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Photophysiological response of glacier ice algae to abiotic stressors

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The glacier ice algae *Ancylonema alaskanum* and *Ancylonema nordenskiöldii* grow in harsh dynamic environments on bare ice surfaces. In these environments, they contribute to the continuous darkening of the ice surface, which in turn accelerates the ice melt. However, investigation into their adaptation and resilience in these environments is necessary in order to understand their robustness and potential for increasing the intensity of blooms. In this study, it was examined how variations in environmental parameters such as pH, salinity, light and temperature impacted the photophysiology of the glacier ice algae during a bloom in Greenland. Through *in situ* incubations and pulse-amplitude-modulation (PAM) fluorometric measurements, the photophysiological responses of algal cells were assessed. Results suggest that light intensity significantly influences glacier ice algae photophysiology, with cells exhibiting better performance (in terms of a higher theoretical maximum light coefficient and maximum quantum efficiency) under lower light intensity. Moreover, while light emerges as the primary driver of photophysiology, glacier ice algae demonstrate tolerance to a broad range of pH and temperatures four times higher than those experienced during Greenland's summer.

KEYWORDS

glacier ice algae, Greenland ice sheet, ancylonema, photophysiology, microalgae, arctic, adaptation, stress

1 Introduction

Glacier ice algae are known to occur in the top centimeters of surface ice in many high-latitude and -altitude areas, such as the Arctic (Yallop et al., 2012; Remias et al., 2012), Alps (Di Mauro et al., 2020), Himalayas (Yoshimura et al., 1997) and Antarctica (Ling and Seppelt, 1990). These algae belong to the Zygnematophyceae (Streptophyta) and are distinct from both snow and sea ice algae. Along the western margin of the Greenland ice sheet (GrIS), extensive algal blooms (~10^4 cells mL^{-1}) that cover 4%–10% of the bare-ice area occur during summer ablation seasons when sunlight and liquid water are available for photosynthesis (Lutz et al., 2018; Williamson et al., 2018; Cook et al., 2020; Shimada et al., 2016). These blooms consist of the unicellular *Ancylonema alaskanum* (Kol) and filamentous *Ancylonema nordenskiöldii* Berggren (Remias et al., 2009; Procházková et al., 2021; Kol, 1942; Berggren, 1871), which both contain secondary phenolic pigments that accelerate surface melt by lowering bare-ice albedo and enhancing the absorption of solar energy (Shimada et al., 2016; Williamson et al., 2020). The reduction in albedo associated with glacier ice algae was estimated to have contributed up to 10%–13% of

the total runoff in southwest Greenland during the summmer of 2017 (Cook et al., 2020). In the Kangerlussuaq region, approximately 58% of the ice was covered with glacier algae, contributing to darkening of the ice surface (Cook et al., 2020). As the melting of the GrIS is the primary cryospheric contributor to global eustatic sea-level rise (Bamber et al., 2018), which has been estimated at 13.7 ± 1.1 mm between 1972 and 2018 (Mouginot et al., 2019), there is an urgent need to improve understanding of how glacier ice algae are adapted to life in ice.

Glacier ice algae, being non-motile (Remias et al., 2009; Dial et al., 2018), must cope with the changes in the environment in which they live. Inhabiting some of the harshest environments on Earth, they must endure extreme conditions promoted by high and fluctuating light intensities, fluctuating pH levels, varying salinity, and temperature extremes (Williamson et al., 2020; Shetty et al., 2019). These environmental factors pose significant challenges to their survival and productivity (Shetty et al., 2019; Baker and Rosenqvist, 2004; Mott and Berry, 1986). For example, during the months of constant light in the Arctic summer season, glacier ice algae are subjected to intense solar radiation, with photosynthetic active radiation (PAR) that can exceed 1700 μmol photons $m^{-2}s^{-1}$, alongside elevated UV radiation (Yallop et al., 2012; Williamson et al., 2020). Additionally, the pH of glaciers and ice sheets where the algae thrive can vary greatly, with measurements between 3.6 and 8.6 (Komárek and Komárek, 2001; Takeuchi and Kohshima, 2004; Remias et al., 2009; Remias et al., 2011). Moreover, changes in temperature (IPCC, 2023) and the potential for algae to be washed out into the ocean with ice melting may significantly impact their distribution and survival (Takeuchi, 2001).

While light serves as the primary energy source for photosynthesis in glacier algae, excessive radiation levels can induce photoinhibition and increase reactive oxygen species, which can damage DNA and proteins within the cell (Aro et al., 1993; Rippin et al., 2019; Williamson et al., 2020). A reduction in incoming light has been shown to alter the photophysiology of glacier ice algae (Williamson et al., 2020). Similarly, the external pH around algal cells plays a role in their photosynthetic performance (Yadav and Singh, 2021). Fluctuations in pH and salinity can disrupt vital cellular processes for algal growth and survival by, for example, damaging the photosystem or affecting the enzymatic processes involved in photosynthesis (Shetty et al., 2019; Mott and Berry, 1986). Additionally, temperature extremes influence enzymatic reactions and metabolic rates, thereby impacting algal productivity (Baker and Rosenqvist, 2004).

Despite the ecological significance of glacier ice algae, substantial gaps persist in understanding their responses to environmental perturbations, particularly regarding their photophysiology and productivity. Bridging this knowledge gap is crucial for predicting future dynamics in glacier ice ecosystems and assessing their vulnerability to ongoing environmental changes. A common and nondestructive method for assessing the fitness of plants and algal cells involves measuring chlorophyll fluorescence to estimate photosynthetic performance (Baker and Rosenqvist, 2004; Baker, 2008). This technique can be implemented using pulse-amplitude-modulation (PAM) fluorometry, which allows for *in vivo* examination of photosystem II (PSII). The electron transport through PSII serves as an indicator of the photosynthetic efficiency of a cell. Additionally, a reduced quantum yield, as indicated by the maximum quantum efficient (F_v/F_m) ratio, may signify damage to PSII and is frequently employed as a proxy for cellular stress (Stibal et al., 2007; Baker and Rosenqvist, 2004).

As a result, the aims of this study were to investigate the impact of light, pH, salinity, and temperature on the photophysiology of glacier ice algae via *in situ* incubations in Greenland, which allows for variable control, and provide valuable insights into their adaptive mechanisms and resilience in the face of changing environmental conditions.

2 Materials and methods

2.1 Study site and sample collection

Surface ice sampling was conducted at an elevation of 617 m a.s.l. ~7.5 km from the S-Greenland ice sheet margin (61°06′05″N, 46°50′53″W) between 13 July and 8 August 2021 (Figures 1A–C). At this location, the microbial community was determined to consist of a diverse range of fungi, bacteria, and algae, including Streptophyte glacier ice algae (Jaarsma et al., 2023), which have been shown to lower bare ice albedo (Williamson et al., 2020). Approximately 100 kg of surface ice (top 1–2 cm) was collected using a field-conditioned metal ice axe. It was then transferred in equal amounts to sterile Whirl-Pak® bags (Madison, WI) before being melted in a tent under low light conditions (8.5 W m^{-2}) at 5°C–10°C over 72 h. Once melted, the samples in each bag were shaken by hand for 1 min. Composite samples were then created by transferring aliquots of 1,000 mL from each bag into fresh Whirl-Pak® bags and shaking them again by hand for 1 min before using them in separate *in situ* incubation experiments.

2.2 Experimental design

In order to comprehensively examine the impact of environmental factors on the photophysiology of the microbial community, a series of controlled incubation experiments were conducted, manipulating pH, salinity, light and temperature. For each parameter, a gradient of five points was systematically created by transferring aliquots of 650 mL from one of the four bags to fresh 2,000-mL Whirl-Pak® bags (Figure 1A). The environmental parameter of interest was then amended in all of the fresh bags except for one per replicate, which served as an unmodified "control", representing the prevailing environmental conditions at the study site.

After determining that the pH of the surface ice was 6.7 ± 0.2 using an Orion™ Star A321 meter (Thermo Fisher Scientific, Waltham, MA), this value was established as a control. Subsequently, a pH gradient (3.58, 4.71, 6.7 [control], 8.07, and 9.21) was created by amending the samples with either sodium hydroxide (Sigma-Aldrich, Burlington, MA) or hydrochloric acid (VWR, Radnor, PA) in accordance with methods outlined by Jensen et al. (2023). A gradient of increasing salinity above baseline conditions (0‰[control], 5‰, 15‰, 35‰and 50‰) was created by adding increasing amounts of sodium chloride (*m*/*v*; Sigma-Aldrich) to the samples. A light intensity gradient was created by wrapping four out of five bags in generic solar control films of different optical performances capable of either excluding UV

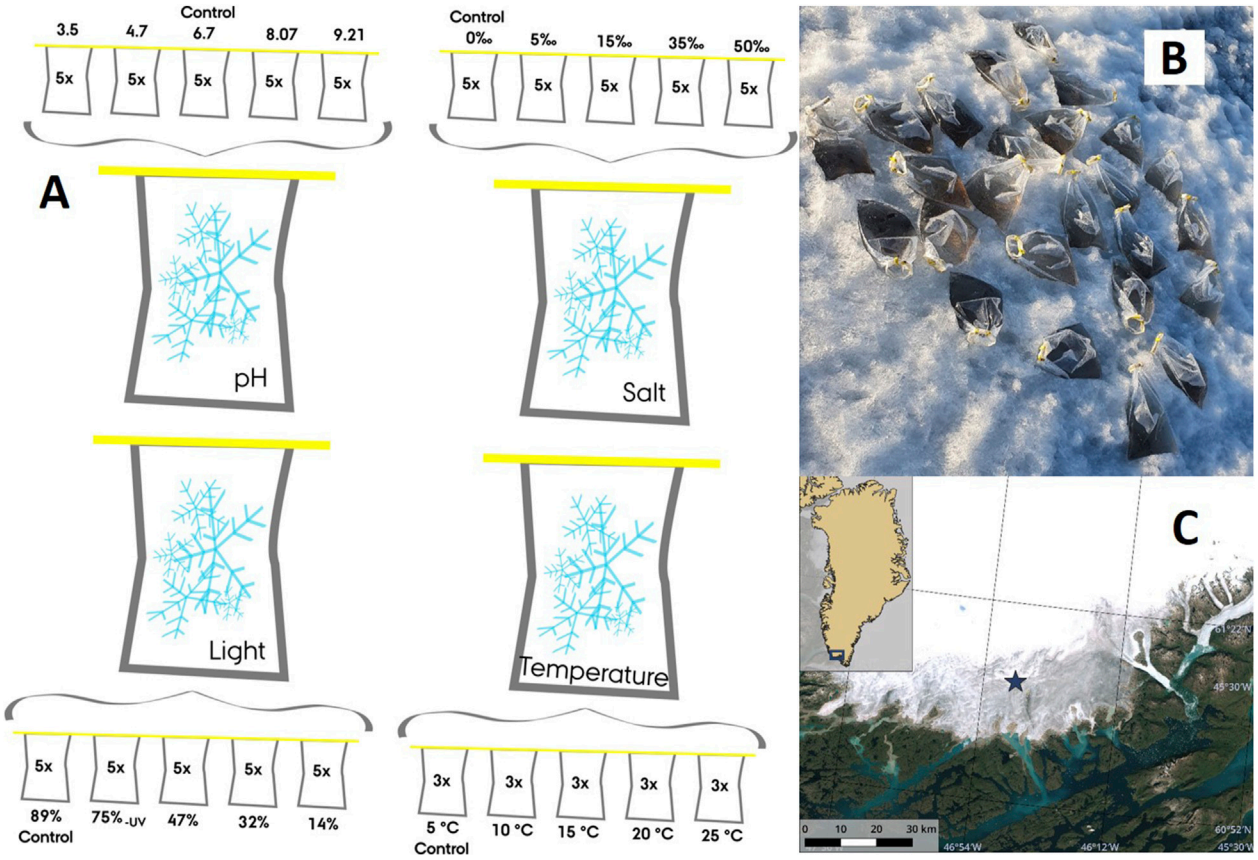

FIGURE 1
An overview of four in situ incubation experiments that manipulated pH, salinity, light intensity and temperature. The 'control' samples represent the prevailing environmental conditions at the study site **(A)**, an example of a field incubation for the pH experiment **(B)**, and Copernicus Sentinel-2 optical satellite imagery of the fieldwork site (denoted by a blue star) in southern Greenland (61°06′05″N, 46°50′53″W) **(C)** during 2021 (The Danish Agency for Data Supply and Infrastructure, 2024).

radiation or reducing incoming light. As confirmed using a UV–vis spectrometer coupled with an F600-VISNIR fiber optic cable (Black Comet CXR-25, StellarNet, Tampa, FL), this resulted in samples within the gradient being exposed to 89% (control), 75% minus UV ($75\%_{-UV}$), 47%, 32%, and 14% ambient irradiance (Supplementary Figure S1; Supplementary Table S1). Measurements made for samples that had been incubated under 14% of ambient irradiance were excluded from further analysis, as they were corrupted by signal noise. Five replicate samples per point within the pH, salinity and light gradients (n = 5) were incubated on the bare ice surface (Figure 1B) under natural light ($105\ W\ m^{-2}$) for 48 h. All reagents used were of analytical reagent grade.

Lastly, a temperature gradient (5°C [control], 10°C, 15°C, 20°C, and 25°C) was created by placing the triplicate bags (n = 3) in heated water baths, where the temperature was manually controlled by adding hot water. Samples incubated at 5°C were considered to be controls, as this was the approximate air temperature (Fausto et al., 2021). Due to the challenges associated with manually maintaining the desired temperatures, the temperature experiments were conducted under low light conditions ($8.5\ W\ m^{-2}$) inside a tent for a shorter period of 4 h.

The photophysiology of the microbial community was assessed immediately within each sample at the end of its incubation period (48 h or 4 h) using the methods described in the following section. Prior to initiating the incubation experiments for pH, salinity, and light, baseline photophysiological measurements were taken for additional control samples that had been prepared using the same methods to establish a reference point after the melting process (T_0). Notably, no baseline for the temperature incubation experiment was needed, as the control sample remained under the initial low-light conditions for the brief 4-h incubation period.

2.3 Bulk community photophysiology

The photophysiology of the microbial community was assessed using pulse-amplitude-modulation techniques following methods described by Perkins et al. (2006). Specifically, variable chlorophyll fluorescence was measured in 3-mL aliquots using a WaterPAM fluorometer combined with a blue emitter/detector cuvette system, with continuous stirring to prevent settling (Walz GmBH, Effeltrich, DE). All samples were dark-adapted for 30 min prior to rapid light curve assessments (RLCs), which consisted of nine 20-s steps of incrementally increasing actinic light intensities that ranged from 33 to 1,306 μmol photons $m^{-2}\ s^{-1}$ followed by a saturating light pulse (~8,600 μmol photons $m^{-2}\ s^{-1}$ for 600 m) at the end of each

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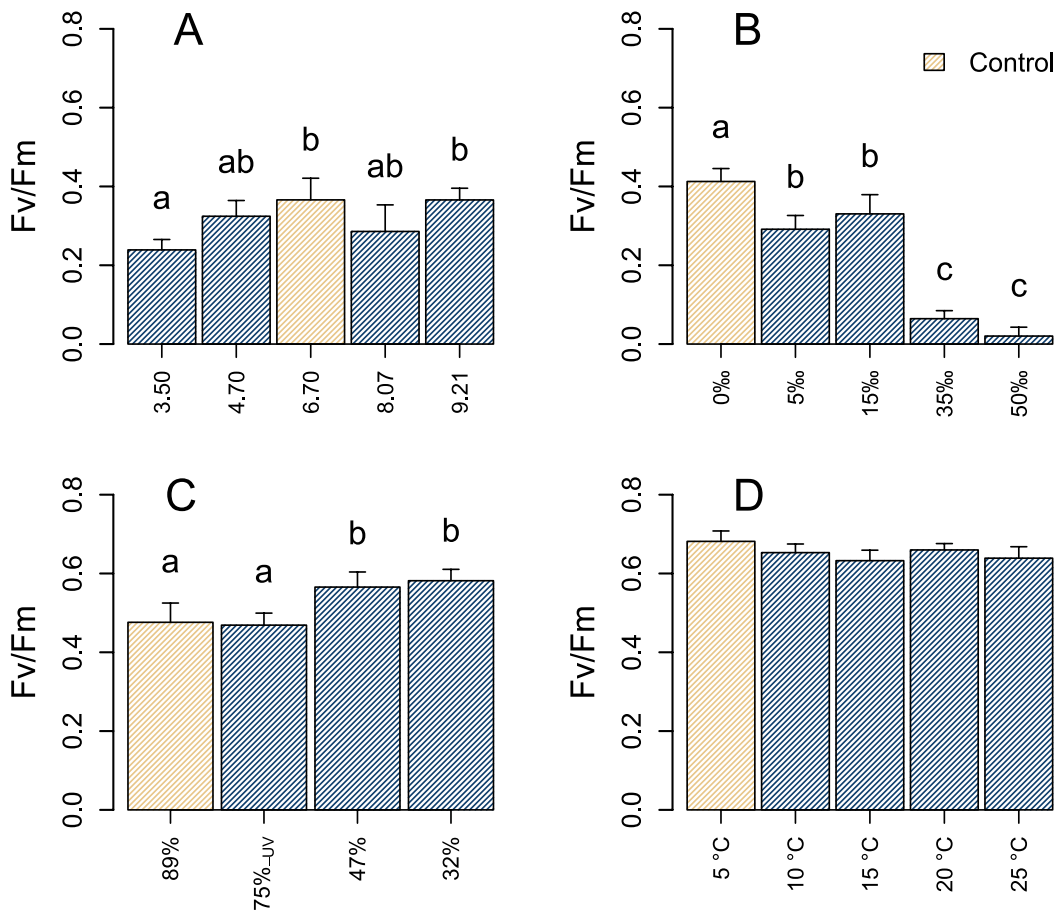

FIGURE 2
Maximal quantum yields (F_v/F_m) of electron transport through photosystem II for glacier ice algae that have been incubated under varying pH **(A)**, salinity **(B)** and light **(C)** conditions for 48 h (n = 5), as well as different temperatures **(D)** for 4 h (n = 3). Gold columns represent control samples reflecting the prevailing environmental conditions of the study site on the Greenland ice sheet (61°06′N, 46°50′W) during 2021. Error bars indicate one standard deviation from the mean. Different lowercase letters denote significant differences at $p < 0.05$ (Tukey's HSD test).

step. Maximum quantum efficiency (F_v/F_m) was calculated as the ratio of variable fluorescence (F_v), which is the difference between the minimum (F_0) and maximum (F_m) fluorescence yields in the dark-adapted state, to F_m. The relative rate of electron transport (rETR) was calculated as the product of the quantum yield of photosystem II (Y [PSII]) multiplied by the incident excitation and 0.5, thereby assuming an equal distribution of absorbed light energy between photosystems I and II. Relative maximum electron transport rates ($rETR_{max}$), theoretical maximum light utilization coefficients (α) and light saturation coefficients (E_k) were derived from RLCs (rETR ~ light intensity) constructed in R version 4.0.2 using an iterative curve fitting approach adapted from Williamson et al. (2020) to apply the Eilers and Peeters (1988) model. Outliers, which were defined as model residuals with z-scores greater than 2 or less than −2, were excluded from the data.

2.4 Statistical analysis

Statistical analysis of the data was performed in R version 4.0.2 using methods outlined by Crawley (2007). Assumptions of normality and homogeneity of variance were assessed through Shapiro-Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) was used to assess differences in photophysiological parameters across each of the gradients. Post-hoc pairwise comparisons were performed using Tukey's HSD test when significant variations were detected. When parametric assumptions were met, the Student's t-test was used to compare means between the T_0 and control samples from the pH, salinity, and light experiments. In cases where assumptions of data normality and equal variance were violated, the Mann-Whitney U test (MW) was employed. For this study, the alpha level was set to 0.05. All errors reported in the text are one standard deviation about the mean.

3 Results

3.1 Technical challenges

Although, a series of incubation experiments aimed to investigate the individual effects of pH, salinity, light, and temperature on the photophysiology of glacier ice algae in south Greenland, the rapid light curve assessments, performed using PAM

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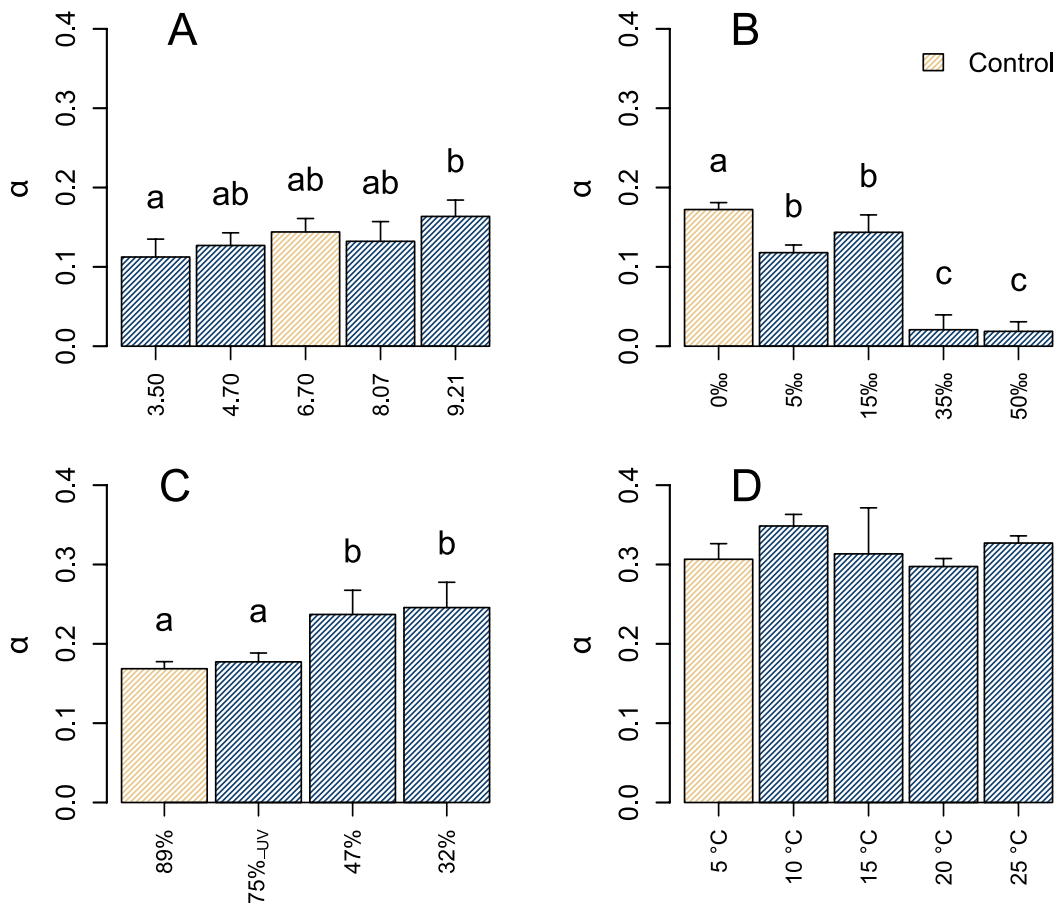

FIGURE 3
Light utilization efficiencies (α) of glacier ice algae that have been incubated under varying pH **(A)**, salinity **(B)** and light **(C)** conditions for 48 h (n = 5), as well as different temperatures **(D)** for 4 h (n = 3). Gold columns represent control samples reflecting the prevailing environmental conditions of the study site on the Greenland ice sheet (61°06′N, 46°50′W) during 2021. Error bars indicate one standard deviation from the mean. Different lowercase letters denote significant differences at $p < 0.05$ (Tukey's HSD test).

fluorometry, were insufficient to fully saturate PSII reaction centers within the microbial community (Supplementary Figures S2, S3). Consequently, it was not possible to derive values for $rETR_{max}$ and E_k from any of the incubation experiments. However, we were able to derive values for F_v/F_m and α.

3.2 Ecophotophysiology

The photophysiological responses of the microbial community to variations in pH, salinity, light, and temperature appeared to differ among the four incubation experiments (Figures 2, 3). Within each specific experiment, both F_v/F_m and α values exhibited similar trends. Most notably, variations in salinity and light exerted a more pronounced influence on algal photophysiology than either pH or temperature.

In the context of salinity, a 5‰ increase above ambient conditions resulted in a decline in F_v/F_m from 0.41 ± 0.03 to 0.29 ± 0.02 and α decreased from 0.17 ± 0.01 to 0.12 ± 0.01 (both Tukey's HSD, $p < 0.05$; Figures 2B, 3B). Subsequently, a 35‰ increase in salinity above ambient levels led to significant decreases in both parameters: F_v/F_m to 0.06 ± 0.02 and α to 0.02 ± 0.02 (both $p < 0.05$). Furthermore, while the exclusion of UV light had no effect on F_v/F_m or α values (both Tukey's HSD, $p > 0.05$; Figures 2C, 3C), both increased respectively from 0.48 ± 0.05 and 0.17 ± 0.01 to 0.58 ± 0.03 and 0.25 ± 0.03 when irradiance was reduced to 32% of ambient conditions (both Tukey's HSD, $p < 0.05$).

The photophysiology of the microbial community remained largely stable in response to variations in pH, with F_v/F_m ranging from 0.29 ± 0.03 to 0.37 ± 0.06 (Figure 2A) and α ranging from 0.13 ± 0.02 to 0.16 ± 0.02 (Figure 3A) between pH 4.7 and 9.21 (both Tukey's HSD, $p > 0.05$). However, F_v/F_m was lower for samples incubated at pH 3.5 (0.11 ± 0.02) relative to those incubated at pH 6.7 (control; $p < 0.05$) and 9.21 ($p < 0.05$). Additionally, temperature elevations showed no discernible impact on either F_v/F_m or α, with values ranging from 0.63 ± 0.03 to 0.68 ± 0.03 for F_v/F_m (Figure 2D) and 0.30 ± 0.01 to 0.35 ± 0.01 for α (Figure 3D), as determined by ANOVA ($p > 0.05$).

3.3 Photoinhibition

The photophysiology of the microbial community was assessed in all control samples. This evaluation occurred immediately after

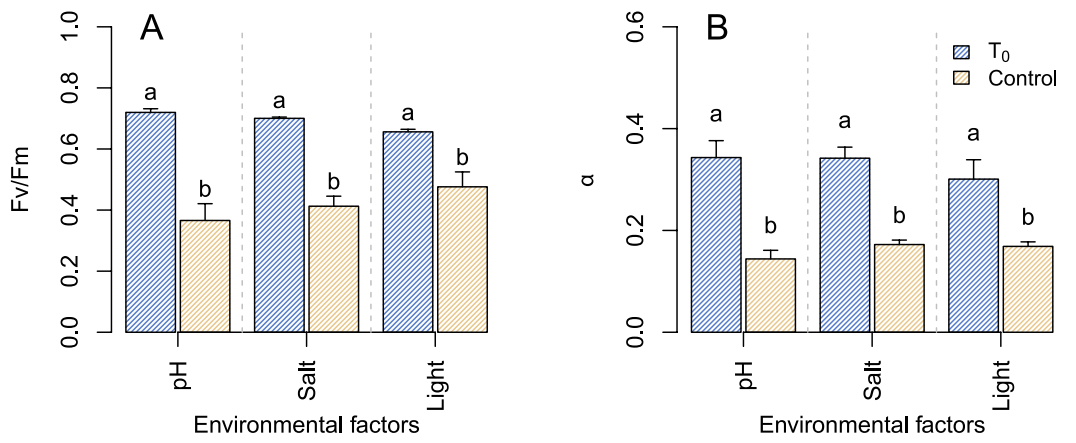

FIGURE 4
Maximal quantum yields (F_v/F_m) of electron transport through photosystem II **(A)** and light utilization efficiencies (α); **(B)** of glacier ice algae from incubation experiments that examined the effects of pH, salinity (salt), and light on algal photophysiology. Incubation conditions reflect those of the study site on the Greenland ice sheet (61°06′N, 46°50′W) during 2021. Measurements were made directly after melting (T_0) and following a 48-h incubation under ambient conditions (control). Error bars indicate one standard deviation from the mean (n = 5). Different lowercase letters denote significant differences at $p < 0.05$ (Student's t-test for all, except Light in A, which used the Mann-Whitney U test).

TABLE 1 Overview of photophysiological parameters from other studies on glacier ice algae based on PAM measurements. All samples were without any treatment. The numbers are the average reported from each study.

Study	RLC	F_v/F_m ratio	Alpha	Condition
Yallop et al. (2012)	15-1800	—	0.20-0.29	Field measurement
Williamson et al. (2020)	0-4000	0.37	0.1	Field incubation
McCutcheon et al. (2021)	0-4000	0.42	-	Field incubation
Procházková et al. (2021) *A. nordenskiöldii*	5-1389	—	0.07	Field measurement
Procházková et al. (2021) *A. alaskanum*	5-1389	—	0.22	Field measurement
Halbach et al. (2022)	0-2064	0.2-0.35	0.05-0.1	Field incubation
Perini et al. (2023)	0-3480	0.67	0.25-0.27	Laboratory incubation
Remias and Procházková (2023) high light	0-2100	—	0.09	Laboratory culture
Remias and Procházková (2023) low light	0-2100	—	0.23	Laboratory culture
Doting et al. (2024)	—	0.31	—	Field measurement
Peter et al. (2024)	—	0.49 and 0.655	—	Field measurement
Control (this study)	33-1306	0.43	0.19	Field incubation
T_0 (this study)	33-1306	0.64	0.35	Field measurement

the melting process (T_0) and subsequently following a uniform 48-h incubation period for the pH, salinity, and light incubation experiments, ensuring consistent conditions across all samples. The F_v/F_m values in the T_0 samples, which ranged from 0.66 ± 0.01 to 0.72 ± 0.01, were consistently higher than in the control samples (0.37 ± 0.06 to 0.48 ± 0.05) after 48 h (Figure 4A) for all incubation experiments conducted (t-test for all, except light, for which MW was used, $p < 0.05$). Similarly, α was significantly lower in the control samples (0.14 ± 0.02 to 0.17 ± 0.01) relative to the T_0 samples (0.30 ± 0.04 to 0.34 ± 0.03; Figure 4B) in the three incubation experiments (t-test for all, $p < 0.05$).

4 Discussion

While glacier ice algae have been observed on glaciers and ice sheets globally (Takeuchi et al., 1998; Takeuchi et al., 2006; Takeuchi and Kohshima, 2004; Remias et al., 2012; Lutz et al., 2018; Di Mauro et al., 2020), very few attempts have been made to examine their photophysiology. Where investigations have been conducted, they have almost exclusively focused on the bulk photophysiology of the whole ice community (Yallop et al., 2012; Williamson et al., 2020; McCutcheon et al., 2021; Halbach et al., 2022; Perini et al., 2023; Doting et al., 2024). However, the photophysiology of both the

unicellular (*A. alaskana*) and filamentous (*A. nordenskiöldii*) algae were analysed separately in a study by Procházková et al. (2021). Photophysiological data from selected studies that utilised PAM fluorometry (Williamson et al., 2020; McCutcheon et al., 2021; Halbach et al., 2022; Doting et al., 2024) have shown that the F_v/F_m ratios for glacier ice algae are often lower than the values typically reported for non-stressed green microalgae and plants (Table 1), which are around 0.7-0.8 (Masojídek et al., 2013; Björkman and Demmig, 1987). A lower F_v/F_m ratio implies low potential maximal photosynthetic activity, indicating potential stress or suboptimal conditions for photosynthesis. In this study, the F_v/F_m ratio of the control samples, incubated under *in situ* light conditions was between 0.37-0.48 for pH, salt, and light experiments, corresponding well with reported values from the literature for naturally occurring glacier algae dominated communities from Greenland, Table 1 (Williamson et al., 2020; McCutcheon et al., 2021; Halbach et al., 2022; Doting et al., 2024; Peter et al., 2024).

From this study, it was determined that light history and intensity prior to and during the experiments exert a greater influence on the photophysiology of glacier ice algae. Three observations in this study supporting this statement: 1) the absolute high F_v/F_m ratios in the temperature experiment, 2) the differences between T_0 and control in the light, pH and salinity experiments, and 3) the light experiment results.

The F_v/F_m ratio for the temperature experiment (F_v/F_m ratio of 0.7–0.8) is higher and similar to the values reported in Perini et al. (2023) and Peter et al. (2024), which was conducted under low light. The higher F_v/F_m ratio during the temperature experiment could be explained by the lower light intensity that the algae received, since these were conducted inside a laboratory tent, compared to the rest of the *in situ* incubation experiments that were outside under *in situ* high light intensity. This observation is also supported by the data from the T_0 measurements (Figure 4A). The samples used for all the experiments were melted in a tent under low light conditions for 72 h, after which T_0 was measured before the start of the light, pH, and salinity experiments, incubated under *in situ* light conditions. T_0 had a F_v/F_m ratio of 0.66–0.72 (close to the ideal ratio of 0.7–0.8), dropping to 0.37-0.48 after incubations (control samples Figure 4A). Finally, in the light experiment, a clear pattern of higher F_v/F_m ratio in samples that received lower light is observed as also observed in Williamson et al. (2020). The glacier ice algae had an increased utilization of the light (α) when exposed to reduced incoming light (Figure 3C). This supports the hypothesis that the glacier ice algae benefit from a lower light intensity compared to the natural irradiation on the ice surface. Additional evidence of the glacier ice algae's higher photosynthetic performance under lower light intensities comes from studies where glacier ice algae are cultured, under lower light intensities (max. 300 μmol photons $m^{-2}s^{-1}$; Remias and Procházková, 2023; Jensen et al., 2023). Production of the purpurogallin pigment under high natural light conditions is suggested to be energetically expensive for the cell and, thus, under low light conditions, photosynthetic performance can be improved (Williamson et al., 2020; Williamson et al., 2021). Besides expending energy on pigment production under increased light intensities, algal cells may also need to manage reactive oxygen species that can damage components such as DNA and proteins within the cell (Rippin et al., 2019). This indicates that, even though glacier ice algae can tolerate irradiation up to 4,000 μmol photons $m^{-2}s^{-1}$ (Williamson et al., 2020; McCutcheon et al., 2021), high light intensities are not their optimal growth condition.

In this study, no significant differences were identified for the F_v/F_m ratio or light utilization efficiency (α) in the pH experiment, indicating that the algae can cope with a broad range of pH conditions, both acidic and alkaline. The pH of the ice at the sampling time was 6.7 ± 0.2. Glacier ice algae have successfully been cultured in media with pH 5 and 6 (Remias and Procházková, 2023; Jensen et al., 2023) and both alkaline (8.6) and acidic (3.6) pH levels have been measured at different glaciers, where glacier ice algae have been observed (Komárek and Komárek, 2001; Takeuchi and Kohshima, 2004; Remias et al., 2011; Procházková et al., 2021), supporting the assertion that glacier ice algae can cope with a broad pH range.

In addition to growth across a broad range of pH, glacier ice algae were also able to withstand increasing temperatures. No significant differences were observed between the increased temperatures (10°C–25°C) and the control (5°C). However, this study was only conducted over a period of 4 h (Figures 2D, 3D). The algae naturally experience a fluctuation in temperature during a day with temperatures fluctuating between 1.5°C at night and 6° during the summer at the field site (Fausto et al., 2021). The maximum temperature for the summer of 2021 at the field camp was 6.21°C (Fausto et al., 2021), indicating that, for at least a short period, the glacier ice algae are still actively photosynthesizing at a temperature four times higher than the maximum air temperature at the study site. Growth and high photosynthetic performance at high temperatures have also been observed in both Arctic and Antarctic microalgae, suggesting that many polar microalgae are psychrotolerant rather than psychrophilic (Chen et al., 2012; Teoh et al., 2004; Stibal and Elster, 2005). The glacier ice algae, however, was strongly impacted by high salinity. Increasing salt concentrations are a known abiotic stressor, where elevated levels of reactive oxygen species interfere with photosynthesis by damaging photosystem I (PSI) and weakening of PSII (Shetty et al., 2019). Furthermore, transcriptomic data from the green microalgae *Chlamydomonas reinhardtii* have shown that downregulation of PSI light-harvesting complex genes leads to reduced photosynthetic performance in the cells (Shetty et al., 2019). Some bacterial and algal species have a cross-tolerance between salt stress and stress from freezing since those stressors are both related to osmotic changes within the cell (Wilson et al., 2012; Schmid et al., 2009; Tanaka et al., 2001; Raymond et al., 2020). However, in this study, the F_v/F_m ratio and the light utilization efficiency (α) significantly dropped with increasing salt concentration (Figures 2B, 3B).

This study was conducted using *in situ* incubations with a single condition change to examine its affect on the photophysiology of glacier ice algae. With fresh cultures of glacier ice algae (Remias and Procházková, 2023; Jensen et al., 2023), it would be possible to conduct a similar incubation study in a more controlled environment for an extended duration, allowing for the investigation of prolonged effects, such as temperature variations on photophysiology. Furthermore, laboratory studies with these cultures could enable the exploration of interactive effects, where multiple environmental parameters, such as increasing light and

temperature, are varied simultaneously to better simulate and understand their combined impact on the algae.

In this study, it was demonstrated that glacier ice algal photophysiology is highly influenced by solar irradiance while performing well under a wide range of other physicochemical conditions. Solar irradiance significantly impacts the photophysiology and fitness of glacier ice algae, potentially serving as the primary driver of photophysiological variability under natural conditions. Despite their sensitivity to light, as expected from a photosynthetic organism, and increasing salt conditions, glacier ice algae were able to sustain *in situ* electron transport under a broad range of pH and temperature conditions. Based on the incubation experiments under different temperature conditions, it was shown that the glacier ice algae may not be truly psychrophilic but rather psychrotolerant. However, these experiments were only conducted for a short period of time and thus, it is possible that more pronounced differences in photophysiology under different temperature conditions could emerge over an extended duration. Despite this, it is argued that the adaptation of glacier ice algae to such a broad range of physico-chemical conditions is advantageous, considering their non-motile nature and the dynamic environment of the ice surface, which can undergo rapid changes over short periods.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MJ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing–original draft, Writing–review and editing. TT-J: Data curation, Formal Analysis, Methodology, Validation, Writing–original draft, Writing–review and editing. MT: Funding acquisition, Project administration, Writing–original draft, Writing–review and editing. LB: Funding acquisition, Project administration, Writing–original draft, Writing–review and editing. AA: Conceptualization, Funding acquisition, Project administration, Supervision, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgeoc.2024.1436488/full#supplementary-material

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