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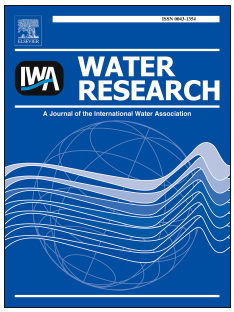

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Research Paper

Influence of parasitic chytrids on the quantity and quality of algal dissolved organic matter (AOM)

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ABSTRACT

Algae-derived dissolved organic matter (AOM) is an important nutrient source for heterotrophic bacteria, while AOM such as humic substances pose significant challenges during water treatment processing. We hypothesized that the parasitic infection of algae could change the composition and concentration of AOM. This study investigated the quality and quantity of DOM and bacterial abundance in diatom (*Synedra*) cultures, with and without-parasitic fungi (chytrids). The quality of DOM was analyzed using three-dimensional excitation-emission matrices combined with parallel factor analysis (EEM-PARAFAC) and was compared to changes in algal and bacterial cell numbers. Bacterial abundance was higher and dissolved organic carbon concentrations were lower in the diatom cultures infected with parasitic fungi. Among the DOM compounds, the concentrations of tryptophan-like material derived from algae were significantly lower and the concentrations of humic substance-like material were higher in the infected treatment. The parasitic fungi may have consumed tryptophan-like material and stimulated the release of humic substances. These results provide the first evidence that fungal infection may modulate algal–bacterial interactions, which are associated with changes in the nature of AOM.

38 **Keywords:** Algal dissolved organic matter (AOM); Parasitic fungi; EEM-PARAFAC;

39 Humic-like substances; protein-like substances; Carbon cycling

40

1. Introduction

Algae-derived dissolved organic matter (AOM) arises extracellularly via metabolic excretion and intracellularly via the autolysis of cells in aquatic and artificial ecosystems (Henderson et al. 2008). The nature of AOM is very complex, but its major fractions include carbohydrates and proteins (Fogg 1983; Myklestad 1995; Henderson et al. 2008), which generally provide good substrates for bacteria, and often exhibit short turnover times (Wetzel 2001). The release of humic substances by growing algae has also been confirmed in nonaxenic (Aoki et al. 2008) and axenic cultures (Romera-Castillo et al. 2010; Fukuzaki et al. 2014). Increasing levels of humic substances pose serious challenges for processing and the commercial supply of drinking water, because the humic substances can be transformed into potentially carcinogenic and mutagenic disinfection byproducts, such as trihalomethanes and haloacetic acids (Richardson et al. 2007; DeMarini 2011; Herzsprung et al. 2012).

Phytoplankton can often be infected by many different parasites, including viruses and fungi (Suttle 2005; Kagami et al. 2007, 2011). Viral infection is known to stimulate dissolved organic carbon (DOC) release from phytoplankton due to cell lysis (Suttle 2005). Whereas cytoplasmic components such as nucleic acids, enzymes, and small proteins will probably be cycled through bacterial uses, some structural materials such as lipid bilayers,

large proteins, and cell walls may be refractory to biological assimilation and cycling. In addition, lipids released by phytoplankton provide precursor materials, which when catalyzed by light, can undergo condensation reactions to form fulvic acid DOM (Harvey et al. 1983; Kieber et al. 1997). Parasitic fungi may also affect AOM, but via different mechanisms than viruses due to their different infection processes. However, the influence of parasitic fungi in AOM has still not been elucidated.

Parasitic fungal infections in phytoplankton have been discovered in not only lakes but also marine ecosystems (reviewed in Frenken et al. 2017). Chytridiomycetes (chytrids) are the main parasitic fungi of phytoplankton. Chytrids penetrate the diatom protoplast using a rhizoidal system (Van Donk and Bruning 1992) and inserting a feeding tube between silicified wall segments after enzymatic digestion of the organic components of the walls (Smetacek 1999). Chytrids would affect AOM in the following ways: 1) chytrid infection could stimulate the release of AOM by causing cell death; 2) chytrid infection could cause the elution of algal cell content-derived DOM by inserting a rhizoid; or 3) chytrids could reduce the release of AOM by consuming algal cell contents. Chytrid infection may also affect bacteria directly or indirectly by changing the composition and concentration of AOM.

In the present study, we investigated the quantity and quality of AOM and bacterial

abundance in diatom cultures with and without parasitic chytrids. The quality of DOM was analyzed using three-dimensional excitation-emission matrices (EEMs) combined with parallel factor analysis (PARAFAC). EEM-PARAFAC has can be used to detect humic-like and protein-like substances with high sensitivity (Yamashita et al. 2010; Senga et al. 2017). We also determined the concentrations of carbohydrates without fluorescence properties using a colorimetric method.

2. Materials and methods

Isolation and maintenance of cultures

Host diatom *Synedra* sp. (Strain S1) and its parasitic chytrid Rhizophydiales were used for the experiments. Both the host and chytrids were isolated from Lake Inba on December 2014 (Kagami et al. in prep.). These clones were maintained in non-axenic batch cultures with CHU-10 medium (Stein 1973) at 18°C with a photoperiod of 12:12 h (light:dark) with a photon irradiance of 20 µmol photons m^{-2} s^{-1} (Kagami et al. 2004).

Incubation experiment

Before the incubation experiments, *Synedra* sp. and parasitic chytrids were grown in batch cultures with vitamin-free Chu-10 medium. First, 4 L of vitamin-free Chu-10

95 medium with 3000 cells mL^{-1} of *Synedra* sp. was prepared and divided into two 4-L
96 polycarbonate bottles. In one bottle, chytrids from a 1-week-old infected culture in which
97 50% of cells were infected were inoculated at a density of 100 spores mL^{-1} (i.e.,
98 diatom-chytrid treatment). In the other bottle, filtrate of infected cultures (0.2 µm pore
99 size) was added in the same quantity as the diatom-chytrid treatment (i.e., diatom
100 treatment). This procedure was followed to avoid the possibility that the chytrid addition
101 itself increased the DOC concentration or changed the DOM compositions. Then, three
102 670-mL aliquots of each treatment were poured into three sterilized polycarbonate bottles.
103 On day 0, the abundances of *Synedra* sp., chytrids, and bacteria, and concentrations of
104 DOC, fDOM-1, -2, -3, -4, and -5, and carbohydrates in the diatom and diatom-chytrid
105 treatments were confirmed to not differ significantly.
106 All bottles were incubated at 16°C under a 12:12 h (light:dark) cycle at 230 µmol
107 quanta m^{-2} s^{-1}. On days 5, 7, 11, and 14 during the incubation period, 100 mL of culture
108 was collected from each bottle to measure the abundance of organisms and DOM
109 concentrations and compositions.
110
111 *Synedra sp., chytrid, and bacteria counting*
112 For the counting, 4.8 mL of each sample was fixed with glutaraldehyde (1% final

concentration) and stored in a refrigerator for the microscopic analysis. The abundances of infected and uninfected *Synedra* sp. were counted under an inverted microscope at a magnification of 400×. The bacterial abundance was counted under a fluorescence microscope with blue fluorescence (wavelength: 460–490 nm) at 1000× magnification after staining with SYBR green (Noble and Fuhrman 1998).

Chemical analysis

DOC concentrations were determined using a total organic carbon analyzer (TOC-2300; Hiranuma Sangyo, Ibaraki, Japan). The soluble carbohydrate carbon concentration was measured according to the anthrone method, using glucose as the standard (Dreywood et al. 1946; Morris 1948). 0.2% anthrone solution was prepared with 90 % sulfuric acid. 1 ml of water sample was mixed with 9 ml of 0.2% anthrone solution in a test tube. The mixture was boiled in a water bath for 15 min. after which it was placed in an ice bath for 10 min. The carbohydrate concentration of the solution was determined by spectrophotometrically at 620 nm.

EEM spectra were obtained using an Aqualog fluorometer (Horiba Scientific, Kyoto, Japan) with a 10 mm × 10 mm quartz cuvette. Each EEM was recorded using an excitation wavelength (Ex) range of 250–600 nm with a step width of 3 nm and an

131 emission wavelength (Em) range of 250–620 nm with a step width of 3 nm. The
132 integration time was 1 s. Each EEM data point was corrected for the inner-filter effect,
133 Rayleigh masking, and Raman scattering using Aqualog v3.6 software. Further details of
134 the procedures used can be found in Senga et al. (2017).

135 To identify the peaks of components from EEM datasets, a PARAFAC analysis was
136 performed using Solo + MIA v7.0.2 software (Eigenvector Research, Manson, WA, USA)
137 at an excitation of 252–573 nm and emission of 252–620 nm. The dataset collected from
138 the samples in the incubation experiments also included the preliminary experiments (n =
139 190). Components with core consistencies > 60 were accepted as the best models for the
140 datasets. The core consistency is a suggested method for determining the correct number
141 of components in multiway models (Bro and Kiers 2003). Using examples from a range
142 of different types of data, it was shown that the core consistency is an effective tool for
143 determining the appropriate number of components in PARAFAC models. Five
144 components, termed fDOM-1, -2, -3, -4, and -5, were identified in this study (Table 1).

145 The relative fluorescence intensity was calibrated using quinine sulfate units (QSU),
146 where 10 QSU corresponded to a fluorescence intensity of a 10 μg L^{-1} quinine sulfate
147 solution in 0.05 M sulfuric acid at Ex/Em = 350/450 nm (Coble et al. 1993).

148

149 *Statistical analysis*

150 Significant differences in parameters, such as the abundance of organisms,
151 concentrations of DOM, and ratios of the concentrations to *Synedra* sp., between the
152 diatom-chytrid and diatom treatments over time and their interaction (time × treatment)
153 were statistically examined using two-way repeated measures analysis of variance
154 (ANOVA) (R statistical software; R Development Core Team 2012). We used a general
155 linear model (LM) to clarify the factors influencing the concentrations of all DOM (DOC,
156 fDOM-1, -2, -3, -4, and -5, and carbohydrates). The response variables included the
157 concentrations of materials with significant differences between the treatments. The
158 explanatory variables included the abundances of organisms (*Synedra* sp., chytrids, and
159 bacteria). We conducted the model selection based on the Akaike information criterion
160 corrected for small sampling sizes (AICc). Model comparisons were based on the delta
161 individual model and the lowest observed AICc value. Models with AICc values differing
162 by < 2 were considered to be equivalent. For the DOC and DOM component
163 concentrations, interactions between bacteria and chytrids were examined with two-way
164 ANOVA. When interaction was detected at significance level ($p < 0.05$), there was an
165 interaction between bacteria and chytrids for DOC and DOM components.
166

3. Results and discussion

The abundance of *Synedra* sp. in the diatom and diatom-chytrid treatments changed significantly over the experimental period, and was significantly higher in the diatom treatment than the diatom-chytrid treatment (Fig. 1a). The abundance of chytrid sporangia increased until day 7, and the percentage of infected phytoplankton cells reached a maximum of ~40% at day 7 (Fig. 1b). The abundance of bacteria was significantly higher in the diatom-chytrid treatment than in the diatom treatment, and increased over time in both treatments (Fig. 1c).

DOC concentrations increased over time in both treatments, and became significantly higher in the diatom treatment than in the diatom-chytrid treatment after day 7 (Fig. 2). The LM model showed that the DOC concentration was correlated positively with diatoms and negatively with chytrids (Table 2). These results indicate that diatoms released extracellular organic matter during growth, and that algae infected with chytrids caused a quantitative reduction in DOC.

In addition to diatoms and chytrids, DOC concentration must have been affected by bacterial (Table 2). DOC concentration was also positively correlated with the abundance of bacteria, but only in the diatom treatment (Fig. 6a). Positive relationship between DOC concentration and the abundance of bacteria could be due to two different processes, i.e.

185 DOC (especially AOM) increased bacteria growth or bacteria increased DOC (especially
186 humic substances). Since the bacterial abundance was higher and DOC concentration was
187 lower in the diatom-chytrid treatment than in the diatom treatment (Fig. 1a, c), it is likely
188 that AOM released by fungal infection would increase the growth of bacteria, and
189 ultimately the AOM concentration will decrease by bacterial uptake. Another study found
190 that viral lysis of *Aureococcus anophagefferens* increased DOC release and bacterial
191 density (Gobler et al. 1997). In addition to the viral lysis of phytoplankton, fungal
192 infection in phytoplankton stimulates the shift of organic carbon from phytoplankton to
193 heterotrophic bacteria (Gobler et al. 1997; Wilhelm and Suttle 1999).

194 Five components, referred to as fDOM-1, fDOM-2, fDOM-3, fDOM-4, and fDOM-5,
195 were identified using EEM-PARAFAC (Table 1). Three components, fDOM-1, -3, and -4,
196 had a single emission maximum and two excitation maxima, while two components,
197 fDOM-2 and -5, had a single emission maximum and a single excitation maximum (Fig. 3).
198 According to previous studies of the spectral characteristics of fluorescent DOM
199 components in various aquatic environments (Cory and McKnight 2005; Stedmon and
200 Markager 2005; Santín et al. 2009; Yamashita et al. 2010; Stubbins et al. 2014), we
201 identified these components by their peak positions (Table 1). The fDOM-1, 3, and 4 were
202 found to be humic-like components and fDOM-2 and -5 protein-like components.

The Ex/Em of fDOM-1 was similar to component 5 identified by Stedmon and Markager (2005), which was determined to be generated though the subsequent microbial processing of AOM in aquatic systems. Moreover, the Ex/Em of fDOM-1 was similar to that of component P3 identified by Stubbins et al. (2014), which had an average molecular weight of 445 Da and a molecular formula with less aromaticity and more nitrogen. The Ex/Em of fDOM-3 was similar to that of Q3 identified by Cory and McKnight (2005) and C4 by Yamashita et al. (2010). Q3 was a microbial precursor material, and was correlated with aliphatic carbon content (Cory and McKnight 2005). Additionally, the microbial humic-like C4 was predicted to be mainly derived from heterotrophic activity (Yamashita et al. 2010). The fDOM-4 peaks were similar to those of SQ1 identified by Cory and McKnight (2005), C2 by Santín et al. (2009), and C5 by Yamashita et al. (2010). SQ1, C2, and C5 were described as terrestrial humic-like or humic acid-like substances. SQ1 (Cory and McKnight 2005) was plant-derived, reduced in quinones, and enriched in aromatic carbon. Considering these characteristics, fDOM-1, -3, and -4 were determined to be humic-like components generated by bacteria using AOM, and fDOM-1 would be less recalcitrant to degradation than fDOM-3 and -4. DOM compounds with less aromaticity and the C/N ratio has been reported to be biologically more labile (Sulzberger and Durisch-Kaiser 2009).

221 The fluorescence intensities (FIs) of fDOM-1, -3, and -4 increased over time (Fig. 4a,
222 c, and d). The FI of fDOM-1 was significantly higher in the diatom-chytrid treatment,
223 while there were no significant differences between treatments for fDOM-3 and -4. It
224 should be noted that the slopes of the regression formula between the FI of fDOM-1 and
225 the bacterial abundance in each treatment were almost the same (Fig. 6b). Additionally, no
226 interaction between bacteria and chytrids was detected ($p = 0.388$). These results
227 suggested that the generation of fDOM-1 was determined by bacteria and was not affected
228 by the presence of chytrids. On the other hand, the slopes of the regression formulae
229 between the FIs of fDOM-3 and -4 and bacterial abundance in each treatment differed
230 substantially (Figs. 6d and e), and interactions between bacteria and chytrids were
231 detected (fDOM-3, $p = 1.77 \times 10^{-4}$, fDOM-4, $p = 4.45 \times 10^{-3}$). This indicates that the
232 generation of fDOM-3 and -4 by bacteria was affected by chytrids. Considering the lower
233 slopes of fDOM-3 and -4 in the diatom-chytrid treatment than in the diatom treatment
234 (Figs. 6d and e), bacteria might have generated less fDOM-3, and -4 in the presence of
235 chytrids. Chytrids may have consumed algal cell contents that acted as precursors of
236 fDOM-3 and -4 by inserting their rhizoids into the host cells before bacteria used.
237 The FIs of fDOM-2, which is a tryptophan-like material (Cory and McKnight 2005;
238 Yamashita et al. 2010), increased over time and were significantly higher in the diatom

239 treatment (Fig. 4b). The LM model showed that the FI of fDOM-2 was significantly
240 positively correlated with diatom abundance (Table 2). It is well known that amino acids
241 can be released by algal activity (Henderson et al. 2008; Li et al. 2016). The FI of
242 fDOM-2 was also significantly positively correlated with bacterial abundance in the
243 diatom treatment (Fig. 6c), indicating that fDOM-2 is a good substrate for bacteria.
244 Additionally, fDOM-2 was negatively correlated with chytrid abundance (Table 2). An
245 interaction between bacteria and chytrids on fDOM-2 was detected ($p = 3.66 \times 10^{-2}$),
246 indicating that chytrids could possibly consume fDOM-2 components before they could
247 be used by bacteria. Chytrids could potentially directly take up the tryptophan-like
248 component via a feeding tube from the bodies of diatoms.
249 The fDOM-5 and carbohydrate concentrations remained almost constant over the
250 experimental period and did not differ significantly between the treatments (Figs. 4e and
251 5). The fDOM-5 components were considered to be tyrosine-like materials (Cory and
252 McKnight 2005; Yamashita et al. 2010). Both could be released during algal activity (Li et
253 al. 2016; Yamashita and Tanoue 2003), and were good substrates for bacteria (Wetzel
254 2001; Pérez and Sommaruga 2006). Moreover, bacteria also release the extracellular
255 polymeric substances (EPS) such as carbohydrates and proteins (Elliott et al. 2006;
256 Salama et al. 2015). Because none of the factors were significantly correlated with the FI

257 of fDOM-5 in the LM model (Table 2), it could be possible that fDOM-5 were not
258 produced or consumed during incubation. While, carbohydrate was significantly
259 correlated with diatom (Table 2), and positively correlated with bacteria in
260 diatom-treatment (Fig 6g). It could be possible that carbohydrates were consumed by
261 bacteria as soon as they were released from the diatoms.

262 Chytrids are ubiquitous in the aquatic ecosystem (Carney and Lane 2014) and play
263 an important role in food web and material cycling, as their zoospores are a good food
264 source for zooplankton (Kagami et al. 2006, 2007). Although the parasites associated with
265 algae have received much interest for their potential economic impact in commercial algal
266 production for biofuels, food, and pharmaceuticals, there is little information regarding
267 their role in artificial ecosystems, including drinking water treatment processes. From the
268 perspective of AOM, parasitic fungi in industrial and commercial water treatment
269 processes require further research.

270 In this study, changes in the DOM quantity and quality in diatom-bacteria cultures
271 with and without chytrids were indicated. We hypothesized that chytrids would affect
272 AOM in the three processes. From our results, the release of AOM seemed to be
273 stimulated by chytrids by 1) causing cell death of diatoms and 2) causing the elution of
274 algal cell content-derived DOM by inserting a rhizoid. AOM released would have

275 increased the growth of bacteria, and ultimately the concentrations of AOM and their
276 components would be altered by bacterial uptake or metabolism. In addition, chytrids
277 could directly take up algal cell contents, such as precursors of fDOM-3 and -4, and the
278 tryptophan-like components (process 3). Recent advancements in the EEM-PARAFAC
279 technique have enabled more rapid and simple analysis of DOM composition with a small
280 sample volume (e.g., Fellman et al. 2010). Many fluorescence characterizations of AOM
281 could be collected during the short incubation period, demonstrating that fungal infection
282 stimulated decreases in DOC and tryptophan-like substances and an increase in the
283 proportion of humic substances by bacterial processing. These results provide the first
284 evidence that parasitic fungi influence the composition and concentration of AOM.
285

286 **4. Conclusion**

287 Parasitic fungal infection decreased AOM concentration. This reason was that
288 abundant bacteria may have actively consumed certain AOM in the infected treatment.
289 The EEM-PARAFAC technique gives some of the first insights into the role of parasitic
290 fungi in aquatic DOM dynamics that fungal infection may modulate algal–bacterial
291 interactions, which are associated with changes in the nature of AOM. Fungal infection
292 stimulated the release of humic-like components, which bacteria processed algal

293 decaying-derived DOM. We also detected the interactions between fungi and bacteria, that
294 fungi chytrids may have consumed certain algal cell contents that acted as precursors of
295 humic-like components faster than bacteria. While, chytrid decreased the tryptophan-like
296 component via direct feeding algal substances.

297 Because fungal infection can cause massive lysis of dominant phytoplankton species,
298 fungal infections can have a considerable effect on AOM and consequently affect carbon
299 cycling. Therefore, both bacteria and fungi should be considered to better understand the
300 dynamics of DOM and carbon cycling in aquatic and artificial ecosystems.

301

302

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Figure captions

Fig. 1 The abundances of (a) diatoms, (b) chytrids and the percentage of infected cells, and (c) bacteria in the diatom and diatom-chytrid treatments.

Fig. 2 DOC concentrations in the diatom and diatom-chytrid treatments.

Fig. 3 Fluorescent DOM components identified using parallel factor analysis. Fluorescence intensity is indicated by the contour color. The identified fluorescent components were (a) fDOM-1, (b) fDOM-2, (c) fDOM-3, (d) fDOM-4, and (e) fDOM-5.

Fig. 4 Fluorescence intensities of (a) fDOM-1, (b) fDOM-2, (c) fDOM-3, (d) fDOM-4, and (e) fDOM-5 in the diatom and diatom-chytrid treatments.

Fig. 5 Carbohydrate concentrations in the diatom and diatom-chytrid treatments.

Fig. 6 Relationships between bacterial abundance and concentrations of (a) DOC, (b) fDOM-1, (c) fDOM-2, (d) fDOM-3, (e) fDOM-34 (f) fDOM-5, and (g) carbohydrates. The x-axis presents the bacterial abundance and the y-axis presents the concentration. The solid and dashed lines indicate the regression lines in the diatom and diatom-chytrid treatments, respectively.

Table 1. Excitation (Ex) and emission (Em) wavelengths and characteristics of the five fluorescent components in this study and from previous studies, as well as the characteristics of carbohydrates in this study.

Components	Ex (nm)	Em (nm)	Characteristics
fDOM-1	< 252 and 348	436	Microbial processing of algae-derived DOM (Stedmon and Markager 2005). Lower aromaticity and higher nitrogen content than terrigenous humic-like components (Stubbins et al. 2014). Aquatic humic-like component from bacterial processing of AOM. Chytrids do not affect the generation of fDOM-1 (this study).
fDOM -2	276	341	Tryptophan-like component (Cory and McKnight 2005; Yamashita et al. 2010). Consumed by bacteria after release from diatoms. Chytrids may consume this component directly (this study).
fDOM -3	< 252 and 312	387	Microbial precursor material with aliphatic carbon content (Cory and McKnight 2005). Heterotrophic microbial activity-derived DOM (Yamashita et al. 2010). Aquatic humic-like component from bacterial processing of AOM. Chytrids may consume algal cell contents, which are precursors of fDOM-3 (this study).
fDOM -4	261 and 384	515	Terrestrial humic-like or humic acid-like component (Cory and McKnight 2005; Santín et al. 2009; Yamashita et al. 2010). Plant-derived reduced quinones, enriched in aromatic carbon (Cory and McKnight 2005). Aquatic humic-like component from bacterial processing of AOM. Chytrids may consume algal cell contents, which are precursors of fDOM-4 (this study).
fDOM -5	270	299	Tyrosine-like component (Cory and McKnight 2005; Yamashita et al. 2010). Used rapidly by bacteria after release from algae (this study).
Carbohydrates			Used rapidly by bacteria after release from algae (this study).

Table 2. The best general linear models explaining the variation in DOC and DOM components.

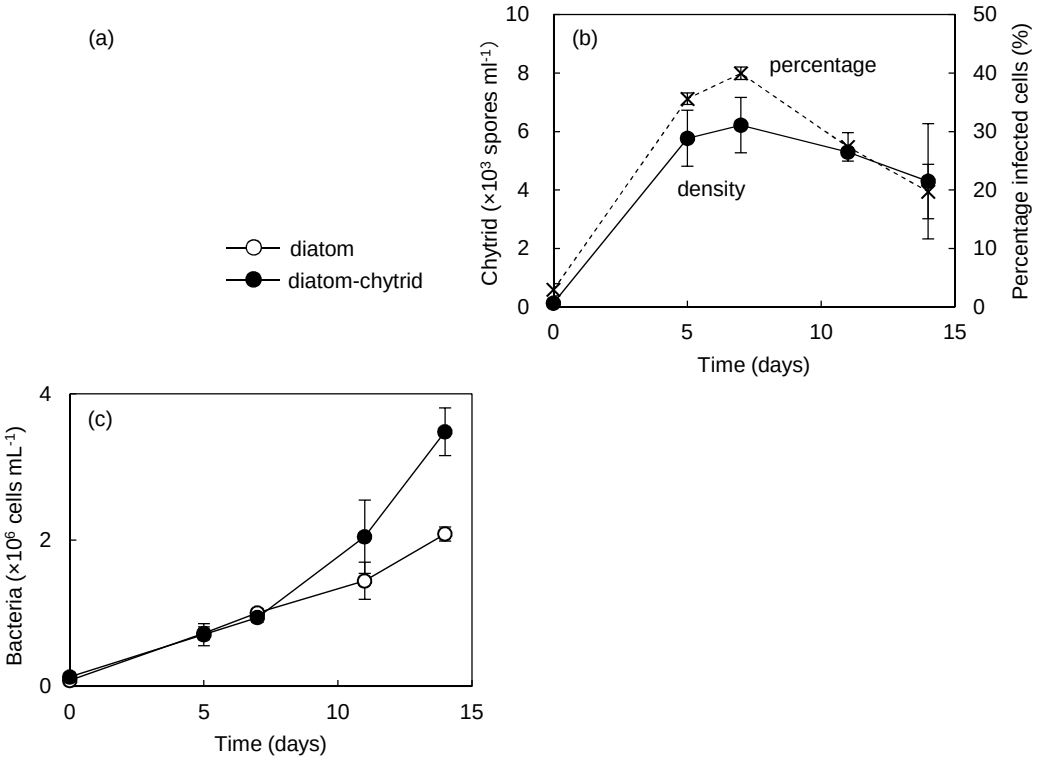
	Model	Diatom		Bacteria		Chytrid					
	Rank	Estimate	*p*	Estimate	*p*	Estimate	*p*	Adj. $R^{2\,a}$	*p*	AICc	ΔAIC
DOC	1	2.49×10^{-5}	**1.85×10^{-2}**	1.93×10^{-7}	9.13×10^{-2}	**-9.30×10^{-5}**	**1.26×10^{-2}**	0.55	**2.75×10^{-5}**	-47.54	
	2	3.64×10^{-5}	**6.03×10^{-5}**			-5.99×10^{-5}	5.78×10^{-2}	0.51	**2.40×10^{-5}**	-46.19	1.35
fDOM-1	1			5.03×10^{-7}	**$<2\times10^{-16}$**			0.94	**$<2.2\times10^{-16}$**	-119.07	
	2	1.27×10^{-6}	0.644	4.96×10^{-7}	**6.30×10^{-16}**			0.93	**$<2.2\times10^{-16}$**	-117.32	-1.75
	3			5.05×10^{-7}	**$<2\times10^{-16}$**	-1.30×10^{-6}	0.893	0.93	**$<2.2\times10^{-16}$**	-117.10	-1.97
-2	1	7.02×10^{-5}	**2.87×10^{-2}**	5.71×10^{-7}	0.102	-3.75×10^{-4}	**1.59×10^{-3}**	0.57	**1.20×10^{-5}**	19.61	
	2	1.04×10^{-4}	**1.32×10^{-4}**			-2.77×10^{-4}	**5.73×10^{-3}**	0.54	**9.10×10^{-6}**	20.76	1.15
-3	1	1.84×10^{-5}	**5.51×10^{-6}**	3.00×10^{-7}	**3.86×10^{-9}**			0.88	**1.04×10^{-13}**	-106.14	
	2	1.86×10^{-5}	**5.42×10^{-5}**	2.99×10^{-7}	**2.07×10^{-7}**	1.02×10^{-6}	0.940	0.88	**1.24×10^{-12}**	-104.15	-1.99
-4	1	9.51×10^{-6}	**7.14×10^{-3}**	2.44×10^{-7}	**3.62×10^{-7}**	-1.92×10^{-5}	0.104	0.83	**9.71×10^{-11}**	-114.24	
	2	1.23×10^{-5}	**2.91×10^{-6}**	2.11×10^{-7}	**3.13×10^{-7}**			0.82	**3.96×10^{-11}**	-113.14	1.10
-5	1	-1.11×10^{-5}	0.134					0.05	0.134	-49.24	0.00
	2*									-48.78	0.46
	3			-8.51×10^{-8}	0.293			0.01	0.293	-47.99	1.25
	4	-1.20×10^{-5}	0.123			-1.40×10^{-5}	0.639	0.02	0.296	-47.49	1.75
	5	-9.71×10^{-6}	0.275	-2.87×10^{-8}	0.762			0.01	0.316	-47.34	1.90
Carbohydrates	1	1.17×10^{-5}	**3.93×10^{-2}**					0.11	**3.93×10^{-2}**	-66.56	
	2	1.10×10^{-5}	0.103	1.35×10^{-8}	0.849			0.08	0.122	-64.60	1.96
	3	1.17×10^{-5}	**4.84×10^{-2}**			-1.49×10^{-7}	0.995	0.08	0.124	-64.56	2.00

Values in bold indicate a significant correlation ($p < 0.05$).

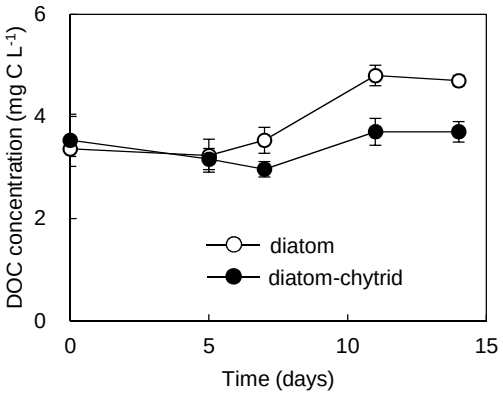
* Null model

[a] Adjusted (Adj.) R^2 is a modified version of the R^2 that has been adjusted for the number of terms in the model.

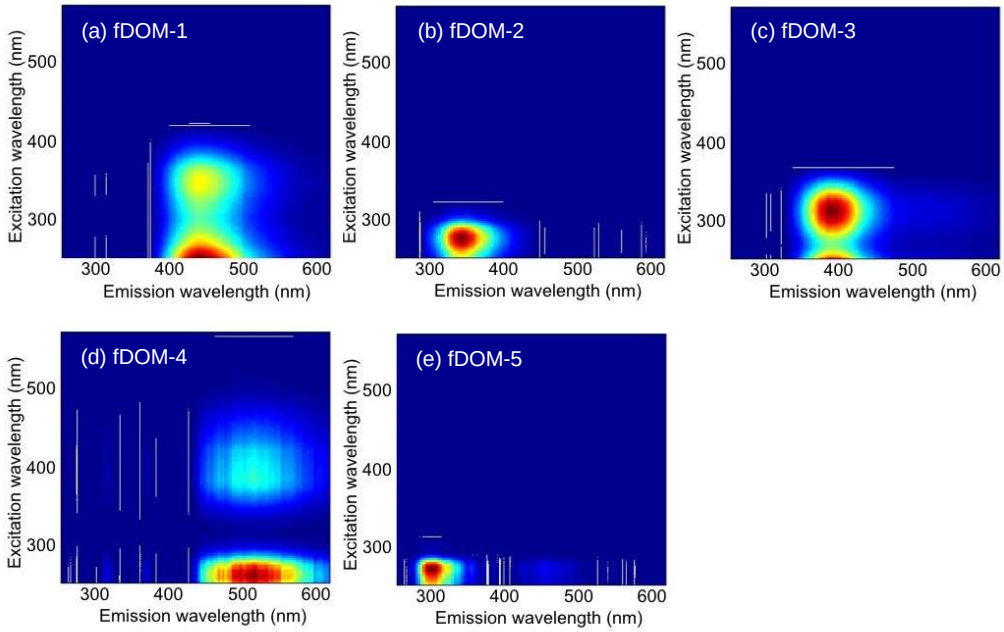
Senga et al., Fig. 1



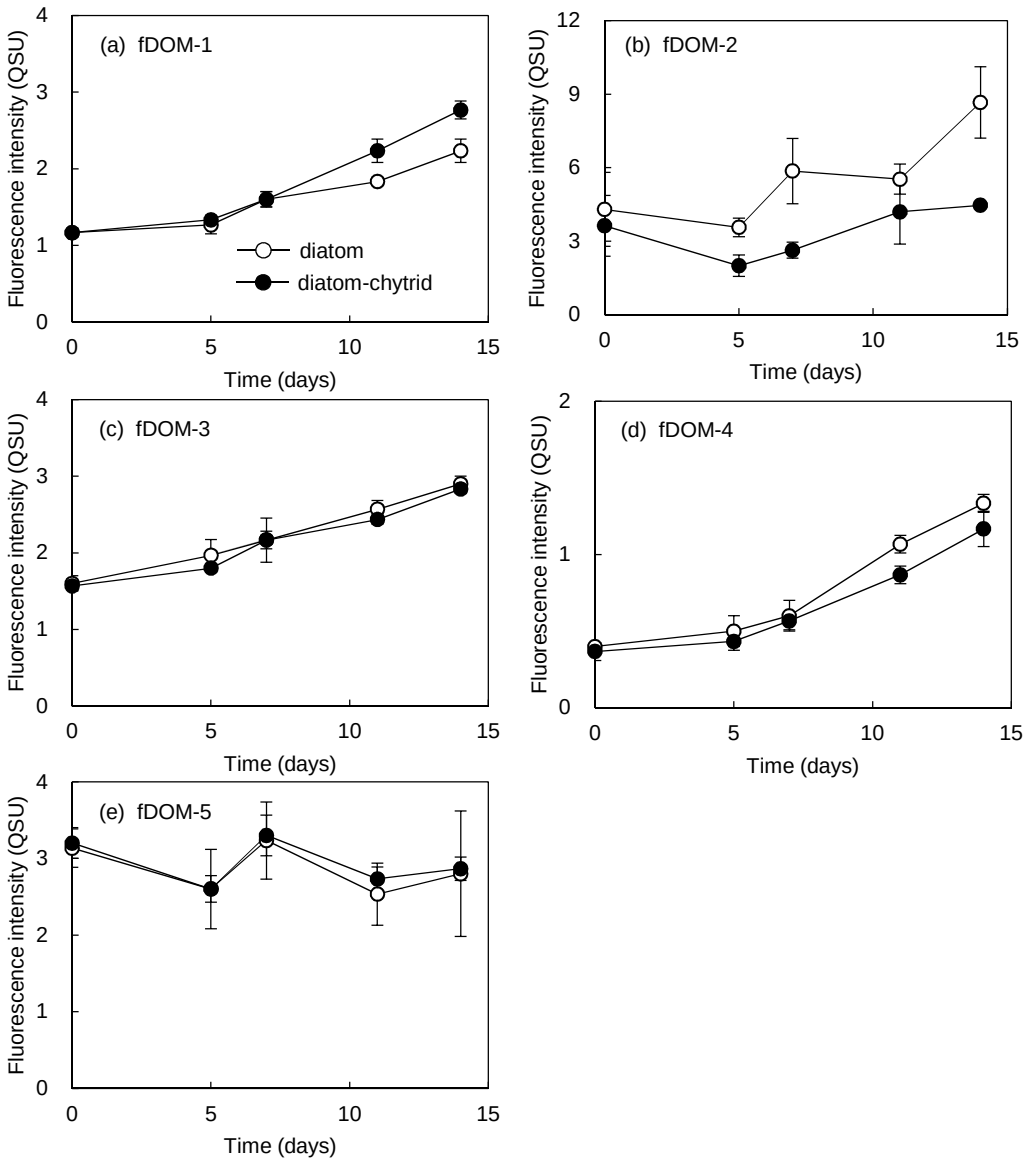
Senga et al., Fig. 2



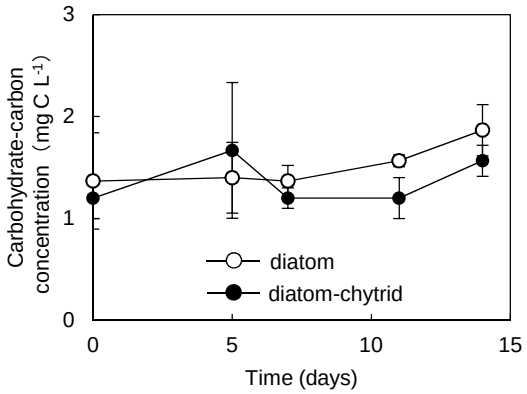
Senga et al., Fig. 3



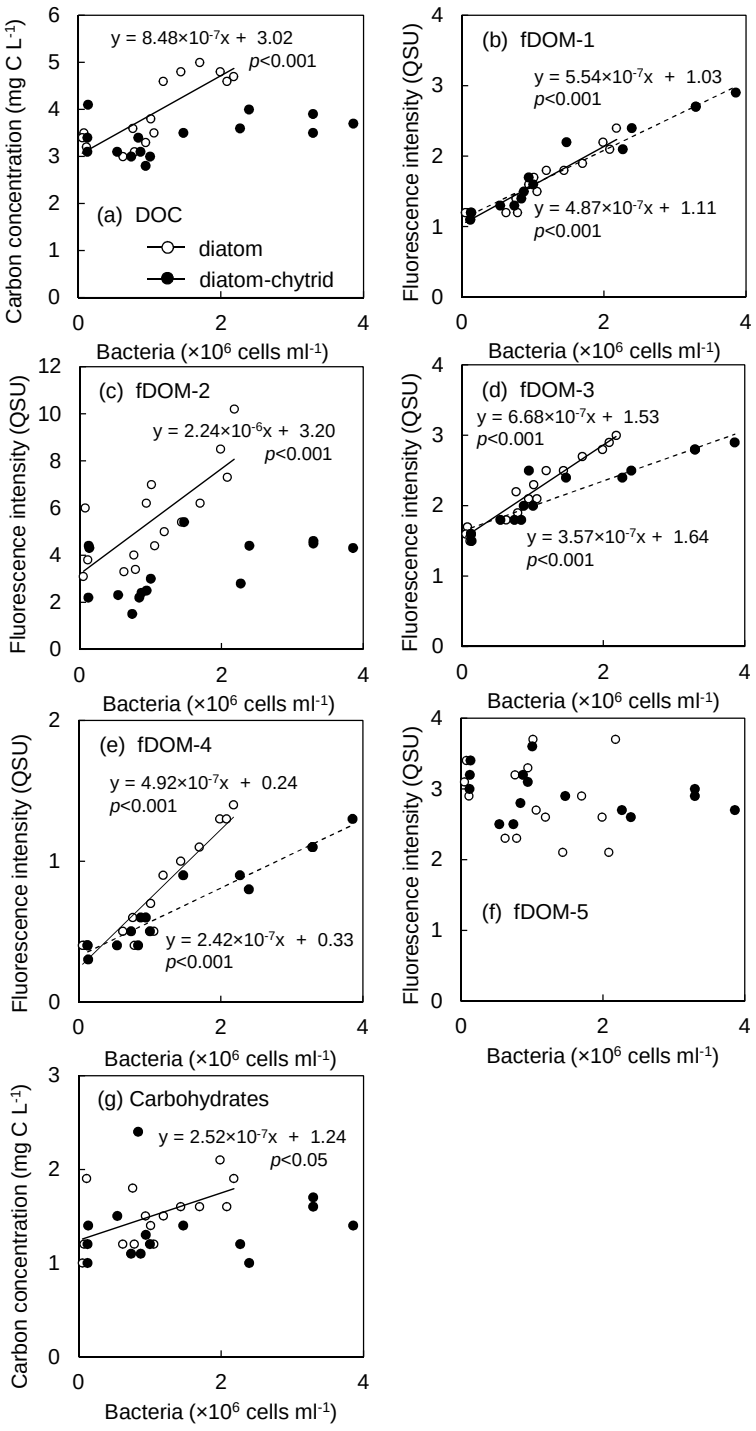
Senga et al., Fig. 4



Senga et al., Fig. 5



Senga et al., Fig. 6



Highlights

- Parasitic fungal infection in phytoplankton affected AOM.
- AOM concentration was lower by fungal infections.
- With EEM-PARAFAC, fungal infections increased humic-like components.
- Fungal infection decreased the tryptophan-like component.
- This study gives the first insights into the role of fungi in AOM dynamics.