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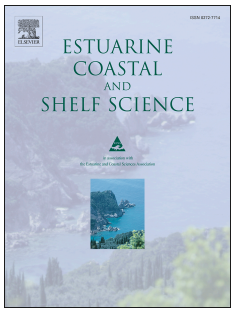

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Influence of allochthonous dissolved organic matter on pelagic basal production in a northerly estuary

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Running title: Coastal primary and bacterial production

Keywords: Primary and bacterial production; Coastal areas; Drivers of pelagic basal production; Allochthonous dissolved organic matter; Northern Baltic Sea

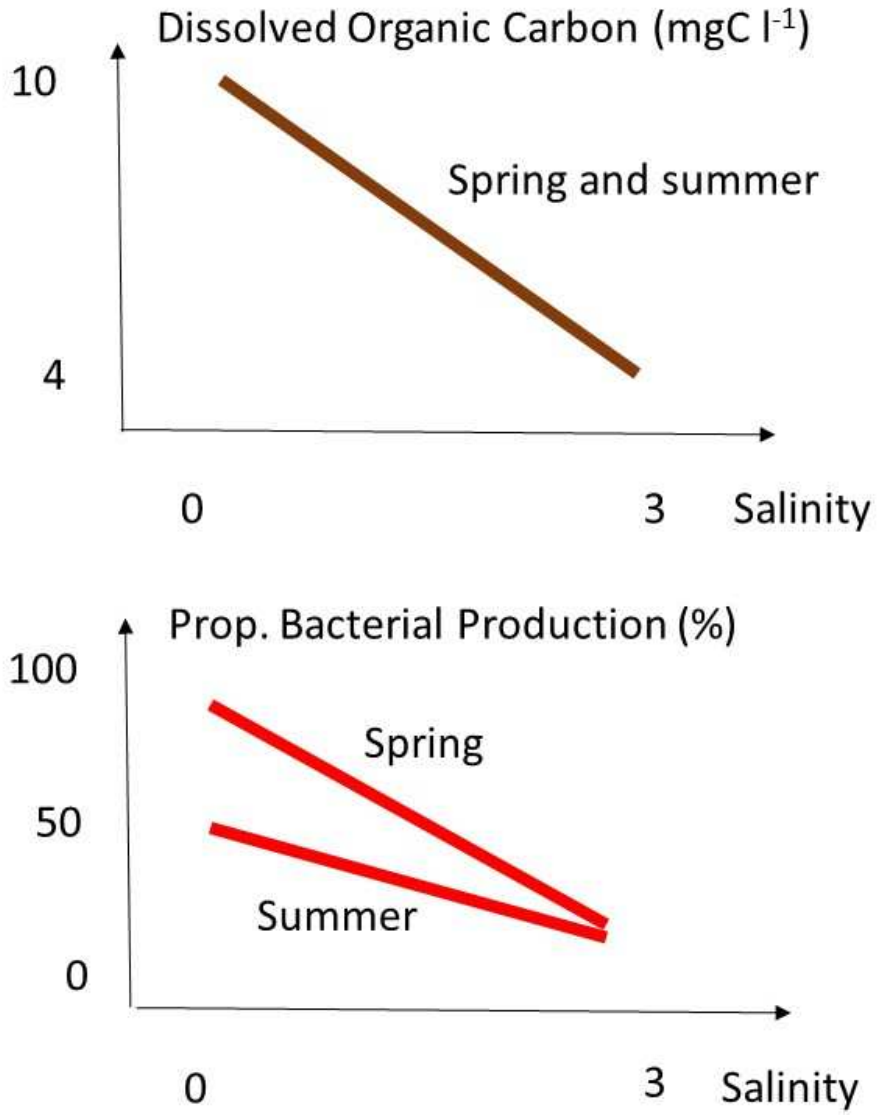
Highlights:

(1) Phytoplankton production is hampered by DOC-shading and P limitation.

(2) Coloured DOC reduces light which decreases the photosynthetic efficiency

(3) Heterotrophic bacterial production and growth are driven by riverine DOC

Graphical abstract:



Abstract

Phytoplankton and heterotrophic bacteria are key groups at the base of aquatic food webs. In estuaries receiving riverine water with a high content of coloured allochthonous dissolved organic matter (ADOM), phytoplankton primary production may be reduced, while bacterial production is favoured. We tested this hypothesis by performing a field study in a northerly estuary receiving nutrient-poor, ADOM-rich riverine water, and analyzing results using multivariate statistics. Throughout the productive season, and especially during the spring river flush, the production and growth rate of heterotrophic bacteria were stimulated by the riverine inflow of dissolved organic carbon (DOC). In contrast, primary production and photosynthetic efficiency (i.e. phytoplankton growth rate) were negatively affected by DOC. Primary production related positively to phosphorus, which is the limiting nutrient in the area. In the upper estuary where DOC concentrations were the highest, the heterotrophic bacterial production constituted almost 100% of the basal production (sum of primary and bacterial production) during spring, while during summer the primary and bacterial production were approximately equal. Our study shows that riverine DOC had a strong negative influence on coastal phytoplankton production, likely due to light attenuation. On the other hand DOC showed a positive influence on bacterial production since it represents a supplementary food source. Thus, in boreal regions where climate change will cause increased river inflow to coastal waters, the balance between phytoplankton and bacterial production is likely to be changed, favouring bacteria. The pelagic food web structure and overall productivity will in turn be altered.

1. Introduction

Phytoplankton and heterotrophic bacteria are key groups at the base of the food web as both assimilate dissolved nutrients and constitute a link between the chemical environment and the food web (e.g. Azam et al. 1983). Their production regulates the energy and nutrients that can be channelled through the food web and thus the production potential of intermediate and higher trophic levels, such as mesozooplankton and fish (e.g. Lefébure et al. 2013, Degerman et al. 2017). However, phytoplankton-based pathways are in many cases more efficient than bacteria-based pathways (e.g. Berglund et al. 2007, Degerman et al. 2017), and therefore environmental conditions leading to a dominance of heterotrophic bacterial production may result in lower food web efficiency and lower top-trophic level production (Berglund et al. 2007, Eriksson-Wiklund et al. 2009, Dahlgren et al. 2011). The fact that bacteria in general also represent a less nutritious resource than phytoplankton for grazers amplifies this issue (Klein Breteler et al. 2004, Dahlgren et al. 2011). It is therefore important to elucidate how environmental changes affect the balance between primary and bacterial production.

Model simulations indicate that climate change will not only cause elevated temperature in high latitude coastal areas but also affect the hydrology (IPCC 2013). For example, in the northern Baltic Sea the surface water temperature is expected to increase ~4°C by 2100, along with a ~30% increase in regional precipitation (Meier 2006, Omstedt et al. 2012, Andersson et al. 2015). This will be accompanied with an increase in run-off of dissolved organic matter (ADOM) from the surrounding terrestrial systems, and consequently of dissolved organic carbon (DOC) (e.g. Stepanauskas et al. 2002, Andersson et al. 2013). Previous studies indicate that phytoplankton might be disfavoured owing to the brown colour of ADOM, while heterotrophic bacteria might be favoured as they can use ADOM as a carbon food source (Andersson et al. 2015, Harvey et al. 2015). In line with this, Wikner and Andersson (2012) showed a negative correlation between the freshwater inflow to the northern Baltic Sea (Gulf of Bothnia) and primary production, and Figueroa et al. (2016) found a negative correlation between DOC concentration and primary production and a positive correlation with bacterial production in a northerly boreal estuary. However, these relationships may have alternative explanations, as for example the dilution of organisms by river discharge. Hence, to get a deeper understanding of the ecological effects of ADOM, it is critical to analyse the relationships between DOC concentrations, photosynthetic efficiency and bacterial growth rate.

ADOM is an environmental stressor in coastal systems, and is likely to affect the food web structure and ecological function of the ecosystem. By promoting bacterial production and disfavouring primary production, additional internal trophic levels will be required to facilitate trophic transfer in a food web predominantly based on smaller organisms. This will increase the energy losses throughout the food web since at each trophic level 70-90% of the energy is lost due to respiration, excretion and sloppy feeding (Straile 1997). Thus, even if the food web length is only slightly increased, the production of higher trophic levels can be substantially decreased (Berglund et al. 2007). Additionally, bacteria are in general of reduced nutritional quality compared to eukaryotic phytoplankton, commonly lacking important lipids and fatty acids that are vital for grazers (Larsson et al. 2000), and having relatively low carbon: nitrogen: phosphorus ratios (C:N:P-ratio 50:10:1, e.g. Fagerbakke et al. 1996, Cotner et al. 2010). On the other hand eukaryotes conform to the Redfield ratio (106:16:1) and are nutritionally more suitable. Consequently, environmental drivers that turn the base of the food web from phytoplankton to bacterial dominance may induce a poorer physiological state of the grazers (e.g. poor fatty acid

content), the effects of which propagate upwards through the food web, also affecting higher trophic levels.

The aim of this study was to find out how inflows of ADOM affect the bacterial and primary production as well as the photosynthetic efficiency and specific growth rate of bacteria in high latitude coastal areas receiving river water from nutrient poor catchment areas dominated by coniferous forests and mires and loads of phosphorus from offshore areas during winter-spring, thus having a pronounced nutrient cycle. We chose the Öre estuary, northern Baltic Sea, as the study system. The Baltic Sea is a brackish semi-enclosed sea where salinity, nutrients and production decrease gradually towards the north. The most limiting nutrient for primary production shifts from nitrogen in the south to phosphorus in the north (Graneli et al. 1990, Tamminen and Andersen 2007). Both phytoplankton and bacteria have been shown to be phosphorus limited in the actual study area (Andersson et al. 1996, Zweifel et al. 1993). Furthermore, the study region is strongly influenced by ADOM-rich and nutrient-poor river discharge (Skoog et al. 2011). We hypothesized that: (1) primary production and photosynthetic efficiency in the upper estuary would be hampered by coloured DOC, while in the lower estuary primary production and photosynthetic efficiency would be governed by phosphorus concentration, and (2) bacterial production and bacterial growth rate would benefit from DOC in the upper estuary due to the large influence of river borne ADOM in this area of the estuary.

2. Material and Methods

The study was performed in the Öre estuary, northern Baltic Sea (Fig. 1). Nineteen stations, radiating from the river to the open sea, were sampled on nine occasions, from May to August 2010 (Suppl. Table 1). The bottom depth in the estuary varies from 5 m at the river mouth (station 2) to 34 m offshore (station 18). The bottom depth at the stations situated on the eastern part of the sampling grid is deeper than at stations located along coast (e.g. stations 5, 8, 12 or 16).

At each sampling occasion, water for all analysis was collected at a depth of 1 meter using a Ruttner sampler, and *in situ* temperature and Secchi depth were recorded (the Secchi disk was not deployed at station 1). For primary and bacterial production estimates water was additionally collected at 3 and 5 m depth, though due to their shallow nature water was only collected at 1 m depth at station 1, and at 1 and 3 m at station 2. Primary production samples were incubated *in situ* (at 1, 3 and 5 m) and other water samples were immediately transported to the laboratory for analysis. Data on river water discharge were obtained from the Swedish Meteorological and Hydrological Institute (SMHI). Surface incident PAR (Photosynthetically Available Radiation) was recorded from May to August at the Umeå Marine Science Center (located 7-10 km from the sampling area) with a Licor LI-193 spherical quantum sensor.

2.1. Physicochemical variables

Maximum light (PAR) at the air-water interface was calculated based on the surface PAR measurements, solar declination, solar elevation and Fresnel's equation (Kirk 2011). PAR at 1 and 5 m depth, and the penetration depth of 1 and 0.1% PAR were calculated based on the PAR at the air-water interface and the Secchi depth (Kirk 2011).

Conductivity and pH were measured using a Mettler Toledo probe at 25°C and recalculated to *in situ* values using the method of Fofonoff and Millard (1983). Salinity was calculated from conductivity as practical salinity units.

Total phosphorus (TP) and total nitrogen (TN) were measured in unfiltered water samples using a Braan and Luebbe TRAACS 800 autoanalyzer, according to standard analytical methods (Grasshoff et al. 1983). Unfiltered samples for humic substances were measured with a Perkin Elmer LS 30 fluorometer at 350/450 excitation/emission wavelengths. Calibration standards were prepared from quinine dihydrogen sulfate dehydrate in 0.05M sulfuric acid (Hoge et al. 1993, Wedborg et al. 1994), and sulfuric acid (0.05M) was used as a blank. Dissolved organic carbon (DOC) analyses were carried out on 0.22 µm filtered (Supor Membrane Syringe Filter, non-pyrogenic; Acrodisc®) and acidified (8 mM HCl final concentration) water samples on a Shimadzu TOC-5000 instrument.

The absorbance of coloured dissolved organic matter (CDOM) was measured on water samples filtered through 0.22 µm polycarbonate membrane filters and stored in amber glass bottles in the dark at 4°C until analysis. Absorbance values were recorded from 300 to 850 nm with a Shimadzu UVPC-2501 scanning spectrophotometer, using ultrapure water as a blank. The absorbance was corrected for the average reading between 700-750 nm according to D'Sa et al. (1999) and the absorption coefficient at 440 nm ($g_{(440)}$) was calculated according Kirk (2011).

Total suspended particulate matter (SPM) was measured using the gravimetric method described by Strickland and Parsons (1972). One litre of sea water was filtered through pre-combusted (450°C) and pre-weighted Whatman GF/F filters (47 mm). Filters were dried for 24 hours at 60°C and re-weighted. Final concentrations of SPM were calculated as the mean of duplicate samples per station.

All physicochemical samples were processed immediately after sampling and completed within ~4 hours of initial water collection.

2.2. Phytoplankton and bacterial biomass

Chlorophyll *a* (Chl *a*) was used as a proxy for phytoplankton biomass. 100 ml samples were filtered onto 25 mm GF/F filters under low vacuum and stored at -80°C. The pigments were extracted in 95% ethanol in the dark at 4ºC overnight. Chl *a* was measured with a Perkin Elmer LS 30 fluorometer (433/674 nm excitation/emission wavelengths) (HELCOM 2014).

Samples for heterotrophic bacteria were preserved with sterile filtered glutaraldehyde (1% final concentration). Preserved samples (1-3 ml) were filtered onto black 0.2 µm 25 mm polycarbonate filters (Poretics) and stained with acridine orange (Hobbie et al. 1977). Prepared slides were analyzed with an epifluoresence microscope using blue excitation light (Nikon TE 300). At least 300 bacterial cells per slide were counted in >20 randomly distributed fields of view. To calculate biomass, a bacterial carbon content of 20 fg C $cell^{-1}$ was assumed (Lee and Fuhrman 1987), which has been shown to be representative for the coastal area (data not shown).

2.3. Primary production and photosynthetic efficiency

Primary production was measured *in situ* at 1, 3 and 5 m depth using the ^{14}C method (Gargas 1975). Five ml of seawater and 0.72 µCi of sodium (^{14}C) bicarbonate (0.1 mCi mmol^{-1}) were added to each of three 20 ml transparent polycarbonate tubes and one dark tube, replicating this set up at each depth. The samples were incubated for ~3 hours around noon. After incubation the samples were immediately transferred to glass scintillation vials and 100 µl 6M HCl were added to stop the reaction. The samples were gently bubbled with air for 30 minutes to get rid of excess ^{14}C. 15 ml scintillation liquid were added (Optiphase Hisafe 3) and the samples were analyzed in a Beckman 6500 scintillation counter. Daily primary production (PP) was calculated using the "light factor method", as described in Andersson et al. (1996), and depth-integrated primary production was calculated by trapezoidal integration.

The ratio between primary production and Chl *a* (PP:Chl *a*) at 1 m was used as a proxy for photosynthetic efficiency, i.e. the production to biomass ratio (P:B ratio).

2.4. Bacterial production and growth rate

Bacterial production was measured at 1, 3 and 5 m depths using the [^{3}H-methyl]-thymidine incorporation method (Fuhrman and Azam 1982). Triplicate 1 ml seawater samples (one control and two samples) were incubated with 0.074×10^{6} Bq (saturation level, 2.81×10^{12} to 3.07×10^{12} Bq mmol^{-1}) of [^{3}Hmethyl]-thymidine at the *in situ* temperature for 1 h (HELCOM 2014). The control sample was killed by the addition of 100 µl ice-cold 50 % trichloroacetic acid (TCA) and a 5 minute incubation at -20°C. After 1 h of incubation thymidine uptake was stopped by the addition of 100 µl of 50% TCA. The samples and controls were then centrifuged, the pellet was washed with 5% TCA, 1 ml of scintillation fluid was added, and the samples were analyzed in a Beckman 6500 scintillation counter. Cell production was calculated using a conversion factor of 1.4×10^{18} cells mol^{-1} of incorporated thymidine (Wikner and Hagström 1999). Daily production rates were calculated assuming stable uptake rates over the day and a bacterial carbon content of 20 fg C cell^{-1} (Lee and Fuhrman 1987), and depth-integrated bacterial production was calculated by trapezoidal integration.

The ratio between heterotrophic bacterial production and bacterial carbon biomass at 1 m was used as proxy for bacterial growth rate, the BP:BB ratio.

2.5. Statistical analyses

Environmental and biological variables were compared between seasons using a Mann-Whitney test. Spearman rank correlation coefficients were estimated between selected variables. Principal component analyses (PCA) were used to visualize the distribution of primary production and biomass, bacterial production and biomass, and photosynthetic efficiency and bacterial growth rate in relation to physicochemical factors. The PCAs were based on matrices of correlation of standardized data, and variables with high correlation were excluded from the analyses. Station 1 (river station) was not included in the analyses. Stepwise multiple linear regressions were performed to elucidate if DOC and TP were drivers of primary production (PP), photosynthetic efficiency (PP:Chl *a*), bacterial production (BP) and bacterial growth rate (BP:BB) in different areas of the estuary (upper estuary stations 2, 3, 4, 5 and 6; lower estuary stations 14, 15, 17, 18 and19; entire estuary (stations 2-19). All data in the regression analysis were ln transformed. The different areas of the estuary were selected from average salinity and variations in salinity: the

upper estuary had low and highly variable salinity (mean 1.7, CV 55%), lower estuary had relatively high and stable salinity (2.5, CV 12%), while the entire estuary (station 2-19) had an average salinity of salinity 2.2 (CV 35%). Data analyses were performed in SPSS Statistics 22 and Canoco 5.

3. Results

3.1. Temporal variation of physicochemical and biological variables

The majority of the variables displayed strong temporal variation, with pronounced seasonal differences between the initial three sampling events and the subsequent period (Suppl. Fig 1, Fig. 2). The three first sampling occasions (May 18th to June 8th) are classified as spring and the remainders are considered as summer (June 22nd to August 31st).

The first sampling occasion coincided with the maximum spring flush of the Öre River (ca. 280 $m^3 s^{-1}$ on May 18th, Suppl. Fig. 2). The river flow decreased within a couple of weeks and remained relatively stable (20-60 $m^3 s^{-1}$) until the end of August (Table 1). The surface temperature increased from May to July (9 to 15°C), remaining high until the end of August when the water temperature decreased to 13°C (mean values presented in Table 1).

Most of the physicochemical variables tightly followed the seasonal pattern of the river flow, showing the highest variation in spring and stabilising during the summer (Suppl. Fig 2). Salinity, Secchi depth and PAR increased from spring to summer before plateauing, fluctuating or steadily decreasing, respectively, during summer (Suppl. Fig 1 A-C). SPM, DOC, humic substances, TN and TP displayed the highest values on the first sampling occasion and generally decreased, stabilizing at lower values in summer (Suppl. Fig 1 D-H). The variation of the CDOM absorption coefficient $g_{(440)}$ closely followed that of humic substances (Table 1, data not shown), and is therefore not described further.

Both the depth-integrated primary production and the respective values at 1 m depth showed a peak on the first sampling occasion (Fig. 2 A and C), declining markedly in the following weeks. Subsequently primary production increased during summer and levelled out in late summer (Fig. 2 A and C). Chl *a* concentrations also displayed maximal values on the first sampling occasion, but remained relatively constant for the rest of the period, at ~2 µg Chl l^{-1} (Fig. 2 E). The ratio of primary production to Chl *a* (PP:Chl *a*) was lowest in the beginning of the sampling period, progressively increased to a maximum at the end of July before subsequently decreasing (Fig. 2 G).

The seasonal variation of bacterial production differed from that of phytoplankton and nearly followed the opposite trend until July. Both the depth-integrated bacterial production and the values at 1 m were high during the spring period, declined until July, before increasing again and stabilizing until the end of August (Fig. 2 B and D). Bacterial biomass also peaked in spring and steadily declined to reach stable numbers by the beginning of July (Fig. 2 F). This resulted in a bi-modal peak of bacterial growth rate (BP:BB), one peak in spring and a second peak at the beginning of August (Fig. 2 H).

3.2. Distribution of physicochemical and biological variables along the river-seaward gradient

The spatial distribution of the variables was mainly driven by the transport of river water within the Öre estuary. The Öre River carried warmer waters into the estuary, especially in July; however the temperature difference between the river mouth and the lower estuary remained below 1.5°C over the entire study period (data not shown). The dominant winds in the area directed the river plume south-westwards, resulting in a stronger influence of freshwater on the western part of the estuary, along the peninsula coast (stations 2, 3, 5, 8, 12 and 16). This is clearly evident in the surface patterns of salinity and DOC, and was also illustrated by the strong difference in salinity and DOC between eastern and western stations situated at the same distance from the river mouth (Suppl. Fig. 3 A, Suppl. Table 2). Over the entire data set, strong and significant linear regressions were observed between DOC and salinity, and DOC and humic substances (Suppl. Fig. 4), highlighting that DOC could be used as measure of allochthonous organic matter (ADOM). Therefore, in order to visualize the influence of river input on the spatial distribution, the variables were plotted against the average DOC concentration at each station (Fig. 3 and Suppl. Fig. 5).

The spatial distribution of most of the variables directly followed the DOC gradient (Figs. 3 and Suppl. Fig. 5). The concentrations of humic substances, SPM, and TN increased along the DOC gradient (Suppl. Fig. 5 E-G), indicative of the terrestrial origin of these compounds. The Secchi depth decreased along the same gradient, as well as PAR levels at 1 and 5 m (Suppl. Fig. 5 B-D), a result of the light attenuation by ADOM. On the contrary, TP was recorded at higher concentrations in the more marine waters characterized by much lower DOC concentrations (Suppl. Fig. 5 H). Both primary production rates (depth-integrated and 1 m) and Chl *a* concentrations decreased at stations with higher DOC concentrations (Fig. 3 A, C and E), however the ratio of primary production to Chl *a* did not display a linear pattern along this gradient (Fig 3 G). The ratio was variable at lower DOC concentrations and decreased at stations with higher DOC concentrations. Primary production profiles showed decreasing values from 1 to 5 m depth, and this vertical pattern was more pronounced in the lower estuary than close to the river mouth (Suppl. Fig. 6). Bacterial production and biomass at 1 m showed a constant increase along the increasing DOC gradient (Fig. 3 D and F), as did the bacterial growth rate (BP:BB) (Fig. 3 H). However, the depth-integrated bacterial production showed a less clear distribution along the gradient (Fig. 3 B), owing to the shallow water column at the river station (~1 m) where the DOC concentrations were highest. Bacterial production showed rather similar values in the depth profiles (Suppl. Fig. 7), except close to the river mouth where the production rates were clearly higher at 1 m than at 3 and 5 m.

Throughout the sampling period bacterial production was highest within the river, where the DOC concentrations were highest, decreasing seawards; while the primary production often showed an opposite trend. The proportion of bacterial production to total basal production (primary + bacterial production) generally showed a positive relationship with DOC concentration (Fig. 4). This pattern was especially observed during the spring period (Fig. 4 A and B), where bacterial production constituted almost 100% of the basal production in the river and upper estuary, with DOC concentrations of ~10 g m^{-3}. Although this DOC-induced

dominance of bacterial production was clearest during spring, it could still be observed during summer (Fig. 4 C and D).

3.3. Factors governing phytoplankton and bacterial production, photosynthetic efficiency and bacterial growth rate

To get an understanding of factors influencing phytoplankton and bacterial production, photosynthetic efficiency and bacterial growth rate, we performed two principal component analyses (PCA). In the PCA including primary and bacterial production, the first two axes summarized 65% of the variance (Table 2). The first axis was mostly driven by DOC, TN and PAR, and the second by PP, Chl *a*. and TP. The PCA indicated a positive relationship between the primary production and TP and a negative relationship with DOC, while bacterial production was positively related to DOC (Fig. 5 A).

In the PCA including phytoplankton and bacterial growth rate (PP:Chl *a* and BP:BB), the first two axes summarized 65% of the variance (Table 2). The first axis was mostly driven by DOC, TN and PAR, and the second axis by pH and TP. The PCA indicated that the photosynthetic efficiency (PP:Chl *a*) was positively related to temperature and negatively to DOC, while the bacterial growth rate (BP:BB) was positively related to DOC (Fig. 5 B).

Multiple linear regressions showed that DOC had a negative effect on primary production and photosynthetic efficiency and a positive effect on bacterial production and growth rate in the estuary (Table 3). These relationships were especially pronounced in the upper estuary (Figure 6). We could also find a positive effect of TP on primary production (Table 3).

4. Discussion

4.1. Drivers of primary production and photosynthetic efficiency

Primary production showed two peaks, one coinciding with the spring flush and one during the summer. Both peaks were driven by the availability of phosphorus, which has been recognized as the limiting nutrient in the study area (Andersson et al. 1996). TP concentrations were generally higher at the more seaward locations throughout the sampling, since river water was relatively deficient in P. This scenario can be attributed to the characteristics of the Öre River catchment, consisting mainly of forests and peatlands (Stepanasuskas et al. 2002, Räike et al. 2012), while the offshore Bothnian Sea contains relatively high P concentrations due to the inflow of P rich seawater from the Baltic Proper (Rolff and Elfwing 2015). However, owing to the high N content of ADOM, the TN concentrations generally decreased from the river towards the more seaward locations. Similar, although less pronounced, distribution patterns of N and P have been found in the Råne estuary situated further north in the Baltic Sea (Figueroa et al. 2016), which can be explained by the stronger influence of Baltic Proper waters in our study region. The second peak in primary production may have been due to predator-induced remineralization of nutrients. During late summer heterotrophic protists and zooplankton have their maximum, remineralizing nutrients which in turn can favour primary producers.

While P was a positive driver of primary production, light attenuation by ADOM and SPM most likely had a negative effect on photosynthesis. Variations of the underwater light field followed a similar spatial pattern across the entire sampling period, where the Secchi depth increased from ~0.5 m at near-shore stations to ~4 m at the more seaward stations, though the strongest spatial gradient was recorded in spring. In general, the photosynthetic efficiency showed positive correlation with Secchi depth (r_s = 0.442 in spring and r_s = 0.386 in summer, $p < 0.05$). Phytoplankton photosynthetic efficiency was hampered by coloured DOC, especially in the upper estuary. However, as TP concentrations were also lowest when the Secchi depth was lower, it is thus difficult to determine if photosynthesis close to the river mouth was constrained by low P concentrations or ADOM-induced light limitation, or a combination of both. Primary production within the sampled region was lower at 5 m depth, compared to 1 m, due to decreasing PAR levels with depth in the water column. However, at 1 m depth the light was not at limiting levels, not even at stations close to the river mouth, while at 5 m depth PAR should have been a strong limiting factor for photosynthesis at stations close to the river mouth (Andersson et al. 1994). The photosynthetic efficiency was lowest in spring and highest in July, which may partly have been driven by the seasonal variations in PAR. This is supported by the multiple regression analysis, showing that DOC had a negative effect on photosynthetic efficiency in the entire estuary.

4.2. Drivers of bacterial production and bacterial growth rate

Heterotrophic bacterial production and bacterial specific growth rate (BP:BB) peaked twice during the sampling period, once during spring and once in summer. However, unlike the patterns observed for phytoplankton, we suggest that these two peaks of heterotrophic bacterial production have different drivers. Throughout the sampling period spatial patterns of bacterial production showed that the highest rates occurred at the river mouth, where DOC concentrations were highest, steadily decreasing at the more seaward stations (i.e. the opposite pattern to primary production). This was especially pronounced in spring, when heterotrophic bacterial production accounted for almost 100% of the basal production in the river mouth and only ~10% at the seaward stations. Thus the voluminous discharge of ADOM-rich river waters, laden with partly bioavailable DOC, was the most likely driver of bacterial production during this initial peak.

The second peak of heterotrophic bacterial production and bacterial specific growth rate in summer occurred concomitantly with a sustained plateau of high primary production and somewhat elevated river discharge. Although ADOM represents a supplementary food source for bacteria it is nevertheless unlikely that ADOM represents a sufficient nutritional supply to sustain the bacterial production levels observed considering the much lower DOC concentrations recorded at this stage of the season. Thus during the summer period in which primary production was high it is likely that phytoplankton production was a major driver of bacterial production. In summer the nutrient concentrations in this sea region are low, as seen here and recorded previously (e.g. Andersson et al. 1996), and under such conditions phytoplankton exudation is generally higher than under nutrient replete conditions (Larsson and Hagström 1982). Since higher rates of primary production occur over a sustained period during summer and phytoplankton exudation levels are also higher, it is therefore likely that phytoplankton production directly sustained the bacterial population. In line with this, we found positive relation between bacterial growth rate and primary production at the most seaward stations (e.g. station 17 r^2=0.87).

Our results are in general agreement with earlier studies performed in diverse estuaries in temperate areas, e.g. in the Scheldt River estuary (Goosen et al. 1997), the Hudson River estuary (Findlay et al. 1991, Sañudo-Wilhelmy at al. 1999), the York River estuary in Chesapeake Bay (Schultz et al. 2003), in tropical (Bega and Clyde River estuaries, SE Australia, Hitchcock et al. 2015), and sub-tropical regions (Fly and Purari Rivers, Gulf of Papua, Robertson et al. 1998). In the Scheldt River estuary, they found a high degree of heterotrophy in the estuarine system, yet the bacterial production also closely followed the peaks of primary production, likely due to the highly bioavailable organic exudates released by phytoplankton (Goosen et al. 1997). During spring, our study system seems to be highly influenced by ADOM, since bacterial production was clearly decoupled from primary production. Similar findings were recorded in a study performed in a more northerly Baltic Sea estuary (Råne), where not only spatial but also temporal decoupling between primary and bacterial production was observed (Figueroa et al. 2016). The patterns we observe in this study are consistent with previous findings, which indicate that in estuaries, especially those entering semi-enclosed seas such as the northern Baltic Sea, allochthonous material can be a crucial component for basal production. Previous studies have shown that bacterial production in coastal waters of the northern Baltic Sea can be both C and P limited (Zweifel et al. 1993, Figueroa et al. 2016) and although riverine DOC is generally of low bioavailability (5-10%, Stepanauskas et al. 2002, Figueroa et al. 2016), the plentiful inflows to coastal areas can promote heterotrophic bacterial production (Figueroa et al. 2016).

In many productive marine and freshwater systems the yearly succession starts with a spring phytoplankton bloom, while bacteria exhibit their maximum during summer, associated with warmer temperatures (e.g. Elmgren 1984, Legrand et al. 2015). Although temperature is undoubtedly important for bacteria, nutrient availability and food resources can also have an influence (Degerman et al. 2013). Exceptions to this “classical” succession pattern have been documented in unproductive brown lakes and sub-Arctic estuaries, where heterotrophic bacterial production exhibits a growth maximum in spring and the highest phytoplankton production occurs in summer (Drakare et al. 2002, Figueroa et al. 2016). In such cases these patterns have been driven by variations in the inflow of ADOM. In the Öre estuary, the bacteria-phytoplankton succession pattern appeared to follow both patterns, with both groups showing maxima in spring and in summer, as observed in the Scheldt estuary entering the North Sea (Goosen et al. 1997). Traditionally, it is anticipated that river discharge causes eutrophication in the recipient waters, but if the ADOM-induced light attenuation is strong the production in the recipient estuary may in fact be hindered (e.g. Andersson et al. 2013). This indicates that elevated riverine inflows rich in ADOM can cause substantial changes in estuarine ecosystem functioning, and that classical assumptions may no longer apply.

4.3. Conclusion

We conclude that ADOM is commonly overlooked as an environmental stressor in estuarine and coastal ecosystems, especially considering climate change projections. Instead of causing phytoplankton blooms and eutrophication in the recipient waters, river waters rich in ADOM can cause a decrease in phytoplankton production, while heterotrophic bacterial production and the microbial food web are favoured. As observed at the stations located closer to the river mouth, the spring river flush reduces the extent of the phytoplankton spring bloom production. This may have a negative effect on higher trophic levels within the pelagic food web and on the benthic

fauna feeding on settling phytoplankton. Although we did not quantify top-down effects, we believe the described patterns to be robust since micro- and mesozooplankton are low in abundance during spring (Elmgren 1984, Samuelsson et al. 2006, Dahlgren et al. 2010), thus only exerting a minor predation-pressure, while their increased presence in summer would be counterbalanced by the regeneration of organic substances and nutrients within the water column (Andersson et al. 1985). Furthermore, our findings may offer an explanation for previously identified trends. For example, during a rainy period with periodically lower primary production in the northern Baltic Sea (Wikner and Andersson 2012) the benthic amphipod *Monoporeia affinis* showed a drastic decrease in the area at a large spatial scale (Eriksson-Wiklund and Andersson 2014). In a low-diversity system such as the brackish Baltic Sea, changes in ADOM inputs will lead to altered balance between primary and bacterial production, which in turn has a potential to propagate to higher levels in the food web and ultimately also affect fish production. Since the resilience of such low-diversity systems can be relatively poor, extended recovery times from such changes may also occur.

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636

Legends to figures

Figure 1. Map of the study area and sampling stations in the Öre estuary northern Baltic Sea.

Figure 2. Temporal variation of (A) depth-integrated primary production (PP), (B) depth-integrated bacterial production (BP), (C) primary production (PP) at 1m, (D) bacterial production (BP) at 1m, (E) Chl *a* concentration, (F) bacterial biomass, (G) PP:Chl *a* ratio and (H) bacterial specific growth rate (BP:BB) in the Öre estuary. Values were averaged per sampling week for all the stations. Error bars denote the standard error.

Figure 3. Distribution of (A) depth-integrated primary production (PP), (B) depth-integrated bacterial production (BP), (C) primary production (PP) at 1m, (D) bacterial production (BP) at 1m, (E) Chl *a* concentration, (F) bacterial biomass, (G) PP:Chl *a* ratio and (H) bacterial specific growth rate (BP:BB) along the DOC gradient in the Öre estuary during spring and summer. Values were averaged per station over the entire sampling period. Error bars denote the standard error.

Figure 4. Contribution of bacterial production to basal production (bacterial + primary production), %BP, along the DOC gradient at selected dates representative of the spring (A) May 18th, (B) May 25th, and of the summer (C) July 20th and (D) August 3rd.

Figure 5. Principal component analyses (projection of the variables and observations) showing the distribution of abiotic (DOC, pH, SPM, T: temperature, TP: total phosphorus, TN: total nitrogen) together with the biotic variables (A) BB: bacterial biomass, BP: bacterial production, Chl *a* concentration, PP: primary production and (B) BB:BP: bacteria specific growth rate, PP:Chl *a*: primary production to Chl *a* concentration ratio for the entire period. Spring samples are indicated by open diamonds, and summer samples by open circles.

Figure 6. Relationship between primary production (PP), photosynthetic efficiency (PP:Chl *a*), bacterial production (BP) and bacterial growth rate (BP:BB) and DOC in the upper estuary (stations 2, 3, 4, 5 and 6).

Supplementary Figures

Supplementary Figure 1. Temporal variation of (A) salinity, (B) Secchi depth, (C) PAR at 1m, (D) SPM concentration, (E) DOC concentration, (F) humic substances concentration (G) TN concentration and (H) TP concentration in the Öre estuary. Values were averaged per sampling week for all stations. Error bars denote the standard error.

Supplementary Figure 2. Daily freshwater discharge from the Öre River during 2010. Vertical lines indicate sampling occasions.

Supplementary Figure 3. (A) Salinity and (B) DOC concentration (average and standard error) against distance from the river mouth. The stations on the western part of the Öre estuary are shown in dark symbols, while the stations on the eastern part are shown in open symbols.

Supplementary Figure 4. Linear regression between (A) salinity, (B) humic substances concentration and DOC concentrations for the entire data set. The equation and determination coefficient of the regression are indicated on each graph.

Supplementary Figure 5. Distribution of (A) salinity, (B) Secchi depth, (C) PAR at 1m, (D) PAR at 5m, (E) humic substances concentration, (F) SPM concentration, (G) TN concentration and (H) TP concentration along the DOC gradient in the Öre estuary during spring and summer. Values were averaged per station over the entire sampling period. Error bars denote the standard error.

Supplementary Figure 6. Vertical profiles of average primary production at each station for the entire study period (error bars denote standard error). The station number is indicated at the lower right corner of each graph.

Supplementary Figure 7. Vertical profiles of average bacterial production at each station for the entire study period (error bars denote standard error). The station number is indicated at the lower right corner of each graph.

700 **Table 1.** Summary of biological and physicochemical variables (mean and range of variation) at
701 all stations studied during spring and summer. * denotes significant ($p < 0.05$) differences
702 between spring and summer.
703

	Spring	Summer
Bacterial production at 1m (mgC m^{-3} d^{-1})	53.7 (0.3-169.3) *	35.8 (9.2-150.5) *
Bacterial biomass (mgC m^{-3})	15.7 (5.9-30.5)	13.3 (3.7-25.4)
BP:BB (mgC mgC^{-1} d^{-1})	3.5 (0.4-9.5)	2.9 (0.6-10.6)
Integrated bacterial production (mgC m^{-2} d^{-1})	186.7 (13.4-504.5)	145.3 (33.0-356.8)
Primary production at 1m (mgC m^{-3} d^{-1})	76.8 (1.2-509.9) *	82.1 (1.9-413.4) *
Chl *a* concentration (mg m^{-3})	6.7 (0.5-57.2) *	2.3 (0.9-7.8) *
PP:Chl *a* (mgC $mgChl^{-1}$ d^{-1})	14.2 (0.7-44.6) *	34.9 (0.8-125.7) *
Integrated primary production (mgC m^{-2} d^{-1})	192.1 (1.2-1058.4) *	282.3 (1.9-1442.3) *
Temperature (°C)	9.9 (6.5-12.3) *	15.1 (10.9-21.0) *
pH	7.7 (6.6-8.6)	7.8 (7.1-8.0)
Salinity	1.4 (0.0-2.5) *	2.4 (0.0-2.9) *
CDOM ($g_{(440)}$) (m^{-1})	4.3 (1.3-8.8) *	1.7 (0.7-7.6) *
Humic substances (g m^{-3})	64 (22-120) *	31 (17-132) *
Secchi depth (m)	2.0 (0.5-4.0) *	3.8 (1.4-6.0) *
PAR 1m (µmol photon m^{-2} s^{-1})	136 (8-307) *	248 (106-378) *
PAR 5m (µmol photon m^{-2} s^{-1})	14 (0-56)	44 (1-107)
Depth 1% PAR (m)	2.3 (0.5-4.7)	4.5 (1.7-7.1)
Depth 0.1% PAR (m)	3.5 (0.8-7.1)	6.7 (2.5-10.6)
DOC (g m^{-3})	6.9 (4.4-10.2) *	4.7 (3.8-9.9) *
TN (mg m^{-3})	309 (119-800) *	206 (79-374) *
TP (mg m^{-3})	18.8 (4.3-114.1) *	8.0 (1.9-3.9) *
SPM (g m^{-3})	5.1 (0.2-35.7) *	1.4 (0.4-5.7) *
River flow (m^3 s^{-1})	143 (29-292) *	26 (11-45) *

704

705 **Table 2.** Variable scores for the first and second components of the PCAs performed with (A)
706 BB, BP, Chl *a* and PP, and with (B) BP:BB and PP:Chl *a* ratios (PP: primary production; BP:
707 bacterial production; BB: bacterial biomass; T: temperature; PAR: PAR at 1m).
708

	Component 1	Component 2
A		
PP	-0.120	-0.752
BP	-0.518	0.585
BB	-0.275	0.508
Chl *a*	-0.562	-0.711
T	0.619	0.040
pH	0.517	-0.649
PAR	0.792	-0.026
DOC	-0.878	0.364
TN	-0.883	-0.205
TP	-0.681	-0.677
SPM	-0.706	0.054
% variance explained	40.58	24.80
B		
PP:Chl *a*	0.564	-0.274
BP:BB	-0.375	-0.596
T	0.648	-0.434
pH	0.549	0.730
PAR	0.802	0.038
DOC	-0.887	-0.239
TN	-0.848	0.202
TP	-0.598	0.562
SPM	-0.753	-0.101
% variance explained	47.28	17.53

709

710

711 **Table 3:** Stepwise linear multiple regression of primary production (PP), photosynthetic
712 efficiency (PP:Chl *a*), bacterial production (BP) and bacterial growth rate (BP:BB) as dependent
713 variables and dissolved organic carbon (DOC) and total phosphorus (TP) as independent
714 (potential explanatory factors) variables (all data ln transformed). Upper estuary (stations 2, 3, 4,
715 5 and 6): average salinity 1.7, CV 55%. Lower estuary (stations 14, 15, 17, 18 and 19): average
716 salinity 2.5, CV 12%. Entire estuary (stations 2-19): salinity 2.2, CV 35%.

717

Area in estuary	**Variable**	**Mod. R^2**	**Model sign.**	**Factor**	**Beta/slope**	**Factor sign**
Upper	PP	0.51	<0.001	DOC	-0.72	<0.001
Lower	PP	0.29	<0.001	TP	+0.54	<0.001
Entire	PP	0.42	<0.001	DOC TP	-0.70 +0.51	<0.001 <0.001
Upper	PP:Chl*a*	0.42	<0.001	DOC	-0.64	<0.001
Lower	PP:Chl*a*	0.29	<0.001	DOC	-0.54	<0.001
Entire	PP:Chl*a*	0.40	<0.001	DOC	-0.63	<0.001
Upper	BP	0.27	<0.001	DOC	+0.52	0.002
Lower	BP	-	-	-	-	-
Entire	BP	0.06	0.006	DOC	+0.24	0.006
Upper	BP:BB	0.22	0.002	DOC	+0.47	0.002
Lower	BP:BB	0.15	0.01	DOC	-0.39	0.010
Entire	BP:BB	0.05	0.013	DOC	+0.22	0.013

718

719

720 **Supplementary Table 1.** Date of each sampling occasion from May to August 2010.
721

Date	Week number
May 18th	1
May 25th	2
June 8th	4
June 22nd	6
July 6th	8
July 20th	10
August 3rd	12
August 17th	14
August 31st	16

722

723

724

725 **Supplementary Table 2.** Salinity and DOC concentration at all 19 stations (average value
726 (minimum-maximum)).

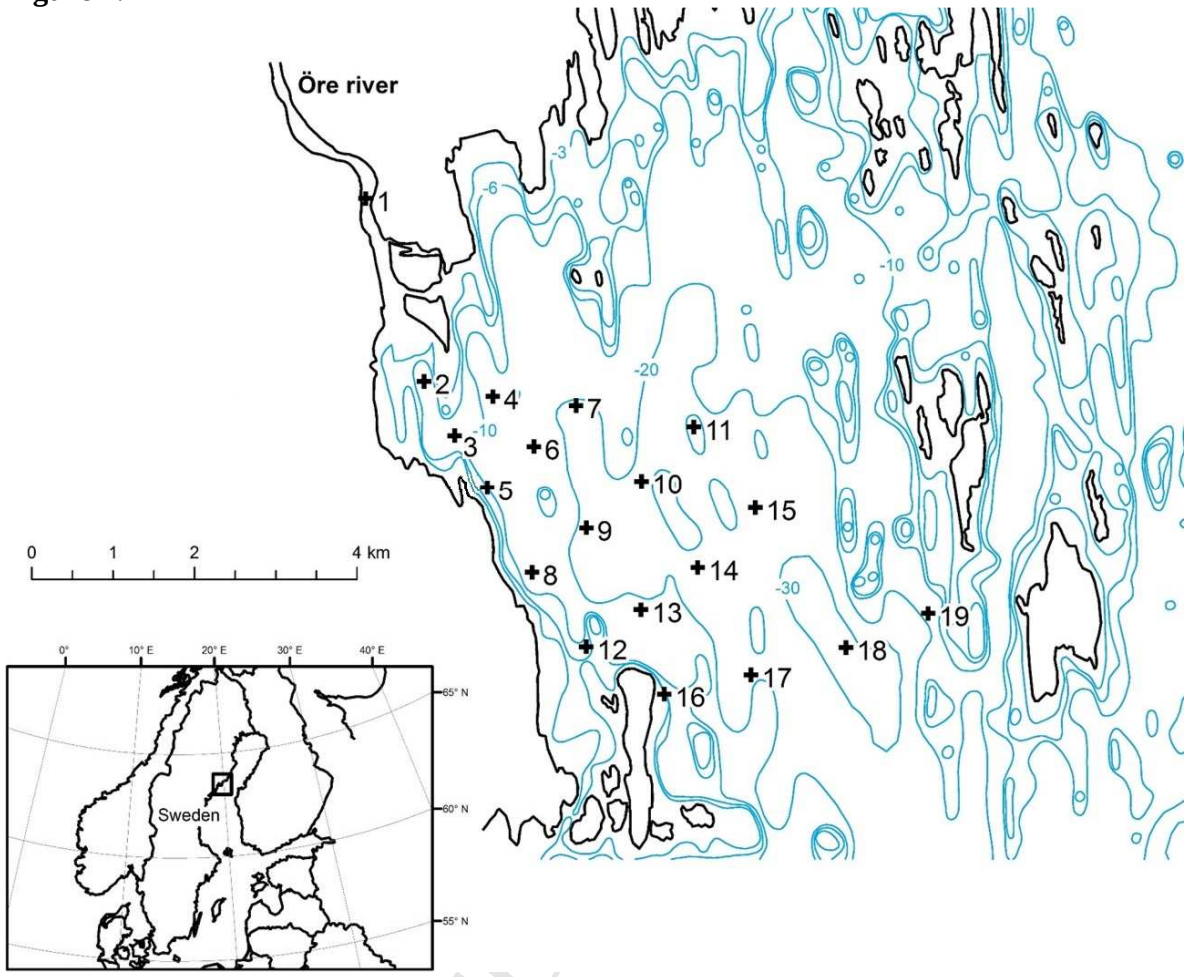
727

Station	Salinity	DOC (g m^{-3})
1	0.0 (0.0-0.0)	8.9 (6.8-10.1)
2	1.4 (0.0-2.5)	6.7 (4.4-9.4)
3	1.4 (0.2-2.7)	6.9 (4.5-10.1)
4	1.9 (0.7-2.6)	5.9 (4.1-8.7)
5	1.6 (0.0-2.6)	6.4 (3.8-10.2)
6	2.2 (1.0-2.7)	5.4 (4.0-8.8)
7	2.4 (1.9-2.8)	4.8 (4.0-6.5)
8	1.7 (0.1-2.7)	6.3 (4.0-10.0)
9	2.4 (1.3-2.8)	4.9 (3.9-7.8)
10	2.5 (1.7-2.8)	4.6 (3.8-6.6)
11	2.5 (2.1-2.8)	4.5 (3.9-5.8)
12	2.1 (0.2-2.9)	5.6 (3.8-10.1)
13	2.3 (0.8-2.9)	5.3 (4.0-9.2)
14	2.5 (2.1-2.8)	4.5 (3.9-5.9)
15	2.6 (2.1-2.9)	4.5 (3.9-5.6)
16	2.3 (0.6-2.9)	5.0 (3.8-8.9)
17	2.4 (1.5-2.9)	4.7 (3.9-6.9)
18	2.6 (2.2-2.9)	4.3 (3.9-4.9)
19	2.6 (2.1-2.9)	4.2 (3.8-5.0)

728

729

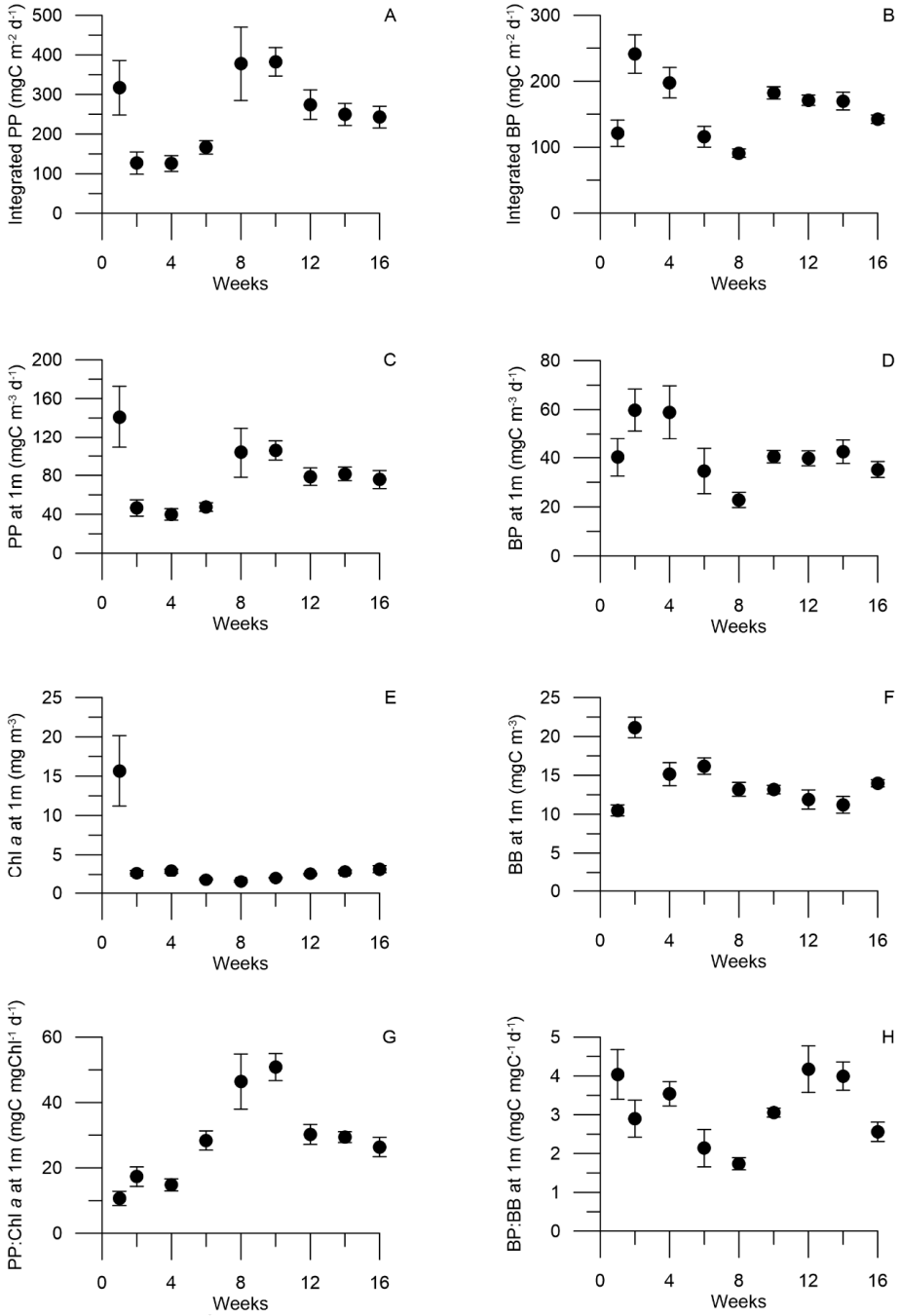
730 **Figure 1.**



731
732
733 **Figure 1.** Map of the study area in the northern Baltic Sea and the sampling stations in the Öre
734 estuary.
735
736

737 **Figure 2.**

738



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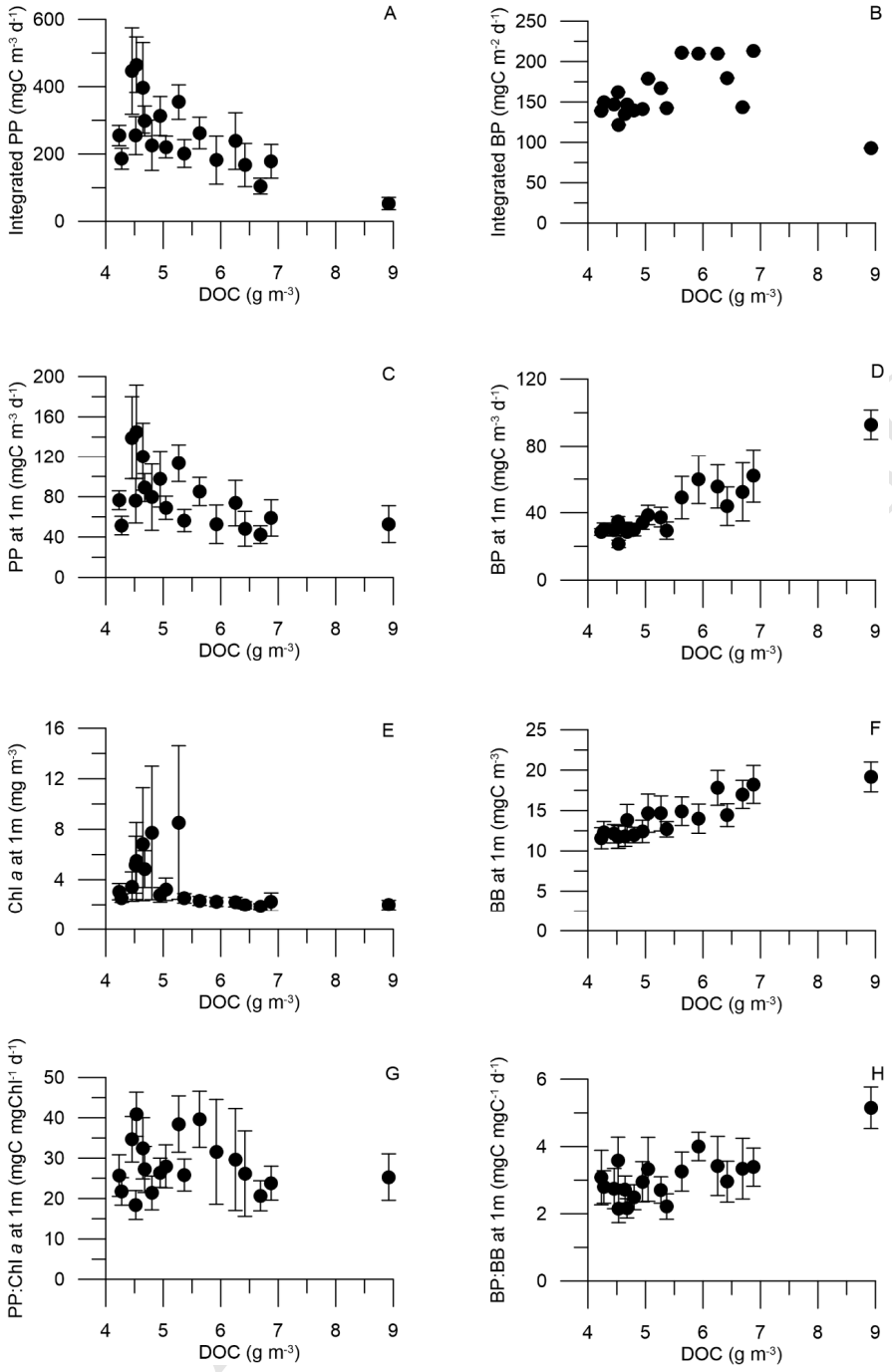
740

741 **Figure 2.** Temporal variation of (A) depth-integrated primary production (PP), (B) depth-
742 integrated bacterial production (BP), (C) primary production (PP) at 1m, (D) bacterial production
743 (BP) at 1m, (E) Chl *a* concentration, (F) bacterial biomass, (G) PP:Chl *a* ratio and (H) bacterial
744 specific growth rate (BP:BB) in the Öre estuary. Values were averaged per sampling week for all
745 the stations. Error bars denote the standard error.

746 **Figure 3.**



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748

749 **Figure 3.** Distribution of (A) depth-integrated primary production (PP), (B) depth-integrated
750 bacterial production (BP), (C) primary production (PP) at 1m, (D) bacterial production (BP) at
751 1m, (E) Chl *a* concentration, (F) bacterial biomass, (G) PP:Chl *a* ratio and (H) bacterial specific
752 growth rate (BP:BB) along the DOC gradient in the Öre estuary during spring and summer.
753 Values were averaged per station over the entire sampling period. Error bars denote the standard
754 error.

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Figure 4.

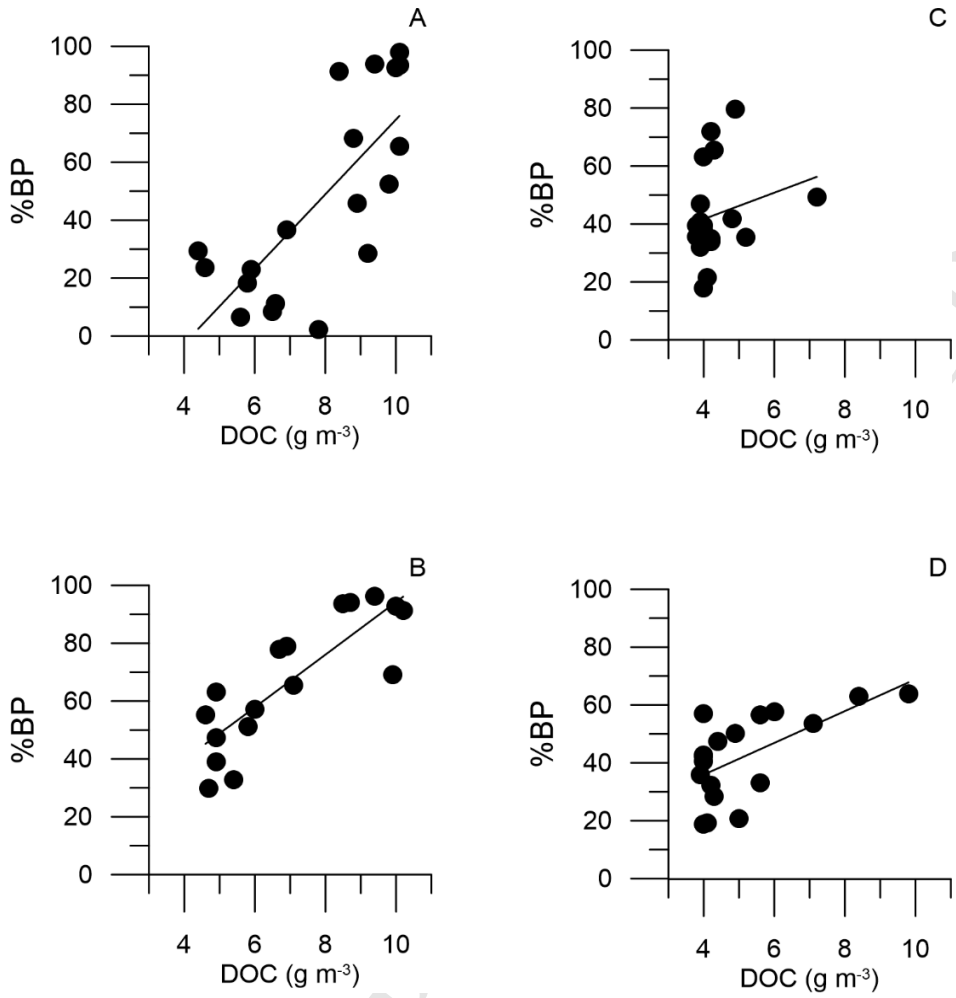
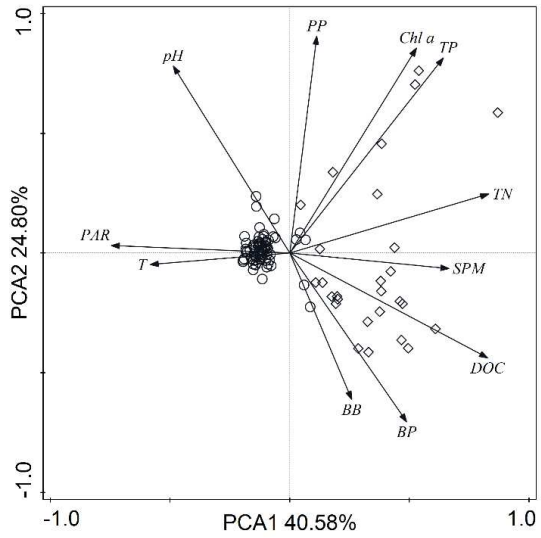


Figure 4. Contribution of bacterial production to basal production (bacterial + primary production), %BP, along the DOC gradient at selected dates representative of spring (A) May 18th, (B) May 25th, and summer (C) July 20th and (D) August 3rd. The lines show the linear regressions between %BP and DOC with equations (A) y = 12.89 x - 54.24, R^2 = 0.56, (B) y = 9.09 x + 3.33, R^2 = 0.70, (C) y = 4.55 x + 23.50, R^2 = 0.05 and (D) y = 5.50 x + 13.89, R^2 = 0.39.

Figure 5.

A



B

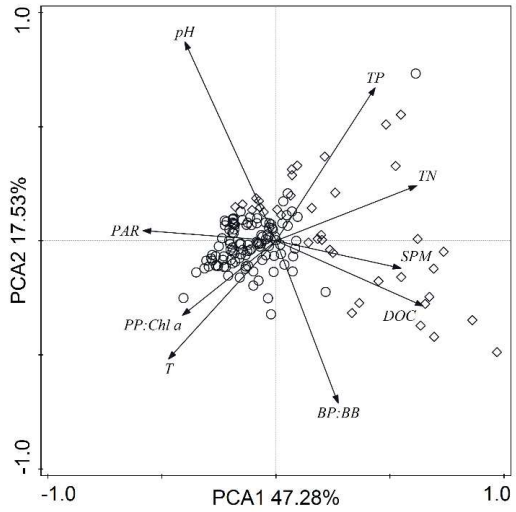
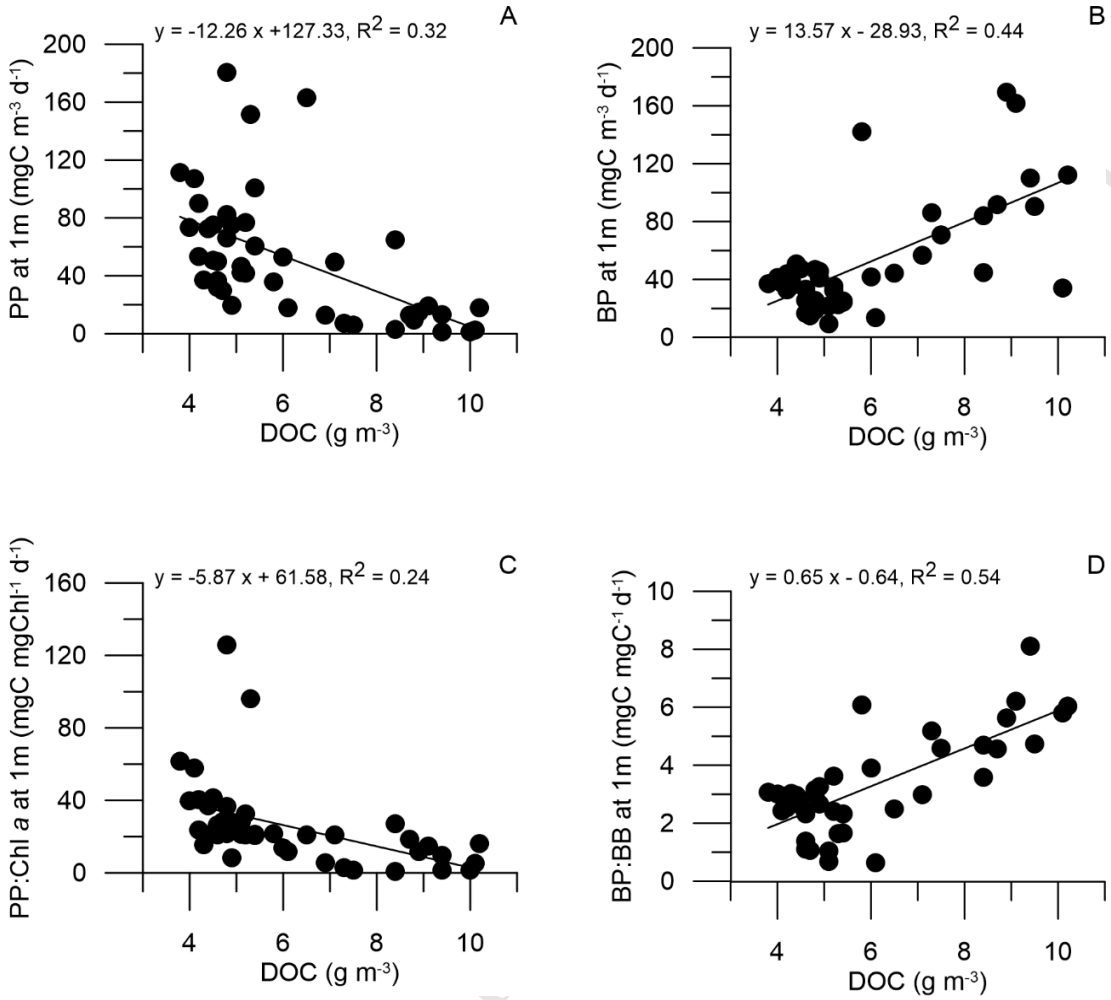


Figure 5. Principal component analyses (projection of the variables and observations) showing the distribution of abiotic (DOC, pH, SPM, T: temperature, TP: total phosphorus, TN: total nitrogen) together with the biotic variables (A) BB: bacterial biomass, BP: bacterial production, Chl *a* concentration, PP: primary production and (B) BB:BP: bacteria specific growth rate, PP:Chl *a*: primary production to Chl *a* concentration ratio for the entire period. Spring samples are indicated by open diamonds, and summer samples by open circles.

784 **Figure 6.**

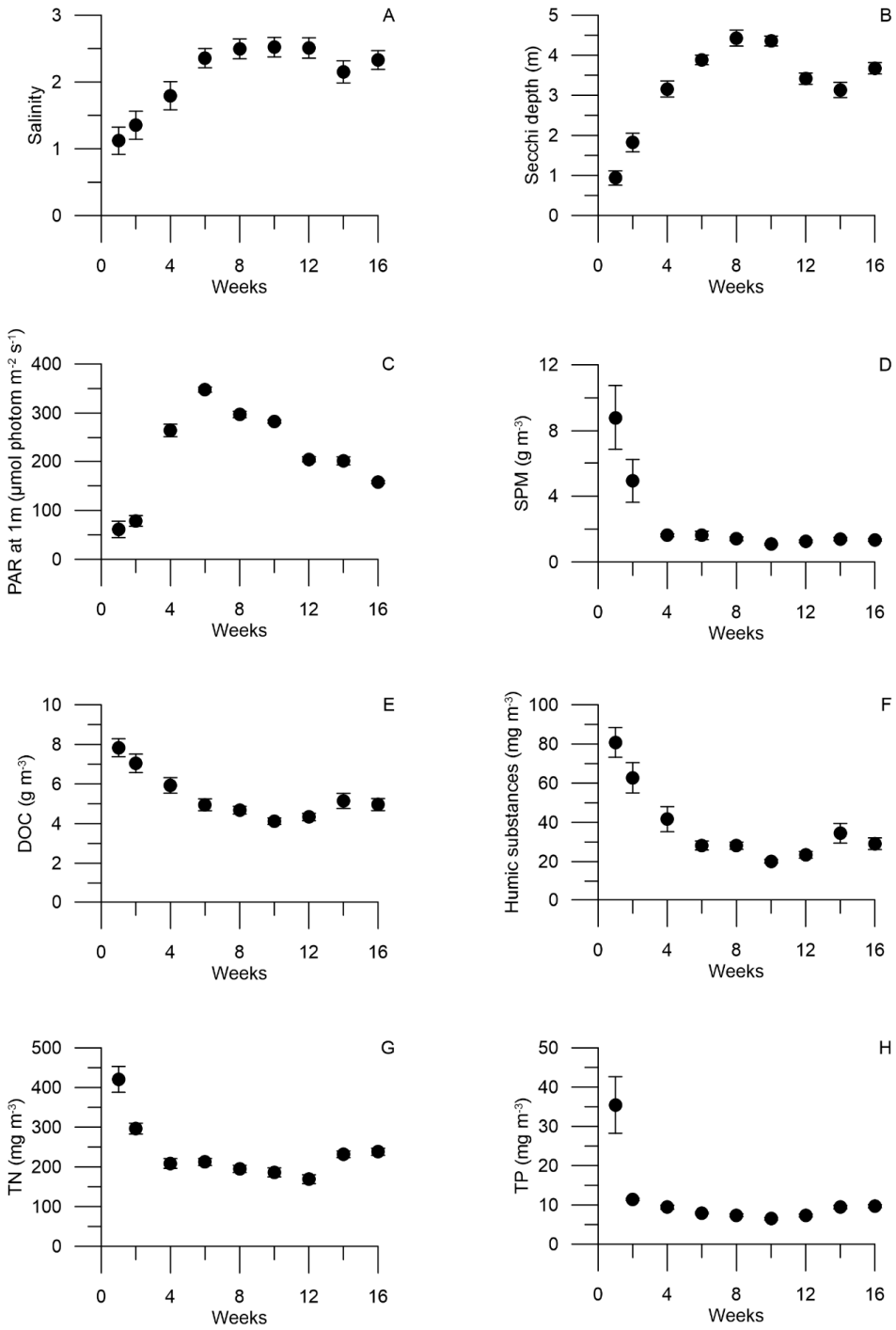


785

786 **Figure 6.** Relationship between primary production (PP), photosynthetic efficiency (PP:Chl *a*),
787 bacterial production (BP) and bacterial growth rate (BP:BB) and DOC in the upper estuary
788 (stations 2, 3, 4, 5 and 6).

789

790 **Supplementary Figure 1.**



791

792 **Suppl. Fig. 1.** Temporal variation of (A) salinity, (B) Secchi depth, (C) PAR at 1m, (D) SPM
793 concentration, (E) DOC concentration, (F) humic substances concentration (G) TN concentration
794 and (H) TP concentration in the Öre estuary. Values were averaged per sampling week for all
795 stations. Error bars denote the standard error.

796 **Supplementary Figure 2.**

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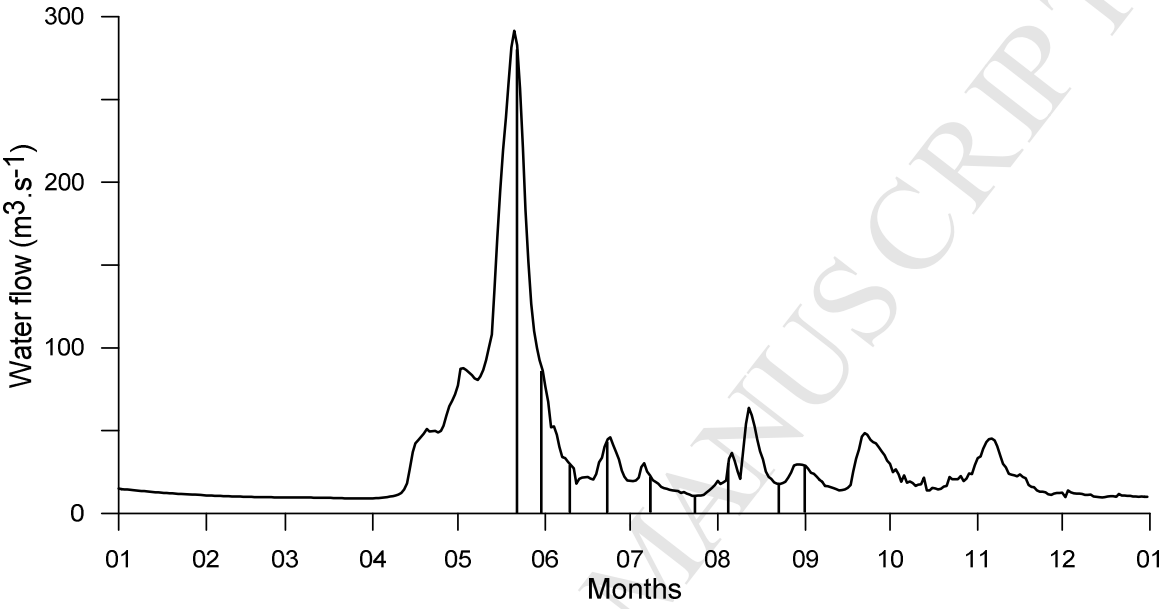
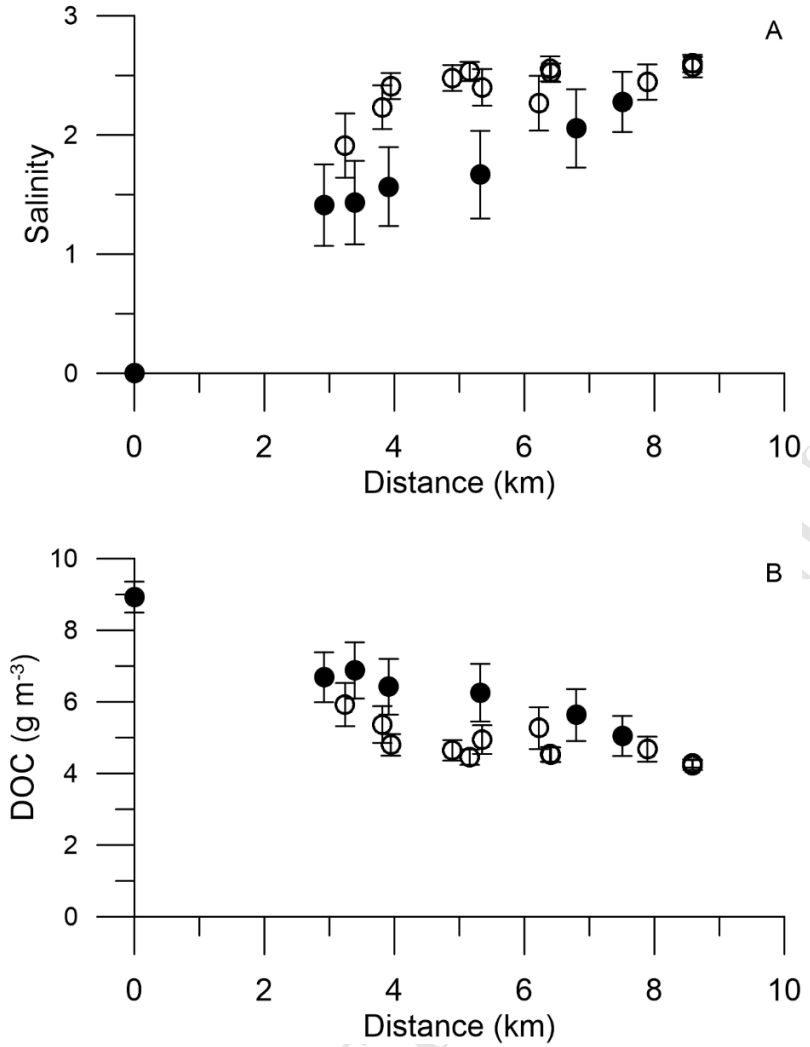


801

802 **Supplementary Figure 2.** Daily freshwater discharge from the Öre River during 2010. Vertical
803 lines indicate sampling occasions.

804

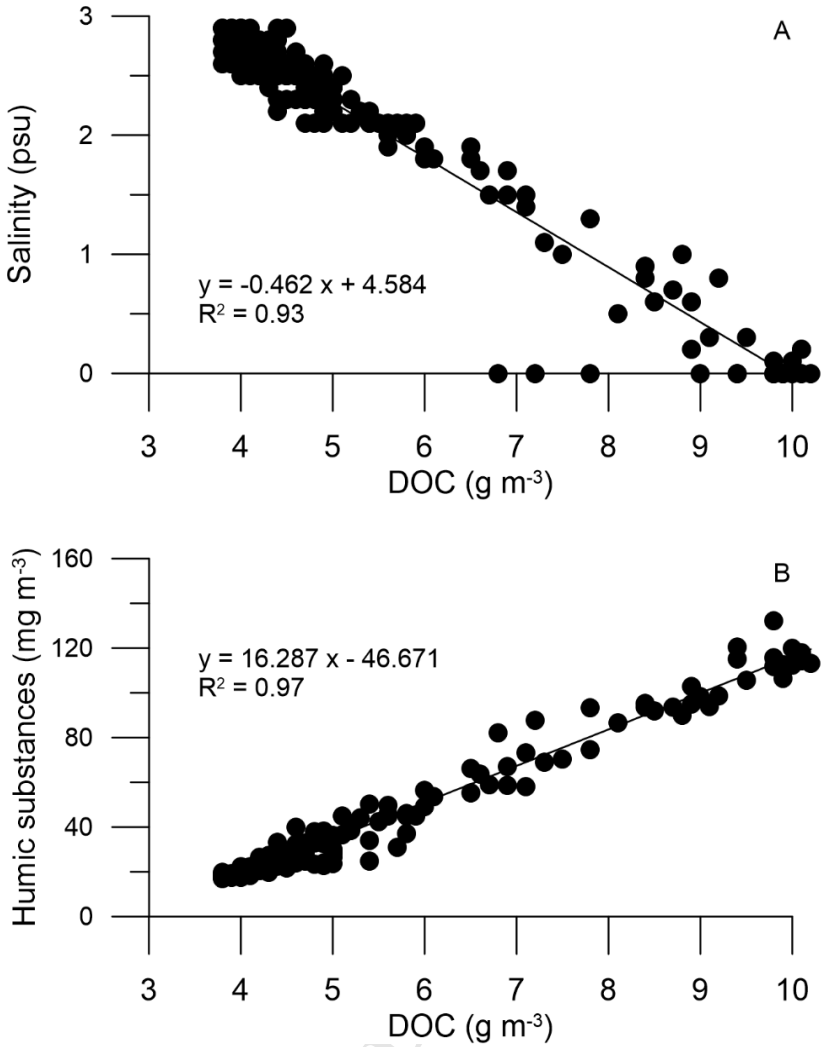
805 **Supplementary Figure 3.**
806



807

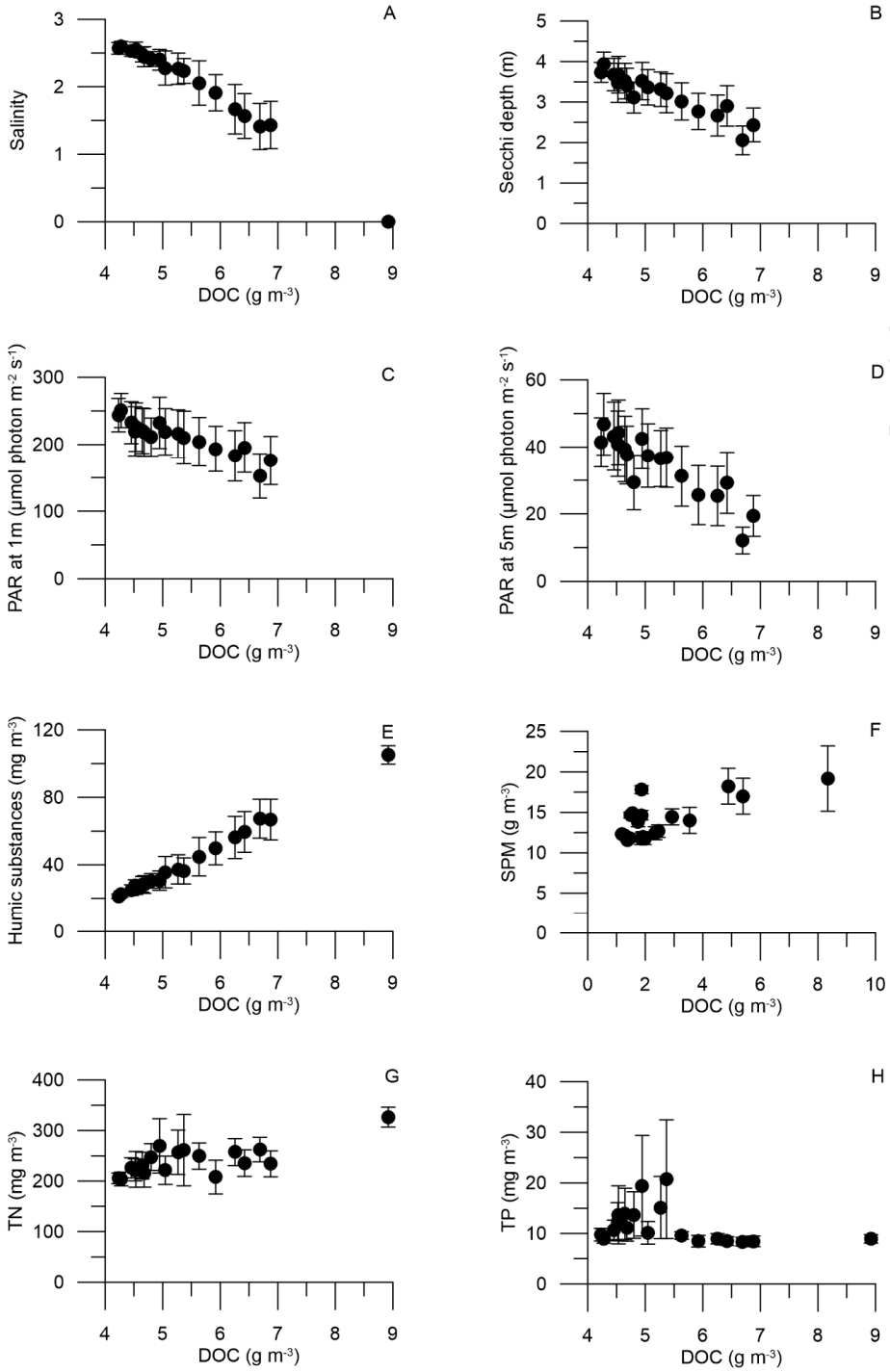
808 **Supplementary Figure 3.** (A) Salinity and (B) DOC concentration (average and standard error)
809 against distance from the river mouth. The stations on the western part of the Öre estuary are
810 shown in dark symbols, while the stations on the eastern part are shown in open symbols.
811

Supplementary Figure 4.



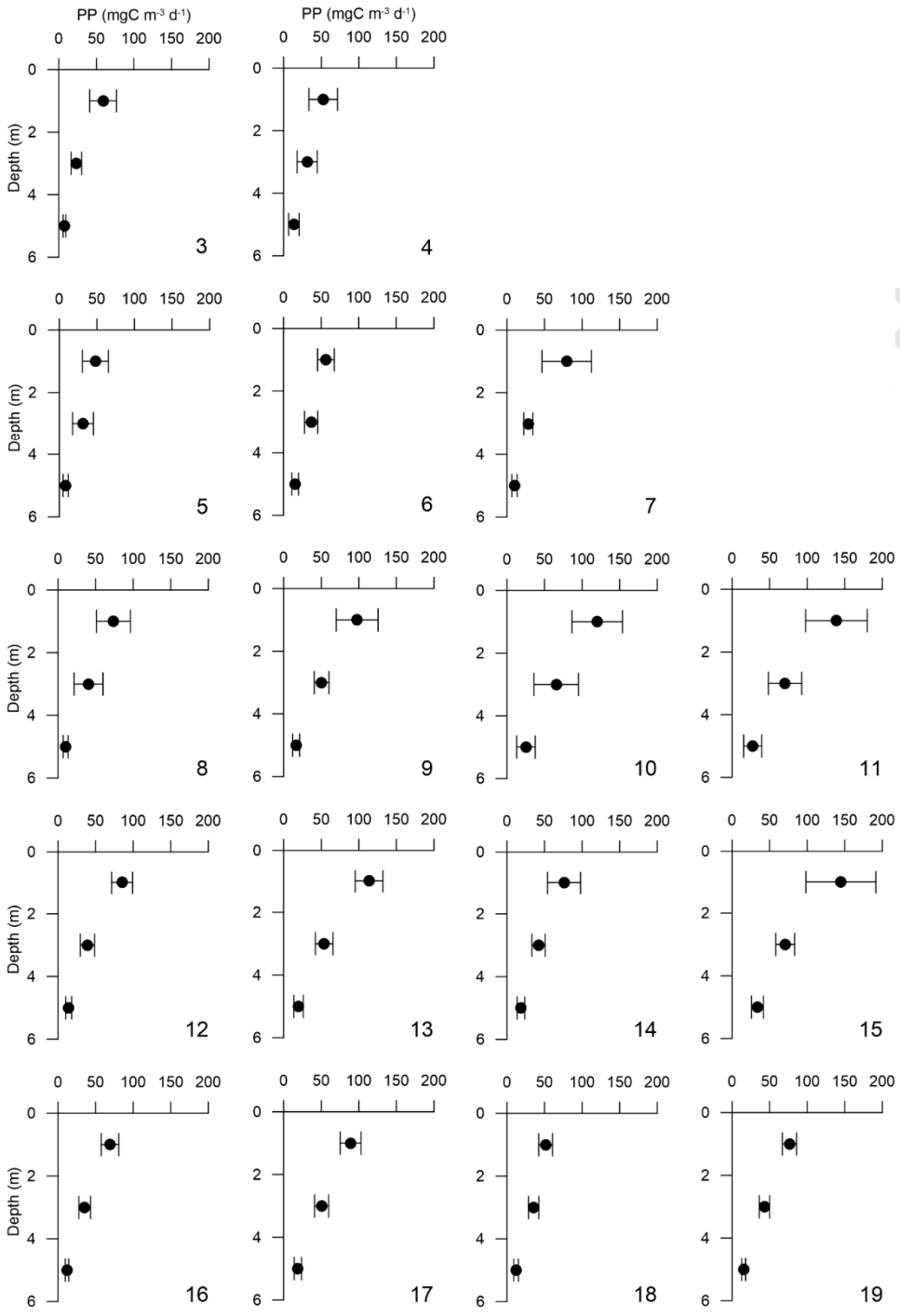
Supplementary Figure 4. Linear regression between (A) salinity, (B) humic substances concentration and DOC concentrations for the entire data set. The equation and determination coefficient of the regression are indicated on each graph.

Supplementary Figure 5.



Suppl. Fig. 5. Distribution of (A) salinity, (B) Secchi depth, (C) PAR at 1m, (D) PAR at 5m, (E) humic substances concentration, (F) SPM concentration, (G) TN concentration and (H) TP concentration along the DOC gradient in the Öre estuary during spring and summer. Values were averaged per station over the entire sampling period. Error bars denote the standard error.

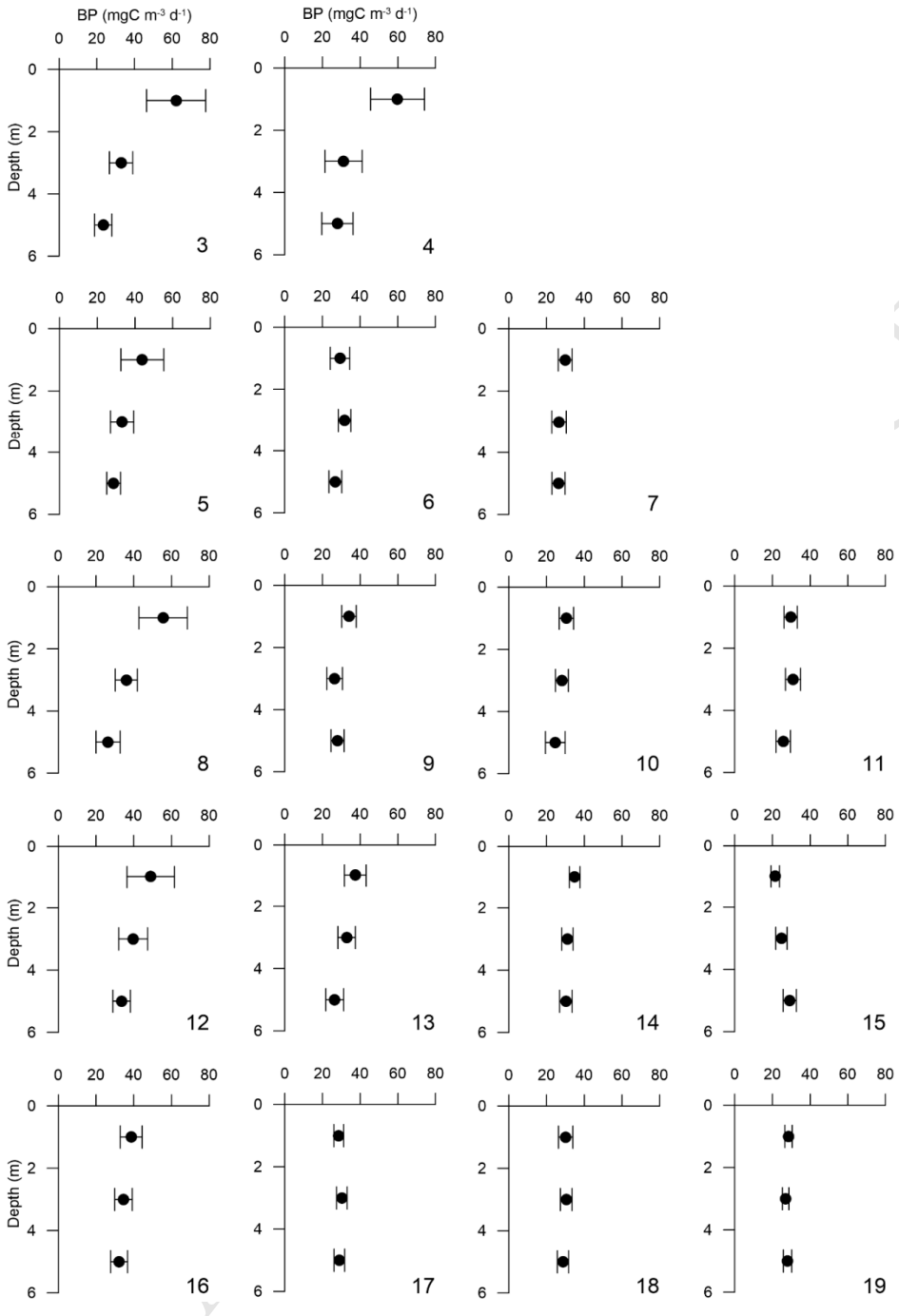
826 **Supplementary Figure 6.**



827

828 **Supplementary Figure 6.** Vertical profiles of average primary production at each station for the
829 entire study period (error bars denote standard error). The station number is indicated at the lower
830 right corner of each graph.

Supplementary Figure 7.



Supplementary Figure 7. Vertical profiles of average bacterial production at each station for the entire study period (error bars denote standard error). The station number is indicated at the lower right corner of each graph.