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ORIGINAL ARTICLE

Carbon dioxide limitation of benthic primary production in a boreal lake

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Abstract

1. Gross primary production (GPP) by benthic microalgae growing on soft sediments is an important contributor to lake productivity in many lakes world-wide. As benthic microalgae have access to nutrients in the sediment they have been regarded as primarily controlled by light, while the role of CO_2 as a limiting factor for benthic GPP in lake ecosystems is largely unknown.
2. In this study, we experimentally tested for CO_2 limitation of benthic GPP by collecting littoral surface sediments, with associated benthic microalgae, from a typical boreal lake. Intact sediment cores were incubated at different depths (light conditions) after addition of dissolved inorganic (bicarbonate) or organic (DOC; glucose) carbon as direct and indirect sources of CO_2, respectively.
3. Benthic microalgal GPP was stimulated by both dissolved inorganic carbon and DOC additions at high, but not at low, light levels.
4. This study shows that benthic microalgal GPP can be CO_2-limited when light is not limiting and suggests that both direct (e.g., via groundwater inflow) and indirect (via mineralisation of DOC) CO_2 supply can stimulate benthic GPP.

KEYWORDS
benthic microalgal gross primary production, CO_2 limitation, dissolved organic carbon, lakes, light limitation

1 | INTRODUCTION

Gross primary production (GPP) by benthic microalgae growing on soft sediments (here *benthic microalgae*) may dominate whole-ecosystem GPP (Ask et al., 2009; Vadeboncoeur et al., 2001) and energy supply for higher trophic levels (Karlsson & Byström, 2005). As benthic microalgae have access to nutrients in the sediment (Bonilla et al., 2005; Daniels et al., 2015) they are regarded as primarily controlled by light availability (Ask et al., 2009; Hamdan et al., 2021; Hansson, 1992). However, experimental studies focusing on GPP in benthic systems are far less numerous than in pelagic systems, and there are still large gaps in our understanding regarding limitations of benthic GPP in lakes.

One potential factor limiting benthic GPP is the supply of inorganic carbon, in particular carbon dioxide (CO_2). CO_2 limitation of GPP has traditionally been regarded as uncommon since atmospheric CO_2

uptake was considered sufficient to support pelagic GPP (Schindler et al., 1972), but recent studies show that high CO_2 concentrations can stimulate pelagic GPP (Hein, 1997; Jansson et al., 2012; Kragh & Sand-Jensen, 2018) and whole-lake GPP (Hamdan et al., 2018), especially in nutrient-rich waters (Hammer et al., 2019; Spijkerman, 2010; Verspagen et al., 2014). In nutrient-poor boreal lakes, however, a positive effect of CO_2 on pelagic GPP is not necessarily observed (Hessen et al., 2017). In benthic habitats, CO_2 limitation might be more pronounced than in the pelagic habitat due to strong boundary layer effects at the water sediment interface that constrain diffusion of CO_2 from the water to the benthic algae (Riber & Wetzel, 1987). However, processes within the sediment (such as methanogenesis) might also provide benthic algae with CO_2 (Gruca-Rokosz & Tomaszek, 2015) although diffusion from such processes should also be constrained by a lack of turbulence. While benthic microalgae in shallow marine systems often show a positive response to CO_2 (Johnson et al., 2013; Vieira et al., 2016), the CO_2 effect on soft-bottom benthic GPP in freshwater systems is largely unknown (Brown et al., 2019). Hence, the extent to which CO_2 availability limits aquatic GPP in different types of ecosystems is complex, which indeed impairs the understanding of factors controlling whole-lake GPP.

The dissolved inorganic carbon (DIC) concentration in water is controlled by a range of processes, including input from the catchment, exchange with the atmosphere, in situ GPP, respiration, and photooxidation (Vonk, 2015). Furthermore, input of allochthonous dissolved organic carbon (DOC) often causes lakes to be CO_2-supersaturated (Cole et al., 1994) via increased heterotrophic respiration and photooxidation (Vachon et al., 2017). Hence, allochthonous supply of both DIC and DOC can be important sources of CO_2 in lakes. Allochthonous DOC is often coloured, and thereby reduces light penetration through the water column (Ask et al., 2009). Thus, it is likely that allochthonous DOC, by increasing the CO_2 supply, could indirectly stimulate GPP at low to moderate DOC concentrations where light is sufficient for photosynthesis, but not at high concentrations where light limitation will be pronounced.

In this study, we tested for CO_2 limitation of benthic microalgal GPP in a freshwater system. We collected lake surface sediment (with associated benthic microalgae) and incubated these at different light levels after addition of a direct (DIC) or indirect (CO_2 production via mineralisation of DOC) source of CO_2. We hypothesised that: (1) benthic GPP is CO_2 limited; (2) CO_2 limitation of benthic GPP can be relieved both by a direct and an indirect supply of CO_2; and (3) the response in benthic GPP to CO_2 addition is dependent on light availability.

2 | METHODS

2.1 | Study design

This experiment was conducted in a typical boreal lake in northern Sweden (Supplementary Table S1) during August 2017. Intact lake surface sediment, with the associated benthic community including microalgae and micro- and meiofauna (macrofauna was not visible in any of the samples), was collected from 1–1.5 m depth using a sediment gravity corer. The sediment was soft, fine-grained, and organic-rich with a highly visible layer (*c.* 5–10 mm thick) of benthic epipelic microalgae on top. The top 10 cm of the sediment and *c.* 10 cm (0.5 L) of overlying water were gently transferred into 2-L transparent acrylic incubation chambers of the same inner diameter (8.6 cm, inner area: 58.1 cm^2) as the collection cores. The amount of available light in the incubation chambers is high; 84.4 ± 3.9 and 81.9 ± 5.3 of photosynthetically active radiation (PAR, µmol m^{-2} s^{-1}) outside and inside of the chamber at 0.5 m depth, respectively. The incubation chambers (32 cm height) were thereafter sealed using bottom and top stopper caps to create a closed incubation unit without an air-filled head space. The chambers were divided into one control and two treatment groups with different sources of CO_2. As a direct source, we increased the concentration of DIC in the water by adding 10 ml of a $NaHCO_3$ solution (12 mM DIC). As an indirect source (i.e., via mineralisation of DOC), we added 20 ml of a glucose solution (7 mM DOC). Glucose was used instead of natural allochthonous coloured DOC to avoid the confounding effects of increased nutrients and decreased light availability. We aimed for an approximately 30% increase in DIC and DOC concentrations compared to the ambient lake water concentrations.

Water samples for DIC, DOC, pH, and nutrients (nitrogen and phosphorus) were taken immediately after the addition of DIC or glucose and at the termination of the incubation. DIC was estimated on acidified water using a headspace equilibration technique (Lundin et al., 2013) and gas chromatography (Perkin Elmer Clarus 500). For DOC, 50 ml of water sample was filtered through combusted (550°C, 4 hr), acid-washed Whatman GF/F filters, then acidified (500 µl 1.2 M HCl) and stored at 4°C until analysis. The pH was measured with a pH-meter (MP220, Mettler Toledo International Inc.). Samples for total phosphorous (TP) and nitrogen (TN) were stored in the freezer (–20°C) until analysis. TP concentration was measured according to Murphy and Riley (1962) by using potassium persulfate for digesting the samples, and DOC and TN were analysed using a combustion chamber (IL550 TOC/TN analyser, Hach Lange GmbH). For nitrate (NO_3), ammonium (NH_4), and phosphate (PO_4), 50 ml of water were filtered through combusted (550°C, 4 hr), acid-washed Whatman GF/F filters to 50-ml Falcon tubes and stored in the freezer until photometric flow injection analysis (Gray et al., 2006).

Oxygen loggers (miniDOT loggers: PME, Inc.) were fixed in the incubation chambers to record temperature and dissolved oxygen concentrations (O_2) every 10 min. The loggers were tightly fixed in the cap with only the sensor tip in the water column to minimise space occupancy inside the chamber. The chambers were then attached to a line hanging freely from an anchored surface buoy and incubated in situ at four epilimnion depths (0.5, 1, 1.5 and 2 m; $n = 3$ for each treatment and depth) to generate a gradient in PAR for benthic microalgae mats at the sediment surface in the incubation chambers but keep temperature constant. In situ incubation was chosen to minimise sediment disturbance. Pelagic GPP in the sampled lake is low and only about 6% of benthic GPP

at 0–1 m depth (Hendricus Verheijen, *personal comment*), assuring that the experimental response in this study will be mainly benthic. Due to limited number of loggers, the experiment was split into two incubation periods, the first for 86 hr (0.5 and 1 m depths) and the second for 64 hr (1.5 and 2 m depths). PAR was measured every second day at every 0.5 m between the surface and the lake bottom using a LI-193 Spherical Quantum Sensor (LI-COR Biosciences). The vertical attenuation coefficient in the lake water (k_d, m^{-1}) was calculated as the slope of the linear regression between the natural logarithm of PAR and water depth. A sensor (ALW-CMP S/No. 221 JFE Advantech Co., Ltd.) at 0.5 m depth recorded PAR ($I_{0.5}$) every 20 min during the experiment. From the k_d and $I_{0.5}$ data, we calculated PAR for the 20-min intervals at each incubation depth as:

$$I_z = I_{0.5} e^{-k_d z}, \quad (1)$$

where I_z is the PAR at depth z (m). The total amount of PAR reaching each incubation depth during the incubations (PAR_{tot}, mol m^{-2} day^{-1}) was obtained by summarising all 20 min PAR values from respective depth.

2.2 | Data analysis

Daily benthic GPP (g O_2 m^{-2} day^{-1}), ecosystem respiration (ER; g O_2 m^{-2} day^{-1}) and net ecosystem production (NEP; g O_2 m^{-2} day^{-1}) was calculated from the diel changes in O_2 by inverse modelling and Bayesian parameter estimation following Hotchkiss and Hall (2014), but excluding air–water exchange, as:

$$mO_i = mO_{i-1} + \left(\frac{GPP}{v} \times \frac{PAR_i}{PAR}\right) + \left(\frac{ER}{v} \times \Delta t\right), \quad (2)$$

where mO_i (g O_2/m^3) is the modelled O_2 at time *i* given parameter estimates of GPP, ER, and NEP; *v* is the volume of water in the chambers (0.5 L); and Δ*t* is the time step (10 min). PAR is the total amount of PAR over 24 hr and PAR*i* is PAR at time *i*. The model used a *random walk* metropolis algorithm and Markov chain Monte Carlo sampling in R (Geyer & Johnson, 2013) to find the best fit between measured and modelled O_2 data. Each parameter estimate was derived from 10,000 model iterations after removing an initial 1,000 iterations of *burn-in* from parameter starting values. We checked for convergence of parameter estimates and removed data points (11%) with negative GPP and with poor fits between measured and modelled O_2. Examples of oxygen curves over time are given in Figure S1.

Photosynthesis–irradiance (P-I) curves were fitted using equation 6 in Webb et al. (1974).

$$\text{Benthic GPP} = P_{max}\left(1 - \exp\left(\frac{-\alpha I}{P_{max}}\right)\right), \quad (3)$$

Photoinhibition was assumed negligible for soft-bottom benthic microalgae (Dodds et al., 1999), hence, benthic GPP in this model is a function of maximum photosynthesis at light saturation (P_{max}) and the initial slope of the curve (α), i.e. photosynthesis per light intensity (*I*).

For statistical analyses (in SPSS 20) all response variables were Log_{10} transformed to achieve homogeneity of variances. *T*-tests evaluated the effects of treatment additions on nutrient and chemical variables (DIC, DOC, and pH) before incubation. Two-way ANOVAs tested the effects of treatments, light (i.e., incubation depth) and their interaction on benthic GPP, ER, NEP, DOC, nutrients, and pH. When interaction terms were significant, one-way ANOVAs tested effects of treatments at each light level, as well as effects of light within each treatment group. Tukey post hoc tested significant differences in responses to treatments.

3 | RESULTS

Average (±1 *SD*) k_d in the lake water was 1.4 ± 0.1 m^{-1}. PAR decreased sharply with depth and most of the light was absorbed in the first 1.5 m (Figure 1). Water temperature inside the chambers did not differ between depths (one-way ANOVA: $F_{3,11} = 3.42$, $p = 0.07$) or between the two incubation periods (first: 16.5 ± 0.1°C; second: 16.3 ± 0.1°C; *t*-test: $df = 5$, $t = 2.43$, $p = 0.06$), nor did the average daily total PAR (PAR_{tot}, Figure 1), implying that the use of two incubation periods had minor effects on the results.

The nutrient concentrations in the water column above the sediments remained constant after the carbon additions, during the incubations (i.e., start vs. end), and were independent of interactions between PAR and carbon additions (Tables S1–S3), suggesting that nutrient availability did not confound the results. DIC and DOC concentrations and pH in the control (i.e. in ambient water) at the start of the experiment were 7.4 ± 0.4 mg/L, 7.5 ± 0.3 mg/L, and 7.2 ± 0.1, respectively (mean ± 1 *SD*; $n = 12$). The DIC addition increased the start DIC concentration (to 10.2 ± 0.9 mg/L), decreased pH (to 6.8 ± 0.0), but had no effect on the DOC concentration (Table 1). The DOC addition increased the start DOC concentration (to 12.6 ± 1.7 mg/L), but had no effect on DIC or pH (Table 1). During the incubations, DOC concentrations in the DOC treatment decreased by 0.4 ± 0.1 mg L^{-1} day^{-1} at 0.5 and 1 m depth and by 0.9 ± 0.04 mg L^{-1} day^{-1} for the incubations at 1.5 and 2 m depth, indicating respiration of the added DOC. DOC also decreased in the control and DIC treatment (Table S1), but the decrease was greater in the DOC treatment than in the control (*t*-test 0.5–1 m depth: $df = 5$, $t = -2.83$, $p = 0.04$; *t*-test 1,5–2 m depth: $df = 5$, $t = -9.45$, $p = 0.0002$). During the incubations, there was an overall decrease in pH (Table S1) but with a greater decrease in the DOC than in the DIC treatment compared to the control (Table S3).

Benthic GPP increased with DIC and DOC additions, but the responses were dependent on light availability (Table 2; Figure 2A). At 0.5 and 1 m incubation depths, benthic GPP in the DOC and DIC

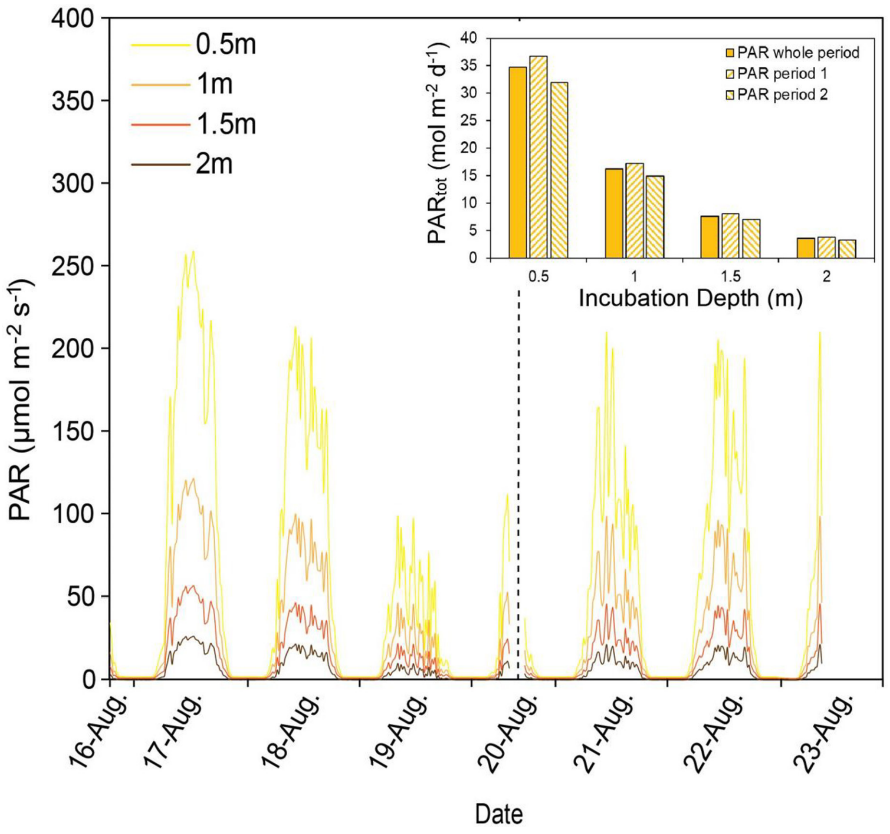

FIGURE 1 Photosynthetically active radiation (PAR) at the different incubation depths during the experiment. The dashed vertical line separates the two incubation periods. The insert shows the average daily total amount of PAR (PAR_{tot}) at each incubation depth for the different incubation periods and over the whole experimental period

TABLE 1 Effects of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) treatments on water chemistry before incubation

	Control vs. DIC treatment		Control vs. DOC treatment	
Variables	***p*-value**	***t*-value**	***p*-value**	***t*-value**
DOC	0.12	−1.65	<0.0001	−9.10
DIC	<0.0001	−11.68	0.39	0.88
pH	<0.0001	18.47	0.33	1.00

Note: Degrees of freedom is 11 for each variable.

treatments were higher than the control, while there was no response to the treatments at 1.5 and 2 m depths (Table 2; Figure 2A). Within each treatment, benthic GPP was also affected by the light availability at the different incubation depths (Table 2; Figure 2A). In both the DOC treatment and the control, there was no difference in benthic GPP between 0.5 and 1 m, but they were both higher than benthic GPP at 1.5 and 2 m (Figure 2A). In the DIC treatment, the highest benthic GPP was at 0.5 m, followed by 1 m, while it was equally low at 1.5 and 2 m (Figure 2A).

Benthic ER was highly correlated to benthic GPP (Control: $r^2 = 0.98$, $p < 0.0001$; DIC treatment: $r^2 = 0.99$, $p < 0.0001$; DOC treatment: $r^2 = 0.98$, $p < 0.0001$), but with some variation in treatment responses (Table 2; Figure 2B). Similar to GPP, benthic ER was stimulated both by DIC and DOC additions. Although ER also decreased with light availability, the carbon additions stimulated ER also in the deep, low light incubations (in contrast to GPP). NEP was positive at shallow (0.5–1 m) depths and close to zero or slightly negative at deeper (1.5–2 m) depths (Table 2; Figure 2C). Although NEP was affected by the carbon additions (Table 2; Figure 2C), the changes were modest.

The estimated P_{max} was highest in the DIC treatment (1.9 g O_2 m^{-2} day^{-1}), followed by the DOC treatment (0.9 g O_2 m^{-2} day^{-1}), and control (0.5 g O_2 m^{-2} day^{-1}; Figure 3). The initial slope of the P-I curve (α) was estimated to be 0.04, 0.05, and 0.03 g O_2 m^{-2} day^{-1} (mol m^{-2} $day^{-1})^{-1}$ in the DIC treatment, DOC treatment, and control, respectively. Even though our estimated P-I curves (Figure 3) were generated from few sampling points, the P_{max} values were similar to those found in other studies (Daniels et al., 2015; Gettel et al., 2013).

4 | DISCUSSION

This study shows that soft-bottom benthic GPP can be stimulated by DIC, either directly (DIC addition) or indirectly following mineralisation of DOC. Since benthic GPP can be substantial in high-light areas of lakes (Puts et al., 2022), and important at the whole-lake scale (Ask et al., 2009; Karlsson et al., 2009), DIC stimulation might be a general, but understudied, feature of northern (including boreal) lakes at low to moderate DOC concentrations.

In the DOC treatment in our study, the pH decreased over the incubation period compared to the control. Since decreased pH induces conversion of HCO_3^- to CO_2, this suggest that the GPP response in the DOC treatments was a result of respiration elevating CO_2 directly as well as indirectly via decreasing pH. Still, given that benthic GPP decreased with increasing incubation depth even when

TABLE 2 Effects of incubation depth (*D*) and treatments (*T*) on benthic gross primary production (GPP), respiration (ER), and net ecosystem production (NEP)

	D		*T*		*D*×*T*	
	F-value $_{(df)}$	*p*-value	*F*-value $_{(df)}$	*p*-value	*F*-value $_{(df)}$	*p*-value
Two-way ANOVA						
GPP	$236.42_{(3,31)}$	<0.0001	$20.64_{(2,31)}$	<0.0001	$6.78_{(6,31)}$	<0.0001
ER	$79.40_{(3,31)}$	<0.0001	$27.42_{(2,31)}$	<0.0001	$6.35_{(6,31)}$	0.001
NEP	$216.59_{(3,31)}$	<0.0001	$11.12_{(2,31)}$	0.001	$20.42_{(6,31)}$	<0.0001
One-way ANOVA						
GPP						
Treatment effect						
0.5 m depth			$35.67_{(2,7)}$	0.001		
1 m depth			$31.38_{(2,8)}$	0.001		
1.5 m depth			$0.54_{(2,7)}$	0.60		
2 m depth			$1.22_{(2,6)}$	0.38		
Depth effect						
Control	$24.06_{(3,8)}$	0.002				
DOC treatment	$137.68_{(3,10)}$	<0.0001				
DIC treatment	$127.39_{(3,11)}$	<0.0001				
ER						
Treatment effect						
0.5 m depth			$9.42_{(2,7)}$	0.02		
1 m depth			$32.04_{(2,8)}$	0.001		
1.5 m depth			$6.26_{(2,7)}$	0.04		
2 m depth			$9.53_{(2,6)}$	0.03		
Depth effect						
Control	$15.36_{(3,8)}$	0.006				
DOC treatment	$82.58_{(3,10)}$	<0.0001				
DIC treatment	$34.53_{(3,11)}$	<0.0001				
NEP						
Treatment effect						
0.5 m depth			$13.79_{(2,7)}$	0.009		
1 m depth			$50.14_{(2,8)}$	<0.0001		
1.5 m depth			$35.17_{(2,7)}$	0.001		
2 m depth			$35.55_{(2,6)}$	0.003		
Depth effect						
Control	$51.24_{(3,8)}$	<0.0001				
DOC treatment	$276.14_{(3,10)}$	<0.0001				
DIC treatment	$77.38_{(3,11)}$	<0.0001				

Note: The differences in degrees of freedom (*df*) are due to the removed data points from the data quality check in the metabolism model.
Abbreviations: DIC, dissolved inorganic carbon; DOC, dissolved organic carbon.

DIC availability was high, stresses the overall importance of light availability for benthic GPP (Ask et al., 2009; Devlin et al., 2016), and shows that surplus DIC cannot be utilised when light is limiting.

Effects of CO_2 on GPP in natural systems may depend on and be obscured by nutrient limitation (Jansson et al., 2012), grazing (Paquette & Beisner, 2018), algal biomass (Spijkerman, 2010), and algal community structure (Hopkinson et al., 2010). In our study, all these factors were probably similar across treatments since the cores were collected in close vicinity to each other. Still, benthic habitats have a high degree of spatial heterogeneity (Fenchel & Glud, 2000) both in terms of sediment characteristics (carbon and nutrient content, and grain type and size) and biomass of benthic primary producers (Vadeboncoeur & Lodge, 2000). This may have induced some initial and background variability in our measures of

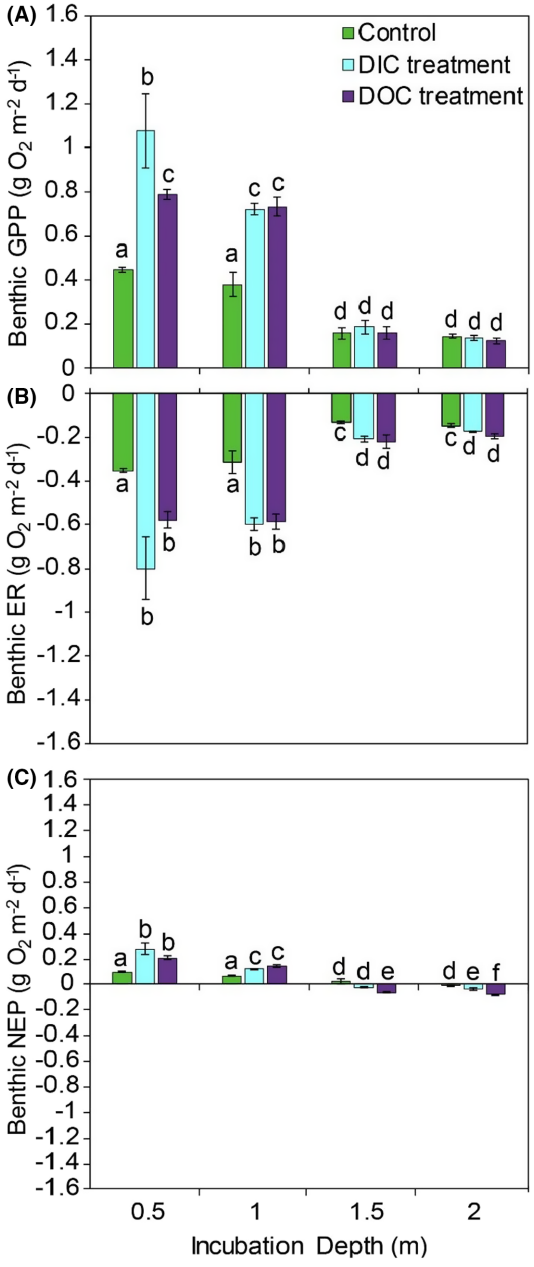

FIGURE 2 Benthic (A) gross primary production (GPP), (B) respiration (ER), and (C) net ecosystem production (NEP) as means ±1 *SD* in control and treatments at the different incubation depths. The letters (a, b, c, d, e, f) inside the panels denote significant differences ($p < 0.05$) between control and treatments as well as between depths. Positive and negative values of NEP indicate a net autotrophic (GPP > ER) or net heterotrophic (GPP < ER) system, respectively, here used to assess the degree of coupling between CO_2 production and consumption. DIC, dissolved inorganic carbon; DOC, dissolved organic carbon

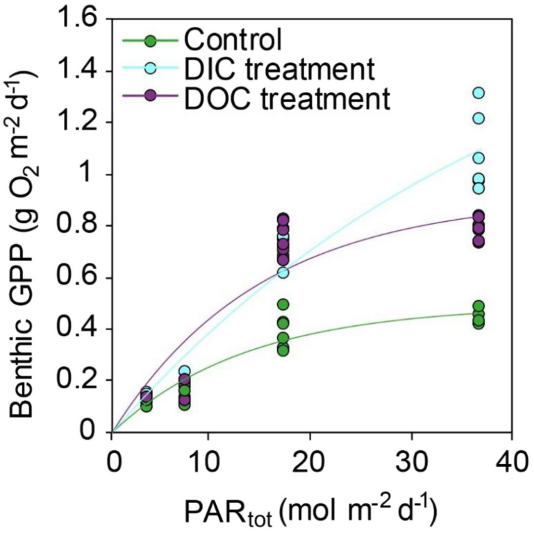

FIGURE 3 Photosynthesis–irradiance curves for control and treatments. The r^2 values for fitted curves are 0.82, 0.83, and 0.92 for control, dissolved organic carbon (DOC) treatment, and dissolved inorganic carbon (DIC) treatment, respectively. GPP, gross primary production; PAR_{tot}, total photosynthetically active radiation

benthic GPP and should, if present, have increased the variation in our measured response variables. However, the low variation within triplicates and the clear treatment responses strongly suggest that the natural benthic heterogeneity did not significantly affect the overall results. Although we cannot rule out the possibility that benthic GPP was nutrient limited (which would have resulted in a weaker response than observed, or no response, to the treatment additions), the strong positive response in benthic GPP to treatments, the fact that soft-bottom benthic microalgae generally obtain nutrients from the nutrient-rich sediments (Bonilla et al., 2005), and the lack of a decline in nutrients in the water above the sediment, suggest that nutrient limitation was unlikely during the extent of our experiment. DIC can also be supplied from sediment pore water or via ground water inflow (Vadeboncoeur & Lodge, 1998); however, this source of DIC was probably minimal, if any, since the sediment inside the chambers were cut-off from ground water input and surrounding sediments. In addition, unlike nutrients, DIC in the overlaying water decreased during the incubation indicating an active uptake and that DIC in the pore water was insufficient for the benthic microalgae in our experimental system. Limitations in general are regulated by the supply of resources necessary for growth and the demand for those resources by the algal biomass pool, implying that limiting substances are not static through space or time. Based on our results, we suggest that the benthic microalgae had enough nutrients supplied from the sediment but were limited by DIC and light. However, since GPP rates and biomass growth operate at different temporal scales (Krause-Jensen & Sand-Jensen, 1998), our results, although relevant for benthic GPP, might not be valid for predicting DIC effects on benthic algal biomass.

In pelagic systems, heterotrophic bacteria can outcompete algae for limiting nutrients when an external carbon source (e.g. allochthonous DOC) is available (Drakare, 2002; Jansson et al., 1999). Bacteria may thus supply algae with CO_2 by mineralising DOC, but at the same time decrease the amount of available nutrients. The net outcome of these processes for GPP in general is virtually unknown. We show a positive effect of DOC addition on benthic GPP,

suggesting that for benthic microalgae, with access to a surplus of nutrients from the sediments, the net role of bacterial metabolism is positive (via production of CO_2) rather than negative (via bacterial nutrient uptake). Interestingly, the DOC addition did not increase ER enough to cause any large decrease in NEP, and the most striking NEP results were that only the two deep incubations had negative NEP values. We interpret this as that, even though the added DOC stimulated ER, the generated CO_2 was largely assimilated by benthic microalgae (i.e., stimulating benthic GPP) and therefore reduced the negative effect of the external DOC supply on NEP. Furthermore, the control incubations were close to metabolic balance and such tight coupling between benthic GPP and ER provide further support for that DIC constrain benthic GPP. The results collaborate with observations of benthic systems in metabolic balance in several northern lakes across a large range of environmental conditions (Ask et al., 2012). In addition, Ask et al. (2012) used a different method with separate light and dark incubations for NEP and ER, respectively, lending support to the conclusions drawn from the method used in this study. Benthic systems in metabolic balance have been attributed to that benthic ER is mainly supported by DOC generated by benthic microalgae, and that respiration of allochthonous DOC is supressed due to poor substrate quality. Our study provides an additional explanation; benthic GPP limited by DIC results in tightly coupled benthic systems where (1) photosynthetic CO_2 fixation will not greatly exceed the respiratory production of CO_2, but also that (2) benthic microalgae will assimilate most of the respired CO_2 and not cause any strong net heterotrophy or net production of CO_2 in benthic systems, provided light is sufficient. Benthic microalgae can generate high amounts of autochthonous DOC, as much as 70% of GPP (Wolfstein et al., 2002; Wyatt et al., 2014), and correspondingly, our mass–balance calculations suggest that a large part of the carbon pool available for respiration in the studied systems is indeed autochthonous (Figure S2).

Although the use of glucose as an external DOC source in our experiment cannot substitute natural allochthonous DOC, the results could help to understand the role of allochthonous DOC for benthic and whole-ecosystem GPP in northern boreal lakes. In particular, the role of allochthonous DOC in providing algae with an additional source of CO_2 via mineralisation is rarely considered and especially not for soft-bottom benthic microalgae. While our results support the general negative impact of light absorption by allochthonous DOC for soft-bottom benthic GPP, they also stress the role of providing an indirect source of CO_2 which stimulates benthic GPP at favourable light conditions. This implies that allochthonous DOC would have a positive effect on benthic GPP in shallow lakes, and in lakes with low to moderate input of allochthonous DOC, while in deep and more DOC rich lakes, light limitation should override any positive effect from elevated CO_2 concentrations and thus constrain benthic GPP. Hence, CO_2 concentrations need to be accounted for to explain variation in benthic GPP at both temporal and spatial scales. Studies on the complex interactions between light, CO_2, and nutrients are thus prerequisites to better understand limitations of benthic GPP and to conceptualise the role of allochthonous DOC in boreal lake ecosystems.

AUTHOR CONTRIBUTIONS

All authors designed the experiment. M.H. and M.J.A. performed the field and lab work. M.H. estimated the metabolism data. The data were analysed by M.H. with help from P.B. and J.A. M.H. wrote the first draft of the manuscript and all authors contributed to the final version.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available by the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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