

Article

Effects of Warming and Increased Dissolved Inorganic Carbon on Phytoplankton Chlorophyll-*a* Concentration in a Freshwater Ecosystem

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Abstract

Freshwater phytoplankton communities are currently being influenced by ongoing climate change. This study aimed to investigate the effects of warming and dissolved inorganic carbon (DIC) on chlorophyll-*a* concentration and physicochemical variables in a freshwater ecosystem in mesocosm conditions. A 21-day experiment was conducted using freshwater collected from the Tigris River. Four treatments were established: control, warming (+3 °C), DIC, and DIC + 3 °C, with three replicates for each treatment. Chlorophyll-*a*, DIC, water temperature, pH, dissolved oxygen (DO), nitrate (NO₃[−]), and phosphate (PO₄^{3−}) were measured throughout the experiment. The results showed that chlorophyll-*a* concentration increased under warming and DIC treatments. The combined treatment (DIC + 3 °C) showed the highest chlorophyll-*a* concentration. Water temperature was significantly higher in the warming treatment compared to DIC treatment. In addition, pH, DO, nitrate, and phosphate varied among treatments and over time. Overall, the combined effects of warming and DIC were greater than those of the individual treatments. These findings indicate that warming and increased inorganic carbon availability may play an important role in influencing chlorophyll-*a* concentration, carbon cycling, nutrient dynamics, and water quality in freshwater ecosystems.

Keywords: climate change; chlorophyll-*a*; DIC; phytoplankton; water temperature

1. Introduction

Phytoplankton serve as foundational primary producers in aquatic ecosystems, making them highly effective bioindicators for monitoring ecological shifts [1]. Within these ecosystems, the dynamics of phytoplankton communities are heavily regulated by a complex interplay of physicochemical factors, notably water temperature, pH levels, dissolved oxygen, and nutrient availability [2]. Temperature is considered one of the major environmental factors regulating metabolic activity and phytoplankton growth, influencing their physiology, cell size, growth rate, and community composition, thereby affecting the structure and functioning of freshwater ecosystems [3]. Previous studies have demonstrated that phytoplankton growth rates and chlorophyll-*a* concentrations are positively correlated with temperature and generally follow the predictions of metabolic theory of ecology [4]. Consequently, climate change and global warming are expected to exert profound impacts on freshwater ecosystems and their ecological processes, including water quality, dissolved oxygen concentrations, and mixing regimes [5,6].



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Dissolved inorganic carbon (DIC) is another essential environmental factor that plays a central role in aquatic carbon cycling and primary production [7]. It represents the major source of inorganic carbon available for photosynthesis in aquatic ecosystems, and its concentration is influenced by biological processes and water chemistry [8]. Furthermore, DIC constitutes a major fraction of riverine carbon export and serves as an important link between terrestrial and aquatic carbon cycles [9]. Inorganic carbon availability should therefore be considered alongside nutrients and light because it can limit phytoplankton biomass and influence carbon dioxide dynamics in aquatic ecosystems [10]. Previous studies have shown that phytoplankton photosynthesis and biomass production may become carbon-limited, indicating that DIC availability is a critical factor controlling primary production in eutrophic freshwater systems [11]. Furthermore, variations in DIC concentrations can significantly modify the abundance of microbial primary producers and regulate overall primary production, emphasizing its critical role under changing environmental conditions [12].

Global warming has been shown to affect carbon cycling in aquatic ecosystems by altering CO₂ fluxes, water chemistry, and biogeochemical processes [13]. Warming can alter the metabolic and photosynthetic rates of phytoplankton as well as the solubility and availability of CO₂ in water [14], whereas DIC determines the amount of inorganic carbon available for carbon fixation and can regulate phytoplankton photosynthesis, growth, and biomass production [15,16]. A recent mesocosm study conducted in Iraq demonstrated that the combined effects of elevated temperature and carbon addition significantly influenced phytoplankton biomass and the physicochemical properties of the water, emphasizing the importance of evaluating multiple environmental drivers simultaneously [17]. Consequently, the interaction between warming and DIC may influence carbon uptake efficiency, phytoplankton productivity, and carbon cycling in freshwater ecosystems [18]. These interactive effects may ultimately alter phytoplankton biomass, chlorophyll-*a* concentration, and ecosystem functioning under changing environmental conditions. Recent studies have also emphasized that warming and carbon-related environmental changes can exert complex and interactive effects on plankton communities and ecosystem functioning, indicating that multiple environmental drivers should be considered simultaneously when predicting ecosystem responses to climate change [19].

Despite the growing number of studies investigating the effects of warming and dissolved inorganic carbon individually, their combined effects on phytoplankton abundance, primary production, and carbon dioxide dynamics remain poorly understood in freshwater ecosystems, particularly under mesocosm experimental conditions [20,21]. This highlighted that the interactions between carbon availability and phytoplankton dynamics deserve further exploration, indicating that important mechanisms regulating carbon cycling under changing environmental conditions remain unresolved. Therefore, there is still a clear need for an integrated understanding of how warming and DIC jointly influence phytoplankton productivity and carbon dynamics in freshwater ecosystems.

Accordingly, this study aimed to investigate the individual and combined effects of warming (+3 °C) and dissolved inorganic carbon enrichment on chlorophyll-*a* concentration and carbon dioxide dynamics in a freshwater mesocosm ecosystem. We hypothesized that warming and DIC enrichment would interact to produce stronger effects on chlorophyll-*a* concentration and carbon dynamics than either factor alone. We predicted that the combined treatment (DIC + 3 °C) would induce greater changes in chlorophyll-*a* concentration and carbon dioxide dynamics than either warming or DIC enrichment alone.

2. Materials and Methods

2.1. Mesocosm Experimental Setup and Design

In January 2026, the experiment was conducted outdoors under natural environmental conditions in an open area exposed to natural sunlight and a natural day–night cycle throughout the experimental period. The water tanks were placed in the same open area to ensure that they were exposed to equal light and identical environmental conditions across all treatments. Water samples were collected directly from the Tigris River between Al-Huwaish and Tarmiyah districts, north of Baghdad (33.690665° N, 44.409992° E). The collected water was transferred immediately to the experimental site using clean containers without filtration to preserve the natural plankton community, and the experiment was initiated on the same day.

Twelve plastic aquaria (20 L; 40 × 25 × 20 cm) were used in the experiment (Figure 1) were used in the experiment. Four treatments were established with three replicates each: Control, Warming (+3 °C), Dissolved Inorganic Carbon (DIC), and the combined treatment (DIC + 3 °C). All aquaria were arranged in the same open area and exposed to natural sunlight and the natural day–night cycle throughout the experimental period to ensure similar environmental conditions among treatments. The experiment lasted for 21 days. Water samples were collected periodically on days 1, 5, 9, 13, 17, and 21 to determine chlorophyll-*a*, dissolved inorganic carbon (DIC), water temperature, pH, dissolved oxygen (DO), nitrate (NO₃[−]), and phosphate (PO₄^{3−}). In addition, the water in each aquarium was mixed manually at regular intervals to minimize phytoplankton settling and maintain a homogeneous distribution of suspended materials throughout the experiment.

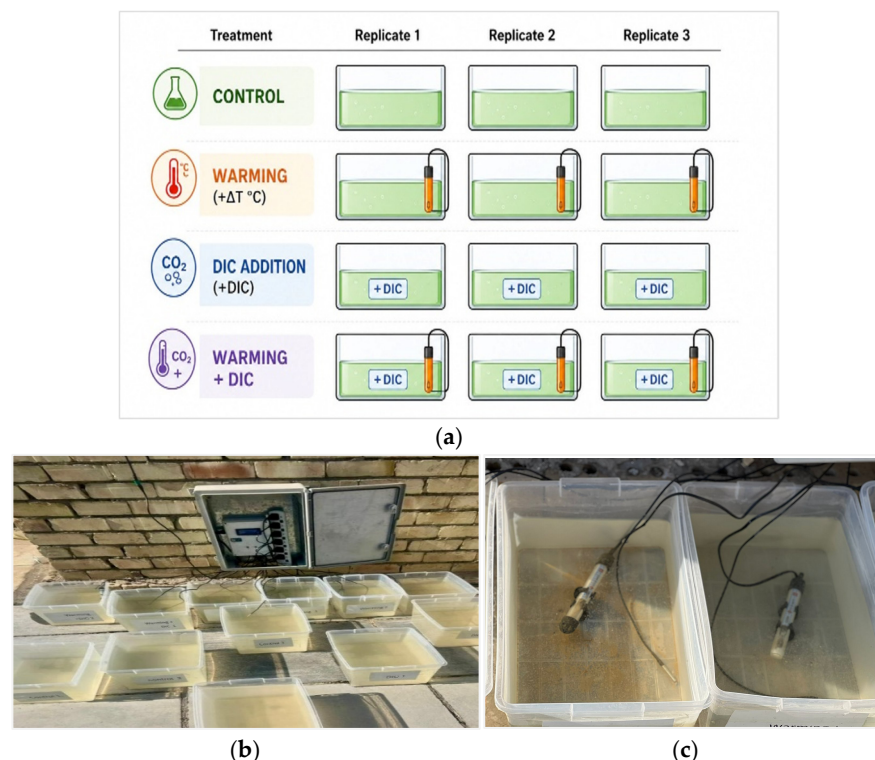


Figure 1. (a) Conceptual model of the mesocosm experiment. (b) Arrangement of the experimental mesocosm tanks. (c) Experimental mesocosm tanks.

Water temperature was continuously monitored and controlled using an automated temperature control system equipped with heaters and temperature sensors. The system was designed and prepared by the technical staff at university of Baghdad. department of Biology. The warming treatments were maintained at approximately 3 °C above ambient

temperature throughout the experiment. Although water temperature fluctuated due to natural variations in environmental conditions, the warming treatments consistently maintained higher temperatures than the control treatment.

The initial concentration of dissolved inorganic carbon (DIC) was 3.08 mM. To increase the DIC concentration to approximately twice the ambient level of 6.16 mM, sodium bicarbonate (NaHCO_3 , Gainland chemical company, Wales, UK) was used as the DIC source. For each aquarium assigned to the DIC and DIC + 3 °C treatments, 5 g of NaHCO_3 was dissolved in a portion of the collected river water, and the resulting solution was added gradually on the first day of the experiment to achieve the target concentration while minimizing abrupt environmental changes.

2.2. Physicochemical Parameters and Laboratory Analyses

Using portable instruments, the water's physical and chemical characteristics were measured, and samples were collected over six trials (from the first to the twenty-first day of the experiment). A [DO9100 dissolved oxygen analyzer, Shanghai Boqu Instrument, Shanghai, China] was used to monitor temperature and DO, while a [Digital pH Pen PH-009, Shenzhen Bestone Industrial Co., Ltd., Shenzhen, China] was used to measure pH. Titration with 0.1 M hydrochloric acid was used to assess alkalinity. To estimate total inorganic carbon and carbonate distribution in water [22], the measured values (temperature, pH, alkalinity, salinity) were entered into CO2SYS software (version 3) in Excel format [23]. DIC concentrations were expressed in Mm throughout the manuscript, assuming a freshwater density of roughly 1 kg L^{-1} [24]. Nitrate (NO_3) was determined using the spectrophotometric method according to [25], by measuring absorption in the ultraviolet field. Phosphate (PO_4) was also estimated using the colorimetric method (Ascorbic acid method) according to [26], based on the formation of a blue complex and measuring it spectrophotometrically. Chlorophyll-*a* (Chl-*a*) was determined using the acetone extraction method following. A 0.5 L water sample was filtered through a $0.45 \mu\text{m}$ filter, and the retained material was extracted with 90% acetone under dark and cold conditions (4 °C). After extraction, the sample was centrifuged, and absorbance was measured at 665 and 750 nm before and after acidification Chlorophyll-*a* concentration was calculated using the following equation [27]:

$$\text{Chl-}a \text{ (}\mu\text{g/L)} = 11.9 \times 2.43 \times (\text{Db} - \text{Da}) \times \frac{V}{L \times \text{Vs}}$$

where Db is the absorbance before acidification, Da is the absorbance after acidification, *V* is the volume of extract (mL), *L* is the path length of the cuvette (cm), and Vs is the volume of filtered sample (L). The acidification coefficient (2.43) is the standard correction factor adopted by [27] which is mathematically derived from the maximum absorbance ratio of pure chlorophyll-*a* before and after acidification (*R* = 1.7) at 665 nm (Factor = $1.7/(1.7 - 1) = 1.7/0.7 = 2.43$) to eliminate pheophytin-*a* interference.

3. Statistical Analysis

The statistical analysis was performed using SPSS version 26. Descriptive statistics, including means and standard deviations, were calculated for all variables. The effects of treatment, time, and their interaction were evaluated using repeated measures ANOVA. When significant differences were detected, Bonferroni post hoc comparisons were performed to identify pairwise differences among treatments. Pearson's correlation coefficient (*r*) was used to examine the relationships among the measured variables. Statistical significance was considered at $p < 0.05$.

4. Results

Before the application of the experimental treatments, all mesocosms were filled with water collected from the same site in the Tigris River. The initial physicochemical characteristics of the source water were as follows: temperature $17.1\text{ }^{\circ}\text{C}$, pH 8.4, dissolved oxygen 8.2 mg L^{-1} , dissolved inorganic carbon (DIC) 3.08 mM , nitrate 2.16 mg L^{-1} , and phosphate 0.31 mg L^{-1} .

Water temperature ranged from $12.20 \pm 0.20\text{ }^{\circ}\text{C}$ in the DIC treatment on day 17 to $23.37 \pm 0.15\text{ }^{\circ}\text{C}$ in the warming treatment on day 9 (Figure 2a). pH ranged from 7.46 ± 0.11 in the DIC + warming treatment on day 21 to 8.86 ± 0.15 in the DIC + warming treatment on day 13 (Figure 2b). Dissolved oxygen (DO) concentrations ranged from $6.26 \pm 1.11\text{ mg L}^{-1}$ in the warming treatment on day 21 to $9.43 \pm 0.49\text{ mg L}^{-1}$ in the DIC treatment on day 13 (Figure 2c). Nitrate (NO_3^-) concentrations ranged from $0.18 \pm 0.06\text{ mg L}^{-1}$ in the DIC treatment on day 17 to $2.85 \pm 0.17\text{ mg L}^{-1}$ in the DIC treatment on day 1 (Figure 2d). Phosphate (PO_4^{3-}) concentrations ranged from $0.09 \pm 0.04\text{ mg L}^{-1}$ in the DIC + warming treatment on day 13 to $0.31 \pm 0.03\text{ mg L}^{-1}$ in the DIC + warming treatment on day 21 (Figure 2e). Dissolved inorganic carbon (DIC) concentrations ranged from $2.066 \pm 0.036\text{ mM}$ in the warming treatment on day 13 to $5.9 \pm 0.1\text{ mM}$ in the DIC treatment on day 1 (Figure 2f). Chlorophyll-*a* (Chl-*a*) concentrations ranged from $0.86 \pm 0.15\text{ }\mu\text{g L}^{-1}$ in the warming treatment on day 21 to $9.63 \pm 1.19\text{ }\mu\text{g L}^{-1}$ in the DIC + warming treatment on day 13 (Figure 2g). The overall mean $\pm$ SD values for all measured variables are summarized in Table 1.

Table 1. Mean $\pm$ SD values of the physical, chemical and biological variables measured during the study period in the mesocosm experiment.

Variables	Control	DIC	Warming	Warming + DIC
WT ($^{\circ}\text{C}$)	16.74 ± 3.07	16.73 ± 3.08	19.72 ± 3.17	19.72 ± 3.11
pH	8.18 ± 0.26	8.35 ± 0.36	8.24 ± 0.23	8.39 ± 0.50
DO (mg L^{-1})	8.11 ± 0.74	8.2 ± 0.83	7.77 ± 0.92	8.05 ± 0.91
NO_3^- (mg L^{-1})	1.45 ± 0.80	1.96 ± 0.96	1.82 ± 0.97	1.93 ± 0.97
PO_4^{3-} (mg L^{-1})	0.21 ± 0.06	0.22 ± 0.06	0.24 ± 0.05	0.23 ± 0.08
DIC (mM)	2.8 ± 0.25	5.3 ± 0.47	2.53 ± 0.34	4.94 ± 0.57
Chl- <i>a</i> ($\mu\text{g L}^{-1}$)	2.55 ± 1.09	3.80 ± 1.77	2.76 ± 1.29	4.83 ± 2.67

As shown in (Table 2), the repeated-measures ANOVA for WT revealed a significant effect of time and treatment ($p < 0.05$), but no significant interaction between them ($p > 0.05$). The research revealed that time, treatment, and their interaction had a significant impact on pH ($p < 0.05$). The only factors that significantly affected DO concentration were time and treatments ($p < 0.05$). The repeated-measures ANOVA revealed significant effects of time, treatment and time $\times$ treatment interaction on NO_3^- concentration ($p < 0.05$), whereas PO_4^{3-} concentration was significantly affected by time and the time $\times$ treatment interaction, while the treatment effect was ($p > 0.05$). Additionally, significant effects of time, treatment and the time $\times$ treatment, interaction were observed for DIC and Chl-*a* ($p < 0.05$).

Following the detection of significant treatment effects in the repeated-measures ANOVA, Bonferroni post hoc analyses were conducted to determine the specific pairwise differences among treatments. The statistically significant pairwise comparisons are presented in Table 3, whereas the remaining comparisons were not statistically significant ($p > 0.05$).

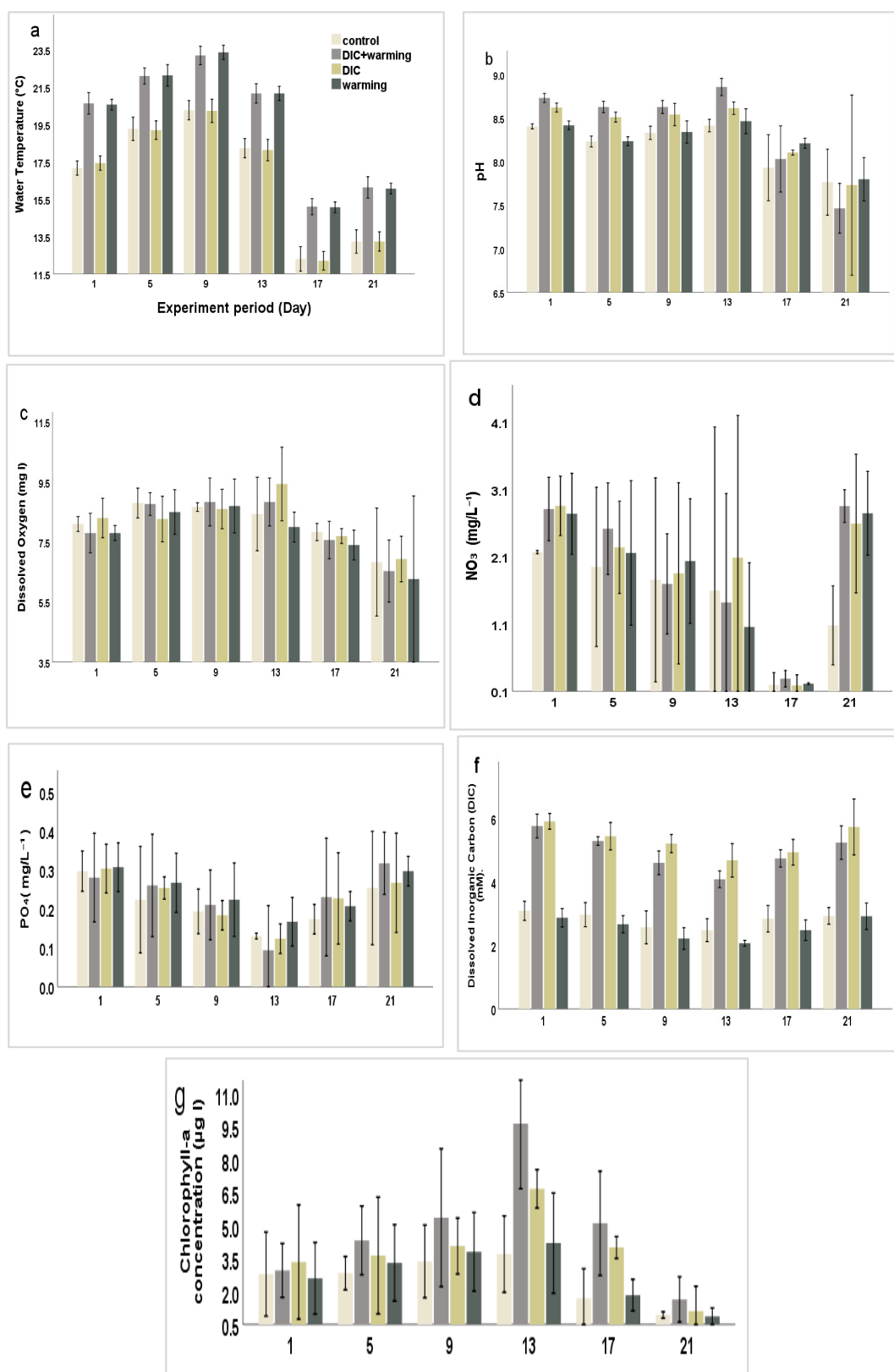


Figure 2. Environmental parameters [(a) water temperature, (b) pH, (c) dissolved oxygen, (d) nitrate (NO₃⁻), (e) phosphate (PO₄³⁻), (f) dissolved inorganic carbon (DIC), (g) chlorophyll-*a*] under four experimental treatments in a mesocosm experiment.

Table 2. Results of the repeated-measures ANOVA demonstrate how time, treatments, and their interactions affect the environmental variables under study.

Variables	Time			Treatment			Time × Treatment		
	<i>df</i>	<i>F</i>	<i>p</i> -Value	<i>df</i>	<i>F</i>	<i>p</i> -Value	<i>df</i>	<i>F</i>	<i>p</i> -Value
WT (°C)	5	3086	<0.0001	3	1932.12	<0.0001	15	1.01	0.456
pH	5	118	<0.0001	3	17.33	0.001	15	5.25	<0.0001
DO (mg L ^{−1})	5	49.86	<0.0001	3	0.014	0.039	15	1.62	0.109
NO ₃ (mg L ^{−1})	5	53.49	<0.0001	3	4.62	0.014	15	2.65	0.007
PO ₄ (mg L ^{−1})	5	70.23	<0.0001	3	0.74	0.37	15	1.93	0.049
DIC (mM)	5	106.91	<0.0001	3	612.46	<0.0001	15	8.8	<0.0001
Chl- <i>a</i> (µg L ^{−1})	5	63.38	<0.0001	3	37.87	<0.0001	15	6.01	<0.0001

Statistical significance was determined at $p \leq 0.05$.

Table 3. Bonferroni post hoc comparisons among treatments for the studied variables.

Variable	Comparison	<i>p</i> -Value	Variable	Comparison	<i>p</i> -Value
WT	Control vs. DIC + Warming	<0.0001	DIC	Control vs. DIC + Warming	<0.0001
WT	Control vs. Warming	<0.0001	DIC	Control vs. DIC	<0.0001
WT	DIC vs. DIC + Warming	<0.0001	DIC	DIC vs. DIC + Warming	0.013
WT	Warming vs. DIC	<0.0001	DIC	Warming vs. DIC + Warming	<0.0001
pH	Control vs. DIC + Warming	0.001	DIC	Warming vs. DIC	<0.0001
pH	Control vs. DIC	0.004	Chl- <i>a</i>	Control vs. DIC + Warming	<0.0001
pH	Warming vs. DIC + Warming	0.014	Chl- <i>a</i>	Control vs. DIC	0.005
DO	Warming vs. DIC	0.016	Chl- <i>a</i>	DIC vs. DIC + Warming	0.016
Chl- <i>a</i>	Warming vs. DIC	0.016	Chl- <i>a</i>	Warming vs. DIC + Warming	<0.0001

The presented values represent *p*-values obtained from Bonferroni post hoc comparisons. Only statistically significant pairwise comparisons are shown, whereas the remaining comparisons were not statistically significant ($p > 0.05$).

Pearson's correlation analysis showed that chlorophyll-*a* concentration was significantly correlated with several environmental variables, including water temperature ($r = 0.456$, $p < 0.0001$; Figure 3a), pH ($r = 0.637$, $p < 0.0001$; Figure 3b), and dissolved oxygen ($r = 0.622$, $p < 0.0001$; Figure 3e). Water temperature was also correlated with pH ($r = 0.670$, $p < 0.0001$; Figure 3c) and dissolved oxygen ($r = 0.637$, $p < 0.0001$; Figure 3d). Furthermore, correlations between chlorophyll-*a* concentration and nitrate ($r = -0.136$, $p = 0.254$; Figure 3f) and chlorophyll-*a* concentration and phosphate concentrations ($r = -0.558$, $p < 0.0001$; Figure 3g) were observed. Dissolved inorganic carbon was correlated with pH ($r = 0.135$, $p = 0.259$; Figure 3h), and chlorophyll-*a* concentration was correlated with dissolved inorganic carbon ($r = 0.167$, $p = 0.160$; Figure 3i).

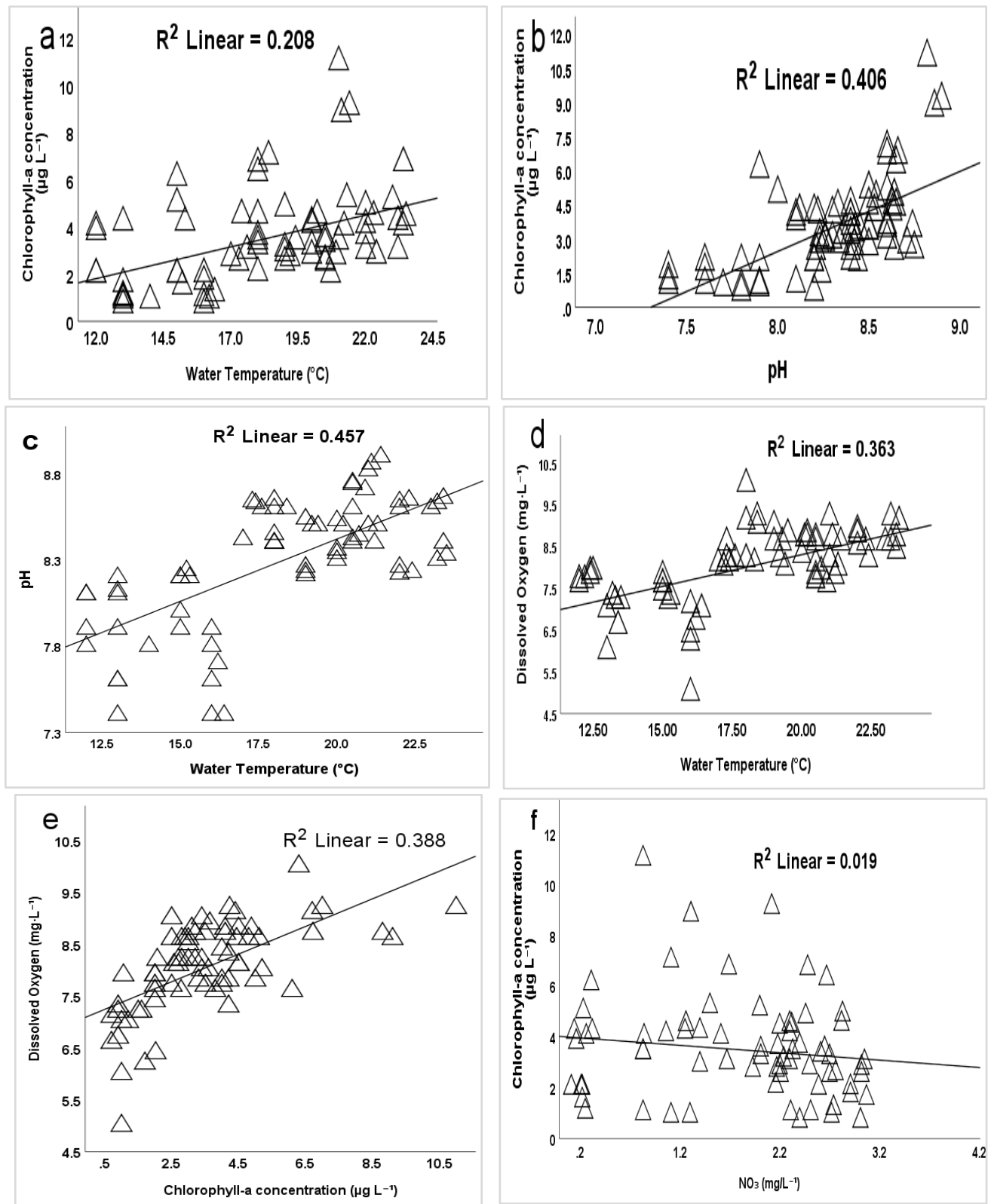


Figure 3. Cont.

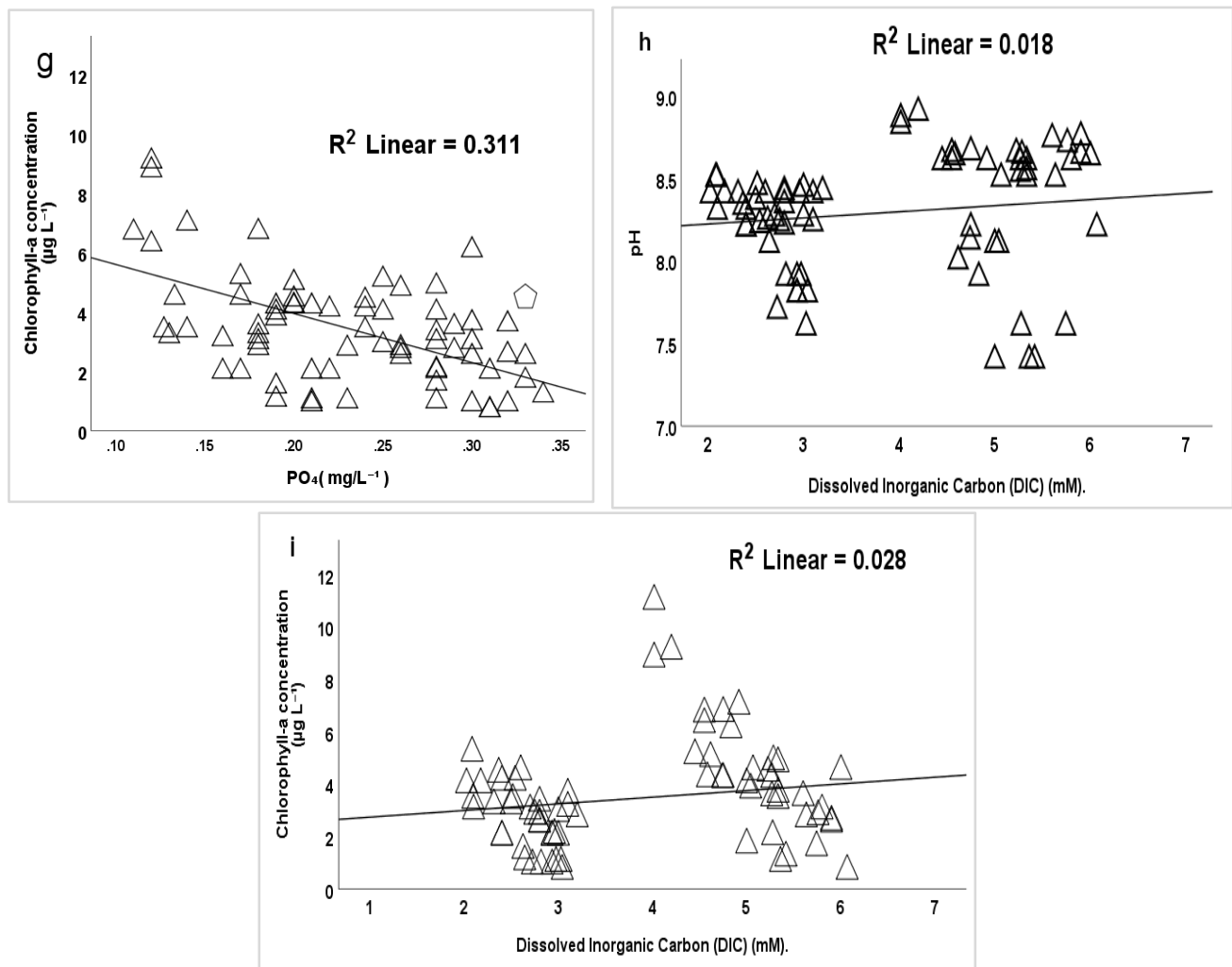


Figure 3. Scatterplots with fitted linear regression lines showing the relationships between: (a) water temperature and chlorophyll-*a* concentration; (b) pH and chlorophyll-*a* concentration; (c) water temperature and pH; (d) water temperature and dissolved oxygen; (e) chlorophyll-*a* concentration and dissolved oxygen; (f) nitrate (NO_3^-), and chlorophyll-*a* concentration; (g) phosphate (PO_4^{3-}) and chlorophyll-*a* concentration; (h) dissolved inorganic carbon (DIC) and pH; and (i) dissolved inorganic carbon (DIC) and chlorophyll-*a* concentration. Triangles represent observed data points, and solid lines represent fitted linear regression lines.

5. Discussion

5.1. Effects of Warming and Dissolved Inorganic Carbon on Chlorophyll-*a* Concentration

The results showed that chlorophyll-*a* concentration changed during the experiment. It gradually increased during the first half of the experiment, reached its maximum around the middle of the trial, and then gradually decreased toward the end. This pattern reflects that the growth of phytoplankton may be stimulated in early stages by the availability of suitable environmental conditions, but its continuity is limited at later stages, perhaps due to nutrient depletion or changes in the physical and chemical properties of water.

The increase in chlorophyll-*a* concentration in the DIC treatments, especially in the DIC + warming treatment, suggests that dissolved inorganic carbon increased chlorophyll-*a* concentration, indicating enhanced phytoplankton growth. DIC is one of the main sources of carbon for photosynthesis, as its increased availability contributes to higher carbon sequestration efficiency and greater biomass production, especially in freshwater ecosystems where carbon may be a growth determinant. These findings are consistent with [28], who

has shown that increased availability of inorganic carbon leads to improved photosynthesis efficiency and enhanced phytoplankton growth by increasing carbon fixation and improving the performance of intracellular carbon concentration mechanisms.

In contrast, the warming treatment alone did not consistently record the highest concentrations of chlorophyll-*a*, suggesting that temperature rise in the absence of sufficient carbon availability may not be enough to achieve maximum growth rates for phytoplankton. Although rising temperatures typically increase metabolic activity and accelerate the enzymatic reactions associated with photosynthesis, their positive impact may be limited by the availability of essential resources, particularly carbon and nutrients. These findings are consistent with [29], showing that the response of phytoplankton to warming is based on the interaction between temperature and environmental resource availability, rather than on the effect of temperature alone.

The combined treatment (DIC + 3 °C) showed a more pronounced response than the warming treatment alone, indicating that the synergy between rising temperature and increased availability of inorganic carbon has provided conditions associated with higher chlorophyll-*a* concentration. This is potentially due to the synergistic effect between operators, where warming leads to an increase in metabolic activity, while DIC contributes to increasing the amount of carbon available for photosynthesis, which is reflected in increased phytoplankton biomass. Ref. [30] reported similar results. They found that the combined effect of higher temperature and increased carbon availability boosted phytoplankton growth to a greater degree than the impact of each individual factor.

Although the results of this current study highlighted the importance of both DIC and warming in influencing chlorophyll-*a* concentration, they differ from the findings of [31], who reported that phytoplankton responses to dissolved inorganic carbon vary according to species-specific carbon acquisition strategies and physiological characteristics. The response depends on the nature of the algal community and the availability of nutrients and prevailing environmental conditions. This variation may be attributed to the variability of the ecosystem used, the duration of the experiment, and DIC concentrations added, as well as differences in the phytoplankton community composition and physical and chemical properties of the water.

The findings of the present study are also in part consistent with [32], who emphasized the importance of carbon availability in controlling carbon dynamics in the Tigris River. However, the present study has shown that a combined effect of warming and DIC may be more effective in increasing chlorophyll-*a* concentration than an individual factor under mesocosm conditions. Overall, the results suggest that phytoplankton response depends on the interaction between temperature, dissolved inorganic carbon, nutrient availability, dissolved oxygen, pH and time. These findings are also ecologically relevant in the context of climate change, where the increasing frequency and intensity of marine heatwaves (MHWs) have emerged as major climate-driven disturbances affecting marine ecosystems [33].

Recent studies have shown that prolonged warming during MHWs can alter phytoplankton biomass [34] and community composition [35], with responses varying according to nutrient availability and local environmental conditions [36].

Although the present study was conducted in a freshwater mesocosm, the observed responses to warming and dissolved inorganic carbon (DIC) provide broader insight into how phytoplankton communities may respond to prolonged thermal stress across aquatic ecosystems under future climate change scenarios.

5.2. Temporal Dynamics of Dissolved Inorganic Carbon (DIC)

Current study results show that the concentration of dissolved inorganic carbon (DIC) experienced significant temporal changes during the trial period, recording a high value at the start of the experiment, then gradually decreasing during subsequent stages before rising again at the end of the trial. This pattern suggests that the dynamics of DIC within an experimental ecosystem were caused by a complex interplay between biological, chemical and physical processes rather than by a single factor. The high DIC at the beginning of the experiment can be explained by the availability of a sufficient amount of dissolved inorganic carbon in water, which provided an initial stock of carbon within the ecosystem. Over time, its concentration gradually decreased and this may be attributed to the consumption of inorganic carbon during photosynthesis processes as well as changes in the chemical balance of carbon forms within water. Also, rising temperatures may affect the solubility of CO₂ and distribution of various carbon species, contributing to changes in DIC concentration over time. The rise in DIC during the later stages of the experiment, however, may be due to a lower CO₂ rate as a result of reduced bioactivity, in addition to continued respiration and organic decomposition processes that lead to re-release of carbon dioxide into the water, thus increasing the concentration of dissolved inorganic carbon once again. Changes in pH values may also affect the chemical balance between different carbon profiles, such as CO₂, bicarbonate and carbonates, which is directly reflected on DIC concentration. These results are consistent with previous studies [37,38], which explained that the carbon dynamics are affected by the interaction between temperature, pH and CO₂ concentration, and that changes in different inorganic carbon species are reflected in DIC concentration in aquatic ecosystems. This is consistent with [39], who reported that temperature significantly influences phytoplankton physiological processes and responses to changing environmental conditions, thereby influencing carbon-related processes in aquatic ecosystems. However, the present findings also indicate that DIC dynamics may vary depending on phytoplankton composition, nutrient availability, and environmental conditions [40,41]. Such variation highlights the complexity of carbon cycling processes in freshwater ecosystems. Broadly speaking, the present findings indicate that DIC is an important dynamic variable in freshwater ecosystems and that its change over time reflects the ongoing interaction of biological, chemical, and physical factors which control the carbon cycle within the ecosystem.

5.3. Changes in Physicochemical Parameters Under Warming and Dissolved Inorganic Carbon (DIC) Treatments

The physicochemical properties of the water responded distinctively to the interactive warming and dissolved inorganic carbon (DIC) treatments. Water temperature remained consistently higher in the warming groups, accelerating metabolic rates and nutrient cycling. This pattern is consistent with the findings of [37,38], which demonstrated that elevated temperatures accelerate metabolic processes and carbon utilization within plankton communities. Concurrently, dissolved oxygen (DO) exhibited substantial temporal fluctuations, reflecting the thermodynamic constraints of temperature on gas solubility balanced against biological oxygen production, as documented by [42,43].

Regarding nutrient dynamics, nitrate (NO₃[−]), and phosphate (PO₄^{3−}), concentrations declined during the middle stages before recovering later in the experiment, a temporal pattern driven by biological uptake and subsequent nutrient recycling. Furthermore, the dynamics of chlorophyll-*a* (Chl-*a*) underscore direct biological feedback on this physicochemical environment. As emphasized by [44], primary producers rely on inorganic carbon and essential nutrients to drive photosynthesis. While standalone warming typically reduces DO availability, the synergistic influence of combined warming and DIC

enrichment observed in this study is consistent with the findings of [45] on the Tigris River, who demonstrated that the co-limitation of temperature and nutrient additions induces the highest biogenic response in Chl-*a* concentrations. Conversely, the depletion of phosphate during peak Chl-*a* phases reflects rapid cellular assimilation, implying a potential limiting effect of phosphorus on phytoplankton dynamics, as highlighted by [46].

Ultimately, these integrated responses suggest that warming and DIC concurrently shape freshwater ecosystems through interconnected pathways rather than through a single environmental driver. The present findings reflect phytoplankton responses under the environmental conditions prevailing during the study period. Because solar radiation naturally varies with season, latitude, and weather conditions, the magnitude of chlorophyll-*a* responses to warming and dissolved inorganic carbon (DIC) may differ under other environmental settings. Future investigations across different seasonal conditions would further extend the applicability of the present findings.

6. Conclusions

This study concluded that warming and increased dissolved inorganic carbon (DIC) significantly influenced chlorophyll-*a* concentration in freshwater ecosystems. The combined effect of these two factors is greater than the individual impact of each factor. The results suggest that future environmental changes may influence carbon cycling, nutrient dynamics, and water quality in freshwater ecosystems. Therefore, further studies under natural conditions and over longer periods are recommended to better understand freshwater ecosystem responses to environmental changes.

Author Contributions: M.H. designed the experiment. T.M. carried out the field and lab work. T.M. analyzed data statistically. T.M. wrote the first draft of the manuscript. M.H. revised the last version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

ANOVA	Analysis of Variance
Chl- <i>a</i>	Chlorophyll- <i>a</i>
CO ₂	Carbon Dioxide
DIC	Dissolved Inorganic Carbon
DO	Dissolved Oxygen
NaHCO ₃	Sodium Bicarbonate
NO ₃ [−]	Nitrate
PO ₄ ^{3−}	Phosphate
WT	Water Temperature

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