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Ocean-related global change alters lipid biomarker production in common marine phytoplankton

Rong Bi[1,2,3], Stefanie M. H. Ismar-Rebitz[3], Ulrich Sommer[3], Hailong Zhang[1,2], Meixun Zhao[1,2*]

[1]Frontiers Science Center for Deep Ocean Multispheres and Earth System, and Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao 266100, China
[2]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China
[3]Marine Ecology, GEOMAR Helmholtz Center for Ocean Research, Kiel, 24105, Germany

Correspondence to: Meixun Zhao (maxzhao@ouc.edu.cn, maxzhao04@yahoo.com)

Abstract. Global change concurrently alters multiple environmental factors, with uncertain consequences for marine ecosystems. Lipids, in their function as trophic markers in food webs and organic matter source indicators in water column and sediments, provide a tool for reconstructing the complexity of global change effects. It remains unclear how ongoing changes in multiple environmental drivers affect the production of key lipid biomarkers in marine phytoplankton. Here, we tested the responses of sterols, alkenones and fatty acids (FAs) in the diatom *Phaeodactylum tricornutum*, the cryptophyte *Rhodomonas* sp. and the haptophyte *Emiliania huxleyi* under a full-factorial combination of three temperatures (12, 18 and 24°C), three N:P supply ratios (molar ratios 10:1, 24:1 and 63:1) and two pCO_2 levels (560 and 2400 µatm) in semi-continuous culturing experiments. Overall, N and P deficiency had a stronger effect on per-cell contents of sterols, alkenones and FAs than warming and enhanced pCO_2. Specifically, P deficiency caused an overall increase in biomarker production in most cases, while N deficiency, warming and high pCO_2 caused non-systematic changes. Under future ocean scenarios, we predict an overall decrease in carbon-normalized contents of sterols and polyunsaturated fatty acids (PUFAs) in *E. huxleyi* and *P. tricornutum*, and a decrease in sterols but an increase in PUFAs in *Rhodomonas* sp. Variable contents of lipid biomarkers indicate a diverse carbon allocation between marine phytoplankton species in response to changing environments. Thus, it is necessary to consider the changes in key lipids and their consequences for food web dynamics and biogeochemical cycles, when predicting the influence of global change on marine ecosystems.

1 Introduction

Ocean phytoplankton has profoundly responded to and driven natural climatic variability throughout Earth's history (Riding, 1992;Falkowski, 2015;Falkowski and Oliver, 2007). In the contemporary ocean, human-induced physical and chemical modifications are complex and concurrent, including warming, acidification, deoxygenation, and changes in nutrient availability (Doney et al., 2012;Moore et al., 2013;DeVries et al., 2017). The ocean-related global change fundamentally affects marine ecosystems (Hoegh-Guldberg and Bruno, 2010). These include especially global

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

phytoplankton biomass decreases (Boyce et al., 2010;Lotze et al., 2019;Moore et al., 2018) and plankton communities changes (Jonkers et al., 2019;Richardson and Schoeman, 2004), which consequently alters food-web dynamics (Kortsch et al., 2015;du Pontavice et al., 2020) and biogeochemical cycles (Doney et al., 2012;Hofmann and Schellnhuber, 2009;Gruber, 2011). A major challenge is the lack of a better understanding of the complexity of biological impacts of global change, which has hindered the prediction of potential feedbacks between marine ecosystems and projected environmental changes. Some of phytoplankton-produced biomolecules (biomarkers), functioning as indicators of nutritional food quality (Müller-Navarra, 2008) and tracers of organic matter sources (Volkman et al., 1998), have provided crucial insight into the trajectory of ecological responses to changing environment along food webs in the present-day ocean (Ruess and Müller-Navarra, 2019), and over geological time (Brocks et al., 2017).

Lipids are amongst the most important and widely used biomarkers, because they have far-reaching biochemical and physiological roles in cells and are sensitive to environmental changes (Arts et al., 2009), but also because of their dominance in the geological record as fossil molecules to reveal life's signatures on Earth (Falkowski and Freeman, 2014). There are also growing applications of lipids as proxies for global climate and marine ecosystem change. Of all biomarkers, fatty acids (FAs), the basic constituents of most algal lipids, have received the most intense attention. Polyunsaturated fatty acids (PUFAs) are essential for many animals and have been applied as nutritional components to study trophic interactions (Kelly and Scheibling, 2012;Dalsgaard et al., 2003;Ruess and Müller-Navarra, 2019;Müller-Navarra et al., 2000;Brett and Müller-Navarra, 1997). The impact of environmental changes on phytoplankton FAs has been well studied, mostly with a focus on the effects of temperature and nutrient changes (reviewed by Guschina and Harwood, 2009;Hixson and Arts, 2016;Galloway and Winder, 2015), while the interplay between different environmental drivers has been recently tested (Bi et al., 2017, 2018;Bermúdez et al., 2015). However, determining how phytoplankton lipids respond to global change still faces substantial challenges, partly because data on other important lipid classes such as sterols and alkenones are scarce. Understanding the impact of environmental change on these lipid classes is critical to achieve a better application of lipid biomarkers to contemporary issues and to the past record of marine ecosystems.

Sterols are tetracyclic triterpenoids present in all eukaryotes (Volkman, 2016, 2003). Sterols function primarily as structural components of plasma membranes, but also play key roles in cellular defense against toxic compounds and signal transduction, and serve as precursors to several important compounds (e.g., steroid hormones in animals) involved in cellular and developmental processes (Hartmann, 1998;Fabris et al., 2012;Guschina and Harwood, 2009). In ecology, sterols have been used as indicators of dietary nutritional quality, because some invertebrates are incapable of synthesizing sterols *de novo* and thus must obtain sterols from their diets (reviewed by Martin-Creuzburg and von Elert, 2009b). In geochemistry, sterols such as 24-methylcholesta-5,22E-dien-3β-ol (brassicasterol/epi-brassicasterol) and 4α,23,24-trimethylcholest-22E-en-3β-ol (dinosterol) have been applied to reconstruct diatom and dinoflagellate production and community structure on historical and geological timescales (Schubert et al., 1998;Volkman, 1986;Zimmerman and Canuel, 2002;Xing et al., 2016). Given the multiple biochemical roles and source specificity of sterols, their composition and biosynthetic pathways in

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020


Biogeosciences Discussions Open Access EGU

phytoplankton have been identified in different phyla (Fabris et al., 2014;Villanueva et al., 2014;Volkman, 2016). It has been observed that sterol contents (per carbon contents or percentage of total sterols) in phytoplankton changed with temperatures (Véron et al., 1996;Piepho et al., 2012;Ding et al., 2019), pCO_2 (Gordillo et al., 1998;Riebesell et al., 2000), nutrient availability (Piepho et al., 2010;Gordillo et al., 1998;Chen et al., 2011), and UV-C radiation (Ahmed and Schenk, 2017). However, the impacts of multiple environmental drivers on phytoplankton sterol contents have not been thoroughly investigated.

Long-chain alkenones are major lipids produced only by certain species of haptophytes, e.g., oceanic species *Emiliania huxleyi* and *Gephyrocapsa oceanica* (Conte et al., 1995;Volkman et al., 1980c) and coastal species *Isochrysis galbana* (reviewed by Conte et al., 1994). Alkenones in *E. huxleyi* are believed to be used for energy storage (Eltgroth et al., 2005;Epstein et al., 2001), while little is known about the entire biosynthetic pathway of alkenones and their evolutionary and ecological functions (Rontani et al., 2006;Kitamura et al., 2018). Alkenones may have fitness and trophic benefit for their producers, because these unusual lipids are not only more photostable than other neutral lipids such as triacylglycerols, but also resistant to digestion, perhaps making alkenone producers less suitable for grazers (Eltgroth et al., 2005). Moreover, alkenones are well preserved in sediments over millions of years and thus their unsaturation ratios [e.g., the $U_{37}^{K'}$ index (= $C_{37:2}/(C_{37:2} + C_{37:3})$) (Prahl and Wakeham, 1987;Brassell et al., 1986)] are widely applied for reconstructing sea surface temperatures (Rosell-Melé and Prahl, 2013;Herbert et al., 2016). A long-standing issue for the use of alkenones to infer paleo-ocean surface temperature is how the production of these compounds is influenced by other environmental factors such as nutrients. Thus, culture studies have been conducted to test alkenone contents (mostly per-cell contents) in several species of haptophytes such as *E. huxleyi* under different growth phases (Pan and Sun, 2011;Wolhowe et al., 2009;Wolhowe et al., 2015), salinity (Sachs et al., 2016), temperature (Ding et al., 2019), and nutrient concentrations (Rokitta et al., 2014;Wördenweber et al., 2018). Conflicting results have been observed in different studies, e.g., independence of C_{37} - C_{39} alkenone contents on temperature (Prahl et al., 1988) *versus* significant responses of C_{37} alkenone contents to temperature changes in *E. huxleyi* (Ding et al., 2019). More empirical evidence appears necessary to determine how the total contents and the ratios of specific alkenone isomers respond to multiple environmental drivers, which would allow us to better understand their roles in ecology and biogeochemistry.

Here, we present data from semi-continuous culture experiments to tackle the question of how important lipid biomarkers (FAs, sterols and alkenones) respond to the changes in multiple environmental drivers (temperature, N:P supply ratios and pCO_2) in three phytoplankton species (the diatom *Phaeodactylum tricornutum*, the cryptophyte *Rhodomonas* sp. and the haptophyte *E. huxleyi*). Specifically, we analyze the changes of carbon-normalized and per-cell contents of major sterols and alkenones in the three species, and compare these responses with those of published FA data from the same experiments. Our aims are to determine (i) how sterols and alkenones respond to the changes of multiple environmental drivers, and (ii) how the responses of sterols, alkenones and FAs differ between each other. The goal of this study is to generate a better understanding of the impact of ocean-related global change on lipid biomarker productions in marine phytoplankton, which

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

will help to quantitatively apply lipid biomarkers as proxies for ecosystem change, and to finally scale up from specific physiological roles of lipids to their effects on energy flow in food webs in the changing ocean.

2 Materials and Methods

2.1 Experimental design

The three phytoplankton species used in the experiments were the diatom *P. tricornutum* [SAG, 1090-1b; isolated from Plymouth, UK (De Martino et al., 2007)], the cryptophyte *Rhodomonas* sp. (isolated from the Kiel Bight, Baltic Sea), and the haptophyte *E. huxleyi* (internal culture collection reference code: A8; isolated from waters off Terceira Island, Azores). *P. tricornutum* and *Rhodomonas* sp. are model species widely used in studies of diatom genomes, cryptophyte photosynthesis and planktonic trophic dynamics (Bi et al., 2017), and *E. huxleyi* is one of the major calcifying organisms in the pelagic ocean (Winter et al., 2014). Over the course of the experiments, the cultures of all species were exposed to a salinity of 37 psu and a light intensity of 100 μmol photons m^{-2} s^{-1} following a light:dark cycle of 16:8 h in temperature-controlled rooms of 12, 18 and 24°C. The culture medium was prepared according to the modified Provasoli's medium (Ismar et al., 2008;Provasoli, 1963), with enrichment nutrient solutions added to sterile filtered (0.2 μm pore size, Sartobran® P 300; Sartorius, Goettingen, Germany) North Sea water. Sodium nitrate and potassium dihydrogen phosphate were added to achieve the molar ratios of 10:1 (35.2 μmol L^{-1} N and 3.6 μmol L^{-1} P), 24:1 (88 μmol L^{-1} N and 3.6 μmol L^{-1} P) and 63:1 (88 μmol L^{-1} N and 1.4 μmol L^{-1} P). Sodium silicate pentahydrate was also added to diatom cultures at a concentration of 88 μmol L^{-1}. Initial pCO_2 was manipulated by bubbling with CO_2-enriched air (560 and 2400 μatm). Subsequently, the culture medium was transferred into sealed cell culture flasks with a 920-mL culture volume. Each treatment was replicated three times. All culture flasks were carefully agitated twice per day at a set time to minimize sedimentation.

At the onset of the experiments, each species was grown in batch cultures across a fully factorial combination of three temperatures (12, 18 and 24°C), three N:P supply ratios (molar ratios 10:1, 24:1 and 63:1) and two pCO_2 levels (560 and 2400 μatm). The chosen levels of temperature, N:P supply ratio and pCO_2 cover the ranges of typical changes of the three factors in natural environments, and they are in general agreements with projections. The temperature regimes broadly conform to sea surface temperatures in the source regions for the three taxa studied: Plymouth, UK for *P. tricornutum* (~9 – 17°C) (Highfield et al., 2010), the Kiel Bight for *Rhodomonas* sp. (~3 – 18°C) (Hiebenthal et al., 2013), and the Azores for *E. huxleyi* (16 – 22°C; http://dive.visitazores.com/en/when-dive). The 6°C elevation also mimicks the largest projected warming under climate change scenarios (Sommer and Lengfellner, 2008). N:P molar ratio of 24:1 was selected as the balanced ratio under which phytoplankton cultures are typically maintained (Guillard, 1975). N:P ratios of 10:1 and 63:1 were selected to bracket almost the full range of values observed in the modern ocean, including coastal and eutrophic regions (Downing, 1997). Nutrient availability in the upper ocean is influenced by several processes, e.g., anthropogenic activities increasing external nutrient supply, and climate change induced stratification causing nutrient deficiency (Moore et

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

al. 2013). Partial CO_2 pressure of 560 μatm is double the pre-industrial value and is a standard level for determining climate model sensitivity to pCO_2 forcing (e.g., IPCC, 2014). The value of 2400 μatm is at the mid-range of the projected values (1371 – 2900 μatm) by 2150 (RCP8.5 scenario; IPCC, 2014). Also, a high pCO_2 has been observed in the areas where one of the studied algae was isolated, e.g., 375 – 2309 μatm in the Kiel Bight (Thomsen et al., 2010).

The observed maximal growth rate (μ_{max}, d^{-1}) was calculated from the changes of cell numbers within the exponential growth phase in batch cultures (Bi et al., 2012). Once the early stationary phase was reached, semi-continuous cultures were started with the algae from batch cultures, and the gross growth rate (μ, d^{-1}) was set as 20% of μ_{max}. We calculated the volume of the daily renewal incubation water by multiplying daily renewal rate [D, d^{-1}; $D = 1- e^{-\mu \cdot t}$, where t is renewal interval (here $t = 1$ day)] with the incubation volume of 920 mL. The difference between the gross growth rate and the loss rate, i.e., the net growth rate [r (d^{-1}); $r = \mu - D$] was used to assess the steady state, at which r was zero, and μ was equivalent to D.

2.2 Sampling and measurements

At steady state in semi-continuous cultures, samples were collected for the measurements of cell density, dissolved inorganic carbon (DIC), total alkalinity, pH, sterols and alkenones. Cell density was measured daily in batch and semi-continuous cultures. Also, pH measurements were carried out daily in semi-continuous cultures, and the electrode was calibrated using standard pH buffers (pH 4 and pH 7; WTW, Weilheim, Germany).

DIC samples were taken on sampling days with 10-mL glass vials (Resteck, Germany) filled using a peristaltic pump and an intake tube containing a single-use syringe filter (0.2 μm, Minisart RC25; Sartorius, Goettingen, Germany). Vials were immediately sealed and stored in the dark at 4°C. DIC was measured according to Hansen et al. (2013) using a gas chromatographic system (8610C; SRI-Instruments, California, USA). For total alkalinity analysis, samples were filtered (GF/F filters; Whatman GmbH, Dassel, Germany) and analyzed with the Tirino plus 848 (Metrohm, Filderstadt, Germany). The remaining carbonate parameter pCO_2 was calculated from DIC and total alkalinity using CO2SYS (Pierrot et al., 2006) and the constants of Hansson (1973) and Mehrbach et al. (1973) that were refitted by Dickson and Millero (1987) (Table S1; Bi et al., 2017, 2018).

Samples for sterol and alkenone analysis were filtered (GF/F filters; Whatman GmbH, Dassel, Germany) and measured according to the procedure of Zhao et al. (2006). Lipids were extracted ultrasonically eight times from the freeze-dried filter samples utilizing dichloromethane and methanol (3:1, vol:vol) as extraction solvent, with C_{19} *n*-alkanol added for quantification. After hydrolysis with 6% potassium hydroxide in dichloromethane, the lipids were separated into a polar fraction and a non-polar fraction using silica gel chromatography. The polar lipid fractions containing sterols from the extracts of all the three species, as well as C_{37} - C_{39} alkenones from the *E. huxleyi* extracts, were eluted with 22 mL dichloromethane and methanol (95:5, vol:vol). Subsequently, the polar lipid fractions were silylated with 80 μL *N,O*-bis(trimethylsilyl)-trifluoroacetamide at 70°C for 1h. The sterol and alkenone fractions were analyzed and quantified using

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

an Agilent 7890A GC with flame ionization detection (50 m HP-1 capillary column, 0.32 mm i.d., 0.17 μm film thickness) by comparing analyte peak area to known amount of the internal standard C_{19} *n*-alkanol. The oven temperature held initially at 80°C for 1 min, increased to 200°C at 25°C min^{-1} and then programmed to 250°C at 4°C min^{-1} and to 300°C at 1.7°C min^{-1} holding for 12 min, and finally to 315°C at 5°C min^{-1} with an 8 min isothermal period.

The identification of brassicasterol/epi-brassicasterol, the major sterol in all three species, was conducted with reference to laboratory standards. In marine systems, the most likely epimer for 24-methylcholesta-5,22E-dien-3β-ol is epi-brassicasterol (24α) synthesized by diatoms (Gladu et al., 1990, 1991), cryptophytes (Goad et al., 1983) and haptophytes (Maxwell et al., 1980). In contrast, 24β-methylcholesta-5,22E-dien-3β-ol (brassicasterol) has been also found, e.g., in a strain of the haptophyte *Isochrysis* (Gladu et al., 1990). As the stereochemistry at C-24 could not be determined in our study, we use the trivial name brassicasterol/epi-brassicasterol for 24-methylcholesta-5,22E-dien-3β-ol.

Alkenones were identified using an Agilent GC 7890B (30 m HP-5MS column, 0.25 mm i.d., 0.25 μm film thickness) connected to an Agilent MSD 5977B mass selective detector (70 eV constant ionization potential, ion source temperature 230°C). The temperature program started with a 1 min hold time at 80 °C and then increased to 200°C at 25°C min^{-1}, followed by a 4°C min^{-1} ramp to 250°C and 1.8°C min^{-1} to 300°C holding for 10 min, and finally increased to 315°C at 5°C min^{-1} holding for 5 min. Alkenone identifications were performed by comparing sample mass spectra generated by GC-MS to previous published EI mass spectra, based on the molecular ion and prominent ions of each alkenone component (Volkman et al., 1980c;de Leeuw et al., 1980;Lopez and Grimalt, 2006). Note that the molecular ions for $C_{39:3}$ ethyl ketone ($C_{39:3}$ Et) (at m/z 556) and $C_{39:2}$ ethyl ketone ($C_{39:2}$ Et) (at m/z 558) were below detection, only prominent ion fragments were detected. In addition, the comparisons of the GC retention time of alkenone molecules between our study and previous work (Volkman et al., 1980c) indicated the presence of $C_{39:3}$ Et and $C_{39:2}$ Et.

2.3 Statistics

Generalized linear mixed models (GLMMs; Bolker et al., 2009) were used to test the effects of temperature, N:P supply ratios and pCO_2 on carbon-normalized and per-cell contents of brassicasterol/epi-brassicasterol and C_{37} - C_{39} total alkenones (as μg mg C^{-1} and pg cell^{-1}), per-cell contents of C_{37} alkenones, C_{38} alkenones, C_{38} ethyl ketones (C_{38} Et) and C_{38} methyl ketones (C_{38} Me), C_{37}/C_{38} alkenone ratios and C_{38} Et/C_{38} Me ratios (C_{38} Et/Me), with temperature, N:P supply ratios and pCO_2 as fixed effects. Target distributions were tested before GLMMs were taken. Subsequently, appropriate link functions were chosen, e.g., identity link function for any distribution except for multinomial, and logit link function for the binomial or multinomial distribution. To find the model that best predicted targets, we tested models containing first order effects, and second and third order interactions of temperature, N:P supply ratios and pCO_2. The Akaike Information Criterion corrected (AICc) was used to select the best model for each response variable, with a lower AICc value representing a better fit of the model. When the changes of AICc values were 10 units or more, it was considered as a reasonable improvement in the fitting of GLMMs (Bolker et al., 2009). In case AICc values were comparable (< 10 units difference), the simpler model was

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

chosen. Based on the differences in AICc values, models containing only first order effects of temperature, N:P supply ratios and pCO_2 were chosen as the best models for all response variables (bold letters in Table S2), while those containing second or third order interactions were not selected.

Principal component analysis (PCA) was conducted to visualize the responses of carbon-normalized contents of brassicasterol/epi-brassicasterol and total fatty acids (TFAs) in the three species, and total alkenones in *E. huxleyi* to the changes of temperature, N:P supply ratios and pCO_2. Data for TFAs were from previous studies (Bi et al., 2017, 2018).

GLMMs were performed using SPSS 19.0 (IBM Corporation, New York, USA). PCA were conducted using R package factoextra (Kassambara and Mundt, 2017) and FactoMineR (Le et al., 2008) in R version 3.5.1 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were conducted at a significance threshold of $p = 0.05$.

3 Results

3.1 Sterol and alkenone composition

Brassicasterol/epi-brassicasterol was the major sterol observed in all the three species, while alkenones were only detected in *E. huxleyi*. The alkenones in *E. huxleyi* consisted of four pairs of homologues, i.e., C_{37} methyl ketones (C_{37} Me including $C_{37:4}$ Me, $C_{37:3}$ Me and $C_{37:2}$ Me), C_{38} Me ($C_{38:3}$ Me and $C_{38:2}$ Me), C_{38} Et ($C_{38:3}$ Et and $C_{38:2}$ Et) and C_{39} ethyl ketones (C_{39} Et including $C_{39:3}$ Et and $C_{39:2}$ Et). C_{37} Me were the most abundant, followed by C_{38} Et, C_{38} Me and C_{39} Et.

3.2 Responses of brassicasterol/epi-brassicasterol to environmental changes

GLMM results showed that per-cell contents of brassicasterol/epi-brassicasterol responded significantly to changes in N:P supply ratios in the three species (bold letters in Table 1). Moreover, per-cell contents of brassicasterol/epi-brassicasterol in *P. tricornutum* also showed significant responses to temperature changes, while non-significant effects of pCO_2 were observed in all species. Specifically, higher per-cell contents of brassicasterol/epi-brassicasterol were observed at higher temperatures and higher N:P supply ratios in *P. tricornutum* (Fig. 1a; Table S3), under the lowest and highest N:P supply ratios in *Rhodomonas* sp. (Fig. 1c), and under higher N:P supply ratios in *E. huxleyi* (Fig. 1e).

Carbon-normalized contents of brassicasterol/epi-brassicasterol responded significantly to pCO_2 in *Rhodomonas* sp., and to temperature and N:P supply ratios in *E. huxleyi* (bold letters in Table 1), but with non-significant responses in *P. tricornutum*. Carbon-normalized contents of brassicasterol/epi-brassicasterol in *Rhodomonas* sp. decreased as pCO_2 increased (Fig. 1d), while those in *E. huxleyi* were generally higher at higher temperatures and under the balanced N:P supply ratio (N:P = 24:1 mol mol^{-1}; Fig. 1e).

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

3.3 Responses of alkenones to environmental changes

Total alkenone content per cell in *E. huxleyi* increased with increasing N:P supply ratios (Fig. 2 a; bold letters in Table 1; Table S4). However, carbon-normalized contents of total alkenones showed non-significant responses to changes in temperature, N:P supply ratios and pCO_2.

C_{37}/C_{38} alkenone ratios responded significantly to all three environmental factors (bold letters in Table 1), showing a clear increase with increasing temperature, a higher value under the lowest and highest N:P supply ratios, and a slight decrease at high pCO_2 (Fig. 2 c and d). C_{38} Et/Me ratios had significant responses only to temperature changes, clearly higher at the highest temperature (Fig. 2 e and f).

3.4 Comparisons of sterol, alkenone and fatty acid responses

PCA extracted four axes with eigenvalues > 1, and the first two axes in PCA explained 44.1% of total variance (Table S5). PC axis 1 explains 26.4% of the total variance and largely differentiates between the highest (N:P = 63:1 mol mol^{-1}) and lower N:P treatments (N:P = 10:1 and 24:1 mol mol^{-1}). Along PC axis 1, N:P supply ratios correlated positively with carbon-normalized contents of TFAs in *P. tricornutum* and *Rhodomonas* sp., but negatively with brassicasterol/epi-brassicasterol in *E. huxleyi* (Fig. 3). PC axis 2 (17.7% of the total variance) differentiates between the highest and both colder temperature treatments, along which temperature showed positive correlations with brassicasterol/epi-brassicasterol in *Rhodomonas* sp. and alkenones in *E. huxleyi*, but a negative correlation with TFAs in *E. huxleyi*. Along PC axis 2, pCO_2 plays a negative role particularly on TFAs in *E. huxleyi*.

4 Discussion

To our knowledge, this is the first study to disentangle the effects of multiple environmental drivers on sterol and alkenone productions, and to compare the responses of sterols, alkenones and FAs in marine phytoplankton. The mean percent changes of the three lipid biomarkers were elucidated (Table 2), particularly showing obvious changes in per-cell lipid contents which underly specific modes of biosynthesis. Furthermore, the PCA results highlighted that carbon-normalized contents of FAs in *P. tricornutum* mainly depended on N:P supply ratios, whereas brassicasterol/epi-brassicasterol in *Rhodomonas* sp. and alkenones in *E. huxleyi* increased at higher temperatures (Fig. 3). *E. huxleyi* FA contents, in contrast, decreased at higher temperatures. These response patterns of lipid biomarkers we observed indicate potential changes of energy flow in marine food webs in response to ocean-related global change.

4. 1 Sterol and alkenone composition

Brassicasterol/epi-brassicasterol was the only major sterol in the three algal species under wide ranges of temperature, N:P supply ratios and pCO_2. It is well established that the diversity of sterols is low in most phytoplankton species (Volkman,

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

2016;Martin-Creuzburg and Merkel, 2016). The predominance of brassicasterol/epi-brassicasterol has been reported for *P. tricornutum* (Rampen et al., 2010;Orcutt and Patterson, 1975;Ballantine et al., 1979), *Rhodomonas* (Chen et al., 2011;Dunstan et al., 2005), and *E. huxleyi* (Riebesell et al., 2000;Volkman et al., 1981). A few other minor sterols have been reported but they were not detected or below detection in our study, such as cholesterol, 24-methylcholest-5-en-3β-ol, and cholesta-5,22-dienol in *P. tricornutum* (Cvejić and Rohmer, 2000;Ballantine et al., 1979;Véron et al., 1996), and cholesterol and 24-methylcholesta-5,7,22-trien-3β-ol in *Rhodomonas/Proteomonas* sp. (Dunstan et al., 2005) and *E. huxleyi* (Mausz and Pohnert, 2015;Sawada and Shiraiwa, 2004;Yamamoto et al., 2000).

Alkenones were only observed in *E. huxleyi* in our study, consistent with previous results showing that these compounds were only synthesized by a few haptophytes including *E. huxleyi* (Volkman et al., 1980b;Volkman et al., 1998). The alkenone composition of *E. huxleyi* was characterized by the presence of four pairs of isomers, including eight alkenone compounds (Table S4) (Marlowe et al., 1984;Sachs et al., 2016;Riebesell et al., 2000). Moreover, higher abundance of several alkenone components have been also observed in some *E. huxleyi* strains under certain culture conditions, e.g., $C_{37:4}$ Me in the strain 1742 (Eltgroth et al., 2005) or at low temperatures (our study; Prahl and Wakeham, 1987). In addition, two other compounds $C_{38:4}$ Et and $C_{38:4}$ Me were also found in one *E. huxleyi* strain (Marlowe et al., 1984).

Collectively, the results above highlight the similarity of sterol and alkenone composition in algal species in our study with those in conspecifics or congeneric phytoplankters in previous studies. Sterol and alkenone composition can vary between algal strains and can be affected by environmental changes (Volkman, 2003;Conte et al., 1994), which may explain the differences between our findings and previous results. In the following section, specific response patterns of brassicasterol/epi-brassicasterol and alkenones are evaluated and quantified, which are further compared with FA responses under changing temperature, N:P supply ratios and pCO_2.

4. 2 Responses of brassicasterol/epi-brassicasterol contents

Increasing temperature caused an overall 12% increase in carbon-normalized contents of brassicasterol/epi-brassicasterol in *E. huxleyi*, but non-significant changes in other two species (Table 1; Table 2). Positive correlations between increasing temperature and sterol contents have been observed in several algal species (Piepho et al., 2012;Ding et al., 2019). High sterol contents at high temperature could be predicted based on its biochemical function, because increasing levels of sterols can reduce membrane fluidity to enable an organism's functional activity as temperature increases (Ford and Barber, 1983).

Enhanced partial CO_2 pressure caused a 21% decrease in carbon-normalized brassicasterol/epi-brassicasterol contents in *Rhodomonas* sp., and non-significant responses in *P. tricornutum* and *E. huxleyi*; however, per-cell contents of brassicasterol/epi-brassicasterol and POC showed non-significant changes in all three species (Table 1; Table 2). Minor effects of CO_2 concentration on per-cell contents of sterols have been observed in another strain of *E. huxleyi* (PML B92/11) (Riebesell et al., 2000) and the Chlorophyceae *Dunaliella viridis* (Gordillo et al., 1998). While the mechanism underlying sterol responses to pCO_2 is still unclear, our results indicate that enhanced pCO_2 did not induce substantial changes in per-

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

cell contents of sterols in phytoplankton due to the role of sterols in membrane composition and functions (Riebesell et al., 2000). Nevertheless, enhanced pCO_2 might change carbon metabolism in phytoplankton (Gordillo et al., 2001), as revealed by variable carbon-normalized contents of brassicasterol/epi-brassicasterol in *Rhodomonas* sp. in our study.

N and P deficiency caused overall 8% and 37% decreases in carbon-normalized brassicasterol/epi-brassicasterol contents, respectively, in *E. huxleyi*, but non-significant changes in other two species (Table 1; Table 2). Carbon-normalized or dry-weight contents of sterols in phytoplankton generally reduced in response to N or P deficiency (Breteler et al., 2005;Ding et al., 2019;Piepho et al., 2010). Furthermore, the relatively higher per-cell contents of sterols in response to P deficiency than N deficiency have been also found in a freshwater diatom *Stephanodiscus minutulus* (Lynn et al., 2000). Lipid modifications triggered by nutrient deficiency have been well studied in the plant *Arabidopsis* and more recently elucidated in typical phytoplankters (Abida et al., 2015;Van Mooy et al., 2009;Shemi et al., 2016). In *P. tricornutum*, N deficiency exerted more severe stress on membrane glycerolipids than P deficiency which caused a stepwise adaptive response, resulting in undetectable phospholipids and instead the increase in the synthesis of non-phosphorus lipids (Abida et al., 2015). Also in plants, P deficiency resulted in the replacement of phospholipids by non-phosphorous glycolipids such as glucosylceramide, sterol glucoside and acylated sterol glucoside (Siebers et al., 2015). Consequently, sterols are synthesized and accumulate in the plasma membrane in response to P deficiency. Thus, N deficiency may inhibit the capacity of the cells to synthesize sterols, while upon P deficiency membrane glycerolipid remodeling with the accumulation of non-phosphorous lipids may explain the relatively higher per-cell contents of sterols in response to P deficiency in our study.

4. 3 Responses of alkenone contents and ratios

Carbon-normalized contents of total alkenones showed non-significant responses to the changes in temperature, N:P supply ratios or pCO_2 in *E. huxleyi* (Table 1), which can be attributed to similar response patterns of per-cell contents of alkenones and POC (Table 2). We observed that per-cell contents of alkenones changed significantly in response to N and P deficiency, but showed non-significant responses to warming or enhanced pCO_2. In the following, the responses of per-cell contents of alkenones and the ratios of certain alkenone isomers are discussed.

N deficiency in semi-continuous *E. huxleyi* cultures grown at 20% of μ_{max} led to a 35% decrease in per-cell contents of alkenones in our study (Table 2). However, all published culture studies we could find in which *E. huxleyi* was grown under N deficiency were performed with batch cultures and reported either an increase in per-cell alkenone contents or a non-significant change (Prahl et al., 2003;Epstein et al., 1998;Bakku et al., 2018;Wördenweber et al., 2018). Several possible explanations exist for these contradictory findings. An increase in growth rate has been shown to reduce alkenone concentrations (ng mL^{-1}) in continuous cultures of *E. huxleyi* (Sachs and Kawka, 2015). Thus, the higher growth rate of *E. huxleyi* (20% of μ_{max}) in semi-continuous cultures in our study is a possible cause of lower alkenone contents compared to the batch culture studies where cells were harvested at or near the stationary phase of growth (i.e., growth rate approaching 0) (Bakku et al., 2018;Epstein et al., 1998;Prahl et al., 2003;Wördenweber et al., 2018). Contradictory results obtained in batch

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

and semi-continuous cultures could indicate different alkenone contents produced by *E. huxleyi* during the bloom period and summertime growth of this species, respectively (Lampert and Sommer, 2007). Another possibility is that gene complements within the species of *E. huxleyi* vary considerably, which may explain different phenotypic variations, including differences in N and P uptake in this species (Read et al., 2013). N deficiency severely impairs the synthesis of nucleotides, amino acids and ultimately all enzymatic machinery, consequently resulting in a decrease of most central metabolites (Wördenweber et al., 2018). Intense lipid turnover with the reduction of most central metabolites have been reported in *E. huxleyi* under N deficiency based on transcriptomic and metabolomic studies (Rokitta et al., 2014;Wördenweber et al., 2018), which may also result in lower per-cell alkenone contents in our study.

In contrast, P deficiency caused an increase of 49% in per-cell contents of alkenones in *E. huxleyi* (Table 2), as well as in other strains and life-cycle stages of *E. huxleyi* (Wördenweber et al., 2018). Experimental data presented here agree with a metabolic model predicted from transcriptomic data (Rokitta et al., 2016), and the findings in a comprehensive metabolome study showing a significant accumulation of several key metabolites, especially neutral lipids such as triacylglycerols, alkenones and alkenes in response to P deficiency (Wördenweber et al., 2018). *E. huxleyi* contains only very small amounts of triacylglycerols and hence alkenones have been suggested to have a storage role (Eltgroth et al., 2005;Bell and Pond, 1996;Volkman et al., 1980c). Our results support this view and suggest that P deficiency can induce the accumulation of alkenones which can serve as storage molecules in *E. huxleyi* cells. The increased abundance of metabolites in response to P deficiency is likely derived from the arrest of cell-cycling due to decreased nucleic acid synthesis, and the reduction equivalents are preserved by lipogenesis as enzymatic functionality (Wördenweber et al., 2018).

The carbon chain-length distribution of alkenones (C_{37}/C_{38} alkenone ratios) showed a 13 – 21% increase from the cold to warm treatments and in response to N and P deficiency, and a slight decrease (6%) with enhanced pCO_2 (Table 1; Table 2). A slight increase in C_{37}/C_{38} alkenone ratios at higher temperatures has been also found in four *E. huxleyi* strains in exponential phase cultures (Conte et al., 1998). In contrast to our results, it has been reported lower C_{37}/C_{38} alkenone ratios occurred under nutrient deficiency at the stationary phase of *E. huxleyi* in comparison to those at the exponential phase (Pan and Sun, 2011;Conte et al., 1998). As discussed above, different culturing approaches may cause conflicting results in different studies, as the effects of nutrient deficiency and growth rate cannot be well distinguished in the batch approach. The proposed biosynthetic pathways of classical C_{37} - C_{40} alkenones show that biosynthesis of C_{37} Me involve chain elongation with malonyl-CoA, while C_{38} Et are formed by the condensation of methylmalonyl-CoA and C_{38} Me are produced after the involvement of an additional α-oxidation (Rontani et al., 2006). Our results suggest that warming, N and P deficiency and enhanced pCO_2 may have independent effects on the synthesis of C_{37} Me and C_{38} alkenones, that ultimately result in changes in C_{37}/C_{38} ratios.

The relative abundance of C_{38} homologs (C_{38} Et/Me ratios) showed an 82% increase from the cold to warm treatments (Table 1; Table 2). The prominent increase in C_{38} Et/Me ratios resulted from non-significant changes in per-cell contents of C_{38} Et and the decrease in C_{38} Me. An increase in C_{38} Et/Me ratios with increasing temperatures has been found in four *E.*

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

huxleyi strains in mid-exponential phase of batch cultures (Conte et al., 1998). More importantly, our experimental results agree well with the findings in the sedimentary records back to ~ 120.5 Ma (Brassell et al., 2004), showing the lack of all C_{38} Me in Cretaceous sediments (warm climate) but the occurrence of $C_{38:2}$ Et from Cretaceous to Quaternary ages (warm to cold climate). The strong increases in C_{38} Et/Me ratios with warming in our study may reflect the differences in biosynthetic pathways between C_{38} Et and C_{38} Me (Rontani et al., 2006). Therefore, the distribution of alkenones in sediments over time can be linked to evolutionary adaption of alkenone biosynthesis in response to global climate change (Brassell, 2014).

The results discussed above show significant changes in per-cell contents of alkenones in response to N and P deficiency, indicating the important role of alkenones as storage molecules. Another biomolecules, alkenoates, have been identified in *E. huxleyi* and may biochemically link with alkenones (Marlowe et al., 1984;Conte et al., 1994). However, alkenoates were converted to FAs by saponification in our sample preparation steps and thus not evaluated. There might be interesting variations in alkenone/alkenoate ratios with changing multiple environmental conditions, which can be assessed in future studies.

4. 4 Implications for ecology and biogeochemistry

There has been evidence that carbon allocation in algal cells is highly responsive to environmental changes (Halsey and Jones, 2015;Palmucci et al., 2011). Our new study demonstrated that, under future ocean scenarios (warming, N and P deficiency and enhanced pCO_2), carbon-normalized contents of brassicasterol/epi-brassicasterol, alkenones and FAs have differential responses, i.e., significant but non-systematic changes in sterols and FAs, and non-significant changes in alkenones (Table 1). Our results further suggest rearrangements of cellular carbon pools under future ocean scenarios, and such variations would have important impacts on marine ecological functions and biogeochemical cycles.

Our study revealed an overall decrease (~ 20%) in carbon-normalized contents of brassicasterol/epi-brassicasterol in *Rhodomonas* sp. and *E. huxleyi* under ocean-related global change scenarios (Table 2). The low availability or absence of dietary sterols has been shown to constrain growth, reproduction and survival in *Daphnia* (Martin-Creuzburg et al., 2005;Martin-Creuzburg and von Elert, 2009a), and development and egg production in copepods (Hassett, 2004;Klein-Breteler et al., 2005). The potential influence of sterol deficiency on ecosystem functioning is the reduction of carbon transfer efficiency across autotroph-herbivore interface, leading to a low production of higher trophic levels (von Elert et al., 2003;Martin-Creuzburg and von Elert, 2009b). Brassicasterol/epi-brassicasterol are cholesterol precursors and can be converted to cholesterol by most crustaceans (Kumar et al., 2018;Martin-Creuzburg and von Elert, 2009b), and thus can efficiently support somatic growth of crustacean zooplankton such as *Daphnia magna* (Martin-Creuzburg et al., 2014). It is therefore possible that reduced brassicasterol/epi-brassicasterol under projected future ocean conditions may have deleterious ecological consequences in plankton communities, particularly where *Rhodomonas* or *E. huxleyi* is dominant.

Carbon-normalized contents of PUFAs showed an overall increase (~ 65%) in *Rhodomonas* sp., and an overall decrease (~ 10 – 20%) in *P. tricornutum* and *E. huxleyi* (Table 2). The responses of FA composition and the relevance of variable

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

PUFAs have been discussed in detail in previous work (Bi et al., 2017, 2018). Here we focus on the comparison of sterol and PUFA responses to environmental changes. Overall, the responses of brassicasterol/epi-brassicasterol were opposite to PUFAs in *Rhodomonas* sp., while both showed an overall decrease in *E. huxleyi* and only brassicasterol/epi-brassicasterol decreased in *P. tricornutum* under future ocean scenarios (Table 2). Co-limitation of sterols and PUFAs has been well studied in a freshwater herbivore *Daphnia magna*, showing a negative effect on the growth of this herbivores (Sperfeld et al., 2012;Martin-Creuzburg et al., 2009;Marzetz et al., 2017). Less is known about how sterols and PUFAs regulate the performance of marine herbivorous zooplankton, although the effects of PUFAs have been reported (Jónasdóttir et al., 2009;Rossoll et al., 2012;Arndt and Sommer, 2014;Chi et al., 2019). The differential responses of sterols and PUFAs we observed may have tremendous implications for the study of marine food webs, especially in habitats where phytoplankton succession is highly dynamic with environmental changes.

Carbon-normalized contents of total alkenones in *E. huxleyi* showed non-significant changes (Table 2). While it is conceivable that alkenones are resistant to digestion (Eltgroth et al., 2005), early studies on copepod feeding clearly showed ingestion of *E. huxleyi* (Volkman et al., 1980a;Nejstgaard et al., 1997;Vermont et al., 2016;Harris, 1994), and excretion of alkenones which are further transported to the deep ocean (Volkman et al., 1980a). We suggest that the response patterns of alkenones can reflect that of POC in variable environmental conditions, indicating quantitative application of alkenones as proxies for *E. huxleyi* biomass in biogeochemical cycles.

Taken together, our data suggest that the variation in temperature, N:P supply ratios and pCO_2 caused dramatic changes in carbon-normalized contents of FAs, smaller changes in sterols, and non-significant changes in alkenones, generally consistent with earlier studies (Riebesell et al., 2000;Volkman, 2016). Such variable responses of the three classes of lipid biomarkers may be attributed to their specific physiological functions and biosynthetic pathways (Riebesell et al., 2000). Carbon-normalized contents of FAs were particularly sensitive to environmental changes, because FAs can be incorporated into different types of lipids, and thus play multiple roles within the cells such as energy storage, membrane components and metabolic regulations (Guschina and Harwood, 2009;Van Mooy et al., 2009). Conversely, less pronounced changes in carbon-normalized contents of sterols and alkenones may reflect their major roles in membrane functions and energy storage, respectively. Also, the lower variabilities in sterols and alkenones provide additional evidence that these can be generally good proxies for paleoenvironmental and paleoecological reconstructions (Volkman, 2016).

5 Conclusions

The responses of sterols, alkenones and FAs to projected future scenario changes in temperature, N:P supply ratios and pCO_2 were experimentally examined in three phytoplankton species. Our results reveal that N and P deficiency had a stronger effect on per-cell contents of the three lipid biomarkers, while the effects of warming and high pCO_2 were relatively moderate. We also show that P deficiency caused an increase but N deficiency led to a decrease in per-cell contents of lipids

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

in most cases. Our results provide important new evidence to previous transcriptomic and metabolomic studies, which showed that key metabolites were up-regulated in response to P deficiency while most central metabolites were down-regulated in response to N deficiency. Future studies are suggested to consider the influence of these and other environmental changes on the composition of major and minor sterols and alkenoates, for example, whether varying environmental conditions influence C-24 alkylation in sterols.

Our study demonstrates that, under future ocean scenarios, the overall carbon-normalized contents of brassicasterol/epi-brassicasterol and PUFAs decreased in most cases in the three algal species; however, non-significant changes were also observed in brassicasterol/epi-brassicasterol and alkenones, and a strong increase was found in PUFAs in one of the three species (*Rhodomonas* sp.). This result highlights that a diverse allocation of carbon would potentially occur between lipid biomarkers and between phytoplankton taxa when they acclimate to large fluctuations in environmental conditions. Such variations in the contents of essential lipids (sterols and PUFAs) and in carbon allocation strategies may influence the structures and functions of food webs and the future ocean ecosystems.

Data availability: Data supporting the conclusions will be publicly available at PANGAEA at the time of publication of this paper.

Author contribution: Rong Bi (RB), Stefanie M. H. Ismar-Rebitz (SI), Ulrich Sommer (US) and Meixun Zhao (MZ) designed the experiments. RB carried the experiments out, with assistance of Hailong Zhang (HZ). RB prepared the manuscript with contributions from all co-authors.

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https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

Biogeosciences Discussions Open Access EGU

Table 1. Results of the selected GLMMs testing for the effects of temperature, N:P supply ratios and pCO_2 on carbon-normalized and per-cell contents of brassicasterol/epi-brassicasterol (brassi./epi-brassi./POC and brassi./epi-brassi./cell; μg mg C^{-1} and pg $cell^{-1}$) in *Phaeodactylum tricornutum*, *Rhodomonas* sp. and *Emiliania huxleyi*, carbon-normalized and per-cell contents of C_{37} - C_{39} total alkenones (Alkenones/POC and Alkenones/cell), per-cell contents of C_{37} alkenones (C_{37}/Cell), C_{38} alkenones (C_{38}/Cell), C_{38} ethyl ketones (C_{38} Et/Cell) and C_{38} methyl ketones (C_{38} Me/Cell), and the ratios of C_{37}/C_{38} alkenones and C_{38} Et/C_{38} Me (C_{38} Et/Me) in *E. huxleyi*. Significant *p* values are shown in bold; T: temperature; N:P: N:P supply ratios.

Species	Variable	Factor	Coefficient	*SE*	*t*	*p*	*n*
P. tricornutum	Brassi./epi-brassi./cell	Intercept	-1.482	0.077	-19.187	< 0.001	50
		T	0.010	0.004	2.842	**0.007**	
		pCO_2	< 0.001	< 0.001	1.656	0.105	
		N:P	0.003	0.001	4.199	**< 0.001**	
	Brassi./epi-brassi./POC	Intercept	7.043	0.820	8.589	< 0.001	52
		T	0.040	0.039	1.021	0.312	
		pCO_2	< 0.001	< 0.001	0.452	0.653	
		N:P	-0.012	0.008	-1.464	0.150	
Rhodomonas sp.	Brassi./epi-brassi./cell	Intercept	-0.388	0.103	-3.750	< 0.001	51
		T	-0.006	0.005	-1.290	0.203	
		pCO_2	< 0.001	< 0.001	0.368	0.714	
		N:P	0.002	0.001	2.092	**0.042**	
	Brassi./epi-brassi./POC	Intercept	1.484	0.186	7.987	< 0.001	54
		T	0.012	0.008	1.435	0.158	
		pCO_2	< 0.001	< 0.001	-2.706	**0.009**	
		N:P	-0.002	0.002	-0.868	0.389	
E. huxleyi	Brassi./epi-brassi./cell	Intercept	-1.189	0.131	-9.085	< 0.001	54
		T	-0.003	0.006	-0.450	0.655	
		pCO_2	< 0.001	< 0.001	1.485	0.144	
		N:P	0.003	0.001	2.367	**0.022**	
	Brassi./epi-brassi./POC	Intercept	5.860	0.709	8.262	< 0.001	54
		T	0.081	0.033	2.447	**0.018**	
		pCO_2	< 0.001	< 0.001	-0.203	0.840	
		N:P	-0.043	0.007	-6.017	**< 0.001**	
	Alkenones/Cell	Intercept	0.143	0.126	1.134	0.263	52
		T	-0.009	0.006	-1.452	0.153	
		pCO_2	< 0.001	< 0.001	1.663	0.103	
		N:P	0.006	0.001	4.973	**< 0.001**	
	Alkenones/POC	Intercept	122.935	25.130	4.892	< 0.001	52
		T	0.468	1.183	0.396	0.694	
		pCO_2	-0.001	0.006	-0.090	0.929	
		N:P	-0.172	0.258	-0.665	0.509	

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

C_{37}/Cell	Intercept	-0.192	0.121	-1.591	0.118	52
	T	-0.004	0.006	-0.772	0.444	
	pCO_2	< 0.001	< 0.001	1.509	0.138	
	N:P	0.007	0.001	5.466	**< 0.001**	
C_{38}/Cell	Intercept	-0.181	0.134	-1.351	0.183	52
	T	-0.012	0.006	-1.922	0.061	
	pCO_2	< 0.001	< 0.001	1.765	0.084	
	N:P	0.006	0.001	4.356	**< 0.001**	
C_{38} Et/Cell	Intercept	-0.509	0.150	-3.396	< 0.001	52
	T	-0.004	0.007	-0.569	0.572	
	pCO_2	< 0.001	< 0.001	1.512	0.137	
	N:P	0.006	0.002	4.054	**< 0.001**	
C_{38} Me/Cell	Intercept	-0.305	0.113	-2.702	0.010	52
	T	-0.032	0.005	-6.045	**< 0.001**	
	pCO_2	< 0.001	< 0.001	2.181	**0.034**	
	N:P	0.005	0.001	4.648	**< 0.001**	
C_{37}/C_{38}	Intercept	0.922	0.084	11.010	< 0.001	54
	T	0.024	0.004	6.094	**< 0.001**	
	pCO_2	< 0.001	< 0.001	-2.179	**0.034**	
	N:P	0.003	0.001	3.348	**0.002**	
C_{38} Et/Me	Intercept	0.895	0.091	9.872	< 0.001	54
	T	-0.028	0.004	-6.524	**< 0.001**	
	pCO_2	< 0.001	< 0.001	0.464	0.644	
	N:P	-0.001	0.001	-0.749	0.457	

775

780

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

785 **Table 2.** The mean percent differences in carbon-normalized and per-cell contents of brassicasterol/epi-brassicasterol (brassi./epi-brassi./POC and brassi./epi-brassi./cell; μg mg C^{-1} and pg $cell^{-1}$), total fatty acids (TFAs/POC and TFAs/cell), polyunsaturated fatty acids (PUFAs/POC and PUFAs/cell), and per-cell contents of particulate organic carbon (POC/cell) in *Phaeodactylum tricornutum*, *Rhodomonas* sp. and *Emiliania huxleyi*; carbon-normalized and per-cell contents of total alkenones (Alkenones/POC and Alkenones/cell), and per-cell contents of C_{37} alkenones (C_{37}/cell), C_{38} alkenones (C_{38}/cell),
790 C_{38} ethyl ketones (C_{38} Et/cell), C_{38} methyl ketones (C_{38} Me/cell) in *E. huxleyi*; C_{37}/C_{38} alkenone ratios (C_{37}/C_{38}) and C_{38} ethyl/C_{38} methyl ratios (C_{38} Et/Me) in *E. huxleyi* between cold and warm treatments, under N and P deficiency (-N and -P) and enhanced pCO_2 conditions. Only significant changes are shown according to GLMMs (Table 1).

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

Biogeosciences Discussions Open Access EGU

Lipids or POC	Species	Effect: Warming	-N	-P	Enhanced pCO_2	Interaction
Brassi./epi-brassi./cell	*P. tricornutum*	+23%	-7%	+37%		
	Rhodomonas sp.		+21%	+51%		
	E. huxleyi		-46%	-3%		
Brassi./epi-brassi./POC	*P. tricornutum*					
	Rhodomonas sp.				-21%	
	E. huxleyi	+12%	-8%	-37%		
Alkenones/cell	*E. huxleyi*		-35%	+49%		
Alkenones/POC	*E. huxleyi*					
C_{37}/cell	*E. huxleyi*		-34%	+59%		
C_{38}/cell	*E. huxleyi*		-38%	+37%		
C_{38} Et/cell	*E. huxleyi*		-47%	+27%		
C_{38} Me/cell	*E. huxleyi*	-39%	-16%	+62%	+25%	
C_{37}/C_{38}	*E. huxleyi*	+15%	+13%	+21%	-6%	
C_{38} Et/Me	*E. huxleyi*	+82%				
TFAs/cell	*P. tricornutum*[a]	+19%	+1%	+77%		
	Rhodomonas sp.[a]		+124%	+218%		
	E. huxleyi[b]	-29%	-11%	+72%		
TFAs/POC	*P. tricornutum*[a]		+2%	+12%		
	Rhodomonas sp.[a]		+78%	+108%		
	E. huxleyi[b]	-22%			-20%	
PUFAs/cell	*P. tricornutum*[a]		-4%	+71%		
	Rhodomonas sp.[a]		+86%	+165%		
	E. huxleyi[b]	-19%	-9%	+93%		
PUFAs/POC	*P. tricornutum*[a]	-20%	-2%	+9%		T × N:P
	Rhodomonas sp.[a]		+58%	+76%		
	E. huxleyi[b]				-24%	
POC/cell	*P. tricornutum*[a]	+17%	-6%	+64%		
	Rhodomonas sp.[a]	-12%	+26%	+59%		T × N:P
	E. huxleyi[b]	-8%	-39%	+50%		T × N:P

Decrease Increase

[a and b] Data are from Bi et al. (2017, 2018), respectively.

795

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

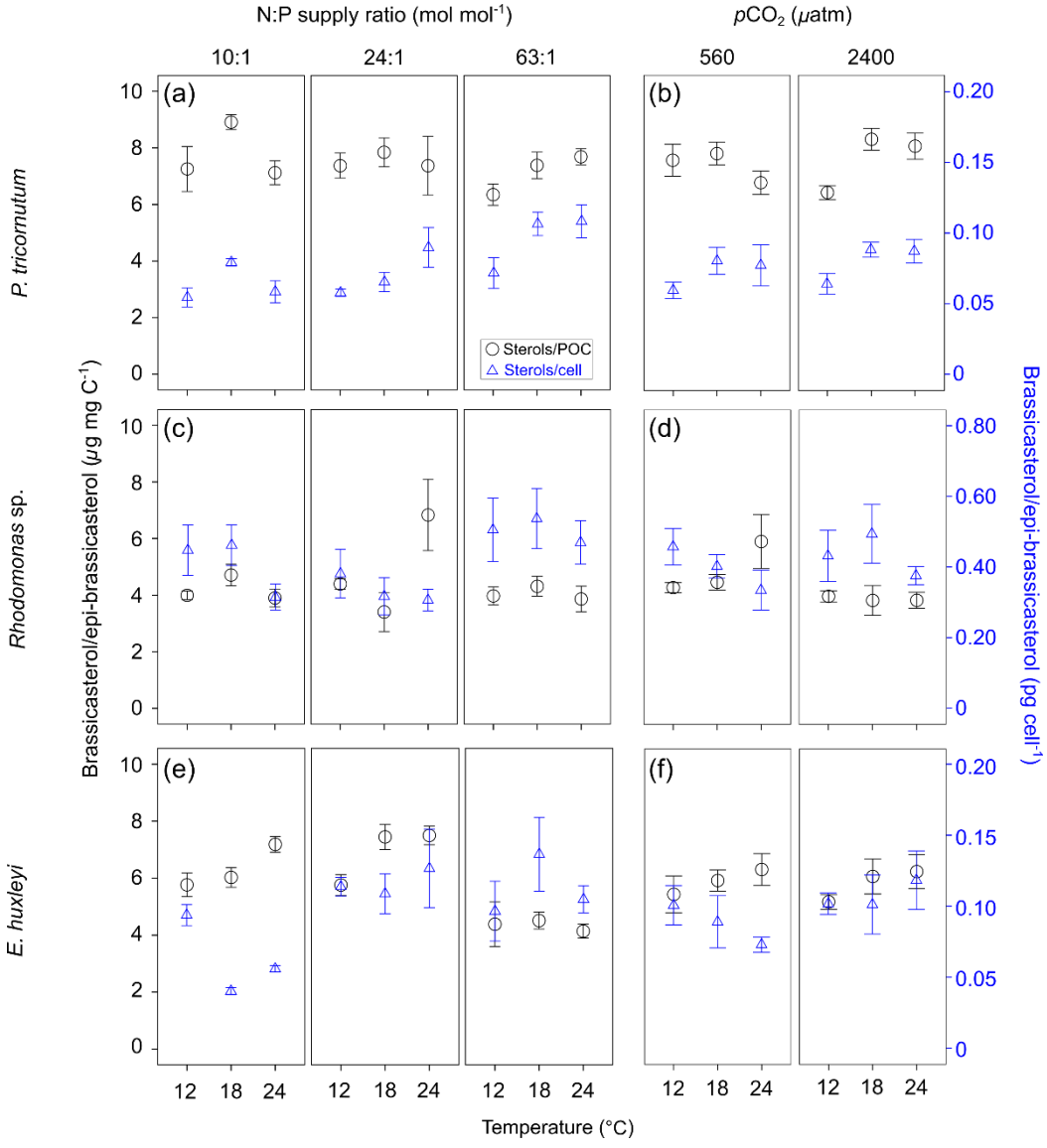
Fig. 1 Carbon-normalized (open circles) and per-cell (open triangles) contents of brassicasterol/epi-brassicasterol (mean ± SE; μg mg C^{-1} and pg $cell^{-1}$, respectively) in response to the changes in temperature, N:P supply ratios and pCO_2 in *Phaeodactylum tricornutum* (a and b), *Rhodomonas* sp. (c and d) and *Emiliania huxleyi* (e and f).

Fig. 2 Carbon-normalized (open circles) and per-cell (open triangles) contents of C_{37} - C_{39} total alkenones (mean ± SE; μg mg C^{-1} and pg $cell^{-1}$, respectively) (a and b), C_{37}/C_{38} alkenone ratios (c and d) and C_{38} ethyl/C_{38} methyl ratios (C_{38} Et/Me alkenone ratios) (e and f) in *Emiliania huxleyi* in response to the changes in temperature, N:P supply ratios and pCO_2.

Fig. 3 PCA biplot based on the carbon-normalized contents of the major sterol (brassicasterol/epi-brassicasterol) and total fatty acids (μg mgC^{-1}) in *Phaeodactylum tricornutum*, *Rhodomonas* sp. and *Emiliania huxleyi*, and C_{37} - C_{39} total alkenones in *E. huxleyi* under different temperatures, N:P supply ratios and pCO_2. Blue, black and red symbols represent 12, 18 and 24°C, respectively. Open triangles, open circles and closed circles represent N:P molar ratios of 10:1, 24:1 and 63:1, respectively. The first two Dims account for 44.1% of the total variance. The length of each vector reflects the combined loading of each variable in the first two Dims (Table S5).

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

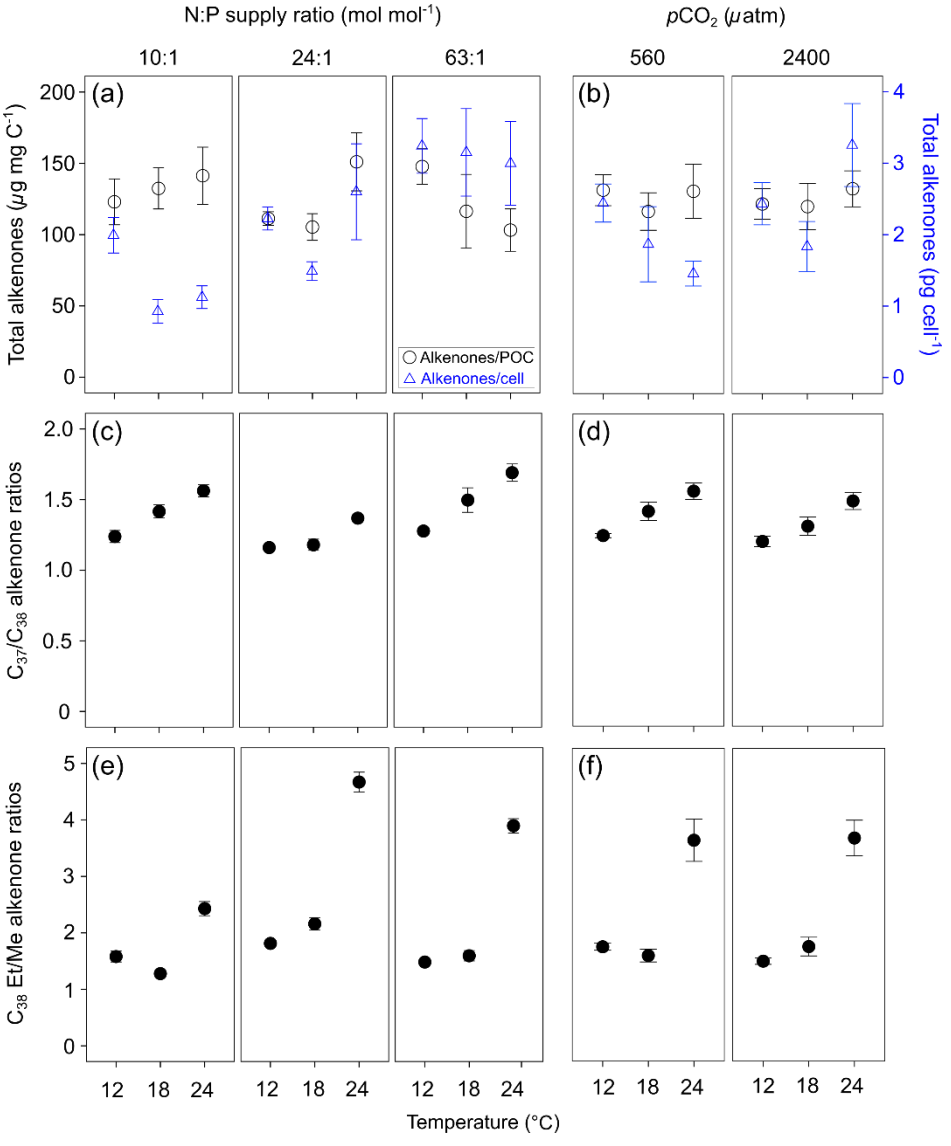
Fig. 1



835

https://doi.org/10.5194/bg-2020-183
Preprint. Discussion started: 8 June 2020

Fig. 2



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845

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Preprint. Discussion started: 8 June 2020

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Fig. 3