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

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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.

Salinity, Temperature

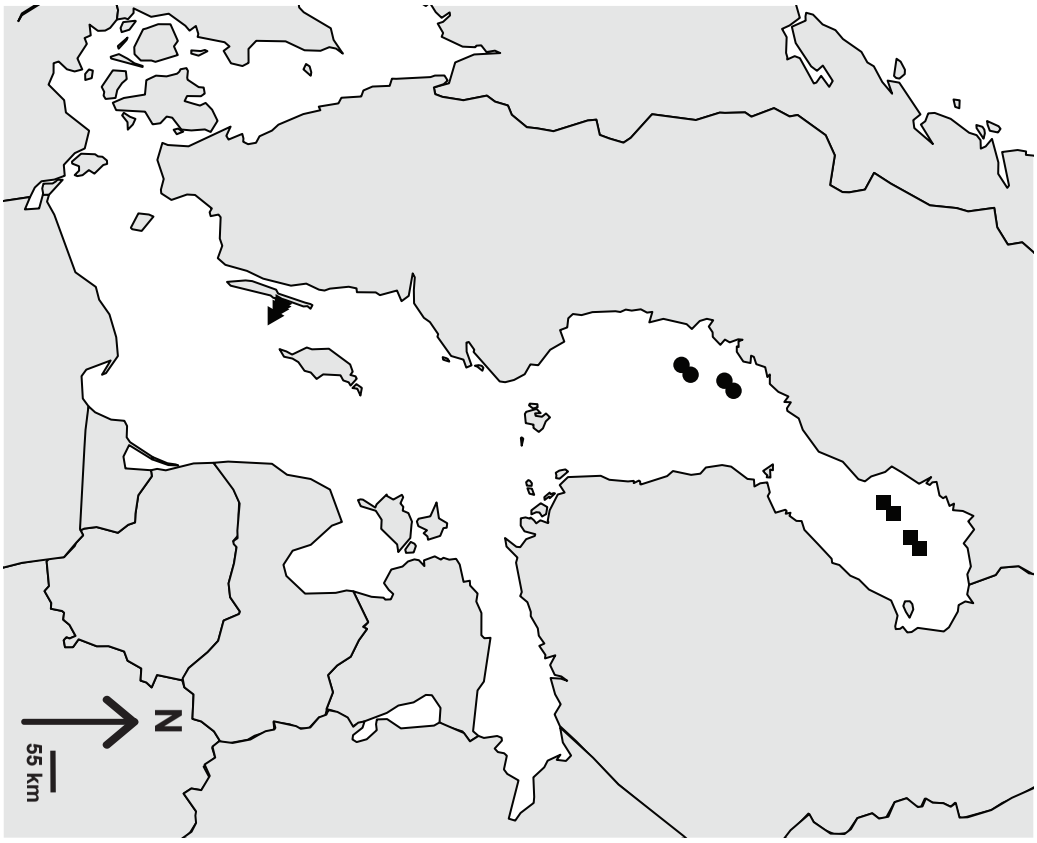
TP and TN

DOC

C:N

N:P

C:P



Baltic Proper Bothnian Sea Bothnian Bay

Molecular weight indicator

Aromaticity ($SUVA_{254}$)

CDOM (a440)

Secondary humic components

Bacterial production

Bacterial growth efficiency

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

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Running head: Brackish water bacteria-DOM interactions

Abstract

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

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

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. Introduction

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

DOM in marine waters is in copious supply (Hedges, 1992; Benner and Amon, 2015). While DOM in open water marine systems is dominantly derived from autochthonous processes (i.e. phytoplankton primary production and related processes: Nagata, 2000), allochthonous terrestrial organic matter can also be an important contributor to the DOM pool. This latter scenario can be especially pertinent in enclosed or coastal waters (Ask et al., 2009; Deutsch et al., 2012; Fleming-Lehtinen et al., 2015). The characteristics of the DOM pool are influenced by its origin (e.g. autochthonous, allochthonous, land use, catchment composition) and these attributes in turn control its bioavailability and fate. These factors influence its potential importance in the ecosystem (Asmala et al., 2013; Boyd and Osburn, 2004; Stedmon et al., 2003). The concentration and properties of the DOM pool can directly influence heterotrophic processes at the base of the food web. Supplementary DOC and allochthonous nutrients may enable bacteria to outcompete autotrophic primary producers (Fandino et al., 2001; Lignell et al., 2008; Sandberg et al., 2004; Smith et al., 1995). Furthermore, DOM can catalyse other concurrent changes, such as controlling the 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; Räike 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 5th) and one in the Gulf of Bothnia
158 (Bothnian Sea and Bothnian Bay, July 19th - 21st). 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.
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163
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165

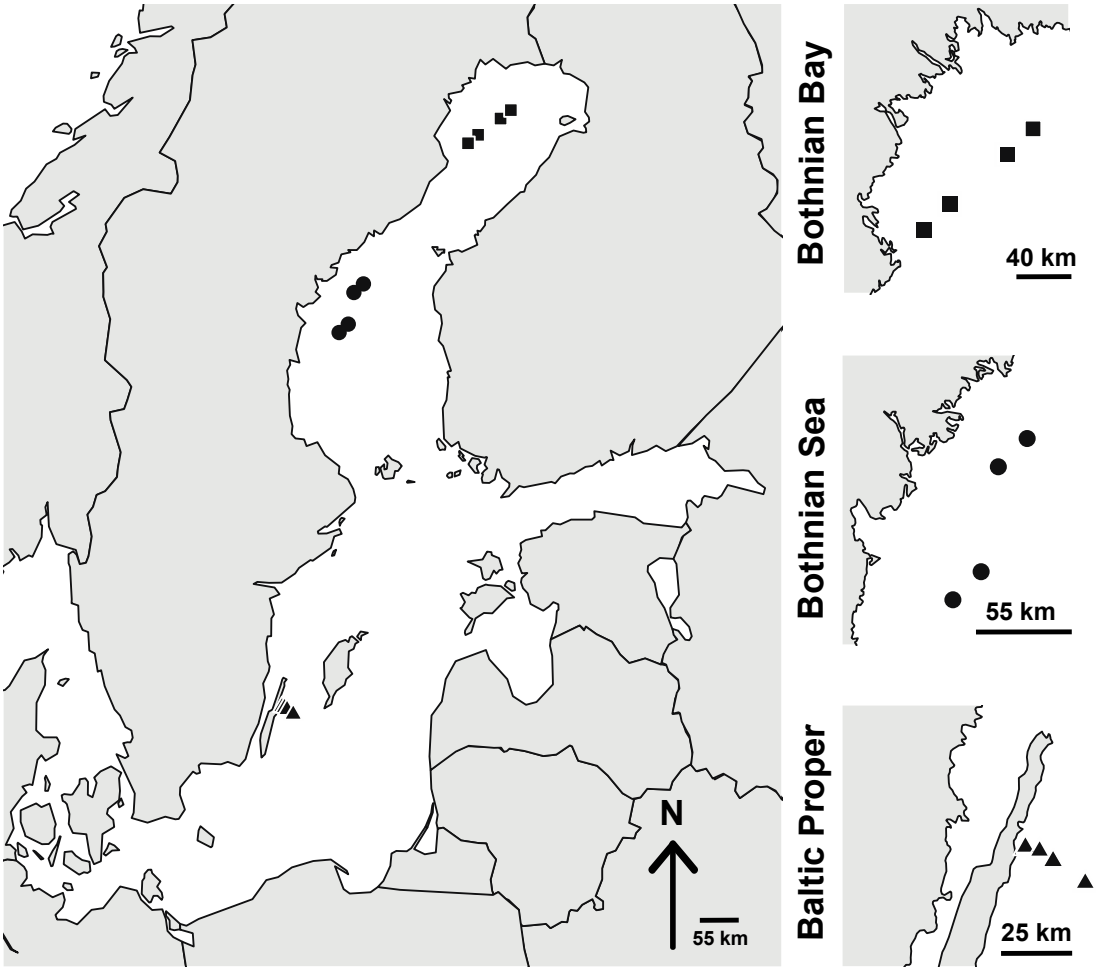

Figure. 1. Map of the Baltic Sea showing sampling locations of the four open sea stations sampled in each of the three major basins (Baltic Proper, Bothnian Sea and Bothnian Bay).

2.3. Preparation of experimental study. To remove larger organisms 10 L of water was passed through a 0.45 µm capsule filter (Millipak-40. Millipore) using gravity filtration. The filter capsule was rinsed with ~1 L of sample water prior to use, and 0.45 µm filtrate was used to rinse the recipient acid-washed plastic carboy. The final 6 L of water passing through the 0.45 µm filter was collected, from here onwards referred to as 'filtrate 1'. Circa 2 L of water was also gravity filtered through a pre-combusted 47 mm GF/F filter, referred to as 'filtrate 2'. This process was repeated for 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 µM N and 3 µM 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 µL 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

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

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

	Temperature (ºC)	pH	Salinity	DOC (µmol C L^{-1})	TP (µmol L^{-1})	TN (µmol 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)

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

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

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

tubes, acidified with 120 µL of 2 M HCl, and stored at 4°C until analysis. DOC samples were analysed using high temperature catalytic oxidation (Shimadzu TOC-5000), as detailed in Traving et al., (2017). DOM fluorescence samples were prepared by collecting a single 40 mL sample that was filtered at low pressure through a pre-combusted GF/F filter into a 50 mL tube and immediately frozen (-20°C) until processing. It should be noted that freezing is not optimal as it may alter DOM fluorescence (e.g. Fellman et al., 2008), potentially in a random manner (Spencer et al., 2007). However the extensive gradient studied and field sampling carried out gave no viable alternative. Since all samples in the present study were treated identically we infer that the observed trends are valid for the direct comparisons carried out. Nevertheless, comparisons of specific values between this and other studies should be done with caution. Samples were acclimated to room temperature on a Horiba Aqualog spectrofluorometer (Horiba Scientific) in a 1 cm quartz cuvette. This instrument simultaneously measures absorption (from 240 nm to 600 nm) and fluorescence (at excitation and emission wavelengths 240 nm to 600 nm) at 3 nm intervals. Correction, calibration and calculation of informative variables were carried out (Asmala et al., 2013; Murphy et al., 2010; Stedmon et al., 2000). The following variables were extracted or calculated: 1. the ratio between $a_{CDOM(254)}$ and $a_{CDOM(365)}$ (referred to as: a254:a365), 2. a slope of the spectra for wavelengths 275-295 nm (slope coefficient, S275-295); both indicators of DOM molecular weight (Asmala et al., 2013; Fichot and Benner, 2012; Helms et al., 2008; Wallin et al., 2015), 3. absorbance at 440 nm ($a_{CDOM(440)}$), referred to as chromophoric dissolved organic matter (CDOM) and indicative of water colour (Harvey et al., 2015), 4. $SUVA_{254}$, indicative of DOM aromaticity (Ripszam et al., 2015; Weishaar et al., 2003), 5. fluorescence peak C (peak C, Ex/Em of 350/420-480 nm), a secondary humic peaks

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

2.7. DOC utilisation, BGE and fluctuation of variables. Calculations of change (increase or decrease, Δ) were carried out between days 0 and 5 ($\Delta_{0\text{-}5}$) and between days 0 and 10 ($\Delta_{0\text{-}10}$), the latter being the full length of microcosm incubations. Trends were generally similar for both incubation time periods examined. However, only data for $\Delta_{0\text{-}5}$ are presented as this represented the more active period of the incubation (see results). Variables for which Δ data are calculated include: BA, DOC, a254:a365, S275-295, $SUVA_{254}$, peak B, peak C, and peak T. Lastly, ΔDOC (or DOC utilisation) was calculated between days 1 and 5 due to missing DOC data at some stations on day 0. Where DOC data was present on day 0 there was no marked decrease in DOC between days 0 and 1. Other calculations reliant on ΔDOC (e.g. BGE) were also calculated using requisite data from the corresponding time period. BGE (%) was calculated as the integrated cumulative bacterial production during days 1-5 ($BP_{cum1\text{-}5}$) divided by the ΔDOC between days 1 and 5 ($\Delta DOC_{1\text{-}5}$), multiplied by 100 (Figueroa et al., 2016).

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 Δ 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 Δ 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

3.1.1. Initial conditions. Clear variation in optical DOM characteristic variables were observed between basins at the start of the microcosm incubation. The a254:a365 ratio was higher in the Baltic Proper and decreased in a northerly direction. $SUVA_{254}$ and CDOM showed the opposite trend, being highest in the Bothnian Bay (Fig. S2). Values for peak B, peak C, and peak T were generally higher in the Bothnian Bay or similar across all basins at the start of the incubations (Fig. S3).

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

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

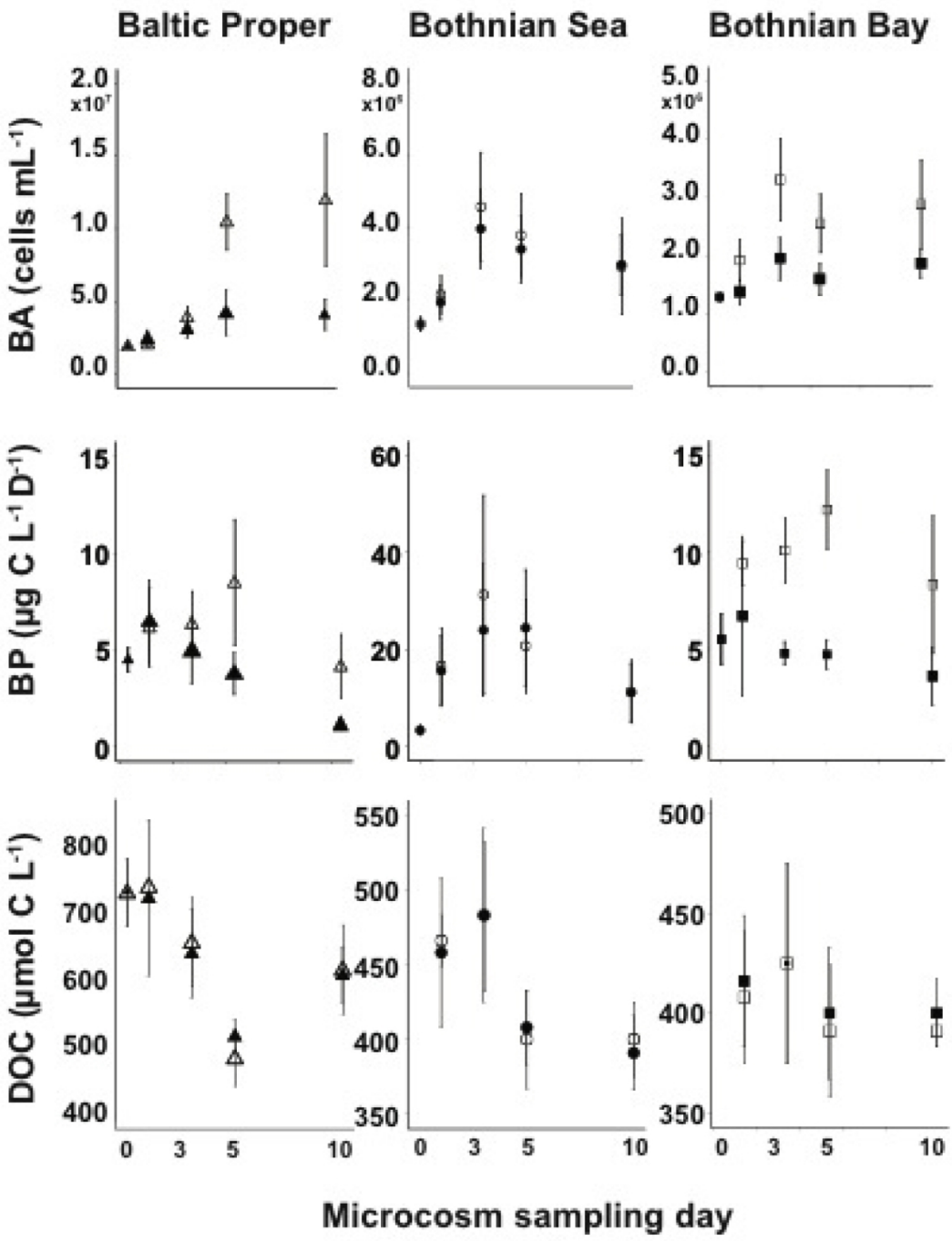

Figure 2. Temporal trends in mean values for bacterial abundance (BA), bacterial production (BP) and dissolved organic carbon (DOC) in microcosm experiments. Data are presented by basin (Baltic Proper, triangles; Bothnian Sea, circles; and Bothnian Bay, squares), with standard (filled symbols) and +NP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

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

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

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 (Δ, %) during the active phase of incubation (standard
389 error). DOC utilisation (ΔDOC), cumulative bacterial production (BP_{cum}, µg 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	
Treatment	-NP	+NP	-NP	+NP	-NP	+NP
BGE_{1-5}	1.4 (0.4)	1.6 (0.4)	16.9 (6.6)	16.0 (4.1)	20.8 (6.7)	30.8 (4.2)
$BP_{cum\ 1-5}$	25.4 (2.4)	32.7 (2.4)	97.5 (2.7)	109.9 (17.1)	27.1 (1.8)	49.2 (1.1)
ΔBA_{0-5}	118.4 (19.9)	444.1 (20.6)	150.6 (13.2)	177.5 (16.6)	18.3 (6.7)	88.6 (11.0)
ΔDOC_{1-5}	-27.6 (3.9)	-33.1 (2.3)	-10.7 (3.9)	-14.2 (2.1)	-3.9 (1.2)	-3.8 (0.5)
$\Delta a254:a365_{0-5}$	8.1 (14.8)	12.5 (15.5)	-2.1 (2.1)	-2.7 (2.1)	-6.9 (3.6)	-4.1 (4.3)
$\Delta S275:295_{0-5}$	4.2	4.6	-5.5	-5.5	-4.3	-4.2

	(2.9)	(3.0)	(0.6)	(0.6)	(1.6)	(2.0)
$\Delta SUVA_{0-5}$	44.1 (3.8)	53.9 (3.9)	2.4 (1.0)	3.8 (2.1)	-1.7 (2.4)	-2.9 (2.7)
$\Delta peak\ B_{0-5}$	1.2 (1.7)	8.2 (2.0)	25.3 (10.2)	11.3 (7.6)	-4.2 (8.7)	-7.7 (9.7)
$\Delta peak\ C_{0-5}$	-4.2 (0.9)	-2.0 (0.8)	8.0 (1.1)	8.1 (1.3)	11.39 (0.8)	10.0 (0.6)
$\Delta peak\ T_{0-5}$	-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_{cum}) 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

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

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

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

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

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

3.4.1. Relative changes (relative Δ values, %, till day 5) in measured variables.

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

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 ΔDOC values (DOC
472 utilisation) during the active phase of the microcosm incubation (DOC v $\Delta DOC_{1\text{-}5}$, *n*
473 = 28, $R^2 = 0.91$, p = <0.001) and with lower BGE ($BGE_{1\text{-}5}$ v DOC, *n* = 23, $R^2 = 0.79$, p
474 = <0.001). However, high DOC utilization correlated with low BGE ($BGE_{1\text{-}5}$ v
475 $\Delta DOC_{1\text{-}5}$, *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 $\Delta BA_{0\text{-}5}$, n = 33, R^2 = 0.65, p = <0.001 and TP v $\Delta BA_{0\text{-}5}$, n = 33, R^2 = 0.64, p =
481 <0.001). Lower starting C:N ratios had a positive effect on BGE (lnC:N v $lnBGE_{1\text{-}5}$, n
482 = 23, R^2 = 0.71, p = <0.001), with the highest BGE recorded at C:N ratios of ~23.
483 However, at higher C:N ratios DOC utilisation was larger (C:N v $\Delta DOC_{0\text{-}5}$, n = 28, R^2
484 = 0.76, p = <0.001).
485
486 Microcosm units exhibiting low BGE values exhibited larger relative increases in
487 $SUVA_{254}$ ($lnBGE_{1\text{-}5}$ v ln normalised % $\Delta SUVA_{0\text{-}5}$, n = 23, R^2 = 0.47, p = <0.001),
488 while those exhibiting higher BGE showed smaller increases in $SUVA_{254}$ or even
489 decreases. The opposite trend was observed for peak C, with relative decreases in
490 peak C at lower BGE values ($lnBGE_{1\text{-}5}$ v ln normalised % Δpeak $C_{0\text{-}5}$, 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 (DOC v Δpeak $C_{0\text{-}5}$, 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.

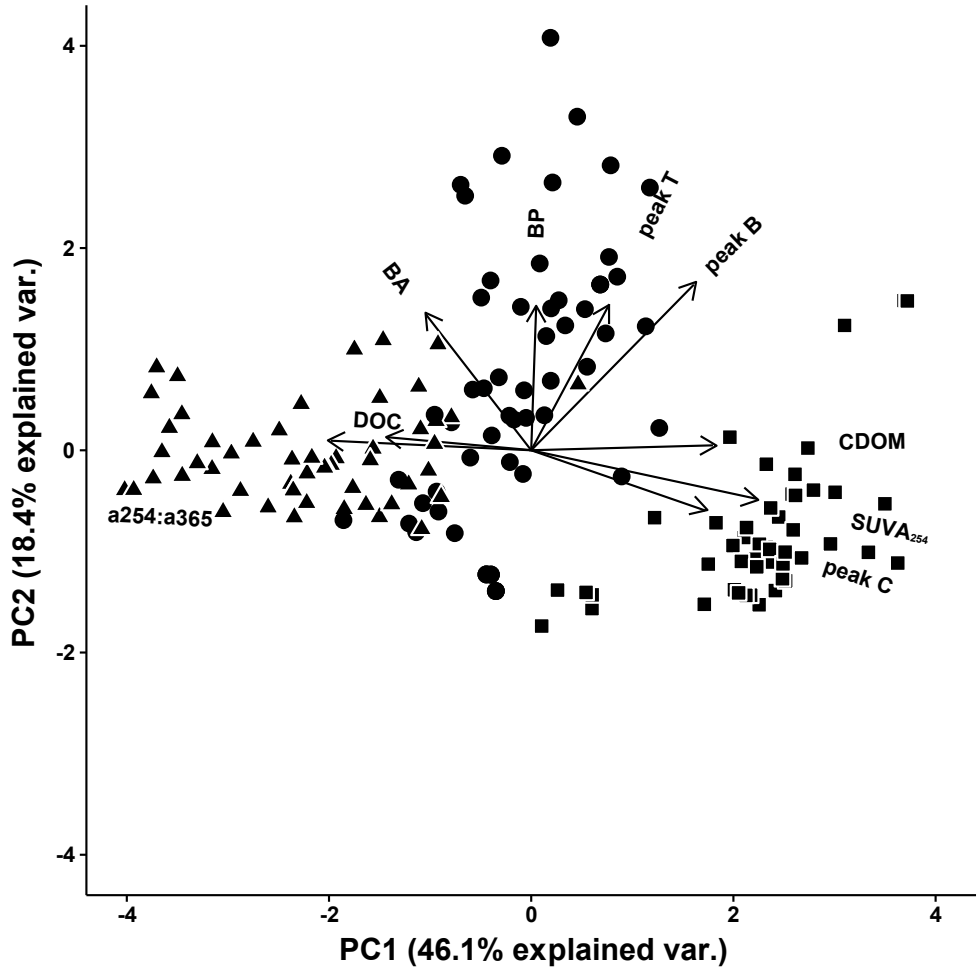

Figure 3. Principal component analysis (PCA) of bacterial and DOM characteristic variables from all standard (+NP excluded) microcosm units and all sampling occasions (Baltic Proper, triangles; Bothnian Sea, circles; and Bothnian Bay, squares). PC1 and PC2 encompass 64.5 % of the cumulative variance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

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 S275-295 and a254:a365
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

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

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Supplementary tables, figures and information from this point onwards:

Table S1. Analysis of variance (ANOVA) for physicochemical variables differences between basins. Key: p = <0.001***, <0.01**, <0.05*, not significant^{ns}.

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

Table S2. Start and end concentrations of TN and TP in microcosm units (n = 11-12, SD) from the Bothnian Sea and Bothnian Bay, standard and +NP treatment.

	TN Start µmol L^{-1}	TN End µmol L^{-1}	TP Start µmol L^{-1}	TP End µmol 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)

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

Dependent variable	d.f.	*F*	Basin	Treatment (+/- NP)	Interaction
BGE_{1-5}	2	15.5	<0.001***	ns	ns
$BPcum_{1-5}$	2	41.0	<0.001***	ns[1]	ns
ΔBA_{0-5}	2	90.2	<0.001***	0.000***	<0.001***
ΔDOC_{1-5}	2	40.6	<0.001***	ns	ns
$\Delta a254:365_{0-5}$	2	1.7	ns	ns	ns
$\Delta s275:295_{0-5}$	2	14.5	<0.001***	ns	ns
$\Delta SUVA_{0-5}$	2	197.4	<0.001***	ns	ns
Δpeak B_{0-5}	2	5.2	0.008**	ns	ns
Δpeak C_{0-5}	2	116.3	<0.001***	ns	ns
Δpeak T_{0-5}	2	6.0	0.004**	ns	ns

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

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

	Comparison	Mean difference	Significance
BP	Baltic Proper – Bothnian Sea	-11.07	<0.001***
	Baltic Proper – Bothnian Bay	-1.97	0.331
	Bothnian Sea – Bothnian Bay	9.11	<0.001***
BA	Baltic Proper – Bothnian Sea	1771896	<0.001***
	Baltic Proper – Bothnian Bay	2579699	<0.001***
	Bothnian Sea – Bothnian Bay	807802	<0.001***
DOC	Baltic Proper – Bothnian Sea	2.28	<0.001***
	Baltic Proper – Bothnian Bay	2.67	<0.001***
	Bothnian Sea – Bothnian Bay	0.39	<0.001***
a254-365	Baltic Proper – Bothnian Sea	3.41	<0.001***
	Baltic Proper – Bothnian Bay	5.50	<0.001***
	Bothnian Sea – Bothnian Bay	2.11	<0.001***
$SUVA_{254}$	Baltic Proper – Bothnian Sea	-1.14	<0.001***
	Baltic Proper – Bothnian Bay	-2.55	<0.001***
	Bothnian Sea – Bothnian Bay	-1.41	<0.001***
peak 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
peak C	Baltic Proper – Bothnian Sea	-0.006	0.158
	Baltic Proper – Bothnian Bay	-0.099	<0.001***
	Bothnian Sea – Bothnian Bay	-0.093	<0.001***

peak T	Baltic Proper – Bothnian Sea	0.008	0.190
	Baltic Proper – Bothnian Bay	0.000	1.000
	Bothnian Sea – Bothnian Bay	-0.008	0.210

Key: p = <0.001***.

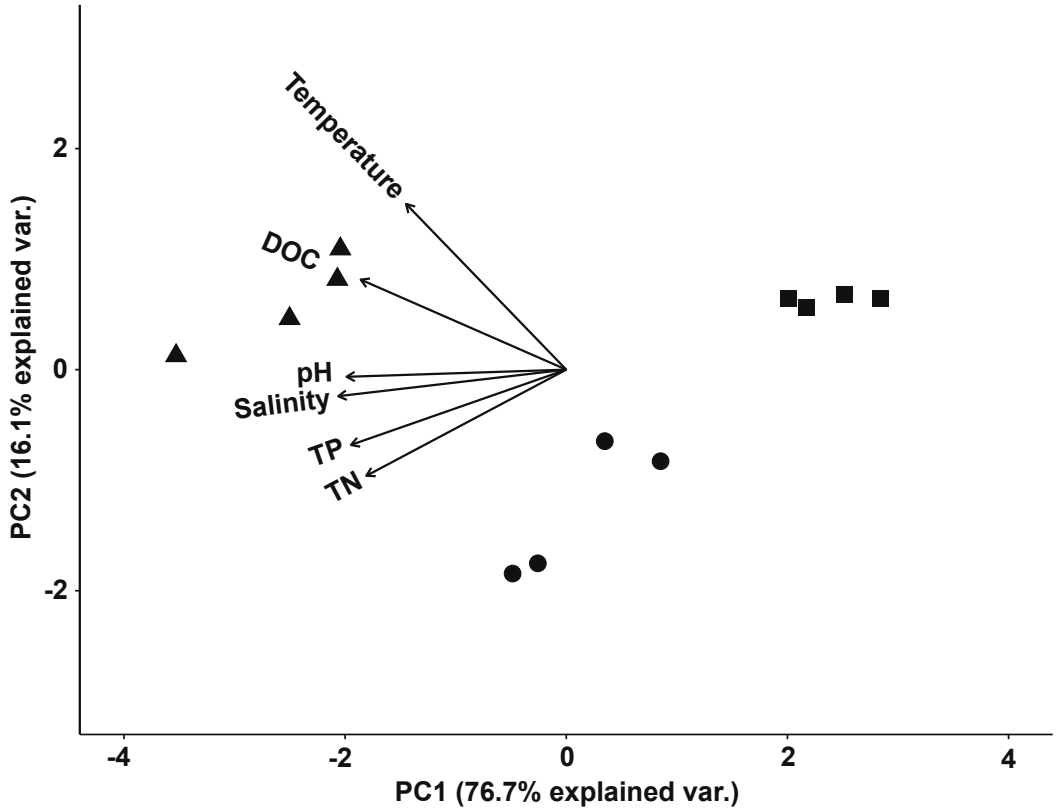

Figure S1. Principal component analysis (PCA) based on in-situ physicochemical variables collected at 12 stations, four per basin (Baltic Proper, triangles; Bothnian Sea, circles; and Bothnian Bay, squares). PC1 and PC2 encompass 93 % of the cumulative variance in the data set. PC1 (77 % of variance)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

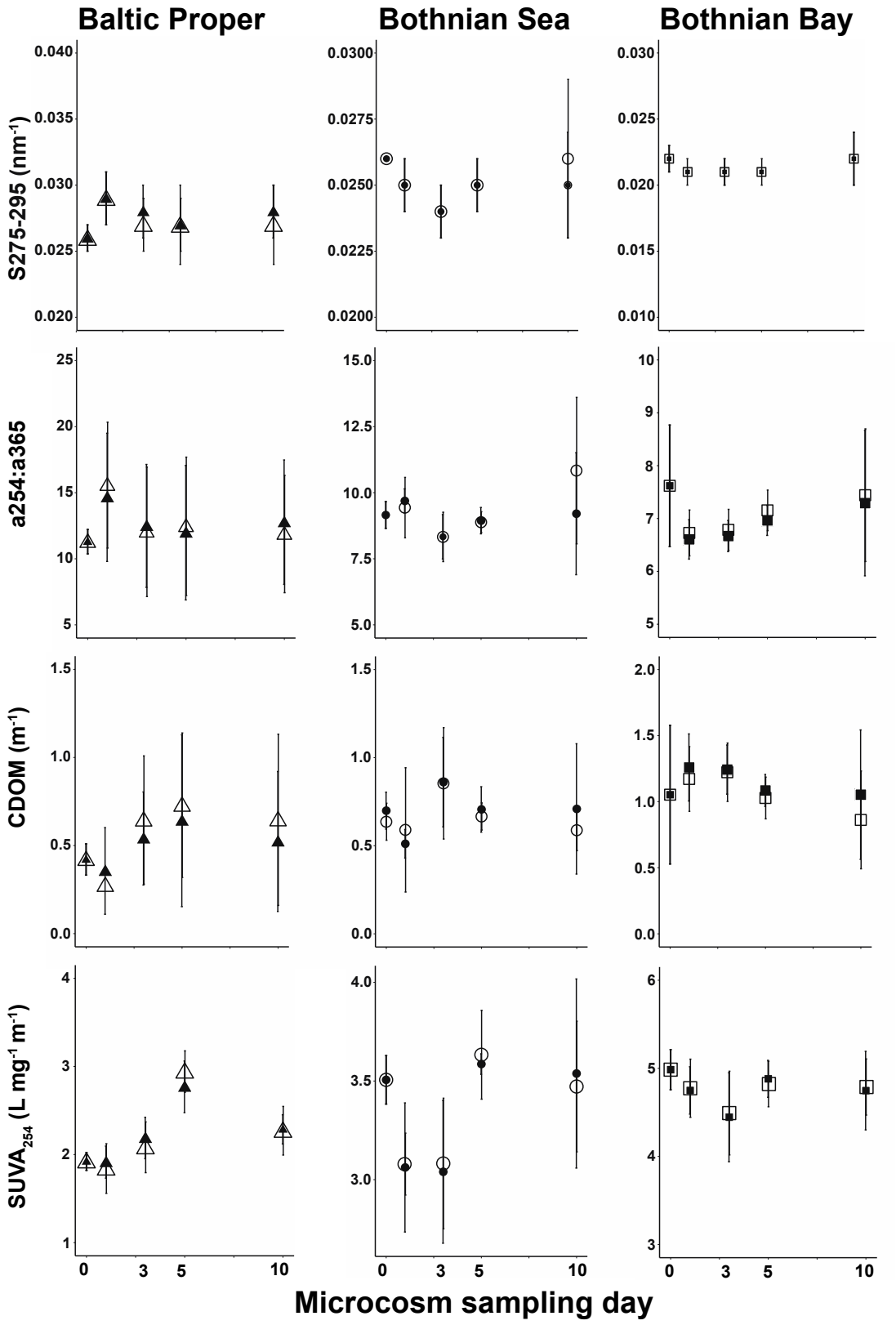

Figure S2. Fluctuation in optical DOM characteristic variables during microcosm incubation. Data are presented by basin (Baltic Proper, triangles; Bothnian Sea,

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

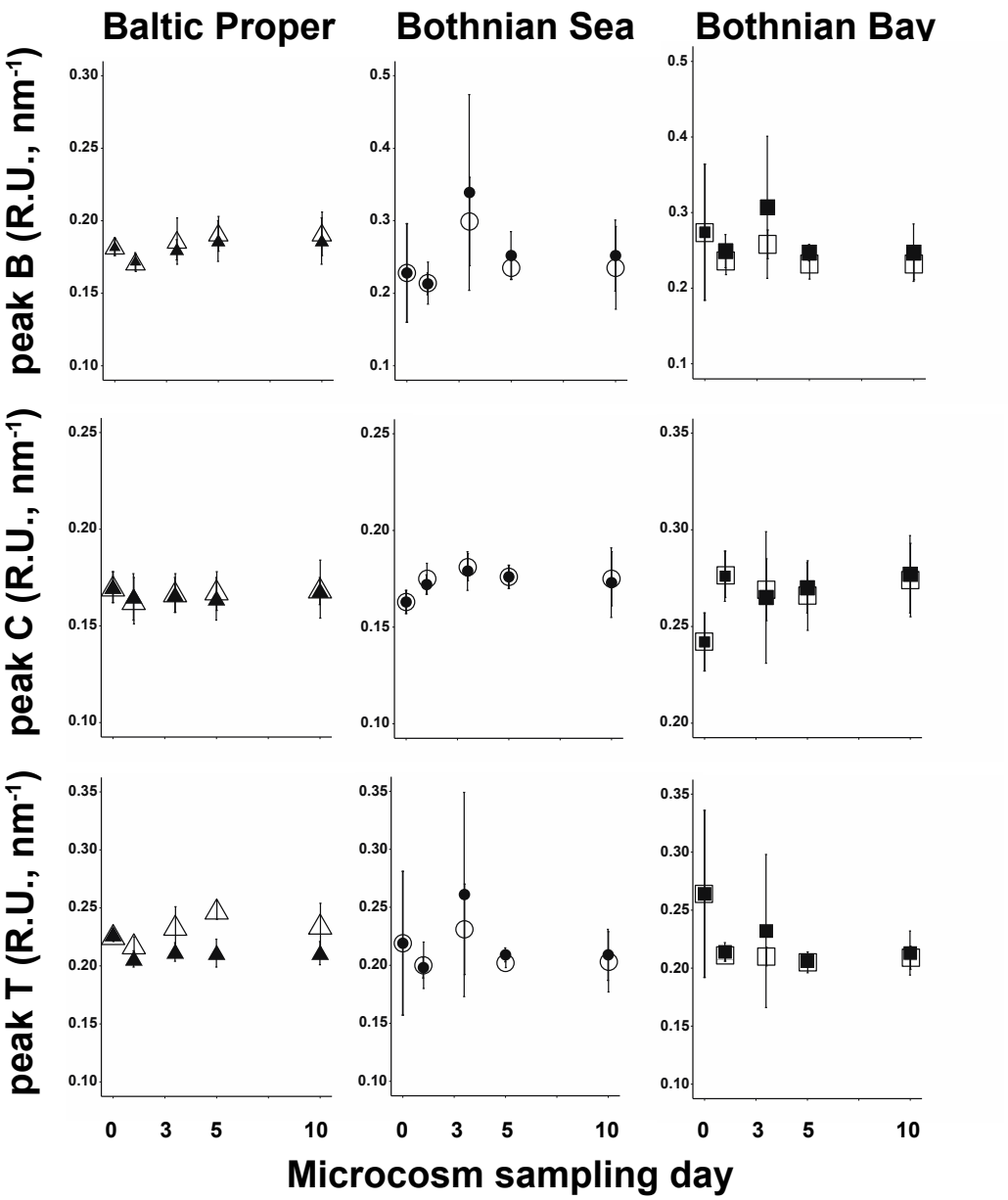

Figure S3. Fluctuation in DOM characteristic fluorescent peaks during microcosm incubation. Data are presented by basin (Baltic Proper, triangles; Bothnian Sea, circles; and Bothnian Bay, squares), with standard (filled symbols) and +NP treatments (open symbols) shown. Mean values are shown with standard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

Supplementary results: other associations between variables.

$SUVA_{254}$ and CDOM correlated spatially (all basins and all microcosm sampling events (ALL): $n = 172$, $R^2 = 0.49$, p = <0.001). Similar but stronger spatial correlations were recorded between S275-295 and $SUVA_{254}$ (ALL: $n = 178$, $R^2 = 0.72$, p = <0.001) and CDOM (ALL, $n = 178$, $R^2 = 0.75$, p = <0.001). A spatial correlation was also found between peak C and $SUVA_{254}$ (ALL: $n = 178$, $R^2 = 0.69$, p = <0.001) and S275-295 (ALL: $n = 178$, $R^2 = 0.69$, p = <0.001).

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

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

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