

Dual-Stressor Effects of Road Salt and Aquatic Herbicide on Freshwater Phytoplankton: Cyanobacterial Tolerance to Salinity

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Abstract

The aquatic herbicide ProcellaCOR (florpyrauxifen-benzyl) is increasingly used for invasive plant control; however, its effects on non-target freshwater phytoplankton have only recently gained scientific attention, particularly under the major co-occurring stressor road salt. To our knowledge, no studies have evaluated their interactive effects on primary producers. We conducted a microcosm study to assess the individual and combined effects of florpyrauxifen-benzyl ($10 \mu\text{g L}^{-1}$ and $50 \mu\text{g L}^{-1}$), road salt ($860 \text{ mg L}^{-1} \text{ NaCl}$), and their mixtures on the green alga *Scenedesmus obliquus* and the cyanobacterium *Synechococcus elongatus*. ProcellaCOR alone did not affect the growth of either taxon. Salinity was the primary driver of responses in green algae, significantly reducing chlorophyll, lipid content, biomass, and cell abundance, with no indication of interactive effects with ProcellaCOR. In cyanobacteria, chlorophyll was affected by the ProcellaCOR $\times$ salinity interaction, with a significant reduction observed only in the $50 \mu\text{g L}^{-1}$ ProcellaCOR $\times$ $860 \text{ mg L}^{-1} \text{ NaCl}$. Salinity also reduced lipid content and biomass in cyanobacteria, whereas cell abundance remained unaffected. Overall, salinity exerted stronger effects than ProcellaCOR on both taxa, and mixture effects were limited to chlorophyll at the highest ProcellaCOR concentration. Across all growth endpoints, cyanobacteria exhibited greater tolerance to salinity than green algae. These findings provide the empirical evidence needed to understand the effects of florpyrauxifen-benzyl on freshwater phytoplankton under environmentally relevant salinity conditions and support the incorporation of multiple stressors into ecological risk assessments.

1. Introduction

Freshwater ecosystems are increasingly threatened by a complex array of natural and anthropogenic stressors (Bănăduc et al., 2022; Orr et al., 2024). Among the most pervasive chemical contaminants in fresh waters are pesticides and road salts, which are widely used in agricultural and urban settings (Hintz & Relyea, 2019; Syafrudin et al., 2021). These pollutants often interact synergistically, antagonistically, or additively, complicating efforts to predict ecological outcomes (Hutton et al., 2021; Ahmed et al., 2026). Despite their ecological relevance, more studies are needed to understand the combined effects of these contaminants, particularly on freshwater primary producers (Ahmed & Schuler, 2026). Factors such as exposure order, duration, and concentration can profoundly influence outcomes, emphasizing the need for integrative, hypothesis-driven research that mirrors real-world scenarios.

In freshwater environments, aquatic herbicides are applied to manage invasive macrophytes, but can negatively affect non-target organisms, such as algae (Staley et al., 2015). Florpyrauxifen-benzyl, the active ingredient in ProcellaCOR EC, is a relatively recent addition to the aquatic herbicide market, approved by the U.S. Environmental Protection Agency in 2017, and is classified within the arylpicolinate group of synthetic auxins (Herrera et al., 2021; Jin et al., 2023). Florpyrauxifen-benzyl mimics the action of endogenous plant hormones such as auxins and is primarily used to target invasive aquatic plants, such as variable-leaf watermilfoil in North America (Haug et al., 2021). Field applications have demonstrated ProcellaCOR EC's efficacy in plant control with reportedly low acute toxicity to non-target freshwater fauna, including sensitive taxa such as unionid mussels (Buczek et al., 2020). Despite these initial findings, the effects of ProcellaCOR on freshwater phytoplankton remain largely unexplored, given the foundational role of algae in aquatic ecosystems and their early exposure to waterborne pollutants.

Herbicides with similar modes of action, such as cycloxydim and fenoxaprop, inhibit acetyl-CoA carboxylase (ACCase), an enzyme that is crucial for fatty acid biosynthesis, and have demonstrated adverse effects on freshwater algae, including disrupted growth and physiological processes (Lichtenthaler, 1990). Therefore, it is important to determine whether florpyrauxifen-benzyl poses similar risks as it gains broader regulatory approval and field application.

Alongside herbicides, road salt is considered a major contributor to freshwater pollution. Road salt, primarily sodium chloride (NaCl), has been extensively used since the 1940s for winter road safety, leading to increased concentrations of sodium (Na^+) and chloride (Cl^-) in surface water and groundwater in urban and suburban areas (Daley et al., 2009; Hintz et al., 2021). Increased salinity can affect algal osmoregulation, with adverse effects on algal growth, community composition, and biodiversity. For instance, increased chloride concentrations may reduce phytoplankton diversity, sometimes shifting communities from dominant green algae to more salt-tolerant cyanobacteria (Urrutia-Cordero et al., 2024). However, responses

to salinity are mixed and species-specific across both cyanobacteria and green algae: some taxa decline sharply under elevated salinity (Ji et al., 2018), while others are resistant (Tonk et al., 2007). These contrasting responses make it difficult to predict when, where, and how road salt pollution will favor either green algae or cyanobacteria.

Phytoplankton taxa differ in their ecological strategies and sensitivity to environmental stress, making comparative studies essential. Green algae, such as *Scenedesmus obliquus*, generally exhibit rapid growth and resilience, whereas cyanobacteria, such as *Synechococcus elongatus*, are often more tolerant of disturbed or eutrophic conditions and can dominate under stress (Mehta et al., 2029; Lin et al., 2020). This taxonomic and ecological diversity makes phytoplankton ideal bioindicators for evaluating environmental stressors in freshwater systems. In this study, we evaluate how florpyrauxifen-benzyl and salinity, individually and combined, affect algal growth, biomass production, and water chemistry across two distinct phytoplankton taxa. By comparing species-specific sensitivities, we will identify vulnerable taxa and potential shifts in community composition, generating data to support environmental risk assessments, ecosystem management, and regulatory guidance for the use of ProcettaCOR EC in saline-impacted freshwater systems. We hypothesize that salinity will act as the primary stressor by disrupting osmotic balance and cellular homeostasis, while florpyrauxifen-benzyl induces context-dependent, threshold-driven mixture effects based on the absence of auxin-regulated growth pathways in non-vascular algae. Furthermore, cyanobacteria will exhibit greater tolerance to salinity than green algae due to their more robust osmoregulatory capacity.

2. Methods and Materials

2.1. Study design

The study was conducted under controlled laboratory conditions using static microcosms. Two algal species representing distinct taxonomic groups were used: the green alga *Scenedesmus obliquus* and the cyanobacterium *Synechococcus elongatus*. Microcosms consisted of 250 mL glass jars (working volume = 0.2 L) containing algal cultures grown under standardized temperature ($25 \pm 2^\circ\text{C}$, using thermal pads) and light conditions (12 h light:12 h dark photoperiod, $60 \mu\text{mol photons/m}^2/\text{s}$).

For each species, eight treatments were established with four replicates per treatment, resulting in 32 microcosms per species and 64 total experimental units. The experimental treatments included a no-chemical control, two environmentally relevant florpyrauxifen-benzyl concentrations ($10 \mu\text{g L}^{-1}$ and $50 \mu\text{g L}^{-1}$), a single NaCl exposure (860 mg L^{-1}), and two combined herbicide–salt treatments. Although we did not continuously monitor pesticide concentrations, we ensured uniform exposure by applying a single dose from the same stock solution at the start of the experiment under identical controlled conditions, with any degradation or changes in bioavailability occurring consistently across all replicates. To account for potential solvent effects, two dimethyl sulfoxide (DMSO) controls corresponding to the herbicide vehicle volumes were included. Algal cultures were inoculated into microcosms and dosed with their respective treatments. The test units were organized following a completely randomized design (CRD). Samples were collected in 48 hours, 96 hours, day 7, day 14, and day 22 after treatment exposure to monitor a suite of biological and chemical parameters. This timeline was chosen to track acute through chronic effects of herbicide and salt exposure.

2.2. Algal culture

Pure strains of freshwater algae, *Scenedesmus obliquus* and *Synechococcus elongatus*, were obtained from the Culture Collection of Algae at the University of Texas at Austin (UTEX, USA), which maintained axenic lines with no history of pesticide or salt exposure, ensuring unconditioned responses to treatments. To prepare the stock solutions for the experiment, concentrated algal cultures were inoculated into the COMBO water medium in 500 mL borosilicate glass Erlenmeyer flasks. The COMBO algal medium was prepared following the standard protocol (Kilham et al., 1998), ensuring that all test organisms were maintained in a nutrient-rich, standardized environment conducive to algal growth. The prepared stock cultures were acclimated for 1 week under controlled laboratory conditions, including a 12 h light:12 h dark photoperiod, with a light intensity of $60 \mu\text{mol photons/m}^2/\text{s}$ to simulate natural light exposure. Cultures were maintained at a constant temperature of $25 \pm 2^\circ\text{C}$ on a hot plate and continuously stirred at 350 rpm using a sterilized magnetic stir bar to ensure uniform nutrient and cell distribution. A continuous supply of oxygen was provided to promote optimal algal growth, facilitating enhanced cellular respiration and

overall culture health. Although we did not quantify the exact initial algal cell density, we ensured comparable starting conditions by inoculating all microcosms with the same volume (10 mL) of a thoroughly homogenized stock culture.

2.3. Data collection

A range of biological and chemical indicators was used to quantify treatment effects on both species. Biologically, measurements included algal cell abundance via flow cytometry, chlorophyll using a Turner Trilogy fluorometer (Turner Designs, San Jose, CA), lipid analysis using a BODIPY 505/515 stain via flow cytometry, and dry-weight biomass after filtration and drying. Physicochemical parameters were also monitored throughout the experimental period to support the interpretation of biological responses. These include temperature, pH, dissolved oxygen (mg/L), electrical conductivity (EC) using calibrated probes, colored dissolved organic matter (CDOM) in RFU measured by a Turner Designs Trilogy Laboratory Fluorometer, as well as total nitrogen (mg/L) and total phosphorus (mg/L) concentrations assessed via spectrophotometric kits following the manufacturer's standard protocols.

2.3.1. Algal cell abundance

Algal cell abundance was quantified using an Attune NxT flow cytometer (Thermo Fisher Scientific), a widely used technique for rapid enumeration of phytoplankton and cyanobacteria in aquatic systems. Flow cytometry allows discrimination of algal populations based on optical properties such as forward scatter (FSC), which is related to relative cell size, and side scatter (SSC), which reflects internal complexity and granularity (Phinney and Cucci, 1989; Dubelaar and Jonker, 2000).

During each sampling event, 1 mL of water sample was collected from each experimental unit and transferred into labeled sample tubes. Samples were preserved immediately by adding 1–2 drops of Lugol's iodine solution, which is commonly used for fixation and preservation of phytoplankton cells before counting (Marie et al., 2005). Before analysis, sample tubes were homogenized using a mechanical shaker to minimize cell settling and ensure uniform cell distribution.

For analysis, a 200 µL subsample was prepared following the tube method. Samples were processed at a low flow rate, and the cytometer was set to acquire 10,000 events using a 50 µL sample volume. Algal populations were identified and gated using FSC vs SSC density plots, which provided size distribution patterns and allowed separation of algal cells from background particles and debris. The primary statistic recorded was cell count per sample, which was used as the measure of algal abundance (Marie et al., 2014).

2.3.2. Lipid analysis

At the end of the experiment (Day 22), lipid content in *Scenedesmus obliquus* and *Synechococcus elongatus* was quantified using BODIPY™ 505/515 fluorescent staining via flow cytometric analysis following the standard protocol (Argyle et al., 2023; Kallau & Yang, 2025). A BODIPY stock solution was prepared by dissolving 2 mg of dye in 1 mL dimethyl sulfoxide (DMSO) and stored at – 20°C in the dark. On the day of analysis, a working dye solution (final concentration: 20 µg/mL) was prepared in COMBO medium.

Live algal cultures (1 mL) were stained with 50 µL of the working dye, gently mixed, and incubated for 10 min at room temperature in the dark. Unstained and solvent (DMSO) controls were included for each treatment and species to account for background fluorescence and solvent effects. Following incubation, samples were analyzed using an Attune NxT flow cytometer (Thermo Fisher Scientific) equipped with a 488 nm laser. BODIPY fluorescence was detected in the BL1 (green) channel. Data were acquired at low flow rates, with 5,000–20,000 events collected per sample.

Cells were first gated using forward scatter (FSC) versus side scatter (SSC) to remove debris and non-cellular particles. Lipid content was quantified as the median BL1 fluorescence intensity of stained samples after subtraction of the corresponding unstained control signal ($Lipid = Stained\ median\ BL1 - Unstained\ median\ BL1$). Fluorescence statistics were exported as median values for subsequent statistical analyses.

2.3.3. Chlorophyll fluorescence and Biomass

Total chlorophyll was measured as *in vivo* fluorescence using a Turner Designs Trilogy Laboratory Fluorometer (Turner Designs, San Jose, CA) equipped with the Chlorophyll *in vivo* module. At each sampling time, 3 mL of water from each experimental unit was transferred to a chlorophyll cuvette and analyzed directly without pigment extraction. Fluorescence measurements were obtained using the “CHL-A” program through the instrument interface. The module excites chlorophyll pigments in intact algal cells using blue light (peak excitation ~ 460 nm; excitation band centered near 441 nm). Emitted red fluorescence resulting from the Stokes shift was detected through an emission filter spanning 660–710 nm. Fluorescence intensity was quantified by the instrument’s internal photodiode detector and recorded as relative fluorescence units (RFU). Because measurements were performed on live, unextracted samples, the signal represents total *in vivo* chlorophyll fluorescence and was used as a non-destructive proxy for algal biomass and photosynthetic activity.

Algal biomass was determined at the end of the experiment (day 22) using a gravimetric method. A 100 mL sample from each of the 64 experimental units was filtered through pre-weighed 47 mm Whatman 934-AH Glass Microfiber Filters. Filters were weighed before filtration to obtain the initial mass. After filtration, filters containing the retained algal material were air-dried for 72 h until a constant weight was achieved and then reweighed. Biomass was calculated as the difference between the final filter mass and the pre-filtration mass and expressed as dry weight (mg/100 mL) per unit volume of culture. This approach provided an integrated estimate of algal standing biomass in each experimental unit.

2.4. Analysis of Temporal Dynamics

Temporal dynamics of algal cell abundance were analyzed using linear mixed-effects models to account for repeated measurements within experimental units. Cell abundance was measured at multiple time points (days) across experimental treatments for each independent experimental unit (Jar). Treatments included control and exposure conditions for both green algae and cyanobacteria systems. Day was treated as a continuous numerical variable, Treatment as a categorical fixed factor, and Jar as a random experimental unit to account for repeated measurements within the same experimental unit over time.

Temporal trends in cell abundance were first visualized using treatment-wise time series plots. For each treatment, mean values and associated standard errors were calculated at each sampling time point. To statistically evaluate temporal responses, linear mixed-effects models were applied separately for green algae and cyanobacteria using the following structure:

Cell Abundance ~ Day × Treatment + (1 | Jar)

where Day, Treatment, and their interaction were treated as fixed effects, and Jar was included as a random intercept to account for repeated observations within experimental units. The significance of fixed effects was assessed using Type III analysis of variance (ANOVA). The main effects of Day and Treatment, as well as their interaction, were evaluated to determine whether temporal trajectories differed among treatments. Model residuals were inspected to ensure appropriate fit and assumptions of homogeneity and normality. All analyses were conducted in R using the *lme4* package.

2.5. Data analysis

All statistical analyses were performed in R (version 4.2.3) (Team, 2022). Normality of all response variables, including biotic parameters (Cell abundance, chlorophyll, lipid content, biomass) and abiotic parameters (CDOM, EC, DO, temperature, pH, TN, TP), was assessed using the Shapiro–Wilk test. Variables that violated normality were rank-transformed using Blom’s rank-based normal quantile transformation before parametric analyses, with transformed variables indicated by the suffix *_ranknorm*. For both green algae and cyanobacteria, the effects of the herbicide ProcettaCOR, salinity, and their interaction on biotic and abiotic variables were evaluated using two-way ANOVA. Where significant main or interaction effects were observed, post-hoc comparisons against the control treatment were conducted by Dunnett’s test using the *glht* function from the *multcomp* package. The control level was specified with the *relevel* function to ensure proper reference for Dunnett’s comparisons. All figures were generated using the *ggplot2* package, with transformed values plotted when appropriate. Significant differences identified from post-hoc analyses were annotated on figures using standard symbols (**p* < 0.05, ***p* < 0.01, ****p* < 0.001).

3. Results

3.1 Biotic Responses

The two-way ANOVA (Table 1) and subsequent Dunnett's post hoc test (Table 2) indicated that green algae were more consistently affected by salt across all biological endpoints, whereas cyanobacteria showed a more variable chlorophyll response and no stressor effects on cell abundance. Chlorophyll concentrations varied among treatments (Fig. 1). In *Scenedesmus obliquus*, chlorophyll decreased with salt ($F_{1,154} = 47.736$, $p < 0.001$), while ProcellaCOR and the interaction with salt had no effect. Dunnett's tests showed lower chlorophyll under salt (adjusted $p < 0.001$) and in both mixture treatments (ProcellaCOR low $\times$ salt: adjusted $p < 0.001$; ProcellaCOR high $\times$ salt: adjusted $p < 0.001$). In *Synechococcus elongatus*, chlorophyll was affected by ProcellaCOR ($F_{2,154} = 6.135$, $p = 0.003$), salt ($F_{1,154} = 5.032$, $p = 0.026$), and their interaction ($F_{2,154} = 5.577$, $p = 0.005$). Dunnett's post hoc results indicate no change under ProcellaCOR-only or salt-only treatments, while chlorophyll decreased only under the high ProcellaCOR $\times$ salt treatment (adjusted $p = 0.006$).

Table 1

Results of two-way analysis of variance (ANOVA) evaluating the main and interactive effects of ProcellaCOR and salt on biotic response variables of Green Algae and Cyanobacteria. For each response variable, the table reports degrees of freedom (df), sum of squares (Sum Sq), mean square (Mean Sq), F values, and corresponding p values for the main effects and their interaction (ProcellaCOR × Salt). Significant effects ($p < 0.05$) are indicated by the reported p values in bold.

Taxa	Variables	Effect	df	Sum Sq	Mean Sq	F value	p value
Green Algae	Chlorophyll	ProcellaCOR	2	172,600,000,000	86,300,000,000	2.097	0.126
		Salt	1	1,964,000,000,000	1,964,000,000,000	47.736	< 0.001
		ProcellaCOR × Salt	2	42,730,000,000	21,360,000,000	0.519	0.596
		Residuals	154	6,337,000,000,000	41,150,000,000		
	Lipid	ProcellaCOR	2	1.607	0.804	2.152	0.137
		Salt	1	18.26	18.26	48.896	< 0.001
		ProcellaCOR × Salt	2	1.18	0.59	1.581	0.225
		Residuals	26	9.709	0.373		
	Biomass	ProcellaCOR	2	38.9	19.4	1.642	0.213
		Salt	1	1557.8	1557.8	131.672	< 0.001
		ProcellaCOR × Salt	2	11.3	5.6	0.476	0.626
		Residuals	26	307.6	11.8		
Cyanobacteria	Cell Abundance	ProcellaCOR	2	2.71	1.354	1.597	0.206
		Salt	1	24.63	24.63	29.048	< 0.001
		ProcellaCOR × Salt	2	0.77	0.387	0.457	0.634
		Residuals	154	130.58	0.848		
	Chlorophyll	ProcellaCOR	2	10.67	5.337	6.135	0.002
		Salt	1	4.38	4.377	5.032	0.026
		ProcellaCOR × Salt	2	9.7	4.852	5.577	0.004
		Residuals	154	133.97	0.87		
	Lipid	ProcellaCOR	2	0.051	0.025	0.058	0.944
		Salt	1	19.133	19.133	43.662	< 0.001
		ProcellaCOR × Salt	2	0.18	0.09	0.205	0.816
		Residuals	26	11.393	0.438		
	Biomass	ProcellaCOR	2	20.1	10.1	0.542	0.588

Taxa	Variables	Effect	df	Sum Sq	Mean Sq	F value	p value
		Salt	1	1058.4	1058.4	57.091	< 0.001
		ProcellaCOR × Salt	2	2.5	1.2	0.067	0.935
		Residuals	26	482	18.5		
	Cell Abundance	ProcellaCOR	2	0.94	0.471	0.463	0.631
		Salt	1	0.7	0.7043	0.692	0.407
		ProcellaCOR × Salt	2	0.24	0.1213	0.119	0.888
		Residuals	154	156.83	1.0184		

Table 2

Adjusted p-values from Dunnett's post hoc tests for biotic responses of Green Algae and Cyanobacteria to various treatments. The four endpoints measured are chlorophyll, lipid content, biomass, and cell abundance. Values indicate the adjusted p-value for each treatment relative to the control. Significant effects ($p < 0.05$) are highlighted in bold.

Taxa	Treatment	Chlorophyll	Lipid	Biomass	Cell Abundance
Green Algae	ProcellaCOR low	0.994	0.232	1	0.944
	ProcellaCOR high	1	1	1	1
	Salt	< 0.001	< 0.001	< 0.001	0.018
	ProcellaCOR low + Salt	< 0.001	0.001	< 0.001	0.002
	ProcellaCOR high + Salt	< 0.001	0.001	< 0.001	0.002
	DMSO low	0.929	0.348	0.998	0.974
	DMSO high	1	0.999	0.871	0.671
Cyanobacteria	ProcellaCOR low	0.999	1	0.972	1
	ProcellaCOR high	1	0.999	0.985	1
	Salt	0.875	< 0.001	0.006	0.982
	ProcellaCOR low + Salt	0.408	0.003	0.008	1
	ProcellaCOR high + Salt	0.006	0.012	0.017	0.868
	DMSO low	1	0.519	0.997	0.705
	DMSO high	1	0.453	0.978	1

A similar pattern was observed for lipid content (Fig. 2). In *S. obliquus*, lipid content decreased with salt ($F_{1,26} = 48.896$, $p < 0.001$), with no effect of ProcellaCOR or the interaction. Treatment-wise, Dunnett's test indicates lower lipid content under salt (adjusted $p < 0.001$) and both mixture treatments (ProcellaCOR low × salt: adjusted $p = 0.001$; ProcellaCOR high × salt: adjusted $p = 0.001$). In *S. elongatus*, lipid content also decreased with salt ($F_{1,26} = 43.662$, $p < 0.001$), with no effect of ProcellaCOR or the interaction. The post hoc comparison of treatments to control indicates reduced lipid content under salt (adjusted $p < 0.001$) and both mixture treatments (ProcellaCOR low × salt: adjusted $p = 0.003$; ProcellaCOR high × salt: adjusted $p = 0.012$).

Biomass followed the same trend (Fig. 3). In *S. obliquus*, biomass decreased with salt ($F_{1,26} = 131.672$, $p < 0.001$), with no effect of ProcellaCOR or the interaction. Dunnett's test results indicate that biomass was lower in salt treatments (adjusted $p < 0.001$).

and mixture treatments (ProcellaCOR low × salt: adjusted $p < 0.001$; ProcellaCOR high × salt: adjusted $p < 0.001$). In *S. elongatus*, biomass decreased with salt ($F_{1,26} = 57.091$, $p < 0.001$), with no effect of ProcellaCOR or the interaction. Dunnett's test results suggest lower biomass under salt (adjusted $p = 0.006$) and both mixture treatments (ProcellaCOR low × salt: adjusted $p = 0.008$; ProcellaCOR high × salt: adjusted $p = 0.017$), while ProcellaCOR-only treatments showed no change.

Cell abundance showed a different pattern (Fig. 4). In *S. obliquus*, cell abundance decreased with salt ($F_{1,154} = 29.048$, $p < 0.001$), with no effect of ProcellaCOR or the interaction. Dunnett's post hoc test results indicate lower cell abundance under salt (adjusted $p = 0.018$) and both mixture treatments (ProcellaCOR low × salt: adjusted $p = 0.002$; ProcellaCOR high × salt: adjusted $p = 0.002$), while ProcellaCOR-only treatments showed no change. In *S. elongatus*, cell abundance showed no change under ProcellaCOR ($F_{2,154} = 0.463$, $p = 0.631$), salt ($F_{1,154} = 0.692$, $p = 0.407$), or their interaction ($F_{2,154} = 0.119$, $p = 0.888$). Dunnett's tests showed no change in any treatment (all adjusted $p \geq 0.705$).

Temporal analysis (Fig. 5) showed distinct differences in cell abundance dynamics between green algae and cyanobacteria across treatments. Green algae exhibited strong temporal changes with clear separation among treatments over time. In contrast, cyanobacteria showed relatively stable trajectories with substantial overlap among treatments and weak temporal variation. For green algae, treatment groups progressively diverged over time, indicating distinct growth and decline patterns under different exposure conditions. Cyanobacteria, however, maintained similar trajectories across all treatments throughout the experimental period. These patterns were supported by linear mixed-effects models (Table S3).

3.2. Abiotic Responses

Abiotic responses were primarily associated with nutrient dynamics. Conductivity (EC) increased under salt treatments, confirming elevated ionic strength, whereas CDOM, pH, dissolved oxygen, and temperature were not significantly affected by the treatments. In green algae, both total nitrogen (TN) and total phosphorus (TP) were significantly affected by salt and ProcellaCOR, with no significant interaction between the two factors (Table S1). In cyanobacteria, TN was significantly influenced by ProcellaCOR and showed a significant ProcellaCOR × salt interaction, whereas salt alone had no effect. Total phosphorus was significantly affected by ProcellaCOR but not by salt or the interaction. Treatment-wise comparisons identified significant differences from the control for selected ProcellaCOR, salt, and mixture treatments (Table S2; Figs. S1–S2).

4. Discussion

4.1. Salinity as the Primary Driver of Algal Growth

Road salt emerged as the primary driver of phytoplankton responses, while ProcellaCOR alone had no direct effects on growth-related endpoints but contributed to interactive effects under saline conditions. Salt reduced chlorophyll content, lipid content, biomass, and cell abundance in *Scenedesmus obliquus*, whereas *Synechococcus elongatus* showed weaker or interaction-dependent responses. Eukaryotic green algae generally maintain intracellular ion homeostasis by actively regulating sodium (Na^+) concentrations. This process requires metabolic energy to exclude excess ions and maintain osmotic balance, potentially diverting resources away from growth and biomass production under saline conditions (Dias et al., 2025). In contrast, prokaryotic cyanobacteria such as *Synechococcus* synthesize compatible solutes, including sucrose, trehalose, and glucosylglycerol, to balance osmotic gradients rather than expending energy on ion exclusion (Liang et al., 2020; Klähn et al., 2021).

Synechococcus elongatus PCC 7942, the model freshwater cyanobacterium closely related to the strain used here, is known to employ ion-induced enzymatic modulation of compatible solute metabolism as a rapid and energetically efficient strategy for adaptation to fluctuating salinity (Liang et al., 2020). This strategy may allow cyanobacteria to maintain cellular function under salinity levels that inhibit many eukaryotic algae. Although *S. obliquus* can partially acclimate through solute accumulation, growth, pigments, and biomass remain suppressed under elevated NaCl (El-Katony & El-Adl, 2020; Gao et al., 2023; Fatemeh, 2024), consistent with our results.

4.2. Effects of ProcellaCOR on non-target Phytoplankton

ProcellaCOR (florpyrauxifen-benzyl) is developed specifically for aquatic use to control invasive submersed macrophytes such as Eurasian watermilfoil (*Myriophyllum spicatum*) and hydrilla (*Hydrilla verticillata*) (Haug et al., 2021; Mudge et al., 2021; Sperry et al., 2021). Our result indicated ProcellaCOR alone did not affect chlorophyll content, lipid content, biomass, or cell abundance in either phytoplankton taxon, supporting its specificity to vascular plant pathways, designed to exploit the evolutionary uniqueness of the auxin signaling pathway in dicotyledonous vascular plants, and its phytotoxic action is correspondingly restricted (Vonk & Kraak, 2020; Netherland & Richardson, 2016; Haug et al., 2021). The auxin-mimicking mode of action of florpyrauxifen-benzyl disrupts processes such as cell elongation and development that are absent in non-vascular phytoplankton, which also lack auxin receptor machinery, such as the TIR1/AFB-type F-box protein receptors (Vonk & Kraak, 2020). However, significant effects on total nitrogen, including reductions in green algae at higher concentrations and non-linear responses in cyanobacteria, indicate that sublethal biochemical interactions can occur even without measurable growth inhibition.

4.3. Non-Additive Interaction Effects Under Combined Stress

ProcellaCOR interacted with salt to reduce cyanobacterial chlorophyll, although neither stressor alone produced a significant effect. Our results support a non-additive interaction pattern under multiple stressors (Velasco et al., 2018; Villar-Argaiz et al., 2018; Liess et al., 2016). Salt stress redirects carbon and nitrogen metabolism toward compatible solute synthesis, which is energetically demanding (Klähn et al., 2021; Liang et al., 2020). The additional metabolic burden associated with sublethal ProcellaCOR exposure may push cellular energy allocation beyond a threshold required to sustain photopigment synthesis. In contrast, for green algae, the absence of a significant ProcellaCOR × salt interaction on chlorophyll is consistent with the interpretation that salt alone was already saturating the stress response capacity of *S. obliquus* at the concentration used, leaving little additional room for herbicide-driven interactive effects. This pattern is consistent with the Stress Addition Model (SAM) framework, which predicts that when one stressor already exerts high general stress, the marginal addition of a second stressor produces limited additional synergism because the organism's total stress capacity is already nearly exhausted (Liess et al., 2016).

4.4. Lipid Responses and Metabolic Reallocation

Salt exposure significantly reduced lipid content in both taxa when measured at the end of the experiment (day 22), which contrasts with the pattern reported in many biotechnology-oriented studies where salinity stress is used deliberately to stimulate lipid accumulation in microalgae for biofuel production (Gour et al., 2020; Pandit et al., 2017; Singh et al., 2023). This apparent contradiction is likely to reflect the cumulative effects of prolonged osmotic stress on cellular metabolism. Under sustained salinity stress, algae must allocate energy toward maintaining osmotic balance and cellular homeostasis, including ion regulation and compatible solute production, processes that require substantial metabolic resources (Dias et al., 2025; Klähn et al., 2021). As a result, carbon and energy that might otherwise support biosynthetic pathways, including lipid production, are redirected toward stress tolerance mechanisms. In addition, salinity stress can impair photosynthetic efficiency and nutrient assimilation, reducing the availability of fixed carbon required for lipid biosynthesis (Singh et al., 2023). The combined effects of reduced photosynthetic activity and increased metabolic costs associated with osmotic regulation likely contributed to the observed decline in lipid content.

4.5. Ecological Implications of Combined Stressors

Road salt is a widespread and increasing freshwater contaminant, and its co-occurrence with ProcellaCOR is likely in managed aquatic systems (Hintz & Relyea, 2019; Dugan & Arnott, 2022; Schuler et al., 2018; Hintz et al., 2022). Our results indicate that ProcellaCOR alone poses limited direct risk to phytoplankton, but its interaction with salinity can produce effects not predicted from single-stressor exposure (Velasco et al., 2018; Liess et al., 2016). In systems where macrophyte removal releases nutrients (Lamb, 2021), salinization may favor cyanobacteria over green algae, increasing the likelihood of community shifts toward cyanobacterial dominance (Kearney, 2022). The observed shifts in total nitrogen and phosphorus further indicate that combined stressors can influence nutrient cycling, with implications for eutrophication dynamics.

5. Limitations and Future Directions

We employed a controlled monoculture laboratory experiment, which provides mechanistic clarity but necessarily abstracts away from the complexity of natural phytoplankton communities where interspecific competition, grazing pressure, and nutrient recycling through the microbial loop mediate responses to stressors (Bonadonna et al., 2020; Hintz et al., 2017). In natural systems, the greater salt tolerance of cyanobacteria relative to green algae can be amplified by indirect effects: salt-induced collapse of herbivorous zooplankton reduces grazing pressure on cyanobacteria, contributing to competitive dominance effects that would not be apparent in single-species toxicity tests (Urrutia-Cordero et al., 2024; Bonadonna et al., 2020). Future mesocosm experiments incorporating multiple phytoplankton taxa, zooplankton, and realistic salt-pulse dynamics representative of spring road-salt runoff events would provide more ecologically relevant risk assessments for co-stressor scenarios. The mechanism underlying the effects of treatments in total nitrogen and total phosphorus remains unresolved. Additionally, the observed effects of ProcellaCOR on cyanobacterial nutrient dynamics at high concentrations, despite the absence of direct growth effects, merit further investigation using metabolomic and transcriptomic approaches to characterize sublethal molecular responses that may influence nutrient cycling at the ecosystem level.

6. Conclusions

Our study demonstrates that road salt (NaCl) was the dominant driver of phytoplankton stress responses, consistently reducing chlorophyll, lipid content, biomass, and cell abundance in the green alga *Scenedesmus obliquus* across all measured endpoints, while eliciting comparatively muted responses in the cyanobacterium *Synechococcus elongatus*. This differential sensitivity is mechanistically consistent with the contrasting osmoregulatory strategies of eukaryotic green algae and prokaryotic cyanobacteria, and with field observations of cyanobacterial competitive advantage in salinizing freshwater ecosystems. ProcellaCOR (florpyrauxifen-benzyl) alone had no significant effect on biotic endpoints in either taxon, which is consistent with its auxin-specific mode of action that lacks a biochemical target in non-vascular primary producers. However, the significant synergistic reduction of cyanobacterial chlorophyll under the high ProcellaCOR × salt mixture, combined with complex herbicide-driven shifts in nitrogen and phosphorus dynamics in both taxa, indicates that combined stressor exposures can produce ecologically meaningful effects not predictable from individual stressor data. These findings have direct relevance for risk assessment of aquatic herbicide applications in watersheds subject to road salt contamination, where the co-occurrence of these stressors may alter phytoplankton community composition and nutrient cycling in ways that warrant integrated management strategies.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Arif Ahmed – Conceptualization, Investigation, Methodology, Validation, Visualization, Software, Formal analysis, Data curation, Writing - original draft, Writing - review & editing.

Averi Loria – Data curation, Writing - review & editing.

Juliana Di Cosmo – Data curation, Writing - review & editing.

Matthew S. Schuler – Methodology, Validation, Writing - review & editing, Project administration, Supervision, Resources.

Ethics approval

No ethical approval was required.

Consent to participate

Not applicable for this study

Consent to publish

Not applicable for this study

Data Availability: The data underlying this study are available upon request from the corresponding author.

Supplementary Information (SI): Table S1-S3, Figure S1-S2.

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Figures

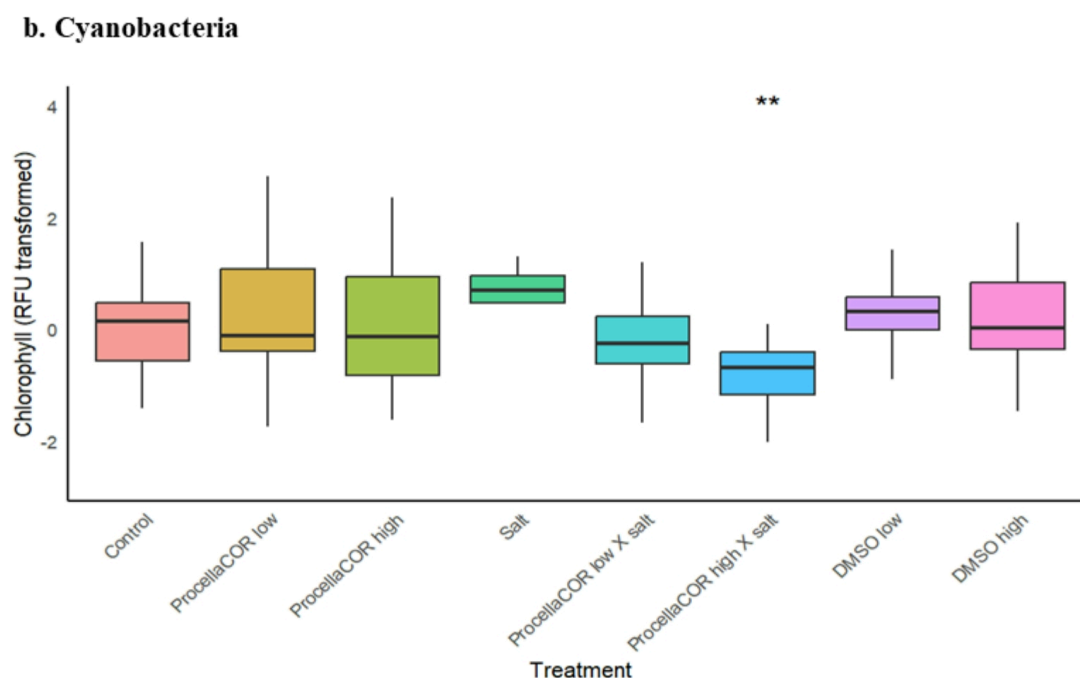
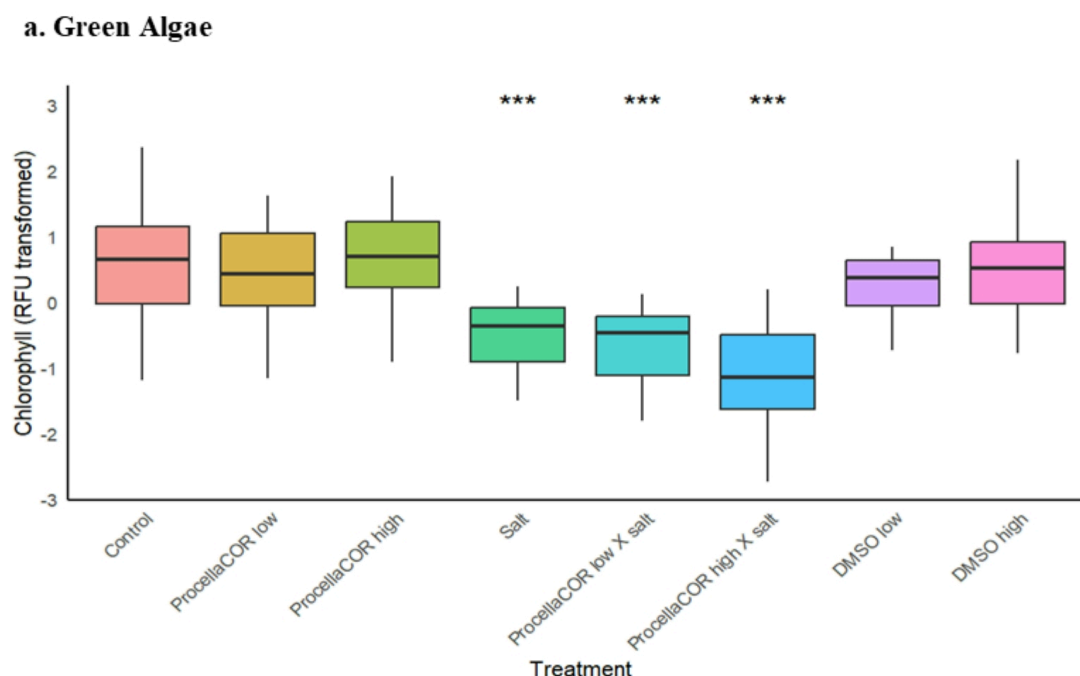


Figure 1

Effects of herbicide ProcellaCOR and salt on total chlorophyll content

Boxplots show the relative fluorescence unit (RFU) of rank-transformed chlorophyll values in both phytoplankton taxa across treatments. Each box represents the interquartile range (IQR), the horizontal line inside the box indicates the median, and whiskers represent the minimum and maximum values. Significant differences from the control are indicated by asterisks (** $p < 0.01$, *** $p < 0.001$).

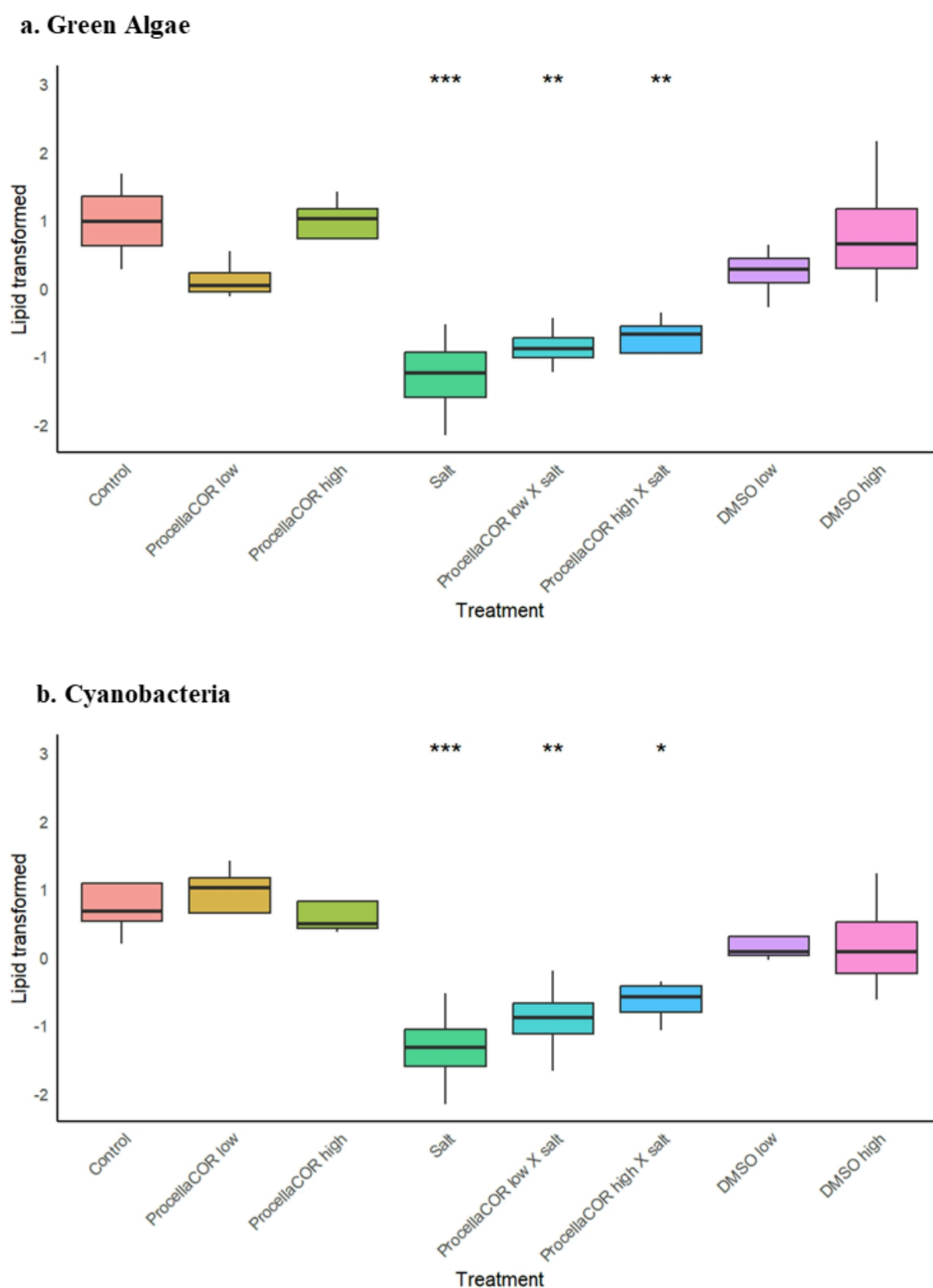
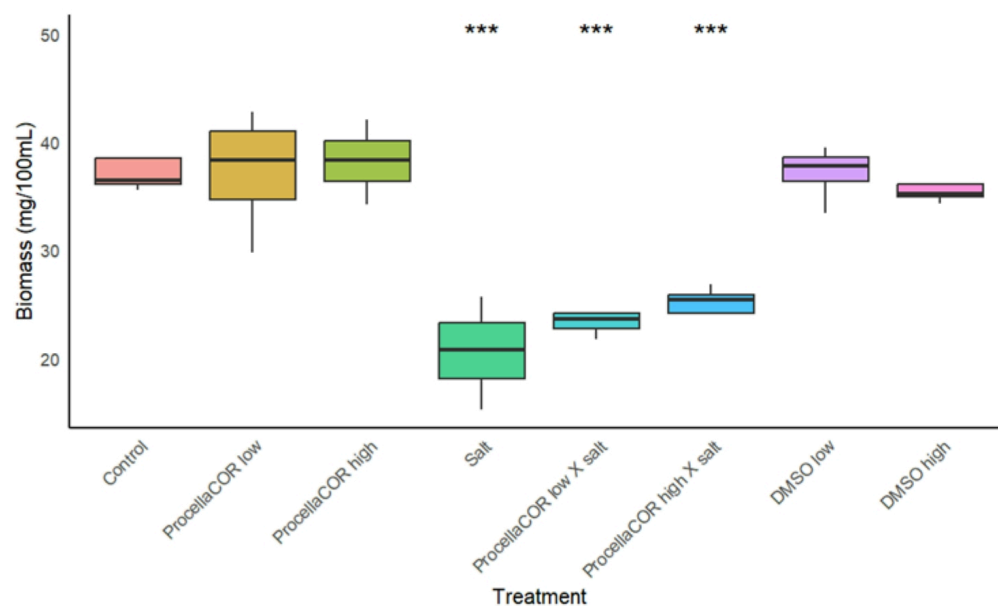


Figure 2

Effects of herbicide ProcellaCOR and salt on Lipid accumulation in green algae and cyanobacteria. The box plots show lipid levels across eight treatment groups, including Control, ProcellaCOR at low and high doses, Salt, combinations of ProcellaCOR with Salt, and DMSO at low and high doses. Each group is color-coded with labels on the x-axis, rank-transformed lipid values on the y-axis, and significance markers (*) indicating notable differences between control and treatments.

a. Green Algae



b. Cyanobacteria

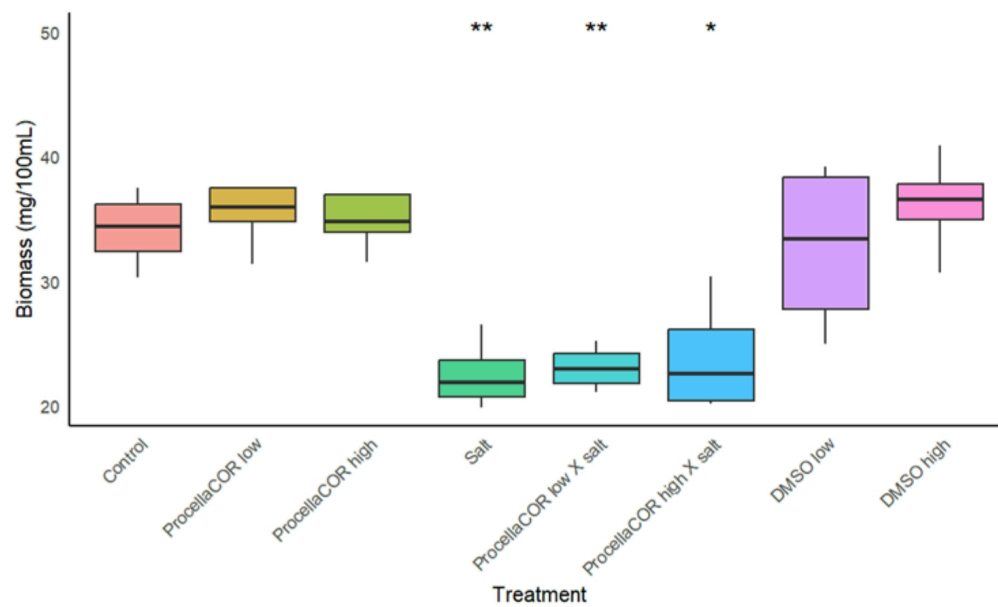
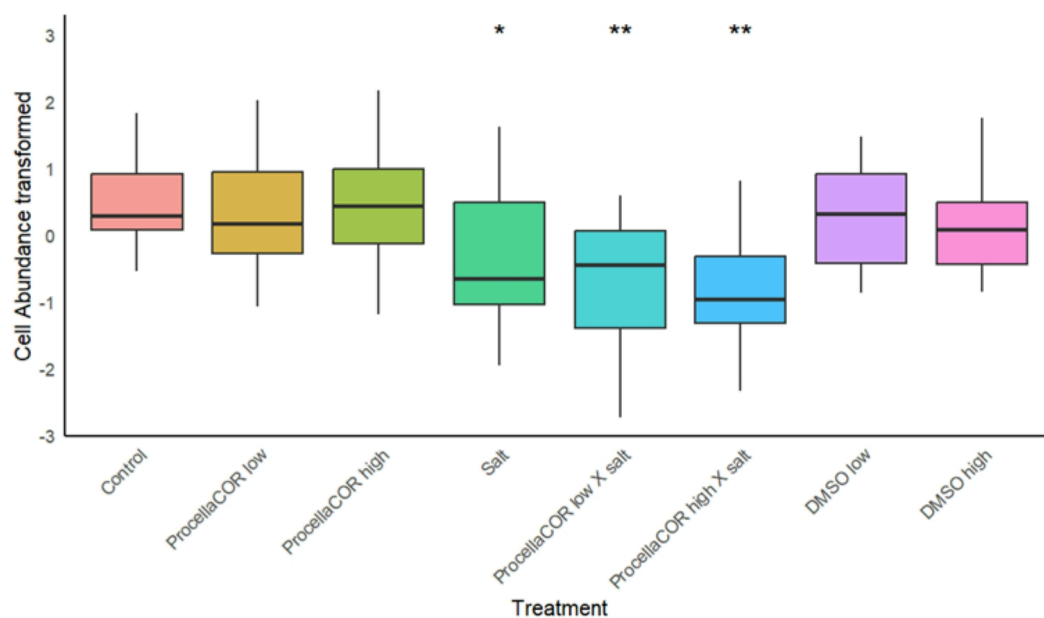


Figure 3

Effects of herbicide ProcellaCOR and salt on biomass production by phytoplankton taxa. Box plot comparing biomass levels (mg/100mL) across eight treatment groups, showing distribution, median, and variability. Significant biomass reduction observed in salt treatments marked by asterisks (***).

a. Green Algae



b. Cyanobacteria

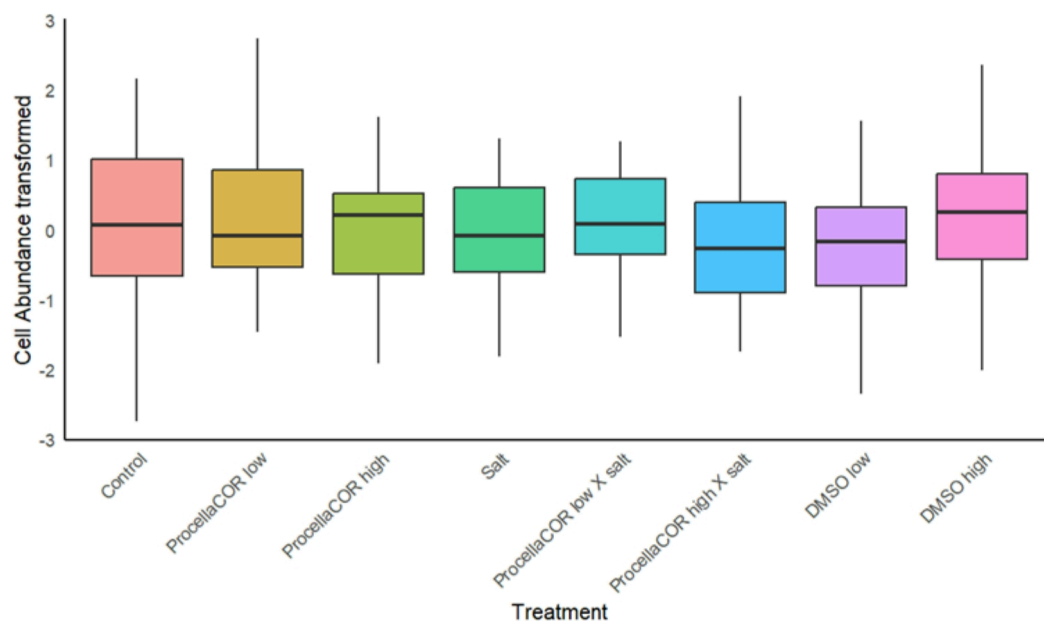


Figure 4

Effects of ProcellaCOR and salt on cell abundance.

The box plots show the (a) green algae and (b) cyanobacteria cell abundance (rank-transformed values) across treatments. Boxes represent the interquartile range (IQR), the center line indicates the median, and whiskers represent the extent. Asterisks denote statistically significant differences relative to control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

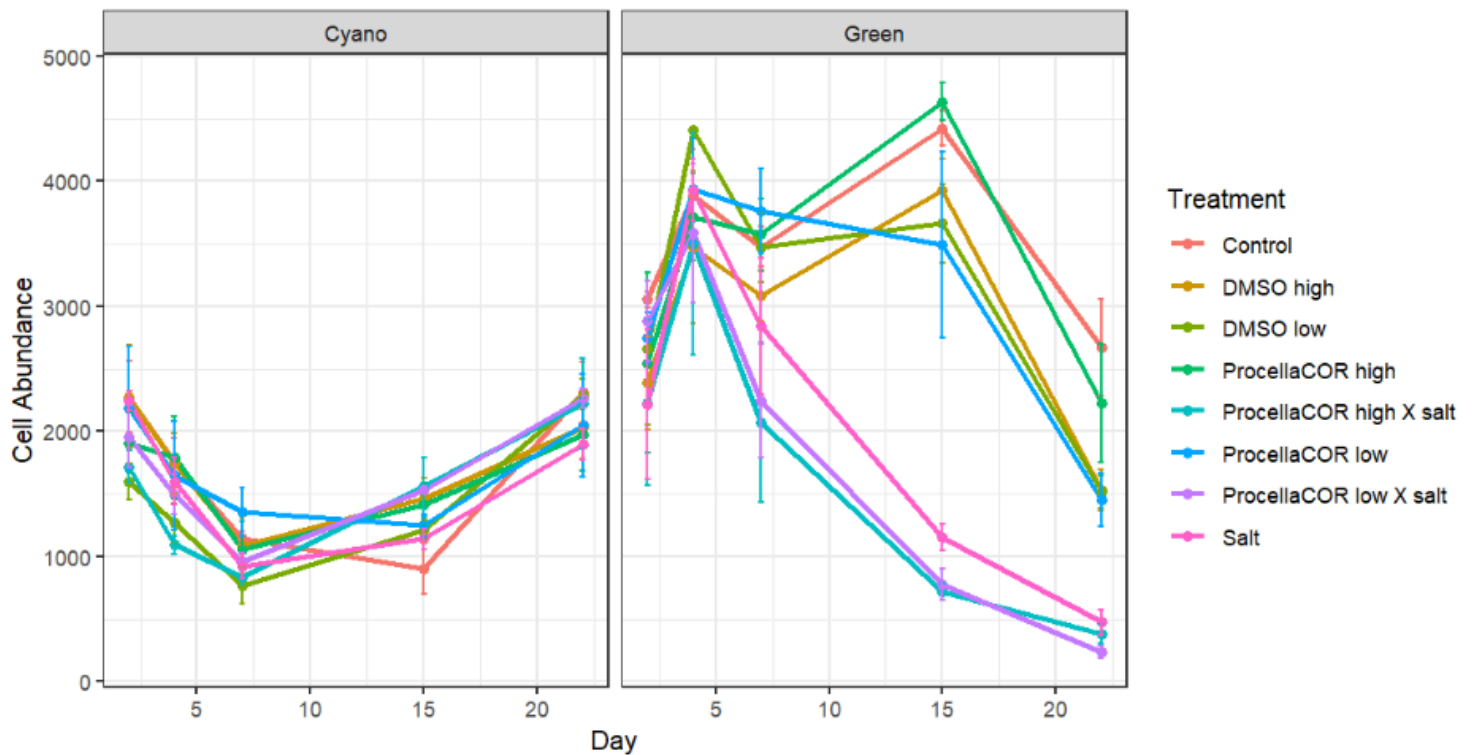


Figure 5

Temporal dynamics of cell abundance in green algae and cyanobacteria under different treatments. Time-series plots show mean cell abundance ($\pm$ standard error) over the experimental period for each treatment condition. Lines represent treatment means, points indicate mean values at each sampling time, and error bars represent $\pm$ standard error. Panels separate green algae and cyanobacteria to allow direct comparison of temporal responses.

Supplementary Files

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