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

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Published in:
Biogeochemistry

Link to article, DOI:
10.1007/s10533-017-0357-2

Publication date:
2017

Document Version
Peer reviewed version

Biogeochemistry

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--Manuscript Draft--

Manuscript Number:	BIOG-D-16-00294R2
Full Title:	Seasonal dynamics and bioavailability of dissolved organic matter in two contrasting temperate estuaries
Article Type:	Manuscript
Keywords:	Bioavailability; DOC; DON; DOP; eutrophication; nitrogen budget
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Funding Information:	Innovation Fund Denmark — Dr. Stiig Markager Statens Naturvidenskabelige Forskningsrad — Professor Lasse Riemann Villum Fonden — Ph.D. Sachia Jo Traving
Abstract:	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,

	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.
Response to Reviewers:	2. Review on manuscript: "Seasonal dynamics and bioavailability of dissolved organic matter in two contrasting temperate estuaries" We have modified Fig. 2 as suggested by splitting the plots of DOC and oxygen concentrations over time into two panel. In addition, we have been through the text again and corrected all misspelling and language problems we could detect.

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

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

Abstract

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, 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.

Introduction

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

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

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

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

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

In addition to freshwater loadings, other nitrogen sources to marine waters include N_2-fixation, atmospheric deposition, and nitrogen from adjacent areas. N_2-fixation can be an important source of nitrogen in coastal waters (Rees et al. 2009; Mulholland et al. 2012; Bentzon-Tilia et al. 2015). The significance of atmospheric nitrogen deposition is highly dependent on the sea surface area relative to the loadings from land but can dominate during summer in open coastal areas (Spokes et al. 2006). Depending on depth, the sediment can play an important role as a nutrient source to the water column and, hence, for phytoplankton production (Fanning et al. 1982; Floderus and Hakanson 1989). However, nutrients from the sediment originate from the water column, so they are not 'new' to the system. The exchange can occur in connection with resuspension events, with a mass flow of water through the sediments, or from diffusive exchange between sediment and water column. Nonetheless, the nutrient pool in the sediment often dominate, e.g. was more than 95 % of the total nitrogen pool (water column plus the upper 20 cm of the sediment) found in the sediment in the Kattegat and Belt

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

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

Methods

Study sites

The estuary Roskilde Fjord (RF) and the strait Great Belt (GB, Fig. 1) are two contrasting systems. RF is a eutrophic 40 km long semi-enclosed estuary with a surface area of 123 km^2 and a residence time of one year (Flindt et al. 1997). The mean depth is only 3 m and the entire water column is well mixed with permanent contact to the sediment. This contact affects the system in several ways, e.g. nutrients are constantly exchanged between the photic zone and the sediment, resuspension of sediments occur regularly and contribute to light attenuation (K_d(PAR) ~ 0.53) (Pedersen et al. 2014; Staehr et al. 2017) and benthic filter feeding is a loss factor for phytoplankton. The sampling station is placed in the middle of the southern broad, south of the narrow strait that divide the estuary in a southern and a northern part. In-depth descriptions of the variability between stations, including maps and an analysis of the development over time is found in Pedersen et al. (2014), Staehr et al. (2017) and references herein. Briefly, the station we have sampled has been monitored since 1980 and is believed to be

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

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

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

in March at both stations with a maximum of 4.7 and 7.3 µg Chl *a* L^{-1} in GB and RF, respectively (Fig. 3).

Sampling

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

Analyzer (Skalar) according to the methods in Grasshoff (1976) and Kaas and Markager (1998). 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, respectively. TN and TP was sampled (either 200 µm pre-filtered *in situ* samples or 0.2 µm filtered + 10 % GF/C filtered bacteria inoculum sample water) in 30 ml brown glass bottles filled with Milli-Q water until sampling and frozen at -20 °C afterwards. After wet oxidation (30 minutes at 121 °C with $K_2S_2O_8$, H_3BO_3 and NaOH), samples were analyzed on an automatic nutrient analyzer as described in Kaas and Markager (1998) with detection limits of 1.0 µM N and 0.1 µM P, respectively. Samples for total organic carbon (TOC) were collected in 60 ml HDPE bottles, acidified with 2 M HCl to a pH of 2 and stored at 5 °C in darkness until measured on a TOC V_{cph} Analyzer (Shimadzu) according to Cauwet (1999). Concentrations were derived from a five-point calibration curve with subtraction of the signal from Milli-Q water and validated with a deep-sea reference sample (Hansell consensus reference material program). Data on Chl *a*, DIN, DIP, DOC, temperature, and salinity are published in Bentzon-Tilia et al. (2015) and for particulate organic carbon (POC) and PON in Mantikci et al. (in revision).

DOM degradation experiments

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

was placed in each bottle to ensure homogeneity of the water. The bottles were filled without headspace and incubated in darkness at 15 °C. At the occasions where the *in situ* seawater temperature was lower than 15 °C, the sample water (for the degradation experiments) was filtered and placed in darkness until it reached 15 °C in order to prevent bubble formation after filling of incubation bottles. At day zero and approximately at day 10, 60, 120, and 180, triplicate bottles were harvested for measurements of DIN, DIP, total dissolved nitrogen and phosphorous, and DOC. The oxygen consumption varied from 45 to 225 µmol O_2 l^{-1}, and the average concentration during the incubations was 195 µmol O_2 l^{-1}. At this level, oxygen was assumed not to limit DOM degradation and denitrification was assumed negligible. An example of oxygen concentrations during two incubations are shown in Fig. 2.

Data Analysis

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

$$DON = TN - DIN - PON. \quad (1)$$

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

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

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

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

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

Results

Annual means of carbon, nitrogen, and phosphorous components

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

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

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

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

Allocation of carbon, nitrogen, and phosphorous into fractions

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

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

Stoichiometry of dissolved organic matter

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

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

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

Seasonal variation

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

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

Discussion

Contrasting but similar systems

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

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

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

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

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

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

Conservative mixing

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

In Markager et al. (2011) two mixing patterns were outlined. Mixing pattern 1 was defined as when conservative mixing dominated and production or degradation rates for DOM were low or insignificant relative to the mixing rate. Mixing pattern 2 means that internal production or degradation processes 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 were 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 depostion 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,

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

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

Budget

The estimated annual average rates for remineralization are 0.76 and 1.0 and mmol N m^{-2} d^{-1} for RF and GB, respectively or 28 and 42 % of the total inputs of bioavailable nitrogen (Table 2). The higher 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 mmol N m^{-3} day^{-1}, compared to 0.073
506 mmol N m^{-3} 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,

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

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

To our knowledge, this is the first time a seasonal study has examined the concentration and 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 (µg 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. Valeus are percentage of TOC, TN, and TP for dissolved inorganic nutrients (DIN and
567 DIP), organic particles (POC, PON, and POP) and bioavialable 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 ligth 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.

Fig. 6 Relationships between concentrations of bioavialable DOM, similar to Fig. 4.

Fig. 7 The relationship between concentrations of DOC, DON, DOP and salinity in Roskilde Fjord and Great Belt. The open symbols denote Roskilde Fjord and the filled symbols Great Belt and the rhombi designate the spring periods – January to April in Roskilde Fjord and January to July in Great Belt. The parameters (± 95% confidence limits) in models with the form Conc. = *intercept* + k_1*salinity + k_2*spring_period were estimated. The explanatory variable 'spring_period' equal 1 or 0, respectivly. The model was fitted for DOC, DON, and DOP concentrations in Great Belt. The dashed and solid lines show the model with and without the offset for the spring period. DOC values are the mean of triplicates with the standard deviation depicted.

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

Acknowledgments

We wish to thank The Danish Nature Agency for help with water sampling. We also thank Dorete Weysmann Jensen for nutrient analysis, Colin Stedmon for help with TOC/DOC analysis, and Ciarán Murray for help with **Error! Reference source not found.**. This study was supported by the project 'Integrated management of agriculture, fishery, environment and economy' (IMAGE) project (09-067259) funded by the Innovation Fund Denmark, and project 11-105450 and 09-066396 from the

Danish council for independent research. SJT was supported by the VKR Center of Excellence on Ocean Life, funded by the Villum foundation.

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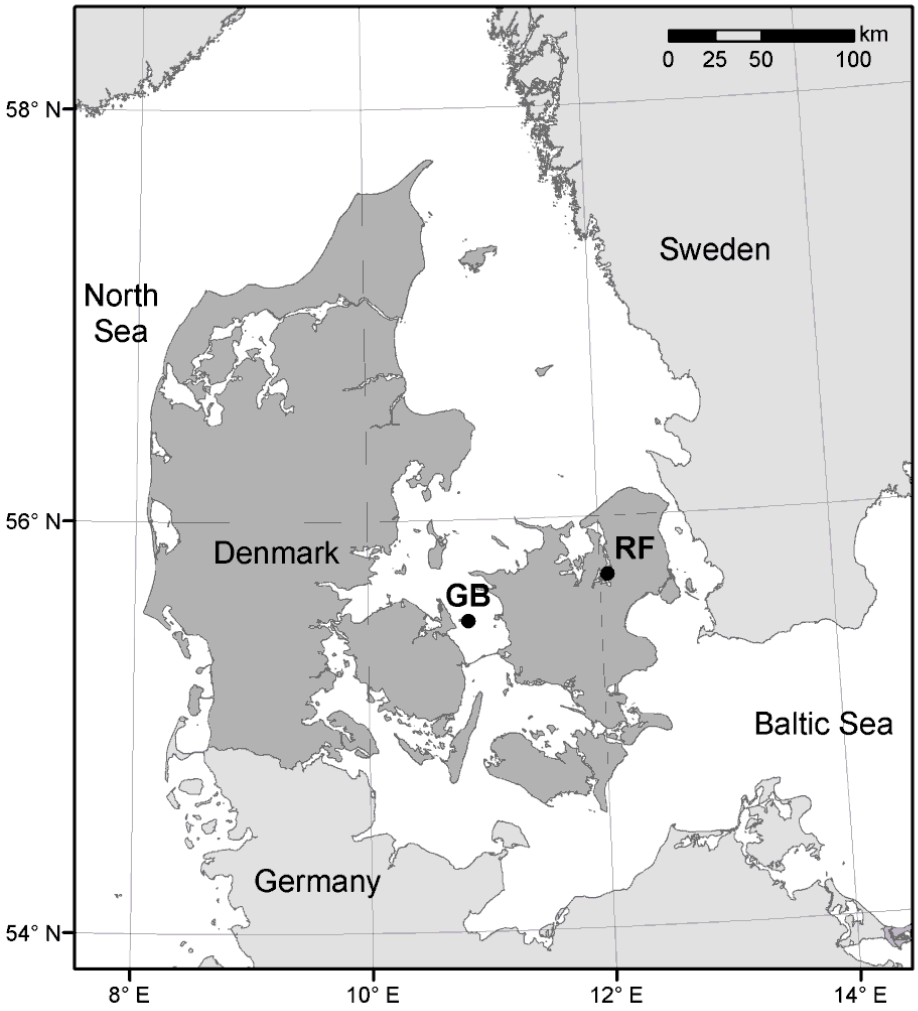

Fig. 1

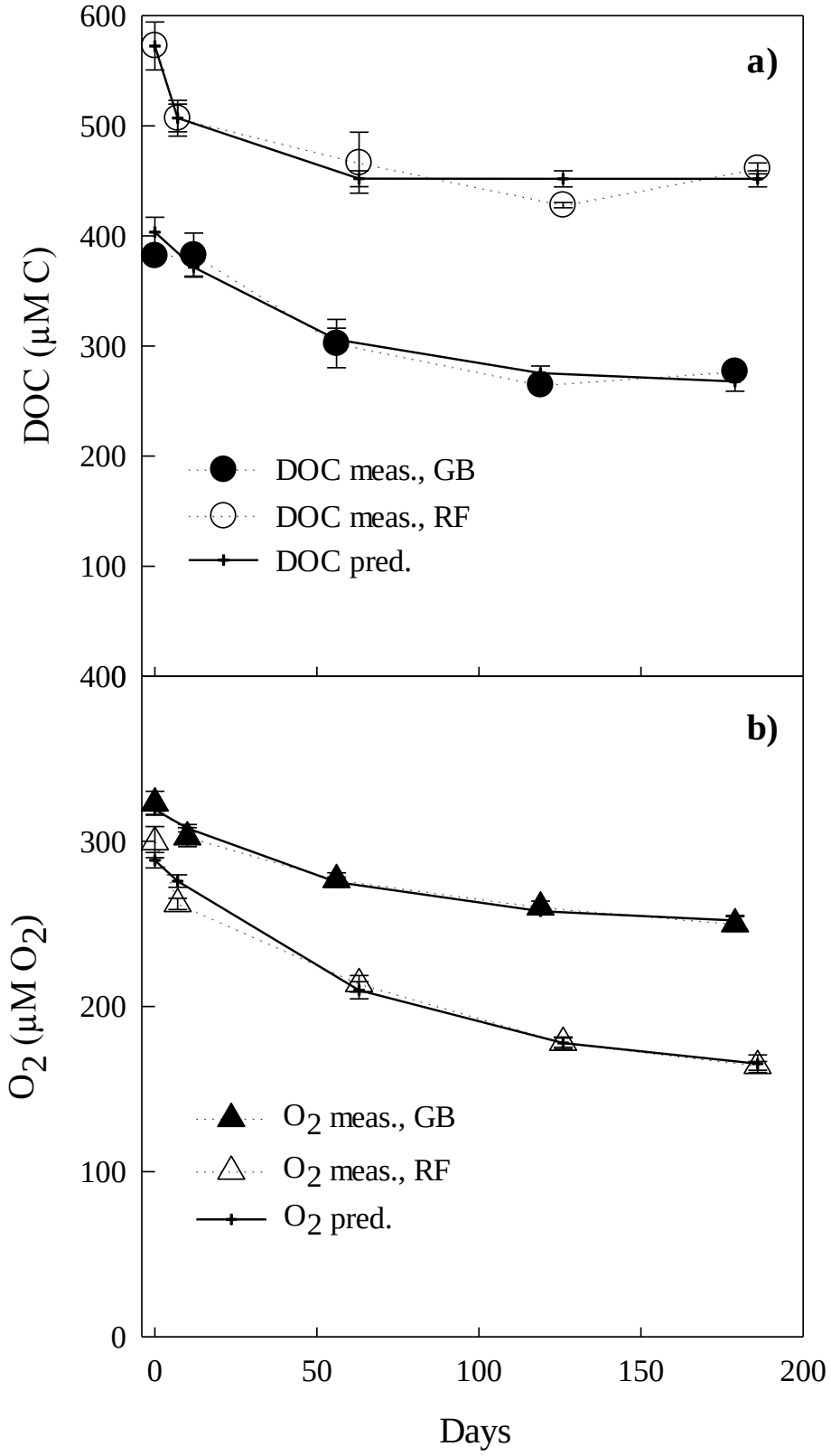
a)
b)
DOC (µM C)
O_2 (µM O_2)
DOC meas., GB
DOC meas., RF
DOC pred.
O_2 meas., GB
O_2 meas., RF
O_2 pred.
Days
600
500
400
300
200
100
400
300
200
100
0
0
50
100
150
200

Fig. 2

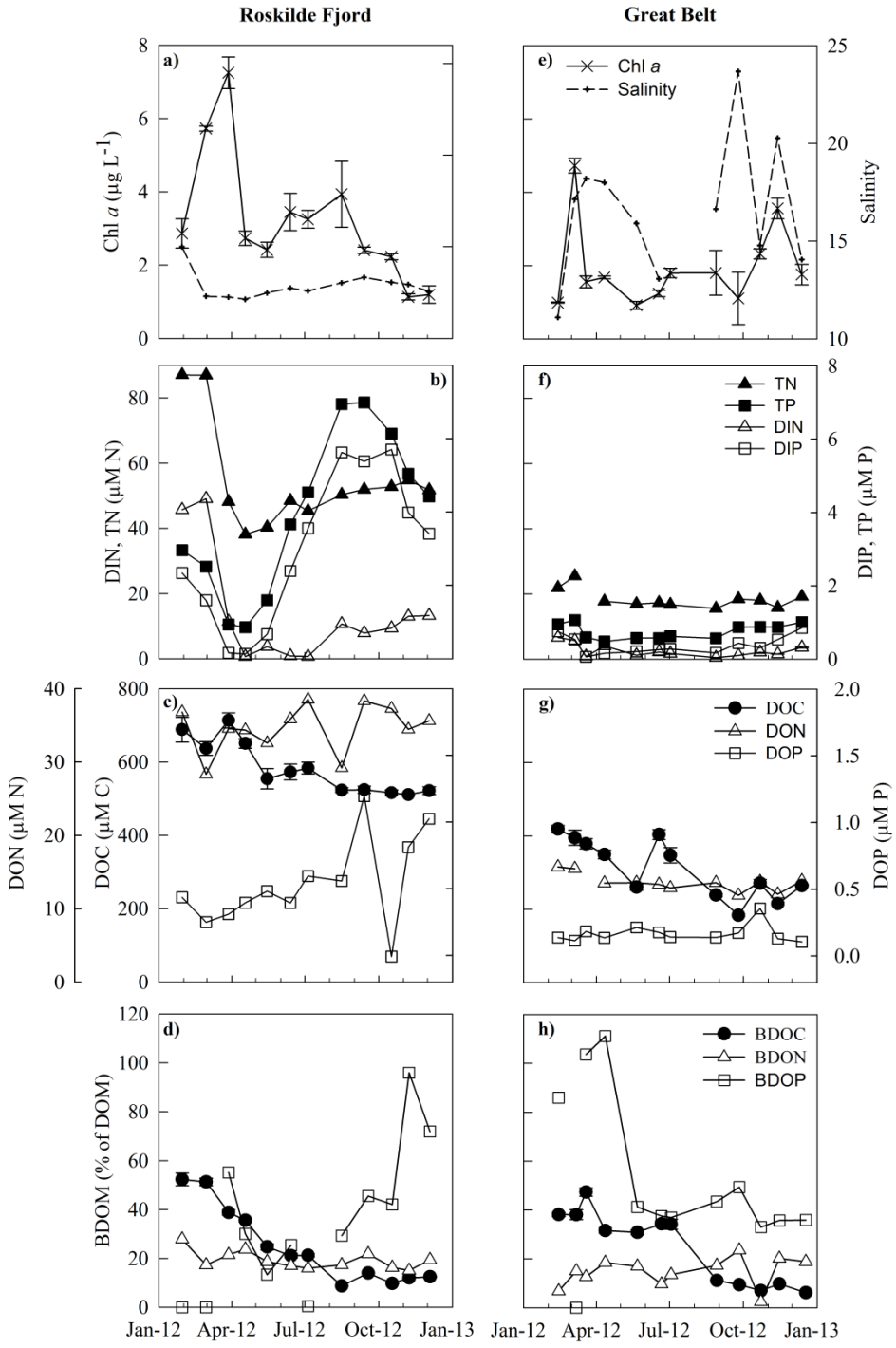
Roskilde Fjord
Great Belt
a)
e)
Chl a
Salinity
Chl a (µg L-1)
Salinity
b)
f)
TN
TP
DIN
DIP
DIN, TN (µM N)
DIP, TP (µM P)
c)
g)
DOC
DON
DOP
DON (µM N)
DOC (µM C)
DOP (µM P)
d)
h)
BDOC
BDON
BDOP
BDOM (% of DOM)
Jan-12
Apr-12
Jul-12
Oct-12
Jan-13

Fig. 3

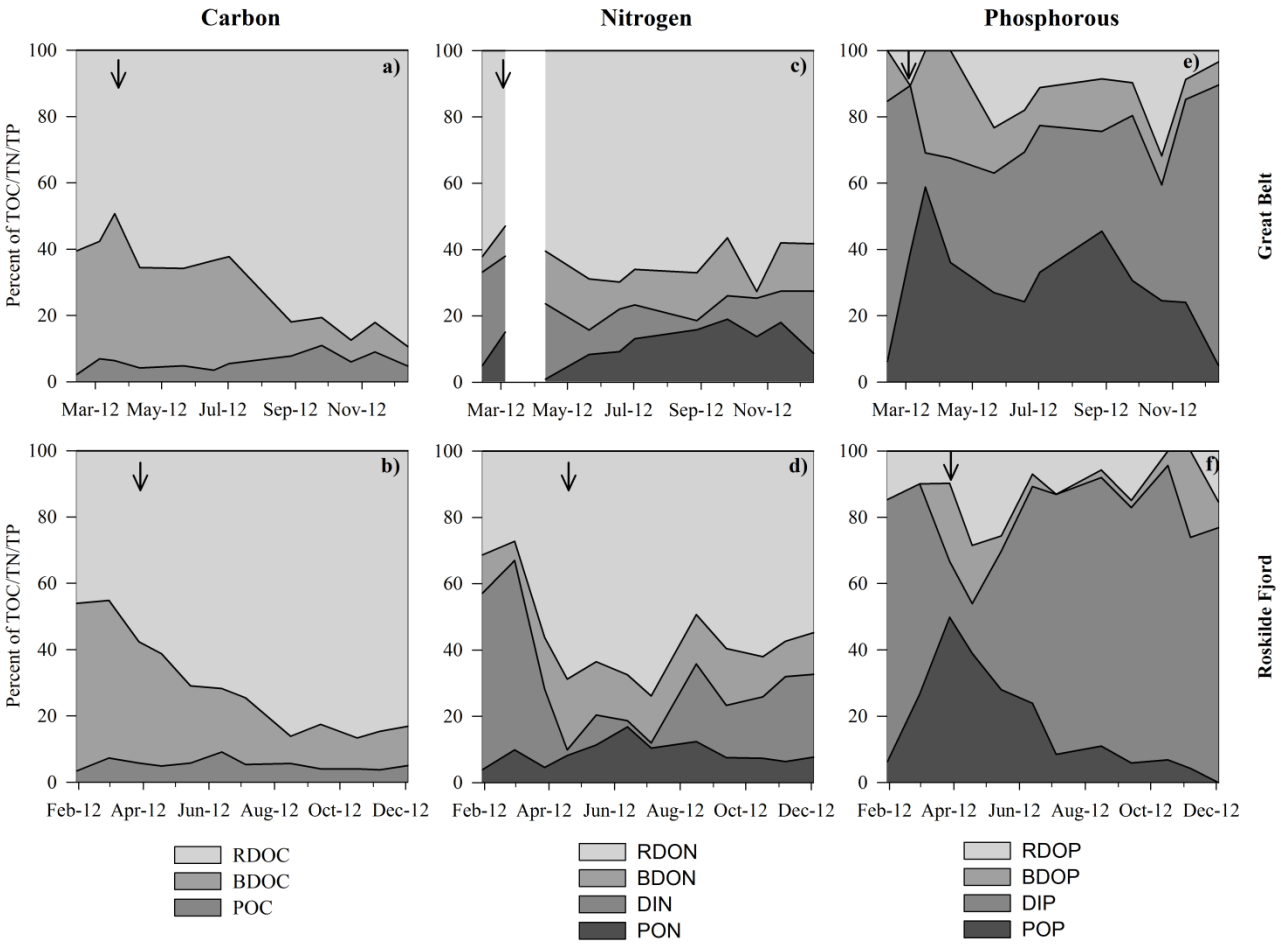
Carbon
Nitrogen
Phosphorous
Great Belt
Roskilde Fjord
Percent of TOC/TN/TP
a)
b)
c)
d)
e)
f)
Mar-12 May-12 Jul-12 Sep-12 Nov-12
Feb-12 Apr-12 Jun-12 Aug-12 Oct-12 Dec-12
RDOC
BDOC
POC
RDON
BDON
DIN
PON
RDOP
BDOP
DIP
POP

Fig. 4

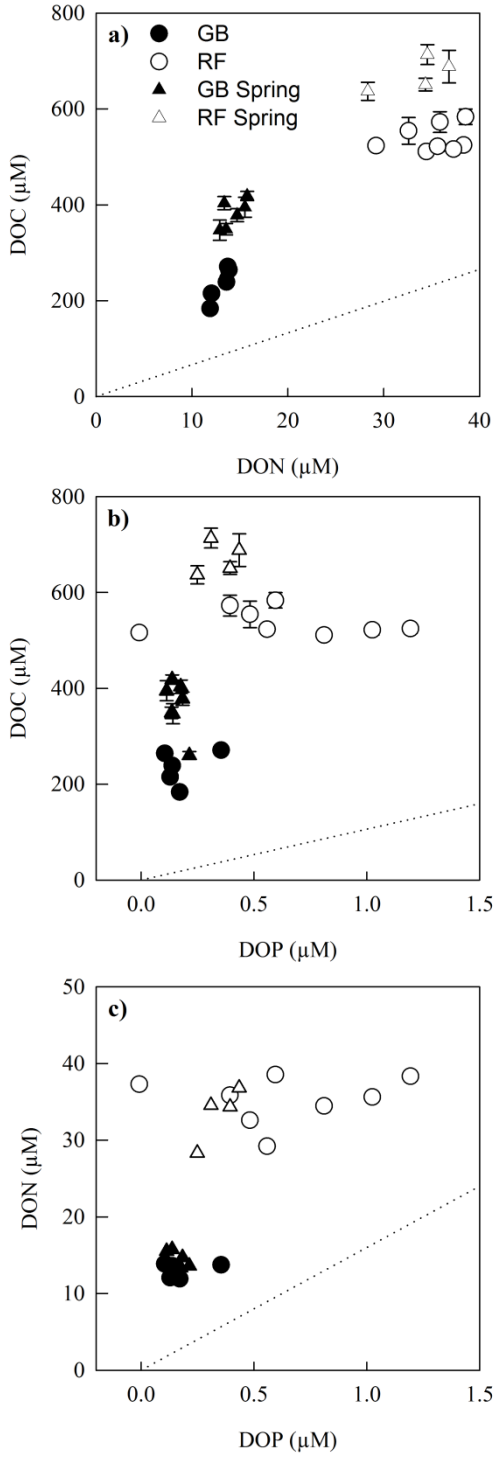
a)
GB
RF
GB Spring
RF Spring
DOC (µM)
DON (µM)
b)
DOC (µM)
DOP (µM)
c)
DON (µM)
DOP (µM)

Fig. 5

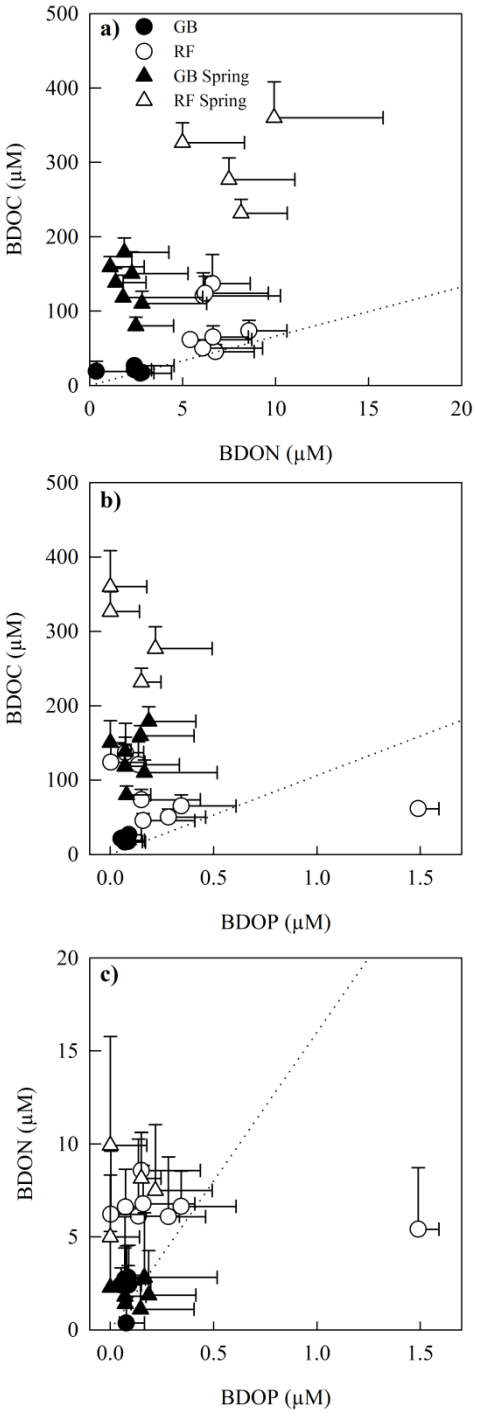
a)
GB
RF
GB Spring
RF Spring
BDOC (µM)
BDON (µM)
b)
BDOC (µM)
BDOP (µM)
c)
BDON (µM)
BDOP (µM)

Fig. 6

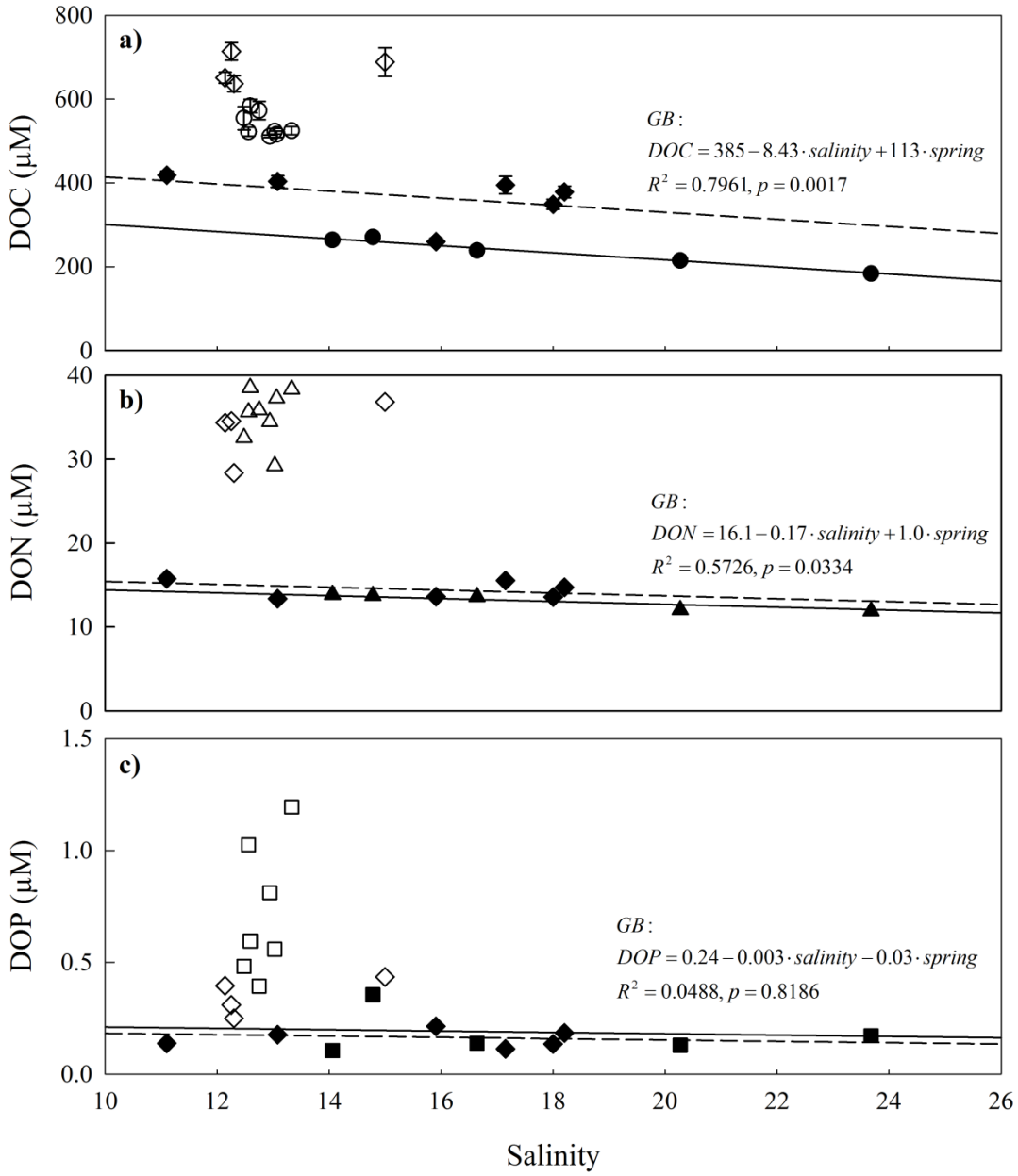
a)
GB :
DOC = 385 − 8.43 · salinity + 113 · spring
R² = 0.7961, p = 0.0017
DOC (µM)
b)
GB :
DON = 16.1 − 0.17 · salinity + 1.0 · spring
R² = 0.5726, p = 0.0334
DON (µM)
c)
GB :
DOP = 0.24 − 0.003 · salinity − 0.03 · spring
R² = 0.0488, p = 0.8186
DOP (µM)
Salinity

Fig. 7

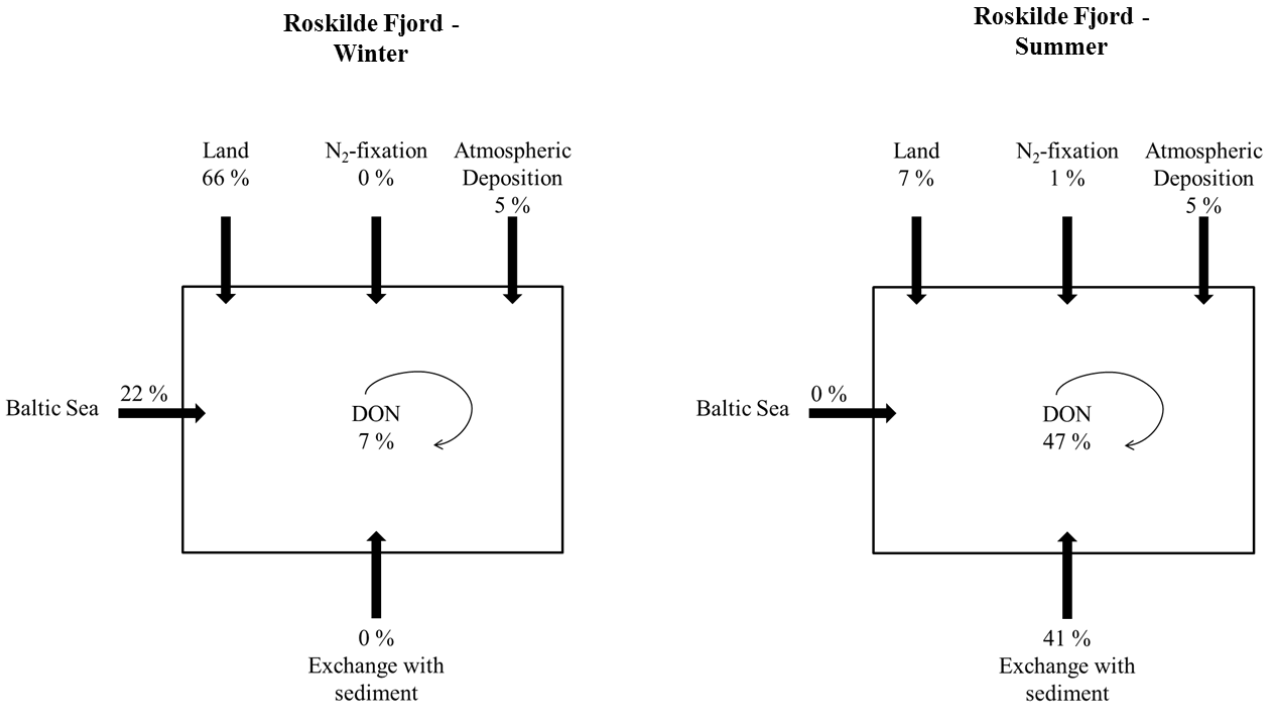
Roskilde Fjord -
Winter
Land
66 %
N_2-fixation
0 %
Atmospheric
Deposition
5 %
Baltic Sea
22 %
DON
7 %
0 %
Exchange with
sediment
Roskilde Fjord -
Summer
Land
7 %
N_2-fixation
1 %
Atmospheric
Deposition
5 %
Baltic Sea
0 %
DON
47 %
41 %
Exchange with
sediment

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 µM	GB µM	RF/GB Ratio	RF %	GB %	RF/GB Ratio
Total	**Nitrogen**	55±16	19±2.9	3.0			
	Phosphorous	3.9±2.2	0.75±0.20	5.1			
Inorganic	**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
Organic	**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
Dynamic	**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)