.3\,d^{-1}$ (two way ANOVA, Holm-Sidak method, $p < 0.001$, Table 1). At $\mu = 0.3\,d^{-1}$, cell normalised pCCHO production was faster at elevated CO_2 while no difference was determined at $\mu = 0.1\,d^{-1}$. Cell normalised HMW-dCCHO production was most affected by elevated CO_2 and by the combined rise in CO_2 and temperature.

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3.3 Carbohydrate composition

Carbohydrate composition was affected by changes in growth rate and by varied CO_2 and temperature conditions, albeit to a lesser extent (Fig. 3).

The pCCHO were generally dominated by Glc. Averaged for all cultures, Glc comprised 78.9 ± 8.0 mol % of pCCHO at $\mu = 0.3\,d^{-1}$ and 68.1 ± 3.2 mol % at $\mu = 0.1\,d^{-1}$.

The decrease in Glc due to the changed growth rate was significant for all cultures (two way ANOVA, $p < 0.001$). Thus, pCCHO composition was shifted to carbohydrates other than Glc, which was significant for Rha ($p \leq 0.015$, except for 300-14 and 900-18), Ara/GalN ($p \leq 0.022$) and Man/Xyl ($p < 0.001$). At $\mu = 0.3\,d^{-1}$, mol % Rha in 550-14 and 900-14 were significantly higher than in 900-18 ($p \leq 0.003$). Mol % of Gal were unaffected by changed growth rates but in 300-14 generally higher than 900-18 ($p = 0.001$). At $\mu = 0.3\,d^{-1}$, mol % of Glc-URA was significantly higher ($p \leq 0.008$) in 300-14 compared to all other cultures. Composition was shifted to significantly ($p < 0.001$) lower proportions of Glc-URA at $\mu = 0.1\,d^{-1}$, and mol % in 300-14 were now comparable to the other cultures.

At $\mu = 0.3\,d^{-1}$, average percentages of pCCHO other than Glc declined in the following order: Gal-URA> Gal> Rha> Glc-URA> Man/Xyl> Ara/GalN> Fuc> GlcN in all cultures. At $\mu = 0.1\,d^{-1}$, composition of pCCHO was clearly shifted to higher proportions of Man/Xyl, yielding after Glc: Man/Xyl> Gal-URA> Gal> Rha> Ara/GalN> Glc-URA> Fuc> GlcN (Fig. 3).

HMW-dCCHO composition was significantly affected by changes in growth rate ($p < 0.001$) (Fig. 3). From averaged 32.7 ± 8.9 mol % at $\mu = 0.3\,d^{-1}$, Glc was reduced to 24.1 ± 6.6 mol % at $=0.1\,d^{-1}$ ($p < 0.001$). The concomitant increase in mol % for HMW-dCCHO monomers other than Glc was significant for Fuc ($p = 0.002$), Rha ($p = 0.002$, except for 900-18), Ara/GalN ($p \leq 0.006$), Gal ($p \leq 0.014$, except for 300-14), Man/Xyl ($p < 0.001$) and Gal-URA ($p = 0.001$). Glc-URA in all cultures was significantly lower at $\mu = 0.1\,d^{-1}$ ($p \leq 0.015$) except for 900-14. For all cultures, the percentages of sugars comprising HMW-dCCHO

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declined for $\mu = 0.3\,d^{-1}$ as follows: Glc> Ara/GalN> Man/Xyl> Glc-URA> Gal> Gal-URA> Rha> Fuc> GlcN and composition was shifted to the following order at $\mu = 0.1\,d^{-1}$: Man/Xyl> Ara/GalN> Glc> Gal> Gal-URA> Glc-URA> Rha> Fuc> GlcN, suggesting selective removal of Glc-URA. HMW-dCCHO were now dominated by comparable percentages of Man/Xyl, Ara/GalN and Glc with 24.1, 22.1 and 19.8 %, respectively (Fig. 3).

3.4 Transparent Exopolymer Particles (TEP)

Different growth-, CO_2- and temperature conditions induced clear changes in the formation of TEP (Fig. 4). At $\mu = 0.1\,d^{-1}$, TEP-C concentrations in all cultures were significantly higher than at $\mu = 0.3\,d^{-1}$ (two way ANOVA, Holm-Sidak method, $p < 0.001$).

Averaging all samplings, throughout both growth rates for each culture, TEP-C concentrations were 36.9 ± 29.3, 36.1 ± 24.1, 44.3 ± 41.5 and $64.2 \pm 36.4\,\mu mol\,l^{-1}$ for 300-14, 550-14, 900-14 and 900-18, respectively. At each growth rate, TEP-C was significantly higher in 900-18 than in 300-14 and 550-14 ($p < 0.002$). In accordance with total concentrations of TEP-C ($\mu mol\,l^{-1}$), cell normalised TEP-C ($fmol\,cell^{-1}$) was significantly higher at $\mu = 0.1\,d^{-1}$ compared to $\mu = 0.3\,d^{-1}$ in all cultures (Two way ANOVA, Holm-Sidak method, $p < 0.001$). Cell normalised TEP-C was highest in 900-18 at both growth rates, although not statistically different from values derived from the other cultures ($p = 0.314$) (Table 1). Throughout the experiment, pCCHO concentrations significantly correlated with concentrations of TEP-C (Fig. 5) (Pearson Product Moment Correlation; $n = 24$, $r^2 = 0.826$, $p < 0.001$).

The decrease in TEP-C formation ($\mu mol\,l^{-1}\,d^{-1}$) was smaller than the decrease in $PO^{14}C$ production ($\mu mol\,l^{-1}\,d^{-1}$) after changing μ from $0.3\,d^{-1}$ to $0.1\,d^{-1}$ leading to generally higher TEP-C:$PO^{14}C$ at $\mu = 0.1\,d^{-1}$. Averaging for all cultures, TEP-C comprised 2.7 ± 1.4 % of $PO^{14}C$ at $\mu = 0.3\,d^{-1}$ and increased significantly to 8.1 ± 2.7 % at $\mu = 0.1\,d^{-1}$ (two way ANOVA, Holm-Sidak method, $p < 0.001$) (Fig. 6).

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At both growth rates, pCCHO:PO^{14}C ranged between 6.0 % and 9.6 % and values increased slightly after changing μ from 0.3 d^{-1} to 0.1 d^{-1} (Table 1). Ratios of pCCHO:PO^{14}C were higher at elevated CO_2 (900-14 and 900-18) at both growth rates.

Ratios of HMW-dCCHO:DO^{14}C were significantly lower at $\mu = 0.1\,d^{-1}$ (23.2 ± 10.0 %) than at $\mu = 0.3\,d^{-1}$ (40.5 ± 10.5%) (two way ANOVA, Holm-Sidak method, $p = 0.002$). At $\mu = 0.3\,d^{-1}$, HMW-dCCHO:DO^{14}C was unaffected by different CO_2 and temperature conditions ($p = 0.664$), and varied between 35.0 % and 43.2 %. At $\mu = 0.1\,d^{-1}$, HMW-dCCHO:DO^{14}C in 900-18 was lowest with 11.9 ± 0.5 % (Fig. 6, Table 1). Thus, high rates of DO^{14}C production observed for $\mu = 0.1\,d^{-1}$ were not reflected in HMW-dCCHO concentration. However, low HMW-dCCHO:DO^{14}C ratios determined for 900-18 were accompanied by highest TEP-C formation and highest ratios of TEP-C:PO^{14}C, suggesting a fast partitioning from DOC to POC at greenhouse conditions (Fig. 6).

4 Discussion

4.1 Particulate and dissolved primary production

While growth of *E. huxleyi* adapted to the nutrient supply (N and P, described in detail with the full record of data in Borchard et al., 2011), primary production, and, therefore the basis for carbon metabolism was clearly decoupled from nutrient assimilation in the present experiment. Primary production (PP) provides dissolved and particulate organic carbon in highly variable ratios, and percentages of extracellular release (PER), depend strongly on environmental conditions that affect the cell's physiology (Myklestad and Haug, 1972; Zlotnik and Dubinsky, 1989; Chen and Wangersky, 1996; Penna et al., 1999; Staats et al., 2000; Wetz and Wheeler, 2003; Magaletti et al., 2004).

During this study, PP of *E. huxleyi* was highest in the 900-18 culture at $\mu = 0.3\,d^{-1}$ (Fig. 1, upper panel), showing a stimulation of PP by the combined rise of CO_2 and temperature. This is in accordance with earlier findings on the effect of elevated CO_2

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(Riebesell et al., 2000) or temperature (Langer et al., 2007) on *E. Huxleyi*. If nutrients are abundant, a linear relationship between PP and extracellular release (ER) is generally assumed (Zlotnik and Dubinsky, 1989; Baines and Pace, 1991; Verity et al., 1991). Nutrient limitation, however, was regularly shown to decouple PP and ER, resulting in higher PER due to a strong relative increase in ER (Myklestad, 1977; Obernosterer and Herndl, 1995; Staats et al., 2000; Lopez-Sandoval et al., 2011). The present experiment was designed to induce enhanced exudation by applying phosphorous limitation at two low growth rates. In agreement with earlier findings, higher PER was determined for conditions of enhanced nutrient stress after changing μ from 0.3 d^{-1} to 0.1 d^{-1}. A direct relationship between PER and growth rate was questioned (Williams, 1990), primarily following a chemostat study with *Thalassiosira pseudonana* (Smith and Platt, 1984) exhibiting very low PER varying in a range of 1.2–2.7 % at growth rates between 0.29 and 2.50 d^{-1}. Data obtained here, however, showed a strong impact of growth rate on PER, as obvious in the pronounced increase of PER from averaged 3.7 % at $\mu = 0.3\,d^{-1}$ to 11.0 % at $\mu = 0.1\,d^{-1}$ (Fig. 1, lower panel).

The effect of elevated CO_2 and temperature on phytoplankton exudation has yet not been directly tested, but ER of organic molecules was hypothesised to be stimulated by either elevated CO_2 (Engel, 2002) or by elevated temperature (Moran et al., 2006; Engel et al., 2010). In the present study, both, PP and ER showed an increasing trend with elevated CO_2 and temperature at a growth rate of $\mu = 0.3\,d^{-1}$ (Fig. 1), and the coupling of PP and ER at $\mu = 0.3\,d^{-1}$ thus yields comparable PER in all cultures.

At the lowered growth rate ($\mu = 0.1\,d^{-1}$), our experiment revealed organic carbon production to be shifted even stronger to the dissolved pool at greenhouse conditions, yielding highest PER of 16.3 % (Fig. 1, lower panel). While the stimulating effect of elevated CO_2 and temperature on POC production was diminished at $\mu = 0.1\,d^{-1}$, DOC production is promoted (Fig. 1, middle panel). Findings obtained from both growth rates thus clearly support the suggested stimulating effect of CO_2 and temperature on ER.

At severe nutrient stress, ($\mu = 0.1\,d^{-1}$), the enhancement of ER is most likely of greater importance for the carbon partitioning due to the enrichment in DOC, while

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POC production may be hampered at the same time. For the future ocean, this implies a potential decoupling of growth, POC production and DOC production. While growth might be slowed down due to lower nutrient availability at the sea surface (Rost and Riebesell, 2004; Sarmiento et al., 2004), primary production may not be limited to the same extend (Banse, 1994). Carbon overconsumption (Sambrotto et al., 1993; Toggweiler, 1993) was proposed as a possible consequence of excess carbon assimilation (relative to nutrients) and potentially results in a higher proportion of organic carbon to be released from the cell as DOC. Data presented here are derived from rate measurements and clearly show a stronger partitioning of PP to the dissolved pool at future ocean conditions.

In natural systems, freshly produced DOC is readily taken up by heterotrophic bacteria (Azam et al., 1983, Martin et al., 1987), and elevated temperature as well as decreased pH have been shown to accelerate enzymatic reactions and to enhance bacterial degradation in marine systems (Piontek et al., 2009, 2010). On the other hand, phytoplankton exudates derived under phosphorous limitation may not be efficiently utilised by heterotrophic bacteria. This was either argued by a severe slowing down of bacterial metabolism due to limiting inorganic and organic phosphorus concentrations (Obernosterer and Herndl, 1995), or by the recalcitrant characteristics of DOC released from phytoplankton under phosphorous limitation (Puddu et al., 2003). A loss of DOC due to heterotrophic activity might thus be minimised and carbon overconsumption and excess exudation may result in higher concentrations of DOC in the future ocean.

4.2 Carbohydrates and linkages to TEP-formation

Compared to $\mu = 0.3\,d^{-1}$, concentrations of HMW-dCCHO, pCCHO and TEP-C in all cultures were higher at $\mu = 0.1\,d^{-1}$. Since cell densities remained constant throughout the experiment, D and μ changed to an identical extend (for details see Borchard et al., 2011). Specific production rates also reached physiological steady states for

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each D, albeit, decoupled from growth rates, resulting in different yields for $\mu = 0.3\,d^{-1}$ and $\mu = 0.1\,d^{-1}$, respectively. Relative to the growth rate, specific production of carbohydrate-rich compounds, was generally higher at $\mu = 0.1\,d^{-1}$, resulting in higher concentrations of all carbon compounds. The different yields of organic carbon in form of carbohydrates and TEP-C were thus not related to the abundance of *E. huxleyi*, but clearly due to changed specific production.

A stronger partitioning of PP to the dissolved pool may reduce carbon export in the future ocean, if DOC is not transferred to the particulate pool via aggregation processes (Kim et al., 2011). Phytoplankton exudation, however, does not only increase DOC concentration, but also provides the pre-cursors for the abiotic formation of transparent exopolymer particles (TEP) (Passow, 2000). The abiotic formation of particles from organic molecules was proposed to be a major mechanism for a loss of dissolved carbohydrates in the ocean (Skoog and Benner, 1997; Skoog et al., 2008; Gogou and Repeta, 2010) and was also observed during this study.

PER in all cultures was higher at $\mu = 0.1\,d^{-1}$ compared to $\mu = 0.3\,d^{-1}$ and highest exudation was clearly determined for the culture grown at elevated CO_2 and temperature (Fig. 1). For the present study, the steep rise in PER, as a consequence of changed μ, or of CO_2 and temperature conditions, was not mirrored in the ratios of HMW-dCCHO:DO^{14}C and HMW-dCCHO:tCCHO but in TEP-C:PO^{14}C (Figs. 2 and 6). This indicates a fast partitioning from HMW-dCCHO to pCCHO as a characteristic fraction of TEP. Compared to all other cultures, however, DOC concentrations in the greenhouse culture in form of HMW-dCCHO were similar, and HMW-dCCHO:tCCHO ratios even lowest in the 900-18 culture (Fig. 2). The transfer of HMW-dCCHO (low HMW-dCCHO:DO^{14}C) and the concomitant relative increase in pCCHO (low HMW-dCCHO:pCCHO) was accompanied by an increase in TEP-C (high TEP-C:PO^{14}C) and was most pronounced at greenhouse conditions (Figs. 4 and 6). Results obtained during this study therefore strongly support the idea of a direct pathway between DOC in form of HMW-dCCHO and POC by physical aggregation, as suggested previously (Chin et al., 1998; Wells, 1998; Engel et al., 2004b; Verdugo et al., 2004, 2008; Ding et

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al., 2009) and shows, that this pathway will be of greater importance under conditions of a simultaneous increase in CO_2 and temperature. The idea of a direct transfer of HMW-dCCHO to TEP is supported by a significant correlation between pCCHO and TEP-C concentration ($r^2 = 0.826$, $p < 0.001$) (Fig. 5).

Lowering the growth rate from $\mu = 0.3$ to $0.1\,d^{-1}$ generally led to a shift in the composition in HMW-dCCHO and pCCHO (Fig. 3). For pCCHO, a particular increase for Man/Xyl was determined and Man/Xyl became the most abundant component of pCCHO after Glc. Compared to $\mu = 0.3\,d^{-1}$, HMW-dCCHO composition at $\mu = 0.1\,d^{-1}$ was dominated by comparable percentages of Man/Xyl, Ara, GalN and Glc and revealed a selective removal of dissolved Glc-URA, accompanied by high Glc-URA in pCCHO and an increase in TEP (Figs. 3 and 4). Throughout the experiment for all cultures, concentration of particulate Gal-URA an acidic sugar which is typical for *E. huxleyi* (Nanninga et al., 1996) correlated significantly with concentrations of TEP-C ($r^2 = 0.827$, $p < 0.001$). Elevated CO_2 and temperature, however, had only a minor impact on the general composition of HMW-dCCHO and pCCHO (Fig. 3).

Thus, low HMW-dCCHO:pCCHO despite the high ER, can be explained by a fast transfer from neutral and acidic HMW-dCCHO to higher proportions of neutral and acidic pCCHO as a characteristic fraction of TEP.

Data obtained here thus show coherence between enhanced exudation of DOC and higher concentrations of pCCHO and TEP-C. A significant proportion of DOC (up to 90 %) derived from phytoplankton exudation was shown to be comprised by carbohydrates (Myklestad, 1995). Here, HMW-dCCHO comprised 12 to 43 % of freshly produced DOC (Fig. 6) and the comparably low proportions indicate a quick transfer of HMW-dCCHO to POC due to aggregation processes in accordance to the model proposed by Engel et al. (2004b) (Figs. 2 and 6).

Earlier studies with *E. huxleyi* reported that TEP-C accounted for 63 % of POC (Engel et al., 2004a) during a mesocosm experiment. In the Bay of Biscay, Harley et al. (2009) observed a 12 % contribution of TEP-C to POC during a bloom of coccolithophores, dominated by *E. huxleyi*. During this study, TEP-C explained on average

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3 % at $\mu = 0.3\,d^{-1}$ and 8 % at $\mu = 0.1\,d^{-1}$ of POC with highest percentages of TEP-C observed in the 900-18 culture.

Thus, compared to field and mesocosm systems, the contribution of TEP-C to POC was relatively low during this chemostat experiment. This suggests that physiological non-steady state situations, such as occurring naturally during bloom situations, are of more relevance to carbon exudation than steady-state growth. Moreover, loss processes of TEP, e.g. bacterial degradation, microzooplankton grazing, and sinking may occur in the field at even lower rates than in our continuous flow system. Clearly, more information on loss processes of TEP in marine systems is needed to evaluate the role of TEP for carbon partitioning in the future ocean.

5 Conclusions

E. huxleyi is one of the most abundant coccolithophore in the global ocean and despite its small size an important contributor to marine primary production. The experimental design applied here, mimics the oligotrophic situations favoring carbon overconsumption (Borchard et al., 2011) and exudation. In the present study, growth rates had the strongest impact on organic carbon partitioning, relative to the influence of CO_2 and temperature. DOC and POC production will therefore potentially vary strongly in the ocean as a consequence of changed nutrient or growth conditions, either regional or during the succession of a bloom. Our data imply that situations favourable for the partitioning of organic carbon to the dissolved pool, might additionally promote DOC production in the greenhouse ocean. Despite the fact, that DOC itself does not sink, an enhanced DOC pool most likely provides the highest concentrations of pre-cursor material for aggregation processes in form of HMW-dCCHO. Although only slightly affected, the composition of HMW-dCCHO might be relevant for an efficient transfer of HMW-dCCHO to the particulate pool. However, TEP-formation in the greenhouse ocean may be primarily contributed to the higher concentration of pre-cursor material due to higher PER.

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The importance of TEP as part of the POC pool is well acknowledged while assessing organic carbon fluxes (Engel and Passow, 2001; Mari et al., 2001; Engel et al., 2004b; Arrigo, 2007; De La Rocha and Passow, 2007; Schartau et al., 2007; Verdugo et al., 2008). A quantitative and qualitative connection between DOC production and TEP-C formation as shown in the present study was proposed earlier (Jackson and Burd, 1998; Engel et al., 2004b). However, timing of the biological process of extracellular release and the abiotic formation of particles may potentially be decoupled. Low HMW-dCCHO:tCCHO, despite high exudation rates may thus point to a fast aggregation of DOC into TEP. Since pre-cursers in form of dissolved CCHO are effectively reduced in the greenhouse culture relative to the other cultures, it can be assumed, that TEP-formation occurs faster at elevated temperature most likely due to physical and chemical properties (McCave, 1984; Claquin et al., 2008). However, the quantitative importance of TEP fuelling the POC pool in the future ocean needs further consideration of the multiple factors and processes of occurring in natural ecosystems.

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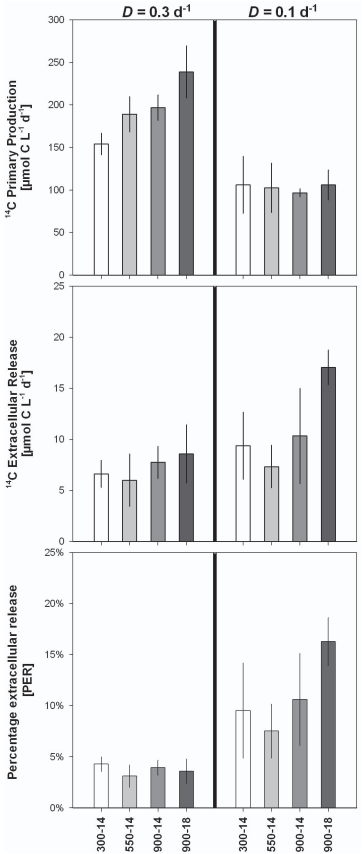

Fig. 1. Primary Production (upper panel), extracellular release (middle panel) and percentage of extracellular release (PER, lower panel) at various growth-, CO_2 and temperature conditions. Mean values and standard deviations were derived from sampling 1, 2 and 3 for $\mu = 0.3\,d^{-1}$ and sampling 4, 5 and 6 for $\mu = 0.1\,d^{-1}$.

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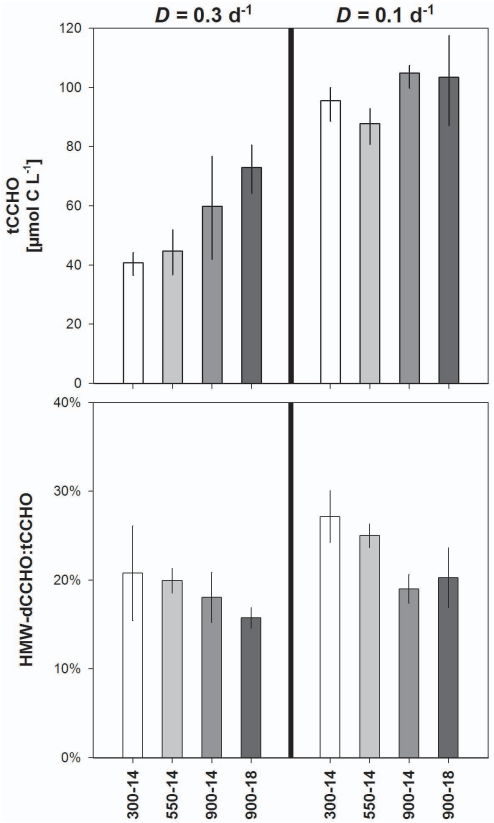

Fig. 2. Total combined carbohydrates (tCCHO, upper panel) determined during this study, and contribution of HMW-dCCHO: tCCHO (lower panel) at various growth-, CO_2 and temperature conditions. Mean values and standard deviations were derived from sampling 1, 2 and 3 for $\mu = 0.3\,d^{-1}$ and sampling 4, 5 and 6 for $\mu = 0.1\,d^{-1}$.

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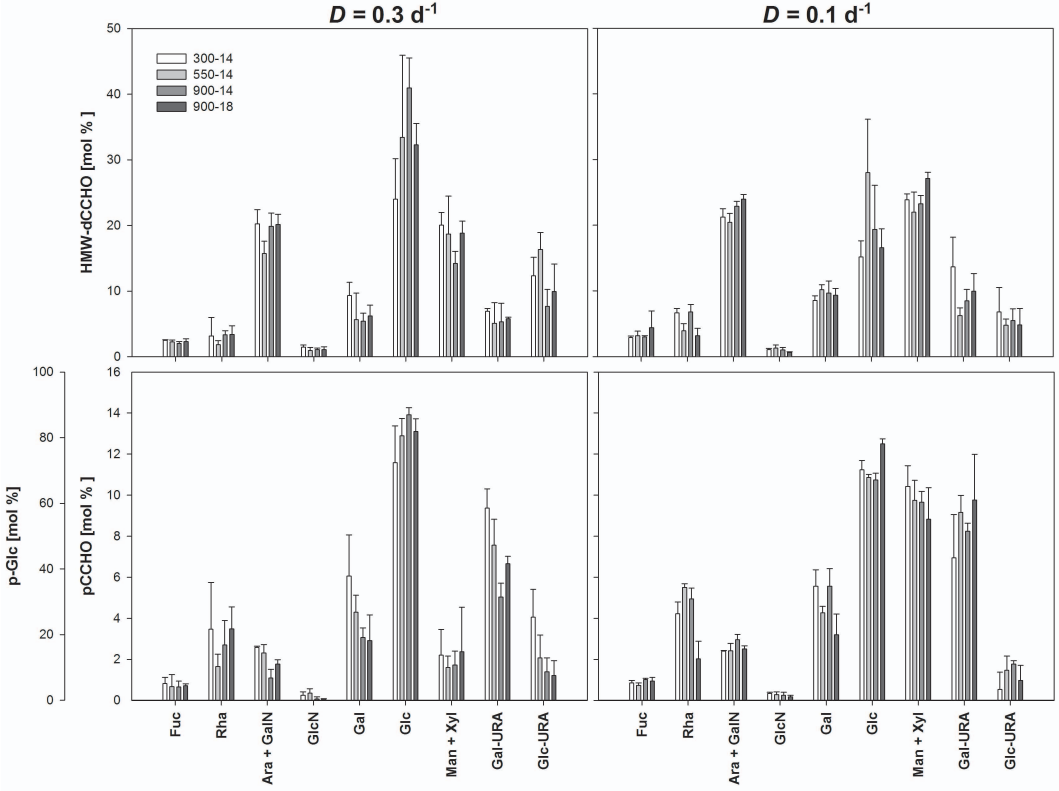

Fig. 3. Composition of HMW-dCCHO, (upper panels) and pCCHO, (lower panels) at various growth, CO_2 and temperature conditions. Mean values and standard deviations are given as mol % for sampling 1, 2 and 3 for $\mu = 0.3\,d^{-1}$ and for 4, 5 and 6 for $\mu = 0.1\,d^{-1}$, respectively.

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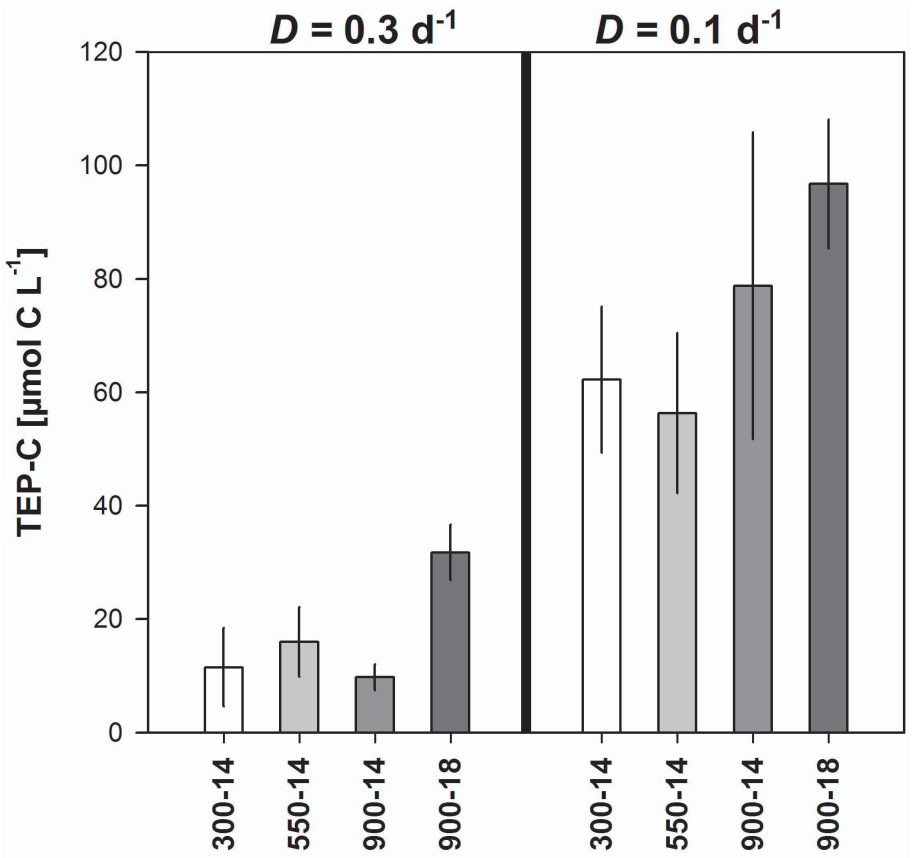

Fig. 4. Concentrations of transparent exopolymer particles (TEP-C) (µmol C l^{-1}) at various growth-, CO_2 and temperature conditions. Mean values and standard deviations are given for sampling 1, 2 and 3 for $\mu = 0.3\,d^{-1}$ and sampling 4, 5 and 6 for $\mu = 0.1\,d^{-1}$.

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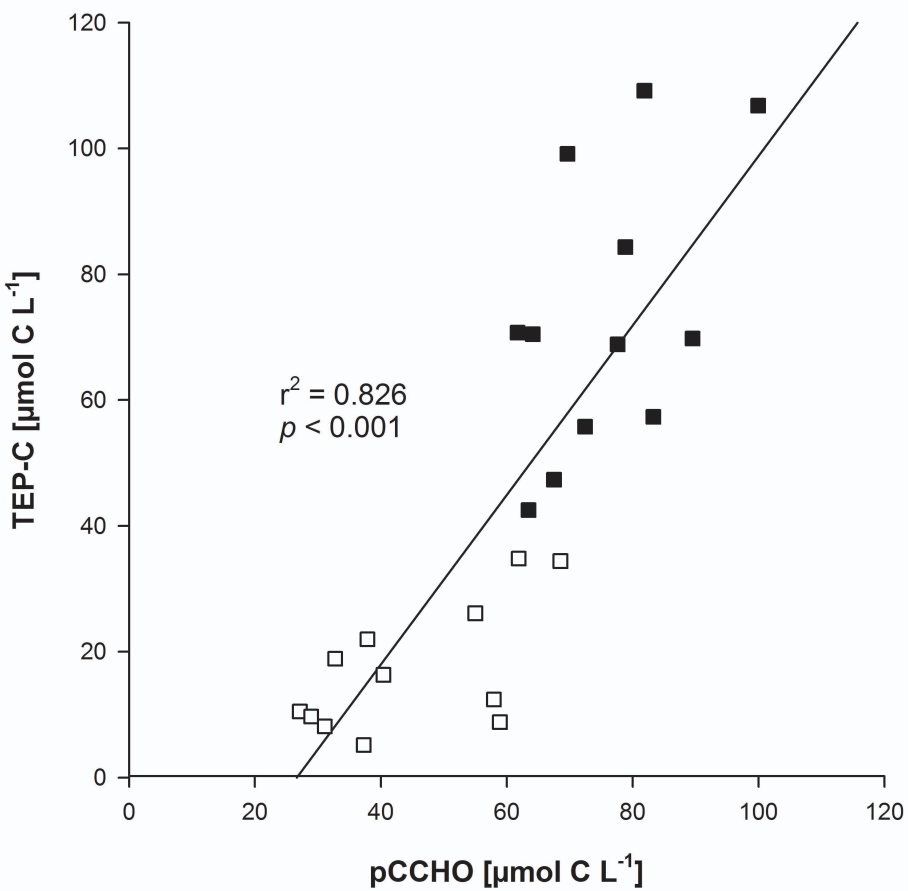

Fig. 5. Correlation between the concentration of pCCHO and TEP-C (Pearson Product Moment Correlation; $n = 24$, $r^2 = 0.826$, $p < 0.001$). Open and closed symbols represent data obtained at $\mu = 0.3\,d^{-1}$ and $\mu = 0.1\,d^{-1}$, respectively.

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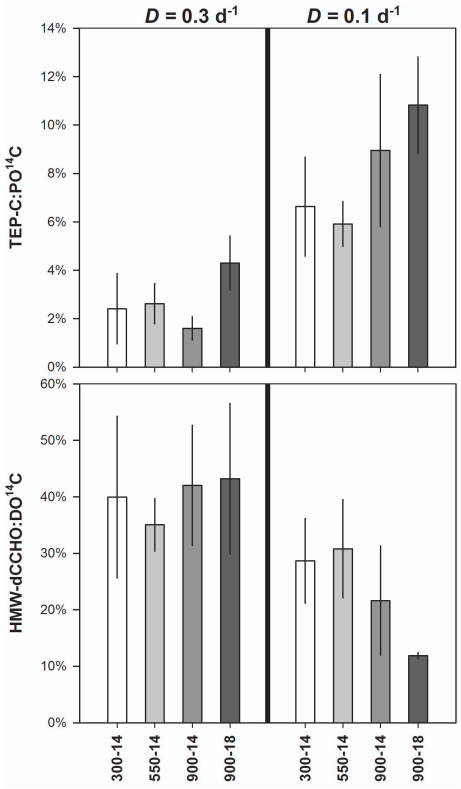

Fig. 6. Carbon partitioning. Contribution of TEP-C: POC as estimated from ^{14}C measurements ($PO^{14}C$) (upper panel) and of HMW-dCCHO: DOC as estimated from ^{14}C measurements ($DO^{14}C$) at various growth-, CO_2 and temperature conditions. Mean values and standard deviations were derived from sampling 1, 2 and 3 for $\mu = 0.3\,d^{-1}$ and sampling 4, 5 and 6 for $\mu = 0.1\,d^{-1}$.

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Table 1. Total concentrations and cell normalised production of particulate (pCCHO) and high molecular weight dissolved combined carbohydrates (HMW-dCCHO), particulate ($PO^{14}C$) and dissolved ($DO^{14}C$) organic carbon at various CO_2, temperature and growth conditions. Cell normalised concentrations for TEP-C, and ratios of pCCHO:$PO^{14}C$ and HMW-dCCHO:$DO^{14}C$ (mol % C) are given. Mean values and standard deviations (in parentheses) are given for sampling 1, 2 and 3 for $\mu = 0.3\,d^{-1}$ and for sampling 4, 5 and 6 for $\mu = 0.1\,d^{-1}$, respectively.

μ [d^{-1}]	Culture	pCCHO [μmol C l^{-1}]	HMW-dCCHO [μmol C l^{-1}]	pCCHO [fmol C cell^{-1} d^{-1}]	HMW-dCCHO [fmol C cell^{-1} d^{-1}]	$PO^{14}C$ [fmol C cell^{-1} d^{-1}]	$DO^{14}C$ [fmol C cell^{-1} d^{-1}]	TEP-C [fmol C cell^{-1}]	pCCHO:$PO^{14}C$ [mol % C]	HMW-dCCHO:$DO^{14}C$ [mol % C]
0.3	300-14	32.4 (5.1)	8.3 (1.4)	37.9 (3.7)	10.2 (4.0)	573 (84)	25.3 (2.1)	44.4 (23.6)	6.7 (0.5)	40.0 (14.3)
	550-14	35.8 (6.0)	8.9 (1.8)	36.5 (3.9)	9.1.8 (0.6)	629 (159)	21.1 (10.7)	51.7 (9.8)	6.0 (1.1)	35.0 (4.7)
	900-14	49.3 (15.8)	10.5 (2.0)	47.0 (4.4)	10.3 (1.0)	639 (235)	25.8 (8.6)	31.8 (7.2)	8.1 (3.0)	42.0 (10.6)
	900-18	61.6 (6.8)	11.5 (1.4)	45.8 (3.7)	8.5 (0.4)	566 (116)	20.8 (6.0)	77.1 (6.9)	8.3 (1.9)	43.2 (13.3)
0.1	300-14	69.8 (7.0)	25.8 (1.4)	22.0 (1.6)	8.2 (0.8)	309 (92)	30.3 (10.4)	199.3 (31.7)	7.4 (1.7)	28.7 (7.5)
	550-14	65.9 (5.7)	21.9 (0.3)	22.3 (2.3)	7.4 (0.3)	333 (112)	25.6 (8.2)	196.7 (58.4)	7.2 (2.4)	30.8 (8.7)
	900-14	84.9 (4.1)	19.9 (1.5)	24.5 (4.1)	5.7 (0.7)	253 (16)	30.5 (13.7)	229.9 (74.1)	9.6 (1.0)	21.6 (9.7)
	900-18	82.8 (15.4)	20.7 (2.1)	20.2 (3.5)	5.1 (0.9)	223 (39)	42.8 (6.9)	240.5 (14.4)	9.1 (0.1)	11.9 (0.5)

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Table 2. Particulate (pCCHO) and high molecular weight dissolved combined carbohydrates (HMW-dCCHO) at various growth-, CO_2 and temperature conditions. Mean values and standard deviations (in parentheses) are given for sampling 1, 2 and 3 for $\mu = 0.3\,d^{-1}$ and 4, 5 and 6 for $\mu = 0.1\,d^{-1}$, respectively.

μ [d^{-1}]	Sample	Culture	Fuc [µmol C l^{-1}]		Rha [µmol C l^{-1}]		Ara/GalN [µmol C l^{-1}]		GlcN [µmol C l^{-1}]		Gal [µmol C l^{-1}]		Glc [µmol C l^{-1}]		Man/Xyl [µmol C l^{-1}]		Gal-URA [µmol C l^{-1}]		Glc-URA [µmol C l^{-1}]	
0.3	pCCHO	300-14	0.271	(0.108)	1.070	(0.491)	0.772	(0.095)	0.078	(0.034)	1.894	(0.574)	23.72	(7.16)	0.592	(0.584)	2.704	(0.655)	1.315	(0.381)
		550-14	0.264	(0.218)	0.588	(0.224)	0.801	(0.409)	0.137	(0.090)	1.580	(0.493)	28.52	(3.79)	0.498	(0.308)	2.581	(1.101)	0.786	(0.463)
		900-14	0.346	(0.215)	1.460	(0.896)	0.904	(0.008)	0.042	(0.052)	1.540	(0.601)	41.58	(12.37)	0.914	(0.742)	2.240	(0.618)	0.620	(0.060)
		900-18	0.444	(0.103)	2.210	(0.811)	1.023	(0.367)	0.044	(0.011)	1.855	(0.951)	49.86	(2.66)	2.832	(1.601)	3.779	(0.451)	0.720	(0.366)
0.1		300-14	0.603	(0.139)	2.995	(0.488)	1.560	(0.200)	0.236	(0.021)	3.968	(0.854)	48.62	(2.68)	6.843	(1.838)	4.557	(1.683)	0.587	(0.547)
		550-14	0.495	(0.122)	3.698	(0.428)	1.503	(0.568)	0.193	(0.097)	2.867	(0.350)	44.52	(3.25)	6.006	(1.256)	5.616	(0.601)	0.991	(0.439)
		900-14	0.878	(0.062)	4.270	(0.513)	2.330	(0.340)	0.214	(0.103)	4.809	(0.702)	56.74	(4.32)	7.634	(0.373)	6.512	(0.163)	1.523	(0.126)
		900-18	0.781	(0.036)	1.625	(0.387)	1.956	(0.578)	0.155	(0.081)	2.593	(0.343)	60.17	(9.57)	7.050	(3.501)	7.695	(2.869)	0.803	(0.543)
0.3	HMW-dCCHO	300-14	0.218	(0.048)	0.296	(0.261)	1.579	(0.080)	0.129	(0.050)	0.824	(0.296)	2.066	(0.425)	1.599	(0.331)	0.553	(0.137)	1.066	(0.200)
		550-14	0.206	(0.027)	0.167	(0.045)	1.368	(0.494)	0.082	(0.030)	0.565	(0.367)	2.929	(0.461)	1.686	(0.965)	0.460	(0.277)	1.471	(0.129)
		900-14	0.222	(0.048)	0.371	(0.116)	2.009	(0.669)	0.114	(0.008)	0.601	(0.204)	4.428	(0.928)	1.379	(0.129)	0.543	(0.278)	0.808	(0.125)
		900-18	0.278	(0.052)	0.406	(0.129)	2.211	(0.464)	0.134	(0.053)	0.750	(0.229)	3.862	(0.680)	2.049	(0.365)	0.631	(0.086)	1.155	(0.296)
0.1		300-14	0.790	(0.012)	1.803	(0.056)	5.280	(0.324)	0.294	(0.014)	2.321	(0.084)	4.142	(0.766)	5.943	(0.222)	3.405	(0.943)	1.856	(0.802)
		550-14	0.723	(0.105)	0.899	(0.152)	4.273	(0.387)	0.290	(0.079)	2.322	(0.079)	6.402	(1.667)	4.604	(0.839)	1.299	(0.159)	1.088	(0.182)
		900-14	0.620	(0.027)	1.414	(0.200)	4.374	(0.263)	0.217	(0.054)	2.027	(0.349)	4.082	(1.404)	4.438	(0.059)	1.613	(0.128)	1.132	(0.193)
		900-18	0.941	(0.346)	0.702	(0.211)	4.776	(0.233)	0.144	(0.016)	2.026	(0.146)	3.588	(0.145)	5.436	(0.775)	2.020	(0.646)	1.070	(0.481)

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