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Brownification reduces oxygen gross primary production and community respiration and changes the phytoplankton community composition: An in situ mesocosm experiment with high-frequency sensor measurements in a North Atlantic bay

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Abstract

In recent decades, the increase in terrestrial inputs to freshwater and coastal ecosystems, especially occurring at northern latitudes, has led to a process of water color darkening known as "brownification." To assess how brownification affects plankton community composition and functioning in northern coastal areas, an in situ mesocosm experiment using a highly colored humic substance to simulate a brownification event was performed in a North Atlantic bay (Hopavågen, Norway) in August 2019. Manual sampling for analyses of nutrient concentrations, phytoplankton pigments and zooplankton abundances was combined with high-frequency (every 15 min) monitoring of key environmental variables to investigate the response of the plankton community in terms of oxygen metabolism and community composition. In response to brownification, the oxygen gross primary production (GPP) and community respiration (R) slowed down significantly, by almost one-third. However, GPP and R both decreased to the same extent; thus, the oxygen metabolic balance was not affected. Moreover, the chlorophyll-*a* concentration significantly decreased under brownification, by 9% on average, and the chemotaxonomic pigment composition of the phytoplankton changed, indicating their acclimation to the reduced light availability. In addition, brownification seemed to favor appendicularians, the dominant mesozooplankton group in the mesocosms, which potentially contributed to lowering the phytoplankton biomass. In conclusion, the results of this in situ mesocosm experiment suggest that brownification could induce significant changes in phytoplankton and zooplankton community composition and significantly alter the overall oxygen metabolism of plankton communities in a northern Atlantic bay.

Freshwater and marine ecosystems are naturally subject to terrestrial inputs bringing humic organic substances and inorganic nutrients during natural rainfall events (Roulet and Moore 2006; Monteith et al. 2007). However, an increase in terrestrial inputs has been observed in freshwater and coastal ecosystems in recent decades (Roulet and Moore 2006; SanClements et al. 2012) being related to changes in land cover and uses (Correll et al. 2001; Clutterbuck and

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Additional Supporting Information may be found in the online version of this article.

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Yallop 2010) and to the intensification and higher frequency of extreme rainfall events due to global climate change (Hongve et al. 2004; Larsen et al. 2011; de Wit et al. 2016). The increase in inputs has led to the darkening of the water color, also known as water "brownification." Brownification is observed most often in northern latitudes (Haaland et al. 2010; Sepp et al. 2018), notably in Scandinavian countries (Solomon et al. 2015), and is predicted to become more common in the upcoming centuries (Larsen et al. 2011; de Wit et al. 2016). Brownification, by increasing humic organic substance concentrations in the water column, alters not only the light conditions but also the availability of organic and inorganic matter (Karlsson et al. 2009; Kritzberg et al. 2020). These changes may affect planktonic communities (Ejankowski and Lenard 2015; Williamson et al. 2015) and therefore the functioning of aquatic ecosystems. For instance, several studies have reported changes in plankton community composition and primary production and therefore in the overall ecosystem functioning of lakes due to brownification (Seekell et al. 2015; Williamson et al. 2015; Lenard and Ejankowski 2017).

Despite the growing interest in studying the effects of humic substance inputs on aquatic ecosystems, because brownification affects mainly freshwater systems, only a few studies have focused on such effects in coastal marine ecosystems (Grytaas 2020; Mustaffa et al. 2020; Paczkowska et al. 2020). Thus, knowledge about the effect of humic substances on marine plankton communities is particularly lacking, though coastal waters are highly exposed to human activities and changes in freshwater runoff and are therefore particularly sensitive to brownification (Mustaffa et al. 2020). This is notably the case for Norwegian coastal waters, which have already darkened over the past decades (Aksnes et al. 2009). HuminFeed® (HuminTech, Germany), a well-characterized, Leonardite-derived, highly colored humic substance (Meinelt et al. 2007), has typically been used under laboratory and in situ conditions to experimentally test the effects of brownification, especially in lakes and rivers in the Northern Hemisphere (Rasconi et al. 2015; Urrutia-Cordero et al. 2017; Lebret et al. 2018). After the addition of HuminFeed®, some studies reported no changes in planktonic metabolism or phytoplankton biomass (Ratcovich 2014; Rasconi et al. 2015; Lebret et al. 2018) while others noted changes in the phytoplankton community composition and diversity (Urrutia-Cordero et al. 2016, 2017; Lebret et al. 2018).

A key parameter used to study the functioning of plankton communities is the oxygen metabolic balance, which provides insights into the metabolic status of an aquatic ecosystem (Robinson and Williams 1999). This balance can be measured by assessing the balance between the oxygen produced by autotrophs via photosynthesis (gross primary production [GPP]) and the oxygen consumed through the entire community (aerobic respiration [R]). Therefore, net community oxygen production (NCP), which corresponds to the difference between GPP and R, is of great importance in understanding and managing aquatic ecosystems (Staehr et al. 2012).

On the one hand, inputs of humic substances to aquatic systems, by reducing the amount of light available for photosynthesis and by changing the light spectrum (Meinelt et al. 2007; Lenard and Ejankowski 2017), can potentially directly or indirectly alter GPP by affecting the phytoplankton biomass and/or community composition. On the other hand, these inputs, by increasing the dissolved organic carbon (DOC) concentration, could potentially enhance the activity and thus the respiration of heterotrophic bacteria. Finally, the addition of humic substances to aquatic systems could potentially affect their metabolic oxygen balance by favoring heterotrophic bacterial respiration while reducing phytoplankton oxygen production, leading to an imbalance between heterotrophic and autotrophic processes.

To study the effects of the addition of HuminFeed® on the functioning of the marine plankton community of a North Atlantic bay (Hopavågen, Norway), six in situ mesocosms were established and sampled according to two sampling procedures during 15 d. Low-frequency sampling was carried out to measure nutrient concentrations, phyto- and zooplankton abundances and phytoplanktonic pigment biomarkers to evaluate the changes in nutrient concentrations, phyto- and zooplankton community composition, and phytoplankton photo-acclimation. High-frequency sampling was carried out to measure the dissolved oxygen (DO) concentration, chlorophyll-*a* (Chl-*a*) fluorescence, water temperature, salinity, and photosynthetically active radiation (PAR) by deploying a set of automated sensors in every in situ mesocosm and was used to estimate GPP, NCP and R with a new method (Soulié et al. 2021) to evaluate the metabolic oxygen balance of this marine system following the addition of humic substances.

Material and methods

Study site and in situ mesocosm setup

An in situ mesocosm experiment was performed in August 2019 in Hopavågen, a landlocked coastal bay in Norway (63°36′N, 9°33′E) with a mean depth of 18 m and a maximum depth of 31 m, subject to limited human activity (van Marion 1996). It is a good outdoor laboratory for hydrographic and biological studies of the coastal waters of central Norway (van Marion 1996).

Six in situ mesocosm enclosures were deployed in Hopavågen Bay for 15 d. The enclosures were cylinders made from 200 μm low density polyethylene (LDPE) at Ludwig-Maximilians-Universität Munich and measured 3.6 m in length and 1 m in diameter, resulting in a 3 m long water column when the enclosures were suspended in water. The mesocosms were moored on a raft located at the center of the bay and were filled on the evening of August 5th by lifting the enclosures from $\sim$ 7 m deep to the surface. Three mesocosms were used as controls. HuminFeed® (HuminTech, Germany) was

added to the other three mesocosms (hereafter referred to as +HF mesocosms). The HuminFeed® was diluted in distilled water before being added to the mesocosms on August 6th (hereafter called d 0) to reach a concentration of 2 mg L^{-1} (corresponding to the addition of 0.8 mg L^{-1} of DOC; Meinelt et al. 2007). HuminFeed® is a well-characterized substance derived from Leonardite that has been used in several studies to study the impact of increased water coloration on aquatic systems (Rasconi et al. 2015; Lebret et al. 2018).

Sensor setup in the in situ mesocosms and high-frequency data acquisition

Each mesocosm was equipped with a set of automated sensors immersed at 1 m depth. The sensors measured biotic and abiotic parameters at high frequency (one measurement every 15 min) during all 15 d of the experiment. The automated sensors that provided the data used in the present investigation were (i) an oxygen optode (Model 3835, Aanderaa) to measure the DO concentration and oxygen saturation, (ii) an electromagnetic induction conductivity sensor (Model 4319, Aanderaa) to measure the conductivity (and therefore the salinity) and water temperature, and (iii) a spherical underwater quantum sensor (Li-193, Li-Cor) to measure the incident PAR. To ensure the accuracy of the sensor data and to avoid any potential drift, every sensor was calibrated before and after deployment (*see* Supporting Information).

Phytoplankton pigment and community composition sampling and analyses

Samples for phytoplankton pigments were taken daily in every mesocosm at 1 m deep using a 5L Niskin water sampler. Pigments were extracted following the method of Zapata et al. (2000) and the detailed protocol of Vidussi et al. (2011), then analyzed by high-frequency liquid chromatography (HPLC, Waters).

Phytoplankton pigment concentrations were used as taxonomic and acclimation biomarkers (Vidussi et al. 2000; Deininger et al. 2016). More specifically, in the present study, fucoxanthin and chlorophyll-*c2* (Chl-*c2*) were associated with Bacillariophyceae (diatoms); chlorophyll-*b* (Chl-*b*) was associated with Chlorophyceae and Euglenophyceae; zeaxanthin was associated with Cyanophyceae (cyanobacteria); and 19′-hexanoyloxyfucoxanthin (19′-HF) was associated with dinoflagellates, mainly Type II and Prymnesiophyceae. It should be noted that Prymnesiophytes were probably present in our experiment but were not identified by inverted microscopy due to their small size. Finally, chlorophyll-*c3* (Chl-*c3*) was assigned mainly to Bacillariophyceae (Roy et al. 2011). Peridinin, which is the major pigment of peridinial dinoflagellates, was not detected during this study, suggesting that peridinial dinoflagellates were absent or present at very low abundances and preventing the identification of peridinial dinoflagellates using their specific pigment biomarker. Furthermore, other accessory pigments are indicators of the photo-acclimation process; thus, the beta-beta-carotene (ββc) : Chl-*a* and diadinoxanthin : Chl-*a* ratios were used to indicate the acclimation of the phytoplankton community to high-light and low-light conditions, respectively (Deininger et al. 2016).

Furthermore, phytoplankton community composition was assessed five times during the experiment (d 0, d 5, d 9, d 11, and d 14). To do so, samples were taken using a 2 m tube sampler (KC Denmark). Samples were stored in 250 mL brown glass bottles, preserved in 1% lugol and counted via light inverted microscopy (Leica DMIL) using Utermölh settling chambers. Identified species were regrouped into five taxa: Bacillariophyceae (*Pseudonitzschia* sp., *Nitzschia longissima*, *Thalassionema* sp., *Dactyliosolen* sp., *Cerataulina* sp., *Skeletonema* sp., *Phaeodactylum* sp.), Prasinophyceae (*Pyramimonas* sp.), Euglenophyceae (*Eutreptiella* sp.), Cryptophyceae (*Teleaulax* sp., *Plagioselmis* sp.), and dinoflagellates (*Ceratium tripos*, *Ceratium fusus*, *Ceratium lineatum*, *Scrippsiella* sp., *Heterocapsa triquetra*, *Dinophysis* sp., *Prorocentrum micans*). Cell abundances were converted into carbon biomass by using specific volumetric conversion factors for each identified species.

Daily light integral calculation using high-frequency PAR measurements

High-frequency PAR data measured at 1 m deep were used to calculate the daily light integral (DLI), which corresponds to the quantity of photosynthetically active photons that are received by a one square meter surface over a 24 h period (Faust and Logan 2018). The DLI (mol m^{-2} d^{-1}) was calculated using the mean PAR (μmol m^{-2} s^{-1}) between sunrise and sunset as follows:

$$\mathrm{DLI} = \frac{\mathrm{Mean\,PAR} \times \mathrm{day\,length} \times 3600}{1 \times 10^{6}} \quad (1)$$

GPP, R and NCP estimations using high-frequency DO data

High-frequency DO data were used to estimate the daily GPP, R during the day (Rdaytime), R at night (Rnight), daily R and NCP, following a new method developed and described in Soulié et al. (2021); this method is derived from the free-water diel oxygen method first describe by Odum and Odum (1955), and was applied for the first time in the present study. A complete and precise explanation of the method is given in Supporting Information.

Statistical analyses

We used repeated measures ANOVA (RM-ANOVA) with treatment as fixed factor and time as random factor (linear mixed-effects models with autoregressive process of order 1, lme(Response ~ Treatment, random = ~ 1|Time, cor = corAR1), *nlme* package, R software) to test the effects of HuminFeed® addition during the entire experiment on physical parameters (water temperature, salinity, DLI, nutrient

concentrations), metabolic parameters (GPP, R), the Chl-*a* concentration, and pigment concentrations and ratios. The significance level was set at 0.05. When the assumptions of the RM-ANOVA could not be met even after transforming the data (logarithmic, square-root or exponential transformations), a nonparametric Kruskal–Wallis test was performed instead. For the abundance of each zooplankton genus obtained on the last day of the experiment, an unpaired *t*-test comparing the mean abundance in the control and in the +HF treatment was performed. To assess the environmental drivers and pigment contributors of the metabolic response to HuminFeed® addition, ordinary least squares linear relationships were assessed between log response ratios (LRR) of metabolic parameters, environmental variables and pigment concentrations. Moreover, Spearman's correlations were performed between dissolved nutrient and Chl-*a* concentrations. All statistical analyses were performed using R software (R-Project, version 4.0.1).

Results

Effects of brownification on high-frequency physical parameters

Overall, the water temperature decreased during the experimental period, ranging from 14.3 ± 0.1°C on d 0 to 13.1 ± 0.1°C on d 14 in the control (Fig. 1a). The water temperature was similar between the +HF treatment and the control (RM-ANOVA, $p = 0.67$, $F_{1,14} = 0.19$). The salinity ranged from 31.09 ± 0.02 (d 13) to 31.19 ± 0.02 (d 6) in the +HF treatment and from 31.10 ± 0.02 (d 12) to 31.17 ± 0.01 (d 6) in the control (Fig. 1b), with a significant but very small treatment effect (RM-ANOVA, $p = 0.02$, $F_{1,14} = 7$). Similarly to water temperature, the DO concentration decreased during the experimental period, and ranged from 291.1 ± 0.1 μmol O_2 L^{-1} to 251.4 ± 1.4 μmol O_2 L^{-1} in the control (Fig. 1c). When considering the entire period, it was not significantly different between treatments (Kruskal–Wallis, $p = 0.96$).

In the control, the DLI was generally above 10 mol m^{-2} d^{-1}, with the highest value on d 0 (17.87 ± 1.43 mol m^{-2} d^{-1}), and the lowest values on d 2 (3.52 ± 0.83 mol m^{-2} d^{-1}) and d 6 (2.68 ± 0.46 mol m^{-2} d^{-1}). As expected, the DLI was significantly lower in the +HF treatment than in the control (RM-ANOVA, $p = 2.4 \times 10^{-3}$, $F_{1,14} = 13.69$), by an average of 23%. The difference in DLI between the +HF treatment and the control varied from 6% (d 8) to 48% (d 11) (Fig. 1d) over the course of the experiment.

Effects of brownification on gross oxygen primary production, community respiration and net oxygen community production

Oxygen gross primary production

The oxygen GPP ranged from 0.11 ± 0.04 (d 2) to 0.63 ± 0.16 gO_2 m^{-3} d^{-1} (d 11) in the control treatment and

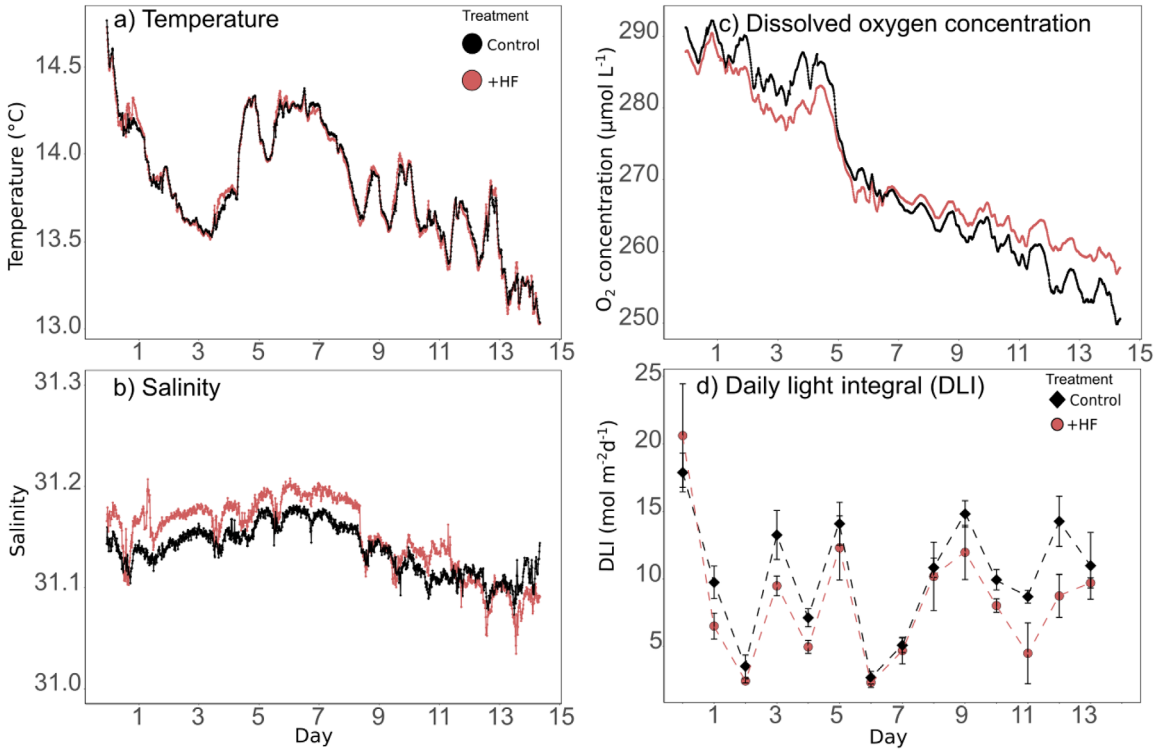

Fig. 1. Parameters based on high-frequency measurements in the control (black) and in the +HF (red) treatment over the course of the experiment. (**a**) Water temperature, (**b**) salinity, (**c**) dissolved oxygen concentration, and (**d**) mean daily light integral (DLI) with standard deviation (error bars).

peaked on d 3, d 9, and d 11 (Fig. 2a). Moreover, GPP was significantly lower in the +HF treatment than in the control ($p = 1.2 \times 10–3$, $F_{1,11} = 18.52$), by an average of 31%. GPP was lower in the +HF treatment than in the control on 11 out of 12 d, with the minimum difference between treatments on d 4 (8%) and the maximum difference on d 3 (62%). The GPP : Chl-*a* ratio, used as proxy of photosynthetic efficiency, followed a similar trend as GPP during the first part of the experiment, with significantly lower values in the +HF treatment than in the control from d 1 to d 3 (Kruskal–Wallis, $p = 0.04$), by an average of 53% (Fig. 2d). However, in contrast to GPP, no significant differences were reported between treatments during the second part of the experiment (from d 4 to d 10, Kruskal–Wallis, $p = 0.66$).

Community respiration

The community respiration (R) ranged from 0.13 ± 0.03 (d 2) to 0.73 ± 0.12 gO_2 m^{-3} d^{-1} (d 4) in the control treatment (Fig. 2b). It was significantly lower in the +HF treatment than in the control ($p = 1.6 \times 10–3$, $F_{1,11} = 17.22$), by an average of 27%. The difference between the +HF and control treatments varied over the course of the experiment, with the minimum difference between treatments on d 4 (6%) and the maximum difference on d 3 (75%).

Net community oxygen production

The NCP, which corresponds to GPP minus R (Fig. 2c), was negative on 11 out of 12 d, with an average value of -0.08 ± 0.12 gO_2 m^{-3} d^{-1}, indicating a globally heterotrophic system (NCP < 0, GPP: R < 1). The NCP was higher in the +HF treatment than in the control by 12% on average, but the difference was not significant ($p = 0.36$, Kruskall–Wallis).

Effects of brownification on the phytoplankton community: Chl-*a* concentration, pigment composition, and light acclimation

Phytoplankton Chl-a concentration, pigment, and community composition

In both the control and the +HF treatments, the Chl-*a* concentration decreased during the first 3 d of the experiment (from d 0 to d 2), stabilized during next 4 d (from d 3 to d 6), and then increased in the middle of the experiment (from d 7 to d 8 in the control) before decreasing again until the end of the experiment (Fig. 3a). The addition of HuminFeed®

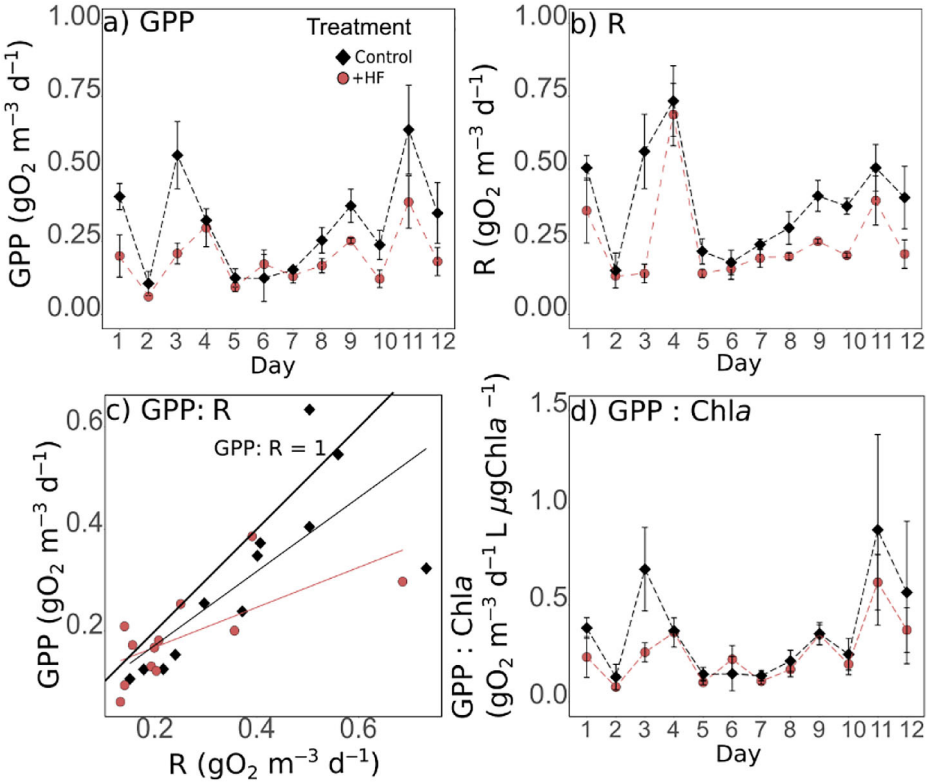

Fig. 2. (**a**) Mean gross primary production (GPP) and (**b**) mean community respiration (**R**) with standard deviations (error bars) over time in the control (black diamonds) and the +HF treatment (red circles); (**c**) gross primary production (GPP): Respiration (R) ratio in the control (black diamonds) and the +HF treatment (red circles). GPP: R > 1 indicates an autotrophic system, while GPP: R < 1 indicates a heterotrophic system; (**d**) mean photosynthetic efficiency (expressed as GPP : chlorophyll-*a* ratio) with standard deviations (error bars) over time in the control (black diamonds) and the +HF treatment (red circles).

significantly reduced the Chl-*a* concentration, by an average of 9%, over the entire experimental period (Supplementary Table S1). However, the effect of HuminFeed® addition was greater after d 8 and until the end of the experiment (Fig. 3a), with the highest difference between treatments occurring on d 9 (30%).

The phytoplankton pigment concentrations showed different trends (Fig. 3). In the control and +HF treatment, the dominant pigments were the Bacillariophyceae-associated pigments fucoxanthin and Chl-*c*2 (Fig. 3b,c), whereas the concentration of remaining pigments were generally lower (Fig. 3d–g).

The fucoxanthin and Chl-*c2* concentrations showed a strong decreasing trend during the experiment in the control (by 1446% and 307%, respectively) as well as in the +HF treatment (by 533% and by 382%, respectively, Fig. 3b,c). Similarly, the zeaxanthin concentration decreased by 718% in the control and by 346% in the +HF treatment (Fig. 3d). In contrast, the Chl-*b*, Chl-c*3*, and 19′-HF concentrations peaked in the middle of the experiment, on d 7 or d 8 depending on the pigment and treatment, in both the control and +HF treatments (Fig. 3e–g).

The concentrations of both pigments associated with Bacillariophyceae (fucoxanthin and Chl-*c2*) as well as of those associated with Cyanophyceae (zeaxanthin) and type-2 Prymnesiophyceae (19′-HF) were not significantly different between the +HF treatment and the control over the entire experimental period. In contrast, the Chl-*b* and Chl-*c3* concentrations were significantly higher in the +HF treatment than in the control (Supplementary Table S1).

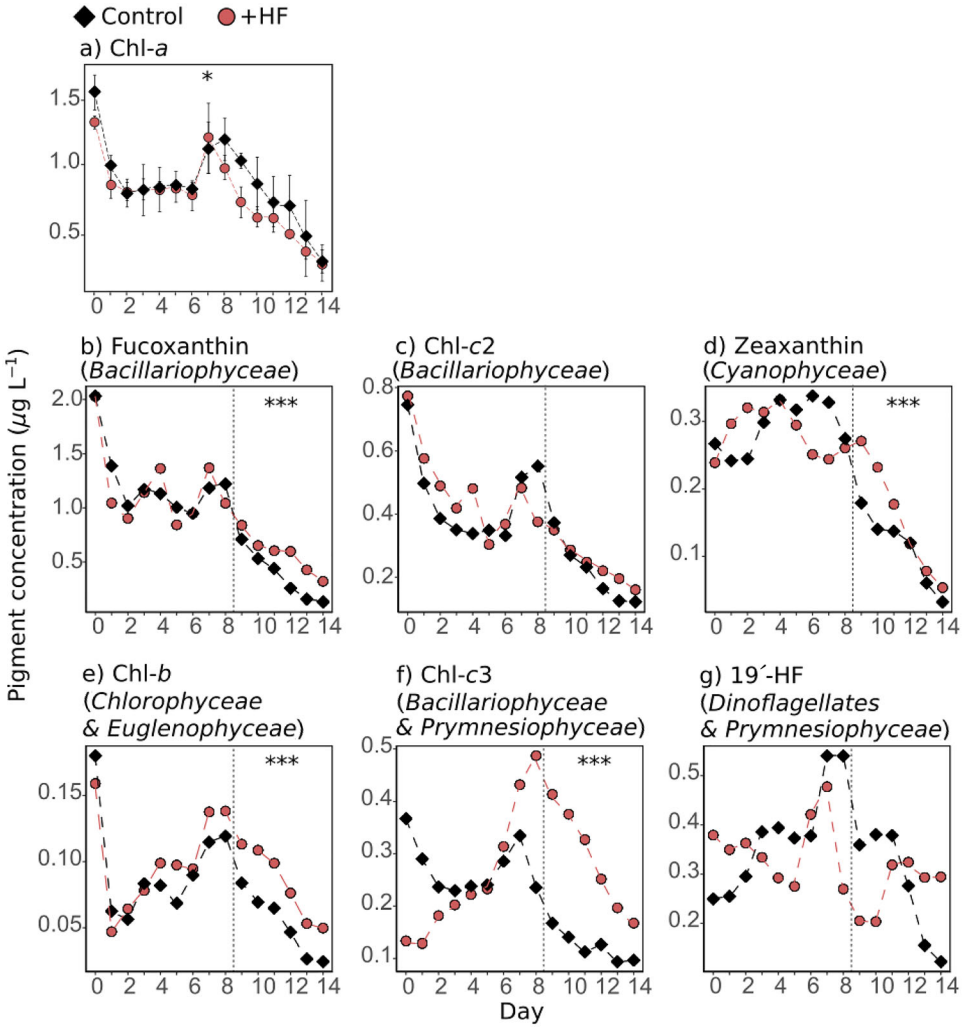

Fig. 3. Mean phytoplankton pigment concentrations (μg L^{-1}) over time in the control (black diamonds) and +HF treatments (red circles). The dashed black vertical line highlights the d 9 to d 14 period, during which a stronger treatment effect was observed. The * symbols represent the result of an RM-ANOVA testing the effect of the +HF treatment at the end of the experiment (from d 9 to d 14; ***$p < 0.001$). Note that the scales of the y-axes are different. (**a**) Chlorophyll-*a* (Chl-*a*); (**b**) Fucoxanthin; (**c**) chlorophyll-*c2* (Chl-*c2*); (**d**) zeaxanthin; (**e**) chlorophyll-*b* (Chl-*b*); (**f**) chlorophyll-*c3* (Chl-*c3*); (**g**) 19′-hexanoyloxyfucoxanthin (19′-HF).

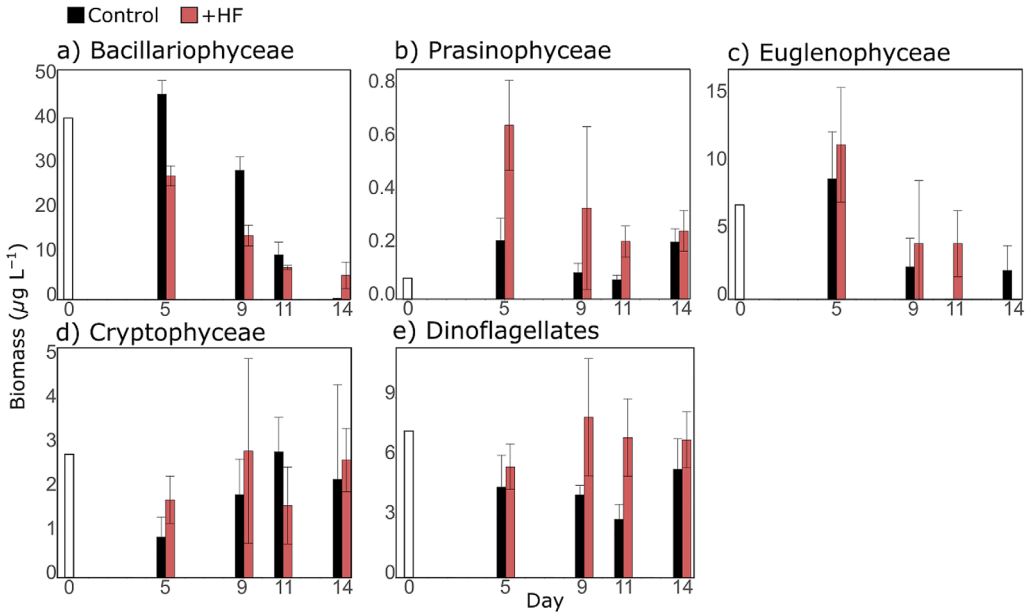

Fig. 4. Mean biomass (μg L^{-1}) for major phytoplankton taxa with standard deviations (error bars) during 5 d of the experiment (d 0, d 5, d 9, d 11, and d 14) in the control (black) and +HF (red) treatments. The sample on d 0 was taken directly into the bay few minutes before filling the mesocosms and is represented in white. Note that the scales of the y-axes are different. (**a**) Bacillariophyceae; (**b**) Prasinophyceae; (**c**) Euglenophyceae; (**d**) Cryptophyceae; (**e**) Dinoflagellates.

Other pigments, such as β-β-carotene (ββc), Chl-*c2* monogalactosyldiacylglyceride ester (Chl-*c2* MGDG), alloxanthin and diadinoxanthin, were present at much lower concentrations, and their concentrations were not significantly different between the +HF treatment and the control over the entire experimental period (Supplementary Table S1).

Interestingly, during the second half of the experimental period (from d 9 to d 14), there were strong, clear differences in almost all pigment concentrations between the +HF treatment and the control. Indeed, except for the Chl-*c2*, ββc and 19′-HF concentrations, all pigment concentrations were significantly higher in the +HF treatment than in the control (Supplementary Table S1). More specifically, the strongest effect was observed on the Chl-*c3* concentration. Finally, the differences between treatments found on d 0 for the chl-c3 and the 19′-HF concentrations might be due to rare planktonic species heterogeneously distributed during the filling procedure.

In both treatments, the Bacillariophyceae were the dominant phytoplankton taxon, for which the biomass strongly decreased over time (Fig. 4a), while other groups were present with a lower biomass. Bacillariophyceae biomass was significantly lower in the +HF treatment compared to the control from d 5 to d 11, by an average of 42% (Kruskal–Wallis, $p = 0.04$), while it was more than 20 times higher in the +HF treatment compared to the control on d 14. The biomasses of Prasinophyceae and dinoflagellates were significantly higher in the +HF treatment, by an average of 142% and 61% respectively between d 5 and d 14 (Kruskal–Wallis, $p = 0.04$ and $p = 0.02$, respectively). The biomasses of other groups were generally higher but not significantly (Kruskal–Wallis, $p > 0.05$).

Phytoplankton pigment ratios

The addition of HuminFeed® significantly increased some pigment ratios during the second half of the experimental period (Fig. 5, Supplementary Table S1). This trend was observed for the Chl-*c2* : Chl-*a* and the fucoxanthin : Chl-*a* ratios, which increased significantly between d 9 and d 14 (42% and 111% higher, on average, in the +HF treatment than in the control, respectively). Similarly, the zeaxanthin : Chl-*a*, Chl-*b* : Chl-*a*, Chl-*c*3 : Chl-*a*, and ββc : Chl-*a* ratios were significantly higher by 69%, 96%, 171%, and 11% in the +HF treatment, respectively. The 19′-HF : Chl-*a* ratio was also higher in the +HF treatment than in the control, but not significantly.

Furthermore, the diadinoxanthin : Chl-*a* ratio, which is an indicator of low-light acclimation, was significantly higher in the +HF treatment than in the control during the last half of the experiment by an average of 133%, indicating that phytoplankton had to acclimate to the low-light conditions that developed at the end of the experiment in the +HF treatment (Fig. 5g). Moreover, the ββc : Chl-*a* ratio decreased strongly but was not significantly different between the two treatments over the entire experimental period (Fig. 5h). However, it was significantly higher in the +HF treatment from d 9 to d 14, but only by an average of 11% (Supplementary Table S1).

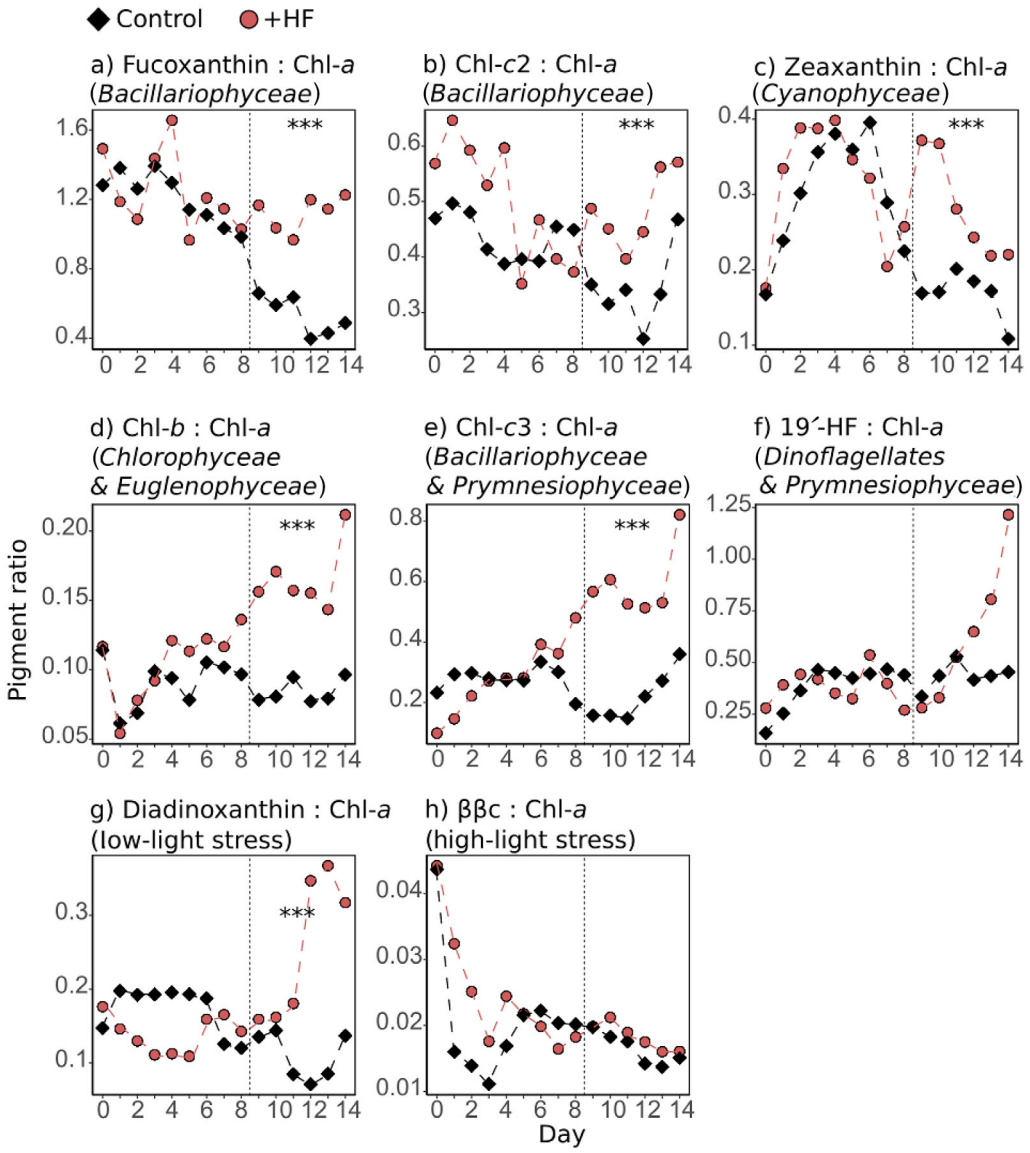

Fig. 5. Mean phytoplankton pigment ratios over time in the control (black diamonds) and +HF treatments (red circles). The dashed black vertical line highlights the d 9 to d 14 period, during which a stronger treatment effect was observed. The * symbols represent the result of an RM-ANOVA testing the effect of the +HF treatment at the end of the experiment (from d 9 to d 14; ***$p < 0.001$). Note that the scales of the y-axes are different. (**a**) Fucoxanthin: Chl-*a*, (**b**) Chl-*c2*: Chl-*a*, (**c**) zeaxanthin: Chl-*a*; (**d**) Chl-*b*: Chl-*a*; (**e**) Chl-*c3*: Chl-*a*; (**f**) 19′-HF: Chl-*a*; (**g**) diadinoxanthin: Chl-*a* (dark acclimation); (**h**) ββc: Chl-*a* (light acclimation).

Environmental and pigment drivers of GPP and R in response to brownification

To assess the potential correlations between biological and environmental variables, ordinary least square linear relationships were assessed between the log response ratios (LRR) of GPP, R, DLI, and pigment concentrations (Supplementary Figure S1). A positive significant relationship was found between GPP and R (Supplementary Figure S1a). In addition, responses of GPP and DLI were positively related (Supplementary Figure S1b), as well as responses of R and Chl-*a* concentration (Supplementary Figure S1e). The only significant relationship with pigments responses was found between GPP and zeaxanthin (Supplementary Figure S1h). In addition, on days when the nutrient concentrations were measured, in both treatments, the Chl-*a* concentration was significantly and positively correlated with the silicate concentration (Spearman's rho = 0.92 and 0.95 in the control and the +HF treatment, respectively, *see* Supporting Information). However, the Chl-*a* concentration was significantly negatively correlated with the ammonium concentration (Spearman's rho = −0.90 and − 0.80 in the control and the +HF treatment, respectively) and with the nitrate concentration only of

the +HF treatment (Spearman's rho = −0.88) (*see* Supporting Information). Finally, the Chl-*a* concentration was not significantly correlated with DLI, as well as with other variables (temperature, salinity) (data not shown).

Discussion

Brownification significantly slowed down both GPP and R

High-frequency sensor measurements allowed us to detect a significant effect of brownification, simulated via HuminFeed® addition, on oxygen metabolism. High-frequency sensors have rarely been used to estimate biological processes in the context of in situ mesocosm experiments (Soulié et al. 2021). However, this technique has many advantages over traditional sampling methods, as it is a noninvasive, less time-consuming way to estimate plankton oxygen metabolism that produces a large amount of data providing details on oxygen dynamics (Staehr et al. 2010).

Brownification resulted in significant reductions in GPP and R. To our knowledge, this is the first time that a significant slowing down of both GPP and R was induced by brownification in a coastal marine plankton community. The reduction in DLI (23%) due to the simulated brownification is one of the factors that altered the GPP, as the effects of the HuminFeed® addition on GPP and on DLI were linearly related (Supplementary Figure S1). The fact that the light availability was the main factor controlling the GPP response to brownification was expected as it has been shown that phytoplankton primary production responds quickly to light fluctuations (Kirk 1983; Morison et al. 2020). Additionally, the photosynthetic efficiency, assessed as GPP normalized by Chl-*a* concentration, was depressed by brownification during the first part of the experiment, before joining the control level during most of the second part of the experiment, in contrast to GPP alone. This implies that the observed decrease of GPP under brownification was certainly due to reduced light availability during the first part of the experiment, while it was mainly related to lower phytoplankton biomass in the second part of the experiment. Other physical, chemical and biological factors combined with light reduction may also have played a role. Indeed, it has been established that brownification could have multiple cascading effects that influence phytoplankton primary production (Solomon et al. 2015). This is especially true in coastal areas, as coastal plankton metabolism can be driven by various factors, among which allochthonous inputs from river loads can play an important role (Duarte et al. 2004).

In addition to a decrease in GPP, brownification resulted in a decrease in community R to a similar extent. This result was unexpected, as the input of humic substances constitutes an input of DOC that becomes available to heterotrophic bacteria (Robinson 2008); this input was expected to increase bacterial respiration and, in turn, the community R. Nevertheless, the lower R reported here is in line with the results of Lebret et al. (2018), in which the addition of HuminFeed® did not enhance total community R in lakes. The addition of 2 mg L^{-1} HuminFeed® corresponded to a small DOC input (0.8 mg L^{-1}), which is lower compared to the DOC concentrations that are naturally present in Hopavågen Bay (around 1.5 mg L^{-1}, Olsen et al. 2006); this may explain why HuminFeed® addition did not considerably increase bacterial respiration. Moreover, bacteria could potentially preferentially use other sources of carbon, suggesting that HuminFeed® represents a DOC source of poor bioavailability for bacteria. Taken together, these results indicate that R was likely controlled by factors other than the DOC concentration alone.

The decrease in R was positively and significantly correlated with the decrease in GPP and in Chl-a concentration (Supplementary Figure S1). This suggests a strong coupling between GPP and R and therefore a strong coupling between phytoplanktonic and bacterial communities. Indeed, in coastal ecosystems, bacterial respiration can contribute up to 76% of the whole community R, and bacterial respiration likely depends mainly on phytoplankton exudates rather than on other carbon sources (Robinson 2008). This is in line with the literature, which states that autotrophic production strongly affects both autotrophic and heterotrophic respiration, particularly in coastal ecosystems (Pringault et al. 2009).

As the decreases in GPP and R were similar in extent (31% and 27%, respectively), the simulated brownification resulted in an NCP that was not significantly different from that in the control. The NCP, which represents the net oxygen balance, was negative throughout almost the entire experiment in both treatments, reflecting a globally heterotrophic system. Regarding the drivers of plankton metabolism, the effects of the simulated brownification on GPP and R were not positively correlated with its effect on the concentration of a particular phytoplankton pigment, suggesting that these GPP and R reductions were not due to the response of one or certain phytoplankton groups but were rather related to the response of the whole phytoplankton community and might occur in other planktonic assemblages. Taking into account that reductions in plankton community metabolism are expected to have cascading consequences on the entire food web as it relates to its fundamental functions (Agusti et al. 2017), the results described in the present study suggest that the entire pelagic ecosystem of Hopavågen Bay could be affected by brownification.

Brownification reduced the Chl-*a* concentration and modified the zooplankton community composition

Brownification significantly reduced the Chl-*a* concentration mostly during the second half of the experiment, in contrast to the rapid decrease in GPP and R. This decrease in Chl-*a* concentration is in line with Nicolle et al. (2012) and Mustaffa et al. (2020), who found similarly lower Chl-*a* concentrations under brownification, but differs from Rasconi et al. (2015) and Lebret et al. (2018), who did not find any

significant effect of the addition of HuminFeed® on Chl-*a* concentrations in lacustrine plankton communities. The decrease in Chl-*a* observed in the present investigation seems not to be driven directly by light availability, as the Chl-*a* concentration was not correlated with DLI. Instead, as the Chl-*a* concentration was negatively correlated with the ammonium and nitrate concentrations, especially in the brownification treatment, bottom-up controls related to the availability of ammonium and nitrate may have driven the decrease in Chl-*a* (*see* Supporting Information). However, these negative relationships could be assessed only at 3 d during the present experiment and may not reflect the dynamics between nutrients and Chl-*a* concentrations during the whole period of the experiment. The reduction in the Chl-a concentration caused by brownification could also be due to grazers and their grazing activity.

The main zooplankton group observed during the experiment, the appendicularian *Oikopleura* sp., as well as all other zooplankton species, had very low abundances at the beginning of the experiment (*see* Supporting Information). The unusual high temperatures of the weeks before the experiment resulted in a strong stratification with high surface temperatures. We suggest that the low initial zooplankton abundances were due avoidance of the upper part of the water column where the mesocosms were filled. Additionally, the Hopavågen bay and its surroundings are known to host large abundances of jellyfishes (Tiller et al. 2014), resulting in a high grazing pressure on meso- and microzooplankton. However, as the abundance of jellyfish was very low in the enclosures, the loss of grazing pressure by large jellyfish may also have contributed to the generally rapid increase of appendicularians and copepods observed across the experiment. Moreover, even if zooplankton was not sampled at the middle of the experiment, it is conceivable that zooplankton abundances increased in the middle of the experiment, which would have led to the decrease in Chl-*a* concentration reported from d 8 in both treatments. In addition, at the end of the experiment, the abundance of the appendicularian *Oikopleura* sp., was significantly higher under brownification than in the control; while it was the opposite for the copepod *Acartia* sp., suggesting a different grazing pressure on phytoplankton between treatments, which could result in the lower Chl-*a* concentration reported. The positive effect of brownification on *Oikopleura* sp. corroborates the results of Kammerlander et al. (2016) and Minguez et al. (2020), who showed that some grazers performed better under low-light, high DOC conditions and that intensified water color coupled with warming could lead to stronger top-down control of the Chl-*a* concentration by grazers (Nicolle et al. 2012). Moreover, the effect of brownification on the zooplankton community structure observed at the end of the experiment might explain the higher Bacillariophyceae and the lower Euglenophyceae found under brownification on d 14, as it has been shown that *Oikopleura* sp. can feed mostly on small-sized organisms (Troedsson et al. 2007), as Euglenophyceae, while *Acartia* can feed upon Bacillariophyceae.

Brownification changed the phytoplankton pigment and community composition, resulting in light acclimation

It should be noted that the timing of the experiment might have already excluded some phytoplankton groups, as the extreme heatwave that occurred a few weeks before the experiment could have affected the phytoplankton community composition. For example, Katechakis and Stibor (2004) reported some Prymnesiophyceae and Chrysophyceae in summer at Hopavågen that were not identified in our experiment. Brownification induced a change in the composition of the phytoplankton community, by depressing Bacillariophyceae biomass in the middle of the experiment and increasing it at the end, and by promoting Prasinophyceae, Cryptophyceae, and dinoflagellates during the second part of the experiment. Brownification also triggered a change in the concentration of pigment-based taxonomic markers, especially during the second half of the experiment, from d 9 to d 14, when the specific biomarker pigments of Bacillariophyceae, Cyanophyceae, Chlorophyceae and Euglenophyceae as well as their respective ratios to Chl-*a* increased in the HuminFeed® treatment compared with those in the control treatment. As pigment concentrations and ratios to Chl-*a* can depict changes in both the community composition and light acclimation, a comparison between phytoplankton biomasses estimated using microscopy and associated pigment concentrations can help disentangle the physiological and the compositional effects of brownification. For example, the higher biomass of Bacillariophyceae at the end of the experiment in the brownified treatment (d 14) is congruent with the increase in fucoxanthin concentration on the same day, suggesting that the latter is related to phytoplankton community composition changes. Conversely, the lower Bacillariophyceae biomass on d 9 and d 11 under brownification was concomitant with higher fucoxanthin concentrations, suggesting a physiological acclimation rather than a change in the community composition. Similarly, the higher Chl-*b* concentration and ratio to Chl-*a* from d 9 to d 14 in the +HF treatment compared to the control do not match with the lower Euglenophyceae biomass on d 14, potentially reflecting a physiological acclimation rather than a community composition change. Hence, the phytoplankton biomass data tend to indicate that Bacillariophyceae were disadvantaged by brownification during most of the experiment, maybe because they were outcompeted by other phytoplankton such as Prasinophyceae, Euglenophyceae and dinoflagellates for which biomass was enhanced in the +HF treatment, thus departing from a study in a freshwater system reporting higher relative abundances of Bacillariophyceae with the addition of HuminFeed® (Lebret et al. 2018). Additionally, the phytoplankton pigment data reported in the present study highlights a potential

physiological acclimation to the lower light conditions of most investigated phytoplankton groups.

Another hypothesis to explain the observed increase in the pigment concentrations and ratios relates to the quality of the available light, not only to its quantity, as discussed previously. Indeed, HuminFeed® has been shown to absorb more light in the UV and blue wavelengths (Meinelt et al. 2007). In a parallel experiment performed at the same time and location as the present work, the addition of different concentrations of HuminFeed® strongly shifted the light spectrum toward red wavelengths (Herwig Stibor pers. comm.). Therefore, the addition of HuminFeed® likely led to a change in the light spectrum available for phytoplankton photosynthesis, which in turn could have shifted the competition within the phytoplankton communities toward groups that are more adapted to red light, such as cyanobacteria (Luimstra et al. 2020) leading to the reported increase in their pigment biomarker. It should be noted that both the light quantity and the light quality hypotheses mentioned above could have occurred synergistically.

Among other ratios, the addition of HuminFeed® significantly enhanced the diadinoxanthin : Chl-*a* ratio during the second half of the experiment. The diadinoxanthin : Chl-*a* ratio is a good indicator of low-light stress, as the diadinoxanthin cycle is the major xanthophyll cycle notably in Bacillariophyceae (Goss and Jakob 2010; Kuczynska et al. 2020). Therefore, the increase in the diadinoxanthin : Chl-*a* ratio further indicate that the phytoplankton in these taxa acclimated to the low-light conditions during the second half of the experiment. The concomitant increase in the fucoxanthin : Chl-*a* ratio observed during the second half of the experiment is also in agreement with previous studies reporting an increase in this ratio under low-light conditions (Schlüter et al. 2000). Surprisingly, while phytoplankton are known to adapt quickly to changes in light conditions (Goss and Jakob 2010), it appears that the light acclimation of the phytoplankton community occurred only during the second half of the experiment, 9 d after the addition of HuminFeed®, conversely to changes in the phytoplankton community that were already noticeable on d 5. This delay in the response of pigment ratios might be explained by the fact that pigment ratios can depend on other parameters rather than on only direct immediate responses to the light availability and spectrum. Pigment ratios can change due to modifications in phytoplankton composition, some ratios varying greatly among species of the same taxon (Mackey et al. 1996), growth phases, some ratios being up to 10-fold higher during the stationary growth phase than during the exponential growth phase (Ruivo et al. 2011), and nutrient conditions, as nutrient depletion can change multiple pigment : Chl-*a* ratios (Goericke and Montoya 1998; Henriksen et al. 2002). Therefore, the fact that pigment ratios seemed to respond to the addition of HuminFeed® only after 9 d of the experiment could be due to several factors that concomitantly affected the pigment ratios and not only to a direct immediate response to the reduction in light availability.

In conclusion, the results presented here, showing the slowing down of plankton metabolic rates and the shift in phytoplankton community and pigment composition in a natural northern-latitude coastal system under simulated brownification, call into question the whole-ecosystem implications of brownification. Although these results come from a single mesocosm experiment and any resultant generalizations must be considered carefully, they stress the importance of considering the brownification-induced modification of these metabolic processes in future modeling projections. If the results of the present experiment can be scaled up to the ecosystem level, they highlight the fact that brownification may affect the capacity of coastal Norwegian waters to both produce oxygen and to release it to the atmosphere. However, as both oxygen production and community consumption were reduced to the same extent under brownification, the net oxygen balance was not significantly altered, suggesting that brownification might not affect the balance between the capacity to consume and to produce oxygen from the water column. In the context of the expected increase in water column deoxygenation in coastal waters as a physical consequence of the increased temperatures caused by global warming, the concomitant slowing down of plankton metabolic rates due to brownification could potentially interact with and alter the oxygen cycle in coastal areas, particularly at those northern latitudes.

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Conflict of Interest

None declared.

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