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1 Pesticide responses of Arctic and temperate microalgae differ in relation to
2 ecophysiological characteristics

3
4 Juan Du¹, Disney Izquierdo², Jonathan Naoum², Leanne Ohlund³, Lekha Sleno³,
5 Beatrix E. Beisner⁴, Johann Lavaud^{5,6} and Philippe Juneau^{2*}

6 ¹ Department of Biological Sciences, Université du Québec à Montréal, GRIL-TOXEN,
7 Succ Centre-Ville, Montreal, Quebec, H3C 3P8, Canada² Department of Biological
8 Sciences, Université du Québec à Montréal, GRIL-EcotoQ-TOXEN, Succ Centre-
9 Ville, Montreal, Quebec, H3C 3P8, Canada³ Chemistry Department, Université du
10 Québec à Montréal, EcotoQ-TOXEN, Succ Centre-Ville, Montreal, Quebec, H3C
11 3P8, Canada

12 ⁴ Department of Biological Sciences, Groupe de recherche interuniversitaire en
13 limnologie (GRIL), Université du Québec à Montréal, Succ Centre-Ville, Montreal,
14 Quebec, H3C 3P8, Canada

15 ⁵ TAKUVIK International Research Laboratory IRL3376, Université Laval (Canada) -
16 CNRS (France), Pavillon Alexandre-Vachon, 1045 av. de la Médecine, local 2064,
17 G1V 0A6 Québec, Canada

18 ⁶ LEMAR-Laboratory of Environmental Marine Sciences, UMR6539, CNRS/Univ
19 Brest/Ifremer/IRD, Institut Universitaire Européen de la Mer, Technopôle Brest-
20 Iroise, rue Dumont d'Urville, 29280 Plouzané, France.

21 ***Corresponding author:**

22 E-mail addresses: juneau.philippe@uqam.ca (P. Juneau)

23 Full postal address:

24 Department of Biological Sciences

25 Université du Québec à Montréal

26 CP8888, Succ Centre-Ville

27 Montréal (QC)

28 H3C 3P8

29 Canada

30

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32 **Highlights**

- 33 ● Arctic microalgae have distinct ecophysiological characteristics relative to their
34 temperate counterparts.
- 35 ● Unexpectedly, one Arctic microalgae was more tolerant to pesticides than its
36 temperate counterparts, which disagrees that the greater ecological risk of
37 pollutants in polar ecosystems is always higher than in temperate regions.
- 38 ● Sensitivity variation to the four pesticides appears to be mainly due to differences
39 in the protective mechanisms between Arctic and temperate microalgae.

40 **Abstract**

41 Polar ecosystems play an important role in global primary production. Microalgae have
42 adaptations that enable them to live under low temperature environments where
43 irradiance and day length change drastically. Their adaptations, leading to different
44 ecophysiological characteristics relative to temperate species, could also alter their
45 sensitivity to pollutants such as pesticides. This study's objective was to understand
46 how different ecophysiological characteristics influence the response of Arctic
47 phytoplankton to pesticides in relation to the responses of their temperate counterparts.
48 Ecophysiological endpoints were related to growth, cell biovolume, pigment content,
49 photosynthetic activity, photoprotective mechanisms (NPQ, antioxidant enzyme
50 activities), and reactive oxygen species (ROS) content. The Arctic species *Micromonas*
51 *polaris* was more resistant to atrazine and simazine than its temperate counterpart
52 *Micromonas bravo*. However, the other Arctic species *Chaetoceros neogracilis* was
53 more sensitive to these herbicides than its temperate counterpart *Chaetoceros*
54 *neogracile*. With respect to two other pesticide toxicity, both temperate microalgae were
55 more sensitive to trifluralin, while Arctic microalgae were more sensitive to
56 chlorpyrifos (insecticide). All differences could be ascribed to differences in the eco-
57 physiological features of the two microalgal groups, which can be explained by cell
58 size, pigment content, ROS content and protective mechanisms (NPQ and antioxidant
59 enzymes).

60 **Keywords:** microalgae, *Micromonas*, *Chaetoceros*, ecophysiology, photosynthesis,
61 pesticides, toxicity mechanism

62

63 Abbreviations:

64 T-CN (Temperate *Chaetoceros neogracile*), T-MB (temperate *Micromonas bravo*) and
65 A-MP (Arctic *Micromonas polaris*), Arctic *Chaetoceros neogracilis* (A-CN), Car,
66 carotenoids; Chl *a*, chlorophyll *a*; PSI, photosystem I; PSII, photosystem II; NPQ, non-
67 photochemical quenching; Q_A, primary electron acceptor of PSII; Q_B, secondary
68 electron acceptor of PSII; RC, reaction center, Φ_M, PSII maximum quantum yield; Φ'_M,
69 PSII operational quantum yield; NPQ_{max}, Maximum ability for dissipation of excess
70 energy; E_k, Light saturation coefficient; α, Maximum light efficiency use ROS, reactive
71 oxygen species; SOD, superoxide dismutase; CAT, catalase; logK_{ow}, a ratio between
72 the concentration of a chemical in the octanol phase to its concentration in the water
73 phase.

74 **1. Introduction**

75 Arctic habitats are subjected to harsh environmental conditions. Nevertheless, they
76 provide a major contribution to global primary production and the Arctic Ocean net
77 primary production has increased recently (Ardyna and Arrigo 2020). More than 75%
78 of the Arctic phytoplankton biomass is composed of diatoms and small flagellate
79 prasinophytes (*Micromonas* sp.) (Balzano et al. 2012, Lovejoy et al. 2007), and they
80 thus play an essential role in the Arctic food web (Frey et al. 2018). Microalgae in polar
81 regions have adaptations that enable them to grow well in these regions where
82 temperatures are permanently low, but irradiance and day length are extremely variable
83 (Handler 2017). Microalgae are impacted by cold environments in many ways,
84 including lower enzyme activity (Wiebe et al. 1992), altered membrane fluidity (White
85 et al. 2000), nutrient availability, balancing the usage and absorption of energy (Parker
86 and Armbrust 2005), and the capacity to grow (Margesin 2007). There are differences
87 in taxonomy, genetics, and ecology between Arctic microalgae and their temperate
88 counterparts, while photo-physiology is not well documented (Lacour et al. 2017).
89 Although there have been great developments in the taxonomy, genetics, and ecology
90 of polar phytoplankton, their photo-physiological properties are not yet well
91 documented, and the understanding of aquatic contaminant effects on their
92 ecophysiology is lacking (Lyon and Mock 2014).

93 Application of pesticides leads to a substantial loss from agricultural fields leading
94 to their detection in the aquatic environment, due to leaching, runoff and spray-drift
95 (Larsbo et al. 2016, Zhang et al. 2018). Some authors have reported that Arctic waters
96 are contaminated with pesticides applied in southern regions due to the long-distance
97 aerial and marine transport of chemicals (Cabrerizo et al. 2019, Ma et al. 2018, Muir et
98 al. 2013, Muir and de Wit 2010). Moreover, owing to the accumulation of pesticides in
99 Arctic ice cover and snow over the years, pesticide concentrations in Arctic waters
100 should increase over time, as accumulated ice is melting at an unprecedented rate due
101 to global warming (Pućko et al. 2017). Chlorpyrifos, diazinon, trifluralin, endosulfan
102 and lindane are some of the typical pesticides found in surface and groundwater near

103 agricultural lands across Canada and USA (Vorkamp and Riget 2014), as well as in
104 Arctic waters (Hoferkamp et al. 2010, Vorkamp and Riget 2014, Weber et al. 2010).

105 Among the main classes of pesticides, herbicides are the most widely used (Balmer
106 et al. 2019). Numerous herbicides have detrimental effects on photosynthesis due to
107 cellular oxidative damage induced by the accumulation of reactive oxygen species
108 (ROS). This accumulation promotes lipid peroxidation, which results in the destruction
109 of membranes, such as the photosynthetic ones (Chalifour et al. 2014, DeLorenzo 2001).
110 Insecticides, although not designed to affect plants, have been demonstrated to have
111 toxic effects on the growth, photosynthesis, biovolume, pigment and lipid contents of
112 phytoplankton (Asselborn et al. 2015, Yadav 2015). It is well known that the sensitivity
113 to pesticides of temperate algal species varies considerably, and several factors may
114 contribute to this species-specificity. For example, damage to photosynthetic apparatus
115 caused by pesticides can be minimized by various photoprotective mechanisms,
116 including antioxidant system designed to eliminate the excess ROS (Medithi et al. 2021)
117 and non-photochemical quenching (NPQ) energy dissipation processes related to the
118 capacity to modulate light absorption and dissipate excess energy as heat (Moustakas
119 et al. 2022). Furthermore, cells can also adjust pesticide uptake by modifying their
120 surface to biovolume ratio (Larras et al. 2013, Tang et al. 1998).

121 Most of our understanding about the physiological characteristics and pesticide
122 effects on microalgae is from temperate phytoplankton species. Indeed, the
123 physiological features and potential impacts of pesticides on Arctic phytoplankton are
124 very scarce (Kottuparambil et al. 2017). However, some authors have predicted that
125 Arctic ecosystems and their organisms are likely more sensitive to contaminants than
126 those at temperate latitudes (CARC 1990, Kottuparambil et al. 2017). In that case,
127 ecophysiological properties of Arctic microalgae evolved under the extreme conditions
128 found in the Arctic Ocean and this result in adaptations that may permit these algae to
129 cope with pesticide stress. Although there have been many reports on the impacts of
130 pesticides on the physiology and growth of temperate phytoplankton (Singh et al. 2016,
131 Vonk and Kraak 2020), very little is known about their potential impacts on microalgae
132 having extreme low temperatures and rapid environmental change due to global

133 warming such as for Arctic phytoplankton. Moreover, the comparison of the tolerance
134 mechanisms and ecophysiological characteristics between the phytoplankton of Arctic
135 with temperate regions is lacking. This knowledge will permit to gain insights for future
136 development of algal bioassays for Arctic regions since using temperate species could
137 not be appropriate. We thus compared pesticide responses in Arctic microalgae and their
138 temperate counterparts to four pesticides (atrazine, simazine, trifluralin and
139 chlorpyrifos). Arctic microalgae grow under the extreme conditions of the Arctic Ocean,
140 and should have different physiological characteristics from temperate microalgae.
141 Therefore, we examined which ecophysiological adaptation might benefit to Arctic
142 organisms by exploring differences in their sensitivities and response mechanisms.

143

144 **2. Materials and methods**

145 2.1 Microalgal species and growth conditions

146 We compared the responses in two temperate species: *Chaetoceros neogracile* (T-
147 CN; CCMP1425), *Micromonas bravo* (T-MB; CCMP1646), and two Arctic species
148 strains *Micromonas polaris* (A-MP; CCMP2099), *Chaetoceros neogracilis* (A-CN;
149 RCC2279). The first three species were purchased from National Contract Management
150 Association (NCMA), while the latter Arctic strain was obtained from the Roscoff
151 culture collections. All species were cultivated in marine L1 medium (Guillard et al.,
152 1993) with a total volume of 100 mL medium in species-specific 250 mL Erlenmeyer
153 flasks. The cultures were grown at $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ under a 14:10 h light: dark
154 illumination cycle with daily gentle shaking. Temperate and Arctic species were grown
155 at 18 °C and 4 °C respectively. Algal cells were periodically transferred (for at least
156 eight generations) into fresh medium to maintain their exponential growth phase. The
157 cell concentrations were measured with a Multisizer 3 Coulter Counter particle analyzer
158 (Beckman Coulter Inc., USA). The growth rate (μ) was determined as follow: $\mu =$
159 $(\ln N_n) - (\ln N_0) / T$, where $\mu =$ Average specific growth rate, N_0 , N_n indicate cell density
160 (cells/mL) at the beginning of test and at the end of the treatment (3 days), T expresses

161 the exposure time (3 days).

162 2.2 Pesticide preparation and treatment

163 All pesticides (Table 1) used in the present study were obtained from Sigma-
164 Aldrich (PESTANAL®, Canada). Pesticides (stock solutions in acetone) were added to
165 the growth media for the exposure experiments at final acetone concentration never
166 exceeding 0.01%, a concentration of acetone not inducing any measurable effect on the
167 parameters assayed. From the original eight pesticides, the impacts of four of them were
168 further investigated (atrazine, simazine, trifluralin and chlorpyrifos), since the other
169 four pesticides showed no (clopyralid-1000 µg/L, metolachlor-3.5 µM/L and lindane-
170 1000 µg/L) or very little (< 10%) toxicity (endosulfan-1000 µg/L) on the photosynthetic
171 activity at very high concentrations for the studied microalgae (data not shown).
172 Concentrations of atrazine measured in the medium at the beginning of the experiment
173 were 0 µg/L, 6.44 µg/L, 25.93 µg/L, 50.68 µg/L, 99.00 µg/L and 245.14 µg/L. For
174 simazine the measured concentrations were 0 µg/L, 5.55 µg/L, 25.23 µg/L, 50.57 µg/L,
175 101.61 µg/L and 248.62 µg/L. We indicated these atrazine and simazine concentrations
176 in the figures and tables as 0 µg/L, 5 µg/L, 25 µg/L, 50 µg/L, 100 µg/L and 250 µg/L.
177 Measured trifluralin concentrations tested were 0 µg/L, 198.47 µg/L and 500.93 µg/L
178 and for chlorpyrifos 0 µg/L, 189.13 µg/L and 475.56 µg/L (noted in the figures and
179 tables as 0 µg/L, 200 µg/L and 500 µg/L). No significant differences in the measured
180 concentrations were observed between the two experimental temperatures for the
181 different pesticides. Concentrations of pesticides were evaluated using a QTRAP 5500
182 mass spectrometer (Sciex, Concord, ON, Canada) according to Chalifour et al. (2016)
183 and Takishita et al. (2021). The four studied pesticides (atrazine, simazine, trifluralin
184 and chlpyrifos) are known to have relatively long half-lives in water, ranging from
185 months to years (Bai et al. 2015, Vonk and Kraak 2020), and therefore are stable and
186 do not degrade during our experiments (Du et al. 2022, in preparation).

187 Exponentially growing microalgae were transferred into 1L Erlenmeyer flasks at
188 a cell density of 2.5×10^5 (*Chaetoceros*) and 2.5×10^6 (*Micromonas*) cells mL⁻¹
189 respectively, and then exposed to different concentrations of pesticides for 72 h. All

190 treatments were done in triplicate. Cell densities and cell biovolumes were assessed at
191 the beginning and the end of the experiment with a particle counter (Multisizer 3
192 Coulter Counter, Beckman Coulter Inc., USA).

193 Table 1. The chemical families and mode of action for pesticides used in this study
194 (adapted from www.irac-online.org).

Class	Substance	Chemical family	Mode of action
Herbicide	Atrazine	Triazine	inhibition of photosynthesis at photosystem II
	Simazine	Triazine	inhibition of photosynthesis at photosystem II
	Trifluralin	Dinitroaniline	inhibition of cell mitosis
	Clopyralid	Pyridinecarboxylic acid	inhibit cell division and growth
	Metolachlor	Chloroacetanilide	inhibition biosynthesis of chlorophyll, proteins, fatty acids and lipids
Insecticide	Chlorpyrifos	Organophosphate	inhibition Nervous System (acetylcholine esterase (AChE))
	Lindane	Organochlorine	inhibition Nervous System (GABA receptor)
	Endosulfan	Organochlorine	inhibition Nervous System (GABA receptor)

195

196 2.3 Pigment measurements

197 Algal cultures (25 mL) were harvested, under dim green light, 72 h after the
198 beginning of the treatments by gentle filtration on 0.8µm filter membrane
199 (Polytetrafluoroethylene; Xingya Purifying Materials Factory; Shanghai, China), and
200 placed in 2 mL Eppendorf tubes covered with aluminum foil, then rapidly immersed
201 into liquid nitrogen and kept at -80 °C until analysis. Extractions of the pigments were
202 done by adding 2 mL of acetone 90% overnight at -20 °C prior to analysis. Ultrasonic
203 probe was used to break the cells (3 W/cm² for 20 s; Sonic dismembrator Model 100,
204 Fisher Scientific). The extracts were centrifuged at 4 °C for 10 min (10000×g) and the
205 supernatant was kept for quantification of chlorophyll (Chl *a*) and carotenoid (Car).
206 Using Cary 300 UV spectrophotometer (Varian, USA) each extract was scanned
207 between 400–750nm. Independent triplicates were sampled for each culture. The
208 contents of Chl *a* and carotenoids were calculated according to Jeffrey and Humphrey
209 (1975) and Seely et al. (1972) respectively.

210 2.4 Fluorescence measurements

211 The photosynthetic light curves were obtained using a PAM fluorometer (Water-
212 PAM, Walz, Germany) according to Du et al. (2019), with saturation pulses (3000 µmol

213 photons $\text{m}^{-2} \text{s}^{-1}$, 800ms) and 8 levels of actinic light intensities (0, 46, 105, 188, 276,
214 427, 635, 906, and 1207 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The samples (3mL) were dark
215 acclimated for 20 minutes before measurements and all samples were measured at their
216 incubation temperature (4 °C and 18 °C). The maximum (Φ_{M}) and operational (Φ'_{M})
217 PSII quantum yields, and the non-photochemical quenching (NPQ) were determined
218 from this light curve when the actinic light was 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for Φ_{M} and 105
219 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for Φ'_{M} and NPQ. Their evaluation was done using the following
220 equations: $\Phi_{\text{M}} = (F_{\text{M}} - F_0) / F_{\text{M}}$ (Kitajima and Butler, 1975); $\Phi'_{\text{M}} = (F'_{\text{M}} - F_{\text{S}}) / F'_{\text{M}}$ (Genty et
221 al., 1989); $\text{NPQ} = (F_{\text{M}} - F'_{\text{M}}) / F'_{\text{M}}$ (Bilger and Björkman, 1990). The maximal electron
222 transport rate (ETR_{MAX}), light saturation coefficient (E_{k}) and light efficiency use (α)
223 was calculated according to Lacour et al. (2017). The Plant Efficiency Analyzer (PEA,
224 Hansatech, Instruments Ltd, UK) was used to determine the polyphasic rise in
225 fluorescence transients. Transients were induced by a 2s red (maximal emission at 650
226 nm) light pulse with 3600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Strasser et al., 1995). The O-J-I-P
227 curves of the microalgae were determined and functional parameters evaluating the
228 PSII energy fluxes under environmental stresses were calculated. All parameter
229 definitions are in the Supplementary Material (Table S1).

230 2.5 Reactive oxygen species (ROS) measurement

231 Intracellular ROS was evaluated by BD Accuri C6 flow cytometer (Biosciences,
232 San Jose, CA, USA) using the fluorescent dye 2',7'-dichlorodihydrofluorescein
233 diacetate (H_2DCFDA) (Molecular probes, Eugene, OR, USA) as described in
234 (Stachowski-Haberkorn et al. 2013). Cells were analyzed by forward and side scatters
235 and measured the fluorescence channel FL1 with excitation wavelength of 488 nm and
236 emission wavelength of 530 nm. To eliminate potential signal alterations brought on by
237 pesticide effects on FL1 fluorescence, we presented the results as FL1 ratios
238 (H_2DCFDA -stained samples FL1 value divided by non-contaminated samples FL1
239 values).

240 2.6 Antioxidant enzyme activity measurements

241 After pesticide exposure (72h), microalgal cultures (50 mL) were centrifuged at
242 15000×g for 25 min at 4 °C, and the pellet was kept into a 2 mL microtube covered
243 with aluminum foil. After adding 1 mL extraction buffer, samples were immediately
244 plunged into liquid nitrogen and kept at -80 °C until analysis. For each extracted sample
245 enzyme activities were determined with Cary 300 UV spectrophotometer (Varian,
246 USA). Cells were broken with the help of liquid nitrogen, grinding one time, and were
247 then centrifuged at 15000×g for 25 min at 4 °C prior to analysis. Each sample was
248 divided into three replicates for analyzing the superoxide dismutase (SOD) and catalase
249 (CAT) according to Vitoria et al. (2001) and Rao et al. (1996) respectively.

250 2.7 Statistical analyses

251 JMP software 10.0 (SAS Institute Inc) was used for statistical evaluations. Data
252 were verified for normality (Shapiro–Wilk test) and homogeneity (Bartlett test) and
253 then statistically evaluated using either one or two-way analysis of variance (ANOVA).
254 Interactions between pesticide concentrations and different species were considered in
255 2-way ANOVA. When there were significant differences in the response variables
256 between treatments, contrast analysis (Tukey's HSD test) was used. The EC₅₀
257 (concentration needed to induce 50% of the maximum effect) values for response
258 variables (growth, Φ_M , and Φ'_M) were calculated from the nonlinear least-square fits by
259 using the inverse of the regression curve (Juneau et al. 2001).

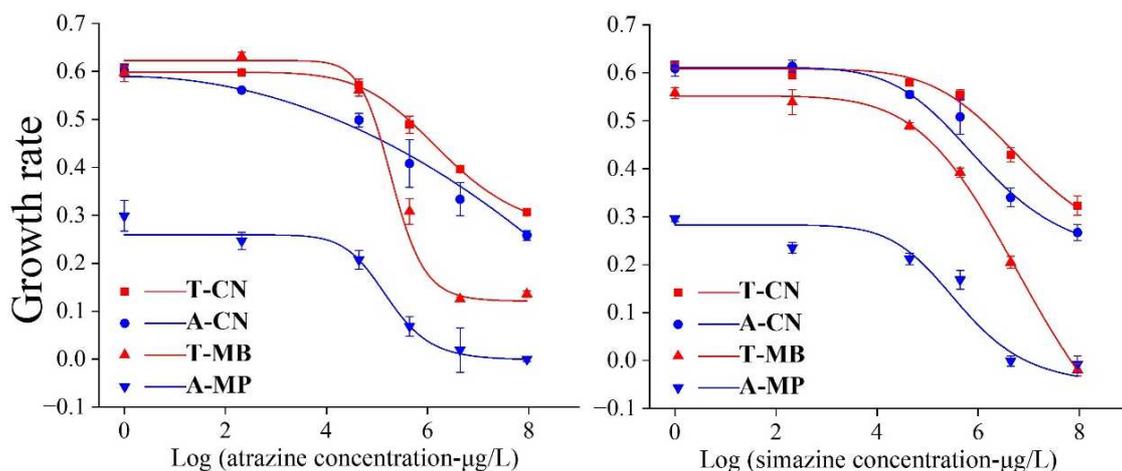
260 3. Results

261 3.1 Effects of pesticides exposure on cell growth and cell biovolume

262 3.1.1 Effects of atrazine and simazine

263 The presence of atrazine and simazine for 72h significantly inhibited the growth
264 of all algal species (Tukey's HSD, $P < 0.05$), a growth inhibition that was further
265 exacerbated at increased atrazine and simazine concentration (Fig. 1). The growth-EC₅₀
266 for temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo*
267 (T-MB) and Arctic *M. polaris* (A-MP) was 143, 86, 52 and 82 µg/L respectively for

268 atrazine, and 166, 171, 69 and 111 $\mu\text{g/L}$ for simazine (Table 2). Cellular biovolume of
 269 the T-CN ($150 \mu\text{m}^3$) was intrinsically nearly three times that of A-CN ($50 \mu\text{m}^3$), while
 270 the biovolume of temperate *M. bravo* was almost the same as Arctic *M. polaris*. The
 271 treatment of atrazine and simazine tend to increase the cell biovolumes of all species
 272 by 2-12%, although not significantly for all studied species/treatments (Tukey's HSD,
 273 $P > 0.05$, Fig. S3).



274

275 Figure 1. The effects of atrazine and simazine on the growth of four species, including
 276 (red color) temperate *C. neogracile* (T-CN), temperate *M. bravo* (T-MB), Arctic *C.*
 277 *neogracilis* (A-CN) and (blue color) Arctic *M. polaris* (A-MP) after 72 h exposure. Data
 278 are expressed as means \pm SD ($n = 6$).

279 Table 2. The EC_{50} of atrazine and simazine based on the growth, the maximal PSII
 280 quantum yield (Φ_M), the operational PSII quantum yield (Φ'_M). The same column
 281 superscript letters (a-d) showed significant differences among strains for EC_{50} . n.d. =
 282 not determined. Data are expressed as means \pm SD ($n = 6$)

Species	Pesticides	EC ₅₀ -Φ _M		EC ₅₀ -Φ' _M		EC ₅₀ -growth rate	
		Atrazine	Simazine	Atrazine	Simazine	Atrazine	Simazine
T-CN		n.d.	n.d.	66±1.4 ^c	142±6.7 ^d	275±10.6 ^d	306±39.2 ^d
A-CN		n.d.	4073±56.3 ^c	37±2.1 ^b	62±2.1 ^c	188±24.3 ^c	128±20.7 ^c
T-MB		104±5.6 ^a	46±1.5 ^a	31±2.3 ^a	49±1.8 ^b	55±3.3 ^a	68±2.7 ^a
A-MP		156±14.3 ^b	703±67.3 ^b	36±1.7 ^b	46±1.9 ^a	75±2.8 ^b	111±6.8 ^b

283

284 3.1.2 Effects of trifluralin and chlorpyrifos

285 The growth of Arctic microalgae (*C. neogracilis*, *M. polaris*) and temperate
286 microalgae (*C. neogracile*, *M. bravo*) was drastically inhibited in the presence of
287 chlorpyrifos at 200 µg/L and 500 µg/L, the exception was for *M. bravo* at 200 µg/L
288 chlorpyrifos (Tukey's HSD, $P < 0.05$). Overall, Arctic microalgae showed greater
289 decline in their growths than their temperate counterparts in the presence of
290 chlorpyrifos (Table S2). The chlorpyrifos treatment induced a significant increase
291 (Tukey's HSD, $P < 0.05$) in the cell biovolume of all species. Overall, the biovolumes
292 of Arctic microalgae increased by more than 100% compared to the temperate
293 counterparts except for the A-CN at 200 µg/L chlorpyrifos (Table S1).

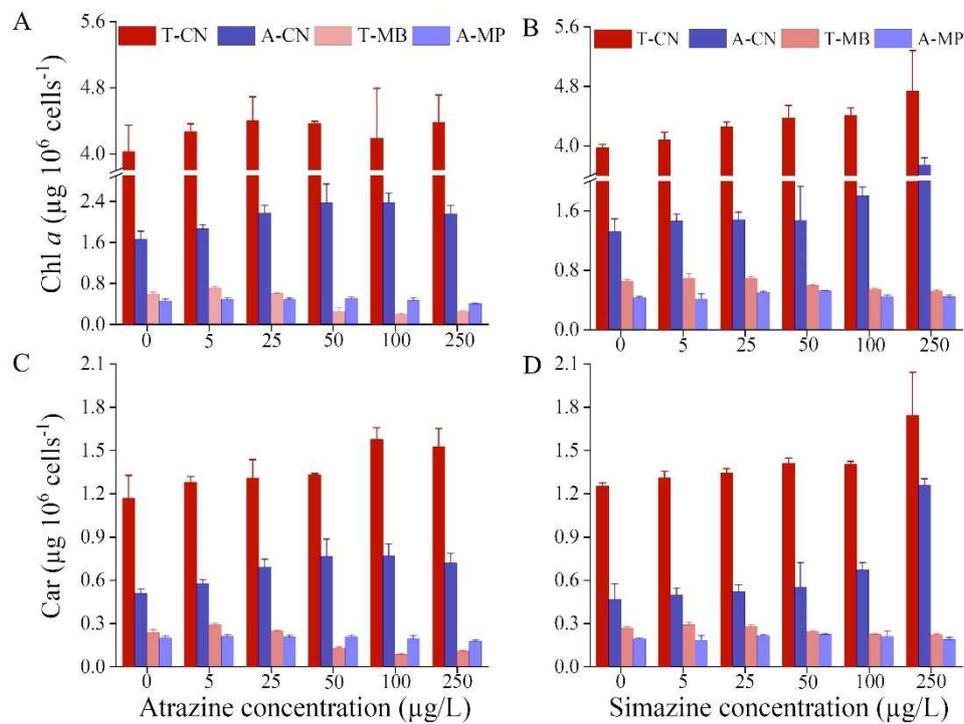
294 The growth of all species was significantly decreased by trifluralin (Tukey's HSD,
295 $P < 0.05$), but Arctic microalgae had smaller growth reductions than did their temperate
296 counterparts in the presence of trifluralin, which is contrary to what was observed in
297 the presence of chlorpyrifos. Cell biovolume of all species except for *M. bravo*
298 increased in the presence of trifluralin (Table S1).

299 3.2 Effects of pesticides on pigment contents

300 3.2.1 Effects of atrazine and simazine

301 Pesticides had various impacts on pigment contents of the four studied microalgae
302 after 72 h treatment. The cellular Chl *a* and Car contents of temperate and Arctic
303 *Chaetoceros* did not show significant changes with increasing atrazine and simazine
304 concentrations, except for simazine at 250 µg/L (Tukey's HSD, $P > 0.05$, Fig. 2),

305 resulting in an unchanged Car/Chl *a* ratio. For *M. bravo*, Chl *a* and Car increased
 306 slightly at low atrazine concentrations and then significantly decreased at higher
 307 concentrations (>100 µg/L, Tukey's HSD, P < 0.05, Fig. 2), while atrazine did not affect
 308 the pigment composition of *M. polaris*. Simazine treatments did not induce a significant
 309 change in Chl *a* and Car contents after 72 h of exposure for the temperate and Arctic
 310 *Micromonas* (Tukey's HSD, P > 0.05). Under control conditions, Chl *a* for the temperate
 311 *Chaetoceros* and *Micromonas* were respectively almost 2.5 and 2.3 times the
 312 concentrations of the Arctic *Chaetoceros* and *Micromonas*, and carotenoids 1.5 and 1.2
 313 times higher.



314
 315 Figure 2. The effects of atrazine (A, C) and simazine (B, D) on the pigment contents of
 316 four species, including (red color) temperate *C. neogracile* (T-CN) and temperate *M.*
 317 *bravo* (T-MB), (blue color) Arctic *C. neogracilis* (A-CN) and Arctic *M. polaris* (A-MP)
 318 after 72 h exposure. Data are expressed as means ± SD (n = 6).

319 3.2.2 Effects of trifluralin and chlorpyrifos

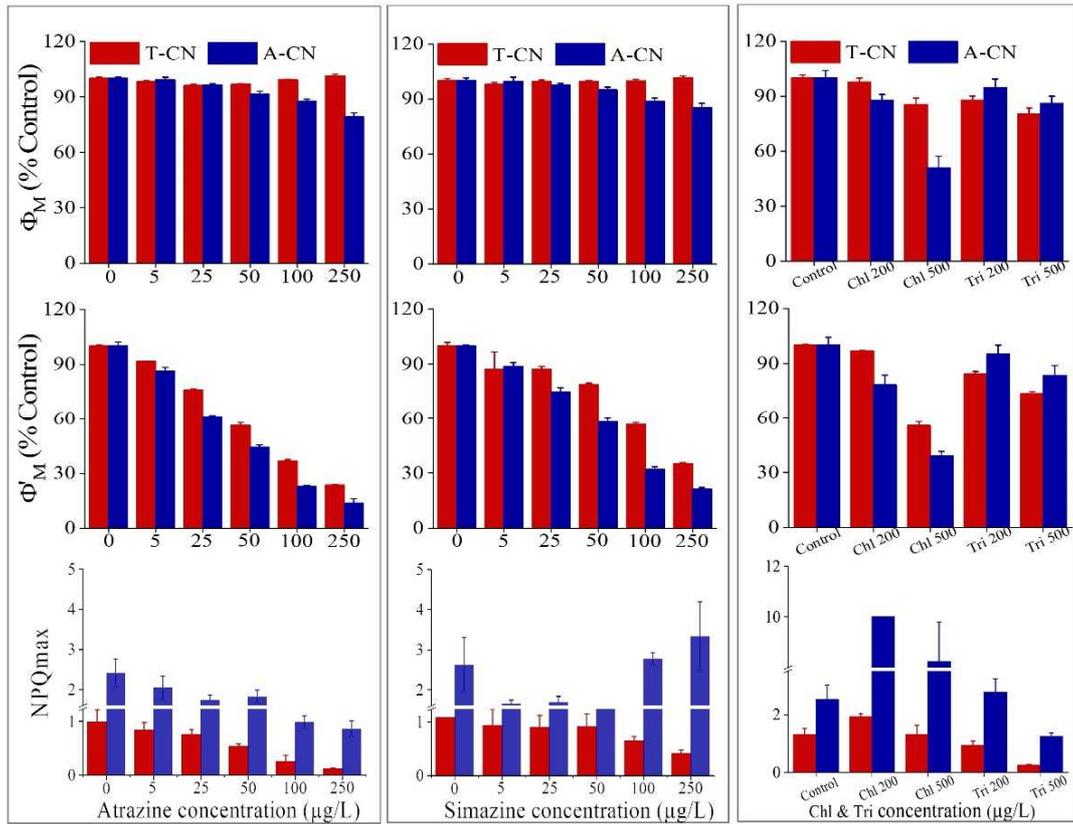
320 Chl *a* and Car contents of both temperate microalgae diminished in the presence of
 321 chlorpyrifos (Table S1). On the other hand, both Arctic microalgae increased their

322 contents in Chl *a* and Car except for *M. polaris* where the Car was unchanged. Car of
323 both diatoms increased and Chl *a* increased for the lower concentrations of trifluralin,
324 but decreased for the higher concentrations. In contrast, both prasinophytes decreased
325 their Car contents, but Chl *a* content was not affected.

326 3.3 Effects of pesticides on PSII activity and the energy fluxes pathways

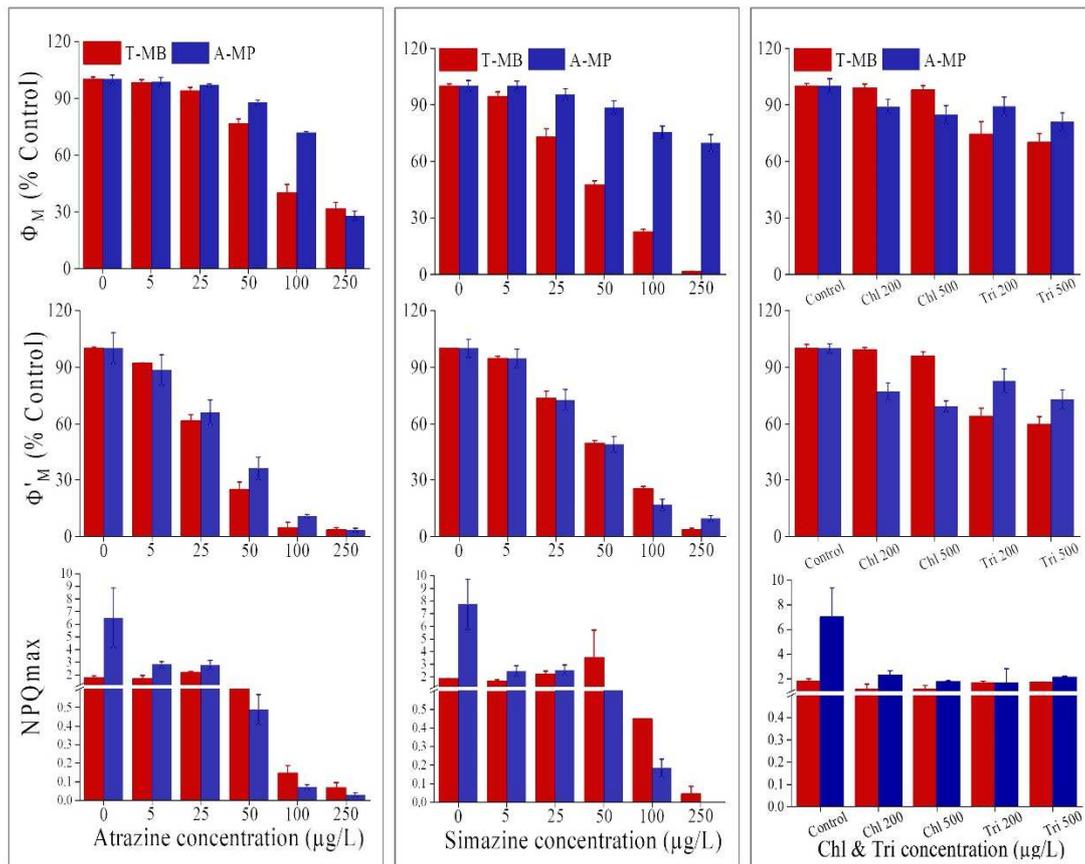
327 3.3.1 Effects of atrazine and simazine on PSII activity and the energy fluxes pathways

328 The maximum PSII quantum yields (Φ_M) of the diatoms (T-CN and A-CN) were
329 not affected by atrazine and simazine except at high concentrations (100 and 250 $\mu\text{g/L}$;
330 Tukey's HSD, $P > 0.05$) (Fig. 3). On the other hand, Φ_M of the two studied prasinophytes
331 (T-MB and A-MP) were reduced (at variable extents) at any tested concentrations
332 except 5 $\mu\text{g/L}$ (Fig. 4). The operational PSII quantum yield (Φ'_M) significantly
333 decreased (Tukey's HSD, $P < 0.05$) with increasing atrazine and simazine
334 concentrations for all studied species (Fig. 3 and 4). Both Φ_M and Φ'_M of A-CN
335 decreased more than T-CN. In contrast, Φ_M and Φ'_M of A-MP decreased less than T-
336 MB. The EC_{50} of Φ'_M and Φ_M also confirmed this result, $\Phi'_M\text{-EC}_{50}$ of T-CN and A-CN
337 are respectively 66.2, 36.7 and 142.2, 62.3 for atrazine and simazine; $\Phi'_M\text{-EC}_{50}$ of T-
338 MB and A-MP are respectively 30.7, 35.8 and 48.7, 45.8 for atrazine and simazine
339 (Table 2). Therefore, similar trend was observed for $\Phi_M\text{-EC}_{50}$ and the $\Phi'_M\text{-EC}_{50}$.



340

341 Figure 3. The effects of atrazine, simazine, chlorpyrifos and trifluralin on the maximal
 342 PSII quantum yield (Φ_M) and the operational PSII quantum yield (Φ'_M) of temperate *C.*
 343 *neogracile* (red color-T-CN) and Arctic *C. neogracililis* (blue color-A-CN) after 72 h
 344 exposure. Chl and Tri represent chlorpyrifos and trifluralin respectively. Data are
 345 expressed as means \pm SD (n = 6).



346

347 Figure 4. The effects of atrazine, simazine, chlorpyrifos and trifluralin on the maximal
 348 PSII quantum yield (Φ_M), the operational PSII quantum yield (Φ'_M) and non-
 349 photochemical quenching (NPQ) of temperate *M. bravo* (red color-T-MB) and Arctic
 350 *M. polaris* (blue color-A-MP) after 72 h exposure. Chl and Tri represent chlorpyrifos
 351 and trifluralin respectively. Data are expressed as means \pm SD (n = 6).

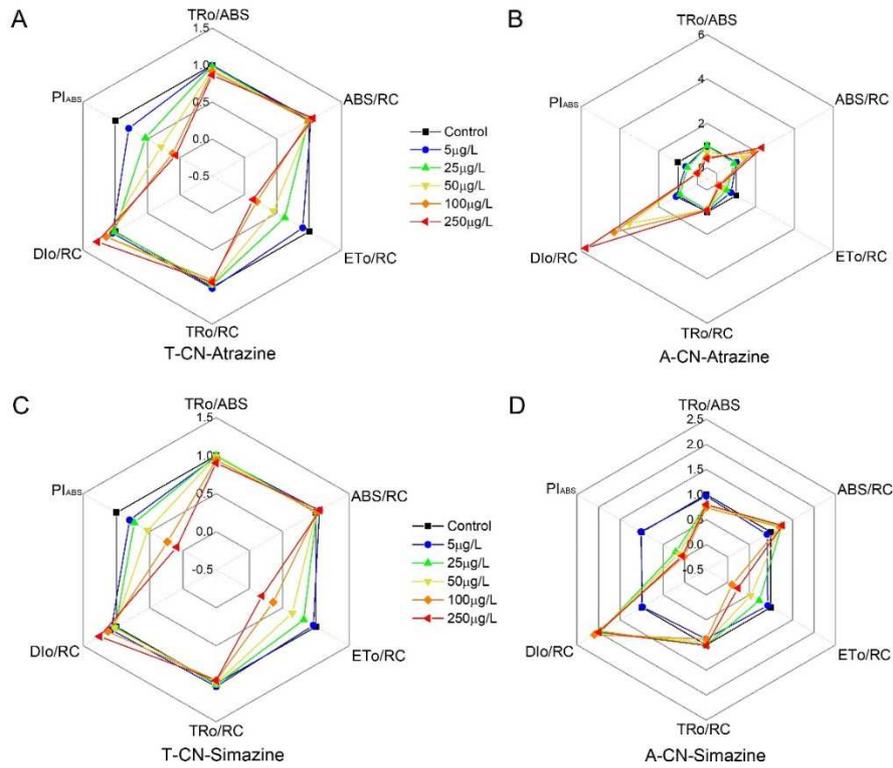
352

353 Table 3. The effects of atrazine and simazine on Φ_M , Φ'_M , α , ETRmax, and E_k of
 354 temperate *C. neogracile* (T-CN), *M. bravo* (T-MB) and Arctic *C. neogracilis* (A-CN),
 355 Arctic *M. polaris* (A-MP) after 72 h exposure. The same column superscript letters (a-
 356 d) showed significant differences among strains under different pesticide
 357 concentrations. ND = not determined (Showing that calculations are strongly
 358 influenced by treatment). Data expressed as means \pm SD (n = 6).

359

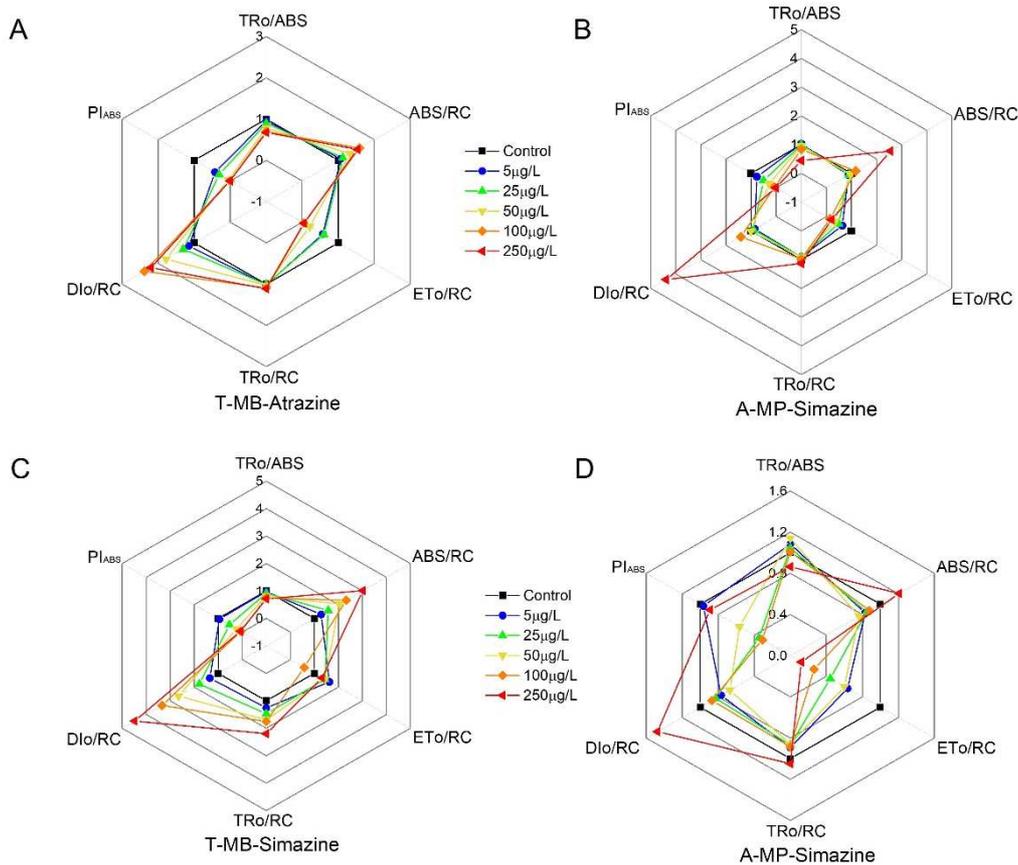
Parameter	Species T-CN						Simazine					
	A-CN	T-MB	A-MP	T-CN	A-CN	A-MP	T-MB	A-CN	A-MP	T-MB	A-CN	A-MP
	Atrazine											
	0	0.65±0.00 ^a	0.58±0.00 ^a	0.65±0.01 ^a	0.62±0.04 ^a	0.66±0.01 ^b	0.58±0.01 ^a	0.64±0.00 ^a	0.63±0.02 ^a	0.64±0.00 ^a	0.58±0.01 ^a	0.63±0.00 ^a
Φ_M	5	0.63±0.00 ^a	0.58±0.01 ^a	0.63±0.00 ^a	0.61±0.00 ^b	0.64±0.01 ^a	0.58±0.00 ^a	0.64±0.00 ^a	0.63±0.00 ^a	0.58±0.00 ^a	0.64±0.00 ^a	0.63±0.00 ^a
	25	0.62±0.01 ^b	0.56±0.01 ^a	0.61±0.01 ^a	0.60±0.00 ^b	0.65±0.01 ^{ab}	0.57±0.00 ^{ab}	0.62±0.00 ^b	0.60±0.01 ^a	0.57±0.00 ^{ab}	0.62±0.00 ^b	0.60±0.01 ^a
	50	0.63±0.01 ^b	0.53±0.01 ^b	0.50±0.02 ^b	0.54±0.00 ^c	0.65±0.01 ^{ab}	0.65±0.01 ^{ab}	0.55±0.00 ^b	0.56±0.00 ^c	0.55±0.01 ^b	0.55±0.00 ^b	0.55±0.01 ^b
	100	0.64±0.00 ^a	0.51±0.00 ^b	0.26±0.03 ^c	0.44±0.01 ^d	0.65±0.01 ^{ab}	0.52±0.00 ^c	0.48±0.00 ^d	0.47±0.01 ^c	0.52±0.00 ^c	0.48±0.00 ^d	0.47±0.01 ^c
	250	0.66±0.00 ^a	0.46±0.01 ^c	0.21±0.02 ^d	0.17±0.02 ^e	0.67±0.00 ^b	0.50±0.01 ^d	0.21±0.01 ^e	0.42±0.01 ^d	0.50±0.01 ^d	0.21±0.01 ^e	0.42±0.01 ^d
	0	0.65±0.00 ^a	0.55±0.01 ^a	0.56±0.00 ^a	0.49±0.04 ^a	0.66±0.01 ^a	0.56±0.01 ^a	0.56±0.00 ^a	0.49±0.02 ^a	0.56±0.00 ^a	0.56±0.00 ^a	0.49±0.02 ^a
Φ'_M	5	0.59±0.00 ^b	0.48±0.01 ^b	0.52±0.01 ^b	0.43±0.00 ^b	0.57±0.06 ^b	0.50±0.01 ^b	0.53±0.01 ^a	0.46±0.01 ^a	0.50±0.01 ^a	0.53±0.01 ^a	0.46±0.01 ^a
	25	0.49±0.01 ^c	0.34±0.01 ^c	0.35±0.02 ^c	0.32±0.01 ^c	0.58±0.01 ^b	0.42±0.01 ^c	0.42±0.02 ^b	0.36±0.01 ^b	0.42±0.01 ^c	0.42±0.02 ^b	0.36±0.01 ^b
	50	0.36±0.01 ^d	0.25±0.01 ^d	0.14±0.02 ^d	0.18±0.01 ^d	0.52±0.01 ^b	0.33±0.01 ^d	0.33±0.01 ^d	0.24±0.01 ^c	0.27±0.01 ^c	0.33±0.01 ^d	0.24±0.01 ^c
	100	0.24±0.00 ^e	0.13±0.00 ^e	0.03±0.02 ^e	0.05±0.00 ^e	0.37±0.01 ^c	0.18±0.00 ^e	0.14±0.01 ^d	0.08±0.01 ^d	0.18±0.00 ^e	0.14±0.01 ^d	0.08±0.01 ^d
	250	0.15±0.00 ^f	0.08±0.01 ^f	0.02±0.01 ^e	0.02±0.01 ^e	0.23±0.00 ^e	0.12±0.01 ^f	0.02±0.01 ^e	0.05±0.01 ^e	0.12±0.01 ^f	0.02±0.01 ^e	0.05±0.01 ^e
	0	0.68±0.01 ^a	0.55±0.02 ^a	0.60±0.02 ^a	0.52±0.05 ^a	0.69±0.01 ^a	0.53±0.01 ^a	0.59±0.02 ^a	0.52±0.05 ^a	0.53±0.01 ^a	0.59±0.02 ^a	0.52±0.05 ^a
a	5	0.62±0.01 ^b	0.46±0.01 ^b	0.54±0.00 ^b	0.48±0.00 ^b	0.63±0.06 ^a	0.47±0.03 ^b	0.52±0.01 ^b	0.50±0.01 ^a	0.47±0.03 ^b	0.52±0.01 ^b	0.50±0.01 ^a
	25	0.51±0.02 ^c	0.33±0.01 ^c	0.35±0.02 ^c	0.34±0.02 ^c	0.60±0.01 ^a	0.41±0.01 ^c	0.43±0.02 ^c	0.37±0.01 ^b	0.41±0.01 ^c	0.43±0.02 ^c	0.37±0.01 ^b
	50	0.36±0.01 ^d	0.23±0.02 ^d	0.13±0.02 ^d	0.17±0.01 ^d	0.53±0.02 ^b	0.31±0.01 ^d	0.27±0.01 ^d	0.24±0.01 ^c	0.31±0.01 ^d	0.27±0.01 ^d	0.24±0.01 ^c
	100	0.22±0.01 ^e	0.11±0.00 ^e	ND	0.06±0.00 ^e	0.38±0.02 ^c	0.17±0.01 ^e	0.14±0.01 ^c	0.09±0.01 ^d	0.17±0.01 ^e	0.14±0.01 ^c	0.09±0.01 ^d
	250	0.11±0.01 ^f	0.10±0.01 ^e	ND	ND	0.20±0.00 ^d	0.11±0.01 ^f	ND	0.05±0.01 ^d	0.11±0.01 ^f	ND	0.05±0.01 ^d
	0	1337±439 ^a	1037±394 ^a	827±227 ^a	404±96 ^a	1366±540 ^a	1147±80 ^a	679±107 ^a	434±163 ^a	1147±80 ^a	679±107 ^a	434±163 ^a
ETRmax	5	1291±515 ^a	869±312 ^a	522±16 ^a	501±35 ^a	1481±833 ^a	801±332 ^a	507±10 ^a	394±40 ^a	801±332 ^a	507±10 ^a	394±40 ^a
	25	1601±804 ^a	708±35 ^a	490±249 ^a	325±70 ^b	1496±305 ^a	741±261 ^a	631±212 ^a	370±103 ^a	741±261 ^a	631±212 ^a	370±103 ^a
	50	1220±102 ^a	394±46 ^b	142±67 ^b	111±10 ^c	1550±491 ^a	595±199 ^b	368±117 ^b	234±24 ^a	595±199 ^b	368±117 ^b	234±24 ^a
	100	479±73 ^b	114±19 ^{bc}	2±1 ^b	28±4 ^c	938±256 ^a	219±17 ^b	131±20 ^c	44±6 ^b	219±17 ^b	131±20 ^c	44±6 ^b
	250	160±13 ^b	18±1 ^c	0±0 ^b	6±2 ^c	378±2 ^b	61±16 ^c	0±0 ^c	14±3 ^b	61±16 ^c	0±0 ^c	14±3 ^b
	0	1972±663 ^a	1872±703 ^a	1389±422 ^a	764±116 ^a	1993±785 ^a	2151±128 ^{ab}	1141±154 ^a	820±229 ^a	2151±128 ^{ab}	1141±154 ^a	820±229 ^a
Ek	5	2065±783 ^a	1898±666 ^a	972±29 ^a	1040±70 ^b	2493±1065 ^a	1695±728 ^{ab}	978±1 ^a	796±66 ^a	1695±728 ^{ab}	978±1 ^a	796±66 ^a
	25	3129±1502 ^a	2138±194 ^a	1416±704 ^a	945±170 ^c	2490±550 ^a	1829±679 ^{ab}	1487±531 ^a	1003±258 ^a	1829±679 ^{ab}	1487±531 ^a	1003±258 ^a
	50	3350±183 ^a	1735±219 ^a	1036±356 ^a	654±30 ^d	2899±882 ^a	1943±657 ^{ab}	1347±385 ^a	980±63 ^a	1943±657 ^{ab}	1347±385 ^a	980±63 ^a
	100	2185±390 ^a	1068±209 ^{ab}	ND	479±79 ^c	2510±790 ^a	1265±785 ^c	974±129 ^a	512±98 ^b	1265±785 ^c	974±129 ^a	512±98 ^b
	250	1476±68 ^a	184±29 ^b	ND	ND	1908±35 ^a	561±183 ^c	ND	320±104 ^b	561±183 ^c	ND	320±104 ^b
	0	0.99±0.22 ^a	2.40±0.35 ^a	1.76±0.17 ^a	6.48±2.36 ^a	1.07±0.25 ^a	2.62±0.68 ^a	1.76±0.17 ^a	0.78±0.07 ^a	2.62±0.68 ^a	1.76±0.17 ^a	0.78±0.07 ^a
NPQmax	5	0.84±0.14 ^{ab}	2.04±0.29 ^a	1.69±0.27 ^a	2.80±0.23 ^b	0.77±0.35 ^a	1.64±0.09 ^a	1.69±0.27 ^a	0.72±0.05 ^b	1.64±0.09 ^a	1.69±0.27 ^a	0.72±0.05 ^b
	25	0.76±0.09 ^{ab}	1.72±0.14 ^a	2.21±0.07 ^a	2.75±0.37 ^{bc}	0.90±0.22 ^a	1.68±0.14 ^a	2.21±0.07 ^a	0.92±0.05 ^{bc}	1.68±0.14 ^a	2.21±0.07 ^a	0.92±0.05 ^{bc}
	50	0.53±0.05 ^b	1.81±0.17 ^a	0.83±0.54 ^b	0.49±0.08 ^{bcd}	0.90±0.24 ^a	1.32±0.18 ^b	0.83±0.54 ^b	0.70±0.08 ^{bd}	1.32±0.18 ^b	0.83±0.54 ^b	0.70±0.08 ^{bd}
	100	0.25±0.12 ^{bc}	4.88±0.51 ^b	0.14±0.04 ^c	0.07±0.01 ^c	0.65±0.08 ^b	2.76±0.15 ^c	0.14±0.04 ^c	0.36±0.04 ^{de}	2.76±0.15 ^c	0.14±0.04 ^c	0.36±0.04 ^{de}
	250	0.12±0.01 ^d	2.32±0.35 ^c	0.07±0.03 ^c	0.03±0.01 ^c	0.40±0.08 ^c	3.33±0.86 ^c	0.07±0.03 ^c	0.22±0.00 ^{de}	3.33±0.86 ^c	0.07±0.03 ^c	0.22±0.00 ^{de}

361 Calculated parameters acquired from the rapid Chl *a* fluorescence kinetics provide
362 useful indication on how pesticides may affect energy fluxes within PSII (Force et al.,
363 2003). The electron transport rate per active reaction center (ET_0/RC) of all species
364 significantly decreased under the treatment of atrazine and simazine by stopping
365 electron flow between Q_A and Q_B (Fig. 5 and 6). The energy conservation parameter of
366 PI_{ABS} also declined under these treatment conditions, but to an extent that was species-
367 dependent. We found that PI_{ABS} of the A-CN was more affected than T-CN (Fig. 5A-
368 D); in contrast, the PI_{ABS} of the temperate *M. bravo* was more affected than in the Arctic
369 *M. polaris* (Fig. 6A-D). The effective dissipation per reaction center (DI_0/RC) of the
370 four species increased because of the high dissipation of the inactive RCs in the
371 presence of atrazine and simazine. Similarly, the absorption flux per reaction center
372 (ABS/RC), a proxy of the PSII antenna size, was increased by up to 202% in the
373 presence of atrazine or simazine, except for the temperate *C. neogracile*. The DI_0/RC
374 was increased in agreement with the change in ABS/RC in presence of atrazine and
375 simazine, and the effect of DI_0/RC on Arctic diatom *C. neogracilis* was stronger (4.5
376 and 1.6 times) than for the temperate *C. neogracile*; for the prasinophytes we observed
377 the contrary (the effect on DI_0/RC of the temperate *M. bravo* was stronger than for *M.*
378 *polaris*). The maximal rate at which excitons are caught by the active reaction centers
379 (TR_0/RC) was only altered by simazine for *M. bravo*.



380

381 Figure 5. The effects of atrazine and simazine on the chlorophyll fluorescence
 382 parameters of temperate *C. neogracile* (T-CN) and Arctic *C. neogracilis* (A-CN) after
 383 72 h exposure.



384

385 Figure 6. The effects of atrazine and simazine on the chlorophyll fluorescence
 386 parameters of temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP) after 72 h
 387 exposure.

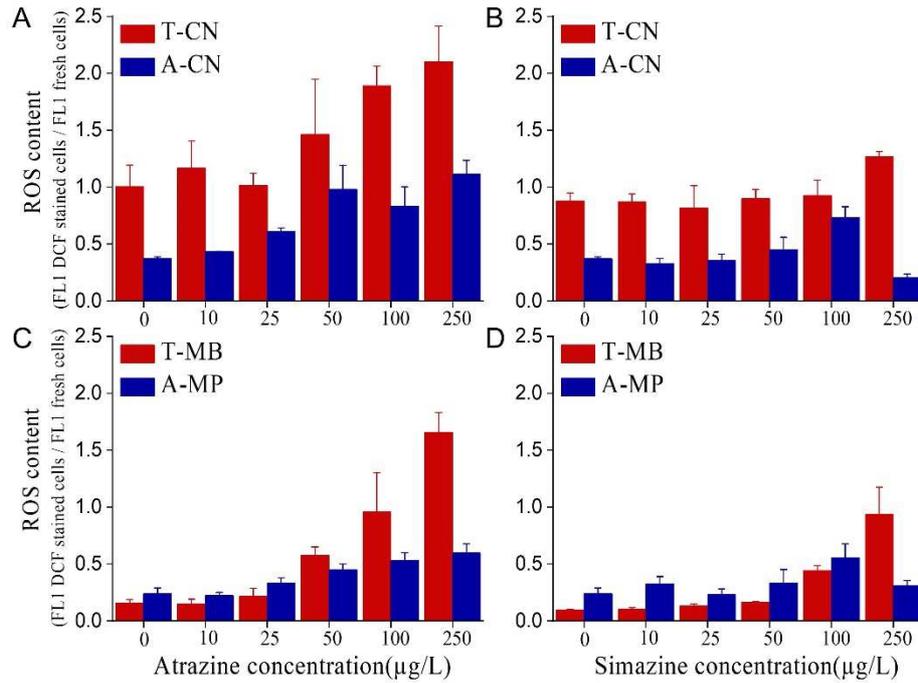
388 3.3.2 Effects of trifluralin and chlorpyrifos on PSII activity and the energy fluxes
 389 pathways

390 The Φ_M and Φ'_M of all species declined in the presence of high concentrations (200
 391 and 500 $\mu\text{g/L}$) of chlorpyrifos and trifluralin (Fig. 3 and 4), but the declines differed
 392 between temperate (*C. neogracile* and *M. bravo*) and Arctic species (*C. neogracilis* and
 393 *M. polaris*). Indeed, both Φ_M and Φ'_M of A-CN decreased more than T-CN when
 394 exposed to chlorpyrifos, but this trend was reversed in the presence of trifluralin. For
 395 the prasinophytes, we observed similar effect where both Φ_M and Φ'_M of Arctic *M.*
 396 *polaris* decreased more in the presence of chlorpyrifos than ones of the temperate *M.*
 397 *bravo*. This trend was reversed in the presence of trifluralin for Arctic *M. polaris* which
 398 declined less than did the temperate *M. bravo*. These results indicate greater sensitivity
 399 of Arctic species to chlorpyrifos than temperate counterparts, and vice versa with

400 trifluralin. In the presence of chlorpyrifos and trifluralin, ET_0/RC and PI_{ABS} of both
401 *Chaetoceros* species decreased with increasing pesticide concentration concomitantly
402 with increasing DI_0/RC (Fig. S1). In response to trifluralin, Arctic and temperate
403 *Micromonas* demonstrated opposite DI_0/RC and ABS/RC trends: these parameters
404 increased for the temperate *M. bravo* and declined for the Arctic *M. polaris* (Fig. S1).
405 Similarly, ET_0/RC and DI_0/RC responses in the two *Micromonas* species showed
406 opposite response to chlorpyrifos.

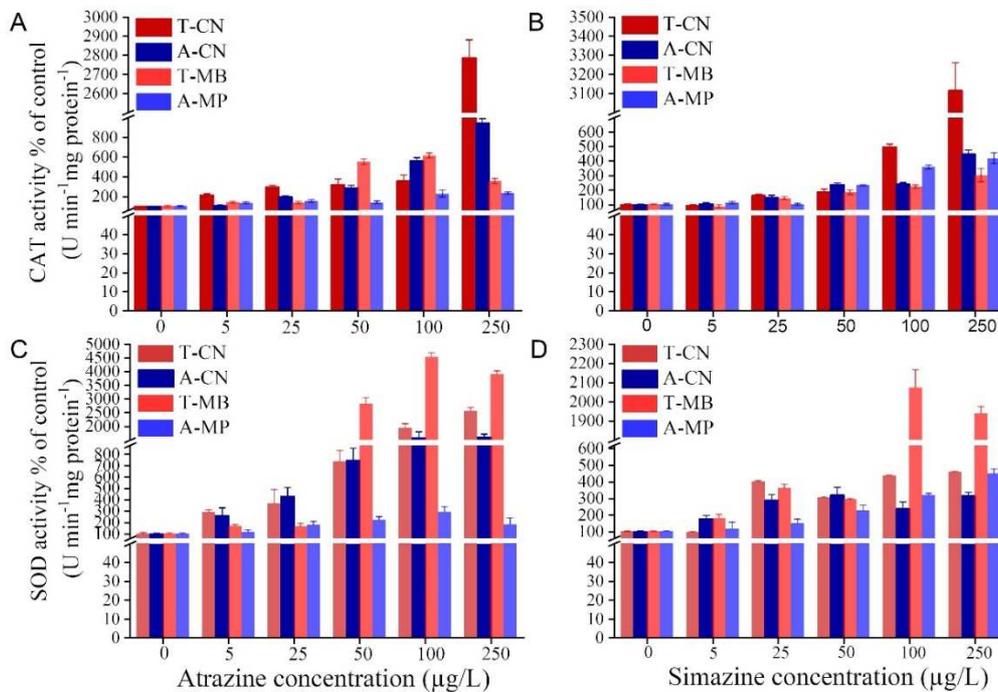
407 3.4 Effects of pesticides on reactive oxygen species, antioxidant enzyme activity and 408 protein content

409 At the lowest concentrations of atrazine and simazine (5 and 25 $\mu\text{g/l}$), the ROS
410 content of all species was unchanged compared to control conditions (Tukey's HSD,
411 $P>0.05$) (Fig. 7). However, the ROS content significantly increased (Tukey's HSD,
412 $P<0.05$) at higher concentrations of pesticides (50, 100 and 250 $\mu\text{g/l}$). Despite similar
413 trends, in the presence of atrazine, simazine and trifluralin, the ROS content of A-CN
414 increased more than for the temperate *C. neogracile*, while the opposite occurred in the
415 presence of chlorpyrifos. We observed a less marked increase of ROS content in the
416 presence of pesticides for the Arctic *M. polaris* compared to the temperate *M. bravo*.
417 Moreover, under control conditions, the ROS content of Arctic microalgae was lower
418 than its temperate counterparts (Table S1). The activity of SOD and CAT of all species
419 strongly increased with atrazine, simazine and trifluralin concentrations (Fig. 8).
420 Concomitantly, the protein concentration per cell significantly decreased in the
421 presence of pesticides, indicating that pesticides induced oxidative stress and a
422 subsequent induction of mechanisms involved in the removal O_2^- and H_2O_2 .
423 Surprisingly, for chlorpyrifos, the activity of SOD and CAT, and the total protein
424 content of temperate *C. neogracile* and *M. bravo* decreased, suggesting that the
425 antioxidant enzyme system was insufficient to cope with the impact of these pesticides
426 or at least was not the main protective measure.



427

428 Figure 7. The effects of atrazine (A, C) and simazine (B, D) on the reactive oxygen
 429 species (ROS) of four species, including (red color) temperate *C. neogracile* (T-CN)
 430 and temperate *M. bravo* (T-MB), (blue color) Arctic *C. neogracilis* (A-CN) and Arctic
 431 *M. polaris* (A-MP) after 72 h exposure. Data are expressed as means \pm SD (n = 6).



432

433 Figure 8. The effects of atrazine (A, C) and simazine (B, D) on the catalase (CAT) and
 434 superoxide dismutase (SOD) of four species, including (red color) temperate *C.*

435 *neogracile* (T-CN) and temperate *M. bravo* (T-MB), (blue color) Arctic *C. neogracilis*
436 (A-CN) and Arctic *M. polaris* (A-MP) after 72 h exposure. Data are expressed as means
437 \pm SD (n = 6).

438

439 **4. Discussion**

440 4.1 Different physiological characteristics between Arctic microalgae and their
441 temperate counterparts

442 It is well accepted that at low temperatures Rubisco activity is the limiting step for
443 growth (Young et al. 2015). Similarly, the cell biovolume of microalgae can be affected
444 by growth temperature (Daufresne et al. 2009), and smaller cells have a larger
445 surface/volume ratio compared to bigger cells, which facilitates better utilization of
446 resources such as light and nutrients for growth (Wirth et al. 2019). In our study, the
447 temperate and Arctic prasinophytes have the same cell biovolume (5-8 μm^3), since
448 prasinophyte morphological and biochemical characteristics are highly conserved and
449 may not be strongly modified by the long term growth conditions (McKie-Krisberg and
450 Sanders 2014). The growth rate of temperate *M. bravo* (T-MB) is almost twice that of
451 the Arctic *M. polaris* (A-MP), which is consistent with previous studies, indicating that
452 growth rates of polar green algae are usually lower than for temperate one
453 (Kottuparambil et al. 2017, Lacour et al. 2017). Thus, not surprisingly, A-MP had lower
454 ETR_{max}, E_k and α compared to T-MB, indicating that the utilization efficiency of light
455 energy and photosynthetic capacity of A-MP were weaker than those of T-MB (Table
456 3). This was also supported by the lower maximal and operational PSII quantum yields
457 (Φ_M and Φ'_M) in A-MP (Table 3). Interestingly, these differences were not found in the
458 comparison between congeneric Arctic (*C. neogracilis*-A-CN) and temperate (*C.*
459 *neogracile*-T-CN) diatoms. Indeed, Arctic and temperate diatoms had similar growth
460 rate, ETR_{max}, E_k and α (Table 3), indicating that A-CN, while growing at a low
461 temperature, has developed some strategies to improve the ability to harvest light
462 energy and light utilization efficiency to optimize growth. However, these strategies did

463 not prevent lower Φ_M and Φ'_M in A-CN compared to T-CN (Table 3), which is partly
464 due to the presence of sustained NPQ (Lacour et al. 2018). Furthermore, A-CN with a
465 much lower cell biovolume compared to T-CN can make better use of resources to
466 benefit growth, which may partially explain the same growth rates of A-CN and T-CN.

467 The growth rates of both diatoms were higher than for *Micromonas*, as previously
468 shown in other species' comparisons (the diatom *Thalassiosira hyalina* with
469 *Micromonas pusilla*) (Hoppe et al. 2018), probably due to the stronger ability of
470 diatoms to increase the Rubisco gene expression when its activity is reduced at low
471 temperature (Young et al. 2015). However, it is uncertain if prasinophytes also exhibit
472 these acclimation responses (Hoppe et al. 2018). Lacour et al. (2017) observed that for
473 a given Chl *a* content, polar diatoms grow more slowly than do temperate ones,
474 suggesting that this difference is related to energy allocation. Interestingly, in our study,
475 A-CN and T-CN had the same growth rate, but A-CN had a much lower Chl *a* content
476 compared to its temperate counterpart. This indicates that different polar diatoms may
477 have developed different adaptation strategies in concordance with their growth
478 environment. Indeed, diatoms have different inherent NPQ abilities in response to their
479 respective habitats (Croteau et al. 2021, Croteau et al. 2022). Lower Chl *a* content in
480 both Arctic microalgae helps reduce excitation pressure on the photosynthetic reaction
481 center, particularly in situations constraining growth, such as cold environments
482 (Halsey and Jones 2015). Recent studies have demonstrated that for the majority of
483 polar microalgal groups, NPQ is an essential element of the species-specific
484 photoadaptive strategies (Croteau et al. 2021, Galindo et al. 2017). Together with low
485 Chl *a*, polar microalgae also can induce high NPQ through an efficient de-epoxidation
486 process to jointly protect the photosynthetic apparatus against environmental stress
487 such as sudden increase in light intensity and temperature modifications (Lacour et al.
488 2020, Ni et al. 2017). In comparison, both Arctic microalgae, having lower ROS content
489 and high CAT and SOD activities, should be more likely to adapt and survive at low
490 Arctic habitat temperatures (Blanc et 2012). In summary, Arctic species appear to have
491 evolved different ecophysiological characteristics than their temperate counterparts:

492 Arctic microalgae have (1) lower Chl *a* and carotenoid contents, (2) a much higher
493 intrinsic NPQ_{max}, (3) lower ROS content and (4) higher CAT and SOD activities,
494 compared to temperate species.

495 4.2 Effects of pesticides on Arctic microalgae and their temperate counterparts

496 4.2.1 Effects of pesticides on diatoms (A-CN and T-CN)

497 PSII inhibiting herbicides (such as atrazine and simazine) that can bind to the Q_B
498 site on the D1 protein of PSII, inhibit the PSII-PSI electron transport resulting in high
499 excitation pressure on PSII and ROS generation (Bai et al. 2015). If the photoprotective
500 processes, NPQ and ROS scavenging system, are ineffective, reduced energy
501 production and cellular damages occur, and ultimately algal growth might be reduced.
502 Our study demonstrated that atrazine and simazine significantly inhibited the growth of
503 both diatoms, as seen previously for *Navicula pelliculosa* (Chalifour and Juneau 2011).
504 Our observations were linked to the significant reduction in Φ'_M and the electron
505 transport rate per active RC (ET₀/RC). Concomitantly, dissipation of excess light
506 energy (DI₀/RC) increased but was not sufficient to protect the PSII since Φ_M , a proxy
507 of the PSII RC integrity, was affected, as previously shown when *Phaeodactylum*
508 *tricornutum* was treated with PSII inhibiting herbicides (Debenest et al. 2010). This
509 result is also supported by the significant decrease in PI_{ABS}, a sensitive indicator of plant
510 health (Bayat et al. 2018) (Fig. 5A-D). The other unchanged PSII energy fluxes
511 (ABS/RC and TR₀/RC) at low pesticide concentrations, together with the minor
512 variations in the carotenoid contents, indicate that the protection of the photosynthetic
513 electron transport chain from the ROS produced in the presence of atrazine and
514 simazine, are likely due to other protective strategies (like antioxidant enzymatic
515 systems). One could expect that, as the first line of defense against the excess of light
516 energy under stressful conditions, NPQ would be activated (Kress and Jahns 2017,
517 Müller et al. 2001). However, NPQ_{max} decreased with increasing atrazine and
518 simazine concentrations (Fig. 3). As shown in previous studies, the decline of NPQ
519 in the presence of pesticides is attributed to the low buildup of the proton gradient across
520 the thylakoid membranes, since electron transport is decreased (Chalifour and Juneau

521 2011, Gomes and Juneau 2017). Therefore, we supposed that the inhibition of the ΔpH -
522 dependant non-photochemical energy dissipation mechanism leads to a reduced ability
523 to decrease excitation pressure at PSII RC, resulting in higher ROS production.
524 Although SOD and CAT activities were significantly increased with increasing atrazine
525 and simazine concentrations, it was not sufficient to cope entirely with the ROS
526 production induced by increasing atrazine and simazine concentrations (Fig. 7).

527 Overall, as shown by the investigation of the physiological parameters in the
528 presence of atrazine and simazine and the determined EC_{50} (Table 2), A-CN was more
529 sensitive than the T-CN to atrazine and simazine. We suspect that the more pronounced
530 PSII RC inactivation of A-CN results from its smaller cell biovolume, thus increasing
531 overall contact with the pesticide molecules and resulting in an enhanced absorption of
532 the contaminants (Weiner et al. 2004). However, the antioxidant enzyme system (SOD
533 and CAT) induced in A-CN was insufficient to cope with the ROS production in the
534 presence of atrazine and simazine. Furthermore, as previously showed in psychrophilic
535 diatoms, PSII repair rates are slower than the ones found in temperate diatoms (Petrou
536 et al. 2010), since lower temperatures decreased enzyme activity and metabolism
537 (Morgan-Kiss et al. 2006). On the contrary, compared to the Arctic diatom, the
538 temperate diatom potentially showed lower absorption of atrazine and simazine, the
539 capacity for an efficient antioxidant enzyme system and probably higher rate of PSII
540 repair cycle, which ultimately lead to its lower sensitivity to these herbicides.

541 For trifluralin, interestingly, we observed that A-CN was more tolerant than T-CN
542 according to the growth, and photosynthetic activity (Φ_M and Φ'_M ; Fig. 3). According
543 to our data (Table S1, Fig. S1), the greater decrease of PI_{ABS} and ABS/RC for T-CN
544 compared to A-CN, indicated that PSII RC was more damaged in T-CN. However, the
545 Arctic diatom has more effective antioxidant capacity (SOD and CAT activities) than
546 its temperate counterpart under the same concentration of trifluralin, indicating that its
547 tolerance to trifluralin seems to mainly depend on the high efficiency of the antioxidant
548 system. Although insecticides are not intended to affect plants and algae, chlorpyrifos
549 has been shown to induce some deleterious impacts at the cellular and population levels

550 (Asselborn et al. 2015), leading to the impairment of cell morphology and growth
551 (Asselborn et al. 2006, Garrido et al. 2019), and the decrease in diversity of diatoms
552 (Stratton, 1987). Similar effects were seen in our study, where chlorpyrifos not only
553 affected the growth, and photosynthesis of both diatoms (Fig. 3; A-CN was more
554 affected than T-CN), but also caused oxidative stress. The observed difference in the
555 sensitivity to chlorpyrifos of diatoms was mainly reflected at the electron transport level,
556 where chlorpyrifos induced the accumulation of Q_A^- and prevented electron transfer
557 downstream of Q_A (revealed by the more pronounced increase in O-J and J-I phases for
558 the Arctic diatom, Fig. S2), also evidence in the significant decrease of Φ'_M , ETo/RC
559 and PI_{ABS} for A-CN (Fig. S1). In comparison, this impact on electron transport was
560 accompanied by a weaker ability to dissipate excess energy (Dio/RC) in the Arctic
561 diatom, resulting in its greater sensitivity to chlorpyrifos. In addition, some authors have
562 suggested that insecticides disturb the cell cycle of *Selenastrum capricornutum* since
563 they observed the inhibition of cell-separation, resulting in the intracellular
564 accumulation of macromolecules, which are responsible for increasing the biovolume
565 in A-CN (Fernandez et al. 2021, Rioboo et al. 2002).

566 4.2.2 Effects of pesticides on prasinophytes (A-MP and T-MB)

567 Prasinophytes responded to atrazine and simazine similarly to diatoms when
568 growth, pigment composition and photosynthetic efficiency were evaluated. In contrast
569 to diatoms, A-MP was more tolerant than T-MB to atrazine and simazine (Table 2 and
570 Fig. 6). The more pronounced damage to the PSII RC (Φ_M), drastic inhibition of
571 photosynthetic electron transport (Φ'_M and ETo/RC) and decreased light conversion
572 efficiency (PI_{ABS}) at the PSII RC level in T-MB eventually induced higher production
573 of ROS, which further damaged these photosynthetic components. We propose several
574 reasons why A-MP was more tolerant to atrazine and simazine. First, A-MP has lower
575 Chl *a* content than T-MB in the absence of pesticides, indicating that A-MP may have
576 lower PSII content and therefore fewer available molecular targets for atrazine and
577 simazine (DeLorenzox et al. 2004). Previous studies have demonstrated that Arctic
578 *Micromonas* have lower active PSII levels than their temperate counterparts (Ni et al.

579 2017). Concomitantly, the lower Chl *a* content in A-MP helps to reduce excitation
580 pressure on the PSII RC caused by atrazine and simazine. Second, the temperate species
581 produced more ROS in the presence of atrazine and simazine compared to the Arctic
582 one, but the induction of CAT and SOD were insufficient to prevent oxidative damage.
583 In comparison, the Arctic species produced lower levels of ROS and have relatively
584 lower CAT and SOD activities compared to the temperate species, which implies that
585 these enzymes can successfully scavenge ROS. ROS has to reach a threshold level to
586 increase the activity of the antioxidant enzyme system (Anu et al. 2016), which may
587 indicate why the temperate species were more affected by the ROS production than the
588 Arctic species in the presence of atrazine and simazine. Finally, A-MP had higher
589 intrinsic NPQ to which was only slightly decreased by atrazine and simazine compared
590 to T-MB (Fig. 4), which would protect PSII (Bai et al. 2015). In summary, T-MB
591 showed higher sensitivity to atrazine and simazine than A-MP, due to the non-
592 effectiveness of its NPQ and antioxidant enzymes to cope with excess light energy and
593 oxidative stress, resulting in photosynthetic damage. In comparison, A-MP seems to
594 mainly rely on NPQ rather than antioxidant enzymes under atrazine and simazine stress.

595 We observed that growth, photosynthetic efficiency, and electron transfer of the T-
596 MB were more sensitive to the herbicide trifluralin than for the A-MP. Furthermore,
597 significantly increased D_{Io}/RC, ABS/RC and decreased E_{To}/RC of T-MB in the
598 presence of trifluralin showed that the excess excitation energy caused by a certain
599 number of inactivated RCs, was mostly dissipated. We propose that the different
600 sensitivities of the prasinophytes to trifluralin are mainly determined by the antioxidant
601 enzyme system, since the Arctic species induced five times higher SOD and CAT
602 activities, even if it produced less ROS than its temperate counterpart (Table S1). The
603 insecticide chlorpyrifos, in a way similar to what was observed for diatoms, inhibited
604 the growth of *Micromonas* (A-MP was more affected than T-MB) and doubled the cell
605 biovolume of A-MP (Table S1), for the same reasons that we proposed for diatoms. For
606 T-MB, chlorpyrifos did not affect the photosynthetic efficiency. Furthermore, the
607 increased PI_{ABS} and E_{To}/RC, and unchanged kinetics of Q_A-Q_B reduction compared to

608 the control (Fig. S1), indicate that PSII RCs and the whole photosynthetic electron
609 transfer chain were protected under exposure to chlorpyrifos. By comparison,
610 photosynthesis of A-MP was strongly affected by this insecticide, as indicated by the
611 strong reduction of the active PSII RC population and suppression of electron transfer
612 between Q_A and Q_B (Fig. S2).

613 4.2.3 Comparative effect of pesticides on Arctic microalgae and their temperate 614 counterparts

615 In our study, based on the EC_{50} values for Φ'_M and growth, the species sensitivity
616 sequence was T-MB>A-MP>A-CN>T-CN in the presence of atrazine and simazine. For
617 trifluralin, based on the impact on Φ_M and Φ'_M (since no EC_{50} value cannot be obtained
618 even at very high concentrations) the species sensitivity sequence was T-MB>A-MP>T-
619 CN>A-CN and A-CN>T-CN>A-MP>T-MB for chlorpyrifos (Fig. 3 and 4). We found
620 that diatoms *Chaetoceros* were more tolerant to atrazine, simazine and trifluralin than
621 the prasinophytes *Micromonas* for both temperate and Arctic strains. Diatoms are
622 known to be dominant in most aquatic environments (Serôdio and Lavaud 2020), likely
623 owing to their specific ecophysiological characteristics, like their high PSII/PSI ratio
624 (Strzepek and Harrison 2004), their high non-photochemical energy dissipation
625 potential (Lavaud and Lepetit 2013), and the presence of fucoxanthin that can prevent
626 photooxidation (Tuchman et al. 2006). Furthermore, diatoms can more efficiently
627 control the ATP/NADPH ratio during photosynthesis compared to other photosynthetic
628 organisms, permitting them to optimize their carbon fixation and growth (Bailleul et al.
629 2015). Indeed, we found that Φ'_M , pigment, ETR_{max}, E_k and α in both diatoms were
630 higher than in prasinophytes, suggesting that diatoms have higher intrinsic
631 photosynthetic capacity and light utilization efficiency. Therefore, if we consider only
632 the impact of pesticides, we propose that *Chaetoceros* would have a greater chance of
633 survival and would become the dominant species in temperate and Arctic ecosystems
634 contaminated with atrazine, simazine and trifluralin in relation to *Micromonas*. On the
635 other hand, according to the EC_{50} of Φ'_M and growth (Table 2), atrazine induced
636 significantly more damage than simazine to all tested algae even though both molecules

637 have the same mode of action on the Q_B site of D1 protein of PSII. Previous studies
638 have shown that irgarol with high octanol/water partition coefficient (logK_{ow}) is more
639 toxic than diuron for marine microalgae as PSII inhibitor, and the higher logK_{ow} of
640 irgarol promotes its affinity for the Q_B binding site, leading to its relative higher toxicity
641 (Coquille et al. 2018, Dupraz et al. 2016, Kottuparambil et al. 2017). Therefore, we can
642 assume that the higher toxicity of atrazine compared to simazine (logK_{ow}=2.3) could be
643 due to its higher logK_{ow} (2.7) (Ronka 2016) and therefore to its higher affinity for the
644 Q_B binding site. Similarly, in the presence of atrazine, CAT and SOD activities were
645 higher and the total protein content decreased more than in the presence of simazine,
646 resulting in a decrease in the available protein for photoprotection processes, such as
647 NPQ and the PSII repair cycle (Bai et al. 2015).

648

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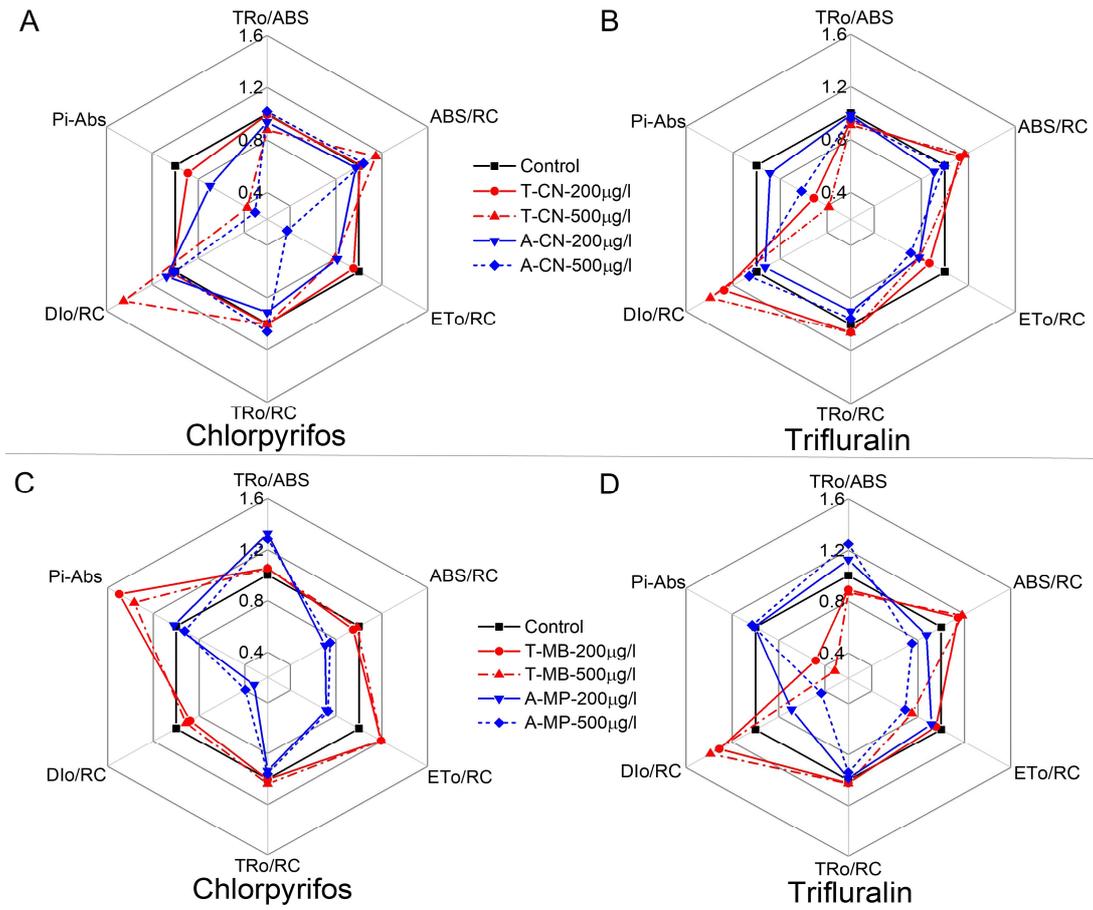
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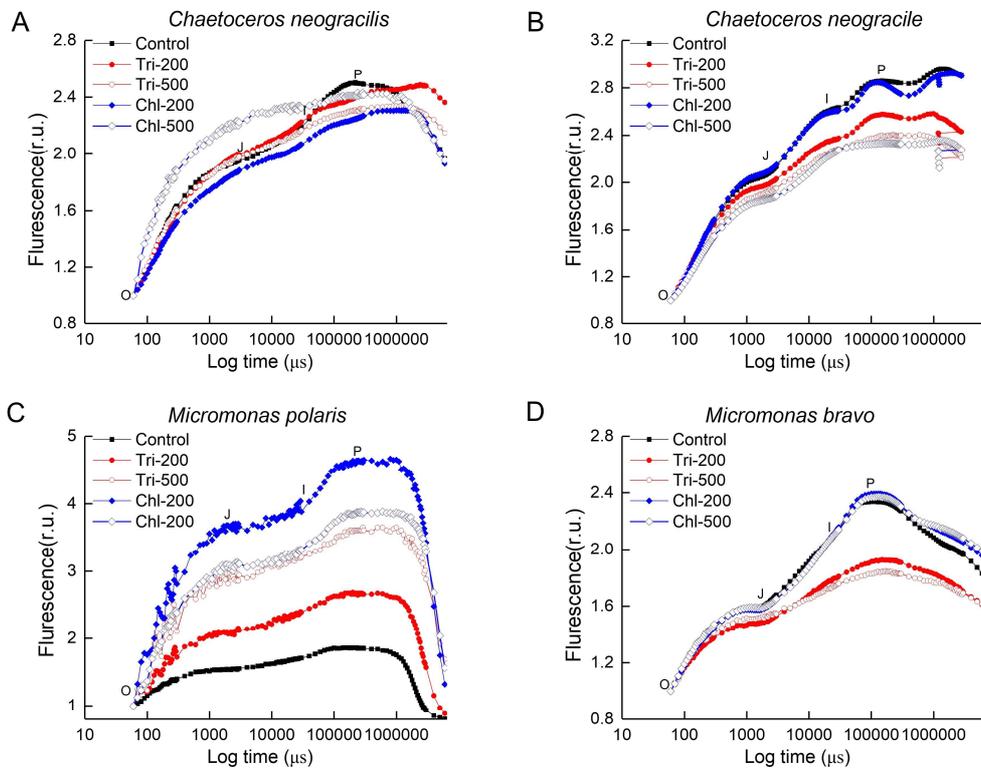
885 **Supplementary Material**

886 Figure S1. The effects of chlorpyrifos and trifluralin on energy fluxes within PSII of
 887 four species, including temperate *C. neogracile* (T-CN), Arctic *C. neogracile* (A-CN)
 888 for A and B, temperate *M. bravo* (T-MB) and Arctic *M. polaris* (A-MP) for C and D
 889 after 72 h exposure. Data expressed as means \pm SD (n = 6).



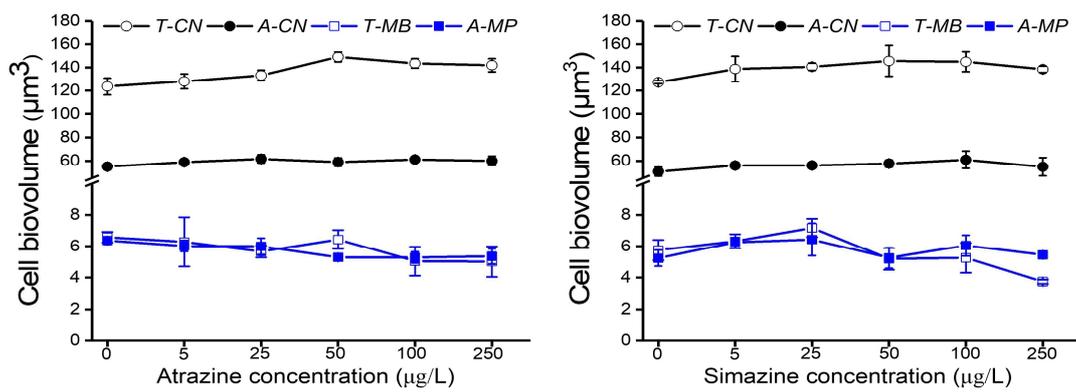
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891 Figure S2. Fast fluorescence kinetics of T-CN (A), A-CN (B), T-MB (C) and A-MP (D),
 892 exposed to chlorpyrifos and trifluralin for 72h.



893

894 Figure S3. The effects of atrazine and simazine after 72 h exposure on the cell
 895 biovolume. Data are means \pm SD. for 72h.



896

Table S1. Definitions for the fluorescence parameters used in this study.

Parameters	Definition
Φ_M	Maximal PSII quantum yield
Φ_M'	Operational PSII quantum yield
NPQ	Non-photochemical quenching
NPQ _{max}	Maximum ability for dissipation of excess energy
rETR _{max}	Maximum relative photosynthetic electron transport rate
α	Maximum light efficiency use
E_K	Light saturation coefficient
Specific energy fluxes (per Q_A reducing PSII RC)	
ABS/RC	Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size)
TRo/RC	Trapped energy flux (leading to Q_A reduction) per RC
ETo/RC	Electron transport flux (further than Q_A^-) per RC
DIo/RC	Dissipated energy flux per RC
Performance index	
$PI_{ABS}=(RC/ABS)$	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors

899 Table S2. The effects of trifluralin and chlorpyrifos after 72 h exposure on the growth
 900 rate, cell volume, Φ_M , Φ'_M , ROS content, and Chl *a* pigment. The percentages of the
 901 control value are shown by the numbers in parentheses. Data are means \pm SD.

Parameter	Pesticide concentration ($\mu\text{g/l}$)	T-CN	A-CN	T-MB	A-MP	
Growth rate	Trifluralin	0	0.622 \pm 0.01 (100)	0.655 \pm 0.01 (100)	0.375 \pm 0.01 (100)	0.245 \pm 0.02 (100)
		200	0.201 \pm 0.04 (28.5)	0.533 \pm 0.01 (69.3)	0.049 \pm 0.04 (37.6)	0.027 \pm 0.02 (52.0)
	Chlopyrifos	500	0.081 \pm 0.08 (22.2)	0.346 \pm 0.15 (60.4)	-0.093 \pm 0.08 (28.2)	-0.195 \pm 0.28 (46.4)
		200	0.608 \pm 0.00 (96.0)	0.523 \pm 0.01 (67.4)	0.382 \pm 0.02 (102)	-0.083 \pm 0.02 (37.4)
		500	0.391 \pm 0.01 (50.1)	0.102 \pm 0.03 (19.1)	0.326 \pm 0.05 (86.9)	-0.098 \pm 0.01 (35.8)
	Volume (μm^3)	Trifluralin	0	144.4 \pm 0.95 (100)	45.8 \pm 2.89 (100)	9.48 \pm 0.20 (100)
200			218.9 \pm 2.51 (151)	53.1 \pm 1.28 (116)	8.43 \pm 0.77 (89.4)	7.62 \pm 0.08 (119)
Chlopyrifos		500	221.8 \pm 20.9 (154)	58.8 \pm 1.66 (129)	8.84 \pm 1.02 (93.3)	8.69 \pm 0.47 (136)
		200	140.7 \pm 1.00 (97.2)	51.2 \pm 0.61 (115)	10.67 \pm 0.04 (112)	12.66 \pm 2.18 (199)
		500	143.8 \pm 5.32 (99.3)	91.3 \pm 9.95 (199)	10.49 \pm 0.21 (111)	12.85 \pm 0.08 (200)
Fv/Fm		Trifluralin	0	0.654 \pm 0.008 (100)	0.624 \pm 0.025 (100)	0.660 \pm 0.002 (100)
	200		0.574 \pm 0.004 (87.8)	0.591 \pm 0.011 (94.8)	0.492 \pm 0.057 (74.5)	0.538 \pm 0.012 (89.1)
	Chlopyrifos	500	0.524 \pm 0.006 (80.2)	0.536 \pm 0.003 (86.0)	0.462 \pm 0.052 (70.0)	0.489 \pm 0.010 (80.9)
		200	0.638 \pm 0.004 (97.6)	0.548 \pm 0.006 (87.8)	0.653 \pm 0.001 (99.0)	0.538 \pm 0.005 (89.0)
		500	0.558 \pm 0.007 (85.4)	0.344 \pm 0.045 (50.8)	0.645 \pm 0.006 (97.9)	0.512 \pm 0.013 (84.7)
	Φ'_m	Trifluralin	0	0.657 \pm 0.003 (100)	0.610 \pm 0.025 (100)	0.555 \pm 0.011 (100)
200			0.552 \pm 0.011 (84.0)	0.579 \pm 0.011 (95.1)	0.356 \pm 0.027 (64.1)	0.406 \pm 0.021 (82.8)
Chlopyrifos		500	0.483 \pm 0.009 (73.5)	0.507 \pm 0.003 (83.3)	0.332 \pm 0.024 (60.0)	0.357 \pm 0.016 (72.9)
		200	0.636 \pm 0.002 (96.7)	0.477 \pm 0.006 (78.4)	0.551 \pm 0.007 (99.3)	0.379 \pm 0.013 (77.2)
		500	0.365 \pm 0.016 (55.6)	0.243 \pm 0.045 (38.9)	0.533 \pm 0.011 (96.0)	0.341 \pm 0.007 (69.4)
ROS content		Trifluralin	0	0.917 \pm 0.236 (100)	0.709 \pm 0.074 (100)	0.128 \pm 0.040 (100)
	200		1.07 \pm 0.052 (117)	0.870 \pm 0.064 (123)	0.508 \pm 0.030 (397)	0.947 \pm 0.197 (393)
	Chlopyrifos	500	1.20 \pm 0.203 (131)	3.46 \pm 0.985 (488)	0.689 \pm 0.125 (539)	0.915 \pm 0.153 (379)
		200	2.16 \pm 0.205 (236)	0.975 \pm 0.120 (138)	1.72 \pm 0.215 (1344)	0.662 \pm 0.151 (274)
		500	2.80 \pm 0.112 (306)	1.02 \pm 0.114 (143)	2.81 \pm 0.581 (2195)	0.492 \pm 0.153 (204)
	Chla (10^6 cells $\mu\text{g/ml}$)	Trifluralin	0	3.818 \pm 0.168 (100)	1.08 \pm 0.060 (100)	0.540 \pm 0.005 (100)
200			12.01 \pm 1.824 (330)	1.21 \pm 0.039 (109)	0.315 \pm 0.008 (58.0)	0.341 \pm 0.018 (110)
Chlopyrifos		500	6.752 \pm 0.468 (145)	1.12 \pm 0.059 (104)	0.745 \pm 0.043 (137)	0.306 \pm 0.097 (94.1)
		200	1.16 \pm 0.053 (30.3)	1.04 \pm 0.027 (96.3)	0.497 \pm 0.059 (91.4)	0.390 \pm 0.036 (124)
		500	1.20 \pm 0.267 (31.8)	1.75 \pm 0.038 (157)	0.424 \pm 0.025 (80.0)	0.388 \pm 0.012 (123)
CAT ($\text{U mg}^{-1}\text{min protein}^{-1}$)		Trifluralin	0	0.090 \pm 0.001 (100)	0.134 \pm 0.003 (100)	0.145 \pm 0.003 (100)
	200		0.101 \pm 0.004 (105)	0.185 \pm 0.004 (138)	0.148 \pm 0.002 (102)	0.353 \pm 0.014 (161)
	Chlopyrifos	500	6.752 \pm 0.003 (113)	0.290 \pm 0.002 (217)	0.281 \pm 0.003 (193)	0.400 \pm 0.087 (182)
		200	0.087 \pm 0.001 (96.8)	0.131 \pm 0.002 (97.6)	0.112 \pm 0.059 (76.6)	0.311 \pm 0.006 (119)
		500	0.057 \pm 0.002 (63.1)	1.089 \pm 0.038 (812)	0.163 \pm 0.025 (112)	0.264 \pm 0.032 (101)