Production of bioavailable and refractory dissolved organic matter by coastal heterotrophic microbial populations

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Abstract

Production of dissolved organic matter (DOM) by heterotrophic microbial communities isolated from Loch Creran (Scotland) was studied in time course incubations in which cells were re-suspended in artificial seawater amended with variable proportions of glucose, ammonium and phosphate. The incubation experiments demonstrated that microheterotrophs released part of the substrate as new DOM, with a production efficiency of 11 ± 1 % for DOC, 18 ± 2 % for DON and 17 ± 2 % for DOP. Estimating the impact of this production in Loch Creran, showed that from 3 ± 1 % (DOC) to 72 ± 16 % (DOP) of DOM could originate from the heterotrophic microbial community. The produced DOM (PDOM) was both bioavailable (BDOM) and refractory (RDOM). Bioavailability as assessed by the difference between the maximum and the end DOM concentration, was generally higher than found in natural systems, with DOP (73 ± 15 %, average ± SD) more bioavailable than DON (70 ± 15 %), and DON than DOC (34 ± 13 %).

The stoichiometry of PDOM was linked to both nutrient uptake and BDOM ratios. Absorption and fluorescence of DOM increased significantly during the incubation time, indicating that microheterotrophs were also a source of coloured DOM (CDOM) and that they produce both bioavailable protein–like and refractory humic–like fluorophores.

Key words: DOM, coloured DOM, bioavailable DOM, refractory DOM, stoichiometry, microbial heterotrophy

1. Introduction

River inputs of inorganic nutrients support high primary production rates in coastal marine ecosystems (Lalli & Parsons, 1997), which lead to the release of significant amounts of autochthonous dissolved organic matter (DOM) (Nagata, 2000). Although heterotrophic microbial communities are the major consumers of this DOM (Seitzinger & Sanders, 1997), considerably less attention has been paid to their ability to produce DOM. Some studies have suggested that microheterotrophs could be a significant but hardly quantified source of bioavailable and refractory DOM in marine systems (Ogawa et al., 2001; Kawasaki & Benner, 2007).

Release of DOM by phytoplankton has been repeatedly observed in both laboratory and field studies, with exudation rates, stoichiometry and availability being related to nutrient levels (e.g. Obernosterer & Herndl, 1995). Some studies have suggested that bacteria are able to alter their C:N:P biomass ratios depending on the organic substrate ratios (e.g. Tezuka, 1990), while others find constant biomass ratios (Golman et al. 1987). However, the effect of changing C:N:P substrate ratios on the heterotrophic microbial production of DOM is largely unknown.

Heterotrophic microbes have been shown to produce chromophoric DOM (CDOM) during mineralization processes (e.g. Rochelle–Newall & Fisher, 2002). CDOM absorbs light in the ultraviolet (UV) region declining to near–zero levels in the red region of the spectrum (Stedmon & Markager 2001). A part of the absorbed light is reemitted at longer wavelengths (FDOM), with two main DOM flourophores being identified: the protein– and humic–like (Coble et al. 1990; 1996). Protein–like fluorescence (FDOMt) has been

suggested as an indicator of freshly produced DOM, while humic–like flourophores (FDOMm) characterize older, more refractory DOM (Coble et al. 1996).

We hypothesised that marine heterotrophic microbial communities could be a quantitative important source of DOM in coastal marine waters, with the stoichiometry of the produced DOM depending on the substrate ratios. This was investigated by isolating coastal heterotrophic microbial populations from Loch Creran, Scotland and growing the community in 0.2 μm filtered artificial seawater, amended with carbon (glucose), inorganic nitrogen (ammonium) and phosphorus (phosphate) as growth media. Over the time course of the experiment dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP) concentrations, as well as DOM fluorescence were monitored as described below.

2. Material and methods

2.1. Incubation experiments

Samples for the microbial inoculum were obtained during winter and spring (16 January and 20 March 2007) from a depth of 5 m in the Scottish fjord Loch Creran. These sampling dates were chosen to investigate the production of DOM by microbial heterotrophic populations collected under different biological and hydrological conditions (See Lønborg et al. 2009).

The artificial seawater was prepared with water treated in a Milli-Q ultraviolet (UV) plus purification unit, which resulted in low carbon and nutrient content. NaCl, KCl, $NaHCO_3$, Na_2SO_4, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$, KBr, H_3BO_3, NaF, $SrCl_2{\cdot}6H_2O$, $Na_2SiO_3{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, $MnSO_4{\cdot}6H_2O$, $ZnSO_4{\cdot}9H_2O$ and $CoSO_4{\cdot}9H_2O$ modified from Harrison et al. 1980, were added to the Milli–Q water to reach salinity levels equal to the

79 microbial inoculum, 28.2 (16 January) and 28.3 (20 March) repectively. NaCl, Na_2SO_4, and
80 KCl were combusted (450°C for 4 hours) before use to remove organic carbon. Glucose
81 ($C_6H_{12}O_6$), ammonium (NH_4Cl) and phosphate (KH_2PO_4) were added as substrate and pH
82 was adjusted to 8.0 with either HCl or NaOH. The microbial inoculum was prepared by
83 gravity filtering the seawater from Loch Creran two times through precombusted GF/F
84 filters. The GF/F filtrate was thereafter inoculated into the media in a 1: 50 dilution. A total
85 of 16 incubations were conducted in duplicate in two litre amber glass bottles and incubated
86 at a constant temperature of 14ºC in the dark. The DOC concentration of the artificial
87 seawater, 20 µmol L^{-1}, originated from the added salts and the inoculum it was considered
88 refractory and was therefore subtracted from all samples. If we consider that the 20 µmol
89 L^{-1} is 100% bioavailable and that the microbes would convert 11% (as found in this study)
90 of this DOC into new DOC, this would lead to an underestimation of the microbial
91 produced pool of ~ 2µmol L^{-1} and 1.5µmol L^{-1} for the refractory pool (considering a
92 bioavailability of 33%).
93 Dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentrations of the
94 artificial seawater were below the detection limit. Glucose, ammonium and phosphate
95 concentrations added to the medium ranged from 163 to 867 µmol L^{-1} of C, 21.4 to 157.8
96 µmol L^{-1} of N, and 1.3 to 13.6 µmol L^{-1} of P (Table 1). C:N ratios of the added substrate
97 varied between 2 and 17, C:P ratios from 32 to 311 and 5 to 57 for N:P. These ratios were
98 chosen to vary widely around the average C:N:P ratio of bacterial biomass (50:10:1)
99 (Fagerbakke et al. 1996).
100 Since dissolved organic nitrogen (DON) and phosphorus (DOP) concentrations were
101 below the detection limit at the beginning of the experiments, it has been assumed that the
102 DON and DOP produced during the experiment was of microbial origin, and was named as

produced DOM (PDOM). Sub–samples were collected at days 0, 3, 11, 30, 50 and 100 after the beginning of the experiments to follow: dissolved organic matter absorbance (CDOM) and fluorescence (FDOM), glucose, DOC, DIN, DIP, total dissolved nitrogen (TDN) and phosphorus (TDP), total organic carbon (TOC), total nitrogen (TN) and phosphorus (TP). Samples for the dissolved phase were filtered through prewashed (> 1 litre of sterile filtered Milli–Q water) 0.2 μm polycarbonate membranes. Samples for CDOM analysis were stored in amber glass bottles at 4°C in the dark. Glucose and FDOM samples (20 mL) were collected in pre–combusted (450°C for 6 hours) glass ampoules and stored frozen (–20ºC) in the dark until analyzed. The effect of freezing the FDOM samples was measured by taking samples over time with no major impact found. Samples (10 ml) for TOC, DOC, TN and TDN analysis were collected in pre–combusted (450°C for 6 hours) glass ampoules and preserved by adding 10 μL 85 % H_2PO_4. Samples for DIN and DIP were stored in acid washed polyethylene bottles and kept frozen (–20ºC). TDP and TP samples were collected in glass bottles and frozen (–20ºC) until analysis. All glassware used was acid–washed (2 μmol L^{-1} HCl) for 24 hours and washed three times with Milli–Q water before use.

2.3. Sample analysis

The absorption of CDOM was measured in four replicates in a Thermo Nicolet Evolution 300 Turn spectrophotometer using Milli–Q water as a blank. Before analysis samples and Milli–Q water were warmed to room temperature. The absorption was measured in a 1 cm quartz cuvette at 375 nm. The absorption coefficients (α_λ) were calculated as follows:

$$\alpha_\lambda = \frac{2.303 \cdot A_\lambda}{L}$$

125 Where A_λ is the optical density and the denominator L is the cell path–length in metres
126 (Stedmon & Markager, 2001).
127 FDOM was measured in duplicate in a Perkin Elmer LS 55 luminescence
128 spectrophotometer. The instrument was equipped with a xenon discharge lamp, using a 1
129 cm quartz fluorescence cell. Milli–Q water was used as a reference, and subtracted the
130 FDOM measurements. Discrete excitation/emission (Ex/Em) pair measurements were
131 performed at peaks T (aromatic amino acids, average Ex/Em, 280/350 nm; FDOMt) and M
132 (marine humic substances, average Ex/Em 320/410 nm; FDOMm), according to Nieto–Cid
133 et al. (2006). Following Nieto Cid et al. (2006), fluorescence units were expressed in ppb
134 equivalents of trypthophan (ppb Trp) for FDOMt and ppb equivalents of quinine sulphate
135 (ppb QS) for FDOMm.
136 Glucose was measured in triplicate at days 0, 3, 11 and 30, using the enzyme assay
137 described by Hicks & Carey (1968). Concentrations were determined using a four-point
138 standard curve following the subtraction of a Milli–Q blank. DOC, TOC, TDN and TN
139 were measured in at least triplicate, using a nitrogen–specific Antek 7020 nitric oxide
140 chemiluminescence detector, coupled in series with the carbon–specific infrared gas
141 analyser of a Shimadzu TOC–5000 organic carbon analyser. Three to five replicate
142 injections of 150 µl were performed per sample. Concentrations were determined by
143 subtracting a Milli–Q blank and dividing by the slope of a daily standard curve. DON
144 concentrations were obtained by subtracting DIN from TDN (DON = TDN – DIN), with
145 the standard error calculated as $SE^2_{DON} = SE^2_{TDN} + SE^2_{DIN}$. DIN (ammonium and
146 nitrate+nitrite) was measured in four replicates with a Lachat (quickchem 500) auto
147 analyzer using standard protocols (Hansen & Grasshoff 1983). Triplicate samples were
148 analyzed for DIP by the standard molybdenum blue technique using a 5 cm cuvette

149 (Koroleff, 1983). TDP and TP were measured in triplicate by oxidation to soluble reactive
150 phosphorous with the addition of sulphuric acid and persulphate (Koroleff, 1983),
151 following autoclaving at 100°C for 90 min. The standard error for DOP was estimated as:
152 $SE^2_{DOP} = SE^2_{TDP} + SE^2_{SRP}$. POM concentrations were calculated as the difference between
153 TOC and DOC for POC, TN and TDN for PON and TP and TDP for POP. The
154 corresponding standard errors where calculated as $SE^2_{POC} = SE^2_{TOC} + SE^2_{DOC}$, $SE^2_{PON} =$
155 $SE^2_{TN} + SE^2_{TDN}$ and $SE^2_{POP} = SE^2_{TP} + SE^2_{TDP}$, respectively.
156 Regression model II was used to examine the linear relationship between pairs of variables
157 (Sokal & Rohlf 1995). In the cases where the intercept was not significantly different from
158 zero, the intercept was set to zero and a new slope was calculated. The confidence level was
159 set at 95% with the statistical analysis conducted in Statistica 6.0.

160 **3. Results**

161 3.1. *Production of DON and DOP by heterotrophic microbial populations*

162 TN (Fig. 1b) and TP (Fig. 1c) remained approximately constant throughout the
163 incubations, TOC decreased rapidly during the first phase of all the cultures (initial 30
164 days) and thereafter more slowly (Fig. 1a). Particulate organic matter (POM) reached
165 maximum values at day 10 with values between 12 and 138 μmol L^{-1} for POC (Fig. 1a),
166 4.7 to 49.3 μmol L^{-1} for PON (Fig. 1b) and 0.6 to 4.4 μmol L^{-1} for POP (Fig. 1c). DIN and
167 DIP concentrations were not significantly different from TN and TP at the beginning of the
168 incubations. The time profile of nutrients concentrations was V–shaped, with a pronounced
169 decrease to a minimum during the first and an increase during the second phase of the
170 cultures (Figs 1b, c). Nitrate+nitrite concentrations were undetectable at the beginning of
171 the incubations, but increased from day 50 onwards in all experiments, concomitantly with

172 the corresponding decrease of ammonium (Fig. 1d). The total uptake of DIN (UDIN) and
173 DIP (UDIP) (Table 1) was calculated as UDIN = DIN_0– LDIN and UDIP = DIP_0 – LDIP,
174 where DIN_0 and DIP_0 are the initial nutrient concentrations and LDIN, LDIP are the
175 minimum DIN and DIP concentrations obtained at the end of the first phase of the cultures.
176 DOM maximum was found at day 30 of the incubations (Fig. 2), associated with complete
177 depletion of the added glucose (data not shown). The produced DOM (PDOM) reached
178 concentrations of 38 to 81 μmol L^{-1} for PDOC, 2.2 to 9.1 μmol L^{-1} for PDON, and 0.24 to
179 0.78 μmol L^{-1} for PDOP (Table 1). Calculating the bioavailability (BDOM) as the
180 difference between PDOM and the refractory DOM (RDOM) at the end of the incubations,
181 it resulted that 33 ± 12 % (average ± SD) of PDOC, 70 ± 16 % of PDON and 79 ± 14 % of
182 PDOP were bioavailable. The PDOM C:N ratios varied between 6 and 16, C:P ratios from
183 20 to 224 and 5 to 21 for N:P. Resulting C:N:P ratios varied between 20: 5: 1 and 225: 14:
184 1 (average 103: 11: 1). The stoichiometry of PDOM was linearly related with the C: N: P
185 ratios of UDOC, UDIN and UDIP (Fig 3a, c, e).

186 *3.3. Production of chromophoric DOM*

187 Table 3a shows how a375 increased from being undetectable at the beginning of the
188 incubations reaching an average value of 0.47 ± 0.26 m^{-1} at the end. In parallel to the
189 production of CDOM, FDOM also increased during the incubations above background
190 levels in all cases, with a mean (± SE) of 12.9 ± 5.6 ppb Trp for FDOMt and 6.4 ± 3.8 ppb
191 QS, for FDOMm (Table 3b, c). The amounts of FDOMt and FDOMm produced increased
192 with substrate uptake and produced DOM (Table 4).

193 **4. Discussion**

194 Single–substrate approaches as used in this study are an obvious simplification of
195 reality, and will not fully represent the natural microbial growth substrate. These
196 experiments should therefore be seen as a first trial to quantify heterotrophic microbial
197 production of DOM.
198 Two contrasting phases have been identified during all the incubations (Fig. 1) : i) an
199 initial net-anabolic phase, when the added glucose was still detectable, as characterised by
200 the net consumption of the glucose, DIN and DIP and a net production of DOM; and ii) a
201 final net-catabolic phase, where glucose was undetectable, characterised by the net
202 production of DIN and DIP and the net consumption of the DOM produced during the
203 anabolic phase of the cultures. Goldman and Dennet (1991) also observed in their cultures
204 that ammonium was taken up as long as a readily assimilable carbon source such as glucose
205 was present, whilst nutrient regeneration was evident only after glucose was completely
206 utilized.

207 *3.1 Production of DON and DOP by heterotrophic microbial populations*

208 POM and inorganic nutrients concentrations showed opposite patterns, POM
209 increased during the net-anabolic phase of the cultures, reaching maximum values at day 10
210 (Fig. 1a-c) and subsequently decreased. The increase in nitrate+nitrite following the
211 decrease in ammonium concentrations (Fig. 1d), suggested, that ammonium was consumed
212 i) as a substrate during the net-anabolic phase of the cultures (Kirchman, 1994); and ii) as
213 an energy source during the net-catabolic phase of the cultures on basis of the complete
214 oxidation of ammonium to nitrate, i.e. nitrification (Wada & Hattori, 1991).
215 In the net-anabolic phase, the use of glucose and inorganic nutrients by the cultured
216 community of microheterotrophs was accompanied by increases in DON and DOP

concentrations (Fig 1a, c). We hypothesise that bacteria take up the dissolved substrate, converting it into organic matter which is subsequently released as DOM by direct extra cellular release and/or by protist grazing and viral lysis (Caron et al., 1985; Riemann & Middelboe, 2002; Kawasaki & Benner, 2007). A DOM maximum was found at day 30 of the incubations (Fig. 2), associated with complete depletion of the added glucose (data not shown). The efficiency of DOM production was found using the slope of the linear regressions between UDOC (glucose) and PDOC (11 ± 1 %), UDIN and PDON (18 ± 3 %), and UDIP and PDOP (17 ± 3 %) (Table 2), which was comparable findings in similar experiments (Kawasaki & Benner, 2007).

In the net-catabolic phase of the incubations, DOM concentrations decreased until the end of the experiments (Fig. 1; Fig. 2). The calculated bioavailability (BDOM) suggest that PDOC and PDON were more bioavailable than found in natural marine waters (9–30 %, DOC; 30–40 %, DON; Hopkinson et al., 2002; Lønborg & Søndergaard, 2008), while PDOP bioavailability was comparable with natural systems (70–80%; Nausch & Nausch, 2006). Thereby PDOP was more bioavailable than PDON, and PDON more than PDOC consistently with findings in natural marine systems (Hopkinson et al., 2002). The slopes of the linear regressions between BDOM and PDOM indicate that most of the variations in PDOM were due to the bioavailable fraction (Table 2). The significant origin intercepts of those regressions together with the significant DOM end levels (Table 1, Fig. 2) showed that part of the PDOM persisted at the end of the incubations, demonstrating that the microbial communities also produce refractory DOM (RDOM) in agreement with previous studies (Ogawa et al., 2001; Kawasaki & Benner, 2007). Estimating the environmental impact of the microbial DOM production, we can use published DOC and nutrient data from Loch Creran (Lønborg et al. 2009), together with average efficiency of DOC (11 ± 1

241 %), DON (18 ± 3 %), and DOP (17 ± 3 %) production found in this study. These
242 calculation showed that the heterotrophic microbial community on average could produce 5
243 ± 2 μM DOC, 0.7 ± 0.5 μM DON and 0.08 ± 0.04 μM DOP, corresponding to 3 ± 1 %, 8 ±
244 7 % and 72 ± 16 % of the measured DOC, DON and DOP in Loch Creran.

245 The average PDOM C:N:P stoichiometry (103: 11: 1), was generally richer in
246 carbon than typically found for bacterial biomass (50: 10: 1; Goldman et al., 1987;
247 Fagerbakke et al., 1996), and more N and P rich than for both algae produced (170: 6.5: 1;
248 Conan et al., 2007) and marine bulk DOM (300: 22: 1; Benner, 2002). The stoichiometry of
249 PDOM was linearly related with the C: N: P ratios of UDOC, UDIN and UDIP (Fig 3a, c,
250 e). The slopes of the linear regressions show that PDOM had C:N and C:P ratios which
251 were 63 ± 13 % and 68 ± 14 % lower than the substrate, while the N:P ratios (105 ± 15 %)
252 were similar, indicating that the stoichiometry of PDOM is linked to the substrate ratios.
253 The fact that the C:N and C:P ratios of PDOM were 30– 40 % lower than the corresponding
254 substrate ratios can be attributed to the bacterial respiration of glucose, which previously
255 has been found to represent 30–60 % of the total consumption (Bianchi et al., 1998). The
256 stoichiometry of BDOM was similar to ranges reported for bacterial C: N: P ratios
257 (Goldman et al., 1987; Fagerbakke et al., 1996). The slopes of the correlation between
258 PDOM and BDOM stoichiometries (Fig. 3b, d, f) indicate that BDOM had C:N and C:P
259 ratios that were 53 ± 19 % and 44 ± 8 % lower respectively, while N:P ratios (92 ± 25 %)
260 were not significantly different from PDOM.

261 *4.2. Production of chromophoric DOM*

262 Recent studies suggest that marine bacteria produce CDOM and FDOM in coastal
263 ecosystems depending on the chemical composition of the substrate (e.g. Rochelle–Newall

264 & Fisher, 2002; Yamashita & Tanoue, 2008). Table 3a shows how a375 increased both
265 during the net-anabolic and net-catabolic phase from being undetectable at the beginning of
266 the incubations reaching higher values (average - 0.47 ± 0.26 m^{-1}) at the end. This increase
267 indicates microbial production of coloured high molecular weight aromatic DOM (Pages &
268 Gadel, 1990). In parallel to the production of CDOM, FDOM also increased during both
269 the net-anabolic and net-catabolic phase above background levels in all cases (Table 3b, c),
270 in agreement with previous studies (Kramer & Herndl, 2004; Yamashita & Tanoue, 2004).
271 The amounts of FDOMt and FDOMm produced increased with substrate uptake and
272 produced DOM (Table 4). Although protein–like fluorophores have been suggested to
273 represent freshly produced DOM (Yamashita & Tanoue, 2004), our study found links
274 between FDOMt and DOM bioavailability, but also the production of refractory FDOMt. It
275 firstly indicates that protein–like fluorophores not only provide a measure of labile
276 material; and secondly suggests that biological processes could be a source of refractory
277 FDOMt. FDOMm was related linearly with both BDOC and RDOC, confirming it as a
278 product of microbial degradation processes (Yamashita & Tanoue, 2004; 2008; Nieto–Cid
279 et al., 2006).

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284

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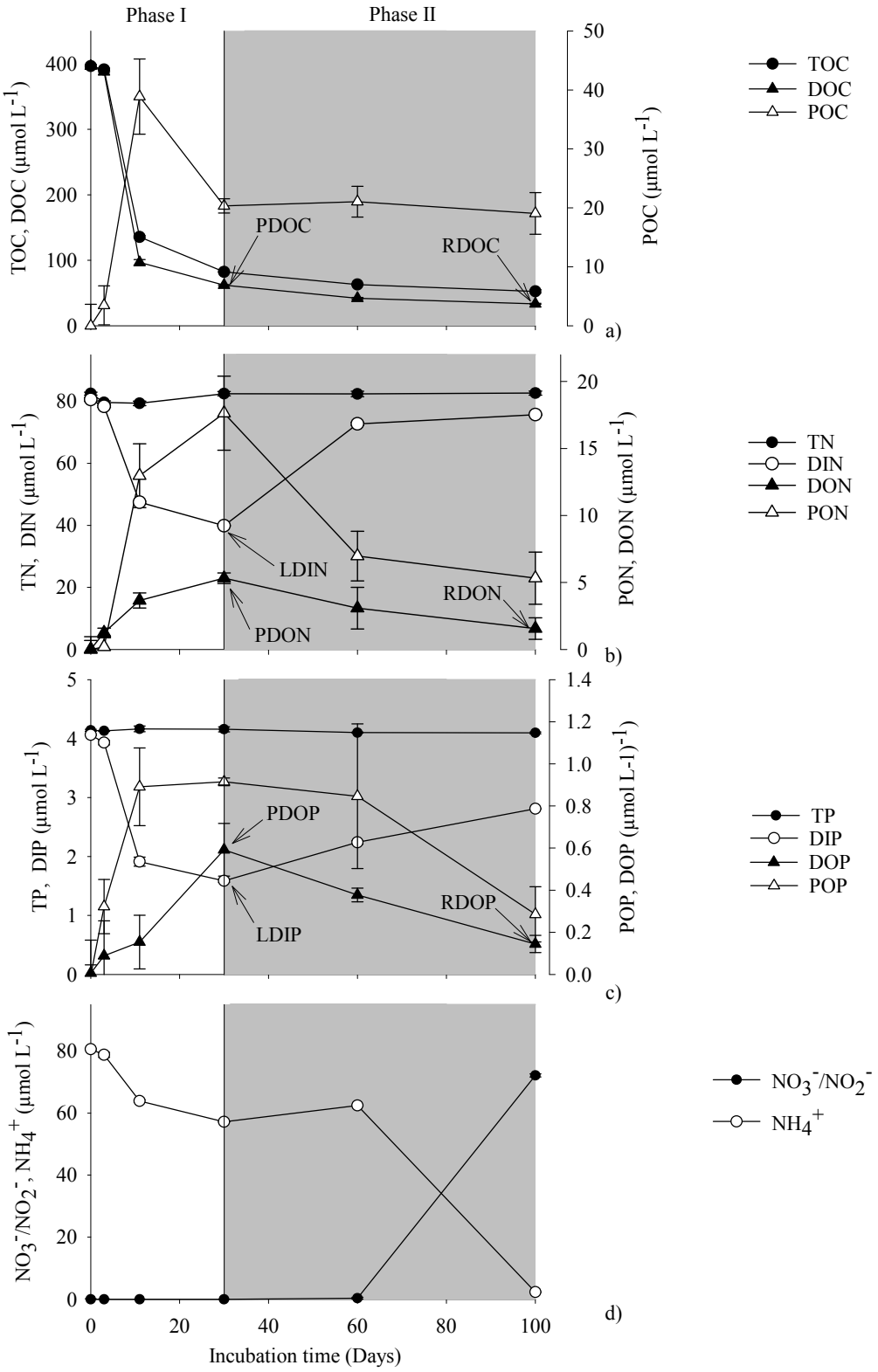
Figure legends

Figure. 1. Examples of time evolution (Exp. 1 shown in table 1), of a) total (TOC) particulate (POC) and dissolved organic carbon (DOC), b) total nitrogen (TN), particulate organic nitrogen (PON), dissolved inorganic (DIN) and organic (DON) nitrogen; c) total phosphorus (TP), particulate organic phosphorus (POP), dissolved inorganic (DIP) and organic (DOP) phosphorus; d) nitrate/nitrite (NO_3^-/NO_2^-) and ammonium (NH_4^+). Error bars represent standard errors.

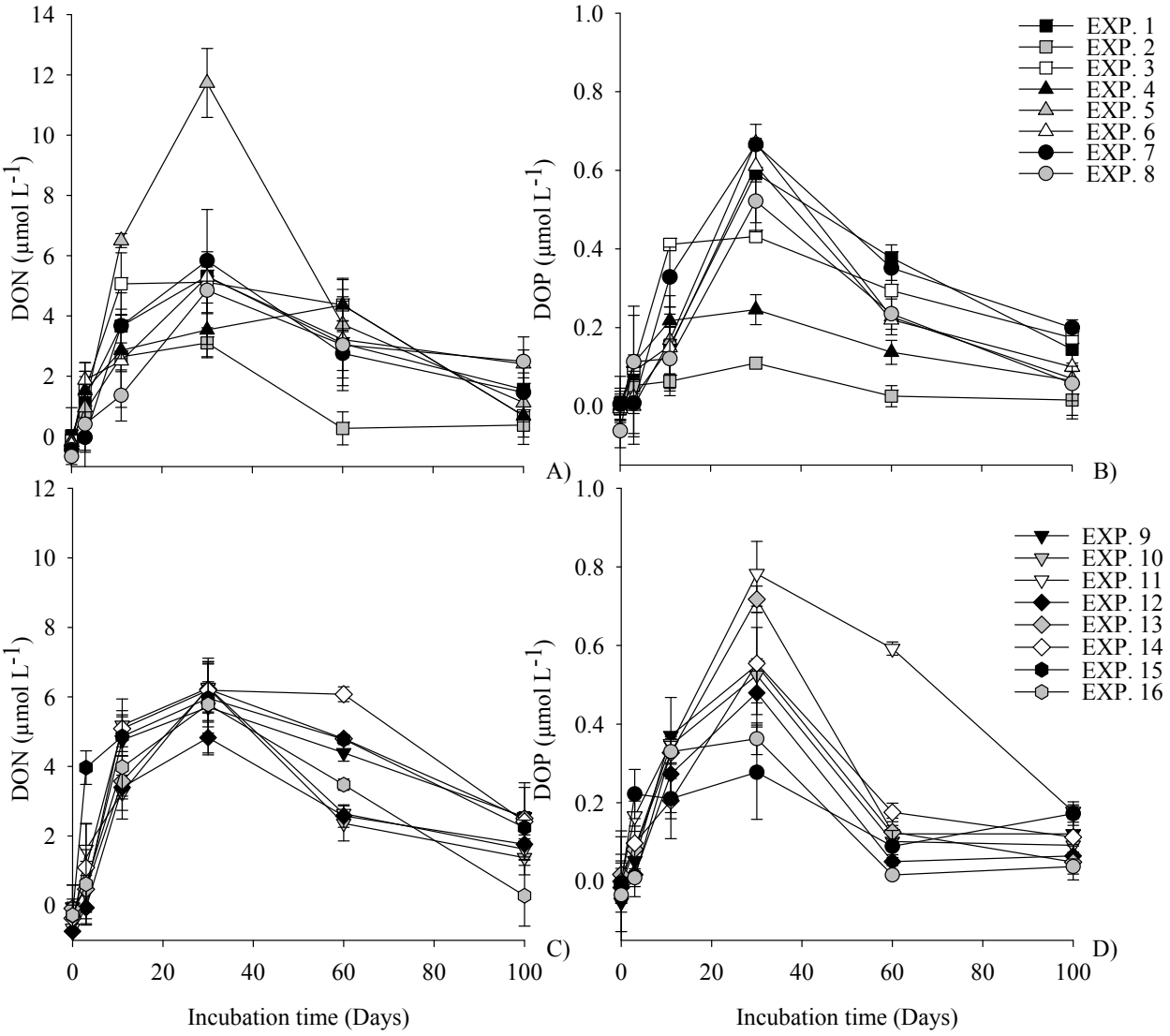
Figure. 2. Production of dissolved organic nitrogen (DON) and phosphorus (DOP) in the 16 experiments, a),b) started in January 2007 and c),d) in March 2007. The experimental numbers 1 to 16 represents the same experiment numbers as shown in table 1.

Figure. 3. Significant regressions between substrate uptake (UDOC, UDIN, UDIP) and produced DOM (PDOM) stoichiometery and the X–Y plots of linear relation between PDOM and bioavailable DOM (BDOM) stoichiometery. With a) UDOC:UDIN with PDOC:PDON, b) PDOC:PDON versus BDOC:BDON, c) UDOC:UDIP versus PDOC:PDOP, d) PDOC:PDOP with BDOC:BDOP, e) UDIN:UDIP versus PDON:PDOP and f) PDON:PDOP with BDON:BDOP. Slope, incept, and standard error are values found by Model II regression. R^2 = coefficient of determination, p = significant levels.

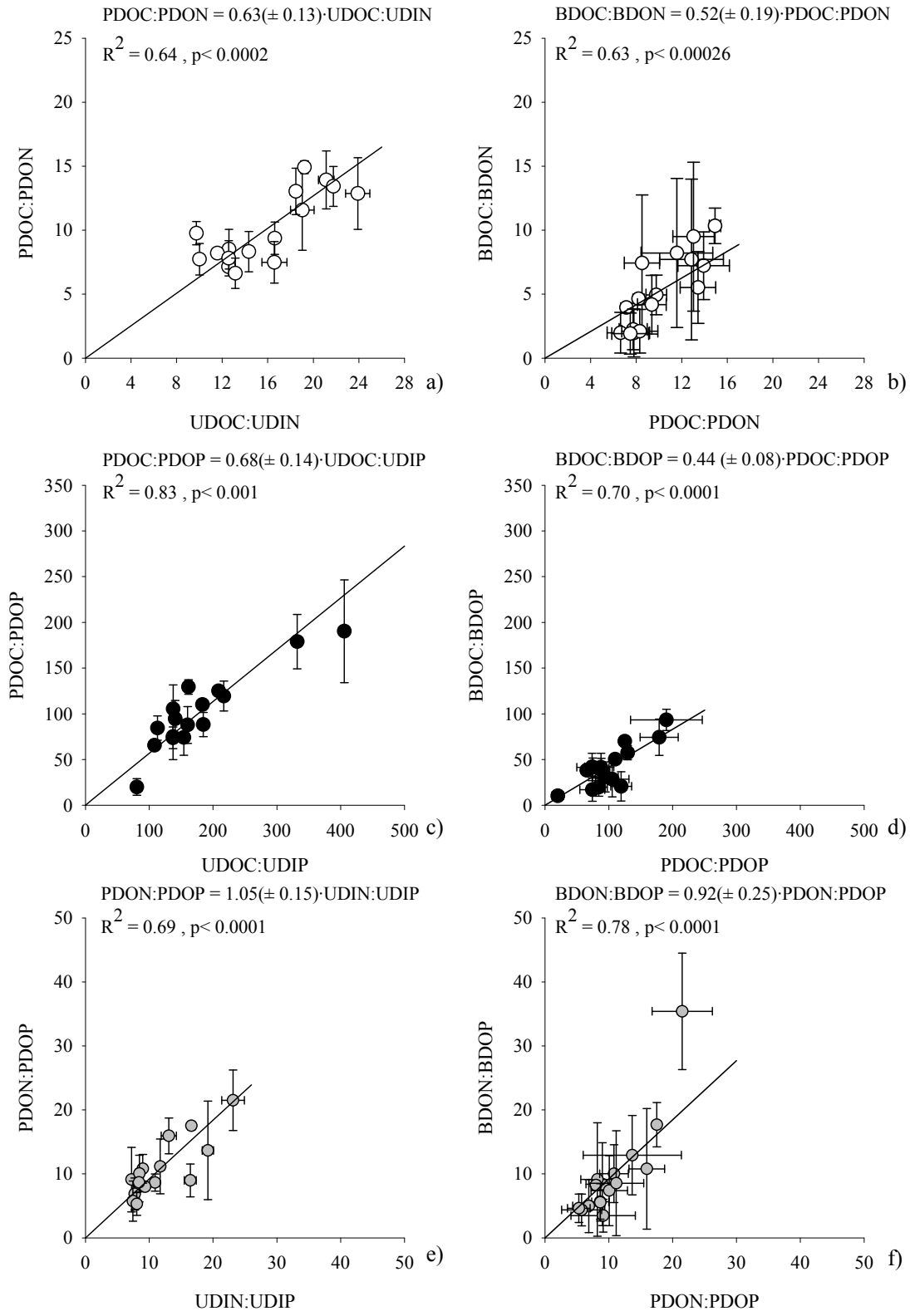

Phase I
Phase II
TOC, DOC (μmol L-1)
POC (μmol L-1)
PDOC
RDOC
TOC
DOC
POC
a)
TN, DIN (μmol L-1)
PON, DON (μmol L-1)
LDIN
PDON
RDON
TN
DIN
DON
PON
b)
TP, DIP (μmol L-1)
POP, DOP (μmol L-1)-1)-1
PDOP
LDIP
RDOP
TP
DIP
DOP
POP
c)
NO3-/NO2-, NH4+ (μmol L-1)
NO3-/NO2-
NH4+
d)
Incubation time (Days)

399

400 Fig.1.


DON (µmol L-1)
DOP (µmol L-1)
A)
B)
C)
D)
EXP. 1
EXP. 2
EXP. 3
EXP. 4
EXP. 5
EXP. 6
EXP. 7
EXP. 8
EXP. 9
EXP. 10
EXP. 11
EXP. 12
EXP. 13
EXP. 14
EXP. 15
EXP. 16
Incubation time (Days)
Incubation time (Days)

401
402 Fig.2.


PDOC:PDON = 0.63(± 0.13)·UDOC:UDIN
R2 = 0.64 , p< 0.0002
PDOC:PDON
UDOC:UDIN
a)
BDOC:BDON = 0.52(± 0.19)·PDOC:PDON
R2 = 0.63 , p< 0.00026
BDOC:BDON
PDOC:PDON
b)
PDOC:PDOP = 0.68(± 0.14)·UDOC:UDIP
R2 = 0.83 , p< 0.001
PDOC:PDOP
UDOC:UDIP
c)
BDOC:BDOP = 0.44 (± 0.08)·PDOC:PDOP
R2 = 0.70 , p< 0.0001
BDOC:BDOP
PDOC:PDOP
d)
PDON:PDOP = 1.05(± 0.15)·UDIN:UDIP
R2 = 0.69 , p< 0.0001
PDON:PDOP
UDIN:UDIP
e)
BDON:BDOP = 0.92(± 0.25)·PDON:PDOP
R2 = 0.78 , p< 0.0001
BDON:BDOP
PDON:PDOP
f)

403

404 Fig.3.

405 Table 1. Concentrations of DOC (UDOC), DIN (UDIN) and DIP (UDIP) utilized during the experiments, DOC (PDOC), DON (PDON), and DOP
406 (PDOP) produced during the experiments, and residual DOC (RDOC), DON (RDON) and DOP (RDOP) at the end of the experiments.
407 Experiments 1 to 8 were commenced in January and experiments 9 to 16 in March 2007. Values are means of three replicates ± standard error
408 given in μmol L^{-1}.

Exp.	DOC	DIN	DIP	UDOC	UDIN	UDIP	PDOC	PDON	PDOP	RDOC	RDON	RDOP
1	396 ± 1	80.6 ± 0.6	4.06 ± 0.02	396 ± 1	40.7 ± 0.9	2.48 ± 0.10	52 ± 1	5.3 ± 1.4	0.59 ± 0.13	33 ± 1	1.6 ± 0.8	0.14 ± 0.04
2	440 ± 6	80.2 ± 0.4	1.42 ± 0.02	440 ± 6	20.8 ± 0.4	1.08 ± 0.08	51 ± 1	5.7 ± 0.8	0.11 ± 0.04	31 ± 1	0.4 ± 0.1	0.02 ± 0.05
3	425 ± 1	25.1 ± 0.1	4.18 ± 0.03	425 ± 1	19.5 ± 0.3	2.32 ± 0.01	47 ± 2	5.1 ± 1.0	0.43 ± 0.04	35 ± 1	1.2 ± 0.2	0.17 ± 0.02
4	163 ± 1	77.6 ± 0.3	4.45 ± 0.02	163 ± 1	8.6 ± 0.4	1.19 ± 0.02	26 ± 2	2.2 ± 0.9	0.25 ± 0.04	21 ± 1	1.6 ± 0.4	0.07 ± 0.05
5	789 ± 3	93.4 ± 0.2	4.73 ± 0.03	789 ± 3	62.8 ± 0.3	3.78 ± 0.08	84 ± 1	11.7 ± 0.8	0.67 ± 0.05	42 ± 1	1.1 ± 0.5	0.07 ± 0.02
6	411 ± 6	157.5 ± 0.4	4.47 ± 0.04	411 ± 6	32.7 ± 0.6	3.00 ± 0.04	45 ± 4	5.3 ± 0.9	0.61 ± 0.06	24 ± 1	2.4 ± 0.5	0.10 ± 0.01
7	396 ± 2	79.2 ± 0.1	8.11 ± 0.06	396 ± 2	34.2 ± 0.3	3.66 ± 0.08	48 ± 1	4.3 ± 0.8	0.67 ± 0.08	28 ± 1	1.5 ± 0.6	0.20 ± 0.02
8	433 ± 1	88.1 ± 0.4	4.59 ± 0.04	433 ± 1	18.1 ± 0.8	2.35 ± 0.10	42 ± 1	4.8 ± 0.8	0.52 ± 0.09	28 ± 1	1.3 ± 0.8	0.06 ± 0.04
9	543 ± 2	84.0 ± 0.6	7.06 ± 0.07	543 ± 2	29.4 ± 0.7	3.95 ± 0.15	74 ± 1	5.7 ± 0.8	0.55 ± 0.10	38 ± 1	1.9 ± 0.9	0.12 ± 0.07
10	440 ± 7	82.1 ± 0.3	4.09 ± 0.02	440 ± 7	35.0 ± 0.8	3.90 ± 0.06	49 ± 3	6.3 ± 0.9	0.52 ± 0.07	39 ± 1	1.4 ± 0.5	0.09 ± 0.03
11	411 ± 9	51.0 ± 0.4	7.69 ± 0.06	411 ± 9	41.1 ± 0.8	5.10 ± 0.07	48 ± 2	6.3 ± 0.8	0.78 ± 0.08	38 ± 1	1.6 ± 0.3	0.18 ± 0.04
12	409 ± 3	88.5 ± 0.2	7.57 ± 0.07	409 ± 3	24.6 ± 0.6	2.91 ± 0.15	45 ± 1	4.8 ± 1.0	0.48 ± 0.09	32 ± 1	1.8 ± 0.4	0.06 ± 0.02
13	867 ± 2	84.1 ± 0.3	7.43 ± 0.10	867 ± 2	45.1 ± 0.6	5.37 ± 0.10	93 ± 2	6.2 ± 0.8	0.72 ± 0.06	54 ± 3	2.5 ± 0.4	0.05 ± 0.03
14	449 ± 2	164.7 ± 0.4	7.28 ± 0.05	449 ± 2	34.1 ± 1.0	2.91 ± 0.09	41 ± 2	6.2 ± 0.9	0.55 ± 0.13	34 ± 1	2.4 ± 1.1	0.11 ± 0.04
15	442 ± 1	74.8 ± 0.4	13.64 ± 0.05	442 ± 1	30.9 ± 0.5	1.33 ± 0.09	50 ± 3	6.0 ± 1.0	0.28 ± 0.12	42 ± 1	2.2 ± 0.5	0.17 ± 0.03
16	448 ± 3	96.5 ± 0.9	7.57 ± 0.03	448 ± 3	27.0 ± 1.6	2.06 ± 0.08	43 ± 1	5.8 ± 1.1	0.36 ± 0.08	27 ± 2	2.3 ± 0.9	0.04 ± 0.02

409

410 Table 2. Significant linear regressions between used DOC (UDOC) and produced DOC (PDOC);
411 bioavailable (BDOC) and PDOC; used dissolved inorganic nitrogen (UDIN) and produced DON (PDON);
412 bioavailable DON (BDON) and PDON); used phosphate (UDIP) and produced DOP (PDOP);, and
413 bioavailable DOP (BDOP) and PDOP. Slope, intercept, and standard error (SE) are values found by Model II
414 regression. R^2 = coefficient of determination, p = level of significance, n.s. – not significant.

415

X	Y	Slope (±SE)	Intercept (±SE)	R^2	p
UDOC	PDOC	0.11 ± 0.01	n.s	0.90	<0.00001
BDOC	PDOC	1.4 ± 0.2	27 ± 3	0.80	<0.00001
UDIN	PDON	0.18 ± 0.02	n.s	0.85	<0.0001
BDON	PDON	1.0 ± 0.2	1.7 ± 0.3	0.92	<0.001
UDIP	PDOP	0.17 ± 0.02	n.s	0.79	<0.00001
BDOP	PDON	1.1 ± 0.1	0.08 ± 0.03	0.82	<0.00001

416

417 Table 3. a) Absorption coefficients (a375) at day 0 ($a375_0$), 30 ($a375_{30}$) and 100 ($a375_{100}$). b) Initial
418 ($FDOMt_0$), produced ($PFDOMt_0$) and refractory ($RFDOMt_0$) protein–like fluorescence; c) Initial ($FDOMm_0$),
419 produced (PFDOMm) and refractory (RFDOMm) marine humic–like fluorescence; and Experiments 1 to 8
420 were commenced in January and experiments 9 to 16 in March 2007, corresponding to the experiments
421 described in table 1. Values are means of two replicates ± standard error.

Experiment	$a375_0$ (m^{-1})	$a375_{30}$ (m^{-1})	$a375_{100}$ (m^{-1})	a)
1	0.05 ± 0.05	0.44 ± 0.08	0.67 ± 0.02	
2	0.01 ± 0.02	0.27 ± 0.03	0.61 ± 0.09	
3	0.06 ± 0.06	0.25 ± 0.10	0.92 ± 0.12	
4	0.07 ± 0.08	0.32 ± 0.08	0.60 ± 0.09	
5	0.01 ± 0.01	0.36 ± 0.03	0.52 ± 0.10	
6	0.07 ± 0.08	0.50 ± 0.05	0.84 ± 0.17	
7	0.01 ± 0.04	0.38 ± 0.10	0.45 ± 0.18	
8	0.02 ± 0.02	0.13 ± 0.07	0.64 ± 0.09	
9	0.07 ± 0.08	0.31 ± 0.08	0.41 ± 0.04	
10	0.08 ± 0.09	0.42 ± 0.10	0.69 ± 0.07	
11	0.08 ± 0.09	0.24 ± 0.05	0.34 ± 0.07	
12	0.03 ± 0.04	0.24 ± 0.04	0.38 ± 0.05	
13	0.02 ± 0.02	0.32 ± 0.08	0.41 ± 0.16	
14	0.04 ± 0.04	0.39 ± 0.04	0.53 ± 0.03	
15	0.01 ± 0.01	0.34 ± 0.05	0.34 ± 0.09	
16	0.01 ± 0.01	0.52 ± 0.03	1.33 ± 0.09	

422

Experiment	$FDOMt_0$ (ppb Trp)	PFDOMt (ppb Trp)	RFDOMt (ppb Trp)	b)
1	3.5 ± 0.6	17.7 ± 0.7	21.1 ± 0.1	
2	3.2 ± 0.6	10.0 ± 0.9	13.2 ± 0.3	
3	2.6 ± 0.4	13.0 ± 1.0	15.6 ± 0.7	
4	2.3 ± 0.2	6.1 ± 0.7	8.4 ± 0.5	
5	2.5 ± 0.2	19.9 ± 0.7	22.4 ± 0.5	
6	3.9 ± 0.1	17.7 ± 0.6	21.5 ± 0.5	
7	3.0 ± 0.3	21.2 ± 0.4	24.2 ± 0.1	
8	1.3 ± 0.5	10.9 ± 0.7	12.2 ± 0.2	
9	2.3 ± 0.6	16.3 ± 0.8	18.6 ± 0.2	
10	3.2 ± 0.3	7.9 ± 0.9	11.1 ± 0.5	
11	2.6 ± 0.3	10.6 ± 0.4	13.2 ± 0.2	
12	2.3 ± 0.6	7.9 ± 1.1	10.2 ± 0.5	
13	4.5 ± 0.9	15.2 ± 1.0	19.7 ± 0.1	

14	2.9 ± 0.1	11.4 ± 0.3	14.3 ± 0.2
15	1.0 ± 0.2	14.5 ± 0.6	15.5 ± 0.4
16	2.3 ± 0.2	9.4 ± 0.6	11.7 ± 0.4

423

Experiment	$FDOMm_0$ (ppb Trp)	PFDOMm (ppb Trp)	RFDOMm (ppb Trp)
1	2.58 ± 0.3	9.0 ± 0.5	11.7 ± 0.1
2	2.5 ± 0.5	10.6 ± 0.8	13.1 ± 0.3
3	2.0 ± 0.6	8.9 ± 0.7	10.8 ± 0.1
4	2.5 ± 0.3	4.7 ± 0.7	7.2 ± 0.5
5	2.9 ± 0.1	14.0 ± 0.2	16.8 ± 0.2
6	2.2 ± 0.3	5.0 ± 0.9	7.2 ± 0.6
7	1.9 ± 0.4	5.4 ± 0.5	7.3 ± 0.2
8	1.8 ± 0.2	4.8 ± 0.2	6.6 ± 0.2
9	2.8 ± 0.3	7.8 ± 1.0	10.5 ± 0.7
10	2.3 ± 0.5	3.7 ± 0.6	6.0 ± 0.1
11	1.8 ± 0.6	4.0 ± 0.7	5.8 ± 0.2
12	2.4 ± 0.3	3.2 ± 1.1	5.6 ± 0.8
13	2.6 ± 0.1	13.1 ± 0.2	15.7 ± 0.1
14	2.2 ± 0.3	2.8 ± 0.5	5.0 ± 0.3
15	2.0 ± 0.4	5.2 ± 0.5	7.2 ± 0.1
16	1.8 ± 0.2	5.1 ± 0.3	6.9 ± 0.1

c)

424

425

426 Table 4. Matrix of the correlation coefficient (R^2) of the significant ($p < 0.05$) linear regressions
427 between DOM and the produced protein– (PFDOMt) and marine humic–like (PFDOMm)
428 flourophores. n.s. – not significant.
429

X–Y	$FDOM_T$	$FDOM_M$
UDIN	0.60	0.33
UDOC	0.53	0.64
RDOC	n.s.	0.45
BDOC	0.77	0.53
BDON	0.30	0.38
BDOP	0.44	n.s.

430