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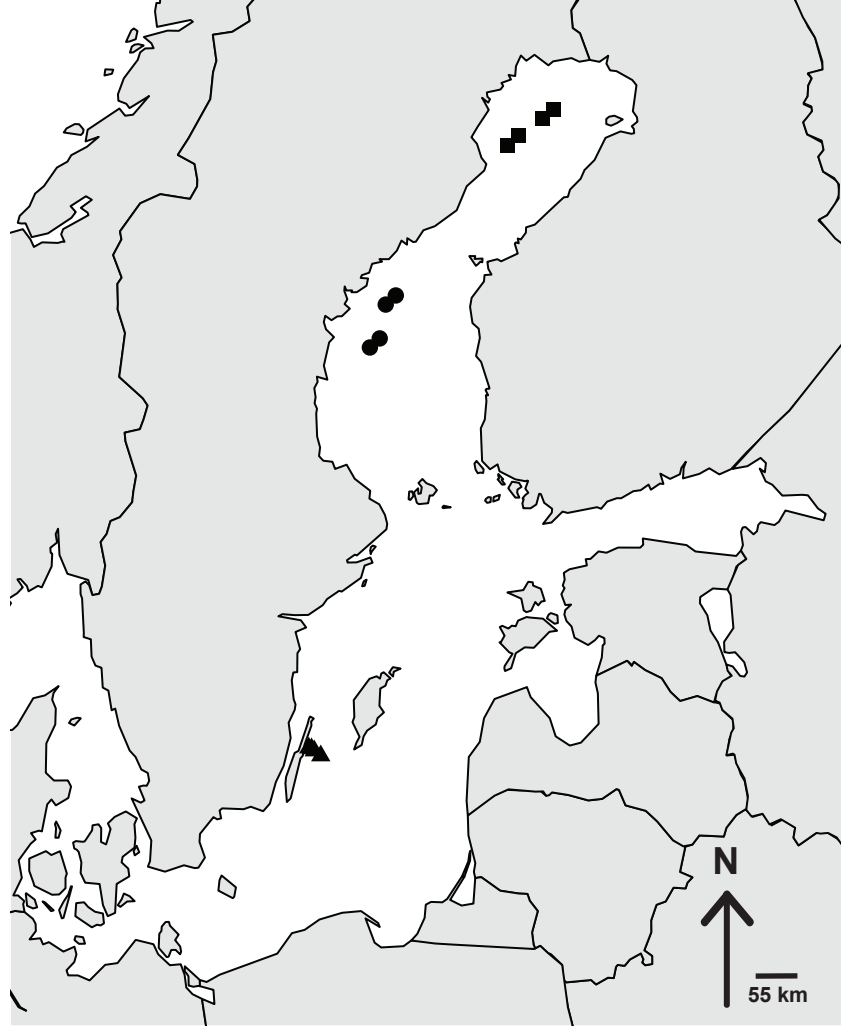
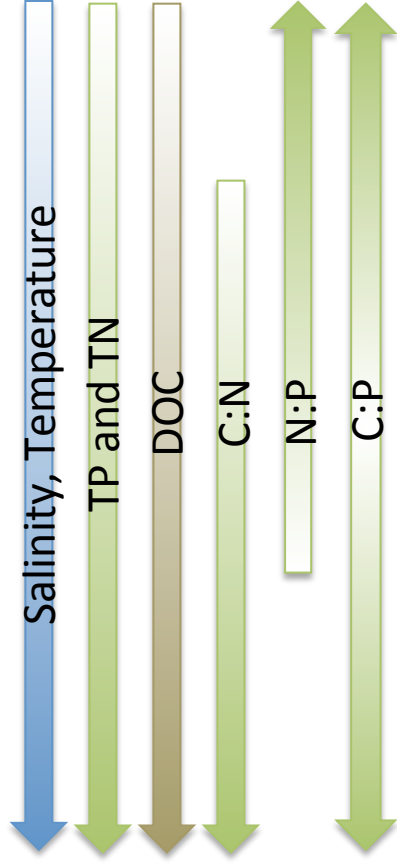
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## **Highlights**

1. Clear spatial differences were seen in DOM characteristics and bacterial response.
2. Bacterial growth and metabolic status have a dual role influencing the DOM pool.
3. Physicochemical and biological processes interact, influencing the carbon cycle.



1 **Major differences in dissolved organic matter characteristics and bacterial**  
2 **processing over an extensive brackish water gradient, the Baltic Sea**

3

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23 Running head: Brackish water bacteria-DOM interactions

24

25

26 **Abstract**

27 Dissolved organic matter (DOM) in marine waters is a complex mixture of  
28 compounds and elements that contribute substantially to the global carbon cycle. The  
29 large reservoir of dissolved organic carbon (DOC) represents a vital resource for  
30 heterotrophic bacteria. Bacteria can utilise, produce, recycle and transform  
31 components of the DOM pool, and the physicochemical characteristics of this pool  
32 can directly influence bacterial activity; with consequences for nutrient cycling and  
33 primary productivity. In the present study we explored bacterial transformation of  
34 naturally occurring DOM across an extensive brackish water gradient in the Baltic  
35 Sea. Highest DOC utilisation (indicated by decreased DOC concentration) was  
36 recorded in the more saline southerly region where waters are characterised by more  
37 autochthonous DOM. These sites expressed the lowest bacterial growth efficiency  
38 (BGE), whereas in northerly regions, characterised by higher terrestrial and  
39 allochthonous DOM, the DOC utilisation was low and BGE was highest. Bacterial  
40 processing of the DOM pool in the south resulted in larger molecular weight  
41 compounds and compounds associated with secondary terrestrial humic matter being  
42 degraded, and a processed DOM pool that was more aromatic in nature and  
43 contributed more strongly to water colour; while the opposite was true in the north.  
44 Nutrient concentration and stoichiometry and DOM characteristics affected bacterial  
45 activity, including metabolic status (BGE), which influenced DOM transformations.  
46 Our study highlights dramatic differences in DOM characteristics and microbial  
47 carbon cycling in sub-basins of the Baltic Sea. These findings are critical for our  
48 understanding of carbon and nutrient biogeochemistry, particularly in light of climate  
49 change scenarios.

50

51 Keywords: Dissolved organic matter, DOC utilization, DOM fluorescence, bacterial  
52 growth efficiency, bacterial production, Baltic Sea.

53

54 **Highlights**

55 1. Clear spatial differences were seen in DOM characteristics and bacterial response.

56 2. Bacterial growth and metabolic status have a dual role influencing the DOM pool.

57 3. Physicochemical and biological processes interact, influencing the carbon cycle.

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## 76 **1. Introduction**

77 The dissolved organic matter (DOM) pool is a complex mixture of molecules of  
78 disparate structure and of diverse origin. The DOM pool incorporates various forms  
79 of elements that are vital for microbial growth, such as: carbon (C), nitrogen (N) and  
80 phosphorus (P). In marine ecosystems the DOM pool, particularly the dissolved  
81 organic carbon (DOC) fraction, represents an important resource for heterotrophic  
82 bacteria (Ducklow et al., 1986; Sherr and Sherr, 1988). Bacteria are in turn  
83 fundamental for the recycling of key nutrients (Hansell and Carlson, 2002).

84

85 DOM in marine waters is in copious supply (Hedges, 1992; Benner and Amon, 2015).  
86 While DOM in open water marine systems is dominantly derived from autochthonous  
87 processes (i.e. phytoplankton primary production and related processes: Nagata,  
88 2000), allochthonous terrestrial organic matter can also be an important contributor to  
89 the DOM pool. This latter scenario can be especially pertinent in enclosed or coastal  
90 waters (Ask et al., 2009; Deutsch et al., 2012; Fleming-Lehtinen et al., 2015). The  
91 characteristics of the DOM pool are influenced by its origin (e.g. autochthonous,  
92 allochthonous, land use, catchment composition) and these attributes in turn control  
93 its bioavailability and fate. These factors influence its potential importance in the  
94 ecosystem (Asmala et al., 2013; Boyd and Osburn, 2004; Stedmon et al., 2003). The  
95 concentration and properties of the DOM pool can directly influence heterotrophic  
96 processes at the base of the food web. Supplementary DOC and allochthonous  
97 nutrients may enable bacteria to outcompete autotrophic primary producers (Fandino  
98 et al., 2001; Lignell et al., 2008; Sandberg et al., 2004; Smith et al., 1995).  
99 Furthermore, DOM can catalyse other concurrent changes, such as controlling the  
100 penetration of UV and visible solar radiation in the surface ocean (Dupont and

101 Aksnes, 2013; Nelson and Siegel, 2013). Thus, any modification of the DOM pool  
102 may result in changes in the balance of basal production (heterotrophic bacterial and  
103 autotrophic algal production) or changes in food web structure. The outcome of such  
104 changes have the potential to influence ecosystem function (Azam et al., 1983; Azam,  
105 1998; Sandberg et al., 2004; Hansson et al., 2013; Lefébure et al., 2013) and the  
106 global carbon cycle (Jiao et al., 2010).

107

108 Since only a limited portion of the DOC pool is available to bacteria (Hoikkala et al.,  
109 2015; Søndergaard and Middelboe, 1995) carbon limitation of bacterioplankton  
110 growth is common (e.g. Carlson and Ducklow 1996; Kirchman and Rich, 1997). To  
111 understand the fate of DOM in marine systems it is therefore important to combine  
112 bacterial utilisation studies with detailed characterisation of the prevailing DOM pool.  
113 By examining DOM absorbance and fluorescence properties it is possible to gain or  
114 infer some important quantitative (e.g. concentrations of chromophoric dissolved  
115 organic matter (CDOM) or humic substances) and qualitative insights, such as:  
116 estimates of molecular weight (Amon and Benner, 1996; Asmala et al., 2013; Wallin  
117 et al., 2015), aromaticity (Weishaar et al., 2003), and DOM origin (e.g. terrestrial,  
118 marine produced or catchment land use). Characteristics of the DOM pool have been  
119 linked to DOC concentration, the potential bioavailability of the DOM, bacterial  
120 growth efficiency (BGE), and biological breakdown and production processes  
121 (Asmala et al., 2013; Benner and Amon, 2015; Fichot and Benner, 2012; Trabelsi and  
122 Rassoulzadegan, 2011). Consequently, knowledge about the characteristics of the  
123 DOM pool, its bioavailability and the efficiency of bacterial utilisation (Asmala et al.,  
124 2013; Dinasquet et al., 2013; Figueroa et al., 2016) is critical for understanding  
125 ecosystem function (Sandberg et al., 2004) and carbon cycling (Bianchi et al., 2013;



126 Jiao et al., 2010). Obtaining such insights appears especially pertinent when  
127 considering climate change predictions (Andersson et al., 2015; Jiao et al., 2010),  
128 particularly those for enclosed water bodies such as the Baltic Sea (Andersson et al.,  
129 2015).

130

131 In this study we examined the bioavailability of DOC in open-sea waters of the three  
132 major basins of the Baltic Sea, and assessed the bacterial-DOM interactions ongoing.  
133 Environmental sampling was combined with DOC utilisation experiments at four  
134 stations in each basin. We explored the influence of DOC concentration and optical  
135 DOM characteristics on bacterial growth and DOC utilisation. We aimed to determine  
136 if: 1) spatial differences in DOC concentration and DOM characteristics occurred  
137 along this latitudinal gradient, 2) differences in DOM influenced the efficiency with  
138 which DOC was utilised, 3) nutrient limitation resulted in decreased DOC utilisation,  
139 and 4) altered DOC utilisation has potential consequences for the Baltic Sea carbon  
140 cycle. We discuss our findings in the context of wider ecosystem function, global  
141 elemental cycles and climate change.

142

## 143 **2. Materials and Methods**

144

145 **2.1. Study system and rationale.** The Baltic Sea is a semi-enclosed sea that is  
146 strongly influenced by an extensive catchment area. DOC concentrations in Baltic Sea  
147 open waters do not differ strongly between the three major basins (Hoikkala et al.,  
148 2015; Ripszam et al., 2015). However, the northern basins are highly influenced by  
149 river discharges of DOC-rich waters (Stepanauskas et al., 2002, Hoikkala et al., 2015,  
150 Fleming-Lehtinen et al., 2015; Reader et al., 2014; Raike et al., 2012), and the salinity

151 and N and P concentrations generally increase in a southerly direction (Andersson et  
152 al., 2015; Hoikkala et al., 2015). These factors are strong drivers of the ecological  
153 gradients that occur in the Baltic Sea.

154

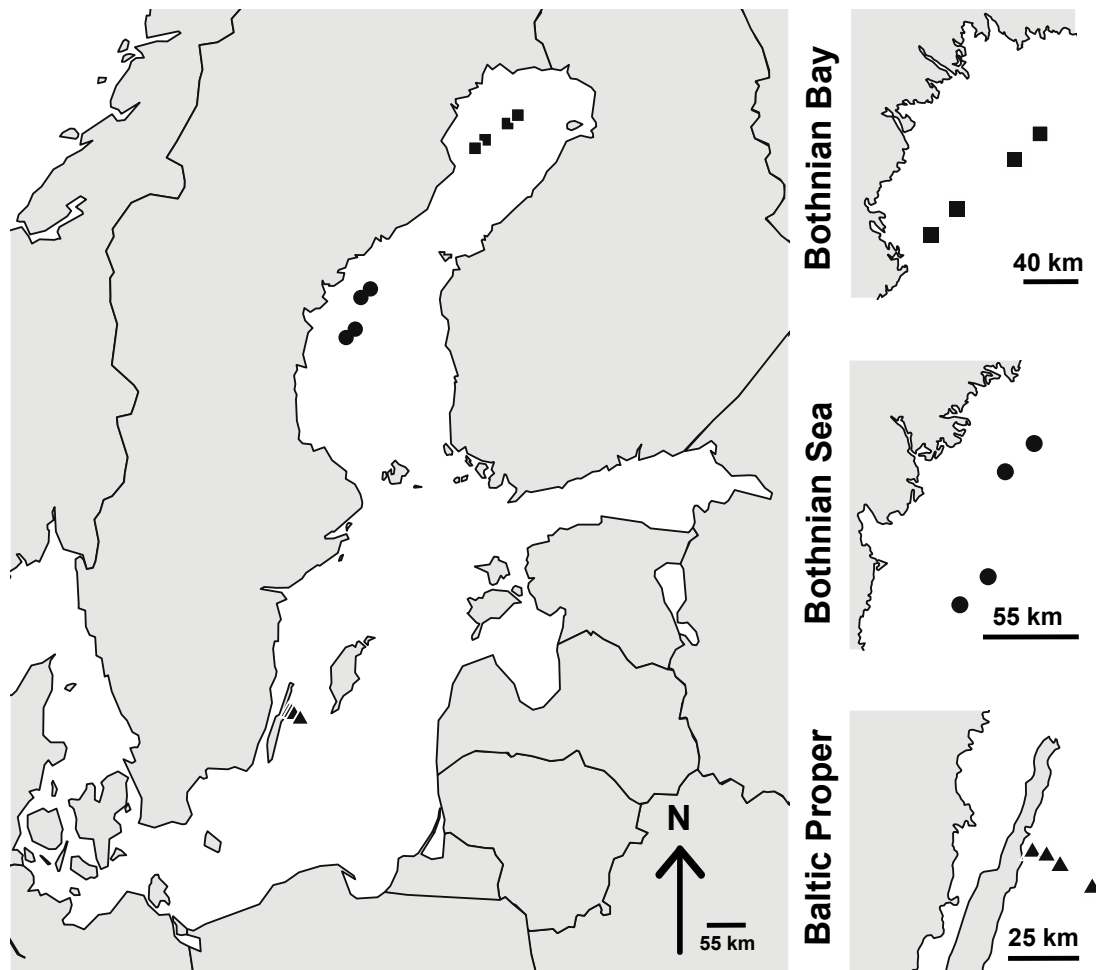
155 **2.2. Sampling and water collection (in-situ).** Sampling was carried out in July 2011  
156 at four stations in each of the three major basins of the Baltic Sea (Fig. 1). Two trips  
157 were made, one in the Baltic Proper (July 5<sup>th</sup>) and one in the Gulf of Bothnia  
158 (Bothnian Sea and Bothnian Bay, July 19<sup>th</sup> - 21<sup>st</sup>). Water was collected from a depth  
159 of 2 m using Niskin bottles and salinity, temperature, pH, total nitrogen (TN), total  
160 phosphorus (TP) and dissolved organic carbon (DOC) were measured, as described  
161 below.

162

163

164

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166

167 **Figure. 1.** Map of the Baltic Sea showing sampling locations of the four open sea  
 168 stations sampled in each of the three major basins (Baltic Proper, Bothnian Sea and  
 169 Bothnian Bay).

170

171 **2.3. Preparation of experimental study.** To remove larger organisms 10 L of water  
 172 was passed through a 0.45  $\mu\text{m}$  capsule filter (Millipak-40, Millipore) using gravity  
 173 filtration. The filter capsule was rinsed with  $\sim 1$  L of sample water prior to use, and  
 174 0.45  $\mu\text{m}$  filtrate was used to rinse the recipient acid-washed plastic carboy. The final 6  
 175 L of water passing through the 0.45  $\mu\text{m}$  filter was collected, from here onwards  
 176 referred to as ‘filtrate 1’. Circa 2 L of water was also gravity filtered through a pre-  
 177 combusted 47 mm GF/F filter, referred to as ‘filtrate 2’. This process was repeated for  
 178 each station using a fresh filter capsule and fresh acid washed containers on each

179 occasion, and the process was completed within ~4 hours. Filtration through a  
180 combusted GF/F filter has been shown to decrease bacterial numbers (Nayar and  
181 Chou, 2003) and this was observed in this study. For example bacterial numbers in  
182 microcosm start waters (a combination of filtrate 1 and 2) were 52 % (SD 7, n = 4)  
183 lower than the *in situ* waters of the Baltic Proper samples (not tested in other basins).  
184 It is possible that the filtration procedure removed larger members of the bacterial  
185 community, possibly altering the natural size distribution at the start of the  
186 experiment.

187

188 **2.4. Microcosm setup and sampling.** At each station six 1 L polycarbonate bottles  
189 (microcosm units) were filled with a combination of 900 mL of filtrate 1 and 100 mL  
190 of filtrate 2. Filtrate 1 and 2 waters were only combined for their respective stations.  
191 A filter-sterilised solution consisting of nitrate, ammonia and phosphate (additions of  
192 20  $\mu\text{M}$  N and 3  $\mu\text{M}$  P, in MilliQ water) was added to three of the microcosm units per  
193 station (+NP treatment) to preclude N or P limitation (as used similarly in Degerman  
194 et al., 2013). In standard microcosm units 200  $\mu\text{L}$  of filter sterile MilliQ water was  
195 added, a volume corresponding to the solution of nutrients added above. Microcosm  
196 units were run in triplicate for each station, making six microcosms per station (three  
197 standard and three +NP treatment), twenty-four microcosms per basin and a total of  
198 seventy-two microcosms units. Acid washed and sterile equipment was used for all  
199 filtration, storage, preparation, incubation and sampling stages.

200

201 Preparation of microcosms was completed within ~6 hours of initial water collection.  
202 All experimental units were immediately incubated in the dark and maintained at 15  
203 °C (Gulf of Bothnia) or 18 °C (Baltic Proper, Table 1). Experimental units were

204 sampled on day 0, 1, 3, 5 and 10 of incubation (removing circa 50 ml on each  
 205 occasion). The Day 0 sample, taken from initial bulk combinations of filtrate 1 and  
 206 filtrate 2 waters (i.e. mixture prior to addition to individual microcosm units), was a  
 207 single sample per station and used to represent the starting values for all treatments  
 208 (i.e. both standard and +NP treatments). Start and end concentrations of TN and TP  
 209 were measured using a Bran & Luebbe TRAACS 800 autoanalyser according to  
 210 Grasshoff *et al.* (1983), following the process described in Traving *et al.*, (2017). Due  
 211 to the nature of the field sampling during which the experiment was carried out, it was  
 212 not possible to monitor inorganic and organic nutrient concentrations. Start C (DOC),  
 213 N (TN) and P (TP) stoichiometric ratios were calculated.

214

215 **Table 1.** Mean values (standard deviation) of in-situ physicochemical variables (n = 4  
 216 independent stations per basin). Nutrient stoichiometry values represent waters from  
 217 standard microcosm at the start of the experiment, expressed as basin mean values (n  
 218 = 12).

	Temperature (°C)	pH	Salinity	DOC ( $\mu\text{mol C L}^{-1}$ )	TP ( $\mu\text{mol L}^{-1}$ )	TN ( $\mu\text{mol L}^{-1}$ )	C:N	N:P	C:P
Baltic Proper	17.6 (0.2)	8.5 (0.1)	6.8 (0.1)	708 (58)	0.21 (0.04)	16.55 (0.91)	31.6 (4.9)	16.6 (3.0)	527.0 (144.2)
Bothnian Sea	14.4 (0.2)	8.3 (0.1)	5.2 (0.1)	466 (42)	0.18 (0.03)	16.16 (1.14)	21.3 (3.1)	18.9 (2.7)	402.6 (78.5)
Bothnian Bay	15.5 (0.1)	8.1 (0.1)	2.8 (0.1)	416 (42)	0.08 (0.01)	13.28 (0.61)	23.1 (1.4)	33.8 (6.5)	780.7 (157.0)

219

220 The following variables were measured on every sampling day and in every  
221 experimental microcosm unit.

222

223 **2.5. Bacterial abundance and production.** Bacterial abundance (BA) samples (1.5  
224 mL) were taken in duplicate 2 mL cryovials, fixed with 0.2  $\mu\text{m}$  filtered glutaraldehyde  
225 (1% final concentration) and flash frozen in liquid nitrogen prior to storage at  $-80\text{ }^{\circ}\text{C}$ .  
226 Samples were stained with SybrGreen (Invitrogen) and cells were counted on a  
227 FACSCantoII flow cytometer (BD Biosciences), as previously described (Gasol and  
228 del Giorgio, 2000). Fluorescent beads (True count beads, Becton Dickinson) were  
229 used to calibrate the flow rate. Bacterial production (BP) was measured by  $[3\text{H}]$ -  
230 thymidine incorporation (Fuhrman & Azam 1982), as modified for  
231 microcentrifugation (Smith and Azam 1992). Triplicate 1.7 ml aliquots were  
232 incubated for 1 hour with  $[\text{methyl-}3\text{H}]$ -thymidine in sterile 2.0 ml capacity  
233 polypropylene tubes at in situ temperature. Saturation curves were used to determine  
234 suitable thymidine concentrations in the Baltic Proper and Gulf of Bothnia regions  
235 separately (20 and 24 nM final concentration, respectively, and a specific activity of  
236  $73.4\text{ Ci mmol}^{-1}$ ) and analysed with a Beckman 6500 scintillation counter. A single  
237 sample per microcosm, killed by adding 5% trichloroacetic acid prior to the addition of  
238 thymidine, served as a blank. Thymidine incorporation was converted to cell  
239 production using  $1.4 \times 10^{18}$  cells  $\text{mole}^{-1}$  (Wikner and Hagström 1999) and  $20.4\text{ fg C}$   
240  $\text{cell}^{-1}$  (Lee and Fuhrman, 1987) to estimate carbon biomass production.

241

242 **2.6. DOC concentration and DOM characteristics.** Duplicate 12 mL samples were  
243 filtered through pre-combusted GF/F filters into 15 ml acid washed polypropylene

244 tubes, acidified with 120  $\mu$ L of 2 M HCl, and stored at 4°C until analysis. DOC  
245 samples were analysed using high temperature catalytic oxidation (Shimadzu TOC-  
246 5000), as detailed in Traving et al., (2017). DOM fluorescence samples were prepared  
247 by collecting a single 40 mL sample that was filtered at low pressure through a pre-  
248 combusted GF/F filter into a 50 mL tube and immediately frozen (-20°C) until  
249 processing. It should be noted that freezing is not optimal as it may alter DOM  
250 fluorescence (e.g. Fellman et al., 2008), potentially in a random manner (Spencer et  
251 al., 2007). However the extensive gradient studied and field sampling carried out gave  
252 no viable alternative. Since all samples in the present study were treated identically  
253 we infer that the observed trends are valid for the direct comparisons carried out.  
254 Nevertheless, comparisons of specific values between this and other studies should be  
255 done with caution. Samples were acclimated to room temperature on a Horiba  
256 Aqualog spectrofluorometer (Horiba Scientific) in a 1 cm quartz cuvette. This  
257 instrument simultaneously measures absorption (from 240 nm to 600 nm) and  
258 fluorescence (at excitation and emission wavelengths 240 nm to 600 nm) at 3 nm  
259 intervals. Correction, calibration and calculation of informative variables were carried  
260 out (Asmala et al., 2013; Murphy et al., 2010; Stedmon et al., 2000). The following  
261 variables were extracted or calculated: 1. the ratio between  $a_{\text{CDOM}(254)}$  and  $a_{\text{CDOM}(365)}$   
262 (referred to as:  $a_{254}:a_{365}$ ), 2. a slope of the spectra for wavelengths 275-295 nm  
263 (slope coefficient,  $S_{275-295}$ ); both indicators of DOM molecular weight (Asmala et  
264 al., 2013; Fichot and Benner, 2012; Helms et al., 2008; Wallin et al., 2015), 3.  
265 absorbance at 440 nm ( $a_{\text{CDOM}(440)}$ ), referred to as chromophoric dissolved organic  
266 matter (CDOM) and indicative of water colour (Harvey et al., 2015), 4.  $\text{SUVA}_{254}$ ,  
267 indicative of DOM aromaticity (Ripszam et al., 2015; Weishaar et al., 2003), 5.  
268 fluorescence peak C (peak C, Ex/Em of 350/420-480 nm), a secondary humic peaks

269 associated with terrestrial origin (Cammack et al., 2004; Coble, 1996; Stedmon and  
270 Markager, 2005), 6. fluorescence peaks B (peak B, Ex/Em of 275/310 nm) and T  
271 (peak T, Ex/Em of 275/340 nm), protein-like peaks of similar structural composition  
272 to tyrosine and tryptophan, respectively (Coble, 1996), 7. fluorescence peaks A (peak  
273 A, Ex/Em of 260/380-460 nm) and M (peak M, Ex/Em of 312/380-420 nm), primary  
274 dissolved humic substances and marine humic associated compounds, respectively  
275 (Coble, 1996), and 8. the fluorescent peaks summed together as total humic-like or  
276 total amino-like peaks.

277

278 **2.7. DOC utilisation, BGE and fluctuation of variables.** Calculations of change  
279 (increase or decrease,  $\Delta$ ) were carried out between days 0 and 5 ( $\Delta_{0-5}$ ) and between  
280 days 0 and 10 ( $\Delta_{0-10}$ ), the latter being the full length of microcosm incubations. Trends  
281 were generally similar for both incubation time periods examined. However, only data  
282 for  $\Delta_{0-5}$  are presented as this represented the more active period of the incubation (see  
283 results). Variables for which  $\Delta$  data are calculated include: BA, DOC, a<sub>254</sub>:a<sub>365</sub>,  
284 S<sub>275-295</sub>, SUVA<sub>254</sub>, peak B, peak C, and peak T. Lastly,  $\Delta$ DOC (or DOC utilisation)  
285 was calculated between days 1 and 5 due to missing DOC data at some stations on  
286 day 0. Where DOC data was present on day 0 there was no marked decrease in DOC  
287 between days 0 and 1. Other calculations reliant on  $\Delta$ DOC (e.g. BGE) were also  
288 calculated using requisite data from the corresponding time period. BGE (%) was  
289 calculated as the integrated cumulative bacterial production during days 1-5 ( $BP_{cum1-5}$ )  
290 divided by the  $\Delta$ DOC between days 1 and 5 ( $\Delta DOC_{1-5}$ ), multiplied by 100 (Figueroa  
291 et al., 2016).

292



293 **2.8. Statistical analyses.** A Kendall-Tau correlation analysis was carried out on in-  
294 situ physicochemical data. A Principal component analysis (PCA) was performed to  
295 examine the similarity and separation of stations within and between the three  
296 different basins. No pre-processing of the data was undertaken. A one-way analysis of  
297 variance (ANOVA) with Tukey's HSD (honest significant differences) post hoc  
298 analysis was also carried out on in-situ data.

299

300 Cumulative bacterial production, BGE and  $\Delta$  data were analysed with a two-way  
301 ANOVA to examine the effects of basin and treatment (+/- NP), and any interaction  
302 between these.

303

304 A Kendall-Tau correlation analysis was performed on the raw data from the  
305 experimental microcosms. All variables measured, on all sampling days, in all  
306 treatments, and from all stations were included. Missing data values (3.5% of all data  
307 values) were imputed as means of replicates. A repeated measures-multivariate  
308 analysis of variance (RM-MANOVA) was performed to examine significant changes  
309 over the duration of the experiment and the influence of treatment and basin. Data  
310 used in the RM-MANOVA analysis did not conform to normality and did not  
311 improve with transformation, however these methods have been shown to be resilient  
312 to violations in normality (Finch, 2005) and have been successfully applied elsewhere  
313 (e.g. Ferrari et al., 2014). A PCA analysis was carried out on the above variables from  
314 standard microcosm data only (i.e. +NP microcosms excluded).

315

316 To explore drivers of specific changes or trends recorded, correlations were carried  
317 out between a selected experimental variables, cumulative data (e.g. cumulative BP),

318 nutrient stoichiometric ratios (e.g. C:N or C:P), and  $\Delta$  data (e.g. BA or BGE). Some  
319 data were normalised (0-1 scale) and others were transformed (ln). In all cases where  
320 such transformations were applied it is defined where the results are presented.

321

322 Statistical analyses and figure production were mainly performed in R Core Team  
323 (2013) using the packages: Rcmdr, prcomp, ggplot2, maps, mapdata and ggbiplot.

324 The RM-MANOVA was performed in SPSS (IBM SPSS Statistics software version  
325 22.0.0.0).

326

### 327 **3. Results**

328 **3.1. Station similarity and basin differentiation.** In-situ physicochemical variables  
329 indicated lower nutrient concentrations (TN and TP), salinity, pH and DOC in the  
330 northerly reaches of the Baltic Sea (the Bothnian Bay), as compared to more southerly  
331 stations (Table 1). Surface water temperature was also lower in the Gulf of Bothnia as  
332 compared to the Baltic Proper. However, during our specific sampling program  
333 temperature was higher in the Bothnian Bay, than the Bothnian Sea. Strong and  
334 significant ( $p < 0.0001$ ) correlations were found between salinity and TP ( $r = 0.7404$ ),  
335 salinity and pH ( $r = 0.8722$ ), TN and TP ( $r = 0.7176$ ), and TP and pH ( $r = 0.7837$ ).

336 The stations within each basin clustered together closely in the PCA analysis, and  
337 clear separation between the three basins was observed (Fig. S1). The global ANOVA  
338 indicated significant differences between the three basins for most in-situ  
339 physicochemical variables measured (Table S1). Stations are thus considered as  
340 replicates within each basin during analysis of the microcosm study.

341

342 **3.1.1. Initial conditions.** Clear variation in optical DOM characteristic variables were  
343 observed between basins at the start of the microcosm incubation. The a<sub>254</sub>:a<sub>365</sub>  
344 ratio was higher in the Baltic Proper and decreased in a northerly direction. SUVA<sub>254</sub>  
345 and CDOM showed the opposite trend, being highest in the Bothnian Bay (Fig. S2).  
346 Values for peak B, peak C, and peak T were generally higher in the Bothnian Bay or  
347 similar across all basins at the start of the incubations (Fig. S3).

348

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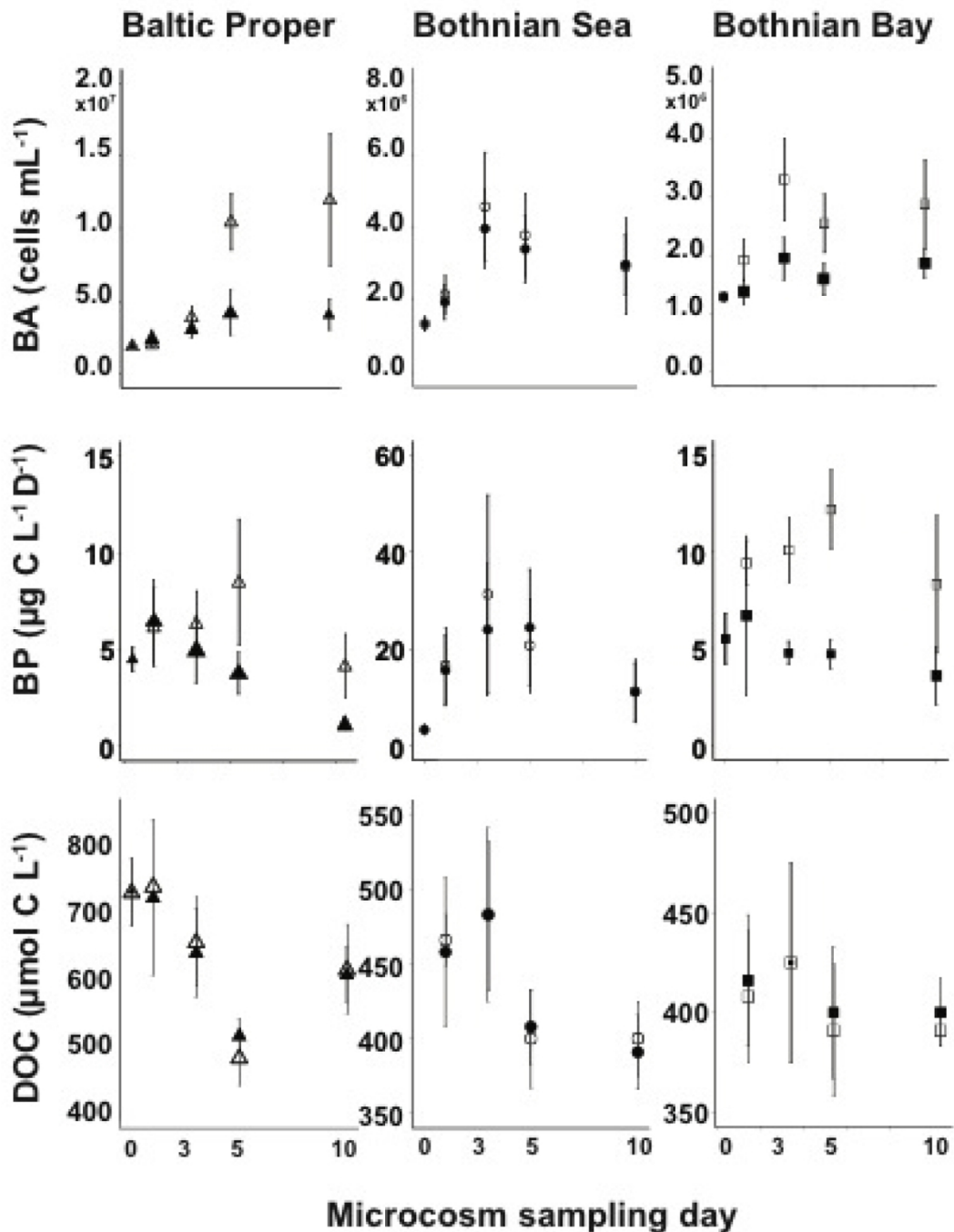
350 **3.2. DOC utilisation, bacterial abundance and bacterial production.** DOC was  
351 utilised and decreased particularly between days 1 and 5 of the incubation. Mean  
352 decreases in DOC were 233  $\mu\text{mol L}^{-1}$ , 58  $\mu\text{mol L}^{-1}$  and 17  $\mu\text{mol L}^{-1}$  (by day 5) in the  
353 Baltic Proper, Bothnian Sea and Bothnian Bay microcosms, respectively (Fig. 2).

354

355 Initial BA and BP rates were similar in all microcosms, however the trends during  
356 incubation differed with basin (Fig. 2). These spatial differences (basin effects) were  
357 significant for most variables, including over the course of the incubation period  
358 (Table 2). BP and BA generally peaked during days 1-5 of the incubation period,  
359 although the correlation between the two measured variables was generally poor. The  
360 highest BA values were recorded in the Baltic Proper microcosms whereas the highest  
361 rates of BP occurred in Bothnian Sea microcosms (Fig. 2). Between days 5 and 10,  
362 BP rates (and BA) generally decreased or plateaued. The initial period of high BA and  
363 BP rates (days 0-5) corresponded with the phase during which DOC decreased. Lower  
364 rates of BP by day 10 coincided with a general increase in DOC at this stage (Fig. 2).

365

366



367  
 368 **Figure 2.** Temporal trends in mean values for bacterial abundance (BA), bacterial  
 369 production (BP) and dissolved organic carbon (DOC) in microcosm experiments.  
 370 Data are presented by basin (Baltic Proper, triangles; Bothnian Sea, circles; and  
 371 Bothnian Bay, squares), with standard (filled symbols) and +NP treatments (open  
 372 symbols) shown. Standard deviation is indicated by error bars where  $n = 12$ . **Note**  
 373 **axis scales are not identical and vary between basins for a single variable.**

374

375 **Table 2.** Between and within subject contrasts from RM-MANOVA carried out on376 microcosm experiment. Statistically significant ( $p < 0.05$ ) are indicated by bold text.

377

	<b>Time</b>		<b>Basin</b>		<b>Treatment</b>		<b>Time</b>		<b>*Basin</b>		<b>Basin</b>		<b>*Treatment</b>		<b>Time</b>		<b>*Treatment</b>	
<b>df</b>	<b>1</b>		<b>2</b>		<b>1</b>		<b>2</b>		<b>2</b>		<b>1</b>		<b>2</b>		<b>1</b>		<b>2</b>	
<b>Variable</b>	<i>F</i>	p	<i>F</i>	p	<i>F</i>	p	<i>F</i>	p	<i>F</i>	p	<i>F</i>	p	<i>F</i>	p	<i>F</i>	p	<i>F</i>	p
<b>BP</b>	24.04	<b>&lt;0.001</b>	47.26	<b>&lt;0.001</b>	4.96	<b>0.029</b>	35.09	<b>&lt;0.001</b>	0.89	0.416	6.60	<b>0.012</b>	4.43	0.160				
<b>BA</b>	340.27	<b>&lt;0.001</b>	142.06	<b>&lt;0.001</b>	105.64	<b>0.0001</b>	112.73	<b>&lt;0.001</b>	39.84	<b>&lt;0.001</b>	86.92	<b>&lt;0.001</b>	66.22	<b>&lt;0.001</b>				
<b>DOC</b>	230.79	<b>&lt;0.001</b>	481.67	<b>&lt;0.001</b>	0.16	0.691	57.67	<b>&lt;0.001</b>	0.38	0.687	0.38	0.538	0.13	0.877				
<b>a254:365</b>	0.02	0.963	154.07	<b>&lt;0.001</b>	0.34	0.563	0.42	0.660	0.05	0.954	0.92	0.763	0.91	0.409				
<b>SUVA<sub>254</sub></b>	42.90	<b>&lt;0.001</b>	3569.4	<b>&lt;0.001</b>	0.027	0.869	37.79	<b>&lt;0.001</b>	0.02	0.980	0.05	0.832	0.26	0.773				
<b>peak B</b>	0.17	0.681	91.17	<b>&lt;0.001</b>	3.96	0.051	5.71	<b>0.005</b>	2.20	0.118	0.36	0.553	0.44	0.645				
<b>peak C</b>	75.01	<b>&lt;0.001</b>	598.51	<b>&lt;0.001</b>	0.07	0.787	30.58	<b>0.003</b>	0.04	0.960	0.01	0.929	0.62	0.539				
<b>peak T</b>	8.03	<b>0.006</b>	2.34	0.104	0.22	0.642	6.54	<b>&lt;0.00</b>	5.81	<b>0.005</b>	0.25	0.620	1.00	0.374				

378

379 **3.2.1. BGE and relative DOC utilisation.** Since BP rates and BA were generally  
 380 highest during the first five days of microcosm incubation (and declined between days  
 381 5-10) we present BGE for this active part of the experiment (i.e. till day 5). Relative  
 382 DOC utilisation was highest in the Baltic Proper (~30 % utilised by day 5) and  
 383 decreased in a northerly direction, ~15 % utilisation in the Bothnian Sea and <5 %  
 384 utilisation in the Bothnian Bay (Table 3). Conversely, BGE showed a clear increase in  
 385 a northerly direction with values of ~1.5, 16 and 26 % for the Baltic Proper, Bothnian  
 386 Sea and Bothnian Bay, respectively (Table 3).

387

388 **Table 3.** Mean relative change ( $\Delta$ , %) during the active phase of incubation (standard  
 389 error). DOC utilisation ( $\Delta$ DOC), cumulative bacterial production ( $BP_{cum}$ ,  $\mu\text{g C L}^{-1}$ )  
 390 and bacterial growth efficiency (BGE) between days 1 and 5. For all values  $n = 7-12$ .

Basin	Baltic Proper (BP)		Bothnian Sea		Bothnian Bay	
	-NP	+NP	-NP	+NP	-NP	+NP
BGE <sub>1-5</sub>	1.4 (0.4)	1.6 (0.4)	16.9 (6.6)	16.0 (4.1)	20.8 (6.7)	30.8 (4.2)
BP <sub>cum 1-5</sub>	25.4 (2.4)	32.7 (2.4)	97.5 (2.7)	109.9 (17.1)	27.1 (1.8)	49.2 (1.1)
$\Delta$ BA <sub>0-5</sub>	118.4 (19.9)	444.1 (20.6)	150.6 (13.2)	177.5 (16.6)	18.3 (6.7)	88.6 (11.0)
$\Delta$ DOC <sub>1-5</sub>	-27.6 (3.9)	-33.1 (2.3)	-10.7 (3.9)	-14.2 (2.1)	-3.9 (1.2)	-3.8 (0.5)
$\Delta$ a254:a365 <sub>0-5</sub>	8.1 (14.8)	12.5 (15.5)	-2.1 (2.1)	-2.7 (2.1)	-6.9 (3.6)	-4.1 (4.3)
$\Delta$ S275:295 <sub>0-5</sub>	4.2	4.6	-5.5	-5.5	-4.3	-4.2

	(2.9)	(3.0)	(0.6)	(0.6)	(1.6)	(2.0)
$\Delta$ SUVA <sub>0-5</sub>	44.1 (3.8)	53.9 (3.9)	2.4 (1.0)	3.8 (2.1)	-1.7 (2.4)	-2.9 (2.7)
$\Delta$ peak B <sub>0-5</sub>	1.2 (1.7)	8.2 (2.0)	25.3 (10.2)	11.3 (7.6)	-4.2 (8.7)	-7.7 (9.7)
$\Delta$ peak C <sub>0-5</sub>	-4.2 (0.9)	-2.0 (0.8)	8.0 (1.1)	8.1 (1.3)	11.39 (0.8)	10.0 (0.6)
$\Delta$ peak T <sub>0-5</sub>	-7.0 (1.6)	8.8 (1.0)	1.6 (6.7)	-2.7 (5.9)	-16.4 (6.5)	-16.3 (7.1)

391

### 392 **3.3 Changes in TN and TP**

393 As expected, TN and TP concentrations were elevated in the +NP treatment.

394 However, over the duration of the experiment no marked changes in microcosm TN  
395 and TP concentrations were observed in either the standard or +NP treatments (Table  
396 S2).

397

398 **3.3.1. Effect of nutrient addition, +NP.** In general nutrient addition increased BA  
399 and BP rate in the Baltic Proper and Bothnian Bay microcosms, as compared to their  
400 respective standard microcosms. However, this effect was only seen in the latter  
401 stages of the overall incubation period (Fig. 2), increasing the respective integrated  
402 cumulative BP (BP<sub>cum</sub>) value (Table 3). No such effect was seen in the Bothnian Sea  
403 microcosms (Fig. 2 and Table 3). Nutrient addition slightly increased the mean  
404 percentage of DOC utilised (~3-5 %) in more southerly basins, although no effect on  
405 DOC utilisation was seen in the Bothnian Bay microcosms (Table 3). Only in the  
406 Bothnian Bay did changes due to nutrient addition translate into increased mean BGE

407 (Table 3). Addition of nutrients had little impact on the optical DOM characteristic  
408 variables measured (Table 3). With the exception of changes in BA and BP, changes  
409 due to the addition of nutrients were not significant (Table 2).

410

411 **3.4 Trends and associations during incubation.** Certain variables in the raw data  
412 were strongly and significantly correlated and therefore removed from the RM-  
413 ANOVA analysis to prevent biasing the result. The variables retained include: BP,  
414 BA, DOC, a254:a365, SUVA<sub>254</sub>, peak B, peak C, and peak T. With the exception of  
415 SUVA<sub>254</sub>-DOC ( $r = -0.68$ ) and SUVA<sub>254</sub>-a254:a365 ( $r = -0.61$ ), correlations between  
416 the retained variables was relatively low ( $r = < +/-0.55$ ).

417

418 During incubation the response of DOM characteristics differed between basins. The  
419 Bothnian Bay exhibited relatively higher levels of peak C than the other two basins at  
420 the start and while it remained relatively constant in the Baltic Proper incubations it  
421 increased markedly during the active phase of incubation (up to day 5) in the  
422 Bothnian Sea and the Bothnian Bay microcosms (Fig. S3). Fluorescence peaks B and  
423 T fluctuated during the incubation period but clear trends were not present (Fig. S3).  
424 The a254:a365 ratio and S275-295 were highest in the southern basin and lowest in  
425 the northern most basin with a minor increase recorded during incubations from the  
426 Baltic Proper and a minor decrease observed during incubation in the northern basin  
427 incubations (Fig. S2). SUVA<sub>254</sub> values increased during the active phase of the  
428 microcosm incubation in the Baltic Proper, however decreased during this phase in  
429 the more northerly basins. A similar trend was observed with CDOM, except for the  
430 Bothnian Sea microcosms in which it fluctuated and appeared to increase, rather than



431 decrease, during the same phase (Fig. S2). Changes over time in the incubations were  
432 significant for the majority of variables (Table 2).

433

434 S275-295 correlated spatially with CDOM, with higher CDOM values corresponding  
435 to lower S275-295 values. The same trend was seen during the microcosm experiment  
436 within each individual basin, suggesting that changes in CDOM during incubation  
437 also correlated with changes in S275-295 ( $n = 54-60$ ;  $R^2 = 0.64, 0.66$  and  $0.74$  for  
438 Baltic Proper, Bothnian Sea and Bothnian Bay, respectively). A similar spatial  
439 correlation was seen between  $\ln\text{DOC}$  concentration and  $\ln\text{SUVA}_{254}$  values ( $\text{ALL}, n =$   
440  $131, R^2 = 0.79, p = <0.001$ ), however, the correlation only remained substantial in the  
441 Baltic Proper when exploring this trend for microcosm units in each separate basin ( $n$   
442  $= 47-51$ ;  $R^2 = 0.68, 0.39$  and  $0.22$  for Baltic Proper, Bothnian Sea and Bothnian Bay,  
443 respectively).

444

#### 445 **3.4.1. Relative changes (relative $\Delta$ values, %, till day 5) in measured variables.**

446 During the active part of the experiment (i.e. till day 5), relative increases in  
447  $\text{SUVA}_{254}$ , S275-295 and a254:a365 were recorded in the Baltic Proper microcosms.  
448 Marginal relative increases or relative decreases were recorded in the Bothnian Sea  
449 microcosms, and relative decreases in the Bothnian Bay microcosms (Table 3).  
450 Relative decreases in peak B and peak T were strongest in the Bothnian Bay  
451 microcosms, while a relative increase in peak C was detected in the Bothnian Bay and  
452 Bothnian Sea compared to a relative decreased in the Baltic Proper (Table 3).  
453 Changes ( $\Delta$  %) were generally significantly different between basins (Table S3).

454

455 **3.4.2. Significance and interaction (time-basin-treatment).** The RM-MANOVA  
456 indicated that basin, treatment and time all contributed to significant differences in the  
457 experimental microcosms (Time\*Basin\*Treatment:  $F_{64,72} = 4.678$ ,  $p = <0.001$ ).  
458 However, the effects of time, basin and time\*basin exhibited higher  $F$  values and  
459 were more significant than any treatment effects (i.e. addition of nutrients, +NP).  
460 Treatment effects (and interactions) were generally only significant for BA (Table 2),  
461 indicating that time (i.e. changes during microcosm incubation) and basin (i.e. origin  
462 of water used in experimental microcosms) were stronger drivers of the significant  
463 differences seen. Mean differences of individual variables between basins and their  
464 significance (post hoc Bonferroni tests) are shown in Table S4.

465

466 **3.4.3. Associations between measured variables.** Since the addition of nutrients had  
467 a limited effect, the following data only encompass the standard microcosm  
468 incubations (without nutrient addition). Other correlations are shown in  
469 supplementary results.

470

471 Higher starting DOC concentrations correlated with higher  $\Delta$ DOC values (DOC  
472 utilisation) during the active phase of the microcosm incubation (DOC v  $\Delta$ DOC<sub>1-5</sub>,  $n$   
473 = 28,  $R^2 = 0.91$ ,  $p = <0.001$ ) and with lower BGE (BGE<sub>1-5</sub> v DOC,  $n = 23$ ,  $R^2 = 0.79$ ,  $p$   
474 =  $<0.001$ ). However, high DOC utilization correlated with low BGE (BGE<sub>1-5</sub> v  
475  $\Delta$ DOC<sub>1-5</sub>,  $n = 52$ ,  $R^2 = 0.78$ ,  $p = <0.001$ ).

476

477 Nutrient concentrations and nutrient stoichiometry at the start of the incubations  
478 varied between basins (Table 1). Higher starting concentrations of TN and TP  
479 corresponded with larger increases in BA during the active incubation period (TN v

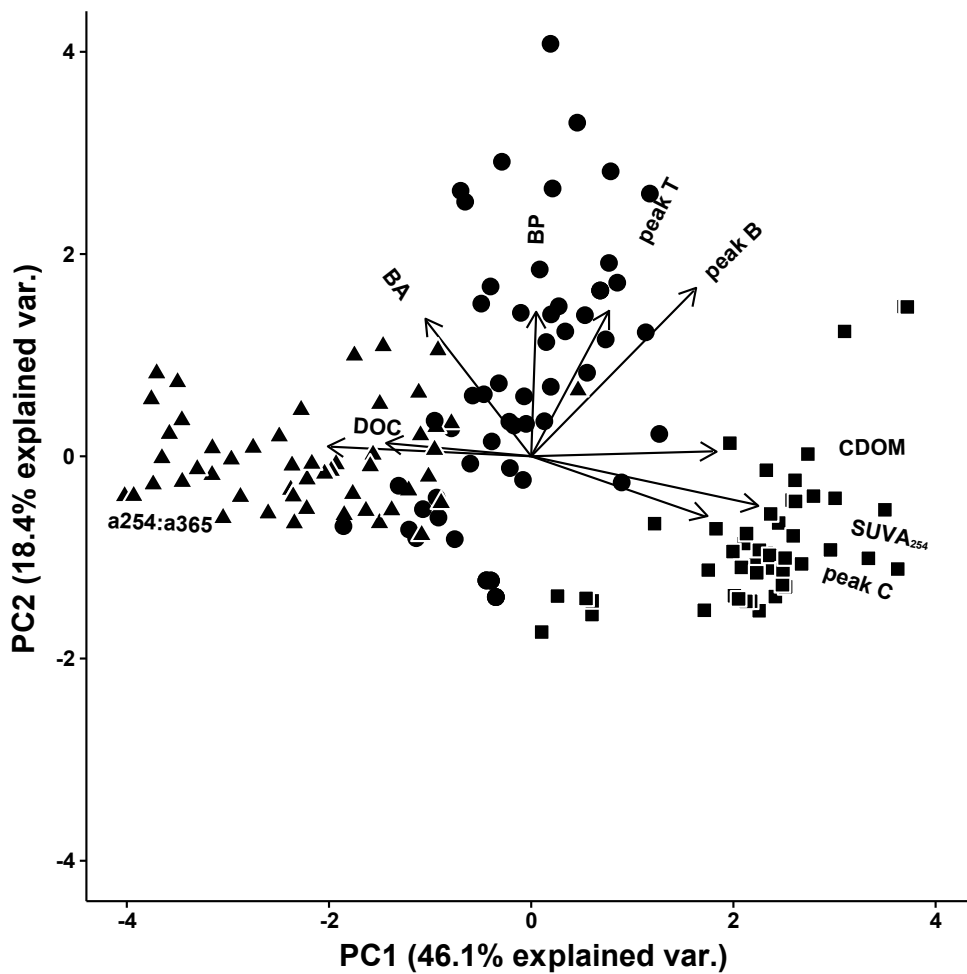
480  $\Delta\text{BA}_{0-5}$ ,  $n = 33$ ,  $R^2 = 0.65$ ,  $p = <0.001$  and  $\text{TP} \nu \Delta\text{BA}_{0-5}$ ,  $n = 33$ ,  $R^2 = 0.64$ ,  $p =$   
481  $<0.001$ ). Lower starting C:N ratios had a positive effect on BGE ( $\ln\text{C:N} \nu \ln\text{BGE}_{1-5}$ ,  $n$   
482  $= 23$ ,  $R^2 = 0.71$ ,  $p = <0.001$ ), with the highest BGE recorded at C:N ratios of  $\sim 23$ .  
483 However, at higher C:N ratios DOC utilisation was larger ( $\text{C:N} \nu \Delta\text{DOC}_{0-5}$ ,  $n = 28$ ,  $R^2$   
484  $= 0.76$ ,  $p = <0.001$ ).

485

486 Microcosm units exhibiting low BGE values exhibited larger relative increases in  
487  $\text{SUVA}_{254}$  ( $\ln\text{BGE}_{1-5} \nu \ln \text{normalised } \% \Delta\text{SUVA}_{0-5}$ ,  $n = 23$ ,  $R^2 = 0.47$ ,  $p = <0.001$ ),  
488 while those exhibiting higher BGE showed smaller increases in  $\text{SUVA}_{254}$  or even  
489 decreases. The opposite trend was observed for peak C, with relative decreases in  
490 peak C at lower BGE values ( $\ln\text{BGE}_{1-5} \nu \ln \text{normalised } \% \Delta\text{peak C}_{0-5}$ ,  $n = 23$ ,  $R^2 =$   
491  $0.63$ ,  $p = <0.001$ ). Furthermore, with higher starting DOC concentrations the  
492 production of peak C was lesser, and at the higher end of DOC concentrations peak C  
493 decreased ( $\text{DOC} \nu \Delta\text{peak C}_{0-5}$ ,  $n = 36$ ,  $R^2 = 0.66$ ,  $p = <0.001$ ).

494

495 The PCA analysis indicated clear clustering of samples from each basin, and clear  
496 separation between samples from each basin (Fig. 3). Moreover, there was a clear  
497 difference in the association of the measured variables to the different basins.



498

499 **Figure 3.** Principal component analysis (PCA) of bacterial and DOM characteristic  
 500 variables from all standard (+NP excluded) microcosm units and all sampling  
 501 occasions (Baltic Proper, triangles; Bothnian Sea, circles; and Bothnian Bay, squares).  
 502 PC1 and PC2 encompass 64.5 % of the cumulative variance in the data set. PC1 (46  
 503 % of variance) was most strongly loaded by SUVA<sub>254</sub> (+0.46), CDOM (0.42), peak C  
 504 (0.40), a254:a365 (-0.39) and DOC (-0.36). PC2 (18 % of variance) was most strongly  
 505 loaded by peak T (0.53), peak B (0.51), BP (0.45) and BA (0.42).

506

507 **4. Discussion**

508 Seawater contains a vast pool of carbon and the concentrations, characteristics, and  
509 bioavailability of this matter can differ seasonally and spatially as it is continuously  
510 altered by degradative and formative physicochemical and biological processes  
511 (Benner and Amon, 2015; Jiao et al., 2010; Nagata 2000). In this study we find that  
512 spatial differences in the nutrient status and DOM characteristics play an important  
513 role in controlling the bacterial utilisation of DOC, thus controlling the BGE and  
514 influencing the DOM pool itself.

515

516 **4.1. Spatial variation and within basin similarity.** The unique hydrology and  
517 extensive latitudinal expanse of the Baltic Sea maintains a high degree of spatial and  
518 seasonal physicochemical variation. Clear differences in biological communities and  
519 processes also exist, including at the basal microbial level (e.g. Andersson et al.,  
520 2015; Herlemann et al., 2011). Within the bounds of each of the three major basins  
521 studied, the sampled stations showed clear physicochemical similarities (Fig. S1,  
522 Table 1) and were in general significantly different from other basins (Table S1). This  
523 affirms spatial physicochemical gradients (Table 1) and validates the consideration of  
524 offshore water-bodies within each basin as single entities for the purpose of this, and  
525 similarly designed studies.

526

527 In contrast to other studies (compiled in Hoikkala et al., 2015) we recorded higher  
528 DOC concentrations at the southerly Baltic Proper stations. This was likely due to the  
529 dual effect of the relative closeness to land of the southern stations sampled and the  
530 presence of an extensive phytoplankton bloom at the time of sampling (Hansson and  
531 Öberg, 2011). Importantly, our data show that the composition of the DOM pool  
532 differed strongly between the studied basins (Fig. S2 and S3) and this is particularly

533 germane for such studies, as these characteristics influence DOM bioavailability or  
534 reactivity (Asmala et al., 2013; Autio et al., 2015; Benner and Amon, 2015). Water  
535 colour (CDOM, Harvey et al., 2015), DOM aromaticity ( $SUVA_{254}$ , Weishaar et al.,  
536 2003) and levels of secondary humic material of terrestrial origin (peak C, Cammack  
537 et al., 2004; Stedmon and Markager, 2005) were all highest in the northern Bothnian  
538 Bay basin and lower in the Baltic Proper. On the other hand  $S_{275-295}$  and  $a_{254}:a_{365}$   
539 were highest in the Baltic Proper, both inversely related to the DOM molecular weight  
540 (Asmala et al., 2013; Fichot and Benner, 2012; Helms et al., 2008; Wallin et al.,  
541 2015). Taken together these data indicate clear spatial trends that are in accordance  
542 with the strong terrestrial influence in the northerly basins (Alling et al., 2008;  
543 Deutsch et al., 2012; Harvey et al., 2015; Stedmon et al., 2007) and are indicative of  
544 more autochthonous DOM sources in the southerly Baltic Proper (Andersson et al.,  
545 2015; Hoikkala et al., 2015; Maciejewska and Pempkowiak, 2014).

546

547 **4.2. Bacterial growth, DOC utilisation and BGE.** BA in all microcosms generally  
548 reached highest levels by day three or five before it plateaued or decreased. Despite  
549 similar starting rates on day zero BP differed strongly between basins, with highest  
550 rates recorded in the Bothnian Sea microcosms. It is possible that this is due to a more  
551 suitable stoichiometric balance of nutrients in the Bothnian Sea (Table 1). This active  
552 phase of the incubation (day 0-5) corresponded with the phase during which DOC  
553 utilisation also took place. During this phase, largest mean DOC utilisation was  
554 recorded in the southerly Baltic Proper basin (~30%) and decreased in a northerly  
555 direction (Bothnian Sea ~12% and Bothnian Bay ~4%), with values being in a similar  
556 range to previous studies (Asmala et al., 2013; Hoikkala, 2015; Zweifel et al., 1993).  
557 Highest DOC utilisation occurred in the region with higher starting DOC

558 concentrations, as Søndergaard and Middelboe (1995) found in a large cross-system  
559 analysis. However, the clear regional differences in the DOM pool characteristics  
560 indicate that the control of bacterial DOC utilisation is a more complex process. The  
561 prevailing conditions resulted in BGE values that were comparable with similar  
562 studies (Asmala et al., 2013; Attermeyer et al., 2014; Figueroa et al., 2016). However,  
563 BGE was negatively correlated with DOC utilisation. BGE values were highest in the  
564 Bothnian Bay basin (~25 %) and decreased in a southerly direction (~16 and ~2 %,  
565 Bothnian Sea and Baltic Proper, respectively). Similar relationships have been  
566 reported recently where higher BGE levels were found in river waters strongly  
567 influenced by humic matter or forested soils, supporting the notion that DOM  
568 characteristics influence bacterial metabolism (Autio et al., 2015; Berggren and del  
569 Giorgio, 2015).

570

571 **4.3. Influence of nutrients on bacterial activity.** The addition of N and P (+NP)  
572 resulted in significantly elevated BA and BP rates in the Baltic Proper and Bothnian  
573 Bay microcosms (Table 2). In essence nutrient addition sustained a longer period of  
574 elevated BA and BP (Fig. 2, and  $BP_{cum}$  Table 3). However, little effect was seen on  
575 DOC utilisation and only in the Bothnian Bay did it result in a markedly different  
576 basin mean BGE (Table 3). This strong increase in BGE in the Bothnian Bay may  
577 relate to the adjusted C:N:P stoichiometric ratios that aligned all basin ratios more  
578 closely in the +NP treatments (basin mean C:N:P = 19-34:2:1), in particular reducing  
579 the C:P ratios that were at their most extreme in the Bothnian Bay natural waters  
580 (Table 1). While stoichiometric ratios of these vital nutrients have been shown to be  
581 important in marine systems (Thingstad et al., 2008; Andersson et al., 2013) the  
582 addition of P would likely have alleviated the major limiting nutrient in the Bothnian

583 Bay (Tamminen and Andersen, 2007; Andersson et al., 2015). Furthermore, nutrient  
584 addition did not induce significant changes in DOM characteristics (Table 3), which  
585 showed stronger and significant changes spatially and over the time period of the  
586 incubation (Table 2). The lack of change in DOM degradation may indicate that  
587 nutrient addition did not strongly alter the bacterial community composition, that  
588 functional redundancy within the local bacterial community strongly determines the  
589 outcome, or that a common pool of generalist bacteria drove the degradation of DOM  
590 at each site (Allison and Martiny, 2008; Attermeyer et al., 2014; Dinasquet et al.,  
591 2013). However specific studies would be required to clarify these issue since our  
592 measurements generally encompass bulk values and net changes during the  
593 experiment.

594

595 Despite the relatively unaltered DOM processing due to nutrient supplementation,  
596 ambient starting nutrient concentrations (and stoichiometric ratios) correlated closely  
597 with changes in BA (standard microcosms only). High starting concentrations of TN  
598 and TP, plus low C:P and N:P stoichiometric ratios resulted in larger increases in BA.  
599 However, no corresponding correlation was found with DOC. While the  
600 concentrations and stoichiometric ratios of these elements at the start of the incubation  
601 are important, and have the potential to limit bacterial growth (Degerman et al., 2013;  
602 Zweifel et al., 1993), the minimal number of close correlations with BP, BGE or  
603 changes in DOM characteristic variables indicate that there are clear differences  
604 between the influence of nutrients on growth (i.e. BA) and the physiological processes  
605 taking place (Guillemette and del Giorgio, 2012). This further supports the reasoning  
606 that changes seen here relate to the physiological capacity of stable local bacterial  
607 communities. However, high C:N starting ratios correlated with largest decreases in



608 DOC during the active phase of the experiment, and with the lowest BGE values. This  
609 supports previous suggestions that in addition to the DOM characteristics and the total  
610 BA or BP capacity, the metabolic balance (i.e. BGE) of the bacterial community is  
611 also vital (Guillemette and del Giorgio, 2012).

612

613 **4.4. DOM characteristics and bacterial interaction.** Clear differences in DOM  
614 characteristics were recorded across the studied gradient, including support for the  
615 hypothesis that DOM would be more strongly autochthonous in the south.

616 However, during the active period of incubation the molecular weight of the DOM  
617 pool (as defined by the S275-295 proxy) decreased in the Baltic Proper, whereas it  
618 increased in microcosms from the two more northerly basins (Table 3). In the Baltic  
619 Proper microcosms a clear increase in CDOM was also observed during incubation  
620 (Fig. S2). This would suggest that larger molecular weight constituents within the  
621 Baltic Proper DOM pool were broken down, whereas DOM components of a larger  
622 size became relatively more dominant in the DOM pool of the northerly basins.

623 Concurrently, bacterial activity contributed to the production of CDOM in Baltic  
624 Proper microcosms, as reported from other systems (Kramer and Herndl, 2004;  
625 Nelson et al., 2004; Yamashita and Tanoue, 2004). However, the exact nature of this  
626 processed portion of the DOM pool, and its interaction with resident biological  
627 communities, is complex. The Baltic Proper DOM pool became increasingly aromatic  
628 in nature during incubation (Table 3), with the relative change in DOC (i.e.  
629 utilisation) and change in aromaticity being associated, and the highest levels of DOC  
630 utilisation corresponding to highest levels of aromaticity increase. Thus, bacterial  
631 activity in the Baltic Proper decreased DOC concentrations, breaking down larger  
632 molecular weight compounds and the processed DOM pool was more aromatic and

633 contributed to increasing water colour. This appears to relate to functional aspects of  
634 the local bacterial community and is not at odds with an earlier study that found  
635 bacteria from the Baltic Proper grew well, if not better than the native bacteria, in  
636 Bothnian Sea water containing natural DOM (Lindh et al., 2015). However, the high  
637 initial DOC concentrations recorded in the Baltic Proper, due mainly to a  
638 contemporary phytoplankton bloom, would also likely have contributed to this trend  
639 (and to the low BGE recorded in this region). This pool of autochthonous DOC would  
640 have been readily available and respired, resulting in extensive carbon losses  
641 (Berggren and del Giorgio, 2015).

642

643 Changes to the intrinsic nature of the DOM pool will influence its subsequent  
644 bioavailability, and have the potential to result in carbon limitation (Carlson and  
645 Ducklow 1996; Figueroa et al., 2016; Kirchman and Rich, 1997). Such carbon  
646 limitation scenarios are likely to contribute to the similar temporal patterns of BP and  
647 BA seen in our experimental microcosms, including the apparently limited influence  
648 of nutrients. It may be that viral lysis also played a role (e.g. Middelboe and  
649 Jørgensen, 2006), though this can not be ascertained directly. In experimental systems  
650 where concurrent physicochemical alteration of a finite DOM pool is limited, and the  
651 bacterial community remains constrained by the starting inoculum, limitation may  
652 appear particularly pronounced. However, in the natural environment the dynamic  
653 nature of these interactions will undoubtedly change this perspective. In the Baltic  
654 Sea, where waters generally transfer between basins in a southerly direction due to the  
655 net freshwater influx in the north, the DOM pool is exposed to an extensive  
656 continuum of biological and physicochemical action. Thus, the patterns of DOM  
657 characteristics (and changes) detailed here could conceivably indicate that the DOM

658 pool, in addition to being altered by bacterial activity, is also a formative driver of  
659 local bacterial community structure (Herlemann et al., 2013; Judd et al., 2006; Lindh  
660 et al, 2015; Logue et al., 2016).

661

662 Samples with high aromaticity or high molecular weight (i.e. from more northerly  
663 basins) generally expressed higher levels of secondary humic matter of terrestrial  
664 origin (peak C: Cammack et al., 2004; Coble, 1996; Stedmon and Markager, 2005).  
665 However, during the microcosm incubation these variables responded very differently  
666 between basins (Table 3). Largest relative increases in aromaticity generally  
667 corresponding with largest decreases in secondary humic matter. Additionally, during  
668 microcosm incubation mean basin changes in DOM molecular weight and secondary  
669 humic matter of terrestrial origin followed latitudinal patterns that were opposite to  
670 each other (Table 3). In the Baltic Proper bacterial activity depleted secondary humic  
671 material of terrestrial origin, resulting in smaller molecular weight DOM that was  
672 more aromatic in nature. Such processes have been observed in the dark ocean where  
673 heterotrophic production was significant (Jørgensen et al., 2011). On the other hand,  
674 in the two more northerly basins the DOM pool became less/less strongly aromatic  
675 and the relative contribution of higher molecular weight secondary humic matter  
676 increased (Table 3). Furthermore, the trends in secondary humic matter correlated  
677 with BGE, where microcosms expressing high BGE showed largest increases in  
678 secondary humic material, whereas microcosms with low BGE expressed smaller  
679 increases or decreases. This is in keeping with a study in lakes, where largest  
680 increases in secondary humic peaks were found in incubations dominated by anabolic  
681 (i.e. high BGE), rather than catabolic (low BGE) processes (Guillemette and del

682 Giorgio, 2012), leading the authors to conclude such factors would also have  
683 importance for the transfer of energy and nutrients within the food web.  
684  
685 Protein-like peaks (peak B and peak T: Coble, 1996), however, responded quite  
686 differently to bacterial activity, and although changes were often significant (Table 2)  
687 the patterns did not follow linearly across the latitudinal gradient studied (Table 3).  
688 We recorded the largest decreases in protein-like fluorescent peaks in the Bothnian  
689 Bay (Table 3), a pattern that has also been observed in lakes (Guillemette and del  
690 Giorgio, 2012). However, in the mid-gradient Bothnian Sea microcosm these two  
691 peaks appeared to be produced, particularly strongly in the case of peak B. It appears  
692 that a different process controls the production or utilisation of protein-like  
693 compounds in this study, with production associated to the BA and BP variables (Fig.  
694 3 and Table 3), potentially representing cell wall proteins (Kawasaki and Benner  
695 2006; Stoderegger and Herndl, 1998; Tanoue et al., 1995) or other structural  
696 components (Kaiser and Benner, 2008; Ogawa et al., 2001).

697

698 **5. Conclusion.** The dual role of bacteria in both utilising and producing DOM, and  
699 the interplay between DOM characteristics, nutrient status, and bacterial metabolism  
700 all determine the fate of DOM and thus the composition of the bulk DOM pool. In  
701 this study we addressed the net balance of these complex processes. Our study  
702 suggests that spatial differences in DOM characteristics, nutrient levels and nutrient  
703 stoichiometric ratios are important factors controlling bacterial growth and BGE, and  
704 that these processes in turn influence the DOM pool. Markedly different DOM-  
705 bacterial interactions were observed in each region of the studied gradient, catalysing  
706 different consequences for the DOM pool. It is clear that bacterial growth and

707 metabolism (e.g. BGE) can alter the characteristics and properties of the DOM pool  
708 and that these modifications can influence bioavailability, have repercussions for long  
709 term carbon sequestration (Brophy and Carlsson 1989; Jiao et al., 2010; Ogawaa et  
710 al., 2001), and can influence the global carbon cycle (Benner and Amon, 2015; Jiao et  
711 al., 2010). Furthermore, climate change scenarios indicate that surface water  
712 warming, elevated rainfall and terrestrial run off, and altered nutrient status within the  
713 studied system are expected (Eilola, 2013; Graham, 2004; Wikner and Andersson,  
714 2012). This will influence the complex DOM-nutrient-bacterial interactions that  
715 currently exist and thereby influence the passage of nutrients and energy to higher  
716 trophic levels.

717

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1117 **Supplementary tables, figures and information from this point onwards:**

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1119 **Table S1.** Analysis of variance (ANOVA) for physicochemical variables differences

1120 between basins. Key: p = <0.001\*\*\*, <0.01\*\*, <0.05\*, not significant<sup>ns</sup>.

	Temperature	pH	Salinity	DOC	TP	TN
BProper - BBay	***	***	***	***	***	**
BSea - BBay	***	*	***	ns	**	**
BSea - BProper	***	*	***	***	ns	ns
Global ANOVA	***	***	***	***	***	**

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1123 **Table S2.** Start and end concentrations of TN and TP in microcosm units (n = 11-12,

1124 SD) from the Bothnian Sea and Bothnian Bay, standard and +NP treatment.

	TN Start $\mu\text{mol L}^{-1}$	TN End $\mu\text{mol L}^{-1}$	TP Start $\mu\text{mol L}^{-1}$	TP End $\mu\text{mol L}^{-1}$
Bothnian Sea	16.14 (1.07)	16.21 (1.14)	0.18 (0.03)	0.19 (0.03)
Bothnian Bay	13.28 (0.57)	13.21 (0.64)	0.09 (0.01)	0.09 (0.01)
Bothnian Sea +NP	36.98 (1.21)	36.77 (1.00)	3.52 (0.12)	3.52 (0.08)
Bothnian Bay +NP	33.91 (0.87)	33.99 (1.00)	3.39 (0.07)	3.39 (0.13)

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1131 **Table S3.** Two-way ANOVA testing effect of basin and treatment (+/- NP) on  $\Delta$   
 1132 values for measured variables, and any interaction between basin and treatment.  
 1133 Subscript indicated the time period (days of microcosm incubation) for which  $\Delta$   
 1134 values are calculated.

Dependent variable	d.f.	<i>F</i>	Basin	Treatment (+/- NP)	Interaction
BGE <sub>1-5</sub>	2	15.5	<0.001***	ns	ns
BPcum <sub>1-5</sub>	2	41.0	<0.001***	ns <sup>1</sup>	ns
$\Delta$ BA <sub>0-5</sub>	2	90.2	<0.001***	0.000***	<0.001***
$\Delta$ DOC <sub>1-5</sub>	2	40.6	<0.001***	ns	ns
$\Delta$ a <sub>254:365</sub> <sub>0-5</sub>	2	1.7	ns	ns	ns
$\Delta$ s <sub>275:295</sub> <sub>0-5</sub>	2	14.5	<0.001***	ns	ns
$\Delta$ SUVA <sub>0-5</sub>	2	197.4	<0.001***	ns	ns
$\Delta$ peak B <sub>0-5</sub>	2	5.2	0.008**	ns	ns
$\Delta$ peak C <sub>0-5</sub>	2	116.3	<0.001***	ns	ns
$\Delta$ peak T <sub>0-5</sub>	2	6.0	0.004**	ns	ns

1135 Key: p = <0.001\*\*\*, <0.05\*.

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1144 **Table S4.** Post hoc Bonferroni tests showing mean differences of individual variables  
 1145 between basins and their significance (microcosm experiment).

	Comparison	Mean difference	Significance
<b>BP</b>	Baltic Proper – Bothnian Sea	-11.07	<0.001***
	Baltic Proper – Bothnian Bay	-1.97	0.331
	Bothnian Sea – Bothnian Bay	9.11	<0.001***
<b>BA</b>	Baltic Proper – Bothnian Sea	1771896	<0.001***
	Baltic Proper – Bothnian Bay	2579699	<0.001***
	Bothnian Sea – Bothnian Bay	807802	<0.001***
<b>DOC</b>	Baltic Proper – Bothnian Sea	2.28	<0.001***
	Baltic Proper – Bothnian Bay	2.67	<0.001***
	Bothnian Sea – Bothnian Bay	0.39	<0.001***
<b>a254-365</b>	Baltic Proper – Bothnian Sea	3.41	<0.001***
	Baltic Proper – Bothnian Bay	5.50	<0.001***
	Bothnian Sea – Bothnian Bay	2.11	<0.001***
<b>SUVA<sub>254</sub></b>	Baltic Proper – Bothnian Sea	-1.14	<0.001***
	Baltic Proper – Bothnian Bay	-2.55	<0.001***
	Bothnian Sea – Bothnian Bay	-1.41	<0.001***
<b>peak B</b>	Baltic Proper – Bothnian Sea	-0.078	<0.001***
	Baltic Proper – Bothnian Bay	-0.071	<0.001***
	Bothnian Sea – Bothnian Bay	-0.003	1.000
<b>peak C</b>	Baltic Proper – Bothnian Sea	-0.006	0.158
	Baltic Proper – Bothnian Bay	-0.099	<0.001***
	Bothnian Sea – Bothnian Bay	-0.093	<0.001***



<b>peak T</b>	Baltic Proper – Bothnian Sea	0.008	0.190
	Baltic Proper – Bothnian Bay	0.000	1.000
	Bothnian Sea – Bothnian Bay	-0.008	0.210

1146 Key: p = <0.001\*\*\*.

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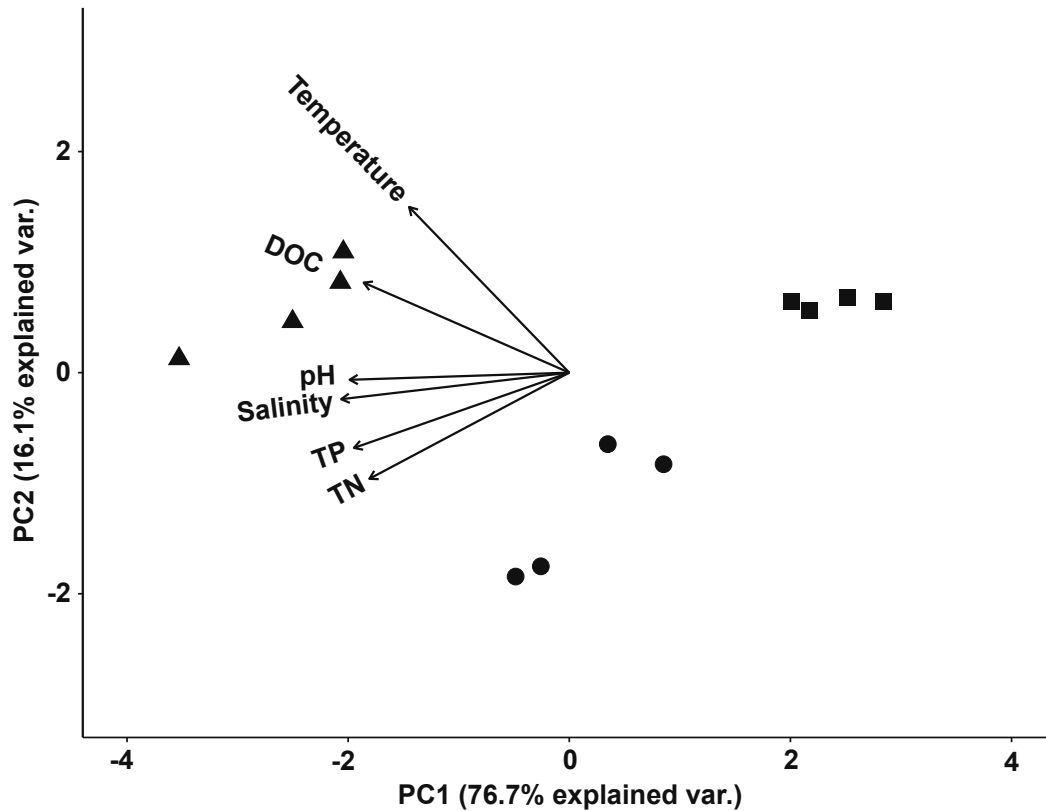
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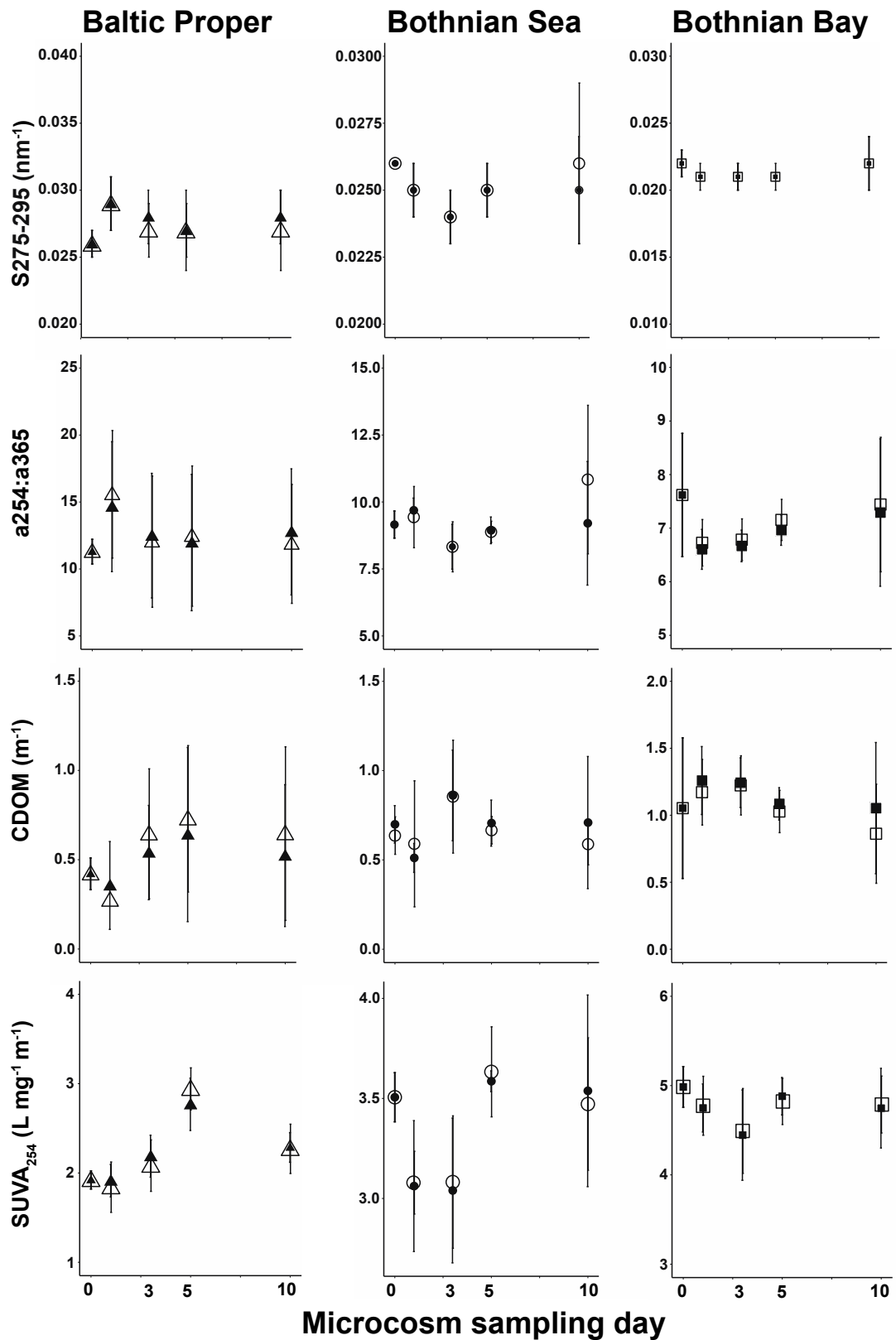
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1169 **Figure S1.** Principal component analysis (PCA) based on in-situ physicochemical  
 1170 variables collected at 12 stations, four per basin (Baltic Proper, triangles; Bothnian  
 1171 Sea, circles; and Bothnian Bay, squares). PC1 and PC2 encompass 93 % of the  
 1172 cumulative variance in the data set. PC1 (77 % of variance) was most strongly loaded  
 1173 by salinity (-0.45), pH (-0.44) and TP (-0.43). PC2 (16 % of variance) was most  
 1174 strongly loaded by temperature (0.72) and TN (-0.46).

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1178 **Figure S2.** Fluctuation in optical DOM characteristic variables during microcosm

1179 incubation. Data are presented by basin (Baltic Proper, triangles; Bothnian Sea,

1180 circles; and Bothnian Bay, squares), with standard (filled symbols) and +NP  
1181 treatments (open symbols) shown. Mean values are shown with standard deviation is  
1182 indicated by error bars ( $n = 12$ ). **Note axis scales are not identical and vary between**  
1183 **basins for a single variable.**

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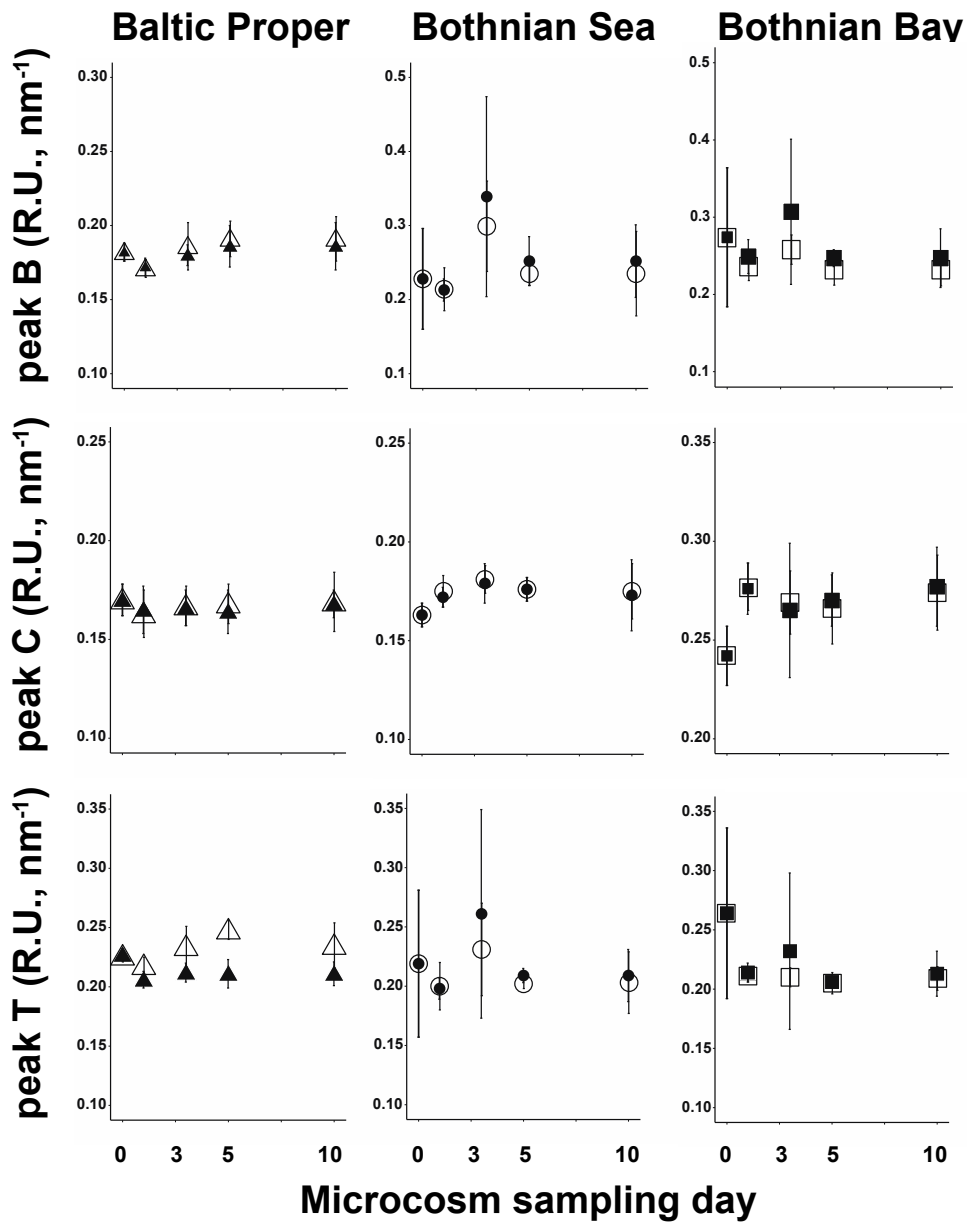
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 1205 **Figure S3.** Fluctuation in DOM characteristic fluorescent peaks during microcosm  
 1206 incubation. Data are presented by basin (Baltic Proper, triangles; Bothnian Sea,  
 1207 circles; and Bothnian Bay, squares), with standard (filled symbols) and +NP  
 1208 treatments (open symbols) shown. Mean values are shown with standard deviation is  
 1209 indicated by error bars ( $n = 12$ ). **Note axis scales are not identical and vary between**  
 1210 **basins for a single variable.**

1211 **Supplementary results: other associations between variables.**

1212 SUVA<sub>254</sub> and CDOM correlated spatially (all basins and all microcosm sampling  
1213 events (ALL):  $n = 172$ ,  $R^2 = 0.49$ ,  $p = <0.001$ ). Similar but stronger spatial  
1214 correlations were recorded between S275-295 and SUVA<sub>254</sub> (ALL:  $n = 178$ ,  $R^2 = 0.72$ ,  
1215  $p = <0.001$ ) and CDOM (ALL,  $n = 178$ ,  $R^2 = 0.75$ ,  $p = <0.001$ ). A spatial correlation  
1216 was also found between peak C and SUVA<sub>254</sub> (ALL:  $n = 178$ ,  $R^2 = 0.69$ ,  $p = <0.001$ )  
1217 and S275-295 (ALL:  $n = 178$ ,  $R^2 = 0.69$ ,  $p = <0.001$ ).

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1219 Lower N:P and C:P ratios (at the start of incubation) had a positive effect on BA  
1220 ( $\ln N:P \ v \ \ln \Delta BA_{0-5}$ ,  $n = 32$ ,  $R^2 = 0.55$ ,  $p = <0.001$  and  $C:P \ v \ \Delta BA_{0-5}$ ,  $n = 33$ ,  $R^2 = 0.52$ ,  
1221  $p = <0.001$ ). Highest increases in BA were recorded at N:P and C:P ratios of  $\sim 13$  and  
1222  $\sim 4-500$ , respectively. Lower starting C:P ratios had a positive effect on  $BP_{cum}$  values  
1223 ( $\ln C:P \ v \ \ln \Delta BP_{cum \ 1-5}$ ,  $n = 34$ ,  $R^2 = 0.51$ ,  $p = <0.001$ ), with the highest  $BP_{cum}$  recorded  
1224 at C:P ratios of  $\sim 3-400$ . Furthermore, larger increases in BA during incubation  
1225 correlated with larger increase in SUVA<sub>254</sub> ( $\Delta BA_{0-5} \ v \ \Delta SUVA_{0-5}$ ,  $R^2 = 0.38$ ,  $p =$   
1226  $<0.001$ ).

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1228 In microcosm units with lower TP or lower DOC starting concentrations there were  
1229 increases in peak C during incubation ( $TP \ v \ \Delta peak \ C_{0-5}$ ,  $n = 36$ ,  $R^2 = 0.52$ ,  $p = <0.001$   
1230 and  $DOC \ v \ \Delta peak \ C_{0-5}$ ,  $n = 36$ ,  $R^2 = 0.66$ ,  $p = <0.001$ ). With higher start TP and DOC  
1231 concentrations the production of peak C was lesser, and at the higher end of TP and  
1232 DOC concentrations peak C decreased. A similar, but weaker, trend was observed for  
1233 C:N ratios, with largest increases in peak C at lower C:N ratios; whereas the largest  
1234 increases in peak C were observed at higher N:P ratios ( $R^2 = 0.39$  and  $0.38$ ,  
1235 respectively). Percentage change in peak C during the active incubation period

1236 correlated with percentage changes in  $SUVA_{254}$ , indicating that where larger increases  
1237 in  $SUVA_{254}$  were recorded a decrease in peak C occurred, whereas where  $SUVA_{254}$   
1238 increases were low (or decreased) peak C increased ( $\Delta\text{peak C}_{0-5}$  v  $\Delta SUVA_{0-5}$ ,  $n = 36$ ,  
1239  $R^2 = 0.57$ ,  $p = <0.001$ ). A similar trend was seen with relative DOC utilisation (i.e. %  
1240 change) where in incubations with largest DOC decreases corresponding relative  
1241  $SUVA_{254}$  values increased ( $\Delta\text{DOC}_{1-5}$  v  $\Delta SUVA_{0-5}$ ,  $n = 28$ ,  $R^2 = 0.46$ ,  $p = <0.001$ )

