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Short-term effects of elevated CO₂ on periphyton community in an artificially constructed channel

Hye-Jin Park¹, Dae-Ryul Kwon², Baik-Ho Kim^{3*} and Soon-Jin Hwang^{1*}

Abstract

Background: Direct impact of inorganic carbon (i.e., carbon dioxide (CO₂)) on the periphyton community is important to understand how and to what extent atmospheric conditions can affect the structure and dynamics of these communities in lotic systems. We investigated the influence of elevated CO₂ concentration on the periphyton community in the artificially constructed channels during the winter period. The channels made of acrylic paneling were continuously supplied with surface water discharged from a small reservoir, which was supported with ground water, at a flow rate of 5 L/min, and water temperature ranging 4–5 °C. The effects of elevated CO₂ concentrations (790 ppm) were evaluated in comparison with the control (395 ppm CO₂) by analyzing pH, water carbon content and nutrients in water, periphyton composition and biomass, chlorophyll-*a*, ash-free dry-matter at 2-day intervals for 10 days.

Results: After the addition of CO₂, significant decreases of pH, NH₃-N, and PO₄-P ($p < 0.05$) and increases of chlorophyll-*a*, ash-free dry-matter, and the cell density of periphyton ($p < 0.01$) were observed, whereas the species composition of periphyton and water carbon content did not change.

Conclusions: These results suggest that elevated CO₂ in flowing water system with low temperature could facilitate the growth of periphyton resulting in biomass increase, which could further influence water quality and the consumers throughout the food web.

Background

Carbon dioxide (CO₂) is a major greenhouse gas with the greatest potential to influence global climate change since the industrial revolution, while the CO₂ emission into the atmosphere has been increasing, and its concentration will reportedly double in the next 50–100 years (Houghton et al. 2001). Atmospheric CO₂ concentrations have risen from 295 parts per million (ppm) to 380 ppm over the last 100 years and have contributed substantially to global warming, climate change, and resultant biological extinctions (Lewis and Nocera 2006; Battisti and Naylor 2008). CO₂ emissions vary with seasonal photosynthesis, respiration of plants, and the amount of fossil fuels consumed (IPCC 2007).

In particular, the atmospheric CO₂ concentration during the winter season typically increases due to the reduction of photosynthesis of plants and an increase in active consumption of fossil fuels (Lewis and Nocera 2006; Sayre 2010). Low water temperature during the winter increases CO₂ solubility (Tortell et al. 2008), which may be expected to affect aquatic ecosystems.

CO₂ gas dissociates into HCO₃⁻, CO₃²⁻, and H⁺ ions in water and may enhance algal growth and production. In general, benthic and planktonic algae use bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) as carbon sources (Falkowski and Raven 2007; Giordano et al. 2005). Almost all studies on algal biomass variations with increased CO₂ have been conducted in marine water in response to a volcanic eruption (Hall-Spencer et al. 2008; Johnson et al. 2011). In contrast, few studies have been performed regarding the impact of increased CO₂ on periphyton structure and function in freshwater ecosystems. One exception is a study that assessed these

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variables on the fish community (Ross et al. 2001). In the freshwater system, the amount of organic carbon supply frequently exceeds inorganic carbon, particularly at high altitudes, because streams receive carbon as particulate organic matter such as dead leaves. These leaves are processed by fungi, bacteria, and benthic macroinvertebrates (Biggs et al. 1998; Tuchman et al. 2002).

Studies considering the direct impact of inorganic carbon (i.e., CO_2) on the periphyton community are important to understand how and to what extent atmospheric conditions can affect the structure and dynamics of these communities. Particularly, in a flowing water system, the periphyton community relies on various physicochemical factors as well as other aquatic organisms. Thus, even a small disturbance in CO_2 can considerably affect the abundance and species composition of periphyton (Hillebrand and Sommer 2000; Villeneuve et al. 2010).

The present study aimed to elucidate the impact of elevated CO_2 concentrations on biomass and species composition of periphyton in an aquatic environment with low temperature, high solubility of CO_2 , and low photosynthesis.

Methods

Experimental design

An artificial channel was made of acrylic paneling ($10\text{ cm} \times 300\text{ cm} \times 20\text{ cm}$), which opened on the topside, and a separate space ($10\text{ cm} \times 10\text{ cm} \times 20\text{ cm}$) that included inlets and outlets for flowing water. Four artificial channels were prepared—two to serve as a control group (no CO_2 addition) and two to serve as a treatment group (CO_2 addition). As a substrate for periphyton colonization, granite was used due to its prevalence in stream soil in

Korea. Fifteen pieces of rectangular granite stones ($10\text{ cm} \times 10\text{ cm} \times 5\text{ cm}$) were scattered on the bottom of each artificial channel (Fig. 1). After setting the substrate, ambient water was supplied for 4 days to build the periphyton community. In a preliminary test, the growth of periphyton nearly reached the stationary phase or mature stage in approximately 8 days, and the majority of the pale green-colored biofilm abruptly underwent destruction at 13–14 days.

The experiment was conducted in the artificial channel for 3 weeks in December when the water temperature was 4–5 °C (2012). The experimental water was transported by an electric pump from a small reservoir to a tank in the laboratory (2 t). The reservoir was located near the laboratory at Konkuk University ($36^\circ 00' \text{ N}$, $140^\circ 02' \text{ E}$). It was 55,661 m^3 in volume with depths of 1.5–2.5 m. This reservoir was ice-covered during the study. For the study, we used the running water from the discharging point of the reservoir. Water was supplied to two buckets (150 L) where CO_2 was injected and pumped into the inlets of each artificial channel. The water flow was controlled at 5 L/min. The atmospheric CO_2 concentration (control group) was automatically measured with a portable meter (Model CGP-1: DKK-TOA, Tokyo, Japan) near the laboratory, and was approximately 395 ppm during the experimental period. For the treatment group, CO_2 was injected to yield a twofold higher concentration—790 ppm. CO_2 gas was purchased from a domestic company (Donghwa Industrial Gas Co. Ltd., Seoul, Korea). Cool-white fluorescent lamps were installed 1 m above the upper part of the artificial channel to deliver a light intensity of $50\ \mu\text{mol}/\text{m}^2/\text{s}$ and a 10-h light and 14-h dark photo-period.

During the experiment, water quality factors including water temperature, electric conductivity, pH, suspended

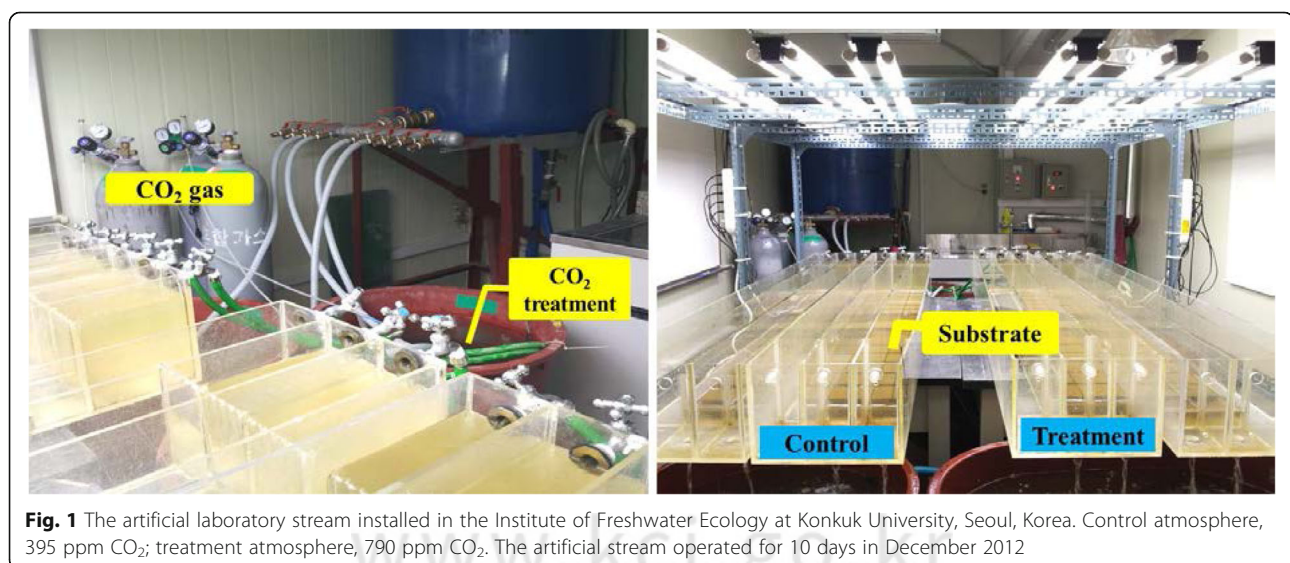


Fig. 1 The artificial laboratory stream installed in the Institute of Freshwater Ecology at Konkuk University, Seoul, Korea. Control atmosphere, 395 ppm CO_2 ; treatment atmosphere, 790 ppm CO_2 . The artificial stream operated for 10 days in December 2012

solids (SS), total carbon, total inorganic carbon, total organic carbon (TOC), chlorophyll (Chl)-*a*, nutrient contents (nitrogen and phosphorus), ash-free dry-matter (AFDM), and periphyton (biomass and species composition) were examined to compare the differences between the control and treatment groups after exposure to CO₂.

Analysis of water quality

Sampling and analysis were performed at 2-day intervals for 10 days and, thus, six times total including day 0. Water temperature, pH, and electric conductivity were measured with a multi-probe detector (YSI 600QS-O-M, YSI Inc., Yellow Springs, OH, USA) at the buckets located at the outlets of each artificial channel. Total carbon, total inorganic carbon, and TOC were measured with a TOC analyzer (Shimadzu, Kyoto, Japan) after injecting CO₂. Chl-*a*, suspended solids, and nutrients were also analyzed. To analyze Chl-*a* concentrations, a water sample was filtered through Whatman GF/F filter paper (Whatman International Ltd., Maidstone, UK) and extracted in the dark at 4 °C for 24 h with 10 mL of 90 % acetone. Then, the sample was centrifuged for 20 min at 1650 rpm (VS-5000 N, Vision Scientific, Seoul, Korea) (APHA 2005) and the supernatant was measured with a spectrophotometer (Optizen 2010 UZ, MECASYS Inc., Seoul, Korea). Suspended solids were measured by first filtering 200 mL of experimental water that had passed through the artificial channel (APHA 2005). The difference was measured between the initial weight (A) and final weight (B) of the filter dried in an oven at 105 °C for 24 h (OF-11, JEIO Tech Inc. Seoul, Korea). The final weight of the filter was measured with the GF/C filter. Nutrients were analyzed by following methods: NO₂-N and NO₃-N by the ultra-violet visible spectrophotometry method, NH₃-N by the indophenol method, total nitrogen (TN) by the absorption metric method, and PO₄-P and total phosphorous (TP) by the ascorbic acid method (APHA 2005).

Analysis of periphyton

The periphyton were sampled by brushing the complete surface of the substrates in each artificial channel. Parts of the sample were used to analyze Chl-*a* and AFDM, and the others were fixed in Lugol solution to identify the species. Chl-*a* formed in the substrate was analyzed by applying the same methods as mentioned previously. The amount of AFDM was obtained from the measured weight difference between A1 (the weight of the sample filtered through the GF/F filter bed and dried in an oven at 105 °C for 24 h) and A2 (the weight of the filter GF/F bed burnt for 2 h in a furnace). Species identification of periphyton were conducted with a ×400–1000 light microscope (Zeiss, Zena, Germany). The abundances of

periphyton were expressed as cell number per area (cells/cm²). As representative references, Patrick and Reimer (1966), Krammer and Lange-Bertalot (1991a, 1991b, 2007a, 2007b), and Chung (1993) were used to identify the periphyton species.

Statistical analysis

Student's *t* test was used to compare differences in water quality variables, relative abundance of periphyton, and species composition between control (without CO₂ addition) and treatment (with CO₂ addition) groups (SPSS v. 18.0.0; SPSS Inc., Chicago, IL, USA) at a *p* < 0.05 significance level.

Results and discussion

Water temperature was 4–5 °C during the experimental period (Table 1). The pH of the water was similar between the groups before injecting CO₂ but decreased significantly in the treatment group compared with the control group over the course of the experiment (*p* = 0.026; Table 1 and Fig. 2). The decrease in pH over time, likely caused by CO₂ addition, is consistent with findings from Min et al. (2011). The electric conductivity, Chl-*a* in the water, and suspended solids were not significantly different between experimental groups (Table 1).

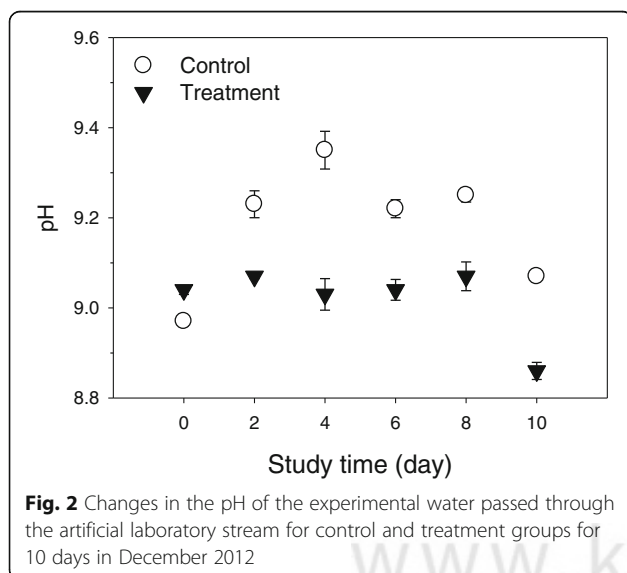
The majority of nutrients (i.e., NO₂-N, NO₃-N, NH₃-N, TN, PO₄-P, and TP) showed a slight decrease in the treatment group compared to the control group, but the difference was not significant (Fig. 3 and Table 1). However, the NH₃-N concentration was significantly lower in the treatment group compared to the control group after increasing CO₂ (*p* = 0.042, Table 1). This result suggests that the increase in CO₂ increased the growth rate of algae that, in turn, readily use NH₃-N. Total phosphate was lower in the treatment group than in the control group 4 days after the CO₂ injection (Fig. 3). PO₄-P showed the largest difference (38 %) in average concentration (1.3 µg/L in the control group and 8 µg/L in the treatment group). Meanwhile, total organic carbon concentrations in the treatment group were slightly higher than the control group, but the differences were not significant (Table 1). Of total carbon, the proportion of total inorganic carbon (IC) and total organic carbon (TOC) was 73 and 27 % in the control group and 70 and 30 % in the treatment group, respectively. These similar ratios suggest that the effect of added CO₂ on carbon content and composition was relatively low.

The periphyton concentrations of Chl-*a* and AFDM were significantly higher in the treatment group than in the control group (*p* < 0.001, Table 1 and Fig. 4). Chl-*a* in the treatment group (6.75 µg/cm²) was 28 % higher than that of the control group, while AFDM was also higher (1.29 mg/cm²) in the treatment group than the control

Table 1 Mean values of experimental parameters in the artificial laboratory stream with control (2 × 6) and treatment groups (2 × 6)

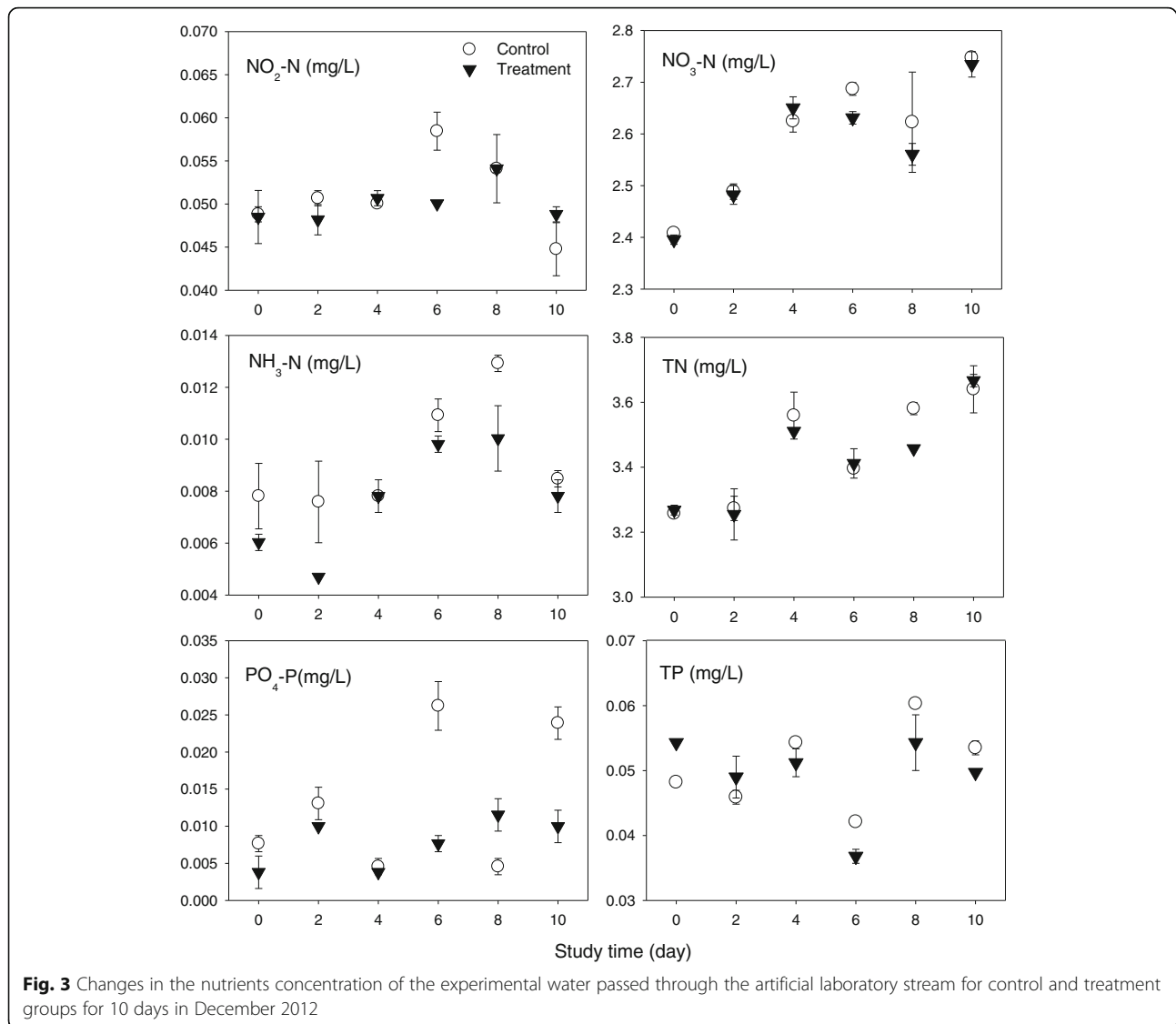
Experimental parameters		Control	Treatment	<i>p</i>
Water	Temperature (°C)	4.8 ± 0.2	4.8 ± 0.2	0.235
	pH	9.18 ± 0.06	9.02 ± 0.03	0.026
	Electric conductivity (µS/cm)	267.8 ± 2.7	268.0 ± 2.8	0.611
	Chlorophyll- <i>a</i> (µg/L)	85.12 ± 11.42	88.99 ± 11.56	0.332
	Suspended solid (mg/L)	23.33 ± 1.22	23.08 ± 3.53	0.932
	NO ₂ -N (µg/L)	51.00 ± 0.40	50.00 ± 0.20	0.550
	NO ₃ -N (mg/L)	2.60 ± 0.05	2.58 ± 0.05	0.184
	NH ₃ -N (µg/L)	10.00 ± 0.20	8.00 ± 0.20	0.042
	Total nitrogen (mg/L)	3.45 ± 0.07	3.43 ± 0.06	0.367
	PO ₄ -P (µg/L)	1.30 ± 0.90	0.80 ± 0.30	0.258
	Total phosphorus (µg/L)	51.00 ± 0.20	49.00 ± 0.30	0.490
	Total organic carbon (mg/L)	5.34 ± 0.34	5.68 ± 0.19	0.319
	Inorganic carbon (mg/L)	14.66 ± 0.64	13.49 ± 0.64	0.112
	Total carbon (mg/L)	20.00 ± 0.65	19.17 ± 0.52	0.253
Substrate	Periphyton chlorophyll- <i>a</i> (µg/cm ²)	5.28 ± 1.29	6.75 ± 1.35	0.008
	Periphyton ash-free dry-matter (mg/cm ²)	1.09 ± 0.15	1.29 ± 0.15	0.000
	Periphyton density (10 ⁶ cells/cm ²)	4.2 ± 0.7	5.6 ± 0.9	0.003
	<i>Fragilaria capucina</i> var. <i>gracilis</i> (10 ⁶ cells/cm ²)	3.2 ± 0.5	4.3 ± 0.7	0.008
	<i>Fragilaria capucina</i> (10 ⁶ cells/cm ²)	0.8 ± 0.2	1.0 ± 0.3	0.095
	<i>Cryptomonas ovata</i> (10 ⁴ cells/cm ²)	5.9 ± 0.5	7.9 ± 0.8	0.016
	<i>Scenedesmus quadricauda</i> (10 ⁴ cells/cm ²)	5.3 ± 0.7	5.0 ± 0.8	0.706
	<i>Aulacoseira ambigua</i> (10 ⁴ cells/cm ²)	3.2 ± 0.6	4.2 ± 0.8	0.468

The artificial stream was operated for 10 days in December 2012. The control stream functioned under atmospheric CO₂ (395 ppm) and the treatment group functioned under added CO₂ (790 ppm)



group (1.09 mg/cm²). Over time, diatoms appeared frequently, comprising about 95 % among all periphyton in both experimental groups. However, cyanobacteria were not observed, perhaps due to the low temperature. The mean cell density of periphyton in the treatment group (5.6×10^6 cells/cm²) was significantly higher than in the control group (4.2×10^6 cells/cm²) ($p = 0.003$, Table 1 and Fig. 5). The major species in both experimental groups were *Fragilaria capucina* var. *gracilis* (Oestrup) Hustedt, *F. capucina* Desmazières, *Cryptomonas ovata* Ehrenberg, *Scenedesmus quadricauda* (Turpin) Brébisson, and *Aulacoseira ambigua* (Grunow) Simonsen.

The present study indicates that the biomass of periphyton slightly increased with a twofold increase in CO₂, but no apparent change was observed in species composition. A recent study demonstrated that a twofold increase in atmospheric CO₂ doubled primary productivity (Schippers et al. 2004). Additionally, Levitan et al. (2007) found a 1.5–3-fold increase in cyanobacteria biomass with a twofold increase in CO₂ from 250 to 500 ppm under constant respiration and photosynthesis. Other studies also showed an increase in algal



density including diatoms with an increase in CO₂ concentrations (Biswas et al. 2011). Johnson et al. (2011) also observed an increase in the amount of pennate diatoms up to sevenfold at 592 and 1611 μatm compared to 419 μatm of CO₂. According to Tortell et al. (2008), *Chaetoceros* spp., which has large cells and forms chains, became dominant and the density of the small-celled *Pseudo-nitzschia subcurvata* decreased rapidly when the CO₂ concentration was increased to 800 ppm.

In contrast, increased CO₂ did not affect algae growth in other studies (Goldman 1999; Tortell et al. 2000, Suffrian et al. 2008). Hargrave et al. (2009) studied the impact of increased CO₂ on periphyton in streams and reported a temporary yet significant difference in primary productivity between 360 and 720 ppm CO₂.

However, they did not see significant changes in biomass or community composition of periphyton. Fu et al. (2007) also reported that *Synechococcus* is sensitive at 380 and 750 ppm CO₂ and appeared stagnant. In the present study, it was presumed that changes in algal biomass were observed due to changes in CO₂. The effects could have been indirect because the winter leads to low levels of nutrients and low light availability, but these effects are also associated with a rise in CO₂ solubility (Hein and Sand-Jensen 1997).

Differences in carbon acquisition efficiency during carbon fixation by algal species influences interspecific competition; thus, increased CO₂ might cause a change in the composition of the algal community (Rost et al. 2003; Fu et al. 2007; Trimborn et al. 2009). The cell membranes of diatoms have high CO₂ permeability, and

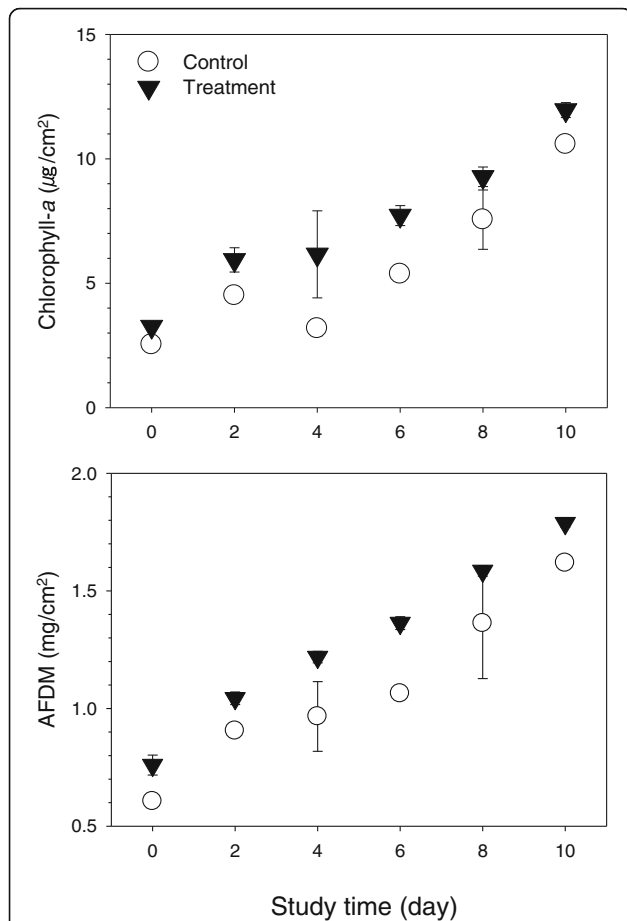


Fig. 4 Changes in periphyton chlorophyll-*a* and ash-free dry-matter (AFDM) on the substrates in the artificial laboratory stream for control and treatment groups for 10 days in December 2012

CO₂ diffusive exchange between cells and the external environment is very high (Hopkinson et al. 2011). Biswas et al. (2011) reported that the algal growth rate doubled when CO₂ pressure increased from 225 to 646–1860 µatm, and the dominant species shifted from

diatoms to cyanobacteria. In this study, no changes were observed in periphyton species composition, perhaps due to the use of cold water with low species richness.

The experimental water in the present study was essentially lake water, not stream water, because the ambient water used was discharged from an outlet of a small reservoir. Also, in this study, we extended the commonly used lake-river hybrid systems or systems of rivers fed by lentic systems. Thus, our results could be useful to understand the dynamics of periphyton or meroplanktonic algae in such aquatic ecosystems. The increased CO₂ could increase the amount of dissolved inorganic carbon in stream water, facilitate the growth of periphyton, and thereby further influence biomass and community composition of macro-invertebrates and fish throughout the food web. Periphyton can also raise the relative rate of carbon assimilation compared to nutrient uptake, which could reduce the nutritional quality of algae for other organisms. This, in turn, can influence the community composition of macro-invertebrate consumers (Hargrave et al. 2009).

Conclusions

Our results clearly showed that the increased CO₂ in low-temperature running water enhanced periphyton development (i.e., biomass, Chl-*a*, and AFDM), but did not shift species composition. In addition, water contents of ammonia and phosphate decreased after addition of CO₂, perhaps due to the increase in the periphyton biomass. However, it remains unknown how CO₂ concentrations may affect the periphyton community in high-temperature conditions. Thus, more research is needed to generalize the impact of CO₂ on various running water systems, including streams with different dimensions and localities (Finlay et al. 1999; Finlay 2003, 2004).

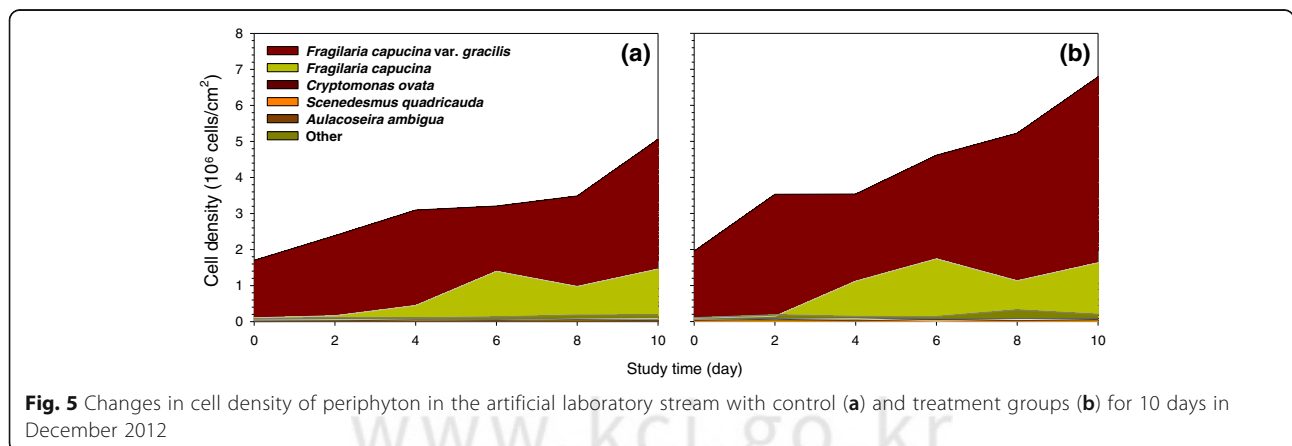


Fig. 5 Changes in cell density of periphyton in the artificial laboratory stream with control (a) and treatment groups (b) for 10 days in December 2012

Abbreviations

AFDM: Ash-free dry-matter; Chl-*a*: Chlorophyll-*a*; IPCC: Intergovernmental Panel on Climate Change; SS: Suspended solids; TN: Total nitrogen; TOC: Total organic carbon; TP: Total phosphorus

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Availability of data and materials

The data in our work will not be shared with a reason that there are no any particular data base and material to uniquely support the work; data and supporting materials are straightforward.

Authors' contributions

HJP performed the experiment and wrote the manuscript. DRK assisted in the experiment and the data analysis. BHK constructed the experimental channel and assisted in the experimental design and the manuscript preparation. SJH supervised the research and assisted in the data interpretation and the manuscript preparation and revision. All authors read and approved the final manuscript

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethical approval and consent to participate

Not applicable.

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