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Abstract:	<p>Production and bioavailability of dissolved organic matter (DOM) were followed during a year in the nutrient-rich estuary, Roskilde Fjord (RF), and the more oligotrophic strait, Great Belt (GB), in Denmark. Bioavailability of dissolved organic carbon (DOC), nitrogen (DON), and phosphorous (DOP) was determined during incubations over six months. Overall, RF had three to five times larger pools of total nitrogen (TN) and total phosphorous (TP) and five to eight times higher concentrations of inorganic nutrients compared to GB. However, the allocation of carbon, nitrogen, and phosphorous into different pools were remarkably similar between the two systems. DON and DOP contributed with about equal relative fractions in the two systems: 72 ± 13 % of total nitrogen and 21 ± 12 % of total phosphorous. The average bioavailability of DOM was 25 ± 15, 17 ± 5.5, and 49 ± 29 % for carbon, nitrogen, and phosphorous, respectively. The observed release of DIN from degradation of DON amounted to between 0.1 (RF winter) and 14 times (GB summer) the loadings from land and contributed with half of the total input of bioavailable nitrogen during summer. Hence, this study shows that nitrogen in DOM is important for the nitrogen cycling, especially during summer. The sum of inorganic nutrients, particulate organic matter, and bioavailable DOM (the dynamic pools of nutrients) accounted for 42 and 92 % of nitrogen, and phosphorous,</p>	

	<p>respectively, and was remarkably similar between the two systems compared to the difference in nutrient richness. It is hypothesized that the pelagic metabolism of nutrients in marine systems dictates a rather uniform distribution of the different fractions of nitrogen and phosphorous containing compounds regardless of eutrophication level.</p>
Response to Reviewers:	<p>2. Review on manuscript: "Seasonal dynamics and bioavailability of dissolved organic matter in two contrasting temperate estuaries"</p> <p>We have modified Fig. 2 as suggested by splitting the plots of DOC and oxygen concentrations over time into two panel.</p> <p>In addition, we have been through the text again and corrected all misspelling and language problems we could detect.</p>



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1 **Seasonal dynamics and bioavailability of dissolved organic matter in two contrasting temperate**
2 **estuaries**

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14 **Keywords:** Bioavailability, DOC, DON, DOP, eutrophication, nitrogen budget

15

16 **Abstract**

17 Production and bioavailability of dissolved organic matter (DOM) were followed during a year in the
18 nutrient-rich estuary, Roskilde Fjord (RF), and the more oligotrophic strait, Great Belt (GB), in
19 Denmark. Bioavailability of dissolved organic carbon (DOC), nitrogen (DON), and phosphorous
20 (DOP) was determined during incubations over six months. Overall, RF had three to five times larger
21 pools of total nitrogen (TN) and total phosphorous (TP) and five to eight times higher concentrations of
22 inorganic nutrients compared to GB. However, the allocation of carbon, nitrogen, and phosphorous into
23 different pools were remarkably similar between the two systems. DON and DOP contributed with
24 about equal relative fractions in the two systems: 72 ± 13 % of total nitrogen and 21 ± 12 % of total
25 phosphorous. The average bioavailability of DOM was 25 ± 15 , 17 ± 5.5 , and 49 ± 29 % for carbon,
26 nitrogen, and phosphorous, respectively. The observed release of DIN from degradation of DON
27 amounted to between 0.1 (RF winter) and 14 times (GB summer) the loadings from land and
28 contributed with half of the total input of bioavailable nitrogen during summer. Hence, this study
29 shows that nitrogen in DOM is important for the nitrogen cycling, especially during summer. The sum
30 of inorganic nutrients, particulate organic matter, and bioavailable DOM (the dynamic pools of
31 nutrients) accounted for 42 and 92 % of nitrogen, and phosphorous, respectively, and was remarkably
32 similar between the two systems compared to the difference in nutrient richness. It is hypothesized that
33 the pelagic metabolism of nutrients in marine systems dictates a rather uniform distribution of the
34 different fractions of nitrogen and phosphorous containing compounds regardless of eutrophication
35 level.

36

37 **Introduction**

38 Many coastal waters and estuaries are struggling with the negative effects of eutrophication due to high
39 nutrient loadings (Nixon 1995; Conley et al. 2000). The negative consequences are numerous including
40 reduced water clarity, hypoxia, and loss of submerged vegetation (Cloern 2001). In Denmark, the first
41 national action plan was initiated in the mid-eighties with the aim to reduce nitrogen and phosphorous
42 loadings to the marine environment with 50 and 80 %, respectively (Kronvang et al. 1993). In recent
43 years, positive effects of these efforts have been observed with a decline in nutrient concentrations
44 (Carstensen et al. 2006) and improvements in water clarity, oxygen concentrations and underwater
45 vegetation (Riemann et al. 2016). Previous studies in this area have shown that the effects of lower
46 nutrient loadings have a time lag of about 15 years before an improved environmental status is
47 noticeable throughout the system (Pedersen et al. 2014; Andersen et al. 2015; Riemann et al. 2016).
48 The reasons for this long time lag can be many, and nutrients accumulated in the sediments are
49 probably important (Jørgensen et al. 2014). However, dissolved organic matter (DOM) is also likely to
50 play a significant role. Previous studies have shown that DOM accounts for considerable parts of the
51 total carbon, nitrogen (TN), and phosphorous (TP) pools (Meybeck 1982; Stepanauskas et al. 2002;
52 Stedmon et al. 2006; Scott et al. 2007; Petrone et al. 2009). However, the DOM pool must be
53 bioavailable to play a role in nutrient cycling and to sustain primary production. Only a few studies
54 have examined the importance of all three components of DOM and their bioavailability on a seasonal
55 scale in relation to nutrient cycling and level of eutrophication (Stedmon et al. 2006; Lønborg et al.
56 2009; Markager et al. 2011). The aim of this study was, therefore, to document and quantify the role of
57 DOM for cycling of nutrients in coastal ecosystems.

58 Nitrogen is considered the limiting nutrient in most marine systems (see e.g. Timmermann et al.
59 2014 for the Danish estuary Limfjorden and Cloern et al. 2001). Anthropogenic sources, especially
60 from agricultural activities, dominate the nitrogen inputs to estuaries and coastal areas through
61 freshwater runoff (Nixon 1995; Howarth et al. 1996; Carstensen and Henriksen 2009; Petrone et al.
62 2009). Nitrogen in freshwater loadings occur either as particles (PON), dissolved organic nitrogen
63 (DON) or dissolved inorganic nitrogen (DIN), and the latter is quickly transformed into particulate
64 organic nitrogen (PON) through phytoplankton growth when it reaches the marine environment and
65 later into DON (Stepanauskas et al. 2002; Stedmon et al. 2006; Markager et al. 2011). Thus, most
66 nitrogen in marine waters is in the form of DON that contributes with up to 90 % of TN (Tamminen
67 and Seppala 1999; Ward and Bronk 2001; Berman and Bronk 2003; Jørgensen et al. 2014). According
68 to Bronk et al. (1994), 25 to 41 % of the DIN uptake by phytoplankton is released as DON within 4 -
69 18 days. Hence, a concurrent seasonal variation can be expected for DON and chlorophyll *a* (Chl *a*)
70 concentrations. To get an insight into the production and turnover of DON, it is, therefore, relevant to
71 study the seasonal variation of DON and its bioavailability.

72 The bioavailability of DOM depends on its origin. Nitrogen (DON) and phosphorous (DOP)
73 compounds in DOM can either be utilized directly by phytoplankton or degraded to inorganic nutrients
74 by heterotrophic microbes. Some of the most labile fractions of DOM are produced by phytoplankton
75 and are degraded on a time scale of hours to a few days (Amon et al. 2001; Davis and Benner 2005).
76 The remaining DOM is more refractory and can e.g. be transported to adjacent areas. Another source of
77 DOM is from land and is considered to have a higher bioavailability than DOM from adjacent seas
78 (Bronk 2002; Jørgensen et al. 2014). Freshwater derived DON was found to have a bioavailability of
79 20-70 % within 15 days when entering marine waters (Seitzinger and Sanders 1997; Stepanauskas et al.

80 1999; Seitzinger et al. 2002; Stepanauskas et al. 2002). In general, studies show that the bioavailable
81 pools of DON (BDON) and DOP (BDOP) are higher than the bioavailable pool of dissolved organic
82 carbon (BDOC) (Lønborg et al. 2009; Petrone et al. 2009; Lønborg and Alvarez-Salgado 2012).

83 In recent years, it has become apparent that several phytoplankton species, including harmful
84 species, directly can assimilate nutrients from DOM (Karl and Björkman 2002; Stolte et al. 2002;
85 Bronk et al. 2007; Sipler et al. 2013; Bronk et al. 2014). This means that DOM can be a supplemental
86 nitrogen source and even in some cases the main source for phytoplankton growth (Berman 2001). The
87 timescale for degradation of DOM is important, and bioavailability must be addressed relative to the
88 water residence time in order to assess its role in the overall nutrient budget of the system (Monbet
89 1992; Seitzinger and Sanders 1997; Nielsen et al. 2001; Jørgensen et al. 2014).

90 In addition to freshwater loadings, other nitrogen sources to marine waters include N₂-fixation,
91 atmospheric deposition, and nitrogen from adjacent areas. N₂-fixation can be an important source of
92 nitrogen in coastal waters (Rees et al. 2009; Mulholland et al. 2012; Bentzon-Tilia et al. 2015). The
93 significance of atmospheric nitrogen deposition is highly dependent on the sea surface area relative to
94 the loadings from land but can dominate during summer in open coastal areas (Spokes et al. 2006).
95 Depending on depth, the sediment can play an important role as a nutrient source to the water column
96 and, hence, for phytoplankton production (Fanning et al. 1982; Floderus and Hakanson 1989).
97 However, nutrients from the sediment originate from the water column, so they are not ‘new’ to the
98 system. The exchange can occur in connection with resuspension events, with a mass flow of water
99 through the sediments, or from diffusive exchange between sediment and water column. Nonetheless,
100 the nutrient pool in the sediment often dominate, e.g. was more than 95 % of the total nitrogen pool
101 (water column plus the upper 20 cm of the sediment) found in the sediment in the Kattegat and Belt

102 Sea area (Jørgensen et al. 2014). This might be an important factor contributing to the time lag for
103 improvements of the environmental status that has been observed during oligotrophication in both
104 study sites (Riemann et al. 2016).

105 The aim of this study was to examine the seasonal importance of DOM as a nutrient source by
106 studying the seasonal dynamics of the different fractions of the DOM pool in the surface water of two
107 temperate coastal systems with contrasting eutrophication levels. By monthly sampling during a year,
108 we determined the concentrations of DOC, DON, and DOP, their seasonal bioavailability and
109 importance relative to other nutrient sources.

110 **Methods**

111 **Study sites**

112 The estuary Roskilde Fjord (RF) and the strait Great Belt (GB, Fig. 1) are two contrasting systems. RF
113 is a eutrophic 40 km long semi-enclosed estuary with a surface area of 123 km² and a residence time of
114 one year (Flindt et al. 1997). The mean depth is only 3 m and the entire water column is well mixed
115 with permanent contact to the sediment. This contact affects the system in several ways, e.g. nutrients
116 are constantly exchanged between the photic zone and the sediment, resuspension of sediments occur
117 regularly and contribute to light attenuation ($K_d(\text{PAR}) \sim 0.53$) (Pedersen et al. 2014; Staehr et al. 2017)
118 and benthic filter feeding is a loss factor for phytoplankton. The sampling station is placed in the
119 middle of the southern broad, south of the narrow strait that divide the estuary in a southern and a
120 northern part. In-depth descriptions of the variability between stations, including maps and an analysis
121 of the development over time is found in Pedersen et al. (2014), Staehr et al. (2017) and references
122 herein. Briefly, the station we have sampled has been monitored since 1980 and is believed to be

123 representative for the open parts of the inner estuary, but gradients exist towards the shore. The estuary
124 is unlike most estuaries in the sense that it does not have a major river entering the inner most part.
125 Instead, freshwater is coming from many smaller streams distributed along the north-south axis. Area
126 specific nutrient loadings and atmospheric deposition are similar between the northern and southern
127 basins (Staehr et al. 2017).

128 The less eutrophic GB (depth 17 m) is the main strait connecting the Baltic Sea with the
129 Kattegat and the North Sea. The residence time is about one month during winter and two to three
130 months during summer (Bendtsen et al. 2009). The water column in GB is permanently stratified due to
131 an outflow of low-saline surface water from the Baltic Sea and an inflow of high-saline bottom water
132 from the Kattegat/North Sea. Hence, a salinity gradient is present from South to North and the
133 sampling station is placed in the middle of this gradient. During our sampling campaign in 2012,
134 stratification occurred almost all year with a halocline at about 13 meters depth (range 7 – 26 m). The
135 surface salinity varied from 11 to 24 depending on current directions (outflow of Baltic Sea water or
136 inflow from the North Sea) with an annual average of 17 (Fig. 3).

137 The 2012 season represents a typical year at both stations. However, nitrogen loadings have, as
138 mentioned above, been decreasing for 18 years, so concentrations of total nitrogen were from 11 to 16
139 % below the average from 2003 to 2015 for GB and RF, respectively. The chlorophyll *a* concentrations
140 were 26 and 6 % below average. Thus, though the precipitation was 30 % above the average during the
141 winter of 2011/12 (Pedersen et al. 2014), nitrogen and also chlorophyll *a* concentrations were lower
142 than previous years in 2012. Since, nitrogen loadings have been increasing, so, overall, the year
143 represents quite well the prevailing conditions over the last decade. The surface water temperature
144 varied between -1 and 20 °C at both stations with an annual mean of 9 °C, and a spring bloom occurred

145 in March at both stations with a maximum of 4.7 and 7.3 $\mu\text{g Chl } a \text{ L}^{-1}$ in GB and RF, respectively (Fig.
146 3).

147 **Sampling**

148 The sampling campaign was part of a larger effort to describe and understand microbial driven
149 processes and nutrient biogeochemistry in the two systems (Bentzon-Tilia et al. 2015; Traving et al
150 2016; Mantikci et al., in revision). Pre-filtered (200 μm) surface water (depth 1 m) was collected
151 monthly with Niskin bottles at stations in GB (55°30.27'N, 10°51.43'E, depth 35 m) and RF
152 (55°42.00'N, 12°04.46'E, depth 4.8 m) in collaboration with the Danish Environmental Agency under
153 the Danish marine monitoring program. Due to ice-coverage in RF, the sampling in January was from a
154 pier 1 km southeast of the station. The sampled water was kept in darkness in 20 liters HDPE Nalgene
155 carboys at a temperature corresponding to the surroundings during the maximum 1.5 hours
156 transportation to the laboratory where environmental variables were sampled, and degradation
157 experiments were initiated in order to measure the pool of bioavailable DOM (BDOM). Water column
158 profiles of salinity, temperature, photosynthetic active radiation (PAR), and chlorophyll fluorescence
159 were collected together with the water samplings. Chl *a* was measured by filtration of 200 or 1000 ml
160 water onto a GF/F filter (Whatman, 25 mm, 0.6 - 0.8 μm particle retention), extracted in 10 ml 96 %
161 ethanol overnight at 4 °C, and measured fluorometrically on a Turner fluorometer (Jespersen and
162 Christoffersen 1987). Samples for POC and PON were filtered onto pre-combusted (3 hours at 450 °C)
163 GF/F filters (Whatman, 25 mm) and frozen at -20 °C until analysis. Filters were dried at 40 °C for 24
164 hours before analysis on a Perkin Elmer 2400 CHNS Analyzer. Samples for inorganic nutrients; nitrite
165 (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), orthophosphate (PO_4^{3-}), and dissolved silica (DSi) were
166 stored frozen in 30 ml acid washed LDPE bottles and later analyzed on a San⁺⁺ Continuous Flow

167 Analyzer (Skalar) according to the methods in Grasshoff (1976) and Kaas and Markager (1998).
168 Detection limits were 0.04, 0.1, 0.3, 0.06, and 0.2 μM for NO_2^- , NO_3^- , NH_4^+ , PO_4^{3-} , and DSi,
169 respectively. TN and TP was sampled (either 200 μm pre-filtered *in situ* samples or 0.2 μm filtered +
170 10 % GF/C filtered bacteria inoculum sample water) in 30 ml brown glass bottles filled with Milli-Q
171 water until sampling and frozen at -20°C afterwards. After wet oxidation (30 minutes at 121°C with
172 $\text{K}_2\text{S}_2\text{O}_8$, H_3BO_3 and NaOH), samples were analyzed on an automatic nutrient analyzer as described in
173 Kaas and Markager (1998) with detection limits of 1.0 μM N and 0.1 μM P, respectively. Samples for
174 total organic carbon (TOC) were collected in 60 ml HDPE bottles, acidified with 2 M HCl to a pH of 2
175 and stored at 5°C in darkness until measured on a TOC V_{cph} Analyzer (Shimadzu) according to
176 Cauwet (1999). Concentrations were derived from a five-point calibration curve with subtraction of the
177 signal from Milli-Q water and validated with a deep-sea reference sample (Hansell consensus reference
178 material program). Data on Chl *a*, DIN, DIP, DOC, temperature, and salinity are published in Bentzon-
179 Tilia et al. (2015) and for particulate organic carbon (POC) and PON in Mantikci et al. (in revision).

180

181 **DOM degradation experiments**

182 A degradation experiment was performed on each sample day at both stations with an average duration
183 of 189 ± 11 days following the methodology described in Hansell and Carlson (2002). In order to
184 sample five times with triplicate bottles each experiment was started with 15 bottles. The degradation
185 experiments were conducted with 0.2 μm (Pall, Supor capsule filter) filtered sample water in 120 ml
186 Winkler bottles. Another part of the sample water was GF/C filtered (Advantec GF-50, nominal pore
187 size 1.2 μm) and used as a bacterial inoculum (10 ml added to each degradation experiment). A magnet

188 was placed in each bottle to ensure homogeneity of the water. The bottles were filled without
189 headspace and incubated in darkness at 15 °C. At the occasions where the *in situ* seawater temperature
190 was lower than 15 °C, the sample water (for the degradation experiments) was filtered and placed in
191 darkness until it reached 15 °C in order to prevent bubble formation after filling of incubation bottles.
192 At day zero and approximately at day 10, 60, 120, and 180, triplicate bottles were harvested for
193 measurements of DIN, DIP, total dissolved nitrogen and phosphorous, and DOC. The oxygen
194 consumption varied from 45 to 225 $\mu\text{mol O}_2 \text{ l}^{-1}$, and the average concentration during the incubations
195 was 195 $\mu\text{mol O}_2 \text{ l}^{-1}$. At this level, oxygen was assumed not to limit DOM degradation and
196 denitrification was assumed negligible. An example of oxygen concentrations during two incubations
197 are shown in Fig. 2.

198 **Data Analysis**

199 The concentrations of DON in samples collected in the field were calculated as

$$200 \quad \text{DON} = \text{TN} - \text{DIN} - \text{PON}. \quad (1)$$

201 as described in Hansell and Carlson (2002). In the degradation experiments, the bioavailable fraction of
202 DON (BDON) was calculated as the net increase in DIN during the incubations. The initial pool of
203 PON was assumed insignificant as particles in the incubations only stemmed from the 10 % inoculum
204 after passing the GF/C filtration, which was 1.4 ± 1.0 % of TN if all particles in the raw samples would
205 pass the GF/C filter. However, it is highly unlikely that the PON pool before and after filtration was
206 equal in size, and we, therefore, considered the PON pool to be insignificant when the degradation
207 experiments were initiated. The pool of DOP was calculated in the same way as DON with PO_4^{3-}

208 representing the pool of DIP and the BDOP pool was calculated as the net increase in DIP during the
209 degradation experiments.

210 All TOC samples, also unfiltered *in situ* samples, were considered a measure of DOC
211 concentrations due to the following reasons. For the degradation experiments, the waters were initially
212 0.2 μm filtered and particles, therefore, only stemmed from microbial growth during the experiments
213 and possible aggregation of DOM. This pool of POC was insignificant compared to the DOC
214 concentration. For the raw samples, the POC concentration was up to 10 % but usually much lower.
215 However, tests (data not shown) revealed that values for filtered and unfiltered samples identical within
216 0.5 % of the mean. This was probably because particles were settling in the bottles during storage or
217 not sampled with the auto sampler needle (in the Shimadzu Analyzer). Thus, the term dissolved organic
218 carbon (DOC) will be used when referring to measurements with the TOC analyzer. The pool of total
219 organic carbon (TOC) was calculated as the sum of the DOC pool and the pool of POC measured on
220 the CHNS analyzer. BDOC during the degradation experiments were calculated using the regression
221 model:

$$222 \quad \text{DOC}(t) = \text{RDOC} + \text{BDOC} \cdot \exp(-D_t \cdot t), \quad (2)$$

223 where RDOC is the refractory pool of DOC, D_t is a time coefficient for degradation of DOC (day^{-1}) and
224 t is time in days. Additional information about the degradation experiments are provided in Knudsen-
225 Leerbeck (2016).

226 **Results**

227 **Annual means of carbon, nitrogen, and phosphorous components**

228 The two systems, RF and GB showed a substantial difference in nutrient richness with the enclosed RF
229 having annual average concentrations of DIN and DIP that were five and eight times higher,
230 respectively, than in GB (Table 1, Fig. 3). This difference was also found for TN and TP concentrations
231 with average concentrations of 55 μM N and 3.9 μM P in RF or three to five times higher than the
232 concentrations in GB. Similar to this, the concentration of organic matter was higher in RF compared to
233 GB. However, the ratio between the concentrations of organic matter in the two locations, RF and GB,
234 was with only 2.3 (mean of RF/GB ratios for the concentrations of dissolved and particulate organic
235 carbon, nitrogen, and phosphorous, Table 1) lower than the inorganic and total pools in RF and GB.
236 The pools of DOC, DON, and DOP ranged between 184 – 418 μM C, 12 – 16 μM N, and 0.1 – 0.4 μM
237 P, respectively, in GB. In RF, the concentrations of DOC, DON and DOP varied between 511 – 714
238 μM C, 28 – 39 μM N, and 0 – 1.2 μM P, respectively, and, hence, were higher than in GB except for
239 one occasion for the DOP concentration.

240 During six months degradation more DOC, DON, and DOP were degraded in RF than in GB
241 (Table 1). Between 46 and 360 μM DOC (on average 156 μM DOC) was degraded in RF, whereas
242 between 16 and 179 μM DOC (on average 86 μM DOC) was degraded in GB. Similarly, the BDON
243 and BDOP pools were about three times higher in RF than in GB. The annual averages were 6.9 and
244 2.0 μM BDON in RF and GB, respectively, with a range of 5.0 – 9.9 μM BDON in RF and 0.4 – 2.8
245 μM BDON in GB. The pool of BDOP was close to the detection limit of DIP with a degradation of
246 0.25 μM DOP (range 0 – 1.49 μM) and 0.09 μM DOP (range 0 – 0.19 μM) in RF and GB,
247 respectively.

248 The ratios of the concentrations of the different nitrogen and phosphorous containing organic
249 compounds were all about three for the two systems – except for PON and particulate organic

250 phosphorous (POP) where the ratios were 2.1 (Table 1). Interestingly, the ratios between RF and GB
251 for the percentages (relative to the TN and TP pools) of the three organic compounds (dissolved,
252 particles, and BDOM) were almost identical with values close to 1 and a range from 0.6 to 1.3. Thus,
253 the higher nutrient richness in RF did not result in a higher relative bioavailability of DON and DOP.
254 The RF/GB ratios for organic carbon concentrations were 1.8 – 1.9 for DOC, BDOC, and POC and,
255 thereby, lower than the ratios for the nitrogen and phosphorous containing fractions (range 2.1 – 3.4,
256 Table 1). The RF/GB ratios of the organic carbon compounds as percentage of the TOC pools were
257 one, like the ratios for the nitrogen and phosphorous pools. Thus, despite RF being more nutrient rich
258 than GB, the relative proportions in the pools were the same.

259 **Allocation of carbon, nitrogen, and phosphorous into fractions**

260 The allocation of the different carbon, nitrogen and phosphorous pools were remarkably constant for
261 the two systems despite the large difference in nutrient richness (Table 1, Fig.4). For both stations,
262 DOC and DON were the major fractions with annual contributions of 94 % of TOC (range 89 – 98 %) and
263 71 % of TN (range 33 – 90 %), respectively. Note that DON is compared to the TN concentration
264 including DIN whereas DOC is only compared to the total organic fraction (DOC + POC) as the pool
265 of dissolved inorganic carbon is large and in equilibrium with the atmosphere. In contrast, DOP
266 constituted only 21 % of TP (range 0 – 46 %) and, hence, much less than for DOC and DON. The
267 dominating phosphorous pool was DIP with 64 and 47 % of TP in RF and GB, respectively (Table 1,
268 Fig. 4). The relative contributions of DOM to the total pool (DOC/TOC, DON/TN, and DOP/TP) were
269 similar at the two sites, and, therefore, the RF/GB ratios were close to one and much lower than the
270 RF/GB ratios for the absolute concentrations of inorganic and organic compounds in the two systems.
271 Like DOM, the relative importance of the POC, PON, and POP pools to the total concentrations were

272 almost identical in RF and GB, but with a tendency to higher fractions in GB. The BDOM fractions
273 were also similar in RF and GB with an average bioavailability of 25, 17 and 49 % for carbon,
274 nitrogen, and phosphorous, respectively. We also calculated the ‘dynamic pool’ of C, N and P in the
275 two systems, i.e. the fraction of TOC, TN and TP participating in biological activity. The dynamic
276 pools were calculated as the sum of inorganic nutrients, particulate organic matter and BDOM. The
277 dynamic pools were on average 32, 42, and 92 % for carbon, nitrogen, and phosphorous, respectively,
278 and the differences between the two systems were only $\pm 0.5 - 5$ % (Table 1).

279 **Stoichiometry of dissolved organic matter**

280 The DOM pool at both stations was depleted in nitrogen and phosphorous compared to the Redfield
281 ratio (106:16:1 on molar basis) and this depletion or enrichment with carbon, became even more
282 pronounced in the spring period. The C:N ratio was 16.7 ± 2.8 (standard deviation) in RF and $22.1 \pm$
283 4.8 in GB or about 2 – 3 times above the Redfield ratio (Fig. 5a, b). A cluster of points with
284 significantly higher values (RF: 20.2 ± 2.6 , GB: 25.7 ± 3.3) all belonged to the spring period (RF:
285 January to April, GB: January to July, t-test, $p < 0.001$, Fig. 5a). The C:P ratios in DOM were 1080 and
286 1823 or 10 and 17 times above the Redfield ratio for RF and GB, respectively, and carbon enrichment
287 relative to phosphorous was also observed for both systems in the spring but was only significant for
288 GB (Fig. 6b). The DOC produced during spring was more bioavailable than the average DOC as these
289 observations showed positive residuals for the regression lines in Fig. 6a. The annual average N:P ratio
290 in the DOM pool was 65 and 82 for RF and GB, respectively, and values from the spring period did not
291 differ from the rest of the year (Fig. 5c). The lower C:P and N:P ratios for DOM in RF were due to
292 higher DOP concentrations in the latter part of the year (Fig. 3c) probably due to release of DIP (Fig.

293 3b) from the sediment caused by hypoxia in some areas, a closer contact between water column and
294 sediment compared to GB and eventually conversion of DIP to DOP.

295 The stoichiometry of BDOM was closer to the Redfield ratio than the total DOM pool (Fig. 6).
296 The main finding is the pronounced carbon enrichment of BDOM during spring of about 130 μM
297 BDOC, whereas the BDOC:BDON ratios for the other months were close to Redfield for both systems.
298 Neither BDOC nor BDOM concentrations were related to BDOP concentrations (Fig. 6b, c). The
299 BDOM pool in RF was enriched with nitrogen compared to the BDOM pool in GB and the Redfield
300 ratio (Fig. 6c, Table 1).

301

302 **Seasonal variation**

303 Concentrations at both stations displayed patterns typical for temperate systems with high seasonal
304 variation (Fig. 3). During the spring bloom, which peaked in March at both stations, a concomitant
305 decrease in DIN and DIP concentrations were observed, as both nutrients were utilized by
306 phytoplankton (Fig. 3b, f and Fig. 3c, g) and incorporated into particulate organic matter. After the
307 bloom, we observed a decrease in the TN and TP pools probably due to sedimentation (Fig. 3b). DON
308 and DOP concentrations were rather constant throughout the year with the exception that DOP
309 approximately doubled from July to December in RF (Fig. 3c and g). In general, the DOC pool
310 decreased during the year and the values did not return to their starting values within the sampling
311 period (Fig 3c, g). The bioavailability of DOC was higher in the spring, 44 ± 9 and 36 ± 6 % for RF
312 and GB, respectively, compared to 15 ± 6 % and 9 ± 2 % during the rest of the year (Fig. 3, Fig. 4).

313 Thus, overall a positive relationship exists between DOC concentrations, BDOC concentrations and
314 BDOC as percentage of DOC.

315 The contribution of the organic pools of nitrogen and phosphorous to the total pools increased
316 in March at the same time as the spring bloom (Fig. 4) and parallel with the decrease in DIN and DIP
317 concentrations, which were highest during winter. In agreement with the absolute concentrations, the
318 fractions of PON and POP increased during the blooms at both stations to 10 – 16 % of TN and 50 –
319 60 % of TP, respectively. The highest contribution of PON to TN was in May – August in RF with 13
320 ± 3 % and in July – November in GB with 16 ± 3 %. The percentage of POP to TP peaked together
321 with the blooms at both stations but also had a second peak during August and September in GB.
322 Similarly, the percentage of DON (in TN) and DOP (in TP) increased during or immediately after the
323 blooms in both GB and RF to 76 and 90 % for DON and 33 and 46 % for DOP. In general, the lowest
324 proportions of DON and DOP, relative to TN and TP, were found in the winter months from November
325 to February when the inorganic fractions were high. No seasonal change could be observed in the
326 contribution of DOC to TOC, but the DOC pool was at all times during the season above 89 % of TOC
327 at both stations.

328 **Conservative mixing and DOM**

329 The DOM components were analyzed using the salinity as a conservative tracer in order to examine the
330 importance of mixing relative to internal processes in the systems. However, the range in salinity in RF
331 was only 12 – 15 (Fig. 7) and, thereby, too narrow for an analysis of conservative mixing. In GB,
332 significant negative relationships were observed between salinity and concentrations of DOC and DON
333 (Fig. 7a, b) and there was a significantly higher concentration of DOC during spring as seen above

334 (Fig. 5a, b). Therefore, the analysis was conducted with the spring period as an explanatory variable
335 (Fig. 7). The model estimated the change in DOC to 8.43 μM pr. unit salinity and a production of 113
336 μM DOC in the spring. With a salinity range of 13 units (from 11 to 24) the salinity driven variation
337 amounted to 110 μM DOC. Thus, conservative mixing and internal processes were of similar
338 importance for the variation of DOC in GB. The same pattern was observed for DON, but in this case
339 conservative mixing accounted for a variability of 2.2 μM DON or about twice the production during
340 the spring period. If the regression line was extrapolated to salinity zero, the estimated freshwater
341 concentration of DOC was between 385 and 498 μM and between 16.1 and 17.1 μM for DON.
342 Contrary to the DOC and DON concentrations, the relationship between the DOP concentration and
343 salinity was insignificant (Fig. 7c).

344 **Discussion**

345 **Contrasting but similar systems**

346 The most remarkable finding in this study is that the allocation of the different carbon, nitrogen, and
347 phosphorous pools were rather similar in the two systems (Table 1, Fig. 4) despite the large differences
348 in nutrient richness and physical structure. RF has five to eight times higher concentrations of inorganic
349 nutrients and three to five times higher concentrations of total nitrogen and phosphorous than GB (Fig.
350 3, Table 1). Moreover, the water column in RF is fully mixed. Therefore, it continuously receives
351 inputs of remineralised nutrients from the sediments there often become anoxic, particularly during late
352 summer and fall (Conley et al. 2000). The higher nutrient levels in RF were associated with twice as
353 much particulate organic matter and a three times larger pool of DOM compared to GB (Table 1, Fig.
354 3). However, the fractions of carbon, nitrogen and phosphorus bound in organic particles were quite the

355 same, although with a tendency toward higher values in GB (Table 1). The latter probably reflecting
356 that particles become more important when the DOM background pool is low. Overall, DOM was the
357 dominating pool of carbon and nitrogen with approximately 95 and 70 % of TOC and TN, respectively,
358 whereas only 20 % of TP was found as DOP. DIP was the major fraction of phosphorous with
359 approximately 50 % (Fig. 4). The higher DOM concentrations in RF led to higher concentrations of
360 BDOC, BDON, and BDOP, but it was striking that the percentage of BDOM relative to the total DOM
361 pool was similar in the two contrasting systems with 25, 17, and 49 % of C, N, and P degraded,
362 respectively (Table 1, Fig. 3, Fig. 4). Just like the BDOM pools, the allocation of all other carbon,
363 nitrogen, and phosphorous pools were similar in RF and GB, and, thus, the ratios for allocation
364 between the two systems were close to one (Table 1, Fig. 4). This was also the pattern observed for the
365 dynamic pools of carbon, nitrogen, and phosphorous which, on average for the two systems, accounted
366 for 32, 42, and 92 %, respectively, of the total pools of these elements. Thus, this study indicates that
367 regardless of large differences in the eutrophication levels, absolute concentrations and physical
368 structure, the pelagic metabolism within the estuaries seems to maintain a uniform distribution of
369 inorganic, organic and dynamic components of carbon, nitrogen, and phosphorous in the water column.

370 The annual average of TN and TP concentrations in the two study areas represents the extremes
371 when comparing with a range of other Danish estuaries and coastal systems. Riemann et al. (2016)
372 examined the nitrogen and phosphorous concentrations in 45 different Danish estuaries and coastal
373 areas during the last 25 years. The levels we measured in GB were similar to the average of measured
374 concentrations of today, whereas the concentrations in RF corresponded to the average nitrogen and
375 phosphorous concentrations in Denmark in the 80's when eutrophication was at its highest. In general,
376 the concentrations measured in RF and GB corresponds to concentrations measured in a wide range of

377 estuaries and coastal areas worldwide (Bronk et al. 1998; Hansell and Carlson 2002; Savchuk 2005).
378 The two systems also differed in the relative importance of nitrogen and phosphorous as limiting
379 nutrient for primary production but they both show a pattern typical for temperate shallow estuaries
380 where phosphorous is limiting in the spring and then replaced by nitrogen limitation (Timmermann et
381 al. 2014). For GB low and probably limiting concentrations of both DIN and DIP were measured from
382 March to October. For RF, DIP concentrations were only potential limiting at one sampling in April
383 whereas DIN was potential limiting from April to the beginning of July.

384 During the spring bloom, a significant increase in production and bioavailability of DOC was
385 observed (Fig. 3) suggesting that phytoplankton primary production was responsible for an enrichment
386 of the DOM pool with labile DOC. This was similar to observations in previous studies (Williams
387 1995; Nagata 2000; Suratman et al. 2009; Markager et al. 2011) that all have shown that carbon
388 fixation continues under nutrient limitation. This creates a carbon surplus inside the algae cells, which
389 eventually can be excreted as DOC, whereas nitrogen is retained inside the cells (Jakobsen and
390 Markager 2016; Markager and Sand-Jensen 1996; Mykkestad 1995; Fajon et al. 1999). Carlson (2002)
391 showed that on average 13 % (up to 55 %) of the primary production was released as DOC. We
392 observed the same phenomenon in earlier studies with plankton from RF where nitrogen depletion
393 caused accumulation of DOC and an increase in the C:N ratio of DOM to above 100 (Knudsen-
394 Leerbeck and Markager, submitted). Nutrient limitation might also explain why we did not observe a
395 concurrent seasonal relation between Chl *a* and DON concentrations like in Bronk et al. (1994). We did
396 observe an increase in the relative contribution of the DON and DOP pools to the total pools of TN and
397 TP during the spring blooms, but this was due to the decrease in DIN and DIP rather than an increase in
398 DON and DOP concentrations. This suggests that DON is retained more efficiently by phytoplankton

399 than DOC and is consistent with observations made by Korth et al. (2012). They found that
400 autochthonous DON is important for phytoplankton nutrition and potentially can be utilized again
401 immediately even before it is observed as a release of DON. Consistent with this, the C:N:P ratios in
402 the DOM pool in both RF and GB were depleted in nitrogen and phosphorous compared to the
403 Redfield ratio (Fig. 5). In spite of different eutrophication levels in the two systems, the C:N:P ratio in
404 DOM was similar in RF and GB and close to ratios observed in other studies (Hopkinson et al. 1997;
405 Lønborg and Søndergaard 2009; Markager et al. 2011). Sometimes the fractions of BDON and BDOP
406 are observed to be higher than the BDOC fraction, which are often explained with selective degradation
407 of nitrogen- and phosphorous-rich compounds (Jackson and Williams 1985; Hopkinson et al. 1997;
408 Hopkinson et al. 2002; Lønborg and Alvarez-Salgado 2012). Nonetheless, in this study the increasing
409 order of the bioavailability was DON, DOC, and DOP. Thus, DON was, with a biodegradable fraction
410 of 17 %, less bioavailable than DOC (25 %) and much less than DOP (47 %). However, the estimated
411 pools of BDON and BDOP might be underestimated if nitrogen or phosphorous is converted to
412 bacterial biomass during the incubation instead of being released as DIN or DIP (Knudsen-Leerbeck
413 and Markager submitted). During the degradation experiments, bacteria were counted and the nitrogen
414 contained in the bacterial biomass was estimated. The bacterial biomass was observed to vary during
415 the degradation experiments (data not shown). The range of the net increase in bacterial biomass was -
416 0.7 $\mu\text{M N}$, i.e. a decrease in the bacterial biomass during degradation.

417 Another factor that can affect the bioavailability of the DOM compounds is photochemical
418 processes. These were not included in this study as the degradation experiments were carried out in
419 darkness. Previous studies have shown that photochemical processes significantly alter DOM
420 bioavailability; photochemical processes can degrade refractory DOM compounds and increase

421 bioavailability (Bushaw et al. 1996; Vahatalo and Zepp 2005; Stedmon et al. 2007) while labile
422 compounds have been shown to become more refractory after photochemical degradation
423 (Obernosterer et al. 1999; Tranvik and Bertilsson 2001).

424 **Conservative mixing**

425 A significant linear relationship was observed between salinity and the DOC and DON concentrations
426 in GB (Fig. 7). In GB, there is an advection of two water masses where high-saline bottom water from
427 the North Sea with relatively low DOM concentrations is mixed with low-saline surface water from the
428 Baltic Sea with higher DOM concentrations. Therefore, conservative mixing was expected to play a
429 significant role for the patterns observed. In contrast, the salinity was almost constant in RF. The
430 results showed as expected that mixing of the different water masses was important for the DOC and
431 DON concentrations in GB and the analysis allows us to quantify the relative importance. For DOC the
432 estimated degradation of 86 $\mu\text{M C}$ (Table 1), the estimated spring production of 113 $\mu\text{M C}$ (Fig. 7a)
433 and the dilution of surface concentrations due to mixing ($13 \text{ salinity units} \times 8.43$ [see Fig. 7a] = 110
434 $\mu\text{M C}$) show that physical mixing and internal processes are of about equal importance for the
435 concentration. For DON, the rates are 2.0, 1.0 and 2.2 $\mu\text{M N}$ for degradation, spring production and
436 mixing, respectively. Thus, the losses processes seem to dominate, indicating that there is a net loss of
437 DON in GB. However, the estimated production from Fig. 7 covers only the spring period.

438 In Markager et al. (2011) two mixing patterns were outlined. Mixing pattern 1 was defined as when
439 conservative mixing dominated and production or degradation rates for DOM were low or insignificant
440 relative to the mixing rate. Mixing pattern 2 means that internal production or degradation processes
441 within a system dominates over mixing. The results from GB indicate a situation in between these

442 extremes for DOC and DON. The results also show that a significant relationship between salinity and
443 DOM should not be interpreted as lack of importance of internal processes. The DOP pool seemed to
444 be governed by production or degradation within the system rather than conservative mixing, but the
445 results for DOP are difficult to interpret due to the low rates.

446 The estimated freshwater concentrations of 385 – 498 μM DOC and 16.1 – 17.1 μM DON with
447 the model for GB (the intercepts in Fig. 7a, b) were lower than concentrations found in Asmala et al.
448 (2012) (1200 μM DOC and 17.3 μM DON for three Finnish rivers), the concentrations of 591 and 84
449 μM DOC and DON, respectively, for the freshwater inflow to a nearby Danish estuary (Markager et al.
450 2011) and 486 and 75 μM DOC and DON, respectively, for RF (Nielsen et al. submitted). However,
451 these studies examined the dynamics in close vicinity to fresh water outlets where an intense activity is
452 often observed. It is therefore to be expected that our estimates are lower and probably reflect the
453 concentration at zero salinity but minus the initial intense degradation in estuaries.

454

455 **Fluxes of bioavailable nitrogen**

456 The importance of remineralization of nitrogen for productivity depends on the relative size of this flux
457 compared to other inputs of bioavailable nitrogen rather than the absolute concentration of BDON
458 provided in Table 1. We have therefore established a budget for bioavailable nitrogen inputs to the two
459 systems annually and for a winter and summer situation, respectively (Fig. 8 and Table 2). This
460 involves compilation of data for different processes where some are difficult to quantify. In addition, the
461 estimation of an instantaneous rate of remineralization is uncertain. However, to our knowledge this is

462 the first time the importance of remineralization of DON is quantified relative to all other sources of
463 bioavailable nitrogen.

464 *Budget data and set up*

465 All external inputs of bioavailable DON and PON were assumed included in the measured
466 remineralization of the DON pool in this experiment, and, therefore, all external inputs only include
467 DIN. Monthly nitrogen fluxes from land to RF were calculated from monthly DIN loadings (Wiberg-
468 Larsen et al. 2012) by dividing with the surface area of the estuary in order to obtain an area specific
469 loading. For GB, the relevant catchment area is less well defined, but a nitrogen budget has previously
470 been established for the entire Kattegat and Belt Sea area (Jørgensen et al. 2014). We have used the
471 same catchment and surface areas as the GB station is situated in the middle of this area in order to
472 calculate the area specific loadings. Fixation of nitrogen by diazotrophic cyanobacteria and
473 heterotrophs was estimated in Bentzon-Tilia et al. (2015) in a study accompanying our campaign and
474 considered representative for the entire estuary. However, for the Kattegat and Belt Sea, where the GB
475 station is situated, the conditions for nitrogen fixation are highly variable due to the salinity gradient in
476 the area. This is accounted for in a model estimate for nitrogen fixation found in Jørgensen et al.
477 (2014), and we have used this as our estimate for GB. The fluxes of nitrogen from atmospheric
478 deposition were available from an atmosphere deposition model there is part of the Danish monitoring
479 program (Ellermann et al. 2013). The DIN fraction of the total deposition was estimated as described in
480 Jørgensen et al. 2014). When estimating the advection of nitrogen from adjacent seas to GB, the
481 continuous inflow of surface water from the Baltic Sea is taken into account (Kaas et al. 1994;
482 Markager et al. 2007). The nitrogen input entering GB from north with the high saline bottom water
483 from Skagerrak is entering the surface water when the bottom water is mixed into the surface water,

484 and the input is accounted for below under exchange with the sediment. In, RF there is an exchange
485 with the adjacent Isefjord/Kattegat. The advective input of DIN was estimated by Staehr et al. (2017)
486 for the period 1998-2005. We used their value and scaled it to the present concentration of DIN in
487 Kattegat. The advective input in summer is negligible as DIN concentrations are close to zero (similar
488 to the results shown in Fig. 3). The nitrogen exchange between the sediment and water column has
489 previously been measured monthly in RF (1999-2000, H. Fossing). In GB, the influx is through mixing
490 of water from below the pycnocline with DIN coming from the sediment, from remineralization of
491 DON in the deep water and from influx of water from Skagerrak as mentioned above. This upward flux
492 is taken from Jørgensen et al. (2014).

493 The initial nitrogen flux from DON remineralization can be difficult to measure experimentally.
494 Therefore, it was estimated by assuming that between 1/100 and 1/1000 of the true BDON
495 concentration is left after 189 days (the average duration of the degradation experiments in present
496 study). Data for oxygen consumption in the bottles and the decrease in DOC concentrations over time
497 at the end of the experiments indicate that the degradation of the DOM pool is negligible at the end of
498 the degradation experiments (Fig. 2). The rates during winter and summer were estimated based on the
499 rates measured at 15 °C, a Q_{10} coefficient of 2.5 and the measured *in situ* temperatures.

500

501 *Budget*

502 The estimated annual average rates for remineralization are 0.76 and 1.0 and $\text{mmol N m}^{-2} \text{d}^{-1}$ for RF
503 and GB, respectively or 28 and 42 % of the total inputs of bioavailable nitrogen (Table 2). The higher
504 areal rate in GB is due to a deeper water column (13.8 m surface layer) compared to 3 m in RF. The

505 volume metric rates of remineralization are highest in RF, $0.25 \text{ mmol N m}^{-3} \text{ day}^{-1}$, compared to 0.073
506 $\text{mmol N m}^{-3} \text{ day}^{-1}$ in GB. The importance of remineralization of BDON is higher in the summer, 47 %
507 (RF) and 55 % (GB) due to a lower run off from land in the summer months, higher temperatures and,
508 hence, higher mineralization rates during summer. Thus, overall BDON provide about half of the
509 bioavailable nitrogen in a summer situation.

510 In RF, the water column is mixed, and the sediment is therefore a potential source of nutrients.
511 Yet in the winter months, there is a net influx of nutrients to the sediment. In addition, remineralization
512 of DON is low and freshwater run off is high so external inputs for land and adjacent seas dominate
513 with 88 % (Table 2 and Fig. 8). This situation is reversed in summer where both remineralization of
514 DON and the contribution from the sediment are high. These are internal sources and together they
515 amount to 88 % in summer. In GB, the situation is more complex as the input from below the
516 pycnocline is due to an inflow of bottom water from the Skagerrak (new nitrogen) as well as nitrogen
517 from the sediments (recycled nitrogen). The sum of the transport up through the pycnocline and from
518 remineralization of BDON in the mixed layer is 50 and 85 % in winter and summer, respectively.
519 Hence, the pattern is similar to the pattern in RF but the contribution from land is relatively lower as
520 other sources (sediment and advection) are higher in GB and the ratio of catchment area to sea surface
521 area is lower.

522 The importance of bioavailable nitrogen from the DON pool were from 0.1 (RF in winter) to 13
523 times the loadings of bioavailable nitrogen from land. Hence, the budgets demonstrate that the BDON
524 pool is an important player in the nitrogen cycling - especially during summer - both in eutrophic
525 estuaries (e.g. RF) as well as in more oligotrophic open areas (GB). As a large fraction of the DON
526 pool is derived from earlier loadings of DIN from land to marine systems (Stedmon et al. 2006,

527 Markager et al. 2011) the calculations demonstrate how the DON pool in the water column, together
528 with nitrogen in the sediments, can keep coastal marine system in a eutrophic state even after
529 significant reductions in nutrient loadings. This can be part of the explanation for an observed time lag
530 in the improvement of the environmental state of estuaries after load reductions (Riemann 2016).
531 Eventually, nitrogen is removed from the systems due to denitrification and fluxing, and from 1988 to
532 2007 the DON concentration decreased with 0.99 $\mu\text{mol pr. year}$ in RF (Petersen et al. 2014) or about
533 35%, concurrent with a decrease in nitrogen loadings a 57%, i.e. the decrease in the DON
534 concentrations is lagging behind the decrease in loadings.

535

536 In conclusion, the total dynamic pools, i.e. the sum of inorganic nutrients, particles, and the
537 bioavailable DOM pools, were found to account for 32, 42, and 92 % of TOC, TN, and TP,
538 respectively. The dynamic pool of nitrogen was 46 and 37 % of TN in RF and GB, respectively, and
539 could potentially fuel a phytoplankton growth of 18-67 $\mu\text{g Chl } a \text{ l}^{-1}$, assuming the Redfield ratio and a
540 carbon:chl *a* ratio of 30. Of this, BDON alone contributed with 28 % of TN equivalent of 2.5 times the
541 maximum Chl *a* concentration measured in RF, which demonstrate the importance of DON
542 mineralization. If the pool of DOM is not utilized in the same area as produced, this pool can be
543 transported to adjacent areas where it can be degraded and utilized by phytoplankton and thereby
544 stimulate new primary production. This in turn implies that eutrophication problems must be
545 considered on a regional scale at least for the more open areas.

546 To our knowledge, this is the first time a seasonal study has examined the concentration and
547 bioavailability of all three components of DOM in two contrasting systems and revealed a uniform

548 distribution of the different components regardless of the eutrophication status. We speculate if this is a
549 general phenomenon, i.e. that the processes responsible for cycling of nitrogen in marine systems tends
550 to end up with about 40 % in a dynamic pool and 60 % in a refractory pool. This study emphasized that
551 nitrogen in DOM is important for the nitrogen cycling, and that nutrients bound in DOM should be
552 taken into account when studying nutrient dynamics in coastal systems and in particular when
553 modelling eutrophication scenarios.

554

555 **Fig. 1** Map of the Baltic Sea transition zone showing the two sampling stations in the nutrient-rich
556 Roskilde Fjord (RF) and the more oligotrophic Great Belt (GB), Denmark.

557

558 **Fig. 2** An example of the DOC degradation (a) and oxygen consumption (b) during the incubations in
559 June and the fitted regression models.

560

561 **Fig. 3** Seasonal variation in Roskilde Fjord and Great Belt for (a, e) salinity and concentrations of Chl *a*
562 ($\mu\text{g L}^{-1}$), (b, f) DIN, DIP, TN, and TP (μM), (c, g) DOC, DON, DOP (μM) and (d, h) BDOC, BDON,
563 and BDOP as the percentage of the concentration of DOM at start of degradation experiments.

564

565 **Fig. 4** Seasonal variations in fractions of carbon, nitrogen and phosphorous for Roskilde Fjord and
566 Great Belt. Values are percentage of TOC, TN, and TP for dissolved inorganic nutrients (DIN and
567 DIP), organic particles (POC, PON, and POP) and bioavailable DOM (BDOC, BDON, and BDOP).
568 Together these fractions constitute the dynamic pool (see text) and are depicted in darker grey shades
569 from the base. The light grey part at the top represents the refractive fractions (RDOC, RDON, and
570 RDOP). Arrows indicate the spring bloom. A lost sample resulted in lack of data in figure c.

571

572 **Fig. 5** Relationships between concentrations of carbon, nitrogen, and phosphorous (μM) for the DOM
573 pool in Roskilde Fjord (RF) and Great Belt (GB). The Redfield ratio is indicated by the dotted line.
574 Values for RF-spring and GB-spring include data from January to July and January to April,
575 respectively. Error bars indicate the standard deviation.

576

577 **Fig. 6** Relationships between concentrations of bioavailable DOM, similar to Fig. 4.

578

579 **Fig. 7** The relationship between concentrations of DOC, DON, DOP and salinity in Roskilde Fjord and
580 Great Belt. The open symbols denote Roskilde Fjord and the filled symbols Great Belt and the rhombi
581 designate the spring periods – January to April in Roskilde Fjord and January to July in Great Belt. The
582 parameters (\pm 95% confidence limits) in models with the form $\text{Conc.} = \text{intercept} + k_1 * \text{salinity} +$
583 $k_2 * \text{spring_period}$ were estimated. The explanatory variable ‘spring_period’ equal 1 or 0, respectively.
584 The model was fitted for DOC, DON, and DOP concentrations in Great Belt. The dashed and solid
585 lines show the model with and without the offset for the spring period. DOC values are the mean of
586 triplicates with the standard deviation depicted.

587

588 **Fig. 8** The relative importance of bioavailable nitrogen input to Roskilde Fjord in a winter and summer
589 situation (see also Table 2).

590

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599

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795



Fig. 1

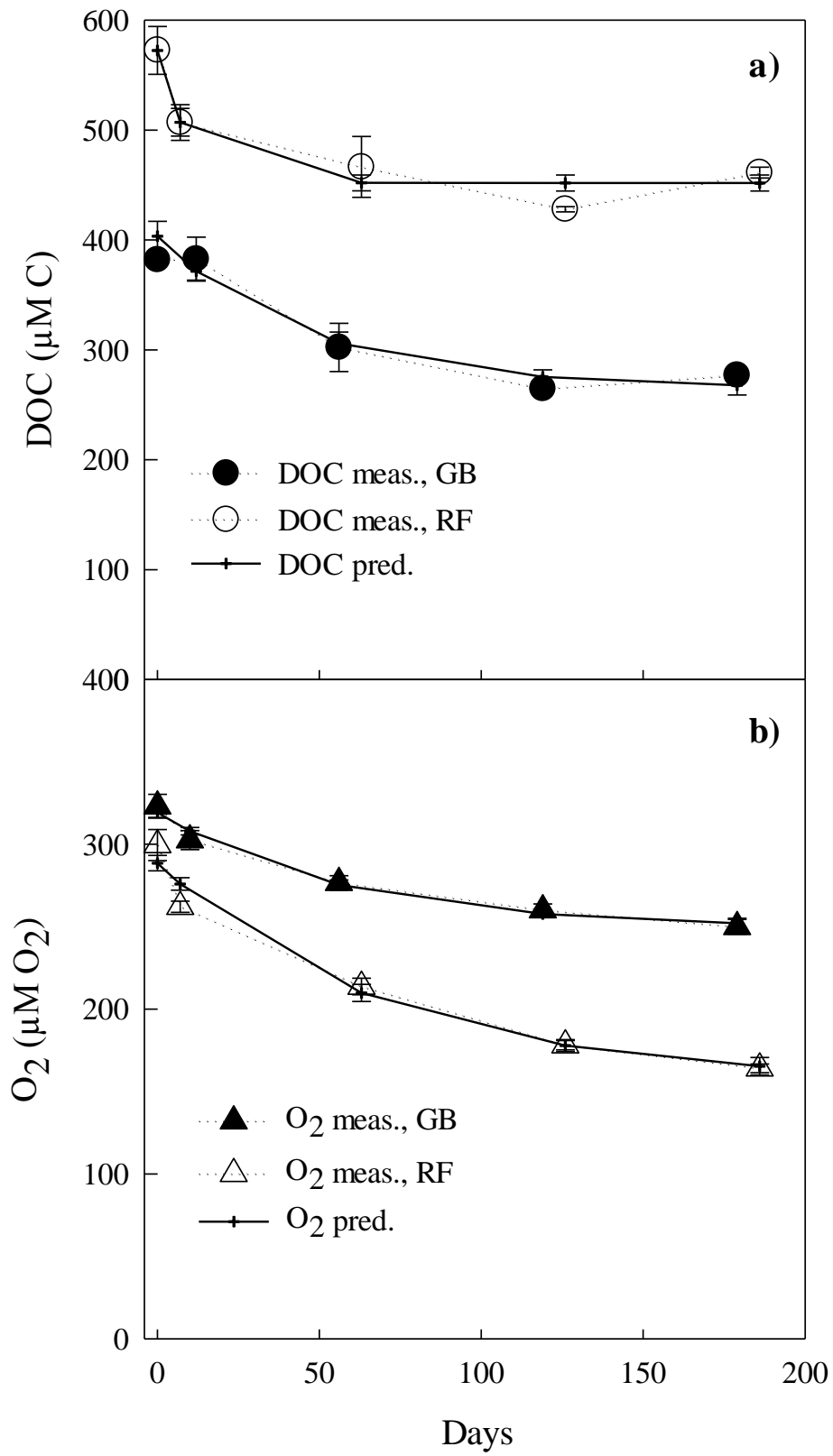


Fig. 2

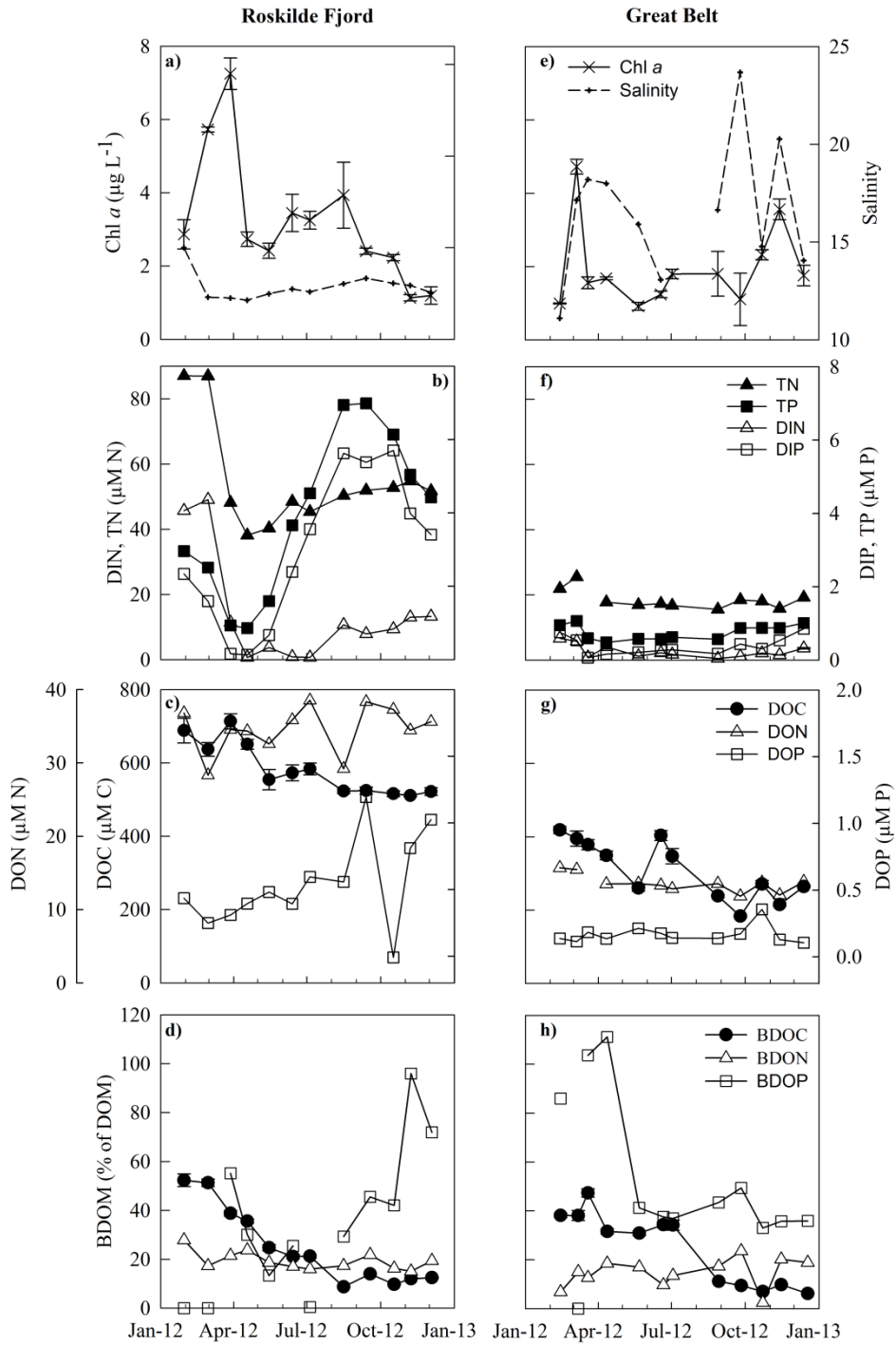


Fig. 3

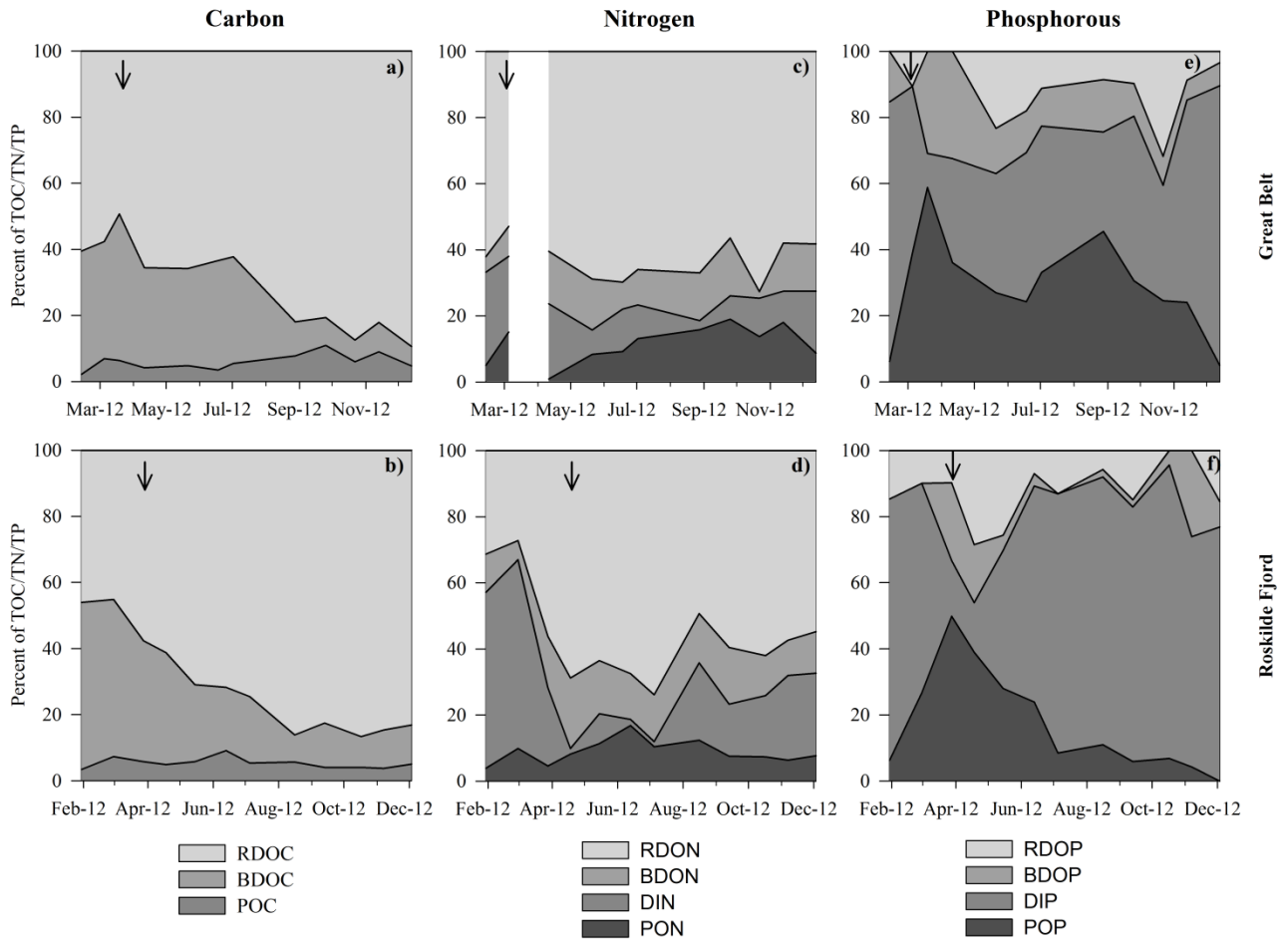


Fig. 4

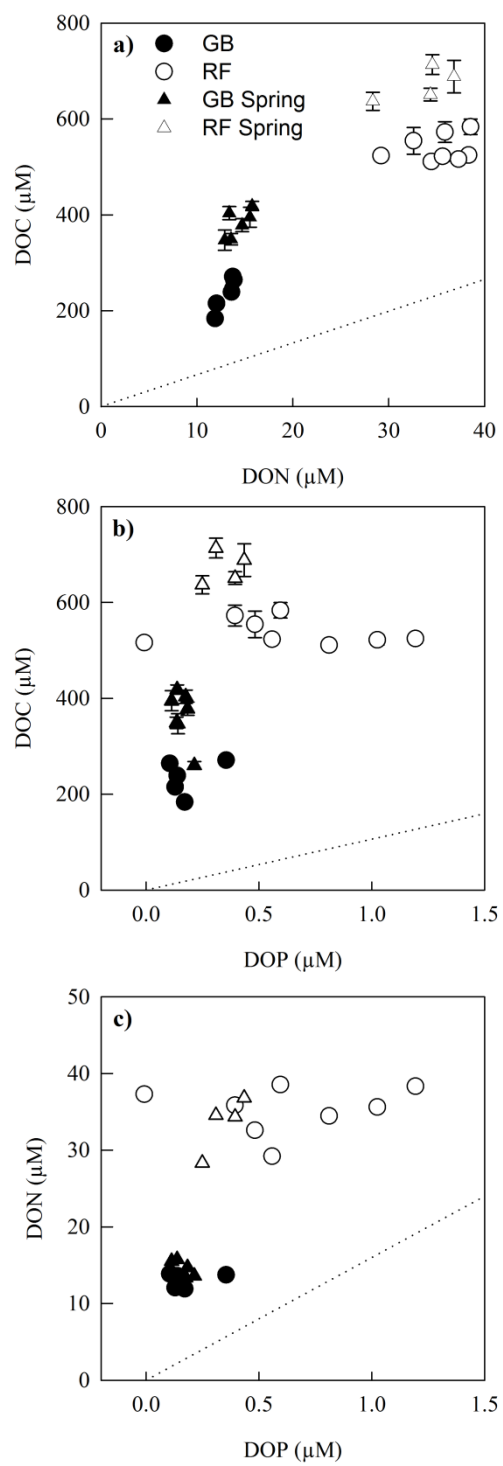


Fig. 5

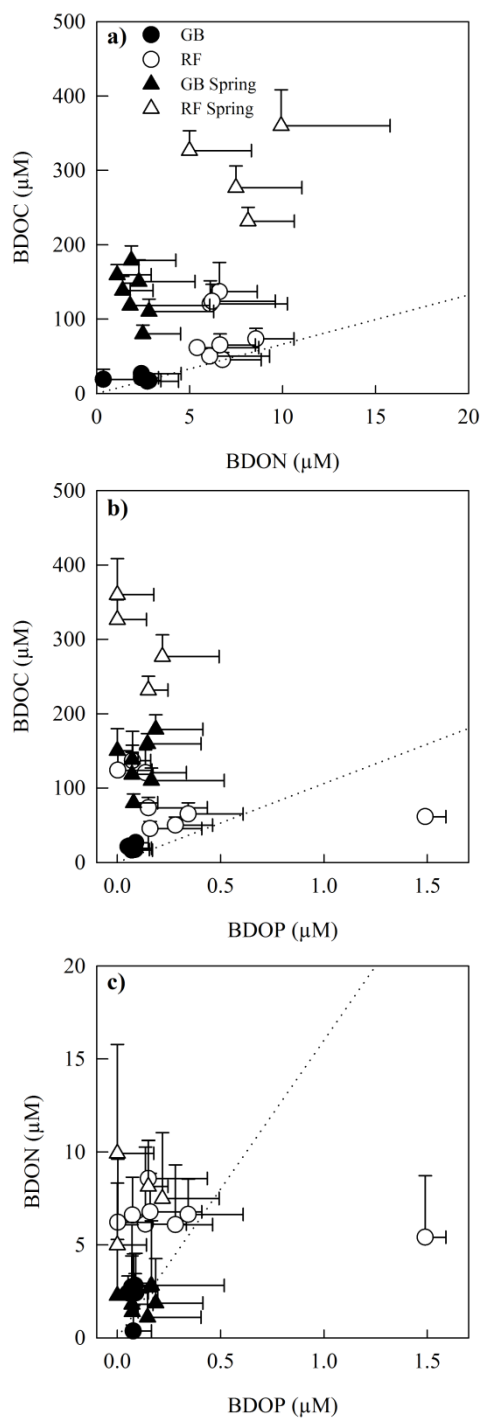


Fig. 6

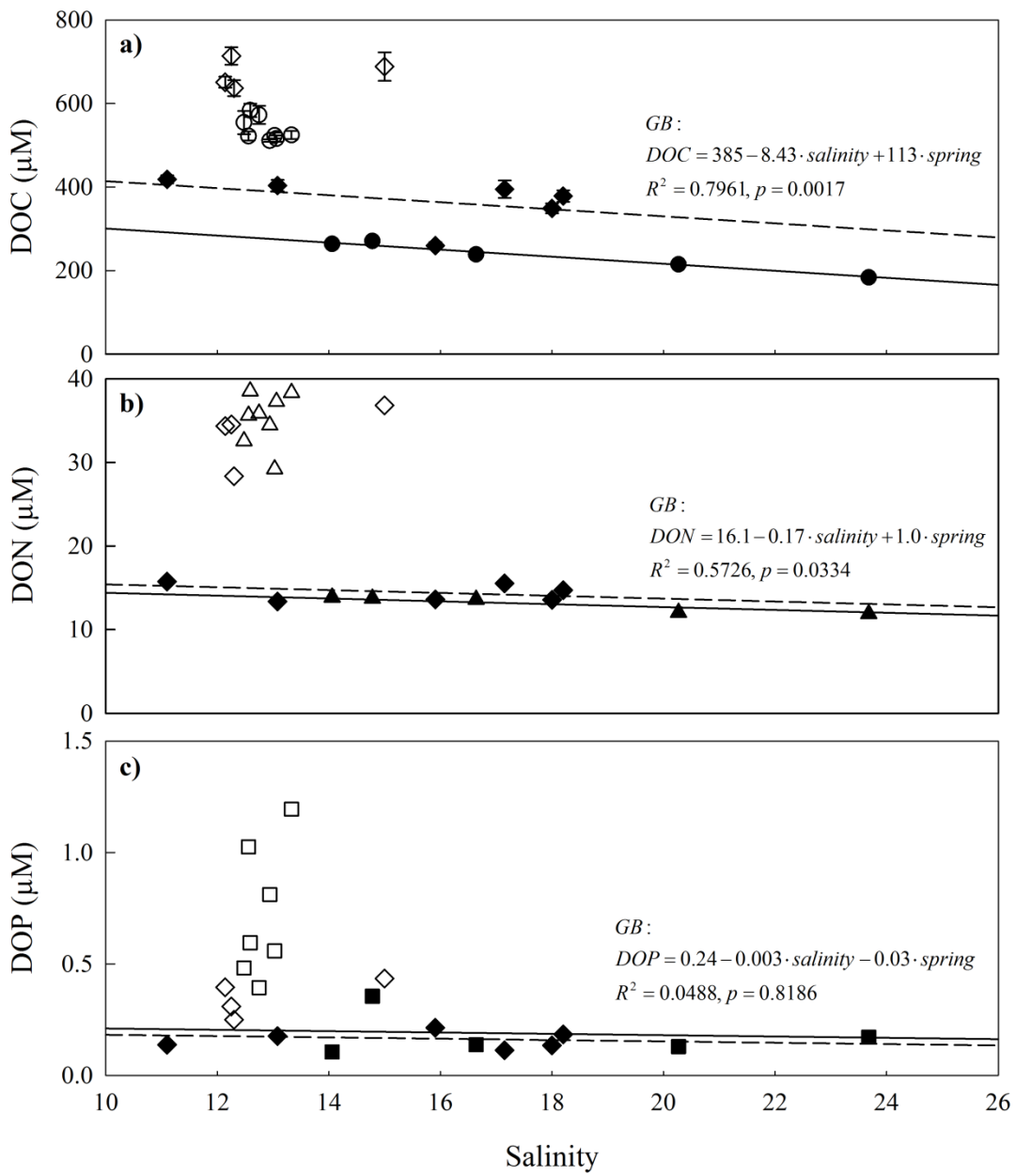
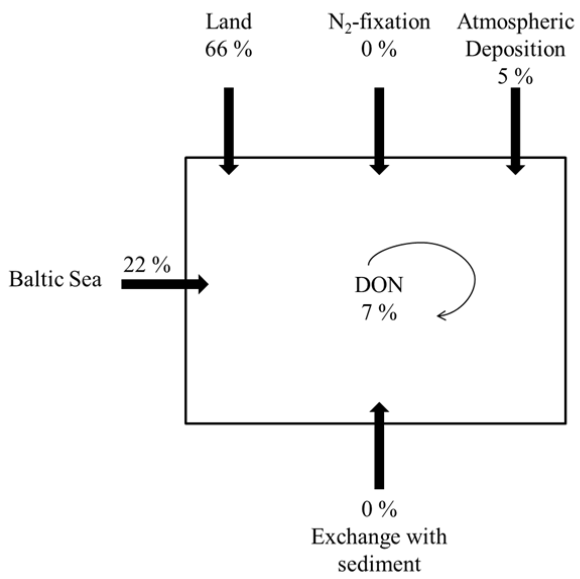


Fig. 7

**Roskilde Fjord -
Winter**



**Roskilde Fjord -
Summer**

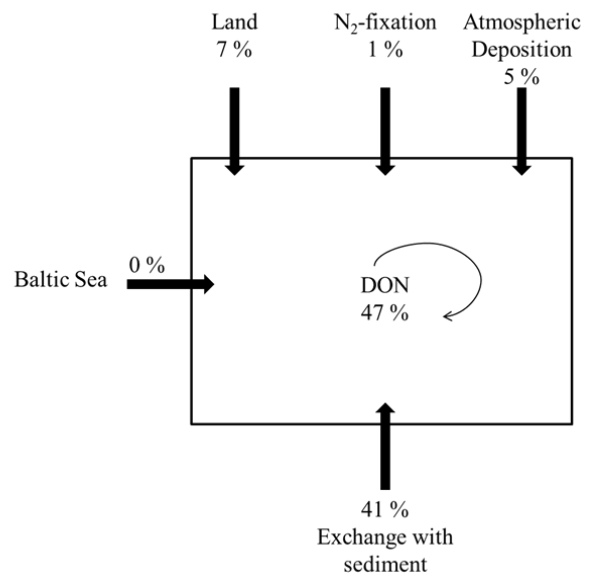


Fig. 8

Table 1 Annual averages concentrations of carbon, nitrogen, and phosphorous containing compounds at the two stations Roskilde Fjord (RF) and Great Belt (GB) all with the unit μM . The ratio between the concentrations in RF and GB are shown. The percentages (RF %, GB %) are calculated as the inorganic, dissolved, and particulate fractions of TOC, TN, and TP, respectively. BDOC, BDON, and BDOP is given as the percentage of DOC, DON and DOP, respectively, degraded during 189 ± 11 days. The dynamic pools were calculated as the sum of inorganic nutrients, particles, and the bioavailable DOM pools. These are given as percentages of the sum of TOC, TN, and TP. The standard deviation is shown (n=12).

		RF	GB	RF/GB	RF	GB	RF/GB
		μM	μM	Ratio	%	%	Ratio
<i>Total</i>	Nitrogen	55±16	19±2.9	3.0			
	Phosphorous	3.9±2.2	0.75±0.20	5.1			
<i>Inorganic</i>	DIN	14±16	2.6±2.0	5.3	21±18	14±8.0	1.5
	DIP	2.9±2.0	0.38±0.25	7.7	64±26	47±21	1.4
<i>Organic</i>	DOC	583±72	310±81	1.9	95±1.6	94±2.4	1.0
	DON	35±3.3	14±1.2	2.6	70±17	74±6.2	0.9
	DOP	0.54±0.33	0.17±0.07	3.3	19±13	24±10	0.8
	POC	33±12	18±5.8	1.8	5.3±1.6	6.0±2.4	0.9
	PON	4.5±1.8	2.1±1.0	2.1	8.9±3.6	12±5.6	0.8
	POP	0.43±0.25	0.21±0.11	2.1	18±16	30±15	0.6
	BDOC	156±113	86±64	1.8	25±16	25±15	1.0
	BDON	6.9±1.4	2.0±0.76	3.4	19±3.8	15±6.0	1.3
	BDOP	0.25±0.41	0.09±0.05	2.7	41±28	56±29	0.7
<i>Dynamic</i>	C	189±114	104±64	1.8	31±19	32±21	1.0
	N	25.2±17	6.8±2.4	3.7	46±33	37±14	1.3
	P	3.59±2.1	0.68±0.27	5.3	93±75	90±44	1.0

Table 2. Fluxes of bioavailable nitrogen (percentage of the total bioavailable nitrogen fluxes and absolute values) from the different sources into the two systems GB and RF during winter, summer, and the entire year. The contribution from external inputs of bioavailable DON and PON were assumed included in the remineralization from the DON pool and therefore not included. Hence, all external inputs represent only the DIN fraction of the total nitrogen input. The sources included were loadings from land, nitrogen fixation, atmospheric deposition, exchange with sediment, remineralization of DON, and advection from adjacent seas. The winter period was from December to February, and the summer period was from June to August. (–) indicate that the net value was negative and that it has not been possible to estimate a gross value, which however was considered insignificant.

	RF			GB		
	Entire year, % (mmol N m ⁻² d ⁻¹)	Winter, % (mmol N m ⁻² d ⁻¹)	Summer, % (mmol N m ⁻² d ⁻¹)	Entire year, % (mmol N m ⁻² d ⁻¹)	Winter, % (mmol N m ⁻² d ⁻¹)	Summer, % (mmol N m ⁻² d ⁻¹)
Land	31 (0.85)	66 (1.97)	7 (0.20)	11 (0.26)	25 (0.53)	4 (0.10)
N-fixation	0 (0.01)	0 (0.01)	1 (0.02)	2 (0.06)	0 (0.00)	5 (0.12)
Atmospheric deposition	5 (0.14)	5 (0.14)	5 (0.14)	6 (0.15)	7 (0.15)	6 (0.15)
Advection Baltic Sea	12 (0.33)	22 (0.66)	0 (–)	8 (0.18)	17 (0.36)	0 (0.00)
Exchange with sediment	23 (0.62)	0 (–)	41 (1.15)	31 (0.73)	31 (0.66)	30 (0.73)
DON	28 (0.76)	7 (0.21)	47 (1.31)	42 (1.01)	19 (0.40)	55 (1.33)