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RESEARCH ARTICLE

Impact of Dissolved Organic Carbon and Temperature on Phytoplankton Biomass in an Outdoor Study

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ABSTRACT

Altered environmental conditions in aquatic ecosystems through Dissolved Organic Carbon (DOC) input and increased temperature influence phytoplankton communities. To assess the impacts of climate change as both single and combined effects of DOC inputs and temperature on phytoplankton growth, we conducted an outdoor experiment including four sets of conditions in triplicate: a) control, b) DOC treatment, c) temperature treatment, and d) DOC and temperature treatment. We added 10 mg/L of glucose ($C_6H_{12}O_6$) as a source of DOC. The warming treatment increased the water temperature by 3°C above the control (p-value < 0.05). The DOC treatment significantly affected pH, CO_2, and DOC (p-values = 0.004, 0.01, and 0.003, respectively). Dissolved oxygen was impacted by both DOC treatment (p-value = 0.0003) and temperature treatment (p-value = 0.003), whereas NO_3^{-1} and PO_4^{-3} were unaffected by either (p-value $>$ 0.05). Both DOC and warming treatments significantly changed the physicochemical properties of the water, influencing phytoplankton biomass. The phytoplankton biomass(chl-*a*) showed significant positive correlations with temperature (r = 0.5, p-value = 0.0002), CO_2 (r = 0.64, p-value < 0.05), DOC (r = 0.6, p-value < 0.05), NO_3^{-1} (r = 0.5, p-value < 0.05), and PO_4^{-3}(r = 0.5, p-value = 0.0001), while being negatively correlated with pH (r = –0.5, p-value = 0.001). Dissolved oxygen also showed a positive correlation with phytoplankton biomass (r = 0.63, p-value < 0.05). We concluded that increasing DOC input is crucial for mitigating the negative effects of warming on phytoplankton growth, as DOC provides a primary source of CO_2 necessary for photosynthesis.

Keywords: Aquatic environment, Biomass, Dissolved organic carbon, Phytoplankton, Temperature

Introduction

Phytoplankton are critically important in freshwater ecosystems, serving as primary producers by supplying essential nutrients and energy to these systems.[1] These organisms can feed themselves by converting chemical energy into organic matter through photosynthesis.[2] Although comprising less than 1% of the Earth's total photosynthetic biomass, phytoplankton produce nearly 50% of the world's net primary productivity, making them crucial to ecological food chains.[3] Phytoplankton communities are dynamic, responding to physical and chemical changes, as well as nutrient inputs, which makes them highly sensitive to environmental variations. Previous research in freshwater ecosystems has shown that phytoplankton composition, community structure, species diversity, and abundance are influenced by multiple environmental factors, including temperature, light availability, key nutrients for algae, pH levels, and zooplankton grazing.[4–7] Warming impacts these factors both directly and indirectly, leading to complex, overlapping effects.[8] Temperature influences phytoplankton community structure through direct effects on organism physiology and metabolic processes such as photosynthesis and

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respiration.[9] Rising temperatures can promote community growth only if nutrients are available, as they may increase bacterial mineralization and photomineralization rates.[10] Higher temperatures can also enhance nutrient use efficiency,[11] resulting in increased phytoplankton growth until an optimal temperature is reached.[12] Conversely, high temperatures may reduce autotrophs' efficiency in carbon fixation.[13] Numerous studies have linked rising temperatures to changes in community structure, often resulting in dominance by a limited set of species with smaller cell sizes, which decreases phytoplankton diversity.[14] Dissolved organic carbon (DOC) significantly influences the food web and ecosystem functions.[15] DOC supports heterotrophic bacteria that depend on organic carbon for energy.[16] Microorganisms decompose DOC into CO_2, raising its levels.[17] Elevated nutrient and CO_2 levels promote phytoplankton growth.[18] CO_2 is the primary inorganic carbon taken up by producers, stimulating photosynthesis.[19] Many studies indicate that the relationship between DOC concentrations and phytoplankton biomass involves trade-offs between light availability and nutrients; as DOC increases, it can negatively impact phytoplankton biomass because DOC absorbs light, hindering photosynthesis and decreasing growth rates, which leads to changes in community composition and productivity.[20–23] Additionally, high DOC levels can create competition between bacteria and phytoplankton, with bacteria becoming more efficient at nutrient absorption, thereby inhibiting phytoplankton growth,[24] resulting in a shift toward mixotrophic species,[25] which can cause oxygen depletion and negatively affect aquatic life.[26] The Tigris River is a vital source of surface water, supporting diverse habitats for various organisms, including fish and plankton.[27] Our current study aims to test the hypothesis that dissolved organic carbon input influences phytoplankton growth across a wide range of temperatures.

Material and methods

To investigate the impact of dissolved organic carbon (DOC) inputs on freshwater phytoplankton growth during ongoing global warming, we conducted an outdoor study spanning 21 days, from December 7 to December 28. We used 12 clean plastic enclosures of 20 liters each to simulate four sets of conditions in triplicate: a) Control (C), b) treated with DOC (DOC), c) subjected to heating (Temperature(T)), and d) treated with both DOC and heating (DOC + Temperature(T)). Enclosures were spiked with 10 mg/L glucose ($C_6H_{12}O_6$) to achieve the desired DOC levels.

The water was heated to 3°C above the average temperature of the control enclosures using an electrical system that included heat exchangers and was equipped with sensors to monitor and achieve the target temperature. Additionally, each enclosure was equipped with an oxygen supply device that was operated at regular intervals during the experiment Fig. 1. The enclosures were filled with fresh water from the Tigris River, and samples were collected every two days from all enclosures to examine the chemical and physical parameters: water temperature (W.T), pH, dissolved oxygen (DO), carbon dioxide (CO_2), nitrate (NO_3^{-1}), reactive phosphate (PO_4^{-3}), dissolved organic carbon (DOC), and Chl-a in both the control and treated enclosures.

Outdoor experiment work

A portable pocket thermometer was used to measure the water temperature (WT). A pen-type pH meter (pH-009(I)A) was used to determine the pH. The instrument was calibrated with standard solutions of pH 4, 4,7, and 9 before testing. The dissolved oxygen levels in the water were measured with a pen oxygen analyzer (Do91Do). Initially, the device was calibrated using 100% atmospheric oxygen. Then, the sensor was placed in the water, and the reading was recorded once it stabilized. The dissolved oxygen results are reported in mg/l. To determine the CO2 concentration, the titration method[28] was used, which involved titrating a 100 ml water sample with a 0.2N sodium carbonate (Na_2CO_3) solution until the pH reached 8.4. Next, an additional 100 ml was titrated with 0.1N hydrochloric acid (HCl) until the pH dropped to 4.2. After these steps, the total CO_2 in the 100 ml water sample was calculated using the provided Eq. (1).

$$CO_2 = (A + B \times 4.4) \times 10) \quad (1)$$

(Where A Volume of titration with (0.2N) Na_2CO_3, and B Volume of titration with (0.1N) HCl. The results are expressed in (μM).

Laboratory measurements

Dissolved organic carbon (DOC) was estimated by acidifying and filtering water samples, followed by chemical oxygen demand (COD) analysis and conversion to DOC using Plutocalc software.[29] Nitrate (NO_{-3}^{-}) was measured spectrophotometrically after adding HCl.[30] Reactive phosphate (PO_4^{3-}) was analyzed using the ascorbic acid method with absorbance measured at 860 nm.[31] Chlorophyll a concentration (phytoplankton biomass) was determined by filtering

(a) **(b)**

Fig. 1. Photos illustrating outdoor experiment: (a) first day of study, (b) during the treatment period.

1000 ml of sample through a 0.45 μm filter, followed by pigment extraction in 90% acetone in the dark for 18–20 hours. Spectrophotometric readings were then taken at 665 and 750 nm, before and after acidification with hydrochloric acid. Chl-*a* concentrations were determined using Eq. (2):

$$\mu g\ \text{Chl}-\text{a per sample} = 11.9\ [2.43\,(\text{Db}-\text{Da})]\ (\text{V/L}) \quad (2)$$

Where Da = the optical density of the chlorophyll extract after adding acid. Db = the optical density of the chlorophyll extract before adding acid. V = the volume of acetone used in the extraction. L = the length of the photocell (cm).[32]

Statistics analyses

All variables were analyzed using SPSS software. A t-test was performed to estimate the differences between treatments. Pearson's correlation coefficient (r) was used to show the relationship between phytoplankton biomass and physicochemical parameters. Linear regression analysis was conducted to assess the significance of these correlations. Additionally, the means and standard deviations for all measured parameters were calculated.

Results and discussion

Water temperature ranged from (5.16 ± 0.09) °C on the 10th day for control enclosures to (17.5 ± 0) °C on the 7th day for warming treatment (T) enclosures, as well as dissolved organic carbon in warming treatment (DOC + T) enclosures Fig. 2a. Warming treatment significantly affected water temperature (t-test; t = –46.24, $p < 0.05$), while DOC treatment did not have an effect (t-test; t = –0.68, p = 0.5).

pH ranged between (7.5 ± 0.14) on the 19th day to (9± 0) on the 4th day for control enclosures Fig. 2b. Warming treatment did not affect pH (t-test; t = –0.7, p =0.4), while DOC treatment affected pH significantly (t-test; t = 3.32, p = 0.004).

Dissolved oxygen (DO) ranged from (5.4 ± 0.008) on the 22nd day for T enclosures to (10.13 ± 0.3) on the 10th day for DOC + T enclosures Fig. 2c. Warming treatment significantly affected dissolved oxygen (t-test; t = 3.5, p = 0.003), and DOC treatment also significantly affected dissolved oxygen (t-test; t = –5.79, $p < 0.05$).

CO_2 ranged between (0.63 ± 0.37) on the 13th day for control enclosures to (272.8 ± 21.8) on the 16th day for (DOC + T) enclosures Fig. 2d. Warming did not affect CO_2 (t-test; t = 0.91, p =0. 3), while DOC treatment significantly affected CO_2 (t-test; t = –2.92, p =0.01).

DOC ranged between (132 ± 24.8) on the 7th day for T enclosures to (11.5 ± 0.86) on the 13th day for DOC + T enclosures Fig. 2e. Warming did not affect DOC (t-test; t = 0.17, p = 0. 8), while DOC treatment significantly affected DOC (t-test; p = –3.22, p = 0.003). NO_3^{-1} ranged between (0) on the 22nd day for T enclosure and DOC T enclosures to (3.21 ± 0.2) on the 1st day for DOC + T enclosures Fig. 3a. Warming did not affect NO_3^{-1} (t-test; t = –0.02, p = 0. 9), and DOC treatment did not affect NO_3^{-1} (t-test; t = 1.84, p = 0.08). PO_4^{-3} ranged between (0) on the 1st day in all enclosures to (0.43± 0.41) on the 13th day for control enclosures Fig. 3b. Warming did not affect PO_4^{-3} (t-test; t = 0.8, p = 0. 4), and DOC

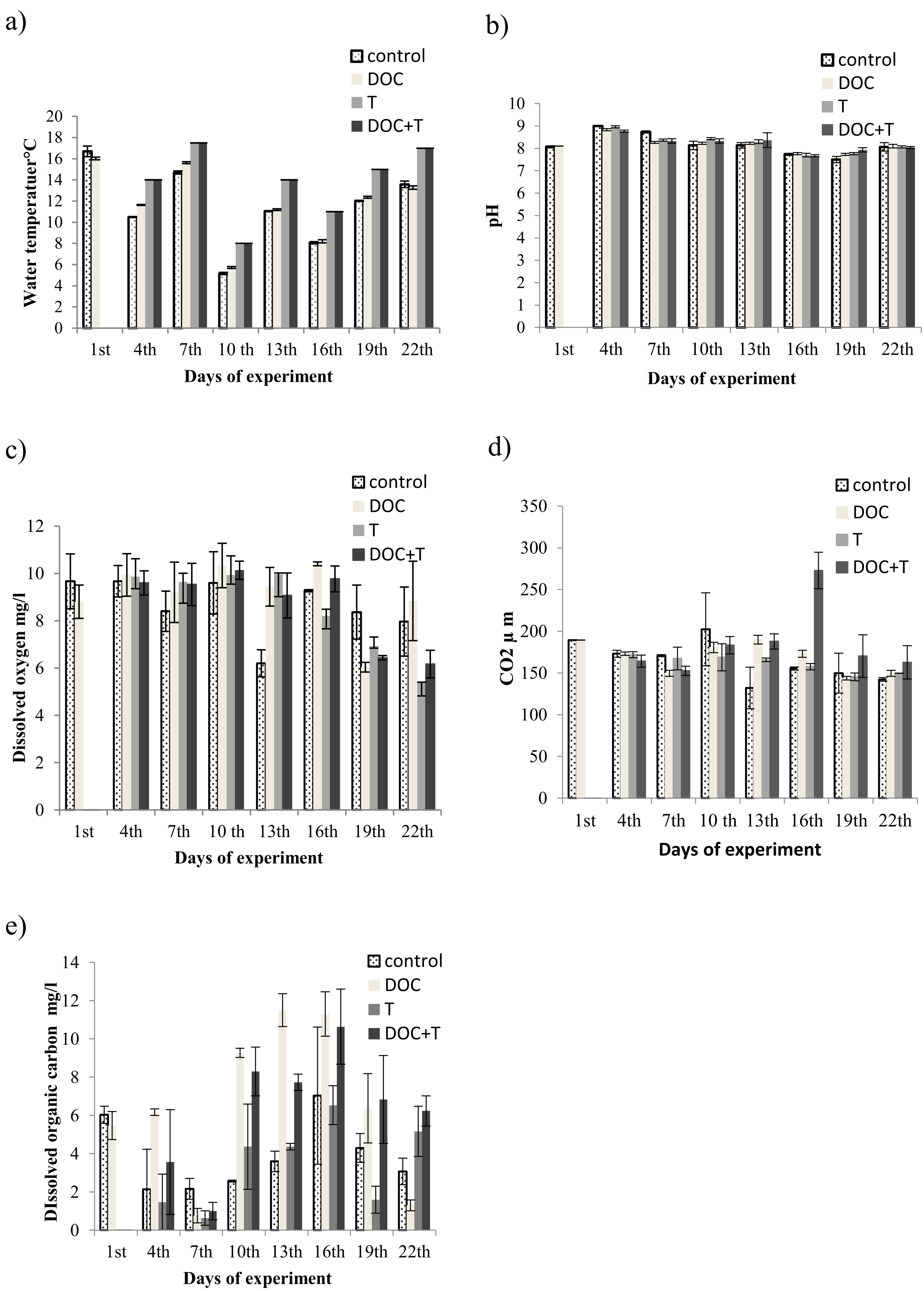

Fig. 2. Physical and chemical parameters (Water temperature, pH, DOC, CO2, dissolved oxygen during study period.

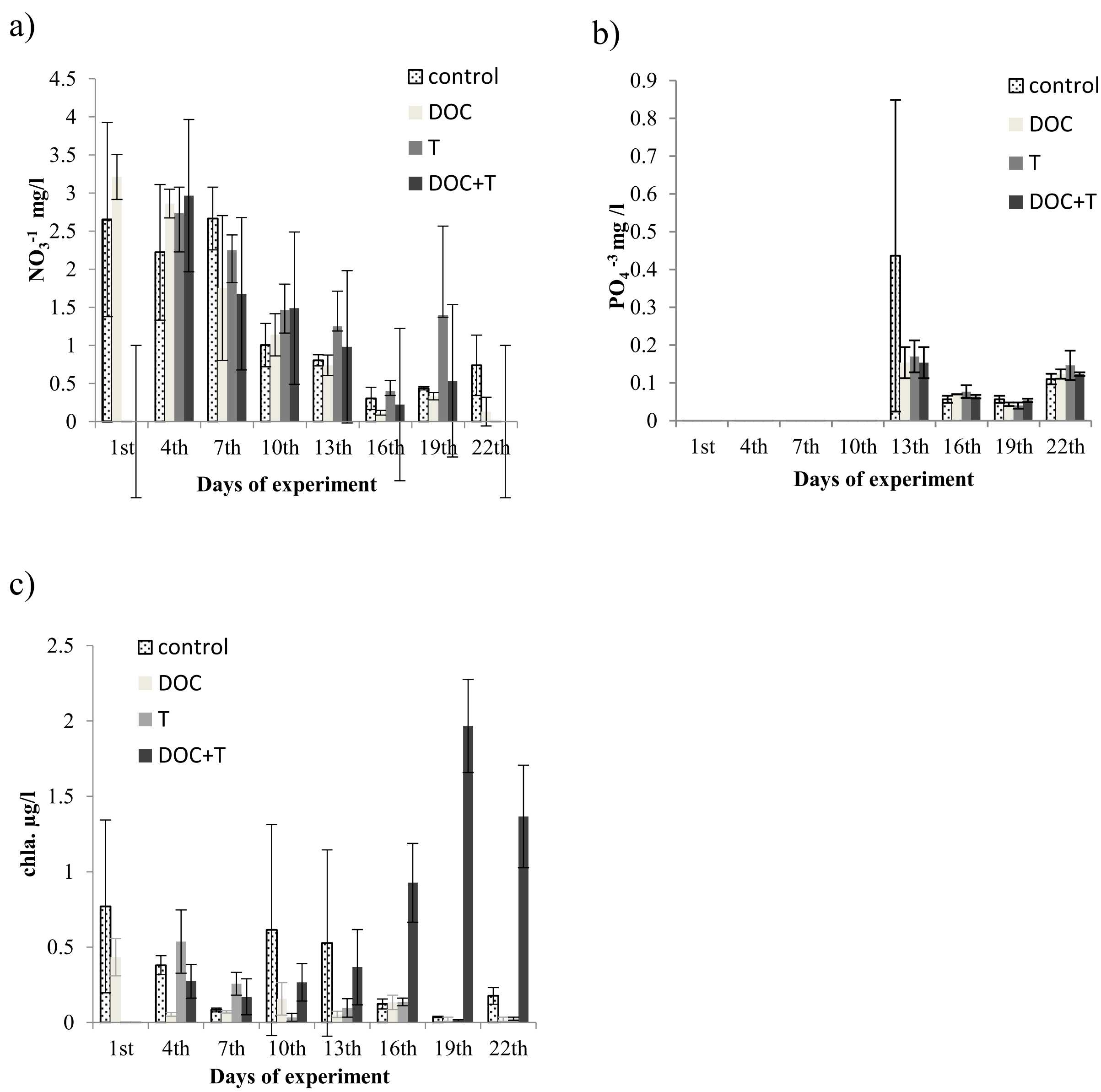

Fig. 3. Changes in Nutrient and phytoplankton biomass during the study period.

treatment did not affect PO_4^{-3} (t-test; t = 0.9, p = 0. 3). Chl-a ranged between (0.014± 0.004) on the 19th day for T enclosures to (1.96 ± 0.3) on the 19th day for DOC + T enclosures Fig. 3c. Warming did not affect Chl-a. (t-test; t = 1.20, p = 0 2), while DOC treatment significantly affected Chl-a (t-test; t = 2.46, p = 0.02). Phytoplankton biomass (Chl-a concentration) was affected by water temperature (r = 0.5 and p-value = 0.0002) Fig. 4a, pH (r = –0.5 and p-value = 0.001) Fig. 4b, CO_2 (r = 0.64 and p-value < 0.05) Fig. 4c, DOC (r = 0.6 and p-value < 0.05) Fig. 4d, NO_3 (r = 0.5 and p-value < 0.05) Fig. 5a, and PO_4^{3-} (r = 0.5 and p-value = 0.0001) Fig. 5b. Dissolved oxygen was affected by chl-a. Concentration (r = 0.63 and p-value,0.05) Fig. 5c.

The study demonstrated that treatment with glucose as a source of dissolved organic carbon (DOC) and temperature (T) had a significant impact on the physical and chemical properties of the water, which in turn affected phytoplankton biomass. Both Chl-a and dissolved organic carbon (glucose) were shown to have a significant positive association, as demonstrated by the findings, as well as Chl-a and CO_2, whereas adding DOC promoted phytoplankton growth. This finding is consistent with a study that found the addition of DOC can

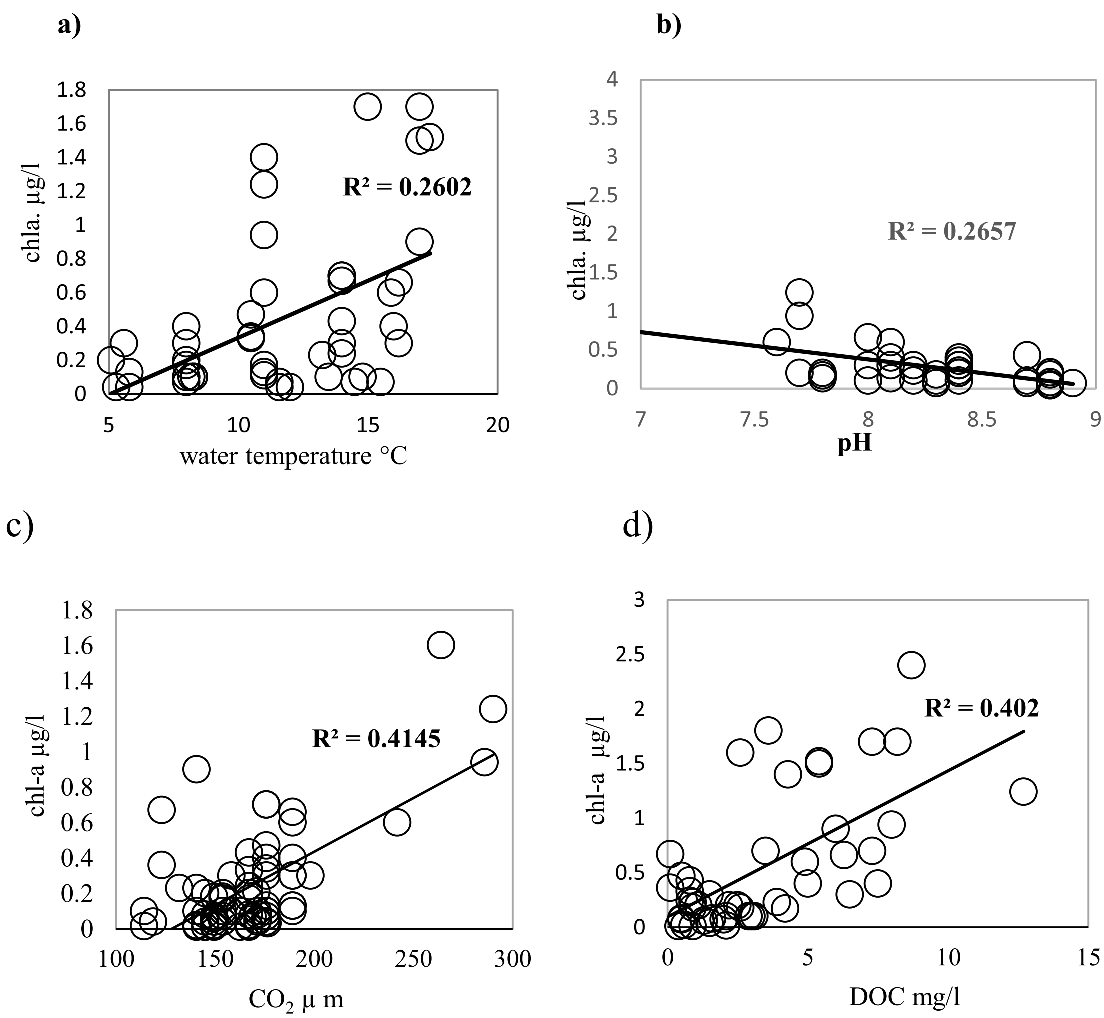

Fig. 4. Relationship between environmental factors and phytoplankton biomass.

stimulate phytoplankton growth, either by enhancing photosynthesis[33] or by increasing CO_2 production from microbial activity.[34] Elevated CO_2 levels lead to increased phytoplankton biomass.[35]

The results indicated that there was no effect for warming treatment alone. However, a 3°C increase in temperature increased the carbon-fixing capacity of phytoplankton, with biomass being higher in the treatment with (DOC + T). This finding is consistent with a previous study, which showed that warming accelerates photosynthesis and increases the rate of cell division in algae.[36] The negative relationship between pH and Chl-a may be a result of increased CO_2 concentration. The phytoplankton benefit from increased carbon dioxide availability in acidic waters.[37] Temperature and DOC treatment directly affected oxygen availability in the water, consistent with recent research demonstrating their complex influence on dissolved oxygen dynamics in aquatic ecosystems. Warming reduces oxygen due to decreased solubility and increased biological respiration,[38] while moderate additions of DOC enhanced primary production, supporting oxygen levels.[39] This explains the positive relationship between Chl-a and oxygen concentration, which reflects photosynthesis, as phytoplankton were found to produce oxygen during the day.[40] While warming and DOC addition had no significant impact on NO_3^{-1} and PO_4^{-3} concentrations in our results, this is consistent with some studies that have found that the effects of warming and DOC on nutrient dynamics may be complex and dependent on a range of environmental and biogeochemical factors.[41] However, the positive relationship between Chl-a and nutrients demonstrates that nutrient availability is a key factor controlling phytoplankton growth.[42,43] These results provide evidence that the addition of dissolved organic carbon mitigated the effects of increased temperature by increasing the abundance of carbon required for photosynthesis.

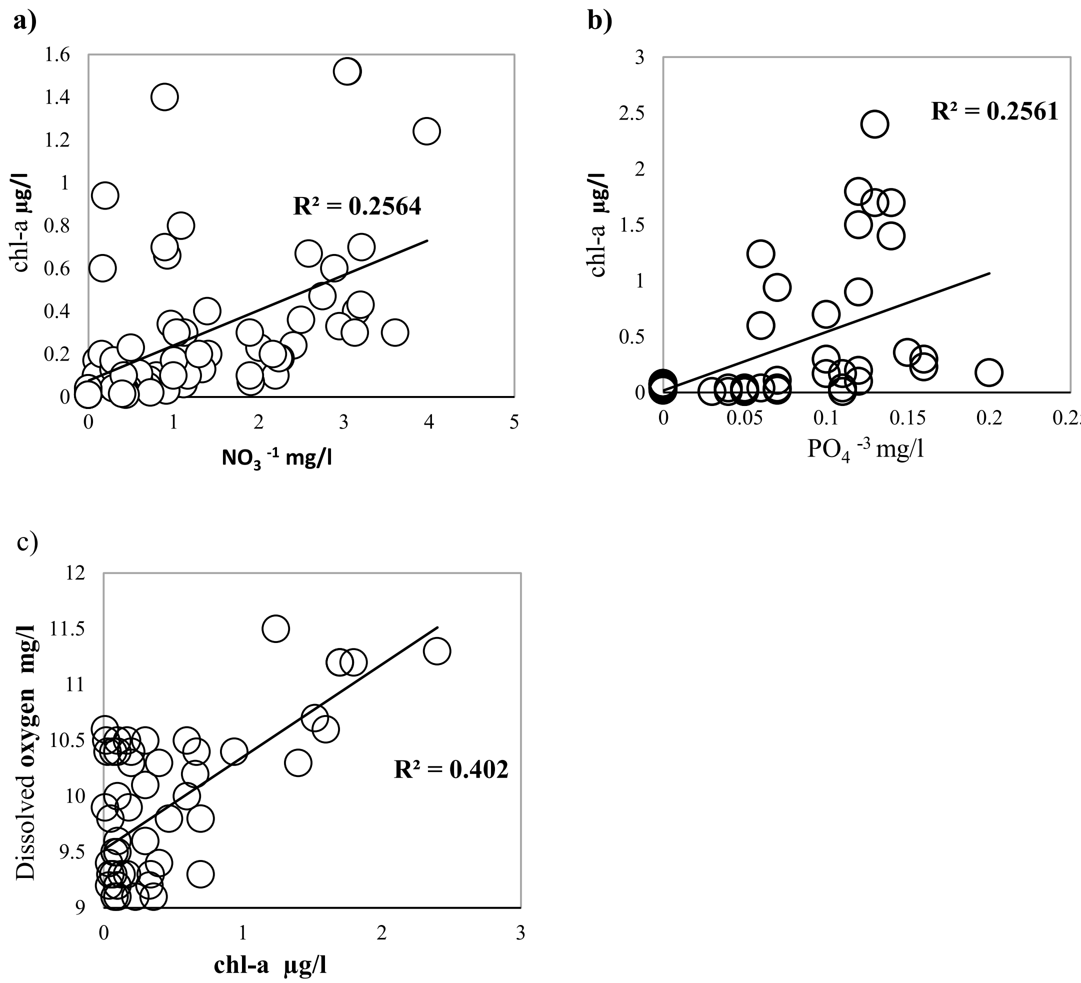

Fig. 5. Relationship between nutrients, dissolved oxygen, and phytoplankton biomass.

Conclusion

In aquatic systems, dissolved organic carbon effectively supports phytoplankton growth, even at high temperatures, by providing the carbon needed for photosynthesis and increasing their biomass. This suggests that the availability of organic carbon and carbon dioxide may determine the effects of warming on primary productivity in freshwater systems, with nutrients and oxygen levels playing important roles as facilitators or limiters.

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at the University of Baghdad.

Authors' contributions statement

M.H. and R.A.L. designed the experiment. R.A.L. carried out the field and lab work. R.A.L. wrote the first draft of the manuscript. M.H. revised the manuscript.

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تأثير الكربون العضوي المذاب ودرجة الحرارة على الكتلة الحية للهائمات النباتية في دراسة خارج المختبر

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الخلاصة

تؤثرالظروف البيئية المتغيرة في النظم المائية بواسطة مدخلات الكربون العضوي المذاب وارتفاع درجة الحرارة على مجتمعات الهائمات النباتية. لتقييم تأثيرات التغير المناخي كتأثيرات منفردة ومشتركة لمدخلات الكربون العضوي المذاب و درجة الحرارة على نمو الهائمات النباتية. أجرينا تجربة خارجية تشمل أربع مجموعات من الظروف في ثلاث نسخ: 1) التحكم و2) المعالجة بالكربون العضوي المذاب، و3) المعالجة بالحرارة و4) المعالجة بالكربون العضوي المذاب والحرارة. أضفنا 10 ملغم/لتر من الجلوكوز ($C_6H_{12}O_6$)) كمصدر للكربون العضوي المذاب.أثرت المعالجة بالحرارة على درجة حرارة الماء بمقدار 3 درجات مئوية فوق درجة التحكم (قيمة p <0.05).اثرت المعالجة بالكربون العضوي المذاب بشكل كبير على درجة الحموضة ،ثاني اوكسيد الكربون،الكربون العضوي المذاب (قيمة 0.003 ،0.01 ،0.004 = p) على التوالي. تأثر الأكسجين المذاب بالمعالجة بالكربون العضوي المذاب(قيمة 0.0003 = p) وبالمعالجة بالحراراة ايضا (قيمة 0.003 = p) ، بينما لم يتأثر NO_3^{-1} و PO_4^{-3}(قيمة p >0.05).المعالجة بالكربون العضوي المذاب والحرارة غيرت بشكل كبير الخصائص الفيزيائية والكيميائية للمياه مماثر على الكتلة الحية للهائمات النباتية(Chl-a). وأظهرت الكتلة الحية للهائمات النباتية ارتباطات إيجابية كبيرة مع درجة الحرارة (r =0.5 وقيمة p = 0.0002)، وثاني أكسيد الكربون (r=0.64 وقيمة p اصغر من0.05)، الكربون العضوي المذاب(r=0.6 و وقيمة p اصغر من0.05) ، NO_3^{-1} (r =0.5 وقيمة p اصغر من0.05) ، PO_4^{-3} (r=0.5 وقيمة p =0.0001) . وارتبط ارتباطًا سلبيًا بشكل ملحوظ مع الأس الهيدروجيني (r = - 0.5 و وقيمة p =0.001). وبالإضافة إلى ذلك، ارتبط الأكسجين المذاب ارتباطًا إيجابيًا بالكتلة الحية للهائمات النباتية (r= 0.63 وقيمة p،0.05).نستنتج ان زيادة مدخلات الكربون العضوي المذاب مهمة لاختزال الاثار السلبية للحرارة على نمو الهائمات النباتية حيث ان الكربون العضوي المذاب هو المصدر الرئيسي لثاني اوكسيد الكربون المطلوب لعملية البناء الضوئي.

الكلمات المفتاحية: البيئة المائية، الكتلة الحية ، الكربون العضوي المذاب، الهائمات النباتية، الحرارة.