Aquatic Sciences

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Methane distribution and methane oxidation in the water column of the Elbe estuary, Germany

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Abstract The River Elbe, as one of the major waterways of central Europe, is a potential source of high amounts of methane into the North Sea. Twelve sampling cruises from October 2010 until June 2013 were conducted from Hamburg towards the mouth of the Elbe at Cuxhaven. The dynamic of methane concentrations in the water column and its consumption via methane oxidizing bacteria was measured. In addition, physico-chemical parameters were used to estimate their influence on the methanotrophic activity. We observed high methane concentrations at the stations in the area of Hamburg harbour ("upper estuary") and about 10 times lower concentrations in the lower estuary (median of 416 versus 40 nmol L^{-1}, respectively). The methane oxidation rate mirrored the methane distribution with high values in the upper estuary and low values in the lower estuary (median of 161 versus 10 nmol L^{-1} day^{-1}, respectively). Methane concentrations were significantly influenced by the river hydrology (falling water level) and the biological oxygen demand while interestingly, no clear relation to the amount of suspended particulate matter (SPM) was found. Methane oxidation rates were significantly influenced by methane concentration and to a lesser extent by temperature. Methane oxidation accounted for 41 ± 12 % of the total loss of methane in summer/fall periods, but for only 5 ± 3 % of the total loss in the winter/spring periods (total loss = methane oxidation + diffusion into the atmosphere). The average sea-air flux of methane was 33 ± 8 g CH_4 m^{-2} y^{-1}. We applied a box model taking into account the residence times of each water parcel depending on discharge and tidal impact. We observed almost stable methane concentrations in the lower estuary, despite a strong loss of methane through diffusion and oxidation. Thus we postulate that losses in the lower Elbe estuary were balanced by additional inputs of methane, possibly from extensive salt marshes near the river mouth.

Keywords Estuary · Methane · Methane budget · Methane oxidation · River Elbe

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Introduction

Estuaries are highly dynamic ecosystems where freshwater meets marine water and diverse biological, physical and chemical parameters constantly change, which makes estuaries to hot-spots of biological production and biodiversity (Bianchi 2007). Due to the physical and chemical mechanisms present in brackish water (e.g. long residence time, low salinity) turbidity zones are produced with high concentrations of suspended particulate matter (SPM). In such zones riverine phytoplankton becomes limited by light and dies, which results in a considerable enhancement of the easily degradable organic matter available to microbes (Cole et al. 1992). This material is subsequently

decomposed and re-mineralized by heterotrophic bacteria resulting in elevated levels of carbon dioxide (CO_2) and methane (CH_4) emissions (Frankignoulle et al. 1998; Borges and Abril 2011), being the two most abundant carbon-containing greenhouse gasses in the atmosphere with a considerable impact on global warming. Estuaries present an interface between rivers and oceans, where rivers act as major sources of CH_4 to the atmosphere (Borges et al. 2015a). The role of the ocean as atmospheric methane source (including geological sources) is estimated to be 16 % of natural sources, which is the same range as freshwater (including lakes and rivers) with 12 % of the natural sources (IPCC 2013).

On the contrary to methane production, microbial CH_4 oxidation via specialized microbes, so called methanotrophs (MOB, methane-oxidizing bacteria) represents a biological sink of methane in aquatic environments. Methanotrophy can significantly reduce the amount of CH_4 escaping from the sediments and subsequently from the water column into the atmosphere (Kankaala et al. 2006; Conrad 2009; Bastviken 2009). Methanotrophs are physiologically specialized groups of methylotrophic prokaryotes capable of utilizing single carbon compounds (methane, methanol and few strains can utilize methylamine and a narrow selection of other C1 compounds; Bowman 2006) as an electron donor and source of cell carbon. Aerobic methanotrophs are widely distributed in nature, typically at anoxic–oxic interfaces (Bowman 2006). They can make an important contribution to the biomass entering the food web and can consume up to 50 % of CH_4 diffusing from the sediments in some estuaries (de Angelis and Scranton 1993; Abril and Iversen 2002). However, the conditions influencing the methanotrophic activity in the water column are still largely unknown, making it even more difficult to uncover the major driving forces of the activity of MOB in such a variable ecosystem as an estuary. For instance, temperature may affect enzymatic activity and temperature variations may also lead to changes in the structure of the methanotrophic community (Sundh et al. 2005; Mohanty et al. 2007). It seems likely that methane oxidation (MOx) is mainly controlled by physical processes, i.e. gas-phase diffusion and transport of CH_4 to the cells, rather than by enzymatic reactions (Urmann et al. 2009). Besides CH_4, the limitation of other simple organic substrates and nutrients may also affect methanotrophic activity (Rudd et al. 1976; Bender and Conrad 1994; Bodelier et al. 2000). King (1990) showed that light strongly controls MOx via photosynthesis by the extension of the oxic zone suitable for methanotrophic activity. On the contrary, the investigations made by Dumestre et al. (1999) in Petit Saut Reservoir and later by Murase and Sugimoto (2005) at mesotrophic Lake Biwa suggested that methane oxidation can be inhibited by visible light. Especially in estuarine habitats, two parameters may strongly affect the MOx—salinity and suspended particulate matter (SPM) content. Whereas salinity has an inhibitory effect on MOx (de Angelis and Scranton 1993; Conrad et al. 1995), SPM shows a positive influence on MOx rate. The MOB may settle on organically rich particles (Zimmermann 1997, 2002) and thus exploit the micropatchiness with locally enhanced CH_4 concentrations to become available for MOB (Abril et al. 2007).

In an effort to clarify the relationship between MOB and their environment, the aims of this study were: (i) to examine the distribution of CH_4 in the water column of the River Elbe estuary, (ii) to assess the activity of MOB as well as major physical and chemical parameters influencing their activities and (iii) to attempt to budget the different methane related processes along a large estuarine ecosystem.

Materials and methods

Study site

The River Elbe rises at an elevation of 1386 m above sea level in the Krkonoše (Giant Mountains) in the northeast of the Czech Republic, flows through the central part of the Czech Republic and central and northern Germany before discharging into the North Sea at Cuxhaven (Fig. 1). The Elbe estuary is a eutrophic ecosystem receiving urban, agricultural and industrial waste. It is an intermediate-turbid, well-mixed estuary with a pronounced estuarine turbidity maximum (ETM; approximately from #659 to #719, see Fig. 1) in the brackish zone of the estuary; note that these station codes are derived from the Elbe-km, which refer to the distance from the Czech-German border (=0 river-km; IKSE 1995). The range of semi-diurnal tides rise from 2 m (at the Geesthacht weir) towards the port of Hamburg where it attains its maximum of 3.5 m. High tidal current velocities (up to 1.8 m s^{-1}; Bergemann and Gaumert 2010) cause a steep horizontal salinity gradient, particularly near Brunsbüttel (#690). The residence time of water varies from 15 to 30 days (Frankignoulle and Middelburg 2002). Based on the different biogeochemical processes and oxygen demands the Elbe estuary can be divided into two sections (according to Amann et al. 2012): (1) the pre-oxygen minimum zone (pre-OMZ; approximately from #585 to #624), and (2) the oxygen minimum zone (OMZ; approximately from #624 to #659), both fall into the tidal freshwater area. The Elbe estuary is a unique, well monitored system with data on physical and chemical parameter available from www.portal-tideelbe.de and www.fgg-elbe.de. Our sampling stations started in the port of Hamburg at station #619 and were spaced at intervals of
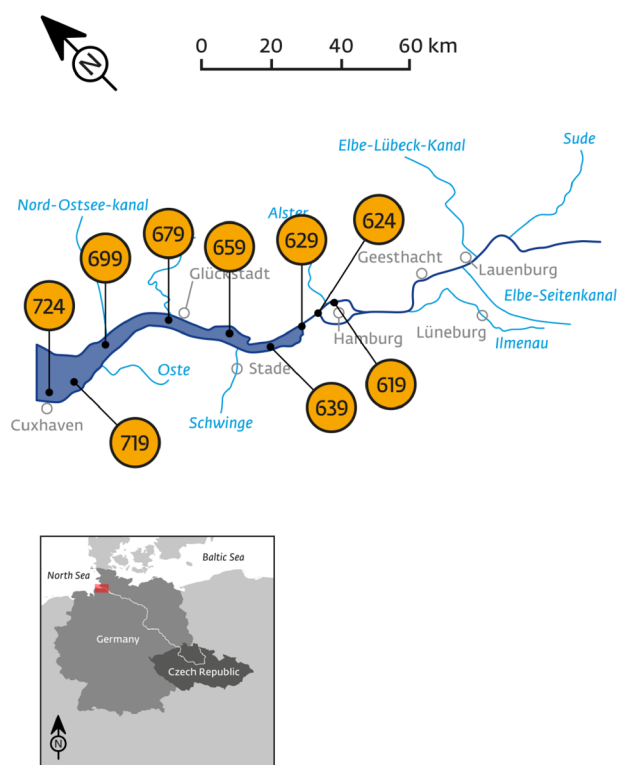
(A)

Station code	Latitude	Longitude
#619 (n=13)	53°31,92 N	10°1,53 E
#624 (n=7)	53°32,59 N	9°57,25 E
#629 (n=10)	53°32,56 N	9°52,77 E
#639 (n=16)	53°33,72 N	9°43,93 E
#659 (n=18)	53°39,99 N	9°30,76 E
#679 (n=18)	53°49,07 N	9°21,50 E
#699 (n=18)	53°52,51 N	9°5,54 E
#719 (n=24)	53°51,10 N	8°47,76 E
#724 (n=20)	53°52,55 N	8°43,94 E

(B)

	Range	Length [km]	Depth [m]	Area [km^2]	Volume [km^3]	Ratio (A / V)
Box 1	#619 - #629	10	8 - 10	3	0.04	97
Box 2	#629 - #639	10	10 - 15	5	0.05	100
Box 3	#639 - #659	20	12 - 15	16	0.13	125
Box 4	#659 - #679	20	12 - 15	25	0.19	134
Box 5	#679 - #699	20	11 - 16	35	0.27	131
Box 6	#699 - #719	20	9 - 16	43	0.34	128
Box 7	#719 - #724	5	9 - 23	23	0.11	204

Fig. 1 Overview of sampling sites in the Elbe River estuary. The station codes are derived from the river-kilometres, which refer to the distance from the Czech-German border (=0 river-km; IKSE 1995). Station coordinates are given in table A. Parameters of used box-model are given in table B Original map source: https://commons. wikimedia.org/wiki/File:Lauf_der_Elbe.png, modified

10 and 20 km, terminated at Cuxhaven with #724 (Fig. 1). For the purpose of this study, the sampling sites were divided "upper estuary" and the "lower estuary": the upper estuary includes sampling sites #619 and #629; the lower estuary part covers the sampling sites from #659 till #724; site #639 was designed as the "transition zone".

Sampling

Twelve sampling campaigns were performed from October 2010 till June 2013. All data are available in the Pangaea data base (www.pangaea.de; Bussmann et al. 2014, Matousu et al. 2015). The water samples were collected in the main channel of the Elbe from the research vessel "Ludwig Prandtl" (Helmholtz-Zentrum Geesthacht, Germany) and were taken from two different depths of the water column: approximately 1 m above the river bed ("bottom") and approximately 1 m below the water surface ("surface"). Samples for CH_4 concentration and CH_4 oxidation rate were transferred directly from a water sampler (Uwitec, Austria) into serum bottles (120 ml). Bottles were overfilled with approximately two volumes of water, capped with black butyl stoppers (Ochs, Germany), and sealed with an aluminum crimp. Care was taken to exclude air bubbles from capped samples. An additional water sample was taken for analyses of other water properties.

Analytical methods

The duplicate methane concentration samples were killed immediately, by injecting 0.3 ml of 5 M NaOH through the septum into the serum bottle filled with water to stop microbial activity. The samples were analyzed within 1 week in the laboratory by using a headspace technique according to McAuliffe (1971): a 20 ml headspace was created by adding N_2 through a syringe causing displacement of 20 ml of water through another needle in the stopper. Afterwards the samples were vigorously shaken and then stored for 24 h at room temperature to allow equilibration of the gases in the headspace. Subsequently the samples were analyzed with a gas chromatograph equipped with a flame ionization detector (GC 2014 Shimadzu Corp). Calibration was performed with 10 ppm and 100 ppm methane standards (Air Liquide). Precision of the analyses was 2 %; the r^2 of the calibration curve >97 %. Dissolved CH_4 concentrations were calculated with the solubility coefficients of Yamamoto et al. (1976).

The CH_4 oxidation rates were determined as outlined in Bussmann et al. (2015). Water samples were processed in triplicates with one "killed control". Immediately after collecting the samples, 100 μl (10 μCi) of ^3H-CH_4 (American Radiolabeled Chemicals, Inc.) was injected into each sample through the septum. Control samples were killed by injecting 200 μl of 5 M NaOH before the tracer

was added. The samples were vigorously shaken for 60 s and incubated in the dark at near in situ temperature. Activities in "live" samples were stopped in the same way as "killed controls", but after approximately 24 h of incubation. Samples were stored in the dark at 4 °C, prior to being analyzed (within 1 week).

In the laboratory, the samples were opened, 2 ml aliquot of each sample was mixed with 5 ml of scintillation cocktail (Ultima Gold^{TM} LLT) and analyzed with a liquid scintillation counter (Tri-Carb® 2910 TR, Perkin Elmer) for estimation of the total radioactivity of the sample, i.e. the labeled CH_4 and labeled produced H_2O (see Eq. 1). Subsequently the samples were sparged with air for 30 min to expel all remaining labeled CH_4. Afterwards, again 2 ml aliquot of each sample was mixed with 5 ml of scintillation cocktail and analyzed with a liquid scintillation counter for estimation of the the microbially produced radioactive water.

The principle of the calculation of the microbial mediated MOx is based on the transformation of added radioactively labeled tracer (^3H-CH_4) into the oxidation product (^3H-H_2O) during incubation:

$$^*CH_4 + 2O_2 \rightarrow CO_2 + 2\,^*H_2O \tag{1}$$
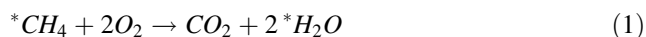
In order to calculate the methane oxidation rate (RO_x), first the fraction of methane that was turned over is calculated:

$$F_{[CH_4]} = \frac{[H_2O]_{produced}}{[CH_4]_{injected}} \tag{2}$$

The fractional turnover rate constant (k) is then determined by dividing it by the incubation time (t):

$$k = \frac{F_{[CH_4]}}{t} \tag{3}$$

Finally the oxidation rate (MO_x) is obtained by multiplication with the in situ methane concentration:

$$MO_x = k \times [CH_4] \tag{4}$$

The inverse of the fractional turnover rate (k) is the turnover time (τ):

$$\tau = \frac{1}{k} \tag{5}$$

The turnover time is the time which it would take to oxidize the ambient amount of CH_4 dissolved in water with a given methane oxidation rate. The turnover time rate is an effective measure of the methanotrophic potential (Heintz et al. 2012).

The "killed controls" served for estimation of abiotically formed radioactive H_2O, which could lead to an overestimation and these (on average about 3.5 % of total transformation) were subtracted.

During the sampling campaigns along the Elbe River temperature, salinity, and oxygen in the water were measured right after sampling using a Universal Pocket Meter (Multi 340i) with a precision of 0.01 for salinity, 0.1 °C for temperature, and 0.5 % for oxygen.

Depending on the load of suspended particulate matter (SPM) 250 ml of water were filtered through prewashed (with MQ water) and pre-weighed Whatman GF/C filters and dried for 24 h at 60 °C, and weighed for determination of the total SPM content.

For nutrient analysis, water samples were filtered through GF/C filters (Whatman^{TM}) immediately after collection. Afterwards the filtrates were transferred into 50 ml Falcon tubes and frozen until further analysis in the laboratory. Samples were analyzed for phosphate, silicate, ammonia, nitrate, and nitrite with an autoanalyzer (AA3 from Seal-Analytical, Germany) and using standardized techniques as detailed in Wiltshire et al. (2010), following techniques described by Grasshoff et al. (1983). The error is estimated to be 10 %.

Additional data for the water level (Ganglinie) were obtained from the "Wasser- und Schifffahrtsverwaltung des Bundes", given through the Bundesanstalt für Gewässerkunde (BfG). We assigned the data from our stations to their locations according to the following: #619 with #623 (St. Pauli), #659 with #660 (Grauerort), #679 with #674 (Glückstadt) and #724 with #724 (Cuxhaven Steubenhöft). We took the original water level of the sampling time and calculated the difference from −10 to +10 min from sampling time and defined this parameter as "delta water level".

Data for the biological oxygen demand in 7 days (BOD-7, in mg O_2 L^{-1}) were obtained from the Elbe data portal (http://www.elbe-datenportal.de). The stations were allocated within 6 km distance to our stations and within 3 days range of our sampling date. Data ranged from 1 to 5.7 mg L^{-1}, n = 24.

Data for the discharge (m^3 s^{-1}) were also obtained from the Elbe data portal (http://www.elbe-datenportal.de). These data were measured at "Pegel Neu Darchau". Data were only allocated to the stations of the upper estuary (#619 and #624), because for the following stations we assume an increasing tidal input. As the water takes about 1 day from Neu Darchau to reach Hamburg, we also took the data from 1 day earlier than the sampling date.

Statistical analysis

To determine the possible dependence of CH_4 concentration and CH_4 oxidation rate on measured physical (water depth, water temperature) and chemical factors (salinity, NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, SiO_4, O_2, SPM) Spearman rank order correlation analyses were performed. Calculations and graphical representation were performed using SigmaPlot for Windows Version 11.0 software. One way

Fig. 2 *Box plots* of the methane concentrations (*grey boxes*) and oxidation rates (*white boxes*) at the river stations in the Elbe estuary during the sampling campaigns from October 2010 to June 2013. We grouped the stations into upper (#619–#629) and lower estuary (#659–#724), with the station #639 as a transition zone. The number of samples ranged from n = 7 at station #624 to n = 24 at station #724. For definition of the *box plot* see "Material and methods"
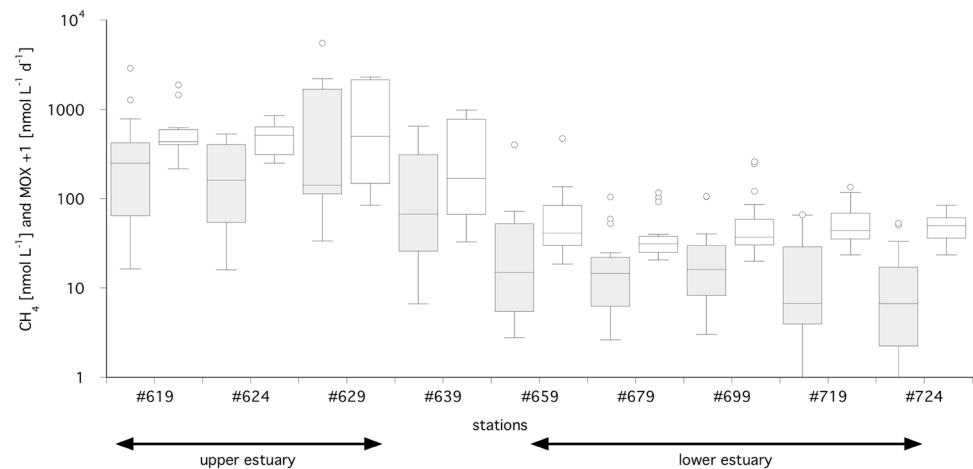
ANOVA, linear correlation after log transformation and Wilcoxon test were performed with Kaleidagraph Version 4.1.3. Redundancy analyses (RDA) were performed using CANOCO program Version 4.5 (Ter Braak and Šmilauer 2002). Environmental parameters were selected using forward selection with 999 Monte Carlo permutations. The results of the RDA were visualized by CanoDraw for Windows. The box plots (Fig. 2) show the median (middle line), the lower and upper quartile (bottom and top of the box) with 25 and 75 % of the data, the minimum and maximum of the data, being the lines extending from the bottom and top of the box. The dots are outliers, whose values are either greater/smaller than 1.5 * upper or lower quartile (Kaleidagraph Version 4.1.3).

Calculations of the sea-air fluxes and the methane budget

To reduce interferences with tidal currents we selected four cruises when the water level was falling during sampling time, i.e. the overall water flow and the ship movement was towards the sea. These cruises were 3. 8. 2011, 12. 4. 2013 and 12. 6. 2013 for stations #619–#679; and 1. 8. 2012 for stations #659–#724. For flux calculation and budgeting we defined boxes between the different stations. With the maps provided by "Wasser- und Schifffahrtsverwaltung des Bundes", given through www.portal-tideelbe.de, we estimated the length and width of the river at each station. The depth of the "navigational channel" was set as 15 m and for the "deep water zone" ranging from 10 to 2 m, an average depth of 6 m was assumed. The boxes were split into different triangles and squares and the respective areas and volumes were calculated (Fig. 1; Supplementary material 1).

Gas exchange across an air–water interface can be described in general by the following function (Wanninkhof et al. 2009):

$$F = k \times (c_m - c_e) \tag{6}$$

where F is the rate of gas flux per unit area, c_m is the methane concentration measured in surface water and c_e is the atmospheric gas equilibrium concentration based on Wiesenburg and Guinasso (1979).

The gas exchange coefficient (k) is a function of water surface agitation. However, in oceans and estuaries k is more determined by wind speed, while in rivers water velocity dominates (Alin et al. 2011). The determination of k is very important for the calculation of the sea–air flux. We decided to calculate k_{600} in the Elbe according to the empirical Eq. 2 from Raymond et al. (2012):

$$k_{600} = 4725 \times (V \times S)^{0.86} \times Q^{-0.14} \times D^{0.66} \tag{7}$$

with V as stream velocity (m s^{-1}), S as slope, Q as discharge (m^3 s^{-1}), and depth (m). Data on stream discharge were obtained from "Pegel Neu Darchau", data on stream velocity were provided by www.portal-tideelbe.de, for the respective stations and dates. The slope of the Elbe estuary at its delta was 0.00005 (tidal range of 5 cm/km for the upper estuary; Vandenbruwaene et al. 2013). For the two lower stations (#719 and #724) we took the empirical model from Borges et al. (2004), which is only wind driven. Wind data were obtained for Cuxhaven from the German Weather Service. Further details are given in Supplementary material 2. The calculated k_{600} (value for CO_2 at 20 °C) was converted to k_{CH4} according to (Striegl et al. 2012):

$$\frac{k_{CH_4}}{k_{600}} = \left(\frac{Sc_{CH_4}}{Sc_{CO_2}}\right)^{0.69} \tag{8}$$

where Schmidt numbers (Sc) are determined by water temperature and salinity, according to (Wanninkhof 1992).

For each station with its "navigational channel" and its "deep water zone", k_{CH4} was determined as described above. The atmospheric equilibrium concentration and difference to the measured methane concentrations were

calculated. With Eq. 6 we calculated the respective sea–air fluxes (mol m^2 day^{-1}). The average of this flux between two neighboring stations was then multiplied with the respective area of the box. Then the sea–air flux for the "navigational channel" and the flux for the "deep water zone" were added, resulting in the total sea–air flux for each box in mol day^{-1}.

For calculation of the total MOx rate, we took the average MOx between two neighboring stations and multiplied it by the total volume of the respective box ($V_{\text{navigational channel}} + V_{\text{deep water}}$). This resulted in the total MOx rate of each box in mol day^{-1}.

Results

Physico-chemical parameters of water

The physical parameters of the water samples are shown in Table 1. The values of the chemical parameters fluctuated considerably (Table 1). Phosphate had a clear seasonal trend with the highest values being in winter at #699 and #719. Oxygen also varied seasonally, with the lowest values being in summer. Other nutrients showed no clear seasonal or spatial pattern, in contrast to SPM with the highest values being in spring at #679 and #699 (i.e., in the estuarine turbidity maximum zone). Salinity increased towards the river mouth with 2.2 ± 1.6 at #699 up to 12.4 ± 6.2 at #724, no clear seasonal pattern was, however, evident.

Distribution of methane in the water column

We observed rather high methane concentrations in the upper estuary (#619–#629; median 416 nmol L^{-1}). At stations #659–#724 CH$_4$ concentrations were about 10 times lower with a median of 40 nmol L^{-1} (Fig. 2). According to this pattern, we grouped our stations into the "upper" and "lower" estuary (one way ANOVA with df = 81, p < 0.001, excluding station #639 as a transition zone). This grouping was also valid for most of the nutrients monitored. The site with the highest CH$_4$ concentration for each cruise moved up- or downstream between #619 and #639. Linear regression analysis (Table 2) and RDA (Fig. 3) revealed no seasonal trend for methane (no CH$_4$—temperature correlation), for either all stations or for the upper or lower estuary.

In the lower estuary, where salinity increases from 5 to 25 PSU towards to the North Sea, CH$_4$ concentrations remain stable and only a slightly negative yet insignificant influence of salinity (dilution) was detected (Fig. 3d), but with no significant correlation.

We found a clear negative correlation between SPM and CH$_4$ for all stations ($r^2 = 0.36$, for log transformed data),

Table 1 Overview of collected data on physico-chemical parameters in the water column of the River Elbe estuary during all sampling campaigns from October 2010 to June 2013: median and the range of the data (in brackets) are given

Station code	Salinity [PSU]	Oxygen [mg L^{-1}]	Temperature [°C]	SiO$_4$ [μmol L^{-1}]	PO$_4^{3-}$ [μmol L^{-1}]	NO$_2^-$ [μmol L^{-1}]	NO$_3^-$ [μmol L^{-1}]	NH$_4^+$ [μmol L^{-1}]
#619	0.4 ± 0.2	8.1 ± 1.5	14.6 ± 5.2	133.6 (0.0–177.0)	1.5 (0.1–4.8)	1.4 (0.6–2.4)	278.2 (196.5–1451.2)	4.9 (3.6–20.3)
#624	0.3 ± 0.2	9. ± 1.4	15.3 ± 5.7	135.2 (129.5–213.3)	1.3 (1.0–5.9)	2.5 (2.0–2.8)	230.2 (216.4–289.9)	9.6 (8.3–11.4)
#629	0.3 ± 0.2	6.9 ± 2.4	12.7 ± 4.7	133.8 (0.0–135.2)	1.5 (0.3–3.7)	1.3 (0.6–2.6)	290.0 (119.3–1386.7)	5.7 (4.2–12.6)
#639	0. ± 0.2	7.6 ± 2.2	13.9 ± 5.4	130.1 (0.0–175.9)	1.5 (1.2–6.2)	1.5 (0.2–2.9)	273.0 (64.7–1490.1)	4.6 (1.3–12.9)
#659	0.5 ± 0.2	7.7 ± 2.0	14.1 ± 4.8	113.1 (1.7–172.5)	1.4 (0.6–6.5)	1.3 (0.1–2.5)	253.1 (136.1–1523.7)	3.2 (1.5–15.1)
#679	0.7 ± 0.5	8.0 ± 1.7	14.2 ± 4.9	118.3 (12.0–169.8)	1.6 (0.8–8.4)	0.3 (0.0–2.7)	253.0 (116.4–1510.0)	2.9 (0.5–15.7)
#699	2.6 ± 2.1	8.3 ± 1.6	14.2 ± 4.7	116.3 (20.8–184.9)	2.5 (1.6–11.9)	0.4 (0.1–1.6)	237.8 (104.4–1618.6)	3.7 (0.9–9.5)
#719	9.9 ± 6.0	8.2 ± 1.5	13.8 ± 4.8	86.2 (17.1–168.5)	2.3 (1.4–14.1)	0.9 (0.4–2.6)	268.2 (40.2–1659.2)	5.5 (2.8–10.7)
#724	12.2 ± 6.5	8. ± 1.8	12.8 ± 4.8	81.0 (26.4–116.4)	1.7 (1.2–2.6)	1.1 (0.5–2.6)	371.0 (60.1–1659.0)	6.8 (2.8–9.6)

Table 2 Linear correlations between methane concentration/methane oxidation rate/methane turnover time and environmental factors, with log transformed data

	Methane concentration		Methane oxidation rate		Methane turnover time	
	Upper estuary $n = 30$	Lower estuary $n = 98$	Upper estuary $n = 30$	Lower estuary $n = 95$	Upper estuary $n = 30$	Lower estuary $n = 95$
Temperature			0.18/0.06	0.13/0.001	−0.47*/<0.001	−0.11/0.002
Salinity				−0.10/0.001		0.16/<0.001
Oxygen					0.28/0.009	
SPM (all stations)	all stations: −0.36/<0.001					
Delta water level	−0.86**/<0.001 (n = 10)					
BOD7	0.80/0.02 (n = 6) all stations (n = 24): 0.29/0.007		all stations (n = 24): 0.31/0.005			
PO_4^{3-}						
NO_2^-	−0.27/0.01					
NO_3^-						
NH_4^+	0.15/0.08				−0.18/0.07	
CH_4			0.55/<0.001	0.49/<0.001		

Shown are the regression coefficients (r^2), positive or negative signs indicate a positive or negative correlation, followed by the respective p value (r^2/p). Notes: * three very high values were omitted; ** two very high values were omitted; when not indicated otherwise the number of sampling occasions (n) ranged from 19 to 24

indicating high methane concentrations at low SPM, i.e. clear water. (Table 2, Fig. 3a). However, when splitting the data into upper and lower estuary, no significant correlation could be found. Using RDA analyses we found a relevant negative correlation only in the case of the transition zone (Fig. 4c), but a slight indication for all stations and even a positive in the upper estuary (Fig. 3b).

The RDA analyses indicated a slight influence of NO_3^- and SiO_4 on the methane dynamics in the upper estuary (Fig. 3b). In the lower estuary, the RDA analysis showed only a minor influence of NO_2^- and water temperature (Fig. 3d). A similar pattern was also evident for the NH_4^+ concentration and we even found a positive correlation between CH_4 and NH_4^+ for the upper estuary (log transformed data, $r^2 = 0.15$), but no correlation for the lower estuary. In the upper estuary, methane concentrations were negatively correlated with nitrite concentrations ($r^2 = 0.27$), but not in the lower estuary.

The water discharge of a river is an important factor in its chemistry. The obtained discharges (see "Materials and methods") can be considered as the total freshwater discharge of the Elbe since the tributary discharges are negligible compared to the discharge of the main channel (factor 100) (Vandenbruwaene et al. 2013). However, no direct correlation between CH_4 concentration and water discharge was detected in the upper estuary.

From the Elbe Daten Portal we obtained data on the BOD-7 and the water level for the respective stations and sampling times. However, these data were not available for all our sampling occasions. When the overall biodegradable material (i.e. BOD-7) in the water was high, we also observed high methane concentrations in the whole estuary ($r^2 = 0.29$, n = 24) and particularly pronounced in the upper estuary ($r^2 = 0.80$, n = 6) but not in the lower estuary. We calculated the difference between the water level 10 min before and 10 min after sampling to get an estimate of tidal influence. We found that when the water level was dropping the higher CH_4 concentrations were observed ($r^2 = 0.86$, n = 10) in the upper estuary, but no correlation was found in the lower estuary.

Methane oxidation and turnover time in the water column

Methane oxidation rates were rather high in the upper estuary (#619–#629) with a median of 161 nmol L^{-1} day^{-1}. In the lower estuary (#659–#724) methane oxidation rates were more than 10 times lower with a median of 10 nmol L^{-1} day^{-1} (Fig. 2). As described for methane concentrations, a one way ANOVA showed that methane oxidation rates and turnover times were significantly different (df = 124, p < 0.001) between the upper (#619–#629) and lower estuary (#659–#724).

We found a positive correlation between MOx and temperature in the upper estuary ($r^2 = 0.18$) and a weaker correlation in the lower estuary ($r^2 = 0.13$, p = 0.06), as can also be seen in the RDA diagrams (Fig. 3a–d). The MOx rate in the upper and lower estuary were both tightly

Fig. 3 The ordination diagrams of the *first* and *second axis* of the redundancy analysis (RDA): **a** whole estuary; **b** upper estuary; **c** transition zone; **d** lower estuary. Note: CH$_4$ oxidation rate and CH$_4$ turnover time (*triangle symbols*) are explained variables; environmental parameters (*arrows*) are explanatory variables. The angle size between variables indicates their interrelationship, while *arrows* pointing in the same direction indicate positive correlations, and *arrows* pointing in opposite directions indicate negative correlations. The length of the *arrows* shows the strength of the environmental variable
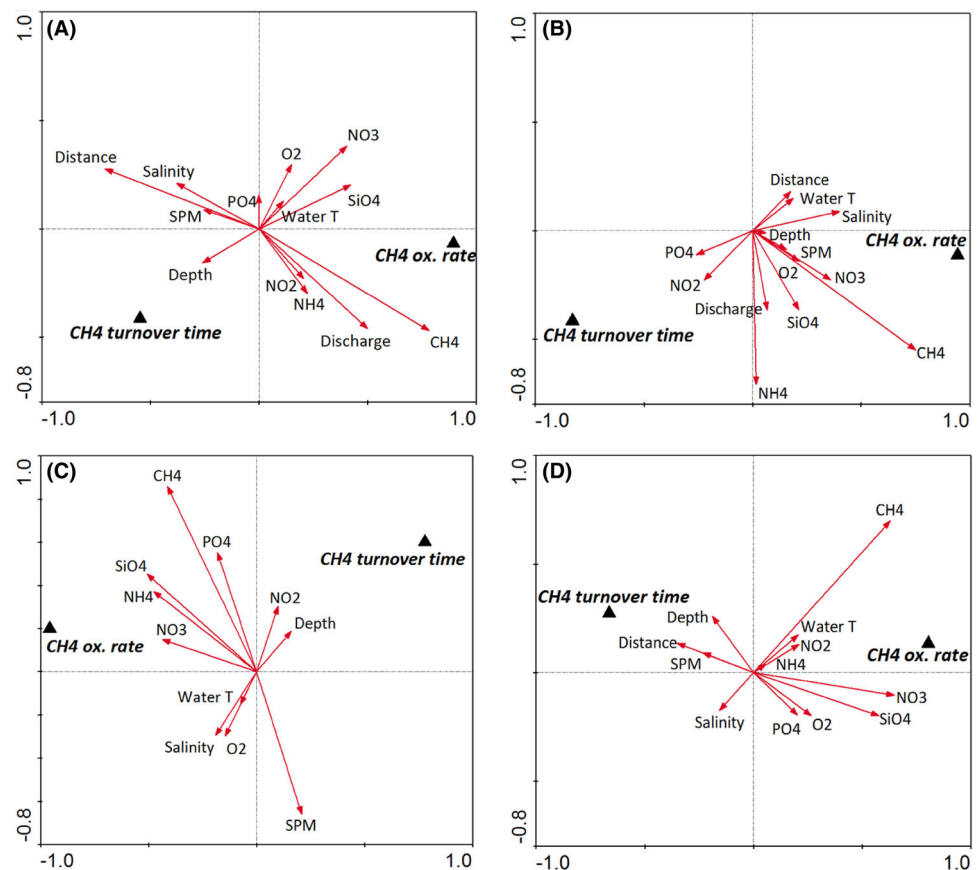
correlated with CH$_4$ concentrations ($r^2 = 0.54$ and 0.66, respectively; both with $p < 0.001$). However, as the MOx is calculated by multiplying the fractional turnover rate constant (k) with the CH$_4$ concentration, this correlation has to be regarded with some caution. For the whole estuary MOx also was correlated with the BOD-7 ($r^2 = 0.31$, n = 24, $p = 0.005$). The analysis revealed no further statistically significant correlation. Only a slight indication of a positive correlation between MOx and some nutrients (NH$_4^+$, NO$_3^-$) was found in the transition zone (Fig. 3c).

Methane turnover times (τ) were rather short at the upper estuary (#619–#629) with a median of 1.6 days and a range of 1–25 days. In the lower estuary, (#659–#724) τ were about twice as long with a median of 3.7 days, ranging from 1 to 66 days (Supplementary Material 6) being, moreover, significantly different (one way ANOVA, df = 124, $p = 0.01$) from the upper estuary.

In the upper estuary, RDA analyses (Fig. 3b) indicated a slightly negative relationship between τ and water temperature ($r^2 = 0.47$, $p < 0.001$), i.e. a slow turnover at lower temperature, and oxygen ($r^2 = 0.28$, $p = 0.009$), i.e. a fast turnover at higher oxygen concentrations. In the transition zone we found the same correlations between τ and water temperature and additionally a weak negative

correlation also with salinity (Fig. 3c). For the lower estuary, we found only a less tight correlation with temperature ($r^2 = 0.11$, $p = 0.002$) and salinity ($r^2 = 0.16$, $p < 0.001$), however, the RDA analyses (Fig. 3d) revealed an influence of the changing nutrient concentrations (NO$_3^-$, PO$_4^{3-}$, O$_2$ and SiO$_4$).

Calculation of the methane sea–air fluxes and budget

The sea-air flux ranged from 6 up to 115 kmol day^{-1} (Table 3). The higher numbers (>60 kmol day^{-1}) were mainly due to high current velocities (100–130 cm^3 s^{-1}) at the respective stations and dates. The total methane oxidation rate ranged from 2 to 49 kmol day^{-1}. For all cruises the total MOx seemed to be most important in box 1 (#619–#629, Hamburg port) with 57 and 61 % of the total loss assigned to MOx. Generally low MOx rates were observed in April 2013 with rather cold water (6.6 °C) compared to the other dates (18–21 °C), thus obviously the low temperature slowing MOx. However, on average 41 ± 12 % (without the April data) of the total loss can be attributed to microbial methane consumption.

In a second step we tried to calculate a budget for the boxes, i.e. to estimate the importance of the input and
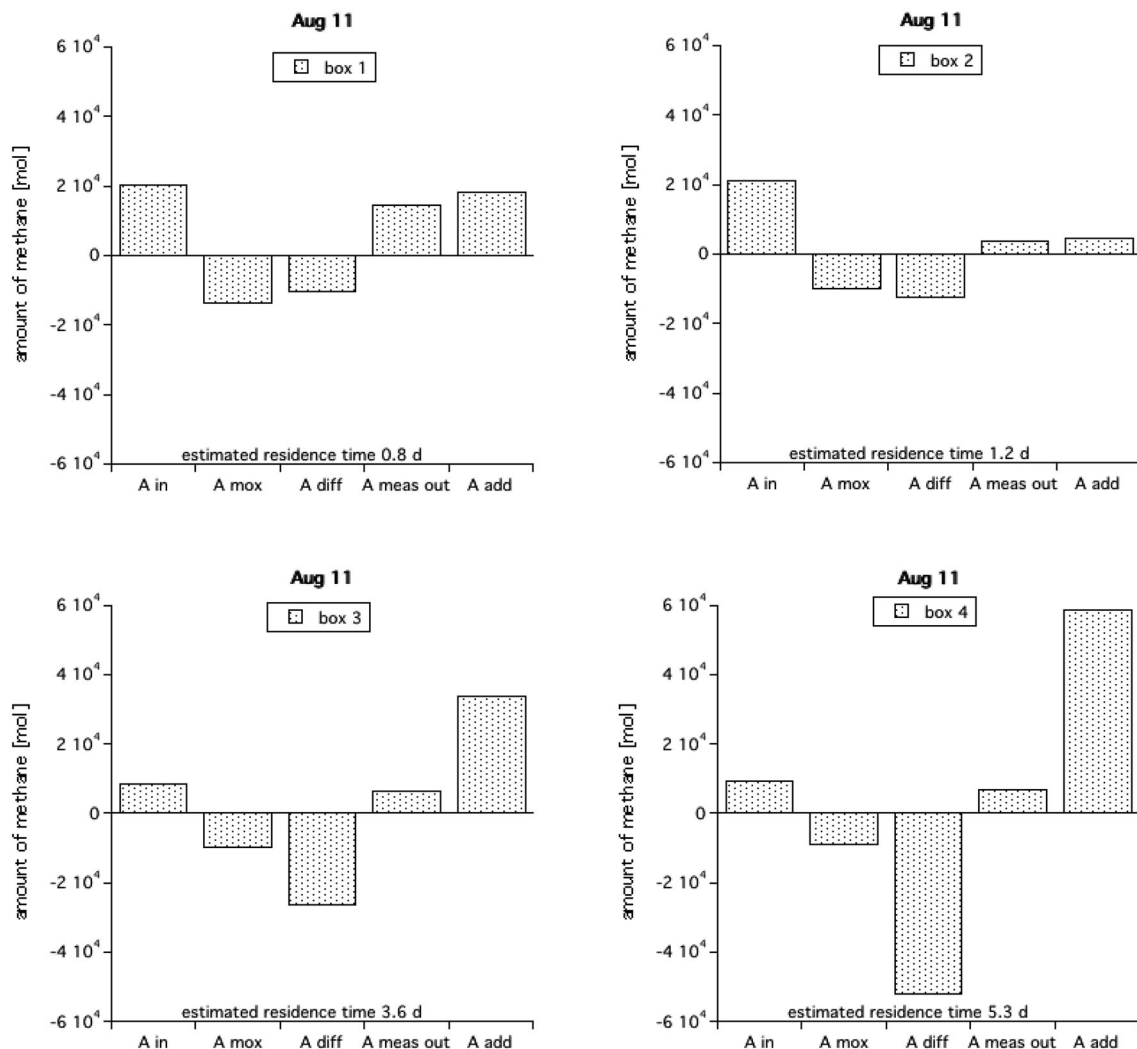
Fig. 4 The amount of methane entering a box (A_{in}) with the methane reducing processes (oxidation A_{Mox} and diffusion into the atmosphere A_{diff}) versus the measured amount of methane "leaving" the box ($A_{meas\ out}$). To obtain equilibrium an additional input (A_{add}) is required. The estimated residence time for each *box* is also given. Data are from 3.8.2011. For details of the calculation see "Results"

Table 3 The loss of methane (in kmol day^{-1}) via diffusive flux (Diff) into the atmosphere and the microbial methane oxidation (MOx) at the different sampling dates and different boxes (the definition of the boxes is given in Fig. 1). The contribution of MOx to the total loss (diffusion + methane oxidation) is also given

	03. 08. 2011			01. 08. 2012			12. 04. 2013			12. 06. 2013		
	Diff	MOx	% MOx	Diff	MOx	% MOx	Diff	MOx	% MOx	Diff	MOx	% MOx
Box 1	13	17	57				33	3	9	13	20	61
Box 2	10	8	44				67	4	5	23	24	50
Box 3	7	3	27				115	2	2	68	49	42
Box 4	10	2	15	6	4	44	35	1	2	76	47	38
Box 5				13	8	36						
Box 6				13	11	47						
Box 7				6	4	37						

output of methane by the river flow, the methane reducing processes (evasion into the atmosphere and microbial oxidation), and finally—when assuming an equilibrium state and a closed budget—an extra input of methane. The amount of methane transported into or out of a box (A_{in} and A_{out} in mol) was calculated by multiplying the CH_4 concentration at the inflowing and outflowing station of the box with the volume of water entering/leaving this box. The residence time of a water parcel in the respective box was estimated from the data given in Bergemann et al.

(1996) at low, medium, and high discharge (see Supplementary Material 7). No information on the error of his estimate is given, thus we assume an error of at least 10 %. The residence time is determined by the discharge of the river as well as the tidal influence, resulting in short residence times near the port of Hamburg. However, with the stronger tidal effects near the coast, the residence time increases. Also the duration of the CH_4 consuming process within one box is determined by the residence time. Thus we multiplied the rate of the removal process, which results in the amount of CH_4 lost through diffusion (A_{diff}) and the amount lost through CH_4 oxidation (A_{MOx}) in this box. In accordance with Anthony et al. (2012) and Bergemann et al. (1996) we then assume that

$$A_{in} - A_{diff} - A_{MOx} = A_{calc-out} \qquad (9)$$

when comparing the calculated amount of CH_4 ($A_{calc\ out}$) with the measured amount of CH_4 ($A_{meas\ out}$) finally gives us the additional input ($A_{additional}$) required to balance the budget for the respective box:

$$A_{calc-out} - A_{meas-out} = A_{additional} \qquad (10)$$

In August 2011, the Elbe discharge was moderate (716 $m^3\ s^{-1}$) and the residence times were estimated to range from 0.8 days in box 1 to 5.3 days in box 4. This contrasts to June 2013 with a very high discharge (4041 $m^3\ s^{-1}$) and a residence time ranging from 0.4 days in box 1 to only 2.9 days in box 4. In August 2012 water discharge was low (409 $m^3\ s^{-1}$) and for the boxes in the lower estuary we estimated residence times of 14 days in box 4 and 25 days in box 6. In Fig. 4 we visualize the different processes for the cruise in August 2011. All processes for all cruises are shown in the Supplementary material 7.

In August 2011, in box 1 the amount of methane leaving the box was only slightly lower than the incoming amount (Fig. 4). With a residence time of 0.8 day, the CH_4 consuming process was about half the amount of the input. The measured output was slightly lower than the input, and "adding" the CH_4 reducing process, we estimated an additional input of the same size as the measured input. As the residence times increases downstream, correspondingly we estimated a residence time of 5.3 days in box 4. Thus the CH_4 reducing processes strongly increased, and whereas the measured input and output were almost the same, the CH_4 reducing processes can only be balanced with additional input about six times as high as the input. A similar pattern was observed for the cruises in April and June 2013 (Supplementary material 7).

In August 2012 the discharge was low and the boxes were nearer to the coast, thus the residence time increased to 14–25 days (Supplementary material 7) and consequently the amount of CH_4 reduction also increased. Even

though the measured input and output of these boxes was about the same, the strong loss of CH_4 can only be accounted for by a very strong additional input (22–33 times higher than the input).

Discussion

Methane distribution in the water column of the Elbe estuary

Our exceedingly high values of methane concentration correspond more or less to tropical/warm water systems and large rivers (Zhang et al. 2008; Borges et al. 2015a, b). Middelburg et al. (2002) measured the freshwater end-member for the Elbe River at a concentration of 111 nmol L^{-1} (salinity of approx. 0.4) and the marine end-member at a concentration of 4–6 nmol L^{-1}. This fits our data well, as at salinities between 0.3 and 0.5 we found a median methane concentration of 127 nmol L^{-1}. Wernecke et al. (1994) determined the CH_4 concentrations to be between 60 and 120 nmol L^{-1} in the main stream of the River Elbe, and up to 1200 nmol L^{-1} in the stagnant water (the upper end of the harbor basin), which is in line with our data. Rehder et al. (1998) estimated the average CH_4 input into the North Sea to be 70 nmol L^{-1} compared to our lower estimates with a median of 50 nmol L^{-1} (for our outmost station #724). In contrast to previous studies (Middelburg et al. 2002; Rehder et al. 1998; Osudar et al. 2015) we observed no correlation between CH_4 and salinity (including for the two outmost stations). This indicates that there is no simple two-compartments based mixing of riverine and marine CH_4.

We found significantly higher CH_4 concentrations at falling water levels in the upper estuary. Grunwald et al. (2009) suggested that CH_4 concentrations usually peaked during low tide, probably due to the CH_4-rich freshwater input and that, conversely, low values may be caused by dilution with CH_4-poor marine water and degassing processes. This was revealed and postulated also by others (Koné et al. 2010; Upstill-Goddard and Barnes 2016). However, we did not find a correlation to water gauge/level, like Grunwald, but with the "falling water level". We assume that our sampling strategy from a moving boat versus fixed stations biased this effect. For the North Sea the tidal flat have been shown to be a methane source (Wu et al. 2015), and we assume that the strong surge at falling water levels results in the release of methane into the river/estuary.

Methane in the whole estuary but more specifically in its upper part is positively correlated with the BOD-7, which represents a measure of the bioavailability of degraded organic matter. The high CH_4 concentrations in the upper

part of the Elbe estuary likely result from the high heterotrophic activity related to remineralization processes of high loads of labile organic matter. The organic material originates mainly from phytoplankton biomass (Kerner 2000), which is subject to enhanced mortality in the upper zone of the Elbe Estuary (area of Hamburg Port) due to higher runoff, deeper water column mixing processes, and high water turbidity (Wolfstein and Kies 1995; Muylaert and Sabbe 1999). No data on waste water input and its influence on the CH_4 concentration in the Elbe were found, however, improvements in wastewater and in industrial inputs led to significant decrease of nitrogen and phosphorus loads since the 1990's (Schlarbaum et al. 2010) and so minor effects are assumed.

Elevated CH_4 concentrations as well as elevated CH_4 oxidation rates are often associated with a high content of SPM or turbidity (Upstill-Goddard et al. 2000; Abril et al. 2007). As most CH_4 is produced in anoxic zones within the sediment, significant CH_4 amounts are also released during particle resuspension (Bussmann 2005). Particle attached bacteria normally show higher activity rates and diversity (Ortega-Retuerta et al. 2013). Additionally, a high variability (from 0 up to 98 %) of the contribution of particle-attached bacteria to total bacterial production is also reported (Garneau et al. 2009). Thus we suggest that MOB may also attach to particles and lead to an elevated particle-attached CH_4 oxidation rate (Abril et al. 2007). However, the described direct relationship does not hold for all rivers and estuaries, as our data do not support these hypotheses, for either CH_4 concentration or MOx. We even observed a negative relationship between CH_4 and SPM for the upper estuary (Fig. 3b). There are also examples in literature where no direct relationship between SPM and methane concentration was found (Grunwald et al. 2009). One possible reason for this inconsistency is that the chemical composition of SPM changes spatially within an estuary (Savoye et al. 2012) and probably also with season. In our data set we observed a great variability of the SPM data, which makes it even more difficult to interpret them. Further, experimental studies would shed light on the relation between SPM and methane, i.e. the composition of the SPM and the status of the particle-attached or free-living MOB being in an active or dormant physiological state (Ho et al. 2013).

Methane oxidation and turnover time in the water column of the Elbe estuary

In our study along the salinity gradient of the Elbe estuary we observed CH_4 oxidation rates from 0.8 nmol L^{-1}-day^{-1} to very high about 5542 nmol L^{-1} day^{-1} (at the Hamburg harbour area; Fig. 2). This variability is in line with the literature data from diverse estuaries (Griffiths

et al. 1982; Scranton and McShane 1991; de Angelis and Scranton 1993; Abril and Iversen 2002). The turnover time varied between 0 and 66 days. Very few investigations included CH_4 turnover time measurements, e.g. a study from the Ogeechee River estuary with very fast turnover time between 2 h and 1 day (Pulliam 1993).

One important factor in both estuary parts was temperature, i.e. higher MOx rates and a fast turnover rate were observed at higher temperatures. This corroborates the observations made by Osudar et al. (2015) and Lofton et al. (2014) but contradicts the observations of Zaiss et al. (1982) and Utsumi et al. (1998), who revealed only a minor influence of temperature on MOx in situ. Additionally Lofton et al. (2014) suggested a specific substrate-temperature interaction, i.e. only methane saturated MOx was influenced by temperature, at limiting methane concentrations temperature had no influence on MOx. These findings are supported by our data on the turnover time, the influence of temperature was much higher in the upper estuary with its higher CH_4 concentrations. As the oxidation of CH_4 also needs oxygen, the process of CH_4 oxidation is also a part of the BOD-7, as is shown by its correlation; however, this fact does not give any further ecological information.

The availability of CH_4, as a sole source of energy for MOB seems to be the key factor influencing their activity. Similar findings are reported for MOx kinetics in the marine water column (Mau et al. 2015). For riverine, but sediment borne CH_4 concentrations of 2000–4000 nmol L^{-1} were still limiting levels (Shelley et al. 2015). Our observed CH_4 concentrations ranged from 18 to 2306 nmol L^{-1} and support the fact that MOx was mostly limited by CH_4 concentration. Type I MOB responds rapidly to substrate availability and is the predominantly active community in many environments (Ho et al. 2013). In the Elbe Estuary a dominance of type I MOB was also observed, with no type II MOB being detected (Hackbusch 2014).

The influence of nutrients showed no clear pattern found, suggesting limited effects of nutrients for MOx as most parts of this in this system have typically high trophic status (Table 2). Other authors also report a complex influence of nutrients (N and P) on MOx (Veraart et al. 2015). Oxygen also had mostly no influence on the microbial parameters. Thus we assume that the minimal oxygen concentrations of 3.4 mg L^{-1} were still sufficient to permit CH_4 oxidation. This is not surprising as aerobic methanotrophs can be active in microoxic conditions (Deutzmann et al. 2014).

The sea-air fluxes of methane and budget calculations

We calculated the diffusive methane flux along the Elbe estuary for four selected cruises when the overall water

movement and the sampling scheme were downstream. For these calculations the values of the gas exchange coefficient (k_{600}) were crucial. They have previously been determined directly via chamber or eddy covariance measurements, or can be alternatively assessed via different models taking into account the stream hydrology. We applied the model from Raymond et al. (2012) and obtained an average k_{600} value of 8.4 m day^{-1} or 35 cm h^{-1}, which is fairly close to the range of 20 and 31 cm h^{-1} previously reported for the Elbe (Abril and Borges 2005; Amann et al. 2015). Thus, we think that our approach is reasonable and our slightly higher values can be related to the rather high water velocities (>100 cm s^{-1}) recorded on some specific occasions.

A direct comparison with flux data from the literature is hindered by the usage of many different units. We calculated an average sea-air flux of CH_4 (for all boxes and all 4 cruises) of 33 ± 8 g CH_4 m^{-2} per year and with a calculated total area of 1.5×10^8 m^{-2} and thus come up with an average CH_4 emission of $5.0 \pm 1.2 \times 10^9$ g per year for the whole Elbe estuary. For the Scottish Tay Estuary 1.02 g C m^{-2} y^{-1} and 5×10^7 g C per year has been calculated (Harley et al. 2015). For both units the flux in the Tay estuary is 30 times and one order of magnitudes lower than in the Elbe, respectively. However, other authors estimated $1.8 - 3 \times 10^{12}$ g CH_4 per year for temperate estuaries, which is much higher than our estimate for the Elbe (Middelburg et al. 2002). For a Baltic estuary CH_4 fluxes were generally below 15 g CH_4 m^2 y^{-1} in the bay (Silvennoinen et al. 2008). For a subtropical estuary CH_4 flux of 0.1–10 g CH_4 m^{-2} y^{-1} is reported (Musenze et al. 2014), which is roughly in line with our estimates. In tropical, African estuaries the flux ranged from 0.1 to 14 g CH_4 m^{-2} y^{-1}, which is less than half of our values (Koné et al. 2010). In a worldwide compilation of CH_4 flux data, the CH_4 flux from estuaries is described as being 539 ± 602 g CH_4 m^{-2} y^{-1} (Ortiz-Llorente and Alvarez-Cobelas 2012), while Borges and Abril estimate the global estuarine CH_4 flux with 6.2 g CH_4 m^{-2} y^{-1} (Borges and Abril 2011). Our data are within the lower range of this data compilation. These examples clearly demonstrate the difficulties of such comparisons. One reason for the variability of the data may be the methods used in calculating the flux, especially the determination of k_{600} (Musenze et al. 2014). Other reasons are certainly the variability of environmental parameters across locations, such as water velocity, tides, river discharge and of course methane contents itself. Also the degree and stability of the stratification, the influence of tidal areas and urban pollution have to be taken into account (Koné et al. 2010; Middelburg et al. 2002; Marwick et al. 2014). Thus the variability of CH_4 emissions is greatest for oceans and estuaries, with no clear seasonal pattern of CH_4 emission

found for estuaries (Ortiz-Llorente and Alvarez-Cobelas 2012).

Another process which eliminates CH_4 from estuaries is microbial MOx. For all cruises the total MOx seemed to be most important in box 1 (Hamburg port). This can be attributed to the morphometry of the boxes, as in box 1 the river is deep and narrow i.e. with a low volume ratio: volume ratio, while the river becomes wider and shallower towards the river mouth i.e. high area: volume ratio. Rather low MOx rates were observed in April 2013 at a water temperature of 6.6 °C. The impeding effect of temperature on MOx also becomes obvious through the negative correlation between temperature and MOx (this study) and a Q_{10} of 1.5 for Elbe water (Bussmann et al. 2015). Thus in warm water i.e. in the summer/autumn on average 41 ± 12 % of the total loss can be attributed to microbial CH_4 consumption and this portion of CH_4 is not lost to the atmosphere. In colder water (winter and spring) we estimate that only 5 ± 3 % of the total CH_4 loss is due to MOx.

In previous studies box models were used to estimate the influence of the different CH_4 related processes in a river (de Angelis and Scranton 1993; Anthony et al. 2012). However, in our study area we also have a strong tidal regime, thus the water is not simply moving downstream. Each day a hypothetic water parcel moves four times up- and downstream. Thus, it takes 12 weeks for a water parcel to get from Geesthacht to Cuxhaven (140 km, with a water discharge of 250 m^3 s^{-1}) (Bergemann et al. 1996). Therefore any CH_4 reducing or producing processes have ample time to act. So we chose a modified box model taking into account the different residence times, depending on the water discharge of the river and the intensity of the tidal regime (Bergemann et al. 1996). Certainly, numerical modeling would give us a better insight into this dynamic estuarine system (Schroeder 1997; Schöl et al. 2014); however, though this approach may be realized in the future, it was beyond the scope of this study.

When the residence time for a box is low, the importance of the CH_4 consuming processes is also correspondingly low. In several cases we observed almost no difference between the amount of CH_4 entering a box (A_{in}) and the amount leaving a box (A_{out}; Fig. 4, box 1). As the CH_4 consuming processes are active at a low level, a low amount of additional CH_4 is required. Near the coast or at times of low discharge the residence time increases and therefore the amount of consumed CH_4 also increases. To balance out this strong loss of CH_4, the additional input also has to increase (Fig. 4, box 4). So even when the CH_4 concentrations and the amount of CH_4 entering and leaving a box seem to be stable or equal, CH_4 reducing processes are active, and to counterbalance them an additional input of CH_4 is required. In box 1, the ratio of A_{in} to A_{add} was 1–2. In contrast to boxes 4–6 where the ratio was 22–44,

indicating a tremendous additional input of methane. As the width of the Elbe increases (from 0.5 km at #629 to almost 7 km at station #724) the bordering tidal marshes could be the source of this additional CH_4 input. For dissolved inorganic carbon (DIC) in the Elbe it is known that marshes contribute up to one-third of the annual DIC excess (Amann et al. 2015). Tidal flats are known to emit CH_4 into the surface water and atmosphere in substantial amounts (Røy et al. 2008; Weston et al. 2014). Due to advective flow, which is of special importance in permeable sandy sediments, pore waters enriched in remineralized nutrients and CH_4 are actively released from sediments into the overlying water column (Beck and Brumsack 2012). Additionally, groundwater also releases CH_4 into estuarine waters (Porubsky et al. 2014).

As the Elbe estuary is surrounded by the tidal flats from the Waden Sea, which are one of the largest sand- and mudflats worldwide (Marencic 2009), we suggest that the influence of these tidal flats is much stronger than the riverine input into the North Sea. Even though the CH_4 concentrations in the upper estuary are very high, this CH_4 will not reach the North Sea (due to oxidation and diffusion on its way). But it is the lower estuary with its lower CH_4 content which is flowing into the North Sea. This is supported by the fact that we did not see any effect of dilution (correlation between salinity and CH_4) in our data. Only at a greater distance from the coast, the CH_4 of the coastal water, being a mixture of riverine and tidal-flat-originated CH_4, becomes diluted with CH_4-poor marine water (Osudar et al. 2015). Detailed isotopic studies could help to clarify this hypothesis.

Conclusions and outlook

Estuaries represent a mosaic of habitats changing from freshwater to marine environment, which can be seen also in relation to methane related processes. High methane concentrations were observed in the upper estuary (Hamburg port), which decreased by one order of magnitude towards the lower estuary and the river mouth. Despite active methane oxidation, the microbial filter was estimated to be responsible for 5–41 % of the methane total loss. The other part was attributed to methane diffusion into the atmosphere. We did not observe any dilution of methane-rich river water with methane poor marine water. On the contrary, we assume that marshes bordering the Elbe estuary release a substantial amount of methane into the river and further into the North Sea.

Our study is based on ship-borne measurements, mostly going downstream. However, it would be interesting to relate this approach and our conclusions with methane data from fixed stations along the river, along which the water and its processes are moving by. The other possibility would be measurements within a "water parcel" as it is moving by river discharge and tidal currents. However, in the Elbe estuary, with its high level of ship-traffic, this would be rather difficult for ship-borne measurements. Permanent recording sensors may help to overcome these logistic restrictions.

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