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Bioavailability of riverine dissolved organic matter in three Baltic Sea estuaries and the effect of catchment land-use

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Abstract

The microbial degradation of dissolved organic carbon and nitrogen (DOC, DON) was studied in three boreal estuaries with contrasting land use patterns (Kiiminkijoki – natural forest and peatland; Kyrönjoki – agricultural; Karjaanjoki – mixed/urban). Bioassays conducted for 12–18 days were used in 3 seasons at in situ temperatures. Besides the bulk parameters, a suite of dissolved organic matter (DOM) quality parameters were investigated, including colored DOM (CDOM), fluorescent DOM and the molecular weight of DOM. Bioavailable DOC and DON pools varied significantly between the estuaries, from 7.9 % in Kiiminkijoki to 10.6 % in Karjaanjoki and from 5.5 % in Kiiminkijoki to 21.9 % in Kyrönjoki, respectively. DOM originating from catchment dominated by natural forests and peatlands had the lowest DOC and DON degradation rates, as well as the lowest proportions of biodegradable DOC and DON. A greater proportion of agricultural land in the catchment increased the bioavailability of DON, but not the bioavailability of DOC. Also DOM quality varied significantly between the estuaries, and DOM originating from the agricultural Kyrönjoki catchment sustained higher DOC and DON degradation rates and higher bacterial growth efficiency (BGE) compared to those of the natural forest and peat dominated Kiiminkijoki catchment. The quality of DOM, indicated by differences in CDOM, fluorescent DOM and molecular weight, varied between estuaries with differing land use and was concluded to be major driver of BGE of these systems and thereafter to the microbial CO_2 fluxes from the estuaries. The differences in BGE resulted in a 5-fold differences in the calculated daily bacterial CO_2-emissions between the study estuaries due to bacterial activity, ranging from 40 kg C d^{-1} in Karjaanjoki estuary to 200 kg C d^{-1} in Kyrönjoki estuary. Two of the study systems (Karjaanjoki, mixed land use; Kyrönjoki, intensive agriculture) in which the DOM pool had lower DOC : DON ratio, smaller molecular weight and higher CDOM absorption spectral slope values resulted in higher proportion of the initial DOC and DON being transferred to microbial growth and therefore to the pelagic food web. The pristine, peatland and forest-dominated Kiiminkijoki catchment had the lowest BGE,

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and therefore proportionally highest CO_2 fluxes. The slope coefficient $S_{275–295}$ was a good proxy of molecular weight across estuaries and seasons, and also for different diagenetic stages of DOM during biological degradation.

1 Introduction

Organic matter consists of particulate and dissolved phases (POM and DOM), which are traditionally separated on the rather arbitrary criteria of filter pore sizes typically ranging between 0.1 to 0.7 µm. Dissolved organic matter is a complex and variable mixture of different organic molecules, which primarily consist of not only carbon, hydrogen and oxygen, but also nitrogen and phosphorus and other elements (Hansell and Carlson, 2002). POM and DOM in aquatic systems are either derived from organisms within the system (autochthonous) or is transported into the system via rivers and atmospheric deposition (allochthonous). The DOM pool is a vital for the functioning of food webs in aquatic systems since it forms the basis for heterotrophic activity and its breakdown is central to the regeneration of inorganic nutrients (Hansell and Carlson, 2002; Søndergaard and Thomas, 2004).

Rivers and estuaries link terrestrial systems and the oceans. Rivers are a major source of allochthonous carbon to the global ocean, transporting an estimated 0.25 Gt of dissolved organic carbon (DOC) annually (Hedges et al., 1997; Jiao et al., 2010). Nitrogen fluxes from terrestrial systems to aquatic systems generally increase with greater degree of anthropogenic disturbance to a catchment (Stedmon et al., 2006), and presently anthropogenically-induced fluxes of nitrogen exceed natural fluxes globally (Vitousek et al., 1997). These fluxes of both organic carbon and nitrogen are essential to the overall productivity of estuarine ecosystems since they are the basis of the heterotrophic food webs (Mann, 1988).

DOM in the estuarine system is susceptible to various transformation and removal processes: adsorption to particulate matter (Krogh, 1931; Gogou and Repeta, 2010), salt-induced flocculation (Sholkovitz et al., 1978; Abdulla et al., 2010), photo-oxidation

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(Miller and Moran, 1997) and utilization by heterotrophs (Sepers, 1977; Elifantz et al., 2007). In this study we concentrate on the latter, and refer to the portion of the DOM pool which is available for heterotrophic consumption (biologically available) as BDOM (Servais et al., 1987).

The properties of DOM pool have been shown to reflect the size of the biologically available fraction of DOM (Lønborg et al., 2009). These qualitative DOM properties can be characterized using various parameters, which are linked to DOM bioavailability: for instance molecular size (Peuravuori and Pihlaja, 1997; Benner et al., 1997; Schwede-Thomas et al., 2005), elemental stoichiometry and bulk chemical properties (Opsahl and Benner 1997; Sun et al., 1997; Kaiser et al., 2003) and structural level features (Fan et al., 2000; Hertkorn et al., 2006; Dittmar and Paeng, 2009).

Variation in DOM quality can explain up to 75 % of the variability of bacterial growth in estuarine waters (Hopkinson et al., 1998). Bacterial growth efficiency (BGE) describes the proportion of carbon that heterotrophic bacterial cells transfer from substrate (DOM) to biomass (del Giorgio and Cole, 1998) and can be used as a metric to characterize bacterial DOM utilization. The quantity of DOM, as a substrate for heterotrophs, has been shown to affect BGE only at very low concentrations (Eiler et al., 2003), whereas the quality of DOM has a stronger influence on BGE, e.g. the DOC : DON ratio being inversely proportional to BGE (Kroer, 1993). A range of other environmental variables have also been reported to affect BGE, including temperature, inorganic nutrient availability, salinity and the DOM source (del Giorgio and Cole, 1998; Wikner et al., 1999).

The DOM pool can be investigated by studying the colored (CDOM) absorption and fluorescent characteristics. These relatively quick and non-destructive measurements reveal details of the whole DOM pool, both quantitatively and qualitatively (Nelson and Siegel, 2013). Absorption and fluorescence properties of CDOM have been linked to DOC concentration (Banoub, 1973; Fichot and Benner, 2011; Asmala et al., 2012; Mann et al., 2012), origin (McKnight et al., 2001; Stedmon and Markager, 2001; Baker and Spencer, 2004) and molecular size (Helms et al., 2008). Molecular size of DOM has implications to the bacterial utilization of the DOM, as generally larger

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molecules support higher bacterial growth and DOM utilization (Amon and Benner, 1996). The continuous degradation of large DOM molecules to smaller ones decreases the bioavailability of DOM, creating a continuum where bioavailability in general decreases with DOM molecular size (Weiss and Simon, 1999).

Combining CDOM with DOC measurements, the resulting DOC-specific absorbance ($SUVA_{254}$) is a proxy of the DOC aromaticity, and is a valuable variable for assessing the quality of the DOM pool (Weishaar et al., 2003). $SUVA_{254}$ has been shown to be inversely proportional to the biodegradability of DOM: increasing SUVA values indicate more aromatic and less biodegradable DOM pool (Kalbitz et al., 2003a; Berggren et al., 2009). Also other optical properties, namely fluorescence properties of DOM can be used to assess its bioavailability. Typically, bacterial degradation increases the amount of humic-like fluorescence in the DOM pool (Boyd and Osburn, 2004).

Land use and land cover changes have significant impacts on riverine, estuarine and coastal water chemistry (Johnes et al., 1996; Jickells, 1998; Sachse et al., 2005). Transport along the hydrological path – streams, rivers and lakes changes the nature of the terrestrially derived DOM through photochemical oxidation and biological transformation (Bertilsson et al., 1999). Further, these changes affect the consequential degradation of DOM down an estuary (Sun et al., 1997). In boreal river systems in general, during this transport the total organic carbon (TOC) concentrations decrease and total organic nitrogen (TON) concentrations increase, leading to decreasing TOC : TON ratios (Mattsson et al., 2005; Kortelainen et al., 2006). This applies to DOC and DON as well, since 94 % of the TOC and 90 % of the total nitrogen (TN) in boreal rivers are in the dissolved form (Mattsson et al., 2005). The retention time of DOM in the hydrological path decreases its bioavailability, and also decreases the total organic matter export from catchment area (Mattsson et al., 2005).

The quality of terrestrially derived DOM in aquatic systems varies among different land use patterns. DOM originating from forested and peatland areas typically have high C : N ratios, up to 66 in boreal catchments (Kortelainen et al., 1997). This DOM pool is dominated by aromatic, humic-like compounds that are inferior substrates for

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heterotrophic utilization compared to non-humic compounds (Moran and Hodson 1990; Hulatt et al., 2013). Agricultural soils on the other hand are sources of highly biodegradable DOM (Boyer and Groffman, 1996; Kalbitz et al., 2003b; van Kessel et al., 2009).

The objectives of this study were: (1) to measure the bioavailability of DOM to heterotrophic bacteria in three Finnish boreal estuaries influenced by contrasting land use; (2) to study the effects of catchment characteristics and seasonal variation on DOM biodegradability with combined effects of salinity and inorganic nutrient manipulations; (3) to link the bioavailability to bulk characteristics of DOM determined by optical and molecular size characteristics. We hypothesize that the differences in land use are reflected to the quality (including bioavailability) of riverine DOM, and subsequently affect the carbon and nitrogen cycling in estuaries.

2 Material and methods

2.1 Study area, catchment characteristics

The three estuaries used for this study were Karjaanjoki, Kyrönjoki and Kiiminkijoki (Fig. 1). The three catchments draining to the estuaries have differing land use and consequently the estuaries exhibit different water properties (Table 1). The Karjaanjoki catchment area is the most urbanized of the three, and has the lowest TOC and TN loadings of the rivers studied (2.0 and 0.18 $t\,yr^{-1}\,km^{-2}$, respectively). The Kyrönjoki catchment is an agriculture-dominated catchment with both fertilized pastures and crops, resulting in high TOC and TN loadings (5.2 and 0.60 $t\,yr^{-1}\,km^{-2}$, respectively). In contrast the Kiiminkijoki catchment consists mostly of peatlands and forests, which results in high TOC and low TN loadings (6.2 and 0.21 $t\,yr^{-1}\,km^{-2}$, respectively).

Samples from both the river and sea end-members of each estuary were collected on four transect sampling trips, and a total of 24 samples were used for the experiments. Sampling campaigns took place in April/May 2010, August 2010, October 2010 and April/May 2011. The Karjaanjoki estuary was the longest of the study estuaries, and

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the distance between river and sea end-member was 38 km with the salinity of the sea being on average 6.3±0.5. In the Kyrönjoki and Kiiminkijoki estuaries the end-members were 36 and 21 km apart and salinity of the sea samples were 2.7 ± 1.1 and 2.3 ± 0.1 , respectively. In all cases the river waters were sampled from the main channels, near to the river mouths. The seawater samples were taken from outer archipelago, where salinities are, or close to those, of the open-sea salinity values. Surface waters were collected, using either a Limnos-type water sampler or polyethylene bucket. The samples were immediately transferred to 30 L polyethylene canisters and stored cool and dark until filtration in laboratory (within 24 h).

2.2 Experimental design

With three estuaries each sampled on four occasions, altogether 12 individual experiments were carried out as illustrated in Fig. 2. Water for each experiment was first filtered through 0.8 µm cartridge filter (Sartoclean CA MidiCaps, Sartorius AG, Goettingen, Germany) to remove most of the particulate matter, including the bacteriovores. After filtration, in addition to the original end-member units a 1 : 1 mix of sea and river water was prepared, creating a three-point experimental gradient with both end-members and the artificial middle point. Synthetic sea salt (Tropic Marin, Dr. Biener GMBH, Wartenberg, Germany) was added to a subset of mix and river water to give a sub-series of these two waters with a salinity of the ambient seawater. The effect of salt addition was tested, since there is evidence of salt-induced flocculation and transformation of DOM in estuaries (Sholkovitz et al., 1978; Abdulla et al., 2010). Also, changes in salinity have been observed to change growth characteristics of bacterial communities in the Baltic Sea estuaries (Langenheder et al., 2003).

The following day, a nutrient addition was made to subsets of the prepared experiment waters, with both nitrate ($NaNO_3$) and phosphate (KH_2PO_4), to reach final concentrations of 5.5 µmol L^{-1} and 1 µmol L^{-1}, respectively. If nutrient levels already exceeded these values, no additions were made. The purpose of nutrient enrichment was to ensure replete nitrogen and phosphorus conditions during the degradation ex-

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periments. The combination of different water types (estuary end-members; sea and river, and 1 : 1 mix of these) and treatments (salt and nutrient addition and the combination of those) created 10 different experiment units (no salt additions to sea units). The newly created experiment waters were left to settle for 3 to 4 h in order to let the units reach gas equilibrium after the physical disturbance and nutrient additions. Subsequently ten 250 mL glass bottles and 3 Winkler-type glass bottles were filled by careful siphoning from each unit. All bottles were submerged in a water bath, and were incubated for between 12 and 18 d (variation in experiment durations due to logistical reasons) in the dark at the in situ temperature of the sampling sites (4 ± 2 °C in Spring, 18±2 °C in Summer and 10±2 °C in Autumn). These seasonal temperatures were used as incubation temperatures for all estuaries in each season.

On each sampling day (typically days 0, 3, 6, 10 and 14), two 250 mL experiment bottles were pooled for further analyses. On every second sampling, also a Winkler-type bottle was taken for dissolved oxygen (DO) analysis. From the pooled samples, subsamples were taken for the following analyses: Flow cytometry (FCM) samples were stored in 2 mL cryo-tubes and fixed with 0.2 µm-filtered electron microscopy-grade paraformaldehyde (final concentration of 1 %) and stored frozen at −80 °C until measurement. Inorganic nutrient analyses (NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}) were done immediately after sampling. For analysis of properties of DOM, a portion of sample water was filtered through pre-combusted (450 °C for 4 h) 0.7 µm GF/F (Whatman). The nominal pore size of GF/F filters decreases with the pre-combustion process reducing the particle cutoff size, thus making the selected filter more suitable for measurement of dissolved parameters (Nayar and Chou, 2003; Hulatt and Thomas, 2010). A subset of these GF/F filtered samples for colored dissolved organic matter (CDOM) and fluorescent dissolved organic matter (FDOM) were stored in acid-washed (10 % HCl) and pre-combusted glass vials at 4 °C until absorbance and fluorescence measurements were made within two weeks from sampling. Another filtered subset of samples for SEC measurement were stored in identical vials at −20 °C until analysis. DOC samples were acidified with H_3PO_4 and stored frozen at −20 °C until measurement.

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2.3 Analyses

Dissolved organic carbon (DOC) concentrations were analyzed by high temperature combustion on an MQ1000 TOC analyzer according to Qian and Mopper (1996). Analysis integrity was tested daily on the certified reference material (University of Miami, Consensus Reference Materials, Florida Straight water, lot #05–10: 41–44 µmol C L^{-1}), the method yielded an average of 42 ± 6.5 (SD) µmol C L^{-1} (n = 213). The inorganic nutrient analyses were carried out according to Grasshoff et al. (1983) where the NH_4^+ analyses were always done manually and for the NO_2^-, NO_3, and PO_4^{3-} analyses an automated flow injection analyzer was used (Lachat QC 8000). Total nitrogen (TN) and total dissolved nitrogen (TDN; from the filtered fraction) were determined following alkaline persulphate oxidation (Koroleff 1977; Grasshoff et al., 1999) followed by automated analysis. Oxidation efficiency of > 90 % performance was tested weekly. Dissolved organic nitrogen (DON) was calculated by subtracting the combined inorganic nitrogen from the TDN.

Spectrophotometric analyses of CDOM samples were performed using PerkinElmer Lambda 650 UV/VIS spectrophotometer with 1 cm quartz cuvette over the spectral range from 200 to 800 nm with 1 nm intervals. Milli-Q (Millipore) water was used as the reference for all samples. Absorbance measurements were transformed to absorption coefficients by multiplying by 2.303 and dividing by the path length (0.01 m). The absorption spectra were characterized by fitting an exponential model with a nonlinear regression as described in Stedmon et al. (2000). A slope of these spectra, S coefficient for wavelengths 275 to 295 nm ($S_{275–295}$) were calculated. These values were used to give an indication of the quality of the CDOM in the samples (Asmala et al., 2012).

Excitation/emission matrices of fluorescence were measured for DOM samples in 1 cm quartz cuvette in a Varian Cary Eclipse fluorometer (Agilent). Bandwidths were set to 5 nm for excitation and 4 nm for emission. A series of emission scans (280–600 nm) were collected over excitation wavelengths ranging from 220 to 450 nm by 5 nm incre-

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ments. Fluorescence spectra were corrected for inner filter effects, which accounted for the absorption of both excitation and emission light by the sample in the cuvette (Mobed et al., 1996). This was done following the methods of McKnight et al. (2001). The FDOM spectra (excitation and emission) were also corrected for instrument biases using an excitation correction spectrum derived from a concentrated solution of Oxazine 1 and an emission correction spectrum derived using a ground quartz diffuser. Fluorescence spectra were Raman calibrated by normalizing to the area under the Raman scatter peak (excitation wavelength of 350 nm) of a Milli-Q water sample, run on the same session as the samples. To remove the Raman signal, a Raman normalized Milli-Q excitation–emission matrix (EEM) was subtracted from the sample data. As the measured signal was normalized to the Raman peak and excitation and emission correction spectra were used, all the instrument specific biases were effectively removed. Rayleigh scatter effects were removed from the data set by not including any emission measurements made at wavelengths ≤ excitation wavelength +20 nm. Fluorescence peaks C, A, M and T were extracted from the EEM data (Coble, 1996). Peak A is a primary fluorescence peak from dissolved humic substances; peak C is a secondary humic substance peak characteristic of terrestrially derived DOM; peak M is a secondary humic substance peak characteristic of marine-derived DOM; peak T is a peak attributable to fluorescence from the aromatic amino acid tryptophan (Coble, 1996).

Bacterial abundance was determined by flow cytometry after Gasol et al. (1999) and Gasol and del Giorgio (2000). Samples were analyzed with an LSR II flow cytometer (BD Biosciences) using a 488 nm laser. Cells were stained with SYBR Green I (Molecular Probes) at a final concentration of 1 : 10 000 for at least 10 min in the dark and analyzed within 30 min of staining. CountBright beads (Molecular Probes) were added to each sample to calculate the volume of sample. Bacterial data were acquired for 1 min, and cell populations identified from bivariate plots of green fluorescence (fluorescein isothiocyanate, FITC) vs. side scatter (SSC). Gating analysis was performed using FACS Diva software (BD Biosciences). Total bacterial abundance in cells mL^{-1}

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were calculated from sample flow rates inferred from sample volumes obtained and number of events recorded. Each experimental set of samples was analyzed during one measurement session.

Bacterial production was measured using both the ^{3}H-thymidine (Fuhrman and Azam, 1980, 1982) and ^{14}C-leucine (Kirchman et al., 1989) incorporation techniques. Dual labeling of 10 mL duplicate samples and an adsorption control was done with [methyl-^{3}H]thymidine (final concentration 20 nmol L^{-1}) and L-[U-^{14}C]leucine (final concentration 160 nmol L^{-1}). The samples were incubated for 2 to 3 h at the same temperature as the experimental units. The incubations were terminated by the addition of formaldehyde, and the samples were refrigerated until further processing. Within 48 h the samples were extracted with cold trichloroacetic acid (TCA, 5 % final concentration) and filtered onto 0.2 µm mixed cellulose ester filters (Advantec MFS, Inc.) in ice-cold conditions. The amount of radioactivity on the filters was measured using a Wallac WinSpectral 1414 Scintillation counter (PerkinElmer) and InstaGel scintillation cocktail (PerkinElmer).

Dissolved oxygen (DO) was measured using a Winkler titration conducted by dynamic endpoint titration with a Metrohm 848 Titrino Plus potentiometric titrator (Metrohm AG). The dissolved oxygen consumption (mg DO L^{-1} h^{-1}) was converted to carbon respiration (mg C L^{-1} h^{-1}) assuming a respiratory quotient (RQ) of 1.

Bacterial growth efficiency (BGE) was calculated by dividing bacterial production (BP) with bacterial carbon demand (BCD), which is the sum of bacterial production and bacterial respiration (BR): BGE = BP/(BCD). Bacterial production was determined in three ways: (1) converting bacterial cell numbers from flow cytometry to carbon, (2) leucine incorporation to carbon and (3) thymidine incorporation to carbon. Cell numbers were converted to carbon units with conversion factor with value 30.2 fg C $cell^{-1}$ (Fukuda et al., 1998). Leucine incorporation was converted to carbon units using the value of 1.5 kg C mol^{-1} incorporated (Simon and Azam, 1989, Kirchman et al., 1989). Thymidine incorporation was converted to carbon units using the conversion factor of 1.10×10^{18} cells mol^{-1} (Riemann et al., 1987) and carbon content of 30.2 fg C $cell^{-1}$

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(Fukuda et al., 1998). The cell to carbon conversion factor used for BGE estimates (30.2 fg C cell^{-1}, Fukuda et al., 1998), is within the range of other cell to carbon values used in the Baltic Sea region (20 to 35 fg C cell^{-1}, Lee and Fuhrman, 1987; Fagerbakke et al., 1996; Søndergaard and Middleboe, 1995). To address the built-in uncertainty of the cell to carbon conversion factors, we used a range of cell carbon values presented by Fukuda et al. (1998) when calculating carbon fluxes from natural systems. This range of ±12.3 fg C cell^{-1} includes these other commonly used conversion factors, and illustrates the effect of varying conversion factors to the final loading values. We used a time-weighted average of leucine and thymidine incorporation rates during the experiment for BGE calculations. BR was calculated from oxygen consumption during the experiment.

We analyzed the molecular size of DOM with size-exclusion chromatography (SEC). The SEC analyzer consisted of an integrated autosampler and pump module (GPC-max, Viscotek Corp.), a linear type column (TSK G2000SW$_{XL}$ column (7.8 × 300 mm, 5 µm particle size, Tosoh Bioscience GmbH) and a guard column (Tosoh Bioscience GmbH) and a UV detector (Waters 486 Tunable Absorbance Detector) set to 254 nm. The flow rate was 0.8 mL min^{-1} and the injection volume 100 µL. The columns were thermo-regulated in a column oven (Croco-cil 100–040-220P, Cluzeau Info Labo) at 25 °C. The data were collected with OmniSEC 4.5 software (Viscotek Corp.). The eluent was 0.01 M acetate buffer at a pH of 7.00 (Vartiainen et al., 1987). Prior to injection, the samples were filtered through a 0.2 µm PTFE syringe filter. The system was calibrated using a combination of standards, as follows: acetone, ethylene glycol, salicylic acid, polystyrene sulfonate (PSS) 3.5 kDa and PSS 6.5 kDa (58, 100, 138, 3610, and 6530 Da, respectively). The calibration curve was linear (R^2 = 0.99) over the apparent molecular weight (AMW) range tested. Comparison between SEC method and absorbance measurements ($a_{(CDOM254)}$) yielded a linear correlation coefficient of 0.92, indicating high qualitative recovery of DOM with the SEC method, as measured from integrated signal from SEC chromatogram. From integrated signal we calculated

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weighted and number averaged apparent molecular weights (AMW_w and AMW_n, respectively) and molecular dispersity ($AMW_n : AMW_w$).

For studying the statistically significant differences between the experimental units, we performed analyses of variance (single factor ANOVA), analysis of covariance, Welch's *t* test and for post-hoc analysis we used Tukey's HSD. All statistical analyses were done using the basic functions of R software (R Core Team, 2012).

3 Results

3.1 DOM degradation during incubations

There were significant differences in the starting DOM quantity and quality between both estuaries and seasons (Table 2). In Table 2 the data of initial conditions is divided between the river and sea end members to assess the differences between the "source" and the "sink" of DOM in the coastal areas. The experimental treatments (salt and nutrient additions and the combination of those) did not have an effect on the DOM characteristics at the start (data not shown).

In the river end member, DOC concentrations were high in forested Kiiminkijoki and agricultural Kyrönjoki, and expressed aromatic, humic-like properties of the bulk DOM, revealed by high $SUVA_{254}$ and fluorescence peak C values. In addition, DON concentrations were substantially higher in Kyrönjoki. Karjaanjoki river end-member had half of the DOC concentration compared to the other two, and the bulk DOM had less pronounced terrestrial signal, indicated by DOM quality parameters. There was also seasonal variability in some of the DOM variables in river end member. In spring, DOC concentration and aromaticity was the lowest. In sea end member, terrestrial signal was strongest in Kiiminkijoki estuary, as indicated by the DOM quality parameters. DON concentration was significantly higher in Karjaanjoki sea end member than in the of other two estuaries. There were no seasonal trends in study variables in the sea end member.

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The differences in DOM quantity and quality led to differential heterotrophic degradation of DOM during the incubations. Also, concentration variables (DOC and DON) and quality variables (CDOM, fluorescent DOM and molecular weight) indicated selective degradation of DOM by heterotrophic bacteria during the experiments.

The bacterial activity during the incubations resulted in variable DOM degradation characteristics between the experimental units (Table 2). DOM degradation is expressed as a difference between the start and the end value of each parameter in incubations and hereafter referred as the Δ value. There were differences between the estuaries in the Δ value of all variables except the molecular weight of the DOM. Seasons did not influence the Δ values of concentration parameters of DOC and DON, but did affect the qualitative variables (CDOM slope and fluorescent DOM). The water type (river water, 1 : 1 mix, seawater) made a difference to the DON degradation. Experimental treatments (nutrient and salt addition and the combination of those) did not have significant differences in Δ values of any study variables (data not shown). Contrary to the expected, seasonal variability turned out to be less significant overall than the difference between estuaries in Δ values for most variables. Since the specific estuary was found to be the key determinant of Δ values of many study parameters, we will mainly focus on the differences between estuaries in this article.

In incubations, both DOC and DON were utilized in varying quantities by bacteria (Table 3). The total amounts of biodegradable DOC among estuaries did not significantly differ, but both the proportion of the biodegradable pool and the daily consumption rates varied significantly among estuaries, being lowest in forest and peatland-dominated Kiiminkijoki estuary and highest in agricultural Kyrönjoki estuary. The sizes of the biologically degradable DON pools, their proportions of the whole DON pools and DON degradation rates were all significantly different among estuaries, being lowest in Kiiminkijoki and highest in Kyrönjoki. Furthermore, the ratio with which DOC and DON were consumed varied significantly among estuaries.

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3.2 Effects of land use and seasonality to DOM degradation

3.2.1 DOC and DON concentrations

The three study estuaries have clearly different land use (Table 1), and differences in bioavailability of DOC and DON (Fig. 3). The Kiiminkijoki estuary catchment is largely dominated by natural forests and peatlands and had the lowest values for the biologically degradable DOC and DON, and also the lowest degradation rates. Kyrönjoki, heavily influenced by agriculture, had the highest proportion of bioavailable DON, and also the highest rates for DOC and DON degradation. Considering the different land cover types, three classes were found to covary with the changes in DOC and DON bioavailability: combined forests and peatlands, lakes and agriculture. A higher percentage of forests and peatlands in the catchment decreased the bioavailability of DOC. Longer retention time in the catchment, as indicated by lake percentage, decreased DON bioavailability, whereas higher agricultural land use increased DON bioavailability.

The DOC : DON ratios varied substantially between estuaries, ranging on average from 21 in Karjaanjoki to 40 in Kyrönjoki (Fig. 4a). In Karjaanjoki and Kiiminkijoki estuaries the variation in DOC : DON ratios was small, but in agricultural Kyrönjoki the variation was considerable (from 4 to 243). This variation was mainly caused by the spring samples, where the lowest DOC : DON values were measured. In other estuaries, this spring effect was not detected.

The ratio of biodegradable DOC (BDOC) and biodegradable DON (BDON) also varied between the estuaries, being 12 in Kyrönjoki, 19 in Karjaanjoki and 52 in Kiiminkijoki (Fig. 4b). In Karjaanjoki and Kiiminkijoki degradation ratios of C : N followed the original DOC : DON ratio, but in Kyrönjoki average degradation ratio was significantly lower than the DOC : DON concentration ratio (12 and 38, respectively).

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3.2.2 DOM quality indicators

Besides the quantitative changes (as indicated by DOC and DON concentrations), there were also changes in DOM quality. We measured significant changes in CDOM and fluorescent DOM properties, but not in molecular weight during the experiments. There were significant differences between estuaries on the Δ values in CDOM in experimental units: DOM aromaticity indicator $SUVA_{254}$ was highest in Karjaanjoki and lowest in Kiiminkijoki (Fig. 5) in all experimental treatments. $SUVA_{254}$ increased and UV-slope of CDOM ($S_{275–295}$) decreased in every estuary and season during the experiment, except in the summer units where the slope increased.

Of the fluorescence peaks measured (A, C, M and T), we chose to concentrate on peak C, since the peak C is a proxy for humic-like, terrestrial substances in the DOM pool (Cammack et al., 2004; Stedmon and Markager, 2005). Also, since peaks A and M co-varied significantly with C peak (R^2 = 0.98), we did not continue analyses of A and M peaks, as they contributed minor additional information. The Δ values of peak C decreased in Kiiminkijoki and Kyrönjoki experiment units, and increased in Karjaanjoki experiment units. Similarly to UV-slope, there was an increase in summer units, and also in autumn units in Δ values of humic-like peak C.

Molecular weight parameters did not change significantly during the incubations, and there were no significant differences in Δ values between estuaries ($P > 0.05$). However, for the whole dataset there were minor average changes in weight-averaged apparent molecular weight (AMW_w), from 2466 Da in the beginning to 2444 Da in the end. The molecular dispersity, i.e. a proxy of heterogeneity of the molecular weights in DOM (Chin et al., 1994), decreased on average from 1.193 to 1.189 in all experiment units. The slope $S_{275–295}$ proved to be a good proxy for the weight-averaged apparent molecular weight (AMW_w) in all of the experimental units (Fig. 6). Previously Helms et al. (2008) had related this slope to the molecular weight of DOM, and recently use of this slope has been expanded to trace terrigenous DOC (Fichot and Benner, 2012).

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There were significant differences in bacterial growth efficiency among experiment variables estuary, water type (end member) and treatment (nutrient addition). Seasonal variation between experiment units was not significant ($P > 0.05$). We measured an average BGE of 0.33, ranging from 0.25 to 0.41 (Fig. 7), which is within the typical range for estuarine datasets (del Giorgio and Cole, 1998). The forested Kiiminkijoki estuary had the lowest BGE, followed by agricultural Kyrönjoki, and Karjaanjoki estuary had the highest BGE. The river end member had lower BGE than the sea end member, and nutrient addition increased the BGE.

When pooling the whole dataset together, we measured multiple significant ($P < 0.001$, $R^2 = 0.14$–0.28) overall relationships between BGE and a selection of independently measured DOM quantity and quality parameters (Fig. 8). These relationships did not vary significantly between estuaries ($P > 0.05$). Weight-averaged apparent molecular weight (AMW_w) had the highest coefficient of determination (R^2), 0.28. BGE decreased with increasing molecular size over the molecular range of 1860 to 3320 Da. Increase in slope coefficient between 275 and 295 nm resulted as an increase in BGE ($R^2 = 0.24$). DOC-specific UV absorption ($SUVA_{254}$) was inversely related to BGE ($R^2 = 0.21$), as well as the humic-like fluorescence peak C ($R^2 = 0.19$). DOC and DON concentrations were less significant drivers of BGE than these quality variables.

Although statistically significant, most of our regression analyses result with coefficients of determination (R^2 values) well below the critical threshold of 0.65 presented by Prairie (1996). This limits the predictive power of our results, but does not prevent the use of the data to describe the complex interdepencies of DOC and DON concentrations, DOM quality and BGE.

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4 Discussion

4.1 DOC and DON degradation

The only experiment variable significantly affecting DOC and DON degradation was the estuary, i.e. the source. This means that the initial quality of DOC and DON (as specified in Table 2), determined by the catchment characteristics, was the major factor defining the biodegradability of DOC and DON, and this agrees with our study hypothesis. Bioavailability of DOM has been previously reported to have strong seasonal variation (Lønborg et al., 2009; Sintes et al., 2010). However, in the experiments presented here the season surprisingly did not have significant effect on DOC and DON degradation. This implies that seasonal variation in DOM quality was balanced by seasonally adapted performance of the bacterial communities resulting as steady total utilization of DOC and DON. The water type (river, sea, 1 : 1 mix) did not affect DOC and DON degradation rates, indicating that the degradable portions of DOC and DON in the river waters were still available in the seawater or the microbial communities in the seawaters were adapted to use different DOM sources than the river communities (Bouvier and del Giorgio, 2002).

We also investigated the importance of inorganic nutrient limitation, and the effects of changes in salinity conditions. The insignificant effect of inorganic nutrient addition on biodegradable DOC and DON amounts suggests that the DOM degradation in the experiments was not limited by inorganic nitrogen or phosphorus availability. This is in contrast to the commonly observed stimulation of heterotrophic DOC utilization by addition of inorganic nutrients (e.g. Kuparinen and Heinänen, 1993; Zweifel et al., 1995). Neither did the addition of salt result in any significant changes in any of the study parameters. We hypothesize this to be the result of the major differences in DOM quality between estuaries, masking the minor changes caused by differences of salt tolerances between bacterial communities. Also, salt-induced changes to DOM bioavailability do not occur when moving from fresh water to low-salinity estuarine conditions (Søndergaard et al., 2003). Furthermore, the amount of salt needed in this study to

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reach Baltic Sea values is considerably smaller than in transects having a full-strength seawater end-member where significant alterations of humic substances have been reported (Kisand et al., 2013).

4.2 Effects of land use and seasonality to DOC and DON degradation

The size of the biodegradable DOC pool was not significantly different between the estuaries (56–79 µmol L^{-1}), but variation in the total DOC pool size led to significant proportional differences between the estuaries. The largest biodegradable DOC proportion (%BDOC) was measured in Karjaanjoki estuary (Table 3), which has the catchment with the least forest and peatland areas of the study estuaries (Fig. 3). The initial DOC concentrations in this estuary were 40–50 % lower than in other two estuaries. Kiiminkijoki, with the forest and peatland-dominated catchment, had the lowest%BDOC. Overall, the differences in %BDOC between estuaries were moderate, regardless of the significant differences in the land use of these estuaries.

The biodegradable DON pool was proportionally largest in the agricultural Kyrönjoki estuary, and lowest in Kiiminkijoki. The range of BDON pools were considerably larger than those of BDOC, and the size of the biodegradable DON pool increased with increasing amounts of agricultural land in the catchment areas (Fig. 3). The variations in the BDOC : BDON ratios followed the patterns of quantity and quality of DON in the estuaries: Kyrönjoki, the predominantly agricultural catchment, had the largest biodegradable DON pool and the highest DON degradation rate, whereas forest and peatland-dominated Kiiminkijoki had the lowest. The relationship between BDOC and BDON amounts was significant only in Kiiminkijoki, implying that in other two systems there is a more complex mix of bioavailable compounds with different C : N ratios (Fig. 4b). The rather homogenous pool of DOM (in terms of DOC : DON ratio) from the Kiiminkijoki catchment also resulted in the tightest coupling between BDOC and BDON, suggesting a homogenous pool of bioavailable compounds in that system.

The amount of forests and peatlands in the catchment decreased the bioavailability of DOC in the estuary. The initial DOM in Kiiminkijoki estuary expressed multiple

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indicators of refractory, humic-like quality: high $SUVA_{254}$, humic-like fluorescence and molecular weight (Amon and Benner 1996; Berggren et al., 2009). These same variables were all lower in Karjaanjoki, which also had the highest BDOC.

The amount of lakes in the catchment area did not influence the DOC bioavailability, but decreased DON bioavailability, suggesting that DON is intensively transformed within waterways (Brookshire et al., 2005; Mattsson et al., 2005). The most bioavailable fractions are subjected to biological uptake already during the riverine transport before entering the estuary, which highlights the role of residence time of terrestrial DOM in lotic systems. Karjaanjoki, with the most lakes (9 %) in its catchment had half the BDON of Kyrönjoki, with only 1 % of lakes in its catchment.

In our study, increased percentage of agricultural land in the catchment evidently increased BDON, but not BDOC. In the Kyrönjoki estuary, where there is the largest proportion of agricultural areas, the biodegradable DON fraction was the highest. The difference between DON concentrations of river and sea members was also greatest in Kyrönjoki estuary, indicating that the high amounts of DON flowing from the river are utilized rapidly in the coastal zone. It is notable that the very low DOC : DON ratios of Kyrönjoki in Fig. 4a were all from spring, when high spring freshet-associated surface runoff over ploughed cropland soil can be expected to transport high amounts of nitrogen into the river.

In the Kiiminkijoki experimental units high BDOC : BDON ratios were measured, implying that the heterotrophs in that system were utilizing DOM of poor quality to meet their energy demand (c.f. Hopkinson et al., 1997). This might be a result of the low concentrations of DON in that particular system. Similar N-poor recalcitrant DOM has been reported to accumulate in oceanic systems (Kähler and Koeve, 2001). Also the refractory nature of forest and peatland-derived DOM in the Kiiminkijoki system may have caused the high C : N ratio, and both low BDOC and BDON degradation rates (Hulatt et al., 2013).

Typically, in pristine areas with low anthropogenic influence organic carbon explains 95 % of organic nitrogen variation (Kortelainen et al., 2006), which is supported by our

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measurements from Kiiminkijoki experiment units, where DOC and DON concentrations were tightly coupled (Fig. 4). On the other hand, in the Karjaanjoki and Kyrönjoki estuaries with higher anthropogenic impact the relationships were weaker, indicating larger variation within the DOM pool in these systems. We found weak relationships between DOC concentration and the proportion of BDOC, and between DON concentrations and the proportion of BDON. From this we conclude that in our study estuaries, the DOM quantity (DOC and DON concentrations) does not determine its bioavailability.

4.3 Changes in DOM quality during incubations

$SUVA_{254}$ increased in all estuaries and seasons during incubations (Table 2, Fig. 5). This increase indicates either the selective utilization of the non-colored or less aromatic DOM by microbes or the production of UV-absorbing compounds by bacteria (Nelson et al., 2004; Ortega-Retuerta et al., 2009). The aromatic compounds, mostly deriving from lignin degradation, are likely to be the most stable and dominant fraction of DOM absorbing at 254 nm (Kalbitz et al., 2003b). As degradation of these compounds is strongly dependent of sunlight (Hernes and Benner, 2003), transformations of these compounds to more readily degradable bioavailable compounds in our dark incubations was not likely. Therefore it follows that the aromatic compounds ($SUVA_{254}$ being the proxy) in the experiment units remained relatively unaffected by bacterial activity compared to non-colored DOM, and in addition newly created CDOM was added to the aromatic pool during the incubations.

The UV-slope $S_{275–295}$ decreased in the experiment units from every estuary, which indicates either a production of material absorbing at higher wavelengths or removal of compounds that absorb at lower wavelengths. However, in summer UV-slope increased. In summer the initial, riverine $SUVA_{254}$ was the highest, which may indicate that the DOM at the river mouth is further in its diagenetic continuum, i.e. processed more by the heterotrophic bacteria in the warm summer temperatures during transport along the hydrological path (Berggren et al., 2009). Additionally, it can be argued

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that the bacterial community have shifted its utilization in summer from aromatic, allochthonous DOM more to the non-aromatic, autochthonous DOM. All in all, the result was that CDOM was not utilized evenly throughout the spectral range.

The fluorescent DOM peak C represents humic-like fluorescent component of the DOM. Increases in the humic fluorescence can be expected if microbial humification processes dominated, with bacteria selectively degrading the bioavailable DOM and in the process generating humic fluorescence (Guillemette and del Giorgio, 2012; Shimotori et al., 2012). In the Karjaanjoki experimental units, the humic-like peak C increased significantly while in the humic-rich estuaries Kiiminkijoki and Kyrönjoki, peak C was consumed. This is evidence that the bacterial communities in these systems are adapted to degrading humic material since it is abundantly available, even though it is not the most favorable substrate for heterotrophic utilization. Peak C increased in summer experiment units of all estuaries and decreased in other seasons, which coincides with the increase of $SUVA_{254}$ and UV-slope in summer units discussed earlier.

We found that treatments (inorganic nutrient, salt addition and combination of these) did not effect the Δ values of CDOM or fluorescent DOM, which is in line with earlier results (Søndergaard et al., 2003) on low-salinity estuarine conditions not influencing the CDOM or fluorescent DOM composition or bioavailability of the whole DOM pool. Also the slight decrease in molecular size distribution during incubations was not significant ($P > 0.05$). This result suggests that the microbial activity did not alter the molecular size distribution notably. This is contrary to the studies in which a linkage between DOM molecular size and its heterotrophic utilization has been established (e.g. Tranvik 1990; Amon and Benner, 1996). However, molecular size had an effect to the bacterial growth efficiency, indicating differing energetic value to the heterotrophs along the DOM size spectrum.

4.4 BGE as a proxy for DOM quality

There were significant differences in bacterial growth efficiency between estuaries (Fig. 7), and these covary with the degradation rates of DOC and DON (Table 3), sug-

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gesting that the faster degradation of DOM and higher BGE resulted from the quality of DOM i.e. its susceptibility to heterotrophic degradation. BGE in the sea end-member experiment units was higher than in the river end-member. We hypothesize that lower BGE in rivers was due to the more refractory status of the riverine DOM compared to the DOM in sea where the DOM has already been exposed to a range of transformative forces (photo-oxidation, salt-induced changes, prolonged microbial activity). In conjunction with the gradual dilution and transformation of the terrestrial DOM in coastal waters, there is also an increase in the relative proportion of autochthonous DOM during mixing which may explain these higher BGE values.

Season did not have an effect on BGE, suggesting that temperature did not effect BGE. This is surprising, since BGE is commonly accepted to be inverse function of temperature (Rivkin and Legendre, 2001). Also, the seasonal changes in DOM quantity and quality evidently did not to affect BGE. Conversely, treatment (addition of inorganic nutrients, salt and combination of these) had an effect, more precisely the inorganic nutrient addition increased BGE. The positive effect of nutrient addition to BGE leads to assume that increased inorganic nutrient availability reduces cost (less extracellular enzymes needed) of nutrient acquisition in general, or DON in other experiment units may have to be used as a source of nitrogen, which requires energy and decreases BGE (Hopkinson et al., 1997). Increased BGE in nutrient added units suggest that increased inorganic nutrient availability increases the amount of organic carbon transferred to aquatic food web instead of respired CO_2 (Pace et al., 2004; Kritzberg et al., 2006; Hitchcock et al., 2010).

Substrate concentrations have been observed to constrain BGE only in oligotrophic conditions (Eiler et al., 2003), and also in this study the DOC concentrations explained the little variation in BGE ($R^2 = 0.14$). Furthermore, there was no significant relationship between the amount of biodegradable DOC and BGE ($P = 0.23$). Instead, the quality of the DOM on the other hand plays a crucial role in determining the BGE of the aquatic system, alongside with other factors such as inorganic nutrient availability and community assembly (Wikner et al., 1999; Berggren et al., 2007).

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The DOM quality properties had significant, albeit weak to moderate relationships to BGE (Fig. 7). Molecular size (as measured by weight-averaged apparent molecular weight) had an inverse relationship to BGE; increasing molecular size led to decreasing BGE (concurring with Tulonen et al., 1992). This relationship arises from the differing quality of DOM along the size gradient, such as C : N ratio decreasing with molecular size and overall larger molecules consist of a larger array of favorable compounds supporting more efficient growth than smaller molecules with less degradable components (Amon and Benner, 1996). BGE increased alongside increasing UV-slope $S_{275–295}$, which can be at least to some extent linked to decrease of refractory biopolymer, lignin (Fichot and Benner, 2012). The negative effect of aromatic, humic-like substances to BGE is supported by DOC-specific UV absorbance ($SUVA_{254}$) and humic-like fluorescence peak C, which were both inversely related to BGE.

All in all, these properties of DOM that most affect BGE can be seen to represent different facets of the same general underlying denominator, as they all can be linked to aromaticity and humic-type characteristics. Humic substances, predominantly formed by the breakdown of terrestrially derived organic matter, dominate as a major carbon fraction in freshwater DOM (Dittmar and Kattner, 2003). They may account for over 80 % of dissolved organic carbon (DOC) leaching from agricultural soils, depending on the prevailing hydrological conditions (Waeles et al., 2013). Such humic substances are generally resistant to biodegradation in estuaries and the greatest changes in their quality and degradation only take place once they reach fully marine conditions (Kisand et al., 2013).

Our results are evidence that the qualitative parameters of DOM (molecular weight, spectral absorption and fluorescence properties) can better explain the variation in BGE instead of the quantitative proxies DOC or DON concentrations. There was variation in DOM quantity and quality throughout seasons studied, and bacterial community was still capable of processing the differing DOM with BGE of same level. Thus, we argue that either each bacterial community in different estuaries was adapted to utilize differing substrates in varying conditions with unchanging BGE, or more likely there

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were seasonal changes in the community composition, which did not change the resulting BGE. The latter view is supported by Langenheder et al. (2005), who found that BGE was determined by growth medium and not the adaptive capacity of the original bacterial community.

4.5 Linking DOM quality and bacterial growth efficiency to CO_2 emissions from estuaries

Since BGE is a metric of the efficiency of bacterial community to transfer carbon into biomass available for transfer into the food webs, we extrapolated CO_2 and bacterial biomass fluxes from the experimental data (Table 4). We also calculated fluxes for nitrogen, excluding the gaseous release of nitrogen (denitrification) by assuming a hypothetical nitrogen transfer efficiency of 1, i.e. all utilized nitrogen being converted to bacterial biomass. This approach can be justified by high C : N uptake ratio and low BGE in the experiment units (Table 2, Fig. 7), which suggests that excess carbon was utilized for sufficient energy and nitrogen uptake.

The carbon and nitrogen assimilation rates used in the calculations of parameters in Table 3 are derived from this study, using the daily average carbon and nitrogen degradation rates from the Δ values. Kiiminkijoki had the lowest assimilation rates for both carbon and nitrogen, and Kyrönjoki had the highest. The lower DOC degradation rate in the Kiiminkijoki units compared to the Kyrönjoki units does not necessarily indicate smaller quantity of bioavailable DOC in the whole DOC pool, but rather a slower utilization of it. This is likely to be due to variability in the quality (bioavailability) of DOM and the ability of each bacterial community to process the given DOM.

The average DOC : DON utilization ratio for the whole dataset was 40, which is 6 to 7 times higher than typical C : N ratios in bacterial cells in aquatic environments (Fukuda et al., 1998; Vrede et al., 2002). The inferred C : N ratio of generated bacterial deviates considerably from the DOC : DON uptake ratio, underlining the role of BGE in microbial carbon budgets. The biomass C : N ratios ranged from 3.5 to 15.8, average value being 8.4. For instance, Fukuda et al. (1998) reported bacterial cell C : N ratio of 5.9 ± 1.1 on

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average, and Vrede et al. (2002) estimated a C : N ratio of 6.1 ± 2.6. However, the C : N ratio is a highly variable parameter, e.g. different growth conditions and growth phases affect the ratio, and in particular nitrogen limitation increases C : N ratio (Vrede et al., 2002). When taking into account that a significant portion of carbon is respired as CO_2 (estimated with BGE), the resulting inferred biomass C : N ratio presented in Table 3 is well within the range of previous estimates of C : N ratio of bacterial biomass.

In all of the study estuaries, the higher BGE in sea end-members than in river end-members resulted in a relatively higher proportion of carbon being transferred to biomass than to CO_2 in sea than in river. However, in addition to the carbon dioxide originating from heterotrophic utilization of DOC estimated in this study, the total CO_2 fluxes change significantly during estuarine transport due to e.g. physical changes in mixing zone, POC remineralization and varying primary production (Frankignoulle et al., 1998). In the system with the highest BGE, Kyrönjoki sea end-member, almost equal amounts of carbon are transferred to biomass and CO_2, 132 and 142 $kg\,C\,d^{-1}$ respectively. The high rates of nitrogen assimilation also led to high amount of N transferred to bacterial biomass in Kyrönjoki system compared to other two estuaries. When comparing the CO_2 fluxes from different estuaries, the differences in river discharge affect the volume-specific CO_2 fluxes, ranging from 28 $\mu g\,C\,L^{-1}$ in the Karjaanjoki sea end-member to 60 $\mu g\,L^{-1}$ in the Kyrönjoki river end-member. The relatively low assimilation rates throughout all the systems means that majority of the terrestrial DOC and DON is transported to the Baltic Sea, unaffected by the heterotrophic processes in the estuaries.

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Table 1. Properties of river catchments and average discharges (Q) and total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) loadings during the study period. "Field" includes pasture and cropland, "open" consists of areas with no vegetation and "water" includes lakes, streams etc. Discharge and loading values are from the database of Finnish Environment (HERTTA, 2012).

River	Karjaanjoki	Kyrönjoki	Kiiminkijoki
Catchment area (km^2)	2046	4923	3814
Land use (%)			
Urban	10	5	2
Field	19	25	2
Forest	46	36	40
Peatland	3	19	40
Open	12	13	14
Water	11	1	3
Mean Q ($m^3 s^{-1}$)	15	37	44
TOC loading ($t yr^{-1}$)	4037	25 401	23 490
TN loading ($t yr^{-1}$)	378	2953	801
TP loading ($t yr^{-1}$)	12	91	42

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Table 2. Initial conditions and cumulative changes (Δ) of DOM parameters during the 12–18 day dark incubations: average DOC and DON concentrations, average absorption coefficients at wavelengths 254 and 440 nm and average slope coefficients between 275–295 and 300–650 nm, fluorescence intensities of peaks C and T and weighted averages of apparent molecular weight. Whole data is divided by study estuaries, seasons and water types. "Estuary" includes all seasons and water types, "Season" includes all estuaries and water types, and "Water type" includes all estuaries and seasons. KA = Karjaanjoki, KI = Kiiminkijoki and KY = Kyrönjoki. Significance level of differences between groups (Sig.) as analyzed by ANOVA is also given: [a] < 0.05, [b] < 0.01, [c] < 0.001, n.s. = not significant. Quantitative variables are bolded.

Initial conditions – river end member								
	Estuary				Season			
	KA	KI	KY	Sig.	Spring	Summer	Autumn	Sig.
DOC (μmol L^{-1})	585 ± 51	1113 ± 230	1283 ± 272	[c]	880 ± 219	1021 ± 372	1192 ± 501	[a]
DON (μmol L^{-1})	27.3 ± 3.4	29.4 ± 4.4	100.2 ± 71.5	[c]	67.5 ± 67.7	33.0 ± 5.5	30.8 ± 4.0	n.s.
SUVA$_{254}$ (mg L^{-1} m^{-1})	3.58 ± 0.33	5.41 ± 0.40	4.51 ± 0.85	[c]	4.31 ± 0.90	5.76 ± 0.47	4.91 ± 0.16	[c]
$S_{275-295}$ (μm^{-1})	16.6 ± 0.2	11.9 ± 0.2	13.5 ± 1.2	[c]	14.1 ± 2.0	12.0 ± 0.2	12.4 ± 0.5	[b]
Peak C (R.U.)	0.78 ± 0.12	1.69 ± 0.21	2.17 ± 0.34	[c]	1.49 ± 0.49	1.65 ± 0.70	1.71 ± 0.78	n.s.
AMW$_w$ (Da)	2374 ± 253	2875 ± 239	2609 ± 274	[c]	2657 ± 292	2484 ± 254	2672 ± 435	n.s.
Initial conditions – sea end member								
	Estuary				Season			
	KA	KI	KY	Sig.	Spring	Summer	Autumn	Sig.
DOC (μmol L^{-1})	363 ± 37	393 ± 27	417 ± 67	n.s.	414 ± 54	372 ± 17	363 ± 46	n.s.
DON (μmol L^{-1})	19.3 ± 1.7	15.0 ± 0.5	12.9 ± 3.5	[c]	15.2 ± 4.0	16.3 ± 2.4	18.1 ± 3.5	n.s.
SUVA$_{254}$ (mg L^{-1} m^{-1})	1.87 ± 0.09	3.47 ± 0.27	2.35 ± 0.18	[c]	2.61 ± 0.85	2.42 ± 1.0	2.82 ± 0.56	n.s.
$S_{275-295}$ (μm^{-1})	24.4 ± 0.2	17.3 ± 1.5	20.5 ± 2.1	[b]	19.5 ± 3.9	22.2 ± 2.0	20.2 ± 2.0	n.s.
Peak C (R.U.)	0.22 ± 0.10	0.43 ± 0.06	0.30 ± 0.08	[b]	0.36 ± 0.14	0.26 ± 0.08	0.33 ± 0.11	n.s.
AMW$_w$ (Da)	2000 ± 31	2393 ± 182	2192 ± 137	[c]	2256 ± 224	2055 ± 79	2166 ± 252	n.s.
Δ(%) – all experiment units pooled								
	Estuary				Season			
	KA	KI	KY	Sig.	Spring	Summer	Autumn	Sig.
DOC	−10.6	−7.91	−9.34	[a]	−7.99	−9.72	−2.79	n.s.
DON	−10.0	−5.46	−21.9	[c]	−13.15	−2.08	1.96	n.s.
SUVA$_{254}$ (mg L^{-1} m^{-1})	17.1	5.8	11.1	[b]	12.1	9.3	6.0	n.s.
$S_{275-295}$	−2.55	−0.03	−0.47	[c]	−1.08	1.27	−1.26	[c]
Peak C	13.0	−2.58	−1.02	[a]	−2.36	7.61	5.77	[a]
AMW$_w$	1.85	0.60	0.27	n.s.	0.77	1.61	0.14	n.s.

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Table 3. Average amounts and standard deviations of biologically degradable DOC (BDOC) and DON (BDON), their proportion of the total DOC on DON pool, the degradation rates during the experiments and the DOC : DON ratios of degraded DOM. Average values of each estuary include every season and every water type. Karjaanjoki catchment has mixed land use with most urban areas of the three catchments, Kiiminkijoki catchment is dominated (80 % of total area) by mostly natural forests and peatlands, and Kyrönjoki catchment is most heavily influenced by agriculture (25 % of total area). Significance level of differences between estuaries (Sig.) as analyzed by ANOVA is also given: $^{a} < 0.05$, $^{c} < 0.001$, n.s. = not significant.

	Karjaanjoki	Kiiminkijoki	Kyrönjoki	Sig.	Mean
BDOC ($\mu mol\,L^{-1}$)	55.6 ± 31.7	64.1 ± 41.8	78.8 ± 45.2	n.s.	65.8 ± 40.8
BDOC (%)	10.6 ± 5.55	7.91 ± 5.13	9.34 ± 5.08	^{a}	8.88 ± 5.24
DOC degradation rate ($\mu mol\,L^{-1}\,d^{-1}$)	4.26 ± 2.64	4.09 ± 2.72	7.14 ± 3.22	^{a}	5.32 ± 2.84
BDON ($\mu mol\,L^{-1}$)	2.63 ± 1.45	1.25 ± 0.71	8.18 ± 7.26	^{c}	3.26 ± 4.61
BDON (%)	10.0 ± 5.17	5.46 ± 2.74	21.9 ± 11.1	^{c}	10.5 ± 9.19
DON degradation rate ($\mu mol\,d^{-1}$)	0.23 ± 0.12	0.08 ± 0.04	0.62 ± 0.52	^{c}	0.24 ± 0.33
DOC : DON degradation ratio	24.1 ± 13.5	57.0 ± 40.6	19.4 ± 16.3	^{c}	40.2 ± 35.7

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Table 4. Daily CO_2 fluxes and bacterial biomass generation from DOC and DON, and inferred biomass C : N ratio in river and sea end-members in each study estuary. Average BGE, BCD, DOC and DON concentrations, and C and N assimilation rates are derived from the results of this study. Range given in BGE values originates from different cell carbon content assumptions (30.2 ± 12.3 fg C cell^{-1}; Fukuda et al., 1998). With BCD and DOC values also standard deviation of averaged dataset is given. *Q* data is averaged over the whole study period from SYKE HERTTA database (HERTTA, 2012). Water volume in sea end-member is equivalent to the river end-members daily discharge for enabling direct comparison of these two. Range given in CO_2 flux and bacterial biomass originates from different cell carbon coefficients.

Estuary End member	Karjaanjoki River	 Sea	Kiiminkijoki River	 Sea	Kyrönjoki River	 Sea
BGE	0.38 ± 0.05	0.45 ± 0.05	0.20 ± 0.03	0.31 ± 0.05	0.30 ± 0.04	0.48 ± 0.04
BCD (μmol C d^{-1})	1.96 ± 0.63	2.10 ± 0.63	2.74 ± 0.94	1.40 ± 0.60	3.57 ± 1.13	3.06 ± 4.57
DOC (μmol L^{-1})	586 ± 42	352 ± 64	953 ± 242	401 ± 29	1273 ± 230	411 ± 60
Q (ls^{-1})	15 000	15 000	44 000	44 000	37 000	37 000
C Assimilation rate (μmol L^{-1} d^{-1})	4.26	4.26	4.09	4.09	7.14	7.14
CO_2 flux (kg C d^{-1})	41.1 ± 3.0	36.4 ± 3.0	149.3 ± 4.7	128.7 ± 8.4	191.7 ± 9.6	142.4 ± 9.6
Bacterial biomass (kg C d^{-1})	25.2 ± 3.0	29.8 ± 3.0	37.3 ± 4.7	57.8 ± 8.4	82.2 ± 9.6	131.5 ± 9.6
Refractory DOC (kg C d^{-1})	9050	5410	43 300	18 100	48 600	15 500
DON (μmol L^{-1})	27.3	19.3	29.4	15.0	100.1	12.9
N Assimilation rate (μmol L^{-1} d^{-1})	0.23	0.23	0.08	0.08	0.62	0.62
Bacterial biomass (kg N d^{-1})	3.6	3.6	3.6	3.6	23.8	23.8
Refractory DON (kg N d^{-1})	421	297	1340	682	3820	472
Biomass DOC : DON	7.0	8.3	10.2	15.8	3.5	5.5

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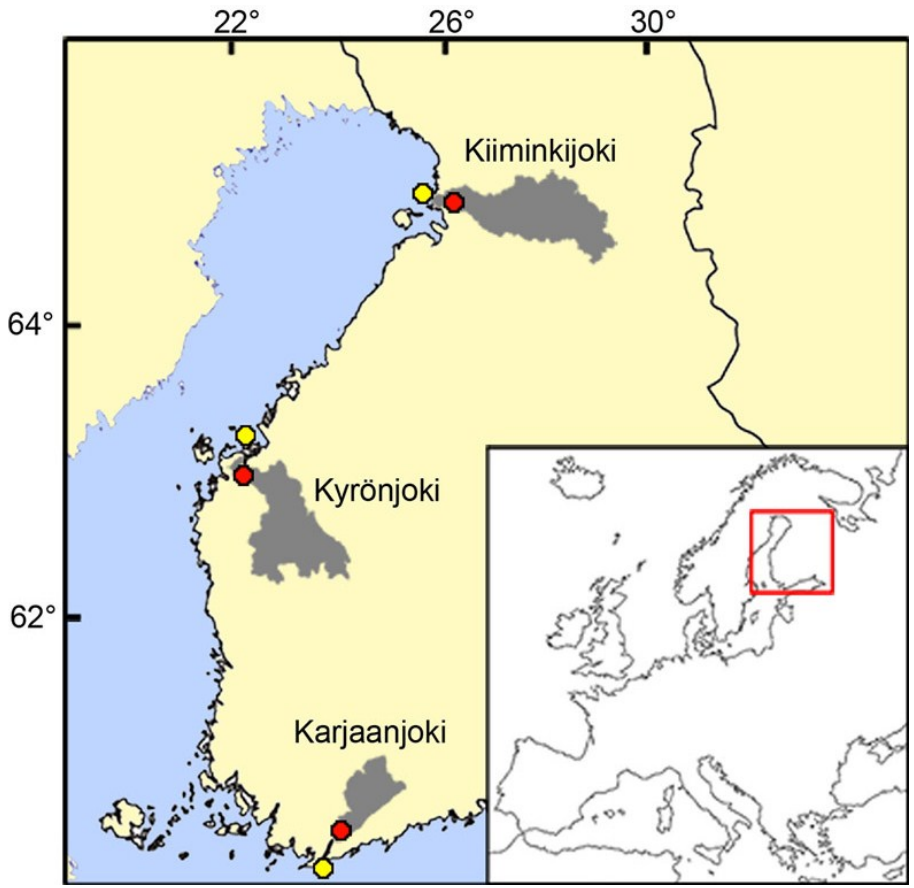

Fig. 1. Map showing the catchment areas (shaded grey) and the sampling locations of study estuaries. Red circles mark the sampling point of each river end-member, and yellow circle the respective sea end-member in Baltic Sea basin.

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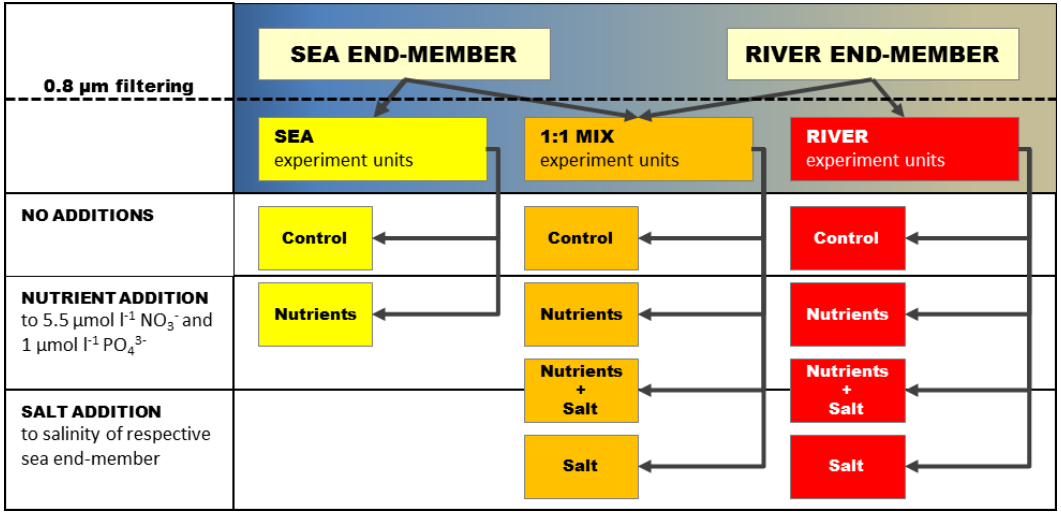

Fig. 2. Schematic representation of experiment design. Experiment following this scheme was repeated on all three estuaries on four different occasions, resulting in 12 individual experiments.

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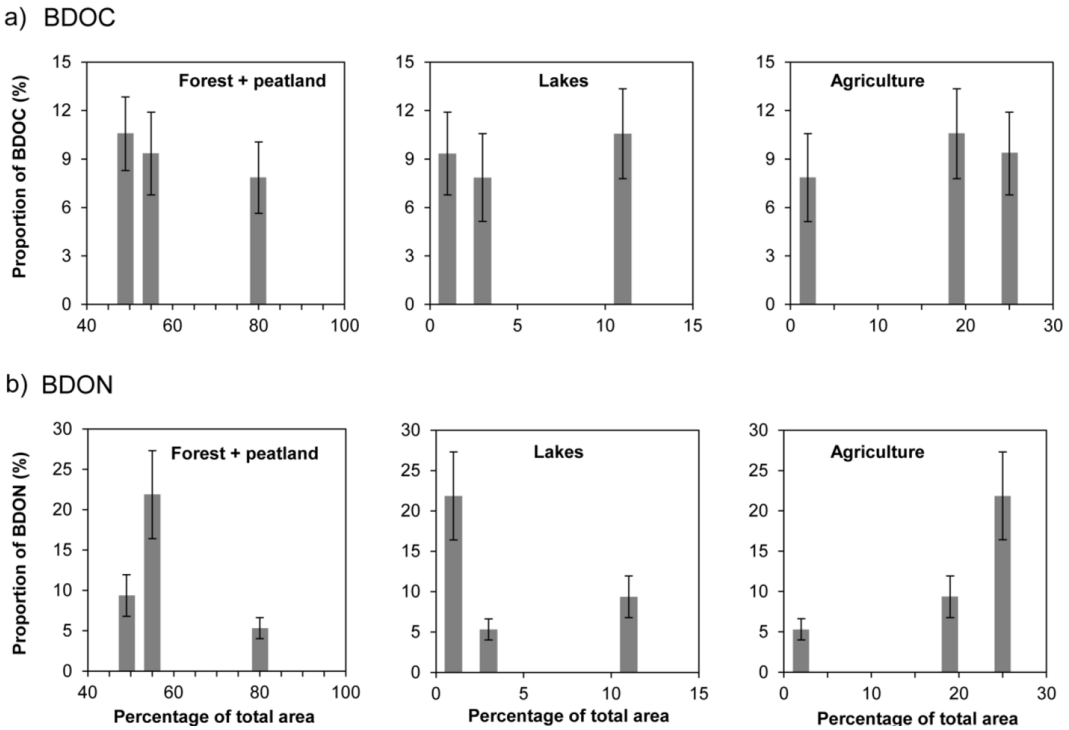

Fig. 3. Relationships between proportional land use categories and biodegradable fractions of DOC and DON of the whole pool (panels **a** and **b**, respectively). Proportion of different land use categories is presented as percentage of total catchment area on x-axis and the biodegradable proportion of DOC and DON is on the y-axis.

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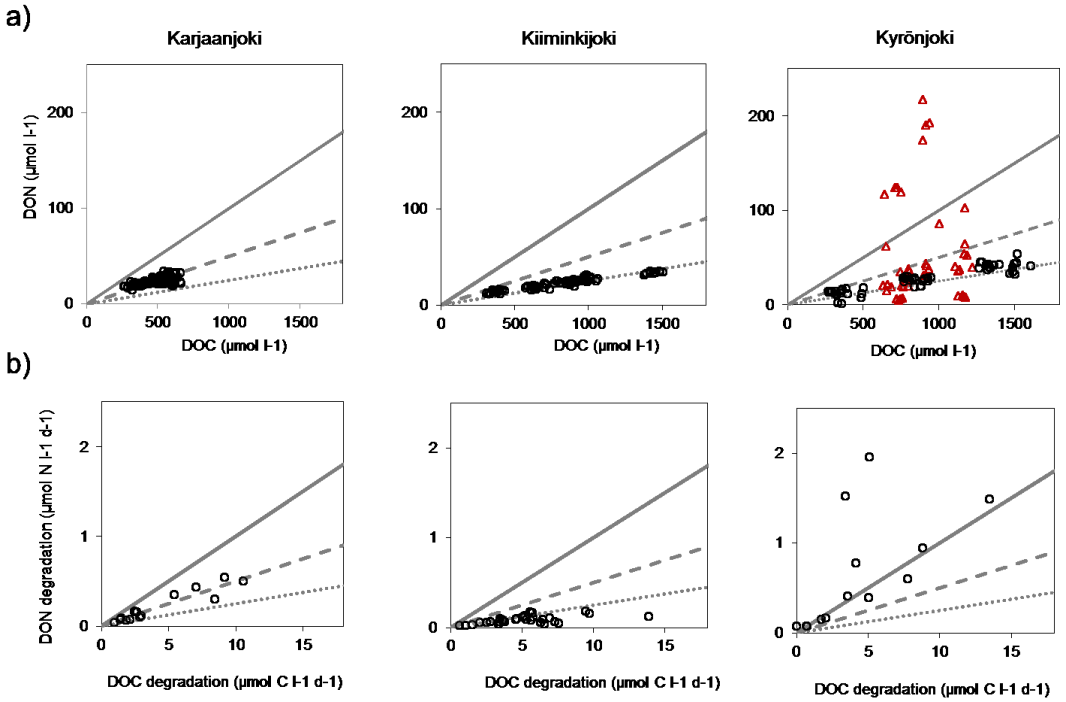

Fig. 4. Relationships between **(a)** DOC and DON concentrations in experiment units, and **(b)** daily degradation rate of DOC and DON in study estuaries. Lines denote fixed C : N relationships: solid line = 10, dashed line = 20 and dotted line = 40. In Kyrönjoki graph **(a)**, the spring values are separated from other seasons as most of them deviate significantly from the average DOC : DON ratio of 40 measured in other seasons in Kyrönjoki. This spring subset is differentiated from the other data with red triangles.

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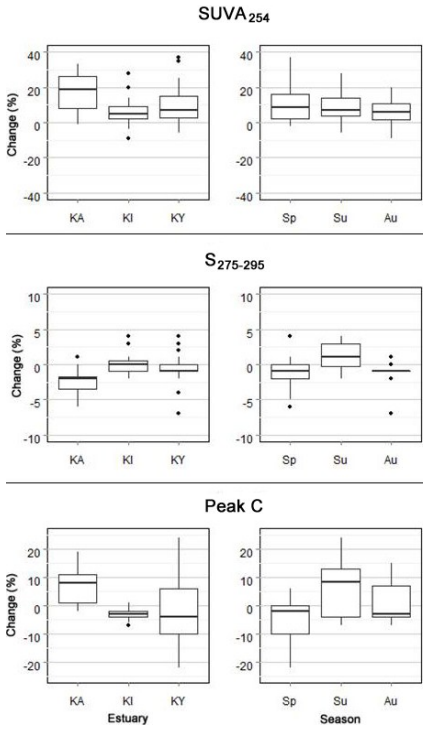

Fig. 5. Boxplots showing Δ values of $SUVA_{254}$, slope coefficient $S_{275–295}$ and fluorescent peak C variables during the degradation experiment. Whiskers indicate the minimum and maximum observations, lower and upper ends of boxes indicate lower and upper quartiles, and thick horizontal line is the mean value of the dataset. Circles denote outliers in the data. Positive values indicate an increase of the variable during the experiment, negative value means loss. On the left side is change between estuaries and on the right side change between seasons. KA = Karjaanjoki, KI = Kiiminkijoki and KY = Kyrönjoki. Au = Autumn, Sp = Spring and Su = Summer.

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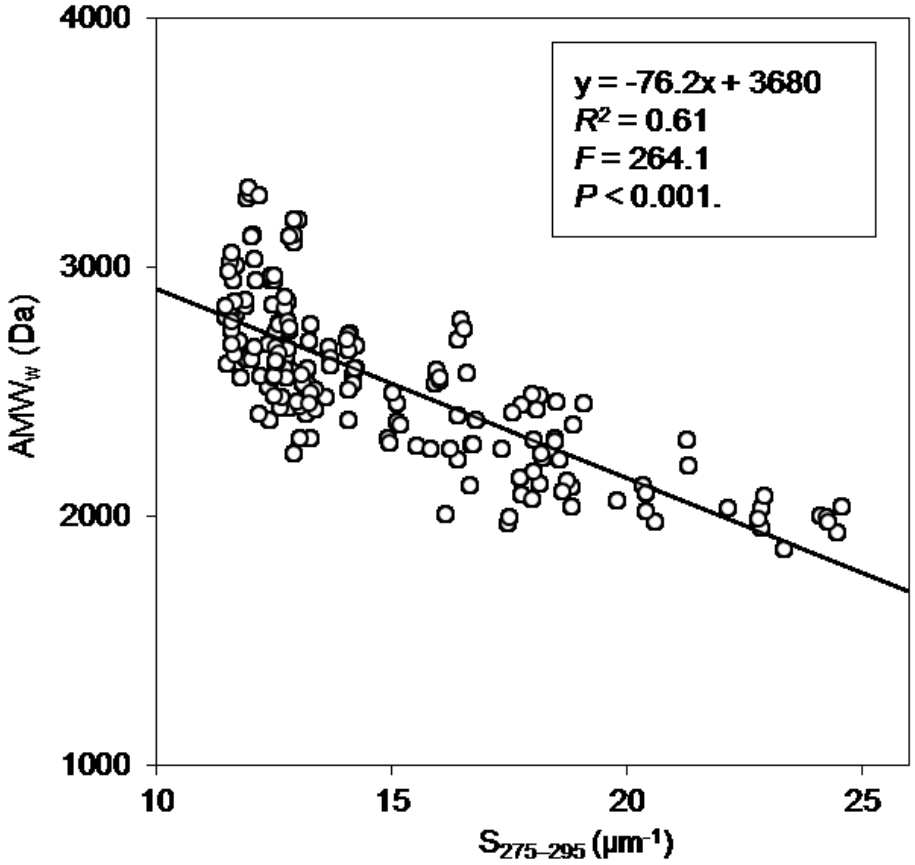

Fig. 6. Regression of weight averaged apparent molecular weight (AMW_w) versus slope coefficient $S_{275–295}$.

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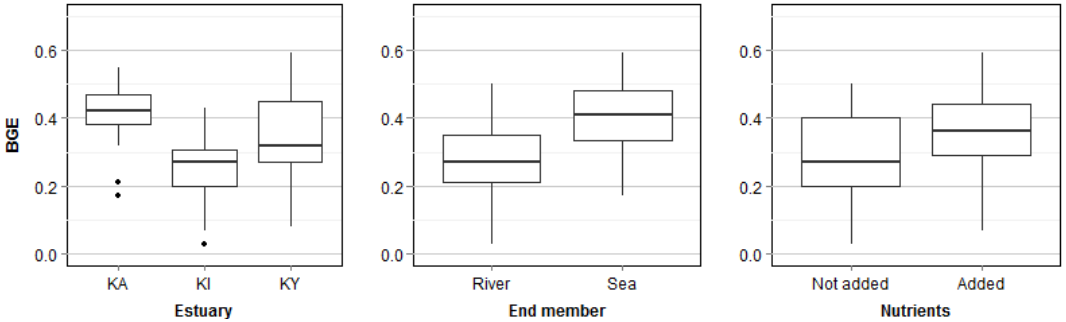

Fig. 7. Boxplots showing variation in bacterial growth efficiencies between experiment units. Whiskers indicate the minimum and maximum observations, lower and upper ends of boxes indicate lower and upper quartiles, and thick horizontal line is the mean value of each dataset. In first panel data is divided by the estuaries: KA = Karjaanjoki, KI = Kiiminkijoki and KY = Kyrönjoki. In second panel the difference between river and sea end members is shown. In third panel the effect of nutrient additions is presented. Differences between estuaries, end members and nutrient manipulations are significant ($P \leq 0.002$).

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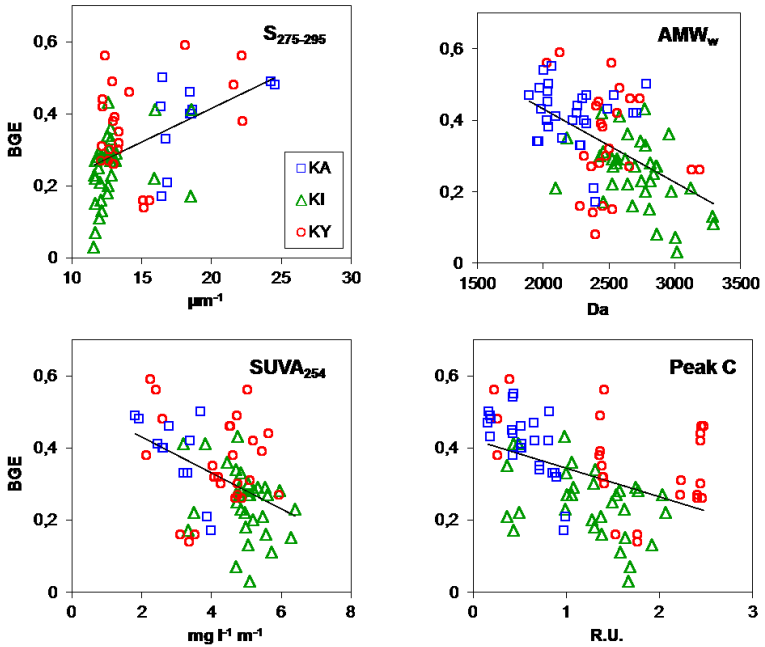

Fig. 8. Relationships between different DOM variables and BGE. AMW_w = apparent weight-averaged molecular weight, $S_{275–295}$ = slope coefficient between wavelengths 275 and 295 nm, $SUVA_{254}$ = carbon specific absorbance at 254 nm and Peak C = humic-like fluorescence peak C. Linear regression for the best-fit line describing relationships between slope BGE and AMW_w: $y = -2.1 \times 10^{-4}x + 0.84$, $R^2 = 0.27$, $F = 33.41$, $S_{275–295}$: $y = 0.018x + 0.43$, $R^2 = 0.23$, $F = 20.59$, $SUVA_{254}$: $y = -0.050x + 0.53$, $R^2 = 0.19$, $F = 17.06$, and Peak C: $y = -0.079x + 0.42$, $R^2 = 0.18$, $F = 19.09$. All F values are significant to $P < 0.001$. Subsets indicate different estuaries: KA = Karjaanjoki, KI = Kiiminkijoki and KY = Kyrönjoki.

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