Limnologica 79 (2019) 125716

Contents lists available at ScienceDirect

Limnologica

journal homepage: www.elsevier.com/locate/limno

Sediment methane dynamics along the Elbe River

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ARTICLE INFO

Keywords:
Methane
River sediments
Methanogenic potential
Methanotrophic potential
qPCR
TRFL-P
Stable carbon isotope
Delta 13C
mcr-A
pmo-A

ABSTRACT

Methane (CH_4) is an important atmospheric trace gas mostly released from wet anoxic soils and sediments. While many studies have focused on relatively homogenous environments like rice fields and lake sediments, the changing contribution of heterogeneous sediments e.g. along the longitudinal profile of a rivers has not been covered very frequently. Here we investigated sediment samples from 11 locations of the Elbe River. Sediments were incubated to measure methanogenic/methanotrophic potentials and contribution of individual methanogenic pathways using isotope analysis of $\delta^{13}C$. Additionally, we determined the diversity of the methanogenic communities (analysis of T-RFLP targeting the mcr-A gene in the sediment samples), while abundances of archaea, methanogens and methanotrophs were determined by qPCR. The CH_4 production was detected in six samples (out of 11 examined) and ranged from 0.12 to 644.72 nmol gDW^{-1} d^{-1}. Methanotrophy was found in all examined sediment samples and ranged from 654 to 10,875 nmol gDW^{-1} d^{-1}. Abundance of methanogens and methanotrophs (Mcr-A and pmo-A gene copy numbers) was not significantly different and quite stable around 10^6 to 10^7 copies gDW^{-1}. The group specific qPCR showed high fluctuations, while the highest counts were reported for *Methanomicrobiales* and *Methanosarcinales* (10^5 to 10^8 copies per gram dry sediment), followed by *Methanobacteriales* (10^3 to 10^5 copies per gram dry sediment). A significant proportion of unidentified methanogens was found in almost every locality. Isotope analysis of $\delta^{13}C$ showed that (CH_4) is produced mainly by hydrogenotrophic methanogens. We see no trend in the studied parameters along the Elbe River. The molecular data showed no spatial characteristics, while we found hotspots of the measured CH_4 processes (CH_4 production and oxidation) due to other local driving factors (e.g. carbon content). Thus, out results indicate that the observed variability of the CH_4 production and oxidation rates is only indirectly linked to the presence or quantities of different microbial guilds.

1. Introduction

Methane (CH_4) is a significant component of the aquatic carbon cycling and is involved in many biogeochemical and physical processes. Since biological methane production is mainly linked to wet anoxic soils and sediments, streams and rivers are one of many sources of atmospheric methane contributing 15–40 % to the total CH_4 efflux of wetlands and lakes (Stanley et al., 2016). Sediments are very important sites of riverine metabolism including their role in methanogenesis (Dahm et al., 1987). Generally, the mineralization of the organic matter under anaerobic conditions is carried out by several microbial organisms and results - in the absence of other electron acceptors like nitrate, iron, manganese etc. - in the release of CH_4 and CO_2 (Zeikus, 1983; Schink, 1997). Two major metabolic pathways of methanogens can be differentiated: acetoclastic (acetate conversion to CH_4 and CO_2) and hydrogenotrophic (H_2 and CO_2 to CH_4 and water). These two pathways can be discriminated using isotopic techniques due to diverse strength of isotopic fractionation during different methanogenic pathways, which lead to different isotopic composition of resulted CH_4 (Conrad, 2005).

The CH_4, which is formed in the sediments is subsequently released via diffusion, ebullition or through plants to the surface water or the

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https://doi.org/10.1016/j.limno.2019.125716
Received 29 October 2018; Received in revised form 29 August 2019; Accepted 29 August 2019
Available online 27 September 2019
0075-9511/

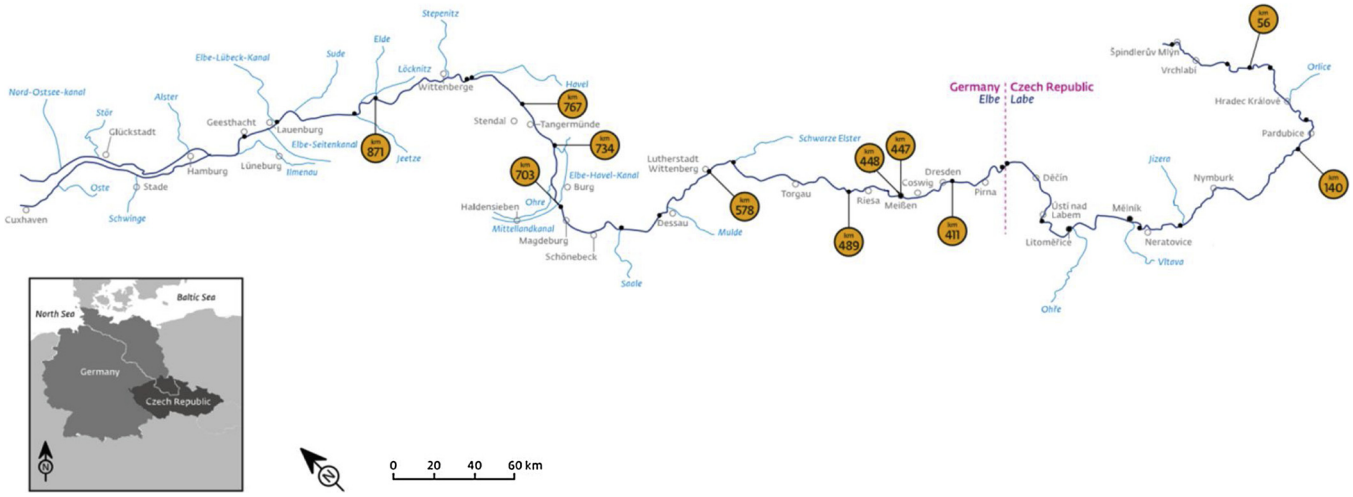

Fig. 1. The sampling sites on the longitudinal profile of the Elbe River. The numbering is determined by the distance from the river source in km (see "River km" in Table 1). Original map: https://commons.wikimedia.org/wiki/File:Lauf_der_Elbe.png; modified.

atmosphere, where it is transported via advection or dispersion, respectively. Simultaneously, the CH_4 is subject of significant oxidation by CH_4 oxidizing bacteria during its transport in aquatic ecosystems. Moreover, all the processes involved in the aquatic CH_4 cycle are subject to large temporal and particularly spatial heterogeneity (Stanley et al., 2016). Understanding the variability of methane-related processes is key factor leading to more precise estimates of lotic ecosystems relevance in the global methane budget, which is recently based on scarce data (Bastviken et al., 2011).

Previous studies conducted in large rivers show, that rivers are mostly oversaturated in dissolved CH_4 with respect to the atmosphere equilibrium (i.e. rivers are a net source of CH_4 to the atmosphere). Frequently observed inverse relationship between discharge and CH_4 concentration is most probably given either by dilution (Kone et al., 2010; Anthony et al., 2012) or by higher temperature during low water periods. Increased temperature further enhances microbial activity and thus decreases oxygen levels (Borges et al., 2018). Notably, CH_4 emissions from rivers may reflect the properties of the surrounding catchments, such as topography, soil type and texture, land use, hydrological connectivity with wetlands and other anthropogenic activities as input of wastewaters (Jones and Mulholland, 1998; Silvennoinen et al., 2008; Yang et al., 2012; Borges et al., 2015). Generally, the studies considering CH_4 in large rivers were focused mainly on its concentration in surface water and its eventual flux to the atmosphere, but the data concerning the sediment related processes are missing (Teodoru et al., 2015; Barbosa et al., 2016). Hence only few data related to CH_4 processes in sediments of large rivers exists and almost no data comes from complex longitudinal studies, despite of fact that river sediments have great potential as source of CH_4 due to high methanogenic biomass (Buriánková et al., 2012).

Many studies examining CH_4 production in stream and rivers confirm that methanogens are ubiquitous members of the microbial community within river hyporheic sediments (e.g. Sanders et al., 2007; Trimmer et al., 2012; Chaudhary et al., 2017). Currently there are seven orders of methanogenic archaea described in the literature: *Methanomicrobiales, Methanosarcinales, Methanocellales, Methanobacteriales, Methanococcales, Methanopyrales* and *Methanomassiliicoccales* (Borrel et al., 2011, 2013; Borrel et al., 2014; Lang et al., 2015). *Methanomicrobiales* and the *Methanosarcinales* followed by *Methanobacteriales* dominate the methanogenic communities in freshwater sediments of lakes and rivers (Chan et al., 2005, Chaudhary et al. 2013). Moreover, *Methanocellales* are common in rice field soils or peats and have rarely been found in lake sediment (Scavino et al., 2013; Galand et al., 2005; Conrad et al., 2010). Our previous studies conducted in another European river (Sitka, Czech republic) revealed three major methanogenic groups using molecular techniques (denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism [T-RFLP], quantitative polymerase chain reaction [qPCR] and cloning): *Methanosarcinales*, *Methanomicrobiales* and *Methanobacteriales* (Buriánková et al., 2013; Brablcova et al., 2014; Chaudhary et al., 2014, 2017). Hence we focused our attempts to clarify the role of these groups using T-RFLP and qPCR in the present study.

In principle, one can raise four hypotheses to describe the turnover of organic matter in river sediments along the longitudinal profile of a river: either (1) decrease or (2) increase of the CH_4 related processes along the river flow; further (3) no correlation with environmental factors but hotspots of the microbial activities due to other local factors (e.g. carbon content etc.), and (4) no obvious impact resulting in comparable process rates along the riverbed. To validate which of these hypothesis may be applied for river systems, our aim was to describe the following processes and elucidate how our results could support the above-mentioned hypothesis: (i) methanogenic and methanotrophic potential of the sediments, (ii) an isotopic signal of CH_4 including determination of methanogenic pathways to the total CH_4 production, (iii) the community composition (TRFL-P) and quantification (qPCR) of archaea, methanogens and methanotrophs in the sediment samples. Samples for this study were taken during a large sampling campaign along the Elbe River carried out in October 2013, from Špindlerův Mlýn (km 8) to Geesthacht (km 948) (more detailed in Matoušů et al., 2018).

2. Material and methods

2.1. Study site

The Elbe River rises at an elevation of 1386 m above sea level in the Krkonoše (Giant Mountains) in the northeast of the Czech Republic, flowing through the central part of the Czech Republic and through central and northern Germany before discharging into the North Sea at Cuxhaven, 110 km northwest from Hamburg. Its total length is 1094 km and its catchment area is 148,268 km^2. Sediment samples for this study were taken at 11 different sites along the river flow in October 2013. Localization of each sampling sites including sediment characteristics are specified in Fig. 1 and Table 1. (Note that we limited the sampling to the freshwater regions of the Elbe River; samples below the weir in Geesthacht are at least partially influenced by the North Sea.)

Table 1
Basic study sites description with sediment characterization (mean values ± SE; n.m. = not measured).

River km	Sampling site	Grain median size (mm)	Water content (%)	Sediment carbon content (%)	Sediment nitrogen content (%)	$\delta^{13}C$ of sediment carbon (‰)	$\delta^{13}C$ of acetate (‰)[a]	Acetate conc. (mM)[a]
56	Verdek	19.3	18.9 ± 1.2	0.19 ± 0.01	0.01 ± 0.0	79.8 ± 60.2[b]	n.m.	n.m.
140	Valy	0.20	31.2 ± 1.4	1.70 ± 0.12	0.13 ± 0.0	−25.6 ± 0.4	−14.5 ± 0.1	1.3 ± 0.7
411	Wachwitz	17.1	19.5 ± 0.6	0.25 ± 0.01	0.01 ± 0.0	−16.7 ± 2.7	n.m.	n.m.
447	Meissen	9.51	17.3 ± 0.6	0.42 ± 0.03	0.03 ± 0.0	−23.2 ± 0.9	−26.3 ± 0.4	3.9 ± 0.6
448	Meissen - harbor	0.32	68.9 ± 2.6	5.70 ± 0.34	0.46 ± 0.1	−27.2 ± 0.4	−17.8 ± 1.1	0.4 ± 0.1
489	Muehlberg	11.4	16.3 ± 0.4	0.12 ± 0.01	0.01 ± 0.0	−0.4 ± 5.7	−18.4 ± 3.8	3.8 ± 2.2
578	Lutherstadt Wittenberg	0.41	18.5 ± 0.9	0.06 ± 0.01	0.004 ± 0.0	−23.6 ± 1.0	n.m.	n.m.
703	Hohenwarthe	0.49	16.2 ± 0.4	0.23 ± 0.01	0.01 ± 0.0	−20.8 ± 2.1	n.m.	n.m.
734	Bittkau	0.56	16.1 ± 0.6	0.17 ± 0.01	0.01 ± 0.0	27.8 ± 26.8[b]	n.m.	n.m.
767	Arneburg	7.49	14.2 ± 0.6	0.03 ± 0.01	0.003 ± 0.0	−10.2 ± 8.3	n.m.	n.m.
871	Domitz	0.91	14.4 ± 0.8	0.08 ± 0.03	0.004 ± 0.0	−11.6 ± 0.0	−27.2 ± 1.8	4.0 ± 1.1

[a] An acetate concentration and $\delta^{13}C$ of acetate were measured after the incubation in samples inhibited by CH_3F.
[b] Observed positive values of $\delta^{13}C$ of sediment carbon are connected with very depleted carbon pool in incubated samples, which leads to significant isotopic enrichment of carbon with ^{13}C.

2.2. Sampling of sediment

Triplicates samples of the upper sediment (down to a depth of 10 cm) were collected by hand shovel near the shore. A bulk sediment was used for a granulometric analysis, while sediment intended for incubation experiments were sieved through a 1-mm sieve immediately after the sampling to remove coarse detritus, stones or invertebrates. Particles < 1 mm are considered for the microbial measurements and for microbial activity measurements since the most of the biofilm is associated with this fraction (Leichtfried, 1988). For the molecular analysis, frost-resistant vials containing 5 g of fresh sediment were put into a liquid nitrogen storage box, sediment samples for nutrient analyses, organic carbon content, methanogenic potential, isotope composition and methanotrophic activity were transferred into 50 ml Falcon tubes and stored in a cool box until further processing.

Sediments for the granulometric analysis were sieved through a system of ten sieves with decreasing mesh sizes. All separate parts of the sediment were weighted and grain median size was analysed using the Gradistat software (version 8.0) (Blott and Pye, 2001). The dry weight of the sample was determined gravimetrically. The carbon and nitrogen contents of the sediments were quantified on a CHNS-element analyzer by the Analytical Chemical Laboratory of the University of Marburg.

2.3. Incubation experiments

For the investigation of methanogenic pathways and methane production potential about 40 g (wet weight) of the sediment were transferred in triplicate into 60 ml sterile serum bottles, flushed with N_2, closed with butyl rubber stoppers and incubated at 25 °C in the dark. At the start of the incubation 5 ml of distilled autoclaved water were added for later sampling of the liquid phase. The gas headspace of half of the bottles was supplemented with 3% CH_3F (v/v) to specifically inhibit acetotrophic methanogenesis (Janssen and Frenzel, 1997). The gas samples were taken repeatedly (twice a week) during the course of incubation (4–6 weeks) and analysed for concentrations of CH_4, carbon dioxide (CO_2) and $\delta^{13}C$ of CH_4 and CO_2. At the end of the incubation, the bottles were sacrificed to determine concentration and $\delta^{13}C$ of acetate.

Methane oxidation potential of sediments was determined in triplicate for each sample. Sterile bottles (250 ml) were filled with 20 g of sediment (wet weight) and closed by a cap with PTFE silicone septa. The headspace (ambient air) was enriched with CH_4 to give a final concentration of 10,000 ppm and incubated at 25 °C in the dark. The concentration of CH_4 in the headspace of each bottle was measured at T_0 h and then nine times during 170 h. The CH_4 production and oxidation potentials were calculated from the linear slope of CH_4 concentration change over time.

In the sediment incubation experiments, CH_4 was analyzed by gas chromatography (GC) using a flame ionization detector (Shimadzu, Kyoto, Japan). CO_2 was analyzed in the same instrument after conversion to CH_4 with a methanizer (Ni-catalyst at 350 °C, Chrompack, Middelburg, Netherlands).

2.4. Isotopic analyses

Isotope measurements of $^{13}C/^{12}C$ in gas samples were performed on a gas chromatograph combustion isotope ratio mass spectrometer (GC-C–IRMS) system (Thermo Fisher Scientific, Bremen, Germany). The precision of repeated analysis was ± 0.2‰ when 1.3 nmol of CH_4 was injected. The principle operation was described by Brand (1996) with details given in several recent publications (Blaser et al., 2013; Penger et al., 2012, 2014). An isotopic analysis and quantification of acetate were performed on a high pressure liquid chromatography (HPLC) system (Spectra System P1000, Thermo Fisher Scientific, San Jose, CA, USA; Mistral, Spark, Emmen, the Netherlands) equipped with an ion-exclusion column (Aminex HPX-87-H, BioRad, München, Germany) and coupled to Finnigan LC IsoLink (Thermo Fisher Scientific, Bremen, Germany) as described by Krummen et al. (2004). Isotope ratios were detected on an IRMS (Finnigan MAT Deltaplus Advantage). The HPLC-C-IRMS system had a detection limit of abou 5 µM and a precision of ± 0.3‰. Details on acetate determination and calculations of isotope fractionation factors and the contribution of hydrogenotrophic methanogenesis have been described in Blaser et al. (2013) or Penger et al. (2014) and it is summarized in the supplementary material (text and Fig. S1).

2.5. Molecular analyses

DNA was extracted from the fresh sediment before the start of the incubation using the PowerSoil DNA Isolation Kit (MO BIO, USA), according to the manufacturer's instructions. The extracted DNA was used to characterize the *mcr-A* gene by T-RFLP (Terminal-restriction lenght polymorphism) according to Chin et al. (Chin et al., 1999; Liu et al., 1997) using the primers *mcr-A* f (TAY GAY CAR ATH TGG YT) and *mcr-A* r (ACR TTC ATN GCR TAR TT) published by Springer et al. (Springer et al., 1995) with a FAM (6-carboxyfluorescein)-label at the forward primer. The *mcr-A* gene amplicons were digested with Sau96I (Fermentas), and the products were size-separated in an ABI 3130 DNA sequencer (Applied Biosystems, Darmstadt, Germany). The normalization and standardization of the T-RFLP profiles was performed according the method from Dunbar et al (2001). To assign the resulting fragments we used published literature values (Chin et al., 2004;

Conrad et al., 2008; Kemnitz et al., 2004; Lueders et al., 2001; Ramakrishnan et al., 2001; Mach et al., 2015) as well as a clone library, which was constructed in our lab in order to thoroughly characterize the methanogenic community at different locations and depth of Sitka stream (Chaudhary et al., 2017).

2.6. Quantitative polymerase chain reaction (qPCR) in sediment samples

In order to quantify the microbial community we used a set of different primers targeting the total archaea (16S *rRNA* genes), methanogenic archaea (*mcrA* gene), three major methanogenic orders *Methanobacteriales* (MBT-set), *Methanomicrobiales* (MMB-set), or *Methanosarcinales* (MSL-set), and methanotrophs (*pmoA gene*) (Øvreås et al., 1997; Luton et al., 2002; Yu et al., 2005) (Table S1). qPCR was performed using the BioRad CFX Connect™ qPCR Detection System (BioRad, USA). The 25 µL real-time PCR mixture was prepared using the Brilliant II SYBR master mix (Agilent Technologies, USA), 12.5 µl of 2x reaction solution, 0.25 µl of each primer (final concentration 0.25 µM), 5 µl of template DNA, and 7 µl of PCR-grade water. The two-step amplification protocol was applied as follows: initial denaturation for 5 min at 94 °C followed by 45 cycles of 30 s at 94 °C and combined annealing and extension for 30 s at X°C (X values are given in Table S1). The fluorescent signal was measured at the end of each annealing/extension step. DNA samples were analyzed in triplicate at each point. Standard curves were generated for the methanogenic strains, by amplifying the target genes with PCR. The PCR products were cloned into the pGEM-T Easy vector (Promega, Madison, WI). The plasmids were extracted, serially diluted, and used as templates in qPCR.

2.7. Statistical analysis

Data analyses were performed by using the STATISTICA 12 software (StatSoft, Inc., 2013). Shapiro-Wilk test was used to test the normal distribution of a data. The Spearman´s correlation analysis of data were used to find the relationships among environmental parameters as independent variables (e.g. carbon content, grain median size) and experimentally measured parameters as dependent variables (e.g. CH_4 production and oxidation potential). All statistical tests used a significance level of 5%.

3. Results

3.1. Methane production and oxidation by sediment

The CH_4 production in top sediments (0–10 cm) was recorded only for six sites (out of 11 examined): Valy (km 140), Meissen – river (km 447), Meissen – harbour (km 448), Muehlberg (km 489), Hohenwarthe (km 703) and Dömitz (km 871) (Fig. 2). Methanogenic potential of these sediments ranged from 0.12 to 644.72 nmol gDW^{-1} d^{-1} with the highest CH_4 production in Meissen - harbour (mean 551.68 ± 46.68 nmol gDW^{-1} d^{-1}). The methanogenic potential was positively correlated with the carbon content of the sediment ($r = 0.64$, $p < 0.05$).

Isotopic analyses of CO_2 and CH_4 were used to calculate the contribution of different methanogenic pathways (Fig. 3). These analyses were performed for CH_4 productive sediments except the site Hohenwarthe (km 703), where the formation of CH_4 was insufficient for isotopic analyses during the incubation (compare Fig. 2). The hydrogenotrophic pathway of CH_4 formation was dominant during the whole incubation for all five sites accounting for 52–78 % of total CH_4 release. Detailed sediment characteristics are provided in Table 1.

The aerobic methanotrophic potential of sediments was recorded for all sites and ranged from 654 to 10,875 nmol gDW^{-1} d^{-1} (Fig. 4). The calculated methanotrophic potential of sediments was always higher than the methanogenic potential at each sampling site. The highest values of methanotrophic potentials were observed at the km 448 (Meissen-harbour). The CH_4 production and oxidation in surface sediments varied spatially along the Elbe River longitudinal profile without any clear trend.

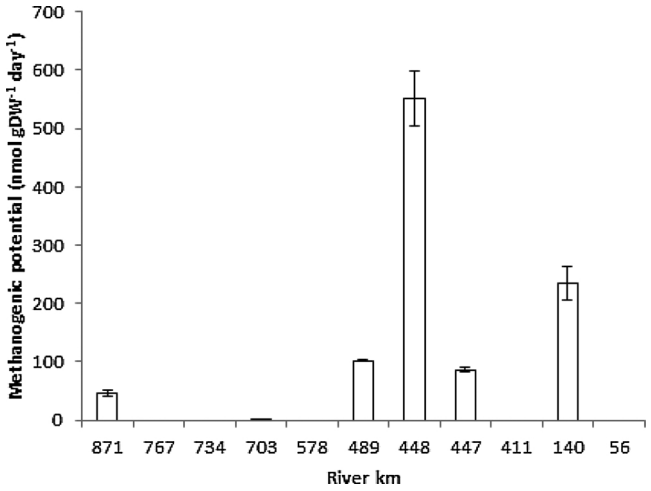

Fig. 2. Methanogenic potential of sediments (mean values ± SE).

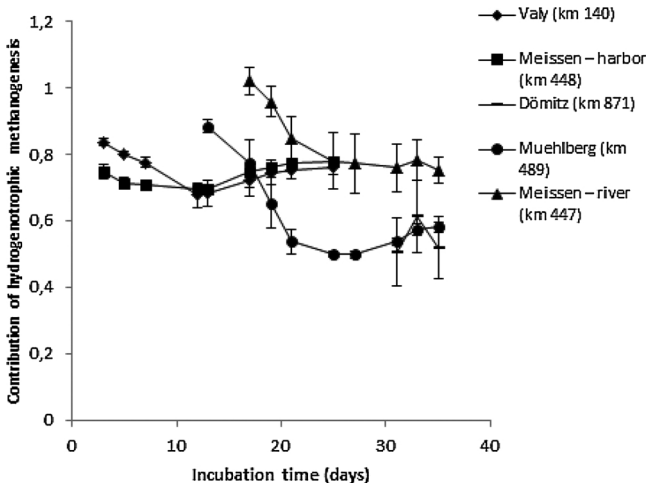

Fig. 3. Contribution of hydrogenotrophic methanogenesis (H_2/CO_2) to total CH_4 production of examined sediments during incubation experiments (mean values ± SE).

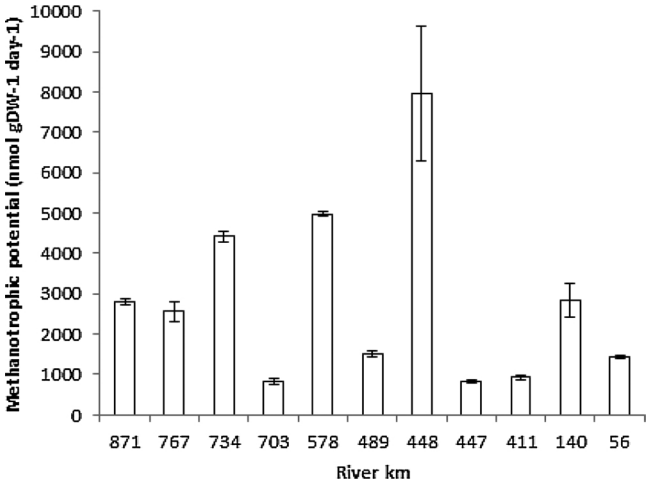

Fig. 4. Methane oxidation potential of sediments (mean values ± SE).

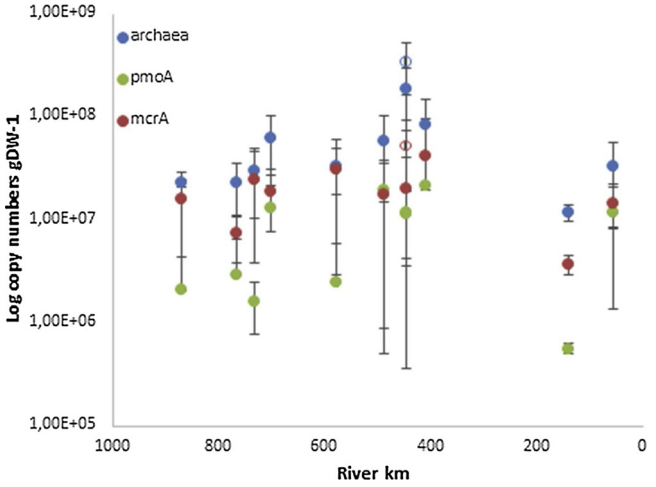

Fig. 5. Abundance (log copy numbers gDW^{-1}) of total archaea, methanotrophs (pmoA) and methanogens (mcrA) in examined sediments (mean values ± SE). Open symbols represent site Meissen – harbour (km 448).

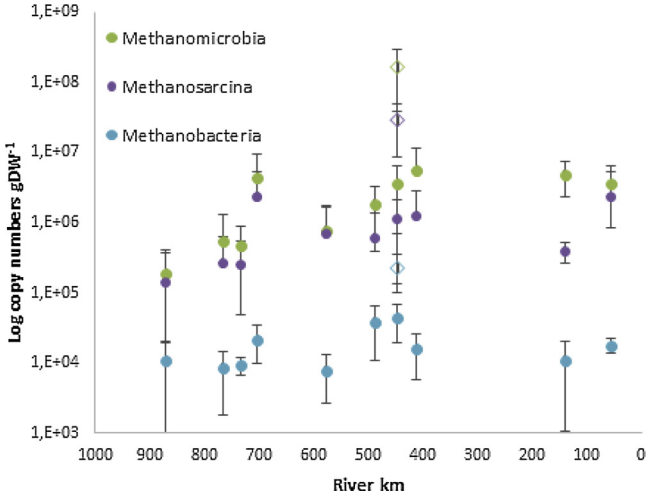

Fig. 6. The abundance (log copy numbers gDW^{-1}) of individual orders of methanogens in the examined samples determined by the group specific qPCR (mean values ± SE). Open symbols represent site Meissen – harbour (km 448).

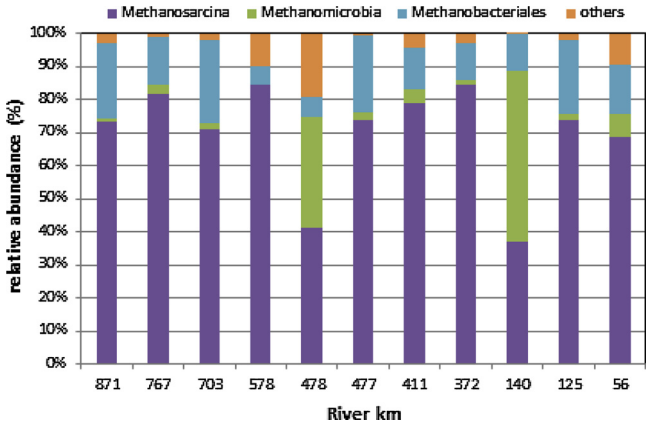

Fig. 7. The relative abundance of individual orders of methanogens in the examined sediments determined by analysis of T-RFLP.

3.2. Population dynamics of archaea (arc), methanogens (mcrA) and methanotrophs (pmoA) in sediments

3.2.1. Quantitative polymerase chain reaction (qPCR)

The abundances of total archaea (16S rRNA gene), methanotrophs (*pmo-A,* coding methane monooxygenases) and methanogens (*mcr-A,* coding for a subunit of the methyl–coenzyme M (CoM) reductase) were determined by qPCR in the fresh samples (Fig. 5). The copy number of archaeal 16S rRNA genes were relatively stable in the range of 10^7 to 10^8 copies per gram dry sediment. The lowest levels were found at the km 140 (Valy), whereas one order of magnitude higher values could be reported from the km 448 (Meissen – harbour) and km 447 Meissen-river sediments. Copy numbers for *mcr-A* and *pmo-A* were 10^6 to 10^7. While *mcr-A* copies were highest at the km 448 (Meissen - harbor), *pmoA* had its maximum at the km 411 (Wachwitz).

The results of the group specific qPCR revealed a similar order of magnitude but showed higher fluctuations. The highest counts were reported for *Methanomicrobiales* and *Methanosarcinales* (10^5 to 10^8 copies per gram dry sediment). Again the Meissen-harbour sample (km 448) showed the highest copy numbers while otherwise there was a slight decrease of copy numbers from spring to mouth for both methanogenic orders. *Methanobacteriales* showed more variability along the river and ranged from 10^3 to 10^5 copies per gram dry sediment (Fig. 6).

3.2.2. Terminal restriction length polymorphism of mcr-A gene

The composition of the methanogenic communities along the Elbe River was determined by an analysis of T-RFLP targeting the *mcr-A* gene in the sediment samples (Fig. 7). All localities were dominated by the T-RF's attributed to *Methanosarcinales* (27–84 %) followed by T-RF's assigned to *Methanobacteriales* (5–60 %). Methanogens belonging to *Methanomicrobiales* were found at a very low level. A number of very long T-RF's of (506-10bp) could not be identified to known T-RF's. Details on TRFLP results are given in the supplementary material (Fig. S2).

4. Discussion

4.1. Methanogenic and methanotrophic potential of the sediments

Despite incubation under wet anoxic conditions, methanogenic activity was detected for roughly half of the samples (six out of eleven sampling sites). This might be caused by lower level of organic substrates in inactive sediments (carbon content below 1%) or by the availability of alternative electron acceptors (dissolved NO_3^-, SO_4^{2-}, Fe^{3+}) in well-oxygenated river surface sediments (Huttunen et al., 2006; Duc et al., 2010). For instance, hardly detectable CH_4 production was also observed in Amazonian white water lakes, probably due to relatively high iron and low organic carbon (below 1.5%) content (Conrad et al., 2014).

The CH_4 formation in the analysed sediments did not show any clear trend along the Elbe River profile. However, sites with high methanogenic potential (Meissen–harbour and Valy) were characterised by fine sediment fraction and high organic carbon content compare to other sampled sites (see Table 1) signifying importance of these local factors in methane production. Many authors (e.g. Sanders et al., 2007; Maeck et al., 2013; Sollberger et al., 2014; Bednařík et al., 2017) indicated that sites, where fine and organic matter rich sediment is accumulated, are particularly active sites of CH_4 production. Moreover, sites with high methanogenic potential (Meissen–harbour and Valy) in our study coincided with high CH_4 concentration in the surface water (for details see Matoušů et al., 2018). This implies that the CH_4 input into the water column may originate in some hot-spots of CH_4 production rather than a continuous supply from the sediment. Despite of nearby existence of large city (Dresden) and its possible anthropogenic pollution, there was not observed unusually increased methanogenic activity in the sediment sample from the river channel in Meissen (km 447) or Wachwitz (km 411). Therefore, increased methanogenic potential in this study is rather linked to local factors allowing accumulation of sediment than to potential pollution from city (Meissen or Dresden). Similarly, there

Table 2
Overview of literature values regarding the isotopic analysis of CH_4 and its production in river sediments.

Site	δ ^{13}C of CH_4 (‰)	f_{mc} (%)*	CH_4 production potential (nmol gDW^{-1} d^{-1})	δ ^{13} C_{org} (‰)	Reference
inflows of Lake Biwa, Japan	−64 to -47	n.s.	n.s.	n.s.	Murase et al., 2003
White Oak River, North Carolina	−70.8 to -65.2	18 to 42	n.s.	n.s.	Avery and Martens, 1999
three streams in eastern Amazonia	−75.1 to -52.7	n.s.	n.s.	−29.7 to -22.8	Moura et al., 2008
five rivers in USA	−56.6 to -36	n.s.	n.s.	n.s.	Sansone et al., 1999
Sitka Stream	−98.6 to -48.2	26 to 51	0 to 960	−26.7 to -25.8	Mach et al., 2015
Elbe River	−71.1 to -54.1	52 to 78	0 to 645	−27.2 to -0.4	Bednařík et al. (this study)
Morava River	−63.9 to -52.5	37 to 89	0 to 1,999	−28.5 to -26.2	Bednařík et al., 2017
River Itchen, U.K.	−58	33	528 to 1,920	n.s.	Shelley et al., 2015
Nine rivers in Germany	n.s.	n.s.	120 to 720	n.s.	Gebert et al., 2006
River Frome, England	n.s.	n.s.	48 to 384†	n.s.	Sanders et al., 2007

n.s. = not specified.
* f_{mc} = part of hydrogenotrophically produced methane.
† nmol CH_4 g^{-1} (wet sediment) h^{-1}.

were not observed significantly different physico-chemical parameters in these sites, which is presented in our previous study (Matoušů et al., 2018). Nevertheless, effect of anthropogenic pollution in rivers on methanogenic activity and river CH_4 concentration exists and it was previously reported for instance by Dzyuban, 2011 in polluted tributaries of the Rybinsk Reservoir or by Alshboul et al. (2016) in effluents and receiving streams downstream of the municipal wastewater treatment plants in Germany.

The methanogenic potential presented in this study for the Elbe River is in the range of the values reported for other streams and rivers (0–1,990 nmol gDW^{-1} d^{-1}; Table 2). Previously reported methanogenic potential from a lower part of the Elbe River by Gebert et al. (2006) reaches a similar range of values as measured in this study. This comparison suggests that obtained results reported here probably correspond to general natural capacity of this environment. It also shows that similar representative results can be reached with different methodological approaches (incubation time, sediment amount). However, methanogenic potential is significantly lower (0.01 and 3.99 µmol gDW^{-1} d^{-1}) compared to CH_4 production in lakes and rice paddy soils (Yao et al., 1999; Conrad et al., 2010; Duc et al., 2010).

Results of this study demonstrate that CH_4 in sediments of the Elbe River is produced predominantly from CO_2 reduction. Although acetoclastically produced CH_4 should theoretically prevail (Conrad, 1999), dominance of hydrogenotrophic methanogenesis is not unusual for freshwater ecosystems (Krüger et al., 2002; Galand et al., 2010; Conrad et al., 2010, 2014). A higher contribution of hydrogenotrophic methanogenesis is probably connected to only a partial oxidation of organic matter as described in more detail in Conrad et al. (2009). Although samples in this study were taken from the sediment surface layer (0–10 cm), generally well oxygenated (i.e. with complete degradation of organic matter), microzones with low oxygen level are likely to occur (Boulton et al., 1998). These anoxic microzones may provide places for anaerobic processes like methanogenesis and for incomplete degradation of organic matter in the surface sediments (Deborde et al., 2010). A more balanced contribution of methanogenic pathways to CH_4 production was found in the Sitka stream sediments, while hydrogenotrophically produced CH_4 reached 36–51 % (Mach et al., 2015). Similarly high contributions of hydrogenotrophic methanogenesis (57–90 %) were detected in the Morava River sediments upstream of the weirs, where fine and organic rich sediments were accumulated (Bednařík et al., 2017).

In contrast, methanotrophic activity (measured under substrate addition) occurred in all samples, while the CH_4 oxidation potential was comparable to other reports from various freshwater ecosystems (Bender and Conrad, 1994; Sanders et al., 2007; Shretsha et al., 2010). However, it should be noted, that the CH_4 production and oxidation rates were not measured under in situ conditions and with addition of substrate in case of CH_4 oxidation measurements. Thus they represent potential rates suitable for comparison of studied sites, but they do not allow mass balance calculation. Moreover, comparison of the CH_4 oxidation rates between different studies is very limited, because obtained results can be highly affected by the diverse incubation setting (e.g. incubation temperature, initial CH_4 concentration) due to strong substrate and temperature dependence of oxidation rates (Shelley et al., 2015).

The limitation of presented study is given by only one sampling campaign (one season, one water discharge). However, the aim was the comparison of different locations along the Elbe River. Changes in the environmental factors associated with different seasons or discharge conditions are less relevant for the sediment analyses based on experimental methods using incubation of samples under stable and same conditions (this study), while it is highly relevant for studies dealing with in-situ measurements of CH_4 concentration in the surface water or its emissions to the atmosphere. These parameters can change rapidly depending on environmental conditions and it is widely discussed in the literature (e.g. Stanley et al., 2016; Borges et al., 2018). Basically, it can be expected that in-situ CH_4 production rates will be supported by increasing water temperature in summer months or due to high sedimentation rate during the low water period. Increasing water discharge can also affect the rate of water exchange between surface water and sediments, while rapid vertical hydrological exchange leads to higher oxygen saturation in the uppermost sediment layer (Malard et al., 2002), which consequently suppresses the methanogenic activity.

4.2. Molecular analyses of sediments

The molecular data suggests that even though the overall numbers on the group level are quite stable, the community composition is much more variable. In general *Methanomicrobiales* and *Methanosarcinales* are the most dominant methanogens detected followed by *Methanobacteriales*. This was supported by the TRFLP results, which also showed the dominance of *Methanosarcina* and *Methanomicrobia*. However, the qPCR results can not directly be compared to T-RFLP since T-RFLP is based on the highly degenerated *mcrA* primers and it gives only relative abundances, while the order specific q-PCR is supposed to provide reasonable estimates of absolute numbers for the respective methanogenic order according to the standards used. The methanogenic community based on T-RFLP of *mcrA* has been so far primarily described for rice field soils (Lueders et al., 2001; Ramakrishnan et al., 2001; Chin et al., 2004; Kemnitz et al., 2004; Conrad et al., 2008). In rice field soil the TRFLP patterns are more diverse and contain additional methanogenic orders (Lueders et al., 2001; Ramakrishnan et al., 2001; Chin et al., 2004; Kemnitz et al., 2004; Conrad et al., 2008). A recent study in river sediments also found *Methanosarcina* as dominant TRF (Chaudhary et al., 2017.)

In general, our results are in good agreement with reported

methanogenic community profiles from other freshwater habitats: *Methanosarcinales* and *Methanomicrobials* have been described as dominant methanogenic members using various archaea/methanogen-specific primers, e.g. from freshwater river and estuarine sediment (Munson et al., 1997; Purdy et al., 2002; Buriánková et al., 2013; Brablcova et al., 2014), as well as from peat bog sites (Galand et al., 2005), freshwater lake sediments (Falz et al., 1999; Koizumi et al., 2004), Florida Everglades wetland soils (Castro et al., 2004), hydrocarbon-contaminated aquifer (Kleikemper et al., 2005) and deep-sea hydrothermal sediments (Dhillon et al., 2005).

When we compare our molecular methods-based results with the activity measurements, it is obvious that they seemingly do not fit together: while we found ten times higher methanotrophic potential compared to the methanogenic potential, the microbial abundance are quite congruent. However, methanotrophs relay on a constant flux of two gaseous substrates: oxygen and methane and hence appear in high quantities at the oxic-anoxic interface where the oxygen and CH_4 gradients overlap. We analysed a sediment mixture of the top 10 cm where the methanotrophs became dispersed, which may explain why they displayed a high activity when stimulated with a specific substrates. In contrast, methanogens are active in all anoxic parts of the sediment and generally more dispersed than methanotrophs.

The second discrepancy is that we found a high contribution of hydrogenotrophic methanogenesis based on our isotope analysis, while our molecular studies revealed high numbers of potentially acetoclastic methanogens (*Methanosarcinales*) and a lower number of the strict hydrogenotrophic methanogens (*Methanobacteriales* and *Methanomicrobiales*). This may have two reasons: On one hand, *Methanosarcinales* can live acetoclastic as well as hydrogenotrophic, and hence as the more substrate versatile microbes may use different substrates according to the environmental conditions. On the other hand, it is generally observed that the microbial abundance and community patterns only rarely correlate with their activity (Mach et al., 2015; Chaudhary et al., 2017). This can be confirmed by our results, demonstrating that the activities (methanogenic and methanotrophic potential rates) correspond only to a certain extent to the molecular data. *Methanosarcina* have been described as relatively oxygen tolerant, containing a series of genes encoding oxygen detoxification (Zhang et al., 2006; Angel et al., 2011).

5. Conclusions

The methanogenic potential of the sediments (using the natural available substrate) showed CH_4 production potential comparable to previously published river systems. However, only approximately half of the samples could be activated (most probably due to substrate limitation) and these samples showed a strong variance (over one to two orders of magnitude). However, all sediment samples showed a methanotrophic potential (under substrate addition), while it differed by one order of magnitude between studied sites.

Molecular analyses of the underlying microbial community revealed constant quantities of several marker genes (16S archea, mcrA, pmoA) over the river continuum. This suggest that the observed variability of the microbial activities (i.e. CH_4 production and oxidation) as well as the resulting CH_4 concentrations in the water column are only indirectly linked to the presence of different microbial guilds, but rather affected by their activity (which has not directly been tested in this study). Further insight into the methanogenic community (TRFLP and the group specific qPCR) revealed large variations in the methanogenic populations of different sediment samples.

Coming back to our original hypothesis mentioned in the introduction, we see no trend in the studied parameters along the Elbe River. However, we found hotspots of the measured CH_4 processes (Figs. 2 and 4) due to other local driving factors (e.g. carbon content, etc.). Therefore, based on results presented in this study, spatial heterogeneity of sediment characteristics (grain median size, carbon content) seems to be more relevant for prediction of methanogenic or methanotrophic activity than only abundance of methanogens or methanotrophs.

Acknowledgements

This project was financially supported by project GAJU 145/2013/D, by project GAČR-13-00243S (PI-K. Šimek), and by project CZ.1.07/2.3.00/20.0204 (CEKOPOT) co-financed by the European Social Fund and the state budget of the Czech Republic. The gratitude belongs to Prof. Jan Kubečka for providing the Thor Heyerdahl research vessel, further to Jakub Matoušů for help with graphic and to Dr. Tomáš Jůza for the organizing and providing the logistical support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.limno.2019.125716.

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