Supplementary Information

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.

Tanguy Soulié, Herwig Stibor, Sébastien Mas, Benjamin Braun, Johanna Knechtel, Jens C. Nejstgaard, Ulrich Sommer, Francesca Vidussi, Behzad Mostajir

Material and Methods

Sensor calibration procedure

The oxygen optodes were calibrated using three different saturation points (0, 50 and 100%) and at two different temperatures (10 and 18°C). The 100% saturation level was reached by bubbling air into a 1 L beaker filled with distilled water, while 0 and 50% saturation were reached by adding potassium metabisulfite. The DO data were also corrected for salinity and temperature, as explained in Bittig et al. (2018). The conductivity sensors were calibrated to three levels of salinity (0, 20 and 35 mg L-1) using sodium chloride in distilled water at two temperatures (10 and 18°C).

Nutrient sampling and analyses

Water samples were taken for ammonium (NH_4^+), phosphate (PO_4^{3-}), nitrate ($NO_3^- + NO_2^-$) and silicate (SiO_4^{4-}) analyses at d 0, d 8 and d 14. For this purpose, depth-integrated water samples were taken with a 2 m tube sampler (KC Denmark). Then, they were filtered through a combusted, acid-washed filter (Whatman GF/F, 25 mm diameter) before being frozen (-28°C)

until further analyses. The dissolved inorganic nutrients were analyzed according to Grasshoff et al. (1999).

Zooplankton sampling and analyses

Zooplankton samples were taken at the beginning (d 0) and the end of the experiment (d 14) using a 250 µm mesh size net (Hydrobios, 25 cm diameter). On d 0, in situ water (707 L) was sampled directly from the bay, just before filling the mesocosms and by lifting the zooplankton net on the same water column as used for filling the mesocosms, from ca. 7 m deep to the surface. At the end of the experiment, after gently mixing each mesocosm to ensure a homogenous distribution of zooplankton organisms, each mesocosm was sampled (442 L) for zooplankton analyses using the net immersed at the middle of the enclosure's water column. Directly after sampling, the samples were fixed with ethanol (50% final concentration) and counted within the next few days. Zooplankton organisms were identified at the genus level and counted using a dissecting microscope (Leica MZ8 binocular, Leica Microsystems, Wetzlar, Germany).

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 was applied for the first time in the present study. The method is derived from the free-water diel oxygen method first described by Odum and Odum (1955), and it takes into account variations in the coupling between the day-night and DO cycles. The method involves several steps. First, the calibrated and corrected DO data were smoothed using a 9-point moving average and a 5-parameter sigmoid model (Soulié et al., 2021). Then, community metabolism was estimated based on the fundamental equation (Odum and Odum 1955; Odum 1956) as follows:

$$\frac{\Delta O_2}{\Delta t} = GPP - R - F - A \qquad \text{Eq. 1}$$

where $\frac{\Delta O_2}{\Delta t}$ is the instantaneous change in DO, F is the physical exchange of oxygen between the water surface and the atmosphere, and A is a term including all other phenomena (chemical and physical phenomena) that affect DO in the considered system. These other phenomena include

nonaerobic DO consumption and horizontal and vertical advection; A is considered negligible in the present study and most other studies (Staehr et al. 2010 ; Mostajir et al. 2013).

The atmosphere-water exchange term F can be calculated as follows:

$$F = (k * (O_2 - O_{2_{Sat}}))/Z_{mix} \quad \text{Eq. 2}$$

where O_2 is the DO, O_{2sat} is the oxygen saturation, k is an air-water constant coefficient and Z_{mix} is the mixing depth of the mesocosm enclosures. In the present work, we used a value of k equal to 0.000387 m min^{-1}, which corresponds to a high value measured as a function of turbulence in mesocosms (Alcaraz et al. 2001) and as proposed in Soulié et al. (2021).

Then, the instantaneous NCP can be calculated as follows:

$$NCP = \Delta O_2 - F \quad \text{Eq. 3}$$

For each DO cycle, the DO and instantaneous NCP data were separated into two periods: one period in which the DO concentration increases (i.e., the NCP was positive), called 'positive NCP periods', and one period in which the DO concentration decreases (i.e., the NCP was negative), called 'negative NCP periods'. Then, the positive and negative NCP periods were used to calculate the daily GPP, Rdaytime, Rnight, daily R and daily NCP.

First, Rdaytime, corresponding to the respiration rate occurring during the day, can be calculated as:

$$Rdaytime = (mean\ of\ instantaneous\ NCP\ during\ Max) * 60 * duration\ of\ positive\ NCP\ period \quad \text{Eq. 4}$$

where Rdaytime is expressed in gO_2 m^{-3} d^{-1}, Max refers to a 1 h period centered on the maximum instantaneous NCP during the negative NCP period, the mean instantaneous NCP is expressed in gO_2 m^{-3} min^{-1}, and the duration of the positive NCP period is expressed in hours.

Rnight, corresponding to the respiration rate occurring at night, can be calculated as:

$$Rnight = (mean\ of\ instantaneous\ NCP\ during\ the\ Negative\ NCP\ period) * 60 * duration\ of\ Negative\ NCP\ Period \quad \text{Eq. 5}$$

where Rnight is expressed in gO_2 m^{-3} d^{-1}, the mean instantaneous NCP is expressed in gO_2 m^{-3} min^{-1}, and the duration of the negative NCP period is expressed in hours.

The daily R (R24h) can simply be calculated as:

$$R24h = Rdaytime + Rnight \quad \text{Eq. 6}$$

The daily GPP (in $gO_2\ m^{-3}\ d^{-1}$) can then be calculated with the following equation:

$$Daily\ GPP = mean\ of\ instantaneous\ NCP\ during\ the\ positive\ NCP\ period * 60 * duration\ of\ positive\ NCP\ Period + Rdaytime \quad \text{Eq. 7}$$

At a result, the daily NCP (in $gO_2\ m^{-3}\ d^{-1}$) can be calculated as:

$$Daily\ NCP = Daily\ GPP - R24h \quad \text{Eq. 8}$$

This method is based entirely on the fact that the DO concentration displayed sufficiently noticeable daily cycles. In the present study, it was possible to estimate these daily metabolic parameters for d 1 to d 12. The data from d 0, d 13 and d 14 could not be used to reliably estimate the metabolic parameters.

Results

Statistical comparisons and difference between treatments expressed in % are presented in **Supp. Table 1**.

Supp. Table 1. Repeated measures analyses of variance (RM-ANOVA) *p*-values for the effect of HuminFeed® addition and percentage changes on pigment concentrations over the entire experimental period (d 0 to d 14) and during the last half of the experimental period (d 9 to d 14). The values in brackets are the F-values. Bold values indicate significant p-values ($p < 0.05$). When homoscedasticity and normality assumptions could not be met despite transforming the data, a nonparametric Kruskal-Wallis (KW) test was used instead.

Pigment or ratio	The entire experiment		From d 9 to d 14	
	p RM-ANOVA	% change	*p* RM-ANOVA	% change
Chl-*a*	**4.5×10^{-2} ($F_{1,74}$=8.57)**	-9	**7.9×10^{-2} ($F_{1,29}$=8.13)**	-24
Chl-*c2*	0.06 ($F_{1,74}$ = 3.51)	5	0.13 ($F_{1,29}$ = 4.46)	7
Fucoxanthin	0.09 ($F_{1,74}$ = 2.99)	4	**5×10^{-4} ($F_{1,29}$ = 15.09)**	54
Zeaxanthin	0.71 (KW)	1	**6.3×10^{-3} ($F_{1,29}$ = 8.68)**	30
Chl-*b*	**1×10^{-4} ($F_{1,74}$ = 16.22)**	25	**$< 1 \times 10^{-4}$ ($F_{1,29}$ = 25.29)**	57
Chl-*c3*	**0.04 ($F_{1,74}$ = 5.22)**	23	**$< 1 \times 10^{-4}$ ($F_{1,29}$ = 77.60)**	131
19'-HF	0.41 ($F_{1,74}$ = 0.68)	-4	0.77 ($F_{1,29}$ = 0.09)	-5
ββc	0.78 (KW)	1	**0.04 ($F_{1,29}$ = 4.84)**	-15

Chl-*c2* MGDG	0.05 ($F_{1,74}$ = 4.52)	-6	**1.4×10^{-3} ($F_{1,29}$ = 12.46)**	-21
Alloxanthin	0.58 (KW)	31	n.d.	n.d.
Diadinoxanthin	0.36 ($F_{1,74}$ = 0.86)	-2	**1.4×10^{-3} (KW)**	66
Chl-c2:Chl-*a*	**7×10^{-4} ($F_{1,74}$=12.63)**	24	**7×10^{-4} ($F_{1,29}$=14.30)**	42
Fucoxanthin:Chl-*a*	**9×10^{-4} ($F_{1,74}$=11.93)**	27	**$< 1 \times 10^{-5}$ ($F_{1,29}$=100.89)**	111
Zeaxanthin:Chl-*a*	**0.01 ($F_{1,74}$=6.52)**	21	**8×10^{-4} ($F_{1,29}$=13.85)**	69
Chl-*b*:Chl-*a*	**9×10^{-4} ($F_{1,74}$=15.24)**	47	**$< 1 \times 10^{-5}$ ($F_{1,29}$=38.45)**	96
Chl-*c3*:Chl-*a*	**0.01 ($F_{1,74}$=6.78)**	61	**8.7×10^{-5} (KW)**	171
19'-HF:Chl-*a*	0.66 ($F_{1,74}$ = 0.19)	19	0.52 ($F_{1,29}$ = 0.42)	46
ββc:Chl-*a*	0.07 (KW)	16	**0.02 ($F_{1,29}$=5.48)**	11
Chl-*c2* MGDG:Chl-*a*	0.79 ($F_{1,74}$ = 0.08)	3	0.58 ($F_{1,29}$ = 0.32)	-4
Alloxanthin:Chl-*a*	0.43 (KW)	41	n.d.	n.d.
Diadinoxanthin:Chl-*a*	0.20 (KW)	26	**1×10^{-4} ($F_{1,29}$ = 20.39)**	133

Effects of brownification on nutrient concentrations

Overall, the nutrient concentrations were low in both the control and the +HF treatment (**Supp. Table 2**). Moreover, the ammonium (NH_4^+), phosphate (PO_4^{3-}), nitrate (NO_3^- + NO_2^-) and silicate (SiO_4^{4-}) concentrations were not significantly different between the +HF and control treatments (**Supp. Table 2**). The ammonium concentration increased over the course of the experiment, reaching 5.1 ± 2.4 µg L^{-1} in the control and 4.3 ± 2.5 µg L^{-1} in the +HF treatment on d 14. Similarly, the nitrate concentration increased during the experiment, reaching 3.6 ± 0.1 µg L^{-1} in the control and 4.2 ± 0.3 µg L^{-1} in the +HF treatment on d 14. Additionally, the phosphate concentration peaked on d 8 in both treatments, reaching 1.2 ± 0.4 µg L^{-1} in the control and 1.5 ± 0.4 µg L^{-1} in +HF. In contrast, the silicate concentration decreased during the entire experiment, from 6.0 (d 0) to 1.7 ± 0.6 µg L^{-1} (d 14) in the control and from 6.0 (d 0) to 1.3 ± 0.6 µg L^{-1} (d 14) in the +HF treatment.

Significant correlations were found in the control and the +HF treatments between ammonium and silicate concentrations (Spearman, ρ = -0.94 and ρ = -0.89), between nitrate and silicate (Spearman, ρ = -0.68 and ρ = -0.78) and between ammonium and nitrate only in the +HF treatment (Spearman, ρ = -0.68).

Supp. Table 2. Mean dissolved inorganic nutrient concentrations expressed in µg L^{-1} (± standard deviations) in the control and in the +HF treatment at d 0, d 8 and d 14 and repeated measures analyses of variance (RM-ANOVA) *p*-values for the effect of the treatment on nutrient concentrations. The values in brackets are the F-values, when applicable. When homoscedasticity and normality assumptions could

not be met even after transforming the data, a nonparametric Kruskal-Wallis (KW) test was used instead (as indicated in the table). On d 0, samples were taken directly from Hopavågen bay. A value of 0 indicates that the concentration was below the detection limit. The * symbol indicates that samples were not taken on triplicates on d 0, thus it is not possible to calculate standard deviation.

		Control	+HF	*p*-value
NH_4^+	d 0	0.00*	0.00*	$p = 0.29$ (KW)
	d 8	2.39 ± 1.72	0 ± 0.68	
	d 14	5.09 ± 2.4	4.32 ± 2.5	
$NO_3^- + NO_2^-$	d 0	3.00*	3.00*	$p = 0.28$ ($F_{1,14} = 1.29$)
	d 8	3.74 ± 0.3	3.56 ± 0.26	
	d 14	3.57 ± 0.11	4.24 ± 0.35	
PO_4^{3-}	d 0	1.00*	1.00*	$p = 0.21$ ($F_{1,14} = 1.73$)
	d 8	1.23 ± 0.38	1.48 ± 0.36	
	d 14	0.85 ± 0.44	0.90 ± 0.17	
SiO_4^{4-}	d 0	6.00*	6.00*	$p = 0.82$ (KW)
	d 8	4.67 ± 0.58	5.33 ± 0.58	
	d 14	1.67 ± 0.58	1.33 ± 0.58	

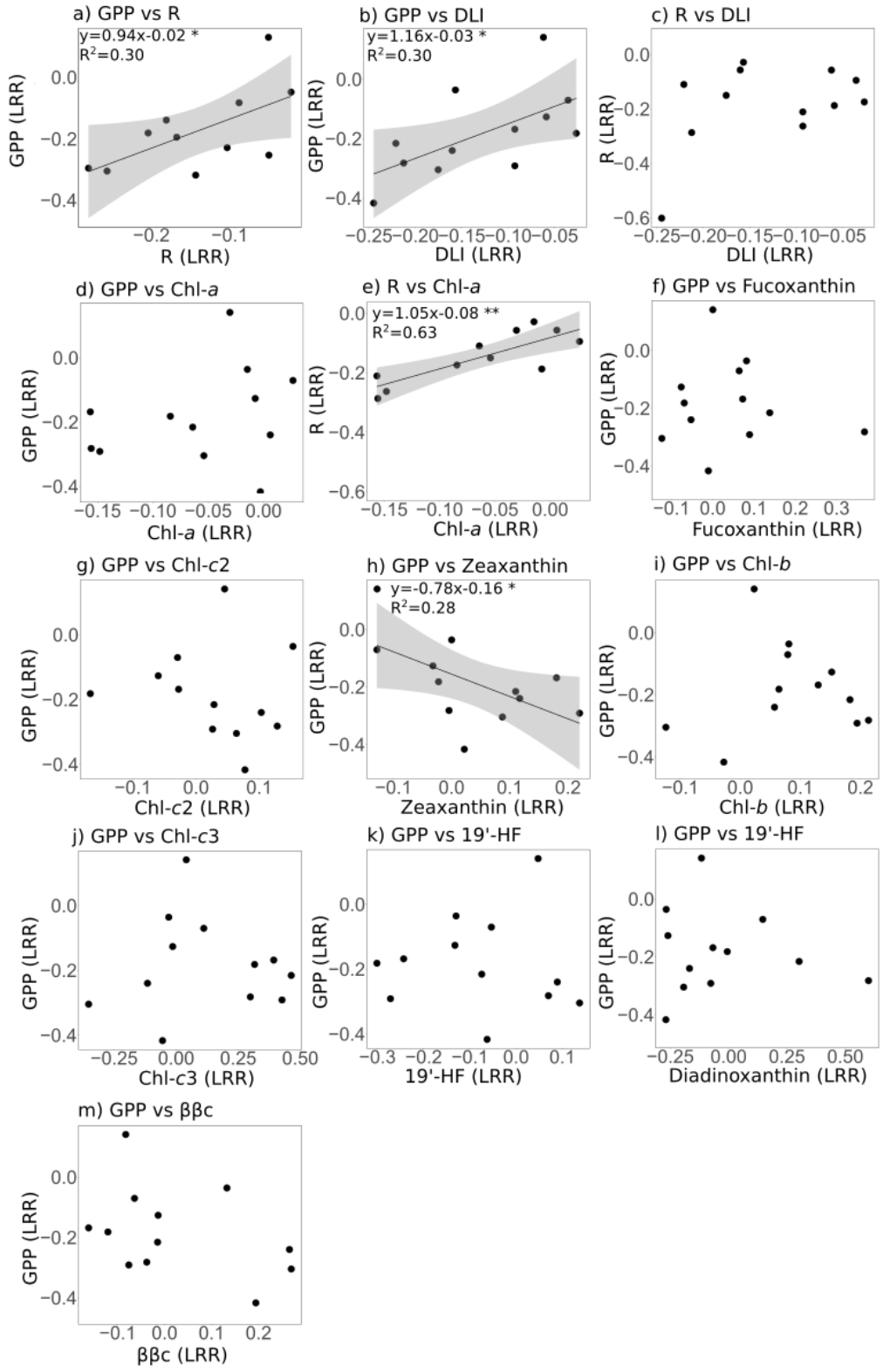
Effects of brownification on zooplankton community composition

At the beginning of the experiment (d 0), the zooplankton abundances in the bay were very low, and the zooplankton community was dominated by the appendicularian genus *Oikopleura*, the copepod genus *Acartia* and the cladoceran genus *Evadne*, with abundances of 93, 34 and 21 ind m^{-3}, respectively. Over the course of the experiment (from d 0 to d 14), the abundances of both *Oikopleura* and *Acartia* increased, by 2678% and 1785%, respectively, in the control and by 3431% and 1076%, respectively, in the +HF treatment (**Supp. Table 3**). The *Oikopleura* abundance was significantly higher in the +HF than in the control treatment, while the *Acartia* abundance was significantly lower in the +HF than in the control treatment (**Supp. Table 3**). In contrast, the abundance of *Evadne* decreased by 76% in the control and increased by 4% in the +HF treatment from d 0 to d 14. Other genera were present at very low abundances, and the effects of the +HF treatment on these genera were therefore not significant and limited.

Supp. Table 3. Mean abundances (± standard deviations), expressed as ind m^{-3}, of the main zooplankton genera at the beginning of the experiment sampled directly from the bay and at the end of the experiment (d 14) sampled from the control and +HF treatments and the results of an unpaired *t*-test comparing the means of the control and +HF treatments on d 14; significance is indicated by * ($p < 0.05$).

Zooplankton genus	In situ d 0 (ind m^{-3})	Control d 14 (ind m^{-3})	+HF d 14 (ind m^{-3})	*t*-test *p* value for d 14
Oikopleura	93	2584 ± 463	3284 ± 220	0.04*
Acartia	34	641 ± 49	400 ± 102	0.02*
Evadne	21	5 ± 3	22 ± 20	0.4
Podon	1	5 ± 3	19 ± 8	0.07
Obelia	8	9 ± 2	7 ± 6	0.57

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



Supp. Figure 1. Ordinary least squares linear relationship between the log response ratios (LRR) of GPP, R, DLI, and pigment concentrations. For significant relationships (p <0.05), the solid black line

represents the least square fit, and the grey area represents the 95% confidence limits. a) GPP vs R; b) GPP vs DLI; c) R vs DLI; d) GPP vs Chl-*a*; e) R vs Chl-a; f) GPP vs Fucoxanthin; g) GPP vs Chl-*c*2; h) GPP vs Zeaxanthin; i) GPP vs Chl-*b*; j) GPP vs Chl-*c*3; k) GPP vs 19'-HF; l) GPP vs Diadinoxanthin; and m) GPP vs ββc. Note that one extremely low point (-0.49 x -0.61) was removed from GPP vs R to avoid a biased relationship.

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