

# Methane formation and consumption by sediments in a cross-channel profile of a small river impoundment

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## ABSTRACT

Rivers are a natural source of methane (CH<sub>4</sub>) into the atmosphere and may contribute significantly to total CH<sub>4</sub> emissions. Even though the details of sources of CH<sub>4</sub> in rivers are not fully understood, weirs have been recognized as a hotspot of CH<sub>4</sub> emissions. In this study, we investigated CH<sub>4</sub> production and consumption in air-exposed river sediments along a cross-channel transect located upstream of a weir. Stable carbon isotopes were used for determination of individual methanogenic pathways. In order to understand the relationship between physicochemical and biological processes, additional parameters such as organic matter, grain median size, and carbon and nitrogen content were characterized as well. Generally, samples from the surface sediment layer (0-10 cm) had higher CH<sub>4</sub> production than sediments from the deeper layer (10-20 cm) during the incubation experiments. Sediments near the bank zones and in the mid-channel were characterized by the highest organic carbon content (6.9 %) as well the highest methanogenic activity (2.5 mmol g<sup>-1</sup> DW d<sup>-1</sup>). The CH<sub>4</sub> production was predominated by H<sub>2</sub>/CO<sub>2</sub> dependent methanogenesis in the surface sediment layer (0-10 cm), while the proportion of acetoclastic and hydrogenotrophic methanogenesis in the deeper sediment layer (10-20 cm) was balanced. The CH<sub>4</sub> oxidation potential of sediments showed the same spatial pattern as observed for the CH<sub>4</sub> production. Our results showed high spatial variability of sediment CH<sub>4</sub> production and oxidation in the cross-channel profile upstream of the weir, whereas the highest CH<sub>4</sub> dynamics were observed in the littoral zones. This variability was closely linked with the carbon and nitrogen content in the sediment samples.

## INTRODUCTION

Inland freshwater habitats including streams and rivers have been recognized to be an important source of methane (CH<sub>4</sub>) into the atmosphere (Bastviken *et al.*, 2011; IPCC, 2013; Deemer *et al.*, 2016). Recent studies show that impounded river zones are CH<sub>4</sub> emission “hotspots”, significantly enhancing our estimations of CH<sub>4</sub> emissions from rivers (Maeck *et al.*, 2013, Wilkinson *et al.*, 2015). River reaches immediately upstream of impoundments are characterized by significantly changed physicochemical parameters of the water, creating transitions between lentic and lotic water ecosystems (Gao *et al.*, 2013). For example, Ogbeibu and Oribhabor (2002) found significantly lower

water transparency, current velocity and concentration of dissolved oxygen in reservoirs compared to rivers. The CH<sub>4</sub> emitted from these sites is mostly derived from sediment CH<sub>4</sub> production resulting from an increased sedimentation rate and poor vertical mixing, causing dissolved oxygen decline and anaerobic sedimentary activity upstream of the impoundments (Barth *et al.*, 2003; Maeck *et al.*, 2013). However, spatial variability of CH<sub>4</sub> production and oxidation in these environments is poorly understood, as is the contribution of individual methanogenic pathways to the total CH<sub>4</sub> production.

In principal, CH<sub>4</sub> is produced during anaerobic degradation of organic matter in freshwater sediments (Zinder, 1993). Anaerobic degradation of carbohydrates results in two dominant intermediates (H<sub>2</sub>/CO<sub>2</sub> and acetate), which are further processed by two different metabolic pathways of CH<sub>4</sub> production - hydrogenotrophic methanogenesis (using H<sub>2</sub>/CO<sub>2</sub>) and acetoclastic methanogenesis (using acetate). Quantification of the relative contribution of both sources can be made by using stable carbon isotopic signals, due to different <sup>13</sup>C/<sup>12</sup>C fractionation during conversion of CO<sub>2</sub> and acetate methyl to CH<sub>4</sub> (Conrad, 2005). Contribution of these methanogenic pathways to total CH<sub>4</sub> production differs in various freshwater habitats. A fraction of hydrogenotrophic methanogenesis is usually prevailing in lake sediments (Conrad *et al.*, 2011), while acetoclastic methanogenesis dominates in rice paddy soils (Scavino *et al.*, 2013) and peatlands (Galand *et al.*, 2010).

Studies dealing with the spatial variability of CH<sub>4</sub> production in the sediments of lakes, reservoirs and rivers

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emphasize littoral zones as main sites of methanogenic activity (Bastviken *et al.*, 2008; Musenze *et al.*, 2014; Yang *et al.*, 2014). Murase *et al.* (2005) found that littoral sediments of lakes can reach substantially higher CH<sub>4</sub> production than profundal sediments, and further served as a source of dissolved CH<sub>4</sub> in lakes together with tributary rivers. Increased CH<sub>4</sub> release from littoral sediments is likely given by (1) greater availability of labile organic matter from the aquatic vegetation, (2) wave turbulence and bottom shear stress, which enhance sediment flux rates, and (3) higher temperatures in summer months, which in turn support higher CH<sub>4</sub> production rates (Bussmann, 2005; Hofmann *et al.*, 2010).

Similarly, there is an evident spatial distribution of surface water CH<sub>4</sub> concentration in large rivers. Richey *et al.* (1988) and Anthony *et al.* (2012) have shown CH<sub>4</sub> cross-channel gradients with increased CH<sub>4</sub> concentrations observed nearby the banks compared to mid-channel, while Sawakuchi *et al.* (2014) observed a different trend in the Amazon and Pará rivers, with high mid-channel CH<sub>4</sub> concentrations and fluxes. However, the cross-channel variability of CH<sub>4</sub> is not usually included in river studies because the mixing of the entire water column in streams and rivers is assumed. Studies dealing with the spatial distribution of CH<sub>4</sub> in the sediments of rivers report higher CH<sub>4</sub> concentration in pore water of nearshore and riparian habitats, while the hyporheic sediments in mid-channel have usually lower CH<sub>4</sub> concentrations (Jones *et al.*, 1995; Crawford *et al.*, 2014). This pattern probably results from the different rate of water exchange between surface water and sediments, leading to oxygen depletion in the uppermost sediment layer of nearshore habitats (Malard *et al.*, 2002), while the sediments on the central river bottom are oxygenated to a large depth due to rapid vertical hydrological exchange with the flowing water column (Fischer *et al.*, 2005). Consequently, oxygen depletion together with high sedimentation rate and supply of allochthonous labile organic matter from the riparian vegetation creates suitable conditions for high methanogenic activity in nearshore sediments (Jones *et al.*, 1995; Jones and Mulholland, 1998; Stanley *et al.*, 2016). However, it should be noted that microbial activity in total (including aerobic and anaerobic bacterial metabolism) is highest in the central channel, only due to connectivity with the surface water, which supplies the organic matter into deeper sediment layers (Fischer *et al.*, 2005).

Our previous study revealed a significant effect of weir impoundments on methane river dynamics (production, oxidation, emission, methanogenic pathways) compared to usual river reaches (Bednařík *et al.*, 2017), but a study describing the cross-channel variability of CH<sub>4</sub> in the sediments upstream of weirs is not known to us. Hence, the overall aim of this study was to get more detailed information about the spatial

variability of CH<sub>4</sub>-related processes in a river impoundment. For this purpose, we examined i) CH<sub>4</sub> production and oxidation rates of the sediments; ii) relationships between environmental variables; and iii) contribution of the methanogenic pathways to total CH<sub>4</sub> production using stable carbon isotopes, all in a cross-channel profile and two different sediment depths of a small river impoundment in Central Europe.

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## METHODS

### Study site and sampling

The study area was located upstream of a weir situated in the Morava River, Czech Republic (Fig. 1; 49°35′12″N, 17°15′43″E). The Morava River is a seventh-order river (according to Strahler, 1957) with a mean annual water discharge of 26.4 m<sup>3</sup> s<sup>-1</sup> in the area of our study site. The impoundment upstream of the weir is 40 m width at its widest point, and has a maximum depth of 3.2 m. The backwater length is approximately 2.6 km. Riparian vegetation is composed mainly by grasses, including reeds and willows. The river channel was without any aquatic macrophytes.

Sediment samples were collected along the cross-section profile of the impoundment (Fig. 2). Triplicates were taken by a piston corer from eight different distances from the bank line to the mid-channel (0 m, 2 m, 4 m, 6 m, 8 m, 10 m, 12 m, 14 m) and from two sediment depths (0-10 cm and 10-20 cm) for each distance. Samples were collected during artificial reduction of the water level in July 2014 caused by weir manipulation, and allowing efficient and accurate sediment sampling when the sediments were shortly exposed to air.

### Incubation experiments

Sediments intended for incubation experiments were sieved through a 1-mm sieve to remove coarse detritus, stones or invertebrates, and stored at 4°C until subsequent analyses and laboratory experiments were carried out. Samples for granulometric analysis were dried and then sieved through a system of ten sieves of decreasing mesh sizes. All separate fractions of the sediment grain sizes were weighed, and grain median size was analyzed using the software Gradistat (ver. 8.0) (Blott and Pye, 2001). The C, N, and H content of the sediments was quantified on a CHNS-element analyzer (vario MICRO cube, Hanau, Germany) by the Analytical Chemical Laboratory of the University of Marburg, Germany. The dry weight of the sample was determined gravimetrically.

For determination of CH<sub>4</sub> production potential and methanogenic pathways, approximately 30 g (wet weight) of the sediments were transferred into 60-mL sterile serum bottles in triplicates, flushed with N<sub>2</sub>, closed with butyl

rubber stoppers and incubated at 25°C in a dark room. At the start of the incubation (before flushing with N<sub>2</sub>), 5 mL of distilled autoclaved water was added into each bottle for sampling of the liquid phase. The liquid phase was sampled at the end of the incubation for analyses of concentration and δ<sup>13</sup>C of acetate. The gas headspace of half of the bottles was supplemented with 3% CH<sub>3</sub>F to specifically inhibit acetotrophic methanogenesis (Janssen and Frenzel, 1997). Gas samples (200 µl) were taken repeatedly (twice a week) during the course of incubation (4-6 weeks) and analyzed for concentrations of CH<sub>4</sub>, CO<sub>2</sub>, and δ<sup>13</sup>C of CH<sub>4</sub> and CO<sub>2</sub>. The CH<sub>4</sub> concentration was analyzed by gas chromatography (GC) using a flame ionization detector (Shimadzu, Kyoto, Japan) and CO<sub>2</sub> concentration was analyzed after conversion to CH<sub>4</sub> with a methanizer (Ni-catalyst at 350°C, Chrompack, Middelburg, the Netherlands).

Twenty grams (wet weight) of sediment samples for determination of the CH<sub>4</sub> oxidation potential were placed in sterile bottles (250 mL) in triplicates, closed by a cap with PTFE silicone septa with ambient air in the headspace and then supplemented with CH<sub>4</sub> to give a final concentration of 10,000 ppm. The incubation was performed at 25°C in a dark room. The concentration of CH<sub>4</sub> in the headspace of each bottle was measured at 0 h and then ten times over 190 h. The CH<sub>4</sub> production and oxidation potentials were calculated from the slope of CH<sub>4</sub> concentration change over time.

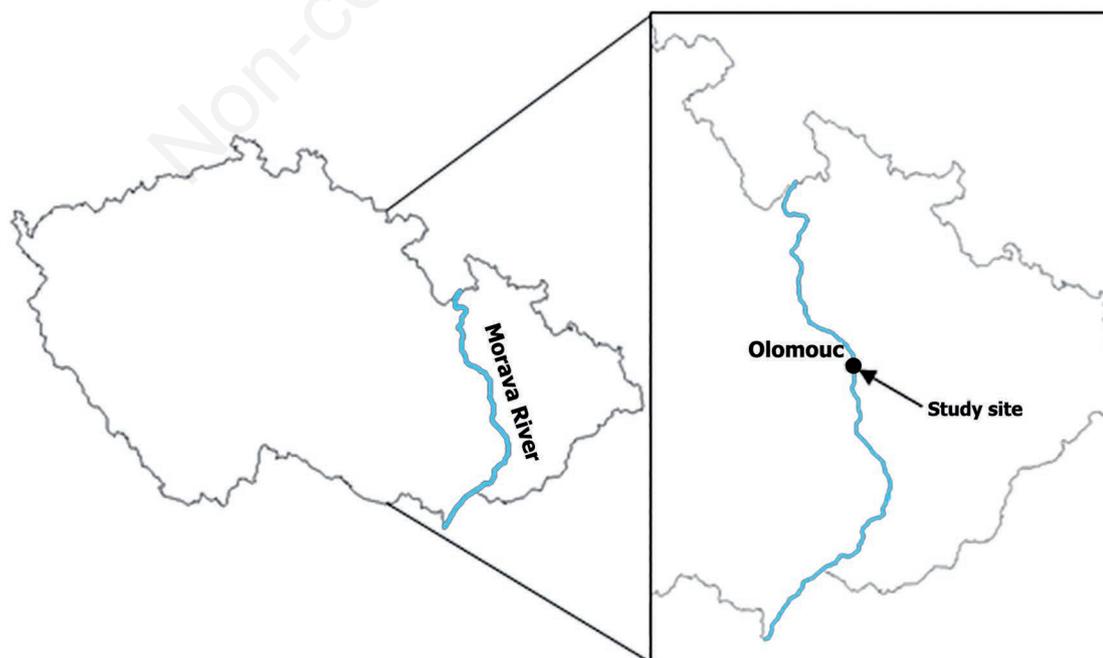
### Isotopic analyses and calculations

Isotope measurements of <sup>13</sup>C/<sup>12</sup>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 principal operation has been described by Brand (Brand, 1996). Other details are given in Penger *et al.* (2012) and Blaser *et al.* (2013). 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; 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).

Isotopic calculations of fractionation factors and estimation of the approximate partition of hydrogenotrophic methanogenesis to total methanogenesis were calculated according to a previously published procedure (Conrad, 2005; Blaser and Conrad, 2016).

In principal, partition of hydrogenotrophic methanogenesis was calculated by the following mass balance equation:

$$f_{mc} = (\delta_{CH_4} - \delta_{ma}) / (\delta_{mc} - \delta_{ma}) \quad (\text{eq. 1})$$



**Fig. 1.** Location of the Morava River in the Czech Republic and position of the study site in the river.

where  $f_{mc}$  is the fraction of hydrogenotrophic methanogenesis;  $\delta_{CH_4}$  is the directly measured isotopic signature of the carbon in  $CH_4$ ;  $\delta_{mc}$  is the carbon isotopic signature of  $CH_4$  solely produced from  $CO_2$  (directly measured from assays inhibited by methylfluoride) and  $\delta_{ma}$  is the carbon isotopic signature of  $CH_4$  solely produced from acetate, the latter calculated from the following equation:

$$\delta_{ma} = (1/\alpha_{ma})(\delta_{ac} + 10^3 - \alpha_{ma}10^3) \quad (\text{eq. 2})$$

where  $\alpha_{ma}$  is the fractionation factor for acetoclastic methanogenesis ( $\alpha_{ma}=1.009$ ; Govert and Conrad, 2009) and  $\delta_{ac}$  is the measured isotopic signal of acetate.

### Statistical analysis

Data analyses were performed using the software STATISTICA 12 (StatSoft, 2013). The Mann-Whitney U test was used for examination of differences between surface and deeper sediment layers, as well as between individual distances from the bank. Spearman's correlation analysis was used to find the relationship between variables. The significance level of  $P < 0.05$  was applied for all statistical analyses.

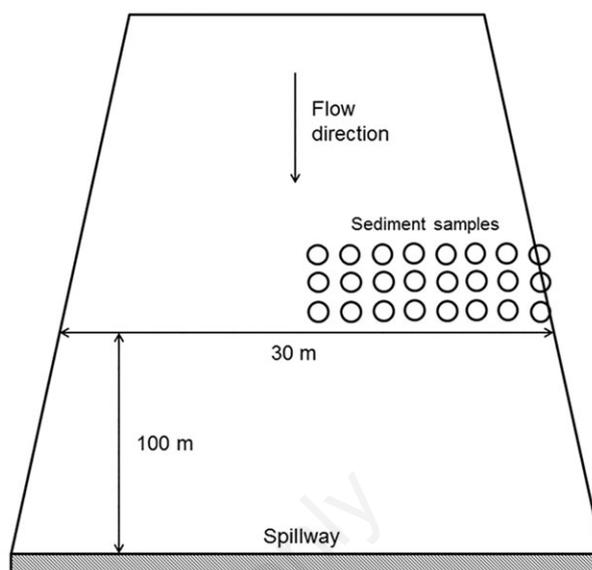
## RESULTS

### Sediment characteristics

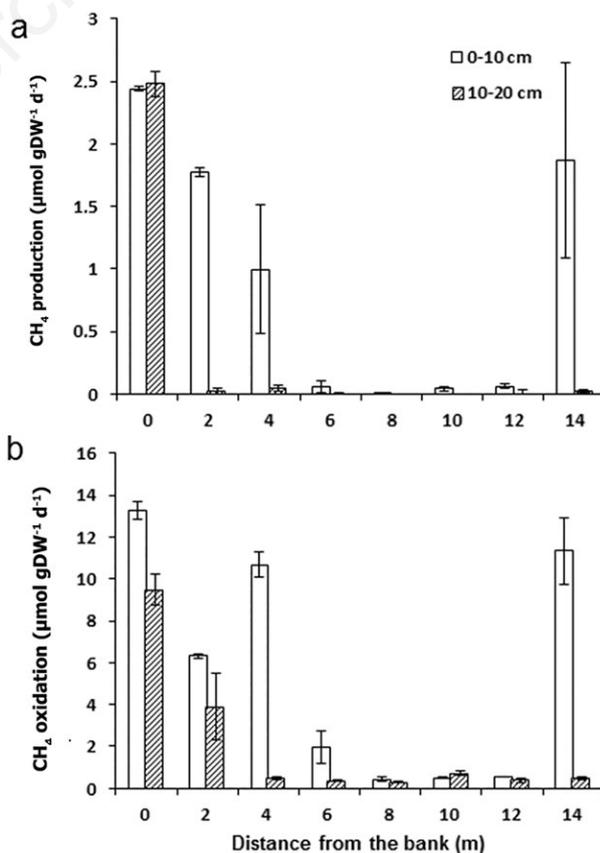
The median grain size of sediments ranged from 0.3 to 9.8 mm, and it was significantly smaller at the surface sediment layer (0-10 cm; mean  $2.4 \pm 0.95$  mm) compared to deeper sediments (10-20 cm; mean  $6.5 \pm 1.3$  mm) ( $P < 0.05$ ). The carbon content ranged from 0.1 to 7.0% and it was significantly higher in the surface sediment layer (mean  $2.5 \pm 0.6\%$ ) compared to deeper sediments (mean  $1.1 \pm 0.5\%$ ) ( $P < 0.05$ ). Similarly, the nitrogen content ranged from 0.01 to 0.67% and was significantly higher in the surface sediment layer (mean  $0.24 \pm 0.05\%$ ) compared to deeper sediments (mean  $0.11 \pm 0.04\%$ ) ( $P < 0.05$ ). The C/N ratio was almost the same in all samples and both sediment depths, with a mean of  $10 \pm 0.1$  (Tab. 1).

### Methane production and oxidation by sediments

Mean  $CH_4$  production potential ranged between 0 and  $2.4 \mu\text{mol gDW}^{-1} \text{d}^{-1}$  (Fig. 3A). The methane production of nearshore sediments (0-4 m; mean  $1.3 \pm 0.3 \mu\text{mol gDW}^{-1} \text{d}^{-1}$ ) was significantly higher compared to the samples (6-14 m; mean  $0.2 \pm 0.1 \mu\text{mol gDW}^{-1} \text{d}^{-1}$ ) ( $P < 0.05$ ). In total, the surface sediment layer (0-10 cm; mean  $0.9 \pm 0.2 \mu\text{mol gDW}^{-1} \text{d}^{-1}$ ) had significantly higher  $CH_4$  production than the deeper layer (10-20 cm; mean  $0.3 \pm 0.2 \mu\text{mol gDW}^{-1} \text{d}^{-1}$ ) ( $P < 0.05$ ). Mean  $CH_4$  oxidation potential of sediments ranged from 0.5 to  $13.3 \mu\text{mol}$



**Fig. 2.** The schematic of the sediment sampling in the cross-channel profile of a small river impoundment. Triplicates were taken from eight different distances from the bank line to the mid-channel (0 m, 2 m, 4 m, 6 m, 8 m, 10 m, 12 m, 14 m).



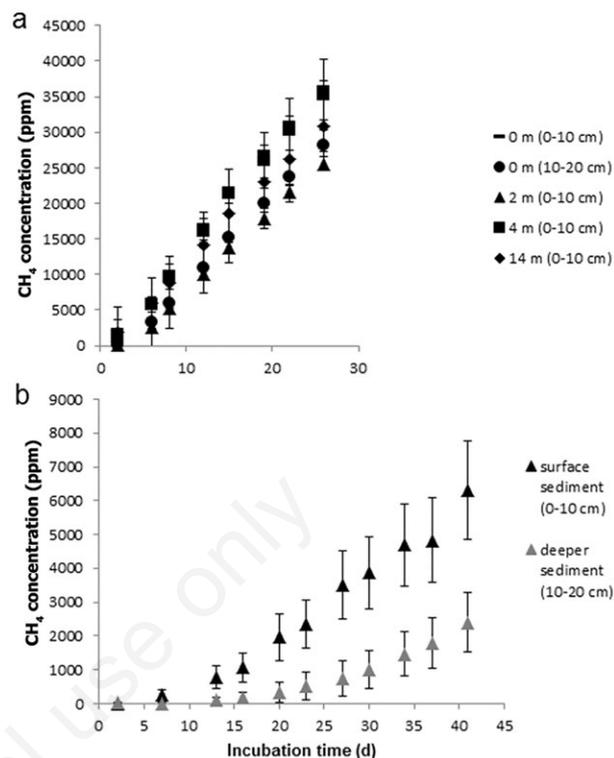
**Fig. 3.** The  $CH_4$  production (a) and oxidation (b) potentials of sediments (mean values  $\pm$  SE).

gDW<sup>-1</sup> d<sup>-1</sup> and showed the same spatial pattern as observed for the CH<sub>4</sub> production potential (Fig. 3B). The nearshore sediments (0-4 m; mean 7.4±1.1 μmol gDW<sup>-1</sup> d<sup>-1</sup>) had higher CH<sub>4</sub> oxidation potential compared to rest of the sampled sediments (6-14 m; mean 1.7±0.6 μmol gDW<sup>-1</sup> d<sup>-1</sup>) (P<0.05). In addition, the surface sediment layer (0-10 cm; mean 5.6±1.1 μmol gDW<sup>-1</sup> d<sup>-1</sup>) reached significantly higher CH<sub>4</sub> oxidation than deeper sediments (10-20 cm; mean 2.0±0.7 μmol gDW<sup>-1</sup> d<sup>-1</sup>) (P<0.05).

Samples with the highest CH<sub>4</sub> production rates were characterized by immediate and linear CH<sub>4</sub> concentration increase during the sediment incubation (Fig. 4A), while the less productive samples were characterized by a lag phase, which takes 10-13 days from the start of the incubation, followed by linear or exponential CH<sub>4</sub> concentration increase (Fig. 4B). The CH<sub>4</sub> concentration in the headspace of the vials incubated for determination of the CH<sub>4</sub> oxidation potential started to decrease after 7 h and continued to decrease linearly over the remaining time of the incubation (Fig. 5). The sediment CH<sub>4</sub> oxidation and production potentials were strongly positively correlated with the carbon content as well as with the nitrogen content in sediments, while it was negatively correlated with the median grain size (Tab. 2).

### Methanogenic pathways

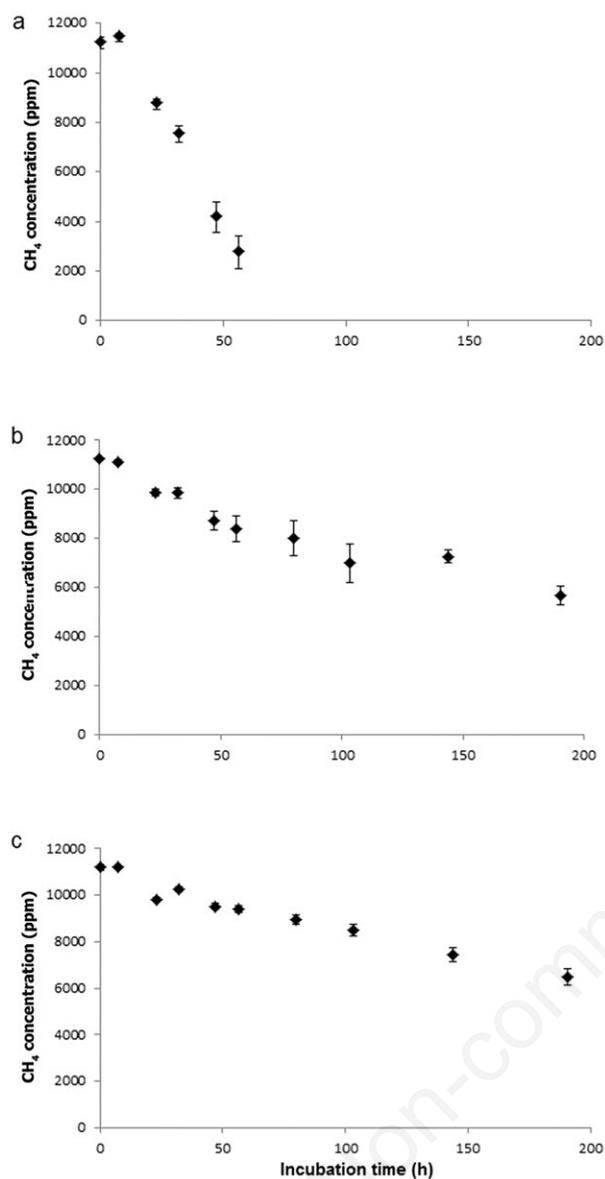
The mean δ<sup>13</sup>C of organic matter in sediments was -27.9±0.4‰ VPDB (n=48) (Tab. 1). The δ<sup>13</sup>C of acetate accumulated in inhibited samples (without acetoclastic methanogenesis) was on average very comparable with



**Fig. 4.** Dynamics of CH<sub>4</sub> formation during the incubation of (a) the most productive sediment samples (mean values±SE), and (b) remaining (*i.e.*, less productive samples characterized by a lag phase lasting more than one week after the start of the incubation) surface (6-12 m; black triangles) and deeper (2-14 m; gray triangles) sediment samples in anoxic conditions (mean values±SE).

**Tab. 1.** Measured characteristics of the sediment samples at two different depths (mean values±SE, n=3).

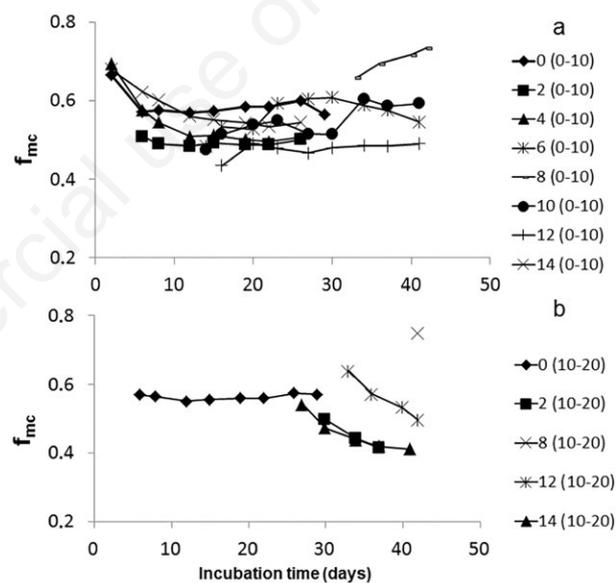
Distance from the bank (m)	Median grain size (mm)	Carbon content (%)	Nitrogen content (%)	C/N ratio	δ <sup>13</sup> C of organic matter
<b>0-10 cm</b>					
0	0.47	6.7±0.1	0.63±0.01	10.6±0.1	-28.3±0.1
2	0.78	5.7±0.1	0.53±0.00	10.6±0.1	-29.3±1.4
4	7.70	2.4±1.3	0.23±0.12	10.2±0.2	-28.1±0.1
6	5.18	0.4±0.1	0.04±0.01	10.1±0.2	-27.2±0.1
8	0.79	0.2±0.0	0.02±0.00	10.1±0.3	-27.1±0.1
10	0.78	0.3±0.1	0.03±0.00	9.9±0.1	-27.4±0.1
12	3.08	0.3±0.0	0.03±0.00	9.8±0.1	-27.2±0.2
14	0.68	4.0±1.6	0.39±0.16	10.1±0.1	-28.1±0.1
<b>10-20 cm</b>					
0	0.26	6.9±0.1	0.66±0.00	10.4±0.0	-27.9±0.1
2	2.45	0.4±0.1	0.04±0.01	10.0±0.1	-27.4±0.2
4	9.75	0.4±0.1	0.04±0.01	10.3±0.4	-27.3±0.0
6	9.36	0.2±0.1	0.02±0.00	9.6±0.2	-26.7±0.1
8	4.46	0.2±0.0	0.02±0.00	10.0±0.1	-26.8±0.1
10	7.93	0.1±0.0	0.01±0.00	8.9±0.2	-26.6±0.3
12	9.21	0.3±0.2	0.03±0.01	9.8±0.3	-27.2±0.3
14	8.86	0.2±0.0	0.02±0.00	10.0±0.2	-27.1±0.1



**Fig. 5.** Methane concentration decrease during the incubation of (a) the most active sediment samples (0 m, 2 m, 4 m, 14 m), (b) remaining surface (0-10 cm) sediments (6-12 m), and (c) remaining deeper (10-20 cm) sediments (6-12 m) in oxic conditions (mean values $\pm$ SE).

$^{13}\text{C}$  of the organic matter with mean  $-26\pm 1.2\%$  VPDB ( $n=25$ ). Acetate did not accumulate in uninhibited samples. The stable carbon isotopic composition of  $\text{CH}_4$  ( $\delta^{13}\text{C}-\text{CH}_4$ ) produced at the end of the uninhibited incubation was on average  $-69.0\pm 1.6\%$  VPDB.

The contribution of hydrogenotrophic methanogenesis ( $f_{\text{mc}}$ ) to  $\text{CH}_4$  production was stable during the whole incubation time and ranged from 41 to 75% for individual samples (Fig. 6). The  $\text{CH}_4$  production was predominated by the  $\text{H}_2/\text{CO}_2$ -dependent methanogenic pathway in the surface sediment layer (0-10 cm) with mean  $f_{\text{mc}}=56\pm 0.02\%$  at the end of the incubation (Fig. 6A). However, the contribution of acetoclastic and hydrogenotrophic methanogenesis to the total  $\text{CH}_4$



**Fig. 6.** The contribution of hydrogenotrophic methanogenesis ( $f_{\text{mc}}$ ) to total  $\text{CH}_4$  production in the samples of (a) the surface sediments (0-10 cm) and (b) the deeper sediment layers (10-20 cm). The missing time points are given by insufficient  $\text{CH}_4$  production rate for stable carbon isotopes measurement of the incubated sample.

**Tab. 2.** Values of the correlation coefficients ( $r$ ) expressing the relationship between the examined variables. All correlations shown are significant at  $P<0.05$ .

Variable	MPP	MOP	Carbon content	Nitrogen content	Median grain size
MPP	1				
MOP	0.67	1			
Carbon content	0.94	0.68	1		
Nitrogen content	0.94	0.70	0.99	1	
Median grain size	-0.62	-0.52	-0.62	-0.63	1

MPP,  $\text{CH}_4$  production potential; MOP,  $\text{CH}_4$  oxidation potential.

production in the deeper sediment layer (10-20 cm) was balanced with mean  $f_{mc}=51\pm 0.05\%$  and prevalence of  $\text{CH}_4$  production from acetate at the end of incubation of the three samples (2 m, 12 m, 14 m; Fig. 6B).

## DISCUSSION

### Spatial changes in $\text{CH}_4$ production and oxidation

In this study, we observed the highest methanogenic potential to be in the surface sediment layer (0-10 cm) at the distance of 0-4 m from the banks. A similar pattern regarding the active littoral sediments has been previously reported for lakes as well as rivers and it is mostly given by hydrological isolation and increased sedimentation rate in the nearshore habitats (Fischer *et al.*, 2005; Murase *et al.*, 2005). Moreover, littoral vegetation not only reduces the flow velocity, but can also serve as an additional source of organic matter (Sanders *et al.*, 2007; Stanley *et al.*, 2016). We expected more homogenous sediment parameters in cross-channel profiles due to the overall decrease of the flow velocity upstream of the weir. However, fine grain size and higher organic carbon content in the nearshore sediments observed in our study indicate a substantially increased sedimentation rate in this habitat. Nevertheless, after the nearshore sediments (0-4 m), we observed high potential  $\text{CH}_4$  formation and oxidation rates in the mid-channel surface sediments (14 m). The best possible explanation is the accumulation of the fresh sediments in mid-channel due to channel morphology, as the mid-channel habitat is the deepest site in the cross-channel profile. In addition, the sediment characteristics (grain median size, carbon and nitrogen content) of the mid-channel samples were very similar to those from the nearshore habitat.

The methanogenic and methanotrophic potentials of the surface sediment layer (0-10 cm) were higher compared to the deeper sediment layer (10-20 cm). Generally, one would expect that better conditions for methanogens might occur in the deeper sediments, where the penetration of dissolved oxygen from the overlying river water is lower and alternative electron acceptors such as dissolved  $\text{Fe}^{2+}$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$  are depleted (Zehnder and Stumm, 1988). However, the results of our study suggested that availability of substrate for methanogens is the main factor driving the rate of  $\text{CH}_4$  production, since a strong positive correlation between  $\text{CH}_4$  production potential and organic carbon content exists ( $r=0.94$ ). High  $\text{CH}_4$  production in the surface sediment layer is not unexpected and has previously been reported in lake sediments (Conrad *et al.*, 2009), river sediments (Mach *et al.*, 2015) and sediments of impounded river zones (Wilkinson *et al.*, 2015).

Another possible factor influencing the  $\text{CH}_4$

production rate is likely the C/N ratio, which is frequently mentioned in studies dealing with the methanogenic activity of sediments. It was found that the total nitrogen content best reflects easily degradable organic substrates available for the methanogens and it is highly correlated with maximum methanogenesis (Yao *et al.*, 1999; Gebert *et al.*, 2006). Duc *et al.* (2010) recognized that the highest potential  $\text{CH}_4$  formation rate is in the sediments with lower C/N ratios ( $<10$ ), while the sediments with higher C/N ratios ( $\sim 20$ ) are characterized by lower  $\text{CH}_4$  formation rates despite the high organic carbon content, which is likely associated with the lability of organic matter. In our study, we observed a similar C/N ratio ( $\sim 10$ ; Tab. 1) across all sediment samples in this study. It may indicate the same source of organic material within all samples, and also confirms that the high variability of methanogenic activity was not caused by the degradability of organic substrates in the examined sediments, but rather by the total amount and the availability of organic substrates for the methanogens.

Despite the higher  $\text{CH}_4$  oxidation potential compared to the  $\text{CH}_4$  production potential of all incubated sediment samples (Fig. 3), sediments represent a source of  $\text{CH}_4$  into the surface water and the atmosphere. As suggested by Bednařik *et al.*, 2017, increased  $\text{CH}_4$  concentrations were observed in surface water, together with high contribution of ebullition to the total  $\text{CH}_4$  emission upstream of the weirs. The discrepancy between  $\text{CH}_4$  production potential and  $\text{CH}_4$  oxidation potential in sediments is given by substrate addition ( $\text{CH}_4$ ) during incubation experiments. Hence, this serves mainly for a comparison of the microbial activity between individual samples and not for calculation of the net  $\text{CH}_4$  flux from the sediments. It would be necessary to measure *in situ*  $\text{CH}_4$  benthic fluxes from the sediments to the surface water using the benthic chamber method in order to determine the net contribution of the sediments to the surface water  $\text{CH}_4$  (Sansone *et al.*, 1998; Bednařik *et al.*, 2015).

We assume that short exposure (several hours) of sediments to air during the reduction of water level upstream of the weir (see Methods, above) had no significant effect on the results presented in this study. Several studies have revealed that methanogenic archaea can survive in aerated and dry soils even in numbers similar to the original state (Mayer and Conrad, 1990; Fetzer *et al.*, 1993). Hernández *et al.* (2019) have recently shown that even after sediment desiccation and rewetting, rates and pathways of  $\text{CH}_4$  production remain similar despite changes in the microbial community composition.

### Methanogenic pathways

Generally,  $\text{CH}_4$  production consists of three distinct phases: i) the first is the lag phase (also reduction phase), during which most of the inorganic electron acceptors in

the sediments, such as nitrate, sulfate or ferric iron, are depleted and only CO<sub>2</sub> is produced; ii) the methanogenic phase, characterized by strong CH<sub>4</sub> formation which maximally depends on the sediment characteristics; iii) in the third phase (the steady state phase), CH<sub>4</sub> production decreases to the stable and long-term level (Yao *et al.*, 1999; Gebert *et al.*, 2006). We observed no lag phase for the most active sediment samples where the CH<sub>4</sub> production was rapid. An absence of lag phase at the start of the CH<sub>4</sub> production could be explained either by i) overlap of the reduction and methanogenic phases (*i.e.*, CH<sub>4</sub> production started at a relatively high redox potential before the full depletion of inorganic electron acceptors; Yao *et al.*, 1999); or ii) inorganic electron acceptors were depleted already before the start of the incubation, because the sediments were fully anoxic (Conrad *et al.*, 2009). However, concentration of the alternative electron acceptors was not measured in our study. Nevertheless, it has been previously shown that the total CH<sub>4</sub> production is strongly negatively correlated with the duration of the lag phase (Yao *et al.*, 1999), which can be completely confirmed by the results of our study.

Despite different rates of CH<sub>4</sub> production, the resulting contribution of individual methanogenic pathways was very similar for all examined samples of surface sediments (0-10 cm). The predominant pathway of CH<sub>4</sub> production in surface sediments was the consistent reduction of H<sub>2</sub> and CO<sub>2</sub> (hydrogenotrophic methanogenesis) throughout the examined samples, which is in agreement with our previous results from sites upstream of the weirs, while the river sections are characterized rather by the predominant acetoclastic methanogenesis (Avery and Martens, 1999; Mach *et al.*, 2015; Bednařík *et al.*, 2017). The stoichiometrically given portion of individual methanogenic pathways (66% from acetate and 33% from H<sub>2</sub>/CO<sub>2</sub>; Conrad, 1999) usually fits well on rice field soils (Conrad *et al.*, 2002; Fey *et al.*, 2004), but it can considerably vary throughout the different freshwater ecosystems (Murase and Sugimoto, 2001; Galand *et al.*, 2010; Conrad *et al.*, 2011). Possible explanations for the deviations in this portion are described for instance in Conrad (1999) and Conrad *et al.* (2009). In the case of higher contribution of CH<sub>4</sub> production from acetate, this can be easily explained by homoacetogenesis (reduction of CO<sub>2</sub> with H<sub>2</sub> via the acetyl-CoA) (Mach *et al.*, 2015), while the higher contribution of the hydrogenotrophic pathway to total CH<sub>4</sub> production can be given by several processes. Basically, we can exclude the not-steady-state conditions because the acetate did not accumulate in the uninhibited samples (without the addition of CH<sub>3</sub>F). Syntrophic acetate oxidation is exceptional in freshwater sediments and unlikely to explain the major part of CH<sub>4</sub> production. The most probable explanation remains incomplete degradation of organic matter, *i.e.*, an additional source of H<sub>2</sub>, which

deflects the resulting contribution of methanogenic pathways, which has been previously observed for lake sediments (Conrad *et al.*, 2009; Conrad *et al.*, 2011).

It is worth noting that hydrogenotrophic methanogenesis was not prevalent in the deeper sediment layer (10-20 cm), where the contribution of the individual methanogenic pathways was equivalent and acetoclastic methanogenesis was prevalent at the end of the incubation experiments of three samples (2 m, 12 m, 14 m). The shift in the contribution of methanogenic pathways was probably not caused by the lability or availability of organic substrate, because of the similarity of these parameters between examined sediment layers (Tab.1). One would expect that the composition of the microbial community can be important for the determination of methanogenic pathways. However, Conrad *et al.* (2011) have shown that the composition of microbial methanogenic communities does not correspond with the resulting contribution of individual pathways of CH<sub>4</sub> production. In spite of the molecular analysis of the methanogenic marker-gene (*mcrA*), which revealed a significantly different methanogenic community for the top layer in contrast to deeper layers, the contribution of individual methanogenic pathways was very similar throughout all examined samples in Mach *et al.* (2015). Similarly, Chaudhary *et al.* (2017) have found no relationship between the absolute numbers of the methanogenic community and the level of CH<sub>4</sub> production. However, studies dealing with the varying contribution of methanogenic pathways in the vertical profile of freshwater sediments are very scarce and deserve to be considered in greater detail in further studies.

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## CONCLUSIONS

We found that the most productive sites in the impounded river zones are littoral sediments; as was previously reported for different freshwater habitats, including lakes and rivers. However, we also observed substantially high CH<sub>4</sub> production in mid-channel sediments, which is likely due to channel morphology causing the accumulation of sediment in this habitat. Hence, the methanogenic and methanotrophic activity of sediments was associated with sites with the finest median grain size of sediments and were best correlated with carbon and nitrogen content. Our results show that it is necessary to consider the sampling location for better representation of particular water habitats. Sediment samples taken only in the littoral zones of water habitats can significantly misrepresent the further extrapolation of obtained results. Considering the substantial sediment CH<sub>4</sub> production potential upstream of weirs, studies focusing on quantification of direct CH<sub>4</sub> fluxes from sediments to surface water and conducted *in situ* are necessary.

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