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Importance of stable carbon isotopes for studying dynamics of methanogenesis in rivers

Ph.D. Thesis

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I, Adam Bednařík, thereby declare that I wrote the Ph.D. thesis myself using results of my own work or collaborative work of me and colleagues and with help of other publication resources which are properly cited.

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Adam Bednařík

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Abstract

Methane is one of the most important greenhouse gases. Despite of recent studies pointing out important contribution of running waters to natural methane emissions to the atmosphere, data concerning the methane sources in rivers are very scarce. This thesis deals with methane dynamic in river ecosystems with special emphasis on an effect of river impoundments on methane related processes. Beside the methane concentrations, oxidation, production and emission to the atmosphere, the changes in contribution of two main methanogenic pathways (hydrogenotrophic and acetoclastic) to the total methane production were determined using the stable carbon isotopes analysis. We found hotspots of the sediment methane production in a river continuum, which are connected with the local driving factors including mainly the existence of artificial barriers as weirs. Changes in rate of individual components of river methane dynamic were further examined in cascade of three weirs and river reaches between them. We found that river impoundments affect the sediment processes in several ways, including changes of the sediment characteristics (fine sediment fraction, higher sediment carbon content), enhanced microbial activities in the sediment (methane production and oxidation), ebullition of methane, and different contributions of hydrogenotrophic methanogens to the released methane. Thus, many parameters found for weir impoundments resemble observations for lake systems. Moreover, remarkable spatial variability in sediment methane production was demonstrated in cross-section profile of the one studied impoundment. Presented studies point to only the part of the samples could be activated for methane production despite of presence of methanogens (most probably due to substrate limitation). This suggest that the observed variability of the microbial activities as well as the resulting methane concentrations in the water column are only indirectly linked to the presence of different microbial guilds, but rather affected by their activity. Altogether our results confirm that the methane dynamics in a river system show a high local variability and that multiple measurements are needed to characterize the sources and fates of the methane. Obtained results might be further used for better estimates of importance of rivers in a global methane budget.

Abstrakt

Metan je spolu s oxidem uhličitým a oxidem dusným řazen mezi nejvýznamnější skleníkové plyny. Navzdory mnoha současným studiím vyzdvihujícím význam říčních ekosystémů jako přírodního zdroje emisí metanu do atmosféry, jsou poznatky týkající se zdrojů metanu v řekách nedostatečné. Předložená práce se zabývá dynamikou metanu v říčních ekosystémech se zvláštním důrazem na vliv umělých příčných bariér (jezů) na procesy spojené s koloběhem metanu. Kromě koncentrací metanu, jeho oxidace, produkce a emisí do atmosféry byl determinován příspěvek dvou hlavních metabolických cest vzniku metanu do jeho celkové produkce, a to s využitím analýzy obsahu stabilních izotopů uhlíku. Na základě provedených měření v říčním kontinuu řeky Labe byly detekovány místa s nezvykle vysokou produkcí metanu v sedimentech, které odpovídaly změnám v lokálních faktorech prostředí spojených především s existencí příčných bariér na vodním toku. Další práce se proto blíže zaměřila na porovnání změn v jednotlivých složkách dynamiky metanu v kaskádě tří jezů a říčních úsecích mezi nimi. Bylo zjištěno, že jezy vyvolávají řadu změn v sedimentačních procesech (usazování jemnější frakce, vyšší obsah organického uhlíku), které se následně projevují v procesech spojených s koloběhem metanu, jako je zvýšená mikrobiální aktivita (produkce a oxidace metanu v sedimentech), vysoký podíl ebulice na celkové emisi do atmosféry (uvolňování metanu ve formě bublin) a rozdílný poměr metabolických cest vzniku metanu. To spolu s ostatními charakteristikami nadjezí vypovídá o tom, že mnoho procesů probíhajících v sedimentech jezových zdrží včetně tvorby bublin a metabolismu uhlíku je lépe srovnatelných s prostředím sedimentů jezer než s říčními sedimenty. Mimo to byla zjištěna také značná variabilita v produkci metanu uvnitř vybraného nadjezí. Další výsledky ukázali, že jen část vzorků inkubovaných v anoxických podmínkách produkuje metan, přestože v nich bylo detekováno srovnatelné množství metanogenních archaea jako v aktivních vzorcích, což bylo s největší pravděpodobností dáno nedostatkem vhodného substrátu. Toto zjištění naznačuje, že pozorovaná variabilita v mikrobiální aktivitě stejně jako výsledné koncentrace metanu ve vodě jsou jen nepřímo řízeny přítomností určitého mikrobiálního společenstva, ale jsou spíše ovlivněny jeho aktivitou. Naše studie tak potvrzuje, že dynamika metanu v říčních ekosystémech vykazuje vysokou prostorovou variabilitu a z toho důvodu lze charakterizovat zdroje metanu a jeho další osud v ekosystému jen s využitím velmi komplexních měření. Získaná data mohou mimo jiné posloužit i jako

cenný údaj pro zpřesnění odhadů významu říčních systémů v bilanci metanu v rámci vnitrozemských vod a v kontextu globální dynamiky metanu.

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Contents

List of publications

The thesis is based on the following papers:

Paper I:

Bednařík A, Blaser M, Matoušů A, Tušer M, Chaudhary PP, Šimek K, Rulík M (2019): Sediment methane dynamics along the Elbe River. Limnologica (*in review*)

Paper II:

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Paper III:

Bednařík A, Blaser M, Rulík M (2019): Methane formation and consumption by sediments in the cross-channel profile of the small river impoundment. Journal of Limnology (*in press*). https://doi.org/10.4081/jlimnol.2019.1898

1. Introduction

1.1. Atmospheric methane cycle

Methane (CH_4) is one of the most potent greenhouse gases with a global warming potential ~28 times higher than carbon dioxide (CO_2) over time horizon of 100 years and represents about 15 % of the anthropogenic greenhouse effect (IPCC 2013). From the mid-Holocene to about 300 years ago atmospheric methane concentration rose steadily by about 25 % (Brook et al. 2000). With human population increase and industrialization, methane concentration is now about 250 % higher than it was in the preindustrial age (Etheridge et al. 1998). Current atmospheric methane concentration is 1858 ppb (Dlugokencky 2018).

While the sinks of the methane in the environment are quite clear (reaction with hydroxyl radicals in the troposphere or oxidation by methanotrophic bacteria), the sources of methane are much more diverse. Among main atmospheric methane sources are included wetlands, ruminants, termites, oceans, freshwater sediments, landfills, biomass burning and fossil methane released during fossil fuel extraction (Wuebbles and Hayhoe, 2002). The most of the methane is produced microbiologically, while contribution of freshwater habitats (wetlands, rice fields) creates ~33 % of the annual atmospheric methane flux (Conrad 2009). Moreover, recent studies estimate that annual methane emission from fluvial ecosystems is equivalent to 20-50 % of lake or wetland effluxes (Stanley et al. 2016). Hence, climate driven fluctuations of methane emissions from natural wetlands are the main drivers of the global inter-annual variability of methane emissions (high confidence), with a smaller contribution from the variability in emissions from biomass burning emissions during high fire years (IPCC 2013). Our incomplete understanding of the global methane budget is in part due to the difficulty in quantifying emissions from all of the diverse methane sources (Saarnio et al. 2009, Bastviken et al. 2011).

Isotopic composition of atmospheric methane is useful for recognition of individual methane sources, when the methane originating from pyrogenic sources reaches values more close to the original source (organic matter) than in case of biogenic methane, which is characterised by high fractionation associated with metabolic processes during its production (Chanton et al. 2005).

The 2000-year methane concentrations together with stable carbon isotopes of methane, which have been derived from ice cores, revealed remarkable correlations between fluctuation of contribution of individual methane sources to the atmosphere

and changes in human population growth, eventually climate change (White et al. 2007). The study of White et al. (2007) demonstrates, that knowledge of $\delta\ ^{13}CH_4$ allows to reveal important changes in the methane budget that are hidden in the simple methane concentration record. Thus, knowledge of the isotopic composition of source of methane emitted from natural and anthropogenic systems is helpful for developing a global budget for methane sources and sinks (Chanton et al. 2005)

1.2. Methane dynamic in lotic ecosystems

1.2.1. Components of methane dynamics in rivers

First comprehensive study summarizing the methane ecology in running waters is a review by Stanley et al. (2016). In short, methane dynamic in lotic ecosystems consists of (1) production of the methane within hyporheic sediments, which are place of anaerobic metabolism and formation of methane, (2) subsequent diffusion of methane to the surface water, where the methane is (3) transported downstream or (4) emitted to the atmosphere. The methane is also a subject of significant (5) oxidation by methane oxidizing bacteria during its transport in lotic ecosystems. Methanotrophs are often found at the anoxic/oxic interface of various habitats including freshwater sediments, where they consume the methane arising from methanogenesis and are thus able to reduce the most of the potential methane flux to surface water (Segers 1998, Trimmer et al. 2010). Moreover, recent research implies that anaerobic oxidation of methane (AOM) might occur in freshwater sediments via denitrification (Ettwig et al. 2010, Norði and Thamdrup 2014), via sulfate reduction (Beal et al. 2011; Norði et al. 2013), and via iron reduction (Norði et al. 2013). It was found that AOM can be widespread in freshwater lake sediments and accounts for one third of the mean total methane produced in surface and near-surface lake sediments (Martinez-Cruz et al. 2018). The most recent study indicates that AOM activity can be important in the reduced sandy riverbeds, where the nitrite-dependent and nitrate-dependent AOM are the dominant AOM pathways (Shen et al. 2018).

This brief description can be further completed by ebullition (escape of methane from sediments directly to the atmosphere in form of bubbles). This process bypasses the importance of oxidation by methanotrophic bacteria and hence, ebullition of sediment gas bubbles is an important transport process accounting up to 60 % of the total methane emissions from the fluvial ecosystem (Wilcock and Sorrel 2008).

Last but not least, methane has been recently recognised as a potentially important carbon and energy source for freshwater food webs due to conversion of methane to microbial biomass by methane oxidation bacteria, which can be highly productive (Jones and Grey 2011). In rivers, grazing methane-oxidizing bacteria could provide the caddis larvae (genuses *Agapetus* and *Silo*) up to 30 % of their carbon (Trimmer et al. 2009).

Important elements modifying the methane dynamics in lotic ecosystems due to accumulation of sediments and organic matter are artificial impoundments (e.g. Maeck

et al. 2013). Moreover, it was found that smaller impoundments have greater sediment accumulation rates per unit area than the large ones, while small impoundments create a significant part of the total area of impoundments (Downing et al. 2006, Downing et al. 2008). These facts further influenced direction of this thesis.

Two main parts of methane dynamics in river ecosystems, methane inputs and outputs, are reviewed with more detail in next two subchapters (1.2.2. and 1.2.3.).

1.2.2. Methane inputs to river ecosystem

Despite of significant contribution of methane emissions from rivers to global methane flux, there is a paucity of data concerning sources of methane to these freshwater ecosystems (IPCC 2013, Bastviken et al. 2011). Recent studies pointing out supersaturation of river water by methane and quantifying emissions from various inland waters deal with methane origin only marginally (e.g. Middleburg et al. 2002, Saarnio et al. 2009, Anthony et al. 2012). Basically, there are three main sources of methane to surface water of fluvial ecosystems: drainage of surrounding methane rich habitats, groundwater input and river sediments, while exact role of each of these sources is not yet quantified in the overall river C budget. Moreover, water inflows enriched in methane from wastewater treatment plants are significant source of methane in the human influenced rivers (Alshboul et al. 2016).

Some studies suggest that an increased concentration of methane in rivers comes from the drainage of the surrounding wetlands. For instance, drainage of methane rich peatland and riparian soils were recognized as a main source of dissolved methane in first-order stream (Hope et al. 2004). Wetlands as an important source of methane to the adjoining rivers were recognized for example by Borges et al. (2015) in the African rivers, when methane was positively related to wetland fraction of the catchment surface. Similarly, spatial heterogeneity of methane concentration in Zambezi River was found to be mainly determined by the connectivity with surrounded floodplains and wetlands (Teodoru et al. 2015).

Groundwater methane input is highly influenced by type of the aquifers and rate of surface-subsurface water exchange. Although methane-rich groundwater was found to significantly contribute to surface water methane concentration (Jones and Mulholland 1998), groundwater with low methane concentration in the shallow and unconfined aquifers (mostly fractured limestones) did not contribute significantly to the high methane levels in surface water of a River Meuse basin (Borges et al. 2018) as well

as groundwater from a chalky areas in UK had marginal effect on the total methane emissions (Gooddy and Darling 2005). All studies point out, that high heterogeneity in methane production and hydrologic exchange between groundwater and surface water results in high spatial variation in methane input from this source to the rivers.

More likely, methane concentration in surface water is rather a result of both the groundwater discharge together with a stream-bed methanogenesis (Atkins et al. 2017, Call et al. 2018). Previous studies showed that methane in hyporheic zone creates substantial part of interstitial DOC and also high methanogenic and methanotrophic biomass were detected through the vertical profile of these sediments (Boulton et al. 1998, Fischer et al. 2005, Hlaváčová et al. 2005, Buriánková et al. 2012, Brablcová et al. 2015). However, role of these microorganisms in direct methane supply to surface water and to whole ecosystem production and respiration remains poorly known (Stanley et al. 2016). Moreover methane input from river sediments is usually calculated from concentration differences corrected by effects of porosity and tortuosity, but only rarely directly measured (De Angelis and Scranton 1993, Huttunen et al. 2006, Sollberger et al. 2014).

Generally, methane production and consumption in river sediments is affected by a number of factors including gradients of dissolved oxygen, temperature, organic matter or sediment deposition (Findlay 1995, Fischer et al. 2005). First of all, methane production potential is significantly affected by organic matter content in sediment (e.g. Conrad et al. 2011, Comer-Warner et al. 2018). Methane production is therefore associated mainly with local patches and deposits of fine sediments, where organic matter is accumulated and dissolved oxygen occurs at low level (Sanders et al. 2007, Baulch et al. 2011). From this point of view, land-use can play important role, as the concentrations of dissolved methane in rivers increased with fraction of agriculture in the catchment owing to a larger delivery of organic matter (Borges et al. 2018) as well as sedimentation rate of organic matter is increased in river impoundments (e.g. Maeck et al. 2013). Increasing temperature can further accelerate the methane production in the river sediments (e.g. Yang 1998, Fey et al. 2004, Wilkinson et al. 2015), which leads to increasing concentration of dissolved methane in surface water during the summer months (Middleburg et al. 2002, Yang et al. 2012) and during the low water level (Borges et al. 2018). Recently, non-linearity and threshold responses of streambed methane production were observed with increased temperature implying the more complex estimation of methane fluxes in future (Comer-Warner et al. 2018). On the

other hand, the calculated kinetic and temperature responses showed that with increasing temperature, methane oxidation has potential to respond rapidly to increasing methane production and thus mitigating efflux of methane diffusing through the anoxic–oxic sediment layer (Shelley et al. 2015). Combination of these multiple controls results in a high spatiotemporal heterogeneity of methane fluxes through the sediment-water interface and their quantification and extrapolation is rather complicated (Bednařík et al. 2015). Nevertheless, methane concentration in surface sediment layer (0-10 cm) appears to be the most important for diffusion of methane through the sediment-water interface (Sollberger et al. 2014).

Usually, deeper sediment layers in streams and rivers are characterized by lower oxygen level and higher methane concentration, which can be oxidized by methanotrophs in the surface sediments or even deep in the sediments (Rulík et al. 2013). Despite of the theoretically better conditions for methanogens in deeper sediment layers of rivers, an analysis of vertical distribution of methanogens show that the methanogenic community in methane-emitting river sediments is relatively stable in absolute numbers along a vertical profile (irrespective of the methane production) not only on the level of total archaea and total methanogens but also on the level of the three dominant methanogenic orders (Mach et al. 2015, Chaudhary et al. 2017). Moreover, it was found that initial steps of organic matter degradation, which are catalysed by hydrolytic and fermenting bacteria, are rate-limiting for methane production and the decrease in bacterial numbers reflects a similar decrease in methane production (Chan et al. 2005). These findings suggest that the sediment methanogenic potential is not only limited by the presence of the different methanogens but also more likely regulated by environmental factors (e.g. substrate availability, metabolic activity of the microbial community) as well as the activity of certain members of the methanogenic community.

1.2.3. Methane emissions from river ecosystem

The largest and most ecologically significant pathways of methane efflux from natural environments to the atmosphere are diffusion, ebullition (escape of methane in gas bubbles directly from the sediments) and passage through vascular plants.

Plant transport is the dominant route for methane release mainly from wetlands dominated by emergent aquatic plants, while it is marginal in the fluvial ecosystems (Bridgham et al. 2013). Nevertheless, macrophytes provide labile organic matter to

microbes (root exudates and root decay) and plant-mediated gas transport to the atmosphere reduces opportunities for methane oxidation at the sediment surface or water column (Yavitt and Knapp 1998, Bhullar et al. 2014). Moreover, vascular plants can trap and accumulate the fine and organic rich sediments that support local methane production in streams (Sanders et al. 2007).

In natural streams and rivers both methane diffusion and ebullition were recognized to contribute significantly to the total methane emissions from these ecosystems. However, compared to other natural ecosystems, very scarce data were available for estimation of their global significance and thus importance of lotic ecosystems contribution to global methane emissions from natural environments were overlooked in the past (Saarnio et al. 2009, Bastviken et al. 2011). Fortunately, a number of studies dealing with quantification of methane emissions from fluvial waters were published since that time.

Methane diffusion from rivers to the atmosphere results from widely occurred oversaturation of surface waters compare to atmospheric equilibrium. Rate of methane diffusion through the water-air interface (gas exchange velocity) is influenced mainly by concentration gradient between dissolved methane in surface water and in an ambient air, flow velocity, water turbulence, temperature, eventually wind speed (Jahne and Haubecker 1998, Natchimuthu et al. 2014, McGinnis et al. 2016, Noss et al. 2018). The methane diffusive fluxes from stream and rivers can reach mean value 8.22 ± 25.50 mmol m^{-2} d^{-1} based on literature reviewed by Stanley et al. (2016). It is significantly lower per unit of area compared to lake methane fluxes (including ebullition) with mean 33 mmol m^{-2} d^{-1} estimated by Bastviken et al. (2004).

Recently, methane ebullition was found to participate significantly on a total methane emission from streams and rivers with wide range of contribution from 0 to 80 % and with the remarkable spatial and temporal heterogeneity of this process (Wilcock and Sorrel 2008, Baulch et al. 2011, Crawford et al. 2014, Spawn et al. 2015). The methane ebullition results from very low solubility of methane in freshwaters (saturation in freshwater is about 1.6 mol m^{-3} at 20 °C, i.e. ~27 times lower solubility than CO_2) and therefore high concentrations of methane lead to production of bubbles and loss to the atmosphere by ebullition (Yammamoto et al. 1976, Sander 2015). Moreover, methane ebullition (as well as plant-mediated fluxes) allows methane leaving a river to bypass zones of aerobic oxidation.

The proportion of methane transported by ebullition is mainly driven by local conditions in rivers, such as sediment accumulation, low water depth, high organic carbon content, high temperature and low oxygen penetration, allowing the development of high methane production and increasing the probability of ebullition occurrence (Baulch et al. 2011). In natural rivers, above described changes can be reached in debris dams (Lancaster and Grant 2006), sediment accumulation due to vegetation (Sanders et al. 2007) or by channel modifications (damming) by beavers (Lazar et al. 2014). For instance, the methane emissions from beaver pond measured by Ford and Naiman (1988) even reached 33-fold higher values than from adjacent river reaches.

In man-altered rivers, artificial impoundments reduced flow water velocity and thus increase water residence time that allows organic matter sedimentation and development of anoxic conditions suitable for methane production (e. g. Maeck et al. 2013, Wilkinson et al. 2015, Crawford et al. 2016). The river impoundments (reservoirs, dams, weirs) have been recognized as significant source of methane emissions to the atmosphere, while they overlap emissions observed in natural lakes (St. Louis et al. 2000). Existence of these artificial barriers play important role in resulted contribution of different methane evasion pathways. Molecular diffusion is usually dominant pathway in rivers (with exceptions described above), while ebullitive emissions are the dominant way for methane emissions from the surface of tropical reservoirs, and it is less significant way for methane emission at the air–water interface in the temperate reservoirs, where the diffusive fluxes are prevailing (Yang et al. 2014).

Impoundments affect also downstream river reaches, where the river water is enriched with methane from the increased methane production in reservoirs, while the most of this methane is degassed at the spillways or the turbine outflows (Abril et al. 2005). This additional release of methane can create the dominant part of total methane emissions and should be further considered in assessments of methane emissions from reservoirs (Li and Zhang 2014, Kemenes et al. 2016).

1.3. Use of stable isotope analysis for determination of methanogenic pathways

1.3.1. Biological methane production and stable carbon isotopes

Biogenic methanogenesis is the terminal step in carbon flow in many anaerobic habitats and hence plays important role in the carbon cycle (Zinder 1993). Generally, methanogenic decomposition of organic matter requires microbial consortia of at least three interacting metabolic groups. In the first step fermentative bacteria degrades polymers to H_2, CO_2, formate, acetate and higher volatile fatty acids. In the second step acetogenic bacteria oxidizes the higher acids to acetate and H_2 or formate. Then, the mentioned substrates are utilized by methane producers, which use two main methanogenic pathways – hydrogenotrophic and acetotrophic methanogenesis (Ferry 1993):

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (1)$$

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \quad (2)$$

Other pathways of methane formation exist (formate or methanol as substrates), but usually create only marginally part of the total methane production (Conrad and Claus 2005, Demirel and Scherer 2008):

$$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O \quad (3)$$

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad (4)$$

Based on the accumulation patterns of carbon dioxide and methane, the reduction process of organic matter might be further divided into three distinct phases: (1) an initial reduction phase during which most of the inorganic electron acceptors are depleted and carbon dioxide production is at its maximum, (2) a methanogenic phase during which methane production is initiated and reached its highest rate, and (3) a steady state phase with constant production rates of methane and carbon dioxide (Yao et al. 1999).

Whole process of organic matter degradation and methane formation is accompanied by isotopic fractionation. Both light and heavy carbon stable isotopes participate in chemical, biological or geochemical reactions freely but there are strong differences in the rate at which they react. The heavier isotopes (^{13}C) react more slowly than lighter isotopes (^{12}C) which lead to isotopic separation or fractionation between the

source and product (Fry 2006). Hence, the stable carbon isotopic composition of methane ($\delta^{13}C\text{-}CH_4$) in a system is dependent on the mechanisms and rates of methane production and consumption and can thus be useful for the study of methane cycling (Whiticar 1986).

An important characteristic of biogenic methane produced in sediments is that with respect to its carbon stable isotope composition it is remarkably ^{13}C-depleted ($\delta^{13}C$ typically around -60 to -80‰; Conrad et al. 2007) compared with either allochthonous terrestrial plant detritus ($\delta^{13}C$ value from C3 plants around -27 ‰; Peterson & Fry 1987) or phytoplankton ($\delta^{13}C$ typically around -25 to -35 ‰; Vuorio et al. 2006). Isotopic fractionation during organic matter decomposition leading to the high ^{13}C depletion of biologically produced methane can be further illustrated by Figure 1.

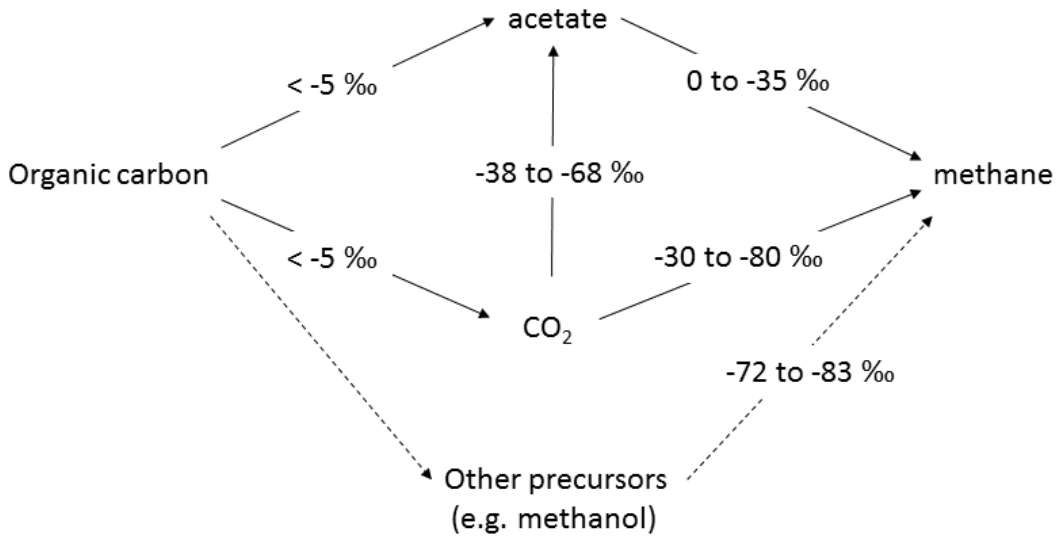

Fig. 1: Scheme of carbon flow and carbon isotope fractionation during the methane production (adapted from Conrad et al. 2014, Blaser and Conrad 2016, and using of additional literature values from: Krzycki et al. 1987, Krüger et al. 2002, Fey et al. 2004, Conrad 2005, Penger et al. 2012, Blaser et al. 2013, Ye et al. 2014).

In addition, bacterial consumption of methane is also associated with kinetic isotope effects, when enrich the residual methane in the heavier isotopes (^{13}C). Carbon fractionation factors related to methane oxidation are generally less than 10 ‰ (Whiticar 1999, Bastviken et al. 2002), but methane oxidation enrichment factor from

18.6 to 21.4 ‰ was observed in boreal reservoirs (Venkiteswaran and Schiff 2005). Methanotrophs therefore appear to be less selective between the lighter and heavier carbon isotopes than the methanogens (Whiticar 1999).

1.3.2. Acetotrophic vs. hydrogenotrophic methanogenesis

Methanogens, which are able to convert acetate to methane, belong to genus *Methanosaeta* and *Methanosarcina*. Hydrogenotrophic methanogens belong to orders Methanobacteriales, Methanococcales, Methanomicrobiales and several genera of the Methanosarcinales (Zinder 1993, Demirel and Scherer 2008). Most of these methanogens are commonly found in river sediments (Brablcová et al. 2013, Buriánková et al. 2013) except a Methanococcales order, which was isolated essentially from marine and coastal environments (Garcia et al. 2000). Nevertheless, the composition of methanogenic community does not have to reflect the contribution of individual methanogenic pathways, as well as abundance of the methanogens does not reflect the methanogenic potential of the sediments (Conrad et al. 2011, Chaudhary et al. 2017).

The $\delta^{13}C$ of methane produced only by acetotrophic methanogenesis vary between -27 and -60 ‰, while the $\delta^{13}C$ of hydrogenotrophically produced methane vary between -45 and -90 ‰ (Conrad 2005). The difference between the two pathways of methane formation leading to the diverse $\delta^{13}C$ values is given by diverse strength of fractionation during different metabolic pathways. The data clearly show that the fractionation factors found for H_2/CO_2-dependent methanogenesis (α_{mc}) are always significantly larger than the fractionation factors found for acetate dependent methanogenesis (α_{ma}), while the α_{ma} range between 1.000 and 1.021 and α_{mc} measured in various cultures of CO_2-reducing methanogens range between 1.031 and 1.077 (Krüger et al. 2002, Fey et al. 2004, Conrad 2005). While the fractionation factors can differ according to environmental conditions (e.g. substrate concentration, different microbial communities), both resulted values additionally depend on $\delta^{13}C$ of organic matter or related processes during its degradation, which affect the $\delta^{13}C$ of the carbon substrate (CO_2 or acetate). Both pathways contribute to methane pool, while resulted $\delta^{13}C$ of methane in the environment is given by different portion of these pathways, $\delta^{13}C$ of methane precursors, eventually by associated processes as oxidation or diffusion. Recent findings regarding the isotopic analysis of methane in various freshwater ecosystems are summarized in Table 1.

Tab. 1: Overview of literature values regarding the isotopic analysis of methane in sediments of inland waters

Site	Ecosystem type	$\delta^{13}C$ of CH_4 (‰)	f_{mc} (%)*	CH_4 production potential (nmol h^{-1} gDW^{-1})	$\delta^{13}C_{org}$ (‰)	Reference
inflows of Lake Biwa, Japan	river	-64 to -47	n.s.	n.s.	n.s.	Murase et al. 2003
White Oak River, North Carolina	river	-70.8 to -65.2	18 to 42	n.s.	n.s.	Avery and Martens 1999
three streams in eastern Amazonia	river	-75.1 to -52.7	n.s.	n.s.	-29.7 to -22.8	Moura et al. 2008
five rivers in USA	river	-56.6 to -36..	n.s.	n.s.	n.s.	Sansone et al. 1999
Sitka Stream	river	-98.6 to -48.2	26 to 51	0-40	-26.7 to -25.8	Mach et al. 2015
Elbe River	river	-71.1 to -54.1	52 to 78	0 to 26.9	-27.2 to -0.4	Bednařík et al. submitted (this study)
Morava River	river	-63.9 to -52.5	37 to 89	0 to 83.3	-28.5 to -26.2	Bednařík et al. 2017 (this study)
River Itchen, U.K.	river	-58	33	22-80	n.s.	Shelley et al. 2015
16 tropical lakes, Brazil	lake	-94.5 to -57.7	50 to 90	4.0 ± 3.8	-32.8 to -25.2	Conrad et al. 2011
Amazonian oxbow lakes, Brazilia	lake	n.s.	>50	0 to 130	-30 to -26	Conrad et al. 2014
Lake Dagow, Germany	lake	-65 to -50	35 to 60	250 to 300	-30.1 ± 0.05	Conrad et al. 2009
two Amazonian clear-water lakes	lake	-70 to -55	53 to 63	45.5 and 63.3	-32	Conrad et al. 2010
Lake Constance, Germany	lake	-85 to -57	n.s.	n.s.	n.s.	Faber 1996
Lake Piaseczno, Poland	lake	-63.1 to -47.5	n.s.	n.s.	n.s.	Jedrysek 1995
Lake Biwa, Japan	lake	-80 to -61	>50	n.s.	-25.6 to -24.7	Murase and Sugimuto 2001
Würmsee, Germany	lake	-61 to - 52	30	n.s.	n.s.	Woltemate et al. 1984
paddy field, Vercelli, Italy	rice field	-80 to -50	33	n.s.	-26.7 ± 0.39	Conrad et al. 2002
paddy field, Vercelli, Italy	rice field	n.s.	33	n.s.	-26 ± 0.3	Fey et al. 2004
paddy field, Vercelli, Italy	rice field	-60 to -40	>50	n.s.	-26.5	Kruger et al. 2002
Uruguay	rice field	n.s.	25 to 42	25 to 35	-21.9 to -17.2	Scavino et al. 2013
swamp forest, Florida, USA	wetland	-63.2 to -37.1	n.s.	n.s.	n.s.	Happel et al. 1994
The Point Pelee Marsh, Ontario, Canada	wetland	-72.3 to -48.2	n.s.	n.s.	-25	Hornibrook et al. 1997
Mizorogaike pond, Kyoto, Japan	wetland	-76.7 to -52.8	n.s.	n.s.	n.s.	Sugimuto and Fujita 2006
Lakkasuo mire complex, Finland	peatland	n.s.	>40	14.9 to 209.7	n.s.	Galand et al. 2005
Lakkasuo mire complex, Finland	peatland	-89 to -58	>40	15 to 210	-27.4 to -26.5	Galand et al. 2010
permafrost region in Siberia (67°N)	peatland	n.s.	30	179.2	n.s.	Metje and Frenzel 2007

* f_{mc} = part of hydrogenotrophically produced methane
n.s. = not specified

Generally, the most of the methane produced in nature originates from acetate, however, the relative amounts of methane produced from the methyl group of acetate or reduction of CO_2 can vary depending on the presence of other metabolic groups of anaerobes and the environment (Ferry 1993). Hydrogen should theoretically account for 33 % of total methanogenesis when carbohydrates or similar forms of organic matter are degraded (Conrad 1999). Many methanogenic environments show both much lower and much higher contributions of H_2 to methane production than is considered normal.

The lower contributions of hydrogenotrophic methanogenesis can be relatively easily explained by the contribution of homoacetogenesis. Homoacetogenic microorganisms oxidize H_2 or formate and reduce CO_2 to acetate. This process increases the amount of methane derived from acetate and the importance of methanogenic acetotrophs (Ferry 1993):

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad (5)$$

Despite of general consideration, that homoacetogenesis is thermodynamically unfavourable in many anaerobic environments and thus its importance is minimal, recent studies shows the significant occurrence of the homoacetogenesis in river sediments or peatland soils (Ye et al. 2014, Mach et al. 2015). Acetogenic bacteria can effectively compete with hydrogenotrophic methanogens (up to ten time faster consumption of H_2 than methanogens) and therefore, acetogens may play an important role in regulating acetate dynamics, methane production, and carbon cycling (Ye et al. 2014). The isotopic effect related to homoacetogenesis (acetyl-CoA pathway) is significantly stronger compared to fermentation and thus leads to depletion in $\delta^{13}C$ of acetate-methyl relative to the soil organic carbon (Blaser et al. 2013). Due to this difference in fractionation between these two acetogenic pathways it was calculated, that homoacetogenesis can contribute up to 40 % of the acetate production in river sediments (Mach et al. 2015).

The mechanisms behind higher contributions of hydrogenotrophically produced methane are mostly unclear despite the fact that dominance of hydrogenotrophic methanogenesis is not unusual for freshwater ecosystems (Krüger et al 2002, Galand et al. 2010, Conrad et al. 2010, Conrad et al. 2014). In methanogenic environments H_2 is rapidly turned over, because its concentration is given by the simultaneous production by fermenting plus syntrophic bacteria and consumption by methanogenic archaea

(Conrad 2002). Conceivable explanations for dominance of hydrogenotrophic methanogenesis in freshwater sediments have been defined by Conrad et al. (2009) and are further discussed for example in Bednařík et al. 2017 (Paper II). Briefly, the most plausible explanation based on several studies is that organic matter in the sediments is incompletely degraded with the preferential production of H_2 and the accumulation of residual organic substances having a higher oxidation state than the original organic carbon (Conrad et al. 2011). End of the reduction process of organic matter characterised by steady state phase with constant production rates of methane and carbon dioxide can result in different rate of methane and carbon dioxide production, when for example oxidation status of soil organic matter changes during decomposition (Yao and Conrad 2000). Different path of organic matter degradation with use of organic compounds as oxidants (thus CO_2 rather than methane is the major degradation product) leading to prevalence of hydrogenotrophic methanogenesis was documented in the oligotrophic environments by Galand et al. (2010).

Moreover, changes in primary productivity, temperature, and hydrology may affect methanogenesis by altering the short-term supply of labile organic substrates to fermentative bacteria in the shallow subsurface. The predominance of methanogenic pathway appears to be determined primarily by the availability of labile substrates and hence the degree of decomposition of organic debris. This may result in prevailing acetoclastic methanogenesis in shallow subsurface, while the methane in deeper and older sediments layers is produced mainly hydrogenotrophically (Hornibrook et al. 1997, Conrad et al. 2009). However, distribution of organic matter in running waters is dependent on many factors such as stream velocity or river bed topography and do not show such a degree of stratification (Malard et al. 2002).

Temperature was recognized as an important factor effecting the stable isotopic composition of produced methane, while the methane was enriched in ^{13}C during the warmer months (Avery and Martens 1999). However, the temperature does not affect the rate of individual fractionation factors during methanogenesis, but increasing temperature was found to induce a change in the microbial community composition or the local substrate concentration and consequently in a change of the methanogenic pathway (Penger et al 2014). The methane produced from acetate reached 85 %, 67 % and 0 % of the total methane production at 10 °C, 20 °C and 50 °C, respectively (Conrad 2002). So, the methanogens are dominated by different species at different temperatures and the different population structures obviously affect the pathway and/or

rate of methane formation. However, methane production is not only affected by the direct methane producers themselves, but also by other microbial populations that influence the availability of methanogenic substrates. Hence, the change in community and pathway induced by temperature must not be mistaken as a direct temperature effect on the fractionation of stable carbon isotopes (Penger et al. 2014).

1.3.3. Experimental determination of particular methanogenic pathways contribution to total methane production

The relative contribution of the two main methanogenic pathways to total methane production can be calculated due to the sufficient difference in isotopic fractionation during both the hydrogenotrophic and acetoclastic methanogenesis. Rough estimation derived only from $\delta^{13}C$ of methane and $\delta^{13}C$ of CO_2 can be calculated as apparent fractionation factor (α_C):

$$\alpha_C = \frac{\delta^{13}C\text{–}CH_4 + 1000}{\delta^{13}C\text{–}CO_2 + 1000} \qquad (6)$$

Resulted values of α_C higher than 1.055 are characteristic for prevailing hydrogenotrophic methanogenesis, while the values lower than 1.055 indicate prevailing acetoclastic methanogenesis (Whiticar et al. 1986, Whiticar and Faber 1986, Whiticar 1999). In addition, apparent fractionation factor is equal to fractionation factor for hydrogenotrophic methanogenesis, if the acetoclastic methanogenesis is not operating and methane is exclusively produced from CO_2 reduction (Conrad et al. 2009).

It is possible to obtain more accurate (in order of percentages) contributions of individual methanogenic pathways to total methane production, if the further stable isotopic signatures and isotopic fractionation factors are known for the examined environmental system: $\delta^{13}C$-CH_4, $\delta^{13}C$-CO_2, $\delta^{13}C$ of acetate-methyl, fractionation factor for the reduction of CO_2 (α_{mc}) to methane and acetate-methyl to methane (α_{ma}) (Conrad 2005). However, experimental determination of fractionation factors in environmental samples is difficult, since either the hydrogenotrophic or the acetoclastic methanogenic pathway must be suppressed in order to determine the isotope fractionation by one of the two pathways specifically. For that reason, it was important milestone, when the methyl fluoride (CH_3F) was reported to be a specific inhibitor of acetoclastic

methanogens, while hydrogenotrophic methanogens were not affected (Janssen and Frenzel 1997) and thus allowing the determination of $\delta^{13}C$-CH_4 specifically produced from CO_2 reduction (δmc) and of fractionation factor for hydrogenotrophic methanogenesis (α_{mc}). Acetate, which is then no longer consumed in inhibited samples, accumulates and allows determination of the isotopic signatures of the fermentatively produced acetate.

Based on inhibition treatment, direct calculation of contribution of methanogenic pathways using the residual methane production (comparison of methane production in the inhibited and uninhibited sample under same conditions) seems to be the simplest way. However, it was found that small part of hydrogenotrophic methanogenesis can be inhibited during incubation with CH_3F in addition to acetoclastic methanogenesis. Extent of the H_2/CO_2-dependent methanogenesis inhibition depends on CH_3F concentration (Conrad and Klose 1999). If the hydrogentrophic methanogenesis is inhibited a little by CH_3F, residual methane production of the samples incubated with CH_3F can be lower than expected values corresponding with part of hydrogenotrophic methanogenesis determined by isotope analysis. Thus, it is more robust to calculate contribution of hydrogenotrophic methanogenesis from the isotopic mass balance, which should not or little be affected, when part of the hydrogenotrophic methanogenesis is inhibited (Conrad et al. 2009).

In order to determine resulted contribution of particular methane production pathways to total methane production, the calculation usually has two more assumptions: (1) $\delta^{13}C$ of acetate is similar to $\delta^{13}C$ of organic matter and (2) $\delta^{13}C$ of acetoclastically produced methane is similar to $\delta^{13}C$ of acetate. Ad 1: Isotopic enrichment factors during the degradation of organic matter to acetate is much smaller than those during hydrogenotrophic and acetoclastic methanogenesis or the intramolecular difference in $\delta^{13}C$ between the carboxyl and methyl group of acetate (Conrad et al. 2014). So, when direct measurement of $\delta^{13}C$ of acetate is not available, it can be assumed, that fractionation during fermentation between organic carbon and acetate-methyl, from which methane is formed, is not significant (Blair et al. 1987, Sugimuto and Wada 1993, Conrad et al. 2009), while the $\delta^{13}C$ of acetate carboxyl group is usually depleted in ^{13}C (Conrad et al. 2009, Conrad et al. 2010). However, possible exchange reactions of the carboxyl group of acetate with the CO_2 pool during incubation may subsequently result to significantly heavier (^{13}C enriched) acetate (DeGraaf et al. 1996). Thus, the higher $\delta^{13}C$ of acetate than the $\delta^{13}C$ of organic carbon

can be theoretically caused by analysis of $\delta^{13}C$ of total acetate, which combines the $\delta^{13}C$ of methyl and carboxyl group of acetate. On the other hand, chemolithotrophic production of acetate (homoacetogenesis) leads to significant depletion of acetate-methyl in ^{13}C due to the strong isotopic effect connected with this metabolic pathway as described above (Blaser et al. 2013).

Ad 2: Preference of the certain metabolic pathway for the lighter carbon isotope (^{12}C), fractionation, is not sufficiently expressed in the substrate limited conditions (Fry 2007). Considering the almost complete consumption of acetate during incubation of samples in steady state conditions, very low or zero fractionation for methane from acetate can be assumed (Sugimoto and Wada 1993). Based on literature, fractionation factor for formation of methane from acetate is usually considered in the range of 1.007–1.035 (Gelwicks et al. 1994, Hornibrook et al. 2000, Penning 2006, Goevert and Conrad 2009).

2. Aims of dissertation thesis

The aims of this thesis were:

1) To characterize the methane production and oxidation potential of the river sediments in longitudinal profile of the Elbe River and to reveal the contribution of individual methanogenic pathways to the total methane production using the stable carbon isotope analysis.
2) To compare the rate of the methane related processes (methane production, oxidation, emissions to the atmosphere) between weir impoundments and free flowing river sections of Morava River. Part of it was also quantification of methane ebullition and extent of methane degassing in the spillways.
3) To determine the proportion of methane production pathways in the sediments of the examined weir impoundments and river sections.
4) To characterize the spatial variability of methane production and consumption by the river sediments including the proportion of the methanogenic pathways in the cross-section profile of weir impoundment.

3. Material and methods

The samples collection and field measurements have taken place at the 11 locations along the Elbe River from river km 8 to river km 948 and in the cascade of three weirs in the 16 km long stretch of Morava River. More detailed characterization of studied sites is included in the Paper I and Paper II, respectively.

Determination of individual components of methane dynamic in river demands involvement of many different methods. Measured components of the methane dynamic are schematically shown in Figure 2.

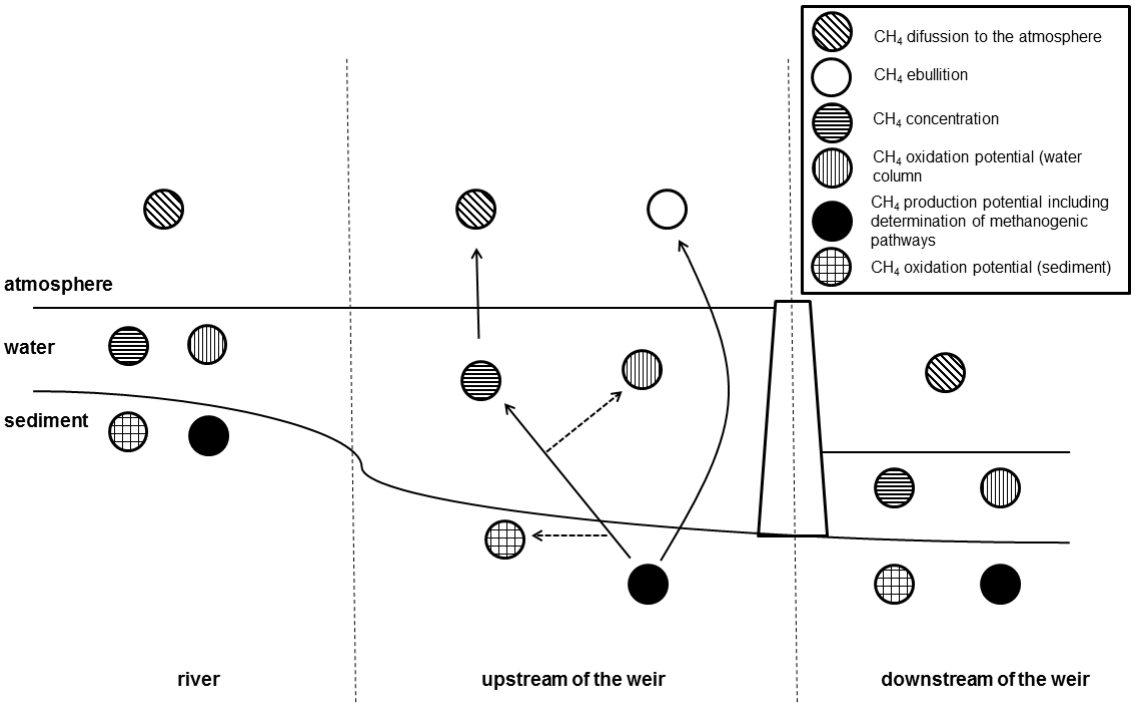

Fig. 2: Measured components of the methane dynamic in the impounded river

Individual methods are described in detail in the attached papers. This chapter therefore contains only an overview of the methods used for this thesis. They are briefly summarized here:

Methane production and oxidation potentials of sediments were measured during incubation experiments in the laboratory. Sediment for measurement of methane production potential was incubated in anoxic conditions (headspace of bottle was flushed with nitrogen) approximately one months. Gas samples from headspace were taken repeatedly during the course of incubation (4-6 weeks) and analysed for concentrations of methane. The rate of methane production was calculated from the

slope of the linear regression given by the graph of methane concentration increase over time. Sediment for measurement of methane oxidation potential was incubated under the oxic conditions (ambient air in a headspace) with addition of methane. Potential methane oxidation rates were obtained from the slope of the methane concentration decrease over time.

Determination of the contribution of individual methanogenetic pathways to total methane production was carried out using the stable carbon isotope measurements. This method is described in more detail in Paper II and schematically illustrated in Figure 3. It is based on effect of methylfluorid (CH_3F), which completely inhibits acetate-dependent methanogenesis. Methane was then exclusively produced by hydrogenotrophic methanogenesis and thus allowed determination of the fractionation factors specific for this methanogenic pathway.

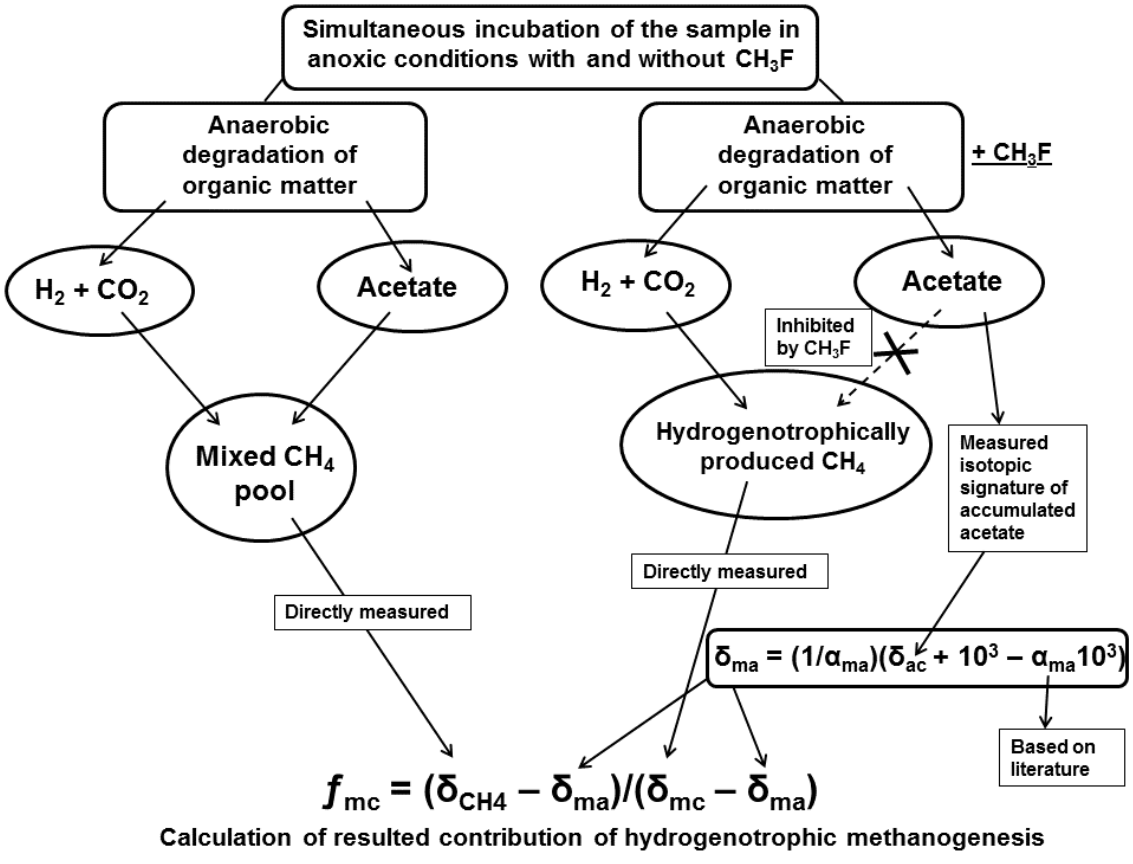

Fig. 3: Scheme of the method for determination of the contribution of two main methanogenic pathways to the total methane production using analysis of the stable carbon isotopes (original Adam Bednařík)

Methane emissions from the surface water to the atmosphere were detected by three different methods fully described in Paper II. First, methane emissions across the air-water interface were directly measured by a floating chamber method. Second, methane diffusion fluxes (i.e. without contribution of the ebullition) to the atmosphere were determined using calculations derived from recent studies and based on the gas transfer velocity and the methane concentration gradient between the river water and the atmosphere (Striegl et al. 2012, McGinnis et al. 2014, Borges et al. 2015, Bodmer et al. 2016). Third, ebullition measurements were carried out using submerged gas funnel traps. Moreover, degassing at weirs was estimated on the basis of methane concentration differences and water discharge.

From comparison of first and second method for measurement of methane emissions in our conditions followed, that we did not observe the increased methane emissions caused by the additional induced turbulence arising from application of anchored chambers described by Lorke et al. (2015). Diffusive fluxes calculated from gas transfer velocity and directly measured emissions by chambers were not different in sites with the marginal contribution of the ebullition.

4. Main results and discussion

4.1. Sediment methane dynamics along the Elbe River (Paper I)

The first study was motivated by a paucity of data on methane dynamic in large rivers, especially those focused on methane-related processes in sediments. Therefore, we have investigated the spatial variability of methane production and consumption by sediments of Elbe River including the differentiation of the methane production pathways (acetoclastic vs. hydrogenotrophic) using the natural abundance of stable carbon isotopes. Additionally, we determined the diversity of the methanogenic communities.

The methane production was detected in six sediment samples (from 11 of total) along the Elbe River, while the methanotrophy was found in all examined sediment samples. The methane production and oxidation differed considerably in the river longitudinal profile without any clear trend and without any correlation with other studied environmental parameters. Moreover, the mcr-A and pmo-A gene copy numbers (genes showing the presence of methanogens and methanotrophs, respectively) were similar and quite stable among the sediment samples. It follows that while it was found hotspots of the measured methane processes, the molecular data showed no spatial characteristics. This observation confirms the more general pattern that the microbial abundance and community patterns only rarely correlate with their activity (Mach et al. 2015, Chaudhary et al. 2017).

On the other hand, several samples of sediments, which did not show any detectable methane production despite of incubation in anoxic conditions and presence of methanogens, were probably limited by substrate (organic carbon) or by the availability of alternative electron acceptors (dissolved NO^{3-}, SO_4^{2-}, Fe^{3+}).

Incubation experiments with isotopic analyses of CO_2 and methane revealed that the hydrogenotrophic pathway of methane formation (CO_2 reduction) was dominant for all examined methane productive sites accounting for 52 to 78 % of total methane release, thus implying the most probably the incomplete degradation of organic matter (see chapter 1.3.2.).

The following research was therefore more focused on detailed characterization of methane dynamic in these hot-spots of methane related processes.

4.2. Effect of weir impoundments on methane dynamics in a river (Paper II)

In order to understand the role of small weir impoundments in river methane dynamics, we measured methane concentration, methane oxidation in the water column as well as sediments, total methane emissions to the atmosphere (diffusion and ebullition) and sediment methane production in three weir impoundments and river reaches between them. Generally, reduced water velocity upstream of dams leads to higher sediment accumulation rates and burial of organic carbon, which subsequently allows development of anoxic conditions suitable for methane production (e.g. Abril et al. 2005; Maeck et al. 2013).

Indeed, we found that river methane dynamics might be highly influenced by weirs, especially by increased methane production and consumption by sediments, followed by increasing methane emissions to the atmosphere. Both methane production and oxidation potential of sediments were higher upstream of the weirs compared to downstream of the weirs or usual river reaches. The total methane emissions to the atmosphere reached the highest values upstream of the weirs, while the ebullition accounted for ~96 % of the total methane emissions. Methane consumption in the sediments together with the microbial methane oxidation in the water column substantially contributed to the methane removal from surface water. Thus, the contribution of the ebullition to the methane emissions in these shallow impoundments was enhanced by bypassing microbial methane oxidation, compared to relatively slow diffusion fluxes. Overall, methane emissions from average weir impoundment can reach up to 42 times higher values than from the river section of an equal area.

In spite of such high emission fluxes including further methane release by degassing in the spillways of the weirs and high methane oxidation, considerable 7.5 times increased of methane concentration in the surface water was observed in the 16 km long examined section, pointing to important methane sources in such a short river reach.

The contribution of H_2/CO_2-dependent methanogenesis to total produced methane tended to be higher for sediments upstream of the weirs, compared to the sediments from river sections or downstream of the weirs. More precisely, hydrogenotrophic methanogenesis contributed 37 to 89 % of the total methane production and was dominant (more than 50 %) in sediments upstream of the weirs, while acetoclastic methanogenesis was probably prevailing in remaining sediments.

Observed predominance of hydrogenotrophic methanogenesis most probably explained by incomplete degradation of organic matter in the sediments with the preferential production of H_2 and the accumulation of residual organic substances having a higher oxidation state than the original organic carbon (Conrad et al. 2011) is frequently reported from lake ecosystems (Murase and Sugimuto 2001, Conrad et al. 2009, Conrad et al. 2014).

These all findings together with other sediment characteristics show that methane related processes upstream of the weirs are more comparable with lake sediments than river sediments.

4.3. Methane formation and consumption by sediments in the cross-channel profile of the impoundment (Paper III)

Previous results encouraged us to have a further insight to the spatial variability of methane production within the weir impoundments. For this purpose, sediments upstream of the weir were sampled in the cross-channel profile and in two different sediment depths. Methane production and oxidation potentials including the contribution of individual methanogenic pathways to total methane production were measured as before.

Samples from the surface sediment layer (0-10 cm) reached higher methane production than sediments from the deeper layer (10-20 cm) during the incubation experiments. The methane oxidation potential of sediments showed the same spatial pattern as observed for the methane production.

We hypothesized that more uniform sedimentation in cross-channel profiles compared to river due to the overall decrease of the flow velocity upstream of the weir will result in more identical rates of methane-related processes through the transect. Instead, we found frequently observed pattern in lakes and rivers, that littoral zones are main sites of the methanogenic activity. Increased methane production in the littoral sediments of lakes is likely caused by greater availability of labile organic matter from the aquatic vegetation and by higher temperatures in summer months, which in turn support higher methane production rates (Bussmann 2005, Murase et al. 2005, Hofmann et al. 2010), while higher methane production in riparian habitats of rivers is probably connected with low water exchange between surface water and sediments, thus, the low oxygen supply to the sediment (Malard et al. 2002, Fischer et al. 2005).

In our study, sediments near the bank zones and in the mid-channel were characterised by the highest organic carbon content (6.9 %) as well the highest methanogenic activity (2.5 mmol g^{-1} DW d^{-1}).

Stable carbon isotopes analysis, used for determination of individual methanogenic pathways, confirms our previous findings that the methane production is dominated by H_2/CO_2 dependent methanogenesis upstream of the weir. However, it was more evident in the surface sediment layer (0-10 cm), while the proportion of acetoclastic and hydrogenotrophic methanogenesis in deeper sediment layer (10-20 cm) was more balanced. This slight shift in the contribution of methanogenic pathways was not caused by the lability or availability of organic substrate because the similarity of these parameters between examined sediment layers, so real causes stayed unclear based on available data.

5. Conclusions

Altogether results presented in this study confirm that river sediments are important place of anaerobic degradation of organic matter with methane as a final product. Moreover, the methane dynamics in a river system show a high local variability, indicating that multiple measurements are needed to characterize the sources and fates of the methane. The methane dynamics along the river continuum is dramatically impacted by the building of small impoundments, which contribute significantly to the total methane production and its subsequent emission disproportionately to their area. Therefore, sampling carried out regardless of occurrence of small impoundments can considerably underestimate methane emissions to atmosphere from lotic ecosystems and the importance of these barriers for river methane dynamic. We found out 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. Modifications of methane related processes in impounded river zones are reflected also by different contribution of individual metabolic pathways of methane production compared to usual river sections.

Recent studies clearly show that rivers may emit considerable amount of methane to the atmosphere. Hence, rivers should be included into the future estimations and models of a global methane budget. Namely, high spatial variability of methane related processes is remarkable in rivers compared to other ecosystems and deserves to be considered in greater details in the further studies. In any case, it is necessary to consider the sampling location of particular water habitats, because sediment samples taken only in the particular zones of water habitats can significantly misrepresent the further extrapolation of obtained results. Considering the considerable sediment methane production upstream of the weirs, more studies focusing on quantification of direct methane fluxes from sediments to surface water and conducted in-situ would also be of great importance.

Finally, I do hope that findings presented here contribute to improving our knowledge about the sources and fate of methane in river ecosystems.

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7. Attached publications

7.1. Sediment methane dynamics along the Elbe River

Bednařík A, Blaser M, Matoušů A, Tušer M, Chaudhary PP, Šimek K, Rulík M (2019): Sediment methane dynamics along the Elbe River. Limnologica (*in review*)

Sediment methane dynamics along the Elbe River

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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 along 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.

Key words: methane, river sediments, methanogenic potential, methanotrophic potential, qPCR, TRFL-P, stable carbon isotope, delta 13C, mcr-A, pmo-A,

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 feeding habits 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 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 (Koné 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 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, Borrel et al. 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. 2003, 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* (Buriankova et al. 2013, Brablcova et al. 2014, Chaudhary et al. 2014, Chaudhary et al. 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).

Material and methods

1.1. Study site

The Elbe River rises at an elevation of 1,386 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 1,094 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.)

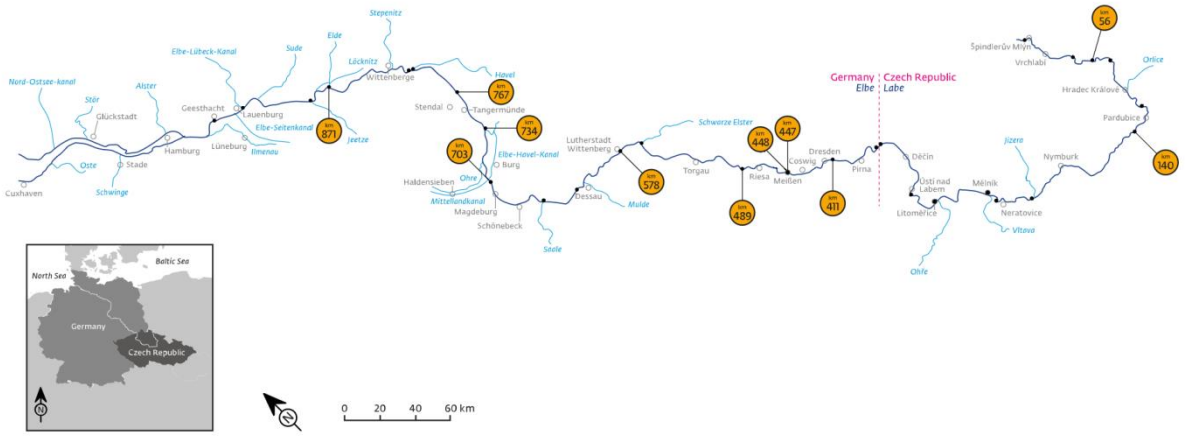


Fig. 1: The sampling sites on the longitudinal profile of the Elbe River. The numbering is determined by the distance from the river source in km (see "River km" in Tab. 1). Original map: https://commons.wikimedia.org/wiki/File:Lauf_der_Elbe.png; modified.

1.2. 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. 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.

1.3. 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).

1.4. 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; Penger et al. 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).

1.5. 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).

1.6. 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*) (Ovreas *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 25µL 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 ºC followed by 45 cycles of 30 s at 94 ºC and combined annealing and extension for 30 s at XºC (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.

1.7. Statistical analysis

Data analyses were performed by using the STATISTICA 12 software (StatSoft 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 %.

2. Results

2.1. 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 ($r = 0.64$, $p < 0.05$).

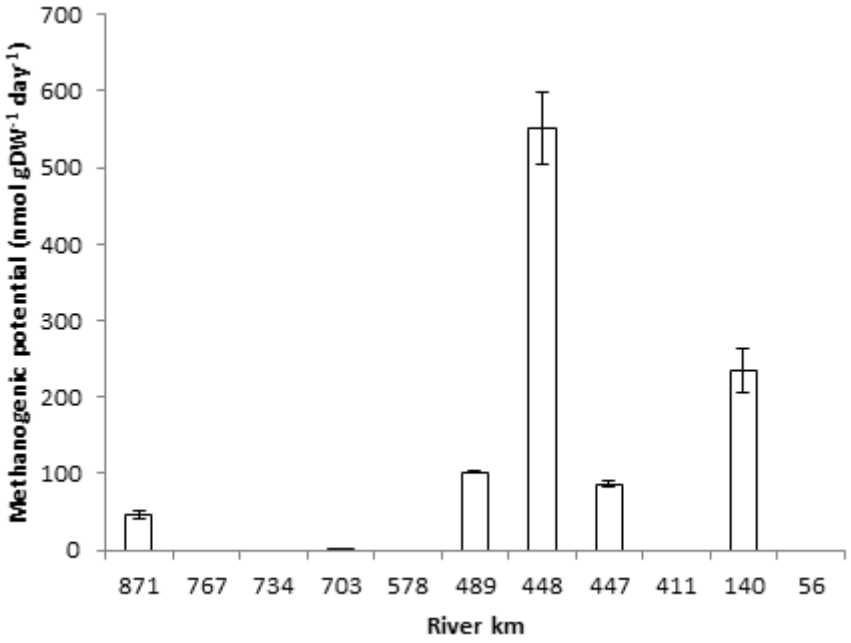
Isotopic analyses of CO_2 and CH_4 were used to calculate the contribution of different methanogenic pathways (Fig. 3). These analyses were performed for CH_4 productive sediments except the site Hohenwarthe (km 703), where the formation of CH_4 was insufficient for isotopic analyses during the incubation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

252 **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	0.00 ± 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	0.00 ± 0.0	-10.2 ± 8.3	n.m.	n.m.
871	Domitz	0.91	14.4 ± 0.8	0.08 ± 0.03	0.00 ± 0.0	-11.6 ± 0.0	-27.2 ± 1.8	4.0 ± 1.1

253 [a] An acetate concentration and $\delta^{13}C$ of acetate were measured after the incubation in samples inhibited by CH_3F

254 [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
255 enrichment of carbon with ^{13}C.



256

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

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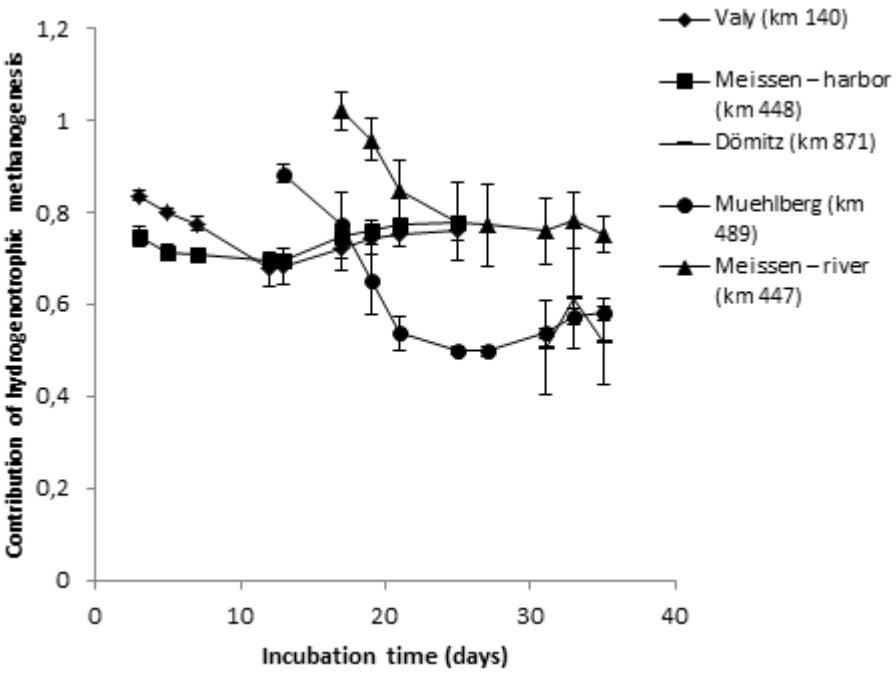

261

262 **Fig. 3:** Contribution of hydrogenotrophic methanogenesis (H_2/CO_2) to total CH_4 production

263 of examined sediments during incubation experiments (mean values ± SE).

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265

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.

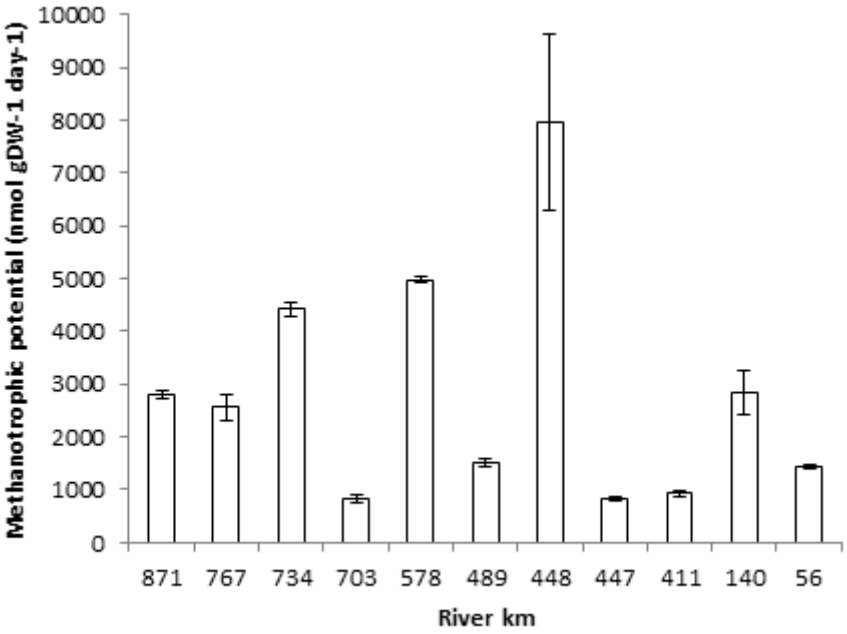
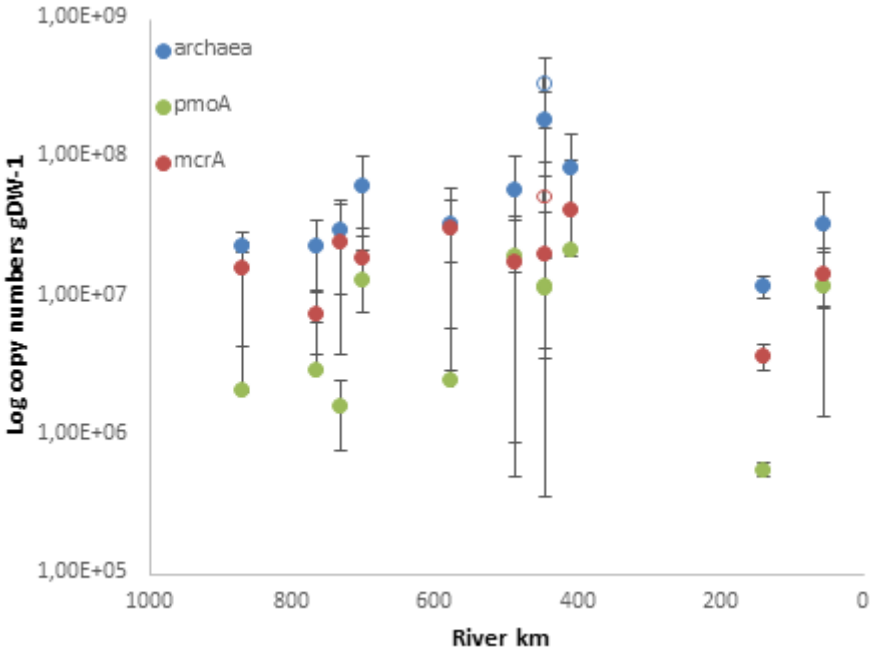


Fig. 4: Methane oxidation potential of sediments (mean values ± SE).

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

2.2.1. 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).



288

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

292

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

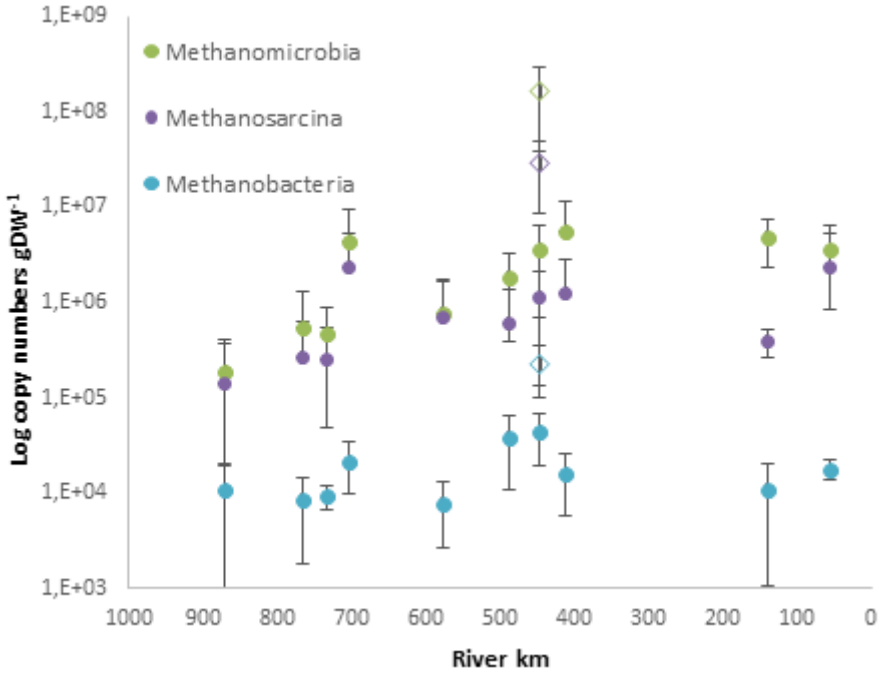
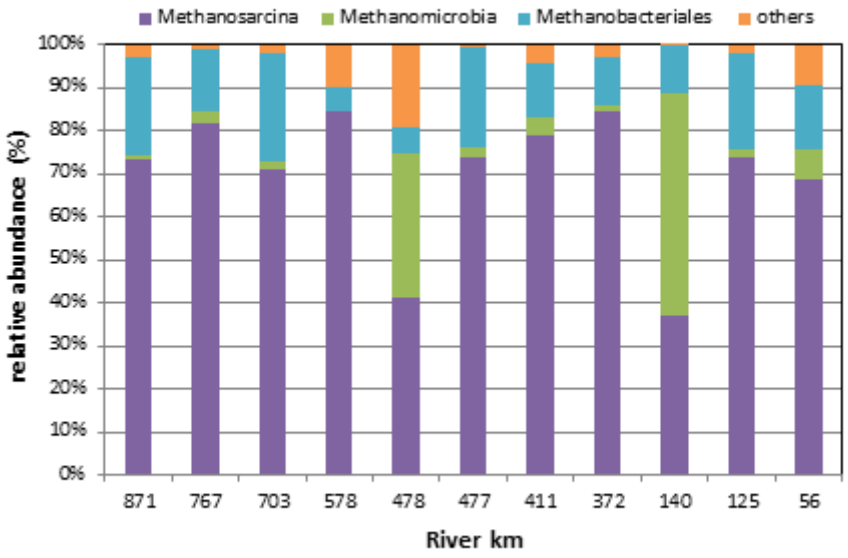


Fig. 6: The abundance (log copy numbers gDW^{-1}) of individual orders of methanogens in the examined samples determined by the group specific qPCR (mean values ± SE). Open symbols represent site Meissen – harbour (km 448).

2.2.2. 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 to 84 %) followed by T-RF's assigned to *Methanobacteriales* (5 to 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).



313

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

316

3. Discussion

3.1. 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 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 and values are not skewed. 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; Conrad et al. 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).

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

Site	$\delta^{13}C$ of CH_4 (‰)	f_{mc} (%)*	CH_4 production potential (nmol gDW^{-1} d^{-1})	$\delta^{13}C_{org}$ (‰)	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

* f_{mc} = part of hydrogenotrophically produced methane

[†] nmol CH_4 g^{-1} (wet sediment) h^{-1}

n.s. = not specified

3.2. 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, Buriankova 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).

4. 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 (Fig. 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.

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Sediment methane dynamics along the Elbe River

Supplementary material

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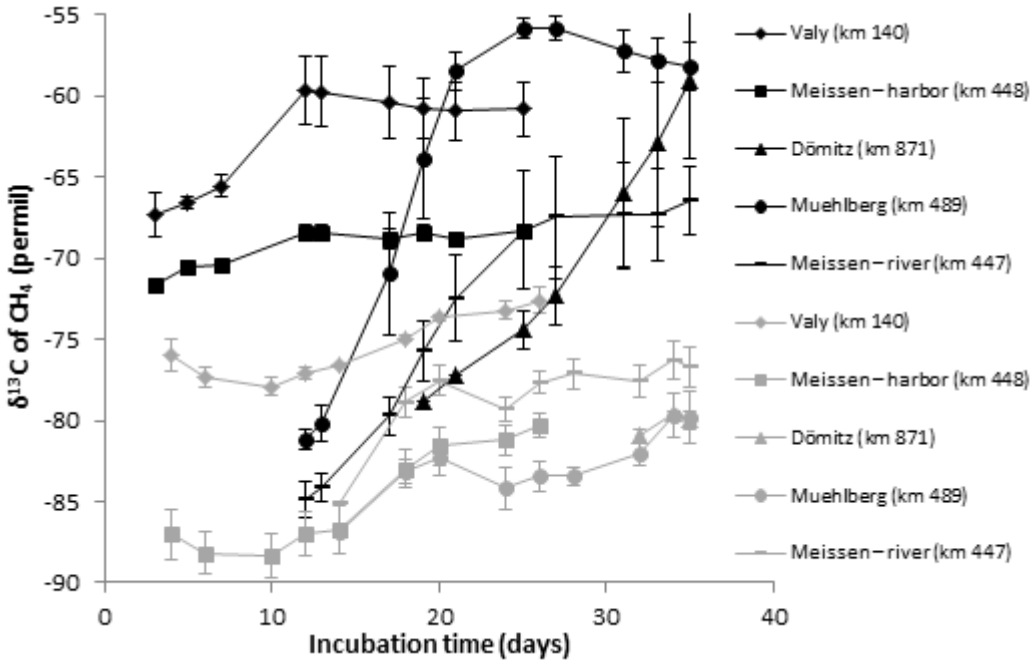

Fig S1: Delta ^{13}C values of methane for sediment incubations of different methanogenic sites. In black incubations under N_2; in grey incubations under N_2 + 3% CH_3F.

In the well studied systems (e.g. rice paddies and lake sediments) methane emission can be linked to two dominating processes: acetoclastic (eq. 1) and hydrogenotrophic (eq. 2) methanogenesis:

$$CH_3COOH \rightarrow CO_2 + CH_4 \quad (1)$$

$$CO_2 + 4H_2 \rightarrow 2H_2O + CH_4 \quad (2)$$

To distinguish the two dominant methanogenic pathways the natural abundance of stable carbon isotopes can be used if the $\delta^{13}C$ of methane and of its precursors and the methanogenic fractionation factors are known (Conrad, 2005). The acetoclastic methanogenesis expresses a smaller kinetic isotopic (α = 1.009 – 1.027) effect (Gelwicks et al., 1994; Goevert and Conrad, 2009; Penning et al., 2006) than the hydrogenotrophic methane formation (α = 1.045 – 1.073) (Valentine et al., 2004). The inhibition of acetoclastic methanogenesis by methylfuoride (CH_3F) allows quantifying the contribution of both pathways (Conrad et al., 2011; Janssen and Frenzel, 1997).

While the acetoclastic pathway is dominating in e.g. rice paddy soils (up to 67% of methane release (Conrad, 1999)) freshwater sediments and gut environments are dominated by hydrogen driven methanogenesis (Conrad, 1999).

Calculations

The carbon isotopic signature was given in the delta notation relative to the Vienna Pee Dee Belemnite (V-PDB) standard. The fractionation factor α for a reaction A → B are defined after (Hayes, 1993):

$$\alpha_{A,B} = (\delta^{13}C_A + 10^3) / (\delta^{13}C_B + 10^3) \quad (3)$$

Isotopic calculations of fractionation factors and estimation of the approximate partition of hydrogenotrophic methanogenesis of the total methanogenesis were calculated according to (Conrad, 2005):

The apparent fractionation factor (α_{app}) for conversion of CO_2 to CH_4 is given by:

$$\alpha_{app} = (\delta CO_2 + 10^3)/(\delta CH_4 + 10^3) \quad (4)$$

where δCO_2 and δCH_4 are directly measured isotopic signatures of the carbon in CO_2 and CH_4, respectively.

Fractionation factor for hydrogenotrophic methanogenesis (α_{mc}) is given by:

$$\alpha_{mc} = (\delta_{CO2} + 10^3)/(\delta_{mc} + 10^3) \quad (5)$$

where δ_{mc} is carbon isotopic signature of methane solely produced from carbon dioxide (directly measured from assays inhibited by methylfluoride). Partition of hydrogenotrophic methanogenesis is calculated by the following mass balance equation (6):

$$f_{mc} = (\delta_{CH4} - \delta_{ma})/(\delta_{mc} - \delta_{ma}) \quad (6)$$

where f_{mc} is the partition of hydrogenotrophic methanogenesis and δ_{ma} is carbon isotopic signature of methane solely produced from acetate. It is calculated from the following equation:

$$\delta_{ma} = (1/\alpha_{ma})(\delta_{ac} + 10^3 - \alpha_{ma} * 10^3) \quad (7)$$

where α_{ma} is fractionation factors for acetoclastic methanogenesis and δ_{ac} is the measured isotopic signal of acetate. Several published α_{ma} have been used to estimate the contribution of hydrogenotrophic methanogenesis e.g. (Gelwicks et al., 1994; Goevert and Conrad, 2009; Penning et al., 2006). We used α_{ma} 1.009 for our calculations which is a somewhat moderate assumption used by several authors e.g. (Aschenbach et al., 2013)

70

71 Table S1: Characteristics of primer sets used in Quantitative PCR

Name	Target Group	Sequence(5´- 3´)	Annealing Temperature (°C)	Amplicon size (bp)	Reference
PARCH340-F **PARCH519-R**	Archaea	CCC TAC GGG GYG CAS CAG TTA CCG CGG CKG CTG	58.3	152	Øvrea´s et al. 1997
pmoA 189-F **pmoA682-R**	Methanotrophs	GGN GAC TGG GAC TTC TGG GAA SGC NGA GAA GAA SGC	56	531	Holmes et al. 1995
MCRA-F **MCRAR- R**	Methanogens	GGT GGT GTM GGD TTC ACM CAR TA TTC ATT GCR TAG TTW GGR TAG TT	55	488	Luton et al. 2002
MBT857-F **MBT1196-R**	Methanobacteriales	CGW AGG GAA GCT GTT AAG T TAC CGT CGT CCA CTC CTT	53.4	342	Yu et al. 2005
MMB282-F **MMB832-R**	Methanomicrobiales	ATC GRT ACG GGT TGT GGG CAC CTA ACG CRC ATH GTT TAC	50.7	506	Yu et al. 2005
MSL812-F **MSL1159-R**	Methanosarcinales	GTA AAC GAT RYT CGC TAG GT GGT CCC CAC AGW GTA CC	52.7	354	Yu et al. 2005

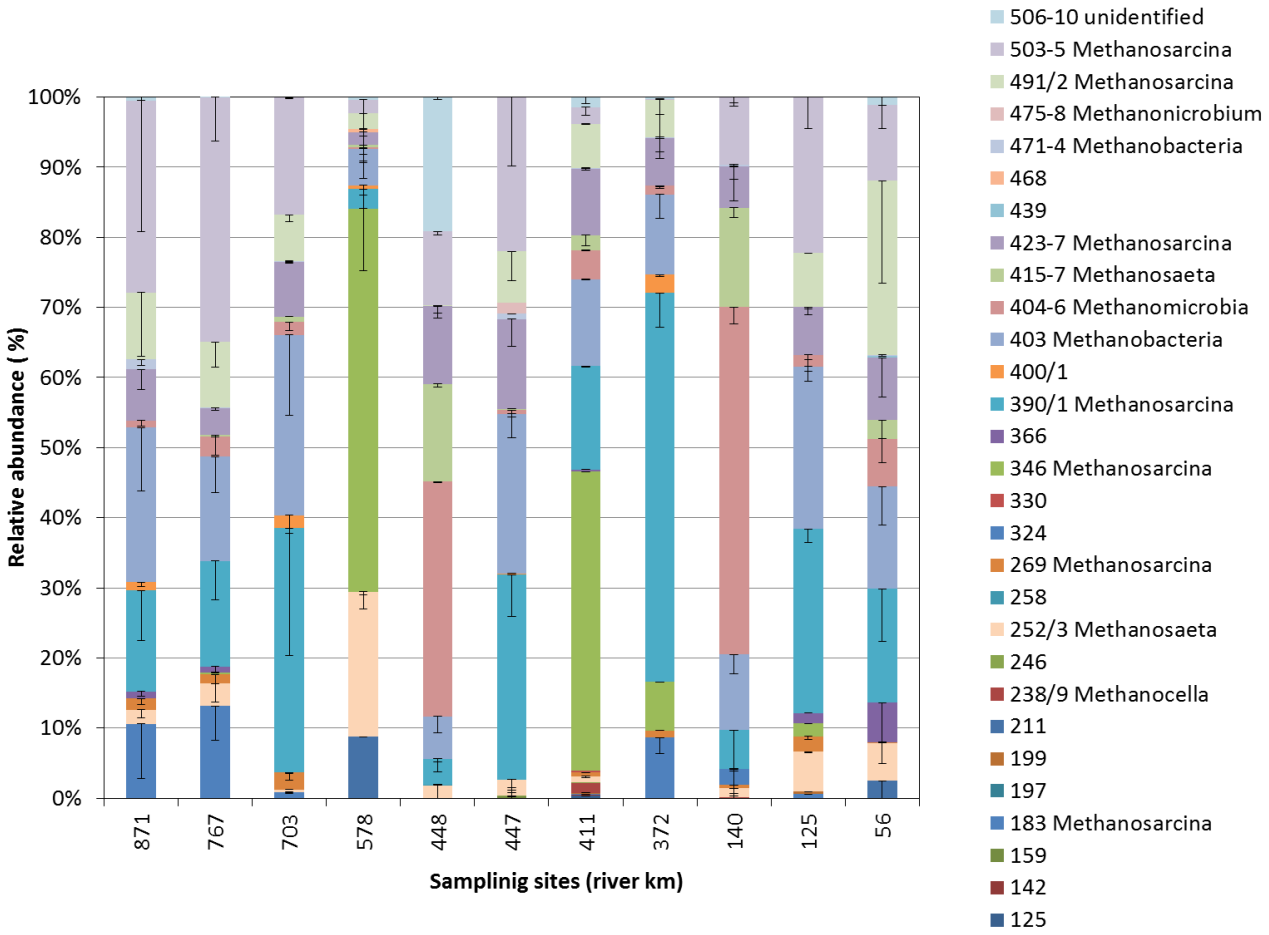

Fig S2: Relative abundance of methanogenic groups as determined by T-RFLP of mcrA in the Elbe River sediments

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7.2. Effect of weir impoundments on methane dynamics in a river

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Effect of weir impoundments on methane dynamics in a river

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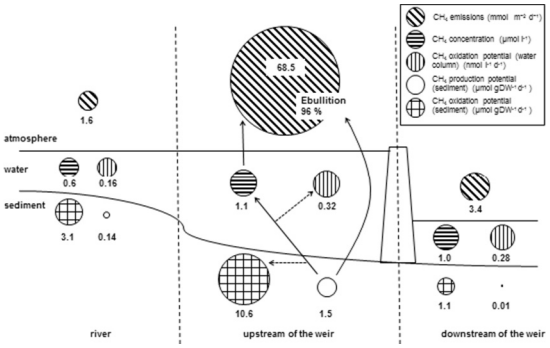
HIGHLIGHTS

- CH_4 emissions were higher upstream of the weirs compared to river reaches.
- Sediments upstream of the weirs resemble many previous observations for lake systems.
- Small impoundments significantly affect the CH_4 cycle in a river.

GRAPHICAL ABSTRACT



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ABSTRACT

We measured CH_4 concentration, CH_4 oxidation in the water column and total CH_4 emissions to the atmosphere (diffusion and ebullition) in three weir impoundments and river reaches between them, in order to understand their role in river methane (CH_4) dynamics. Sediment samples were also collected to determine CH_4 consumption and production potentials together with the contribution of individual methanogenic pathways. The CH_4 surface water concentration increased 7.5 times in the 16 km long river stretch. Microbial CH_4 oxidation in the water column reached values ranging from 51 to 403 nmol l^{-1} d^{-1} and substantially contributed to the CH_4 removal from surface water, together with CH_4 emissions. The total CH_4 emissions to the atmosphere varied between 0.8 and 207.1 mmol CH_4 m^{-2} d^{-1} with the highest values observed upstream of the weirs (mean 68.5 ± 29.9 mmol CH_4 m^{-2} d^{-1}). Most of the CH_4 was transported through the air-water interface by ebullition upstream of the weirs, while the ebullition accounted for 95.8 ± 2.0% of the total CH_4 emissions. Both CH_4 production and oxidation potential of sediments were higher upstream of the weirs compared to downstream of the weirs. The contribution of hydrogenotrophic methanogenesis to total CH_4 sediment production was 36.7–89.4% and prevailed upstream of the weirs. Our findings indicate that weirs might influence river CH_4 dynamics, especially by increased CH_4 production and consumption by sediments, followed by increasing CH_4 emissions to the atmosphere.

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1. Introduction

Methane (CH_4) emissions from inland freshwater ecosystems (lakes, reservoirs and rivers) are believed to be to important contributions to global CH_4 flux (Ciais et al., 2013; Bastviken et al., 2011). It has been estimated that CH_4 effluxes from lakes or wetlands are equivalent to ca. 20–50% of global CH_4 emission, but fluvial systems are one of the least studied freshwater types (Stanley et al., 2016). The controlling factors of CH_4 fluxes from rivers and the characteristics of their spatial and temporal heterogeneity are still poorly understood (Saarnio et al., 2009; Ortiz-Llorente and Alvarez-Cobelas, 2012).

One major factor affecting the CH_4 dynamic in rivers is the presence of dams. In general, impoundments can cause significant changes to river habitats, morphology, sediment transport and physicochemical properties of the water (Ogbeibu and Oribhabor, 2002; Rickard et al., 2003; Gao et al., 2013; Kattel et al., 2016) and is also known to be important in greenhouse gas dynamics (Louis et al., 2000; Sobek et al., 2012). Impoundments in central Europe are environments with a high organic carbon burial rate; with smaller impoundments having greater deposition and accumulation rates per unit area (Downing et al., 2008). There is a high contribution of small impoundments to the total area of impoundments, making it obvious that small aquatic bodies can play an important role in the global carbon cycles (Downing et al., 2006; Holgerson and Raymond, 2016).

The microbial processes related to CH_4 production occur to a large extent in the hyporheic zone of streams and rivers. Here anaerobic carbon cycling is the prevailing process and CH_4 is one of the major components of interstitial dissolved organic carbon (Dahm et al., 1991; Baker et al., 1999; Fischer et al., 2005).

Generally, H_2/CO_2 and acetate have been recognized as the two dominant substrates for methanogenic Archaea in freshwater ecosystems. When carbohydrates are anaerobically degraded to CH_4 and CO_2, acetoclastic methanogenesis (using acetate) would theoretically contribute 67% of CH_4 production following the stoichiometry of the degradation, while the contribution of hydrogenotrophic methanogenesis (using H_2/CO_2 as substrate) would contribute the remaining 33%. This holds true for rice field soils (Conrad et al., 2002; Fey et al., 2004; Scavino et al., 2013), but recent studies from numerous freshwater ecosystems show various contributions of the two dominating methanogenic pathways. Low contributions of hydrogenotrophic methanogenesis (using H_2/CO_2) have been reported for rivers – 18–45% (Avery and Martens, 1999; Mach et al., 2015). Much higher contributions of hydrogenotrophic methanogenesis have been frequently reported for lakes – 50–90% (Murase and Sugimuto, 2001; Conrad et al., 2011; Conrad et al., 2014) and peatlands – 46–89% (Galand et al., 2005; Galand et al., 2010). Nevertheless, very few data are available concerning spatial variations of methanogenic pathways in freshwater sediments, particularly those from rivers.

Damming reduces the flow velocity of the water, which in turn decreases the oxygen availability. Hence CH_4 produced in the sediment is less likely to be oxidised in the sediment and the water column. This has two consequences: (1) the increased production and reduced oxidation of CH_4 results in a supersaturation and increased ebullition events and (2) the supersaturated water increases the CH_4 concentration in downstream water sections.

Sites with increased sedimentation and CH_4 concentration are thus considered to be 'hot spots' of CH_4 ebullition (Sobek et al., 2012; Maeck et al., 2013). Bubble release is a frequent transportation pathway of CH_4 from lakes and reservoirs (Bastviken et al., 2004; DelSontro et al., 2010), while it is considered to be less important in natural streams and rivers, where most of the emission measurements have focused on transport through the air-water interface by diffusion (Striegl et al., 2012; Yang et al., 2012; Silvennoinen et al., 2008). There is now good evidence however, that bubble-mediated fluxes are an important CH_4 emission mechanism in running waters (Baulch et al., 2011; Crawford et al., 2014; Sawakuchi et al., 2014).

The aim of this study was to quantify the effect of three weirs and weir impoundments on individual components of the CH_4 dynamic in a 16 km river reach. Our hypotheses were that impoundments cause changes in sediment composition that leads to increased rate of CH_4 production and reduced rate of CH_4 consumption; these changes lead to increased CH_4 emissions to the atmosphere. We examined 1) changes in river CH_4 concentrations, 2) emissions to the atmosphere (diffusion and ebullition), 3) methanotrophic activity in water column and 4) CH_4 production and consumption by sediments including the determination of methanogenic pathways using stable carbon isotope values.

2. Material and methods

2.1. Study site

The study river was the Morava (Czech Republic), where it flows through Olomouc city (from 49°36.8″ N, 17°15.2″ E to 49°29.7″ N, 17°16.7″ E). The Morava is a second-order tributary of the Danube with a mean annual discharge of 26.4 $m^3\ s^{-1}$ at our study site. The Morava river catchment is characterised by the development of the relief features on the marginal West-European platform, young Carpathian folded mountain ranges and of the Pannonian basin. The present georelief has been characterised by alternating period of quiet development and by periods of abrupt changes. About 54% of the catchment area is agricultural land (45% arable), 34% is covered by forest, 1.5% urbanized areas and 1.4% is covered by water. The main sources of nutrients are municipalities and agricultural activities. Coniferous forests prevail in the upper part of the catchment. The river upstream from the study area meanders through the floodplain forests of the Protected Landscape Area Litovelské Pomoraví, however our study reach of Morava River is straightened, its cross section is modified and its river banks are stabilized.

Three weirs are situated in this 16 km stretch of the river (Table 1), constructed for stabilization of the vertical alignment and of the riverbed, energy production using small hydropower plants, and retention of surface water for water supply. Nine sampling points were chosen along the river (Fig. 1). Three sites were located in river parts unaffected by impoundments, between the weirs (R 1–3); three sites were directly in impoundments upstream of the weirs (UW 1–3) and three sites were situated just downstream of the weirs (DW 1–3). All samples were collected twice in the summer months of 2014, one week, in mid-July and one in mid-August. Measurements of all parameters were performed simultaneously for each sampling site. Data from both sampling periods are presented together.

2.2. Sediment samples and incubation experiments

Triplicate samples of surface sediment layer (0–10 cm) from each site were collected in July 2014 by scuba diving. Sediments were then sieved through a 1-mm sieve and stored at 4 °C until subsequent analyses and laboratory experiments. Sediments for the granulometric analysis were sieved through a system of ten sieves of decreasing mesh sizes. All separate parts of the sediment were weighted and grain median size was analyzed using the software Gradistat (version 8.0) (Blott and Pye, 2001). The dry weight of the sample was determined gravimetrically. The carbon content of the sediments was quantified on a CHNS-

Table 1
Basic parameters of weirs.

Weir	Backwater length (m)	Volume of reservoir (m^3)	Weir length at overflow edge (m)	Maximum water depth (m)	Height of weir(m)
W1	2600	139,000	40.8	3.2	2.7
W2	2000	160,000	40.0	4.1	2.4
W3	5290	450,000	50.6	2.7	3.7

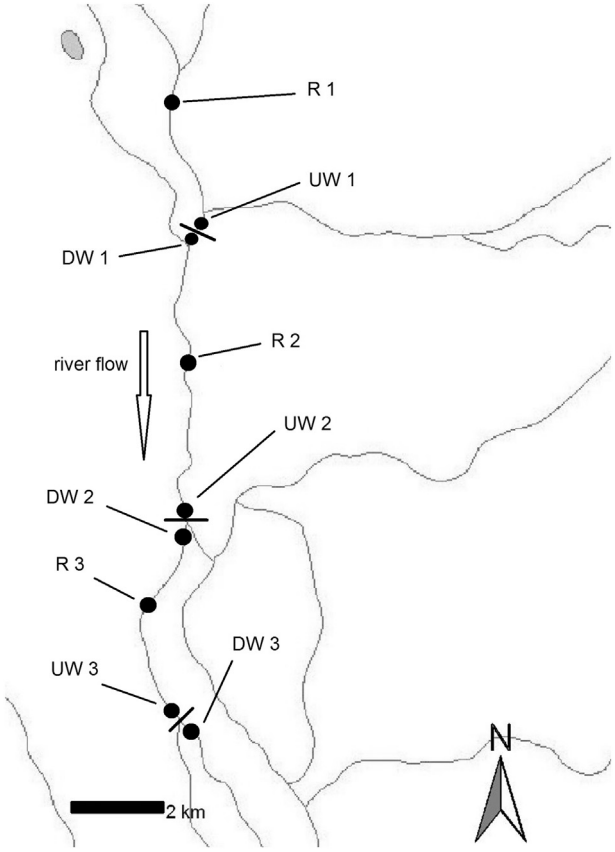

Fig. 1. Schematic map identifying the nine sampling sites along the studied section of Morava river (UW = upstream of the weir, DW = downstream of the weir, R = river section).

element analyzer by the Analytical Chemical Laboratory of the University of Marburg.

Investigation of methanogenic pathways and methane production potential used approximately 30 g (wet weight) of the sediment in duplicate, transferred into 60-ml sterile serum bottles, flushed with N_2, closed with butyl rubber stoppers and incubated at 25 °C in the dark. Five ml of distilled autoclaved water was added into each bottle at the start of the incubation for later sampling of the liquid phase. The gas headspace of half of the bottles was supplemented with 3% CH_3F 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_4, CO_2 and $\delta^{13}C$ of CH_4 and CO_2. At the end of the incubation, the bottles were sacrificed to sample the liquid phase, stored frozen (−20 °C) for later analyses of concentration and $\delta^{13}C$ of acetate The rate of CH_4 production was calculated from the slope of the linear regression given by the graph of CH_4 concentration increase over time.

Methane oxidation potential of the sediment 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 dark. The concentration of CH_4 in the headspace of each bottle was measured at 0 h and then nine times during 185 h. Potential CH_4 oxidation rates were obtained from the slope of the CH_4 decrease over time.

In the sediment incubation experiments, CH_4 was analyzed by gas chromatography (GC) using a flame ionization detector (Shimadzu, Kyoto, Japan), while CO_2 was analyzed after conversion to CH_4 with a methanizer (Ni-catalyst at 350 °C, Chrompack, Middelburg, Netherlands). 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 principal operation was described by Brand (Brand, 1996). Other details are given in Penger et al., 2012; Blaser et al., 2013; Penger et al., 2014.

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 (Krummen et al., 2004). Isotope ratios were detected on an IRMS (Finnigan MAT Deltaplus Advantage).

The carbon isotopic signature was given in the delta notation relative to the Vienna Pee Dee Belemnite (V-PDB) standard. The fractionation factor α for a reaction A → B is defined after (Hayes, 1993) as:

$$a_{AB} = \left(\delta^{13}C_A + 10^3\right) / \left(\delta^{13}C_B + 10^3\right) \quad (1)$$

Isotopic calculations of fractionation factors and estimation of the approximate partition of hydrogenotrophic methanogenesis to total methanogenesis were calculated according to Conrad (2005):

The apparent fractionation factor (α_{app}) for conversion of CO_2 to CH_4 is given by:

$$\alpha_{app} = \left(\delta_{CO_2} + 10^3\right) / \left(\delta_{CH_4} + 10^3\right) \quad (2)$$

where δCO_2 and δCH_4 are directly measured isotopic signatures of the carbon in CO_2 and CH_4, respectively.

Fractionation factor for hydrogenotrophic methanogenesis (α_{mc}) is given by:

$$\alpha_{mc} = \left(\delta_{CO_2} + 10^3\right) / \left(\delta_{mc} + 10^3\right) \quad (3)$$

where δ_{mc} is carbon isotopic signature of methane solely produced from carbon dioxide (directly measured from assays inhibited by methylfluoride). Partition of hydrogenotrophic methanogenesis is calculated by the following mass balance equation:

$$f_{mc} = (\delta_{CH_4} - \delta_{m\alpha}) / (\delta_{mc} - \delta_{m\alpha}) \quad (4)$$

where f_{mc} is the partition of hydrogenotrophic methanogenesis and δ_{ma} is carbon isotopic signature of methane solely produced from acetate, the latter calculated from the following equation:

$$\delta_{m\alpha} = (1/\alpha_{ma}) \left(\delta_{ac} + 10^3 - \alpha_{ma} 10^3\right) \quad (5)$$

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

2.3. Physical parameters of water

All physical parameters were measured at the depth from which the water samples were collected (10 cm below the water level), except flow velocity, which was calculated from several measuring depths. Concentration of dissolved oxygen together with temperature was measured using an oximeter Eutech DO 450. Conductivity was measured using a Hanna DiST 3 conductivity meter HI 98303. Flow velocity was measured using a portable flowmeter (Marsh-McBirney model 2000 Flo-Mate).

2.4. Water samples

Surface water samples (n = 4–8 per sampling site) for analysis of dissolved CH_4 were collected at a depth of 10 cm below the water

level, in vials (40 ml) with screw-tops, covered by a polypropylene cap with PTFE silicone septa. They were immediately treated by injecting concentrated sulfuric acid (200 μl) to stop the microbial activity (final concentration ca. 90 mM). All samples were transported to the laboratory in a cool box.

Concentrations of dissolved CH_4 in the surface water were measured using headspace equilibration. Dissolved CH_4 was extracted from the water by replacing 15 ml of water with N_2 and then vigorously shaking the vials for 30 s. to release the supersaturated gas from the water to facilitate equilibration between the water and gas phases. All samples were equilibrated at laboratory temperature. CH_4 was analyzed from the headspace by injecting 0.2 ml of gas sub-sample with a gas-tight syringe with valve into a 6890 N (Agilent, USA) gas chromatograph, equipped with a flame ionization detector, with a 0.53 mm × 30 m GS Alumina column. Gas concentration in water was calculated using Henry's law.

Dissolved organic carbon (DOC) was analyzed in samples filtered through glass-fibre filters of 0.4-μm nominal pore size (GF-5, Macherey-Nagel, Düren, Germany) with a Shimadzu TOC-5000A analyzer (Shimadzu, Japan).

The CH_4 oxidation rate was determined as outlined in Bussmann et al. [2015]. Briefly, water samples were processed in triplicates with two killed controls. Immediately after collecting the samples we injected 100 μl (10 μCi) of ^{3}H-CH_4 (American Radiolabeled Chemicals, Inc.). The samples were vigorously shaken for 60 s and incubated in the dark at near in situ temperature. Control samples were killed by injecting 200 μl of concentrated sulphuric acid before the tracer was added. Activities in "live" samples were stopped the same way, but after approximately 24 h. Samples were stored in the dark at 4 °C, prior to being analyzed within 1 week. In the laboratory the samples were opened, and the total radioactivity of the sample - including the labelled CH_4 and labelled produced H_2O - measured immediately by mixing a 1 ml aliquot of each sample with 5 ml of scintillation cocktail (Ultima Gold™ LLT) and analyzed with a liquid scintillation counter (Tri-Carb® 2910 TR, Perkin Elmer; or Tri-Carb® 2900 TR, Packard). Subsequently the samples were sparged with air for half an hour to expel all remaining CH_4. Afterwards the microbially-produced radioactive water was analyzed in the same way, by mixing a 1 ml aliquot of each sample with 5 ml of scintillation cocktail and analyzed on liquid scintillation counter as described above. The calculation of the microbial mediated CH_4 oxidation is based on the transformation of added radioactively marked tracer (^{3}H-CH_4) into the oxidation products (^{3}H-H_2O) during a timed incubation (detailed in Bussmann et al., 2015).

2.5. Emissions to the atmosphere

Methane emissions (diffusion + ebullition) across the air-water interface were determined by a floating chamber method. The open-bottom floating polyethylene chambers (volume 3.1 l covering an area of 0.024 m^2) were deployed on the water surface (see Rulík et al., 2013; Bednařík et al., 2015). The chambers (n = 5 per sampling site) were anchored and allowed to float on the water's surface for a period of 3 hours. Pre-incubations were performed to assess linearity of gas concentrations in headspace of the chambers and establish the incubation time required for reliable flux measurements. Samples of headspace gas were collected through the rubber stopper inserted at the chamber's top using a 100 ml gas-tight syringe. The syringe was pumped two times to mix the chamber content before withdrawing 50 ml of gas from the chamber. Samples of ambient air were collected in each study point for determining the initial background concentrations. Emissions were calculated as the difference between initial background and final concentration in the chamber headspace, and expressed according to Eq. (6):

$$E = [(c_I - c_R) \times V \times 24/(t \times 1000)]/p \quad (6)$$

where E is gas emission in mmol m^{-2} day^{-1}; c_I is concentration of the methane in the chamber headspace in μmol l^{-1}; c_R is concentration of the methane in background air in μmol l^{-1}; V is volume of the chamber in L; t is time of incubation in hr.; p is the area of chamber expressed in m^2.

We determined CH_4 diffusion fluxes to the atmosphere using calculations derived from recent studies (Striegl et al., 2012; McGinnis et al., 2014; Borges et al., 2015; Bodmer et al., 2016).

The air-water CH_4 diffusion flux (F) was computed according to

$$F = k(c_w - c_a) \quad (7)$$

where k is the gas transfer velocity for water-air gas exchange (m d^{-1}), $c_w - c_a$ is the gas concentration gradient between the river and the atmosphere (mol m^{-3}).

For the river flux calculations k is defined as (Fortescue and Pearson, 1967)

$$k = 1.46(DV/h)^{-0.5} \quad (8)$$

where D is the molecular diffusion coefficient of CH_4 in the water (Broecker and Peng, 1974), V is the water velocity (m s^{-1}) and h is the water depth (m).

In addition to that, we used E (Eq. (6)) to calculate diffusive gas transfer velocity (k) by inverting the equation for Fick's law of gas diffusion, as follows:

$$k = E/(c_w - c_a) \quad (9)$$

where k is the gas transfer velocity (m d^{-1}), E is taken from Eq. (6) and $c_w - c_a$ is the gas concentration gradient between the river and the atmosphere (mol m^{-3}).

For better comparison of k with different studies, we standardized k of CH_4 to $k600$ (equivalent to k of CO_2 at 20 °C) computed according to:

$$k_{600} = k(600/S_c)^{-0.5} \quad (10)$$

where k is the calculated from Eq. (8), Sc is the Schmidt number for CH_4 in situ temperature (Wanninkhof, 1992).

Ebullition measurements were carried out, using triplicate submerged gas traps located at three sites upstream of the weirs (UW 1–3) and one river stretch (R 3) on Morava River. The other sites did not allow the installation of the bubble traps due to shallow water depth. The traps consisted of inverted funnels (0.65 m internal diameter, covering an area 0.332 m^2) sealed with butyl-rubber septum. The bottom of the funnel was situated 1.5 m below the water level. The funnels remained on site for 24 h. Accumulated gas volumes during the sampling period were collected manually through a butyl-rubber septum using a 100 ml gas-tight syringe at the end of the experiment. The total volume of gas collected was estimated from an external scale on each funnel. Methane concentration in bubbles was multiplied by the volume of accumulated gas over the sampling period to determine CH_4 ebullition fluxes. Care was taken to avoid disturbing the benthic substrate during funnel traps operation. All gas samples were stored in 12 ml evacuated soda glass vials (Labco Limited UK) until analysis.

Degassing at weirs was estimated on the basis of methane concentration difference and water discharge according to the calculation given by Maeck et al. (2013) (Eq. (8)).

$$E_{\text{degas sing}} = [(c_{uw} - c_{dw}) \times Q]/P_{oe} \quad (11)$$

where $E_{degassing}$ is the emissions at the weir (mol m^{-2} d^{-1}), c_{uw} is methane concentration upstream of the weir (mol m^{-3}), c_{dw} is methane concentration downstream of the weir (mol m^{-3}), Q is a water discharge (m^3 d^{-1}) and P_{oe} is an area of overflow edge (m^2).

2.6. Statistics

Data analyses were performed using the software STATISTICA 12 (StatSoft, 2013). Differences between groups of data were examined using a Kruskal–Wallis test. Multiple regression analysis and Spearman's correlation analysis of data were used to find the relationships among variables. All statistical tests used a significance level of 5%.

3. Results

3.1. Environmental parameters

Water temperature (mean 20 ± 0.1 °C), conductivity (mean 319.2 ± 3.2 μS cm^{-1}) and dissolved organic carbon (mean 2.7 ± 0.2 mg l^{-1}) were relatively stable along the river profile without significant differences between each type of observed sites (Table 2). Mean values of dissolved oxygen concentrations and flow velocity ranged from 5.0 to 12.1 mg l^{-1} and from 0.06 to 0.6 m s^{-1}, respectively, with values significantly lower for sections upstream of the weirs compared to river sections downstream of the weirs and unaffected river sections (K-W test; $p < 0.01$) (Table 2). Grain median size was the smallest in sediments upstream of the weirs (mean 1.8 ± 0.9 mm), whereas the biggest grain median size was observed in the river sections with mean 15.2 ± 3.7 mm. Total sediment carbon content was significantly highest upstream of the weirs (mean 5.5 ± 0.8%) compared to other studied sites (K-W test; $p < 0.001$).

3.2. Methane oxidation and production by sediments

Consumption of CH_4 by sediments during the sediment incubation experiments was observed in all samples, with mean values in the range of 0.6–11.8 μmol gDW^{-1} d^{-1} (Fig. 2a). The CH_4 oxidation potential of sediments was an order of magnitude higher upstream of the weirs (mean 10.6 ± 1.7 μmol gDW^{-1} d^{-1}) compared to sites downstream of the weirs (mean 1.1 ± 0.2 μmol gDW^{-1} d^{-1}) and three times higher than the river sections (mean 3.1 ± 1.5 μmol gDW^{-1} d^{-1}) (K-W test; $p < 0.001$).

Formation of CH_4 by sediments wasn't observed in all samples, unlike CH_4 consumption, and mean values ranged from 0 to 2 μmol gDW^{-1} d^{-1}. The CH_4 production potential was the highest for sites upstream of the weirs (mean 1.5 ± 0.4 μmol gDW^{-1} d^{-1}), followed by the river sections (mean 0.14 ± 0.07 μmol gDW^{-1} d^{-1}), however without significant difference (Fig. 2b). The lowest values of CH_4 production were observed downstream of the weirs (mean 0.009 ± 0.003 μmol gDW^{-1} d^{-1}) (K-W test; $p < 0.05$). Both CH_4 formation and consumption were strongly positively correlated with sediment carbon content ($r = 0.63$, $p < 0.05$; $r = 0.80$, $p < 0.05$, respectively).

The $\delta^{13}C$ of organic matter in sediments was −27.1 ± 0.3‰ ($n = 27$) (Table 3). The $\delta^{13}C$ of acetate was ^{13}C enriched compared to the organic matter with mean − 22 ± 2.4‰ ($n = 9$) in inhibited samples, while there was no observed acetate accumulation in uninhibited samples. The $\delta^{13}C$ of CH_4 measured in the end of the uninhibited incubation was on average 6.4 ± 1.8‰ more depleted in ^{13}C than the $\delta^{13}C$ of CH_4 from bubbles trapped in situ. Detailed results of ^{13}C measurements of sediment samples with sufficient CH_4 production are given in Table 3.

Contribution of hydrogenotrophic methanogenesis (f_{mc}) to CH_4 production was calculated for samples with sufficient CH_4 formation during the incubation experiment (UW 1–3, DW 1, R 1, R 3). In general, the time courses of the contribution of H_2/CO_2-dependent methanogenesis to total produced CH_4 were relatively constant for individual samples and tended to be higher for sediments upstream of the weirs compared to the sediments from river sections or downstream of the weirs (Fig. 3). Hydrogenotrophic methanogenesis contributed 36.7 to 89.4% of the total CH_4 production and was dominant (>50%) in sediments upstream of the weirs, while acetoclastic methanogenesis was probably prevailing in remaining sediments.

Despite the different contribution of methanogenic pathways between the sites, the $\delta^{13}C$ of CH_4 released in the end of incubation and in situ (bubbles), the apparent fractionation factor (α_{app}; using Eq. (2)) and fractionation factor for hydrogenotrophic methanogenesis (α_{mc}; using Eq. (3)) were approximately the same for all locations (see Table 3).

3.3. Methane in surface water

Methane concentration in the surface water tended to increase along the river stretch (Fig. 4a) with significantly higher concentrations in surface water upstream of the weirs (mean 1145.3 ± 127.5 nmol l^{-1}) compared to river sections (mean 617.2 ± 139.5 nmol l^{-1}), while methane concentrations in sites downstream of the weirs were not significantly different (mean 1060.6 ± 171.8 nmol l^{-1}) (K-W test; $p < 0.05$). Measured CH_4 surface water concentration at R1 (the most upstream location) was 7.5 times lower than at location DW 3 (the most downstream location). Multiple regression analysis of the data did not reveal any relationship between CH_4 concentration in surface water and measured environmental parameters in Table 2 ($F = 2.32$, $p = 0.26$).

Mean aerobic CH_4 oxidation potential in the water column of the Morava River varied from 51 to 403 nmol l^{-1} d^{-1} (Fig. 4b). Consequently, mean potential CH_4 turnover time in surface water (black squares, Fig. 4b) was constant across the studied locations with values between 3.9 and 5.6 days, except one location (DW 2), where the oxidation was slower (potential turnover time 15 days). Hence, methanotrophy in the water column was an important process in the CH_4 budget of most locations.

3.4. Methane emissions to the atmosphere

The CH_4 emissions were determined by direct measurements with floating chambers and bubble traps. However, considering the high contribution of bubbles to total CH_4 emissions upstream of the weirs, we also calculate the CH_4 diffusion through the air-water interface using the gas transfer velocity (k) and CH_4 concentration difference between

Table 2
Selected physicochemical properties of studied sites (mean values ± SE) (R = river; UW = upstream of the weir; DW = downstream of the weir).

Site	Flow velocity (m s^{-1})	Dissolved oxygen (mg l^{-1})	Dissolved oxygen saturation (%)	Temperature (°C)	Conductivity (μS cm^{-1})	Dissolved organic carbon (mg l^{-1})	Grain median size (mm)	Sediment carbon content (%)
R 1	0.35 ± 0.03	9.7 ± 0.3	109 ± 5	20.6 ± 0.7	305 ± 15	2.5 ± 0.1	19.41	0.2 ± 0.0
UW 1	0.06 ± 0.01	8.4 ± 0.2	95 ± 3	20.5 ± 0.3	319 ± 7	2.5 ± 0.2	2.12	4.9 ± 1.1
DW 1	0.56 ± 0.03	12.1 ± 1.8	135 ± 20	20.1 ± 0.6	320 ± 10	2.7 ± 0.5	8.19	0.2 ± 0.0
R 2	0.35 ± 0.03	9.8 ± 0.6	108 ± 5	20.1 ± 0.7	308 ± 11	2.7 ± 0.3	7.85	0.2 ± 0.0
UW 2	0.10 ± 0.01	7.9 ± 0.1	88 ± 1	20 ± 0.1	316 ± 1	2.8 ± 0.2	0.13	6.2 ± 0.1
DW 2	0.33 ± 0.02	7.7 ± 0.4	86 ± 5	19.9 ± 0.1	316 ± 0	3.1 ± 0.1	1.76	0.3 ± 0.0
R 3	0.18 ± 0.00	8.7 ± 0.1	94 ± 1	19.3 ± 0.2	320 ± 5	2.7 ± 0.2	18.37	4.0 ± 0.7
UW 3	0.07 ± 0.01	5 ± 0.2	56 ± 2	19.9 ± 0.4	340 ± 3	2.7 ± 0.1	3.16	5.3 ± 1.3
DW 3	0.24 ± 0.02	7.6 ± 1	82 ± 11	20.2 ± 1.6	330 ± 8	2.4 ± 0.1	10.02	0.2 ± 0.0

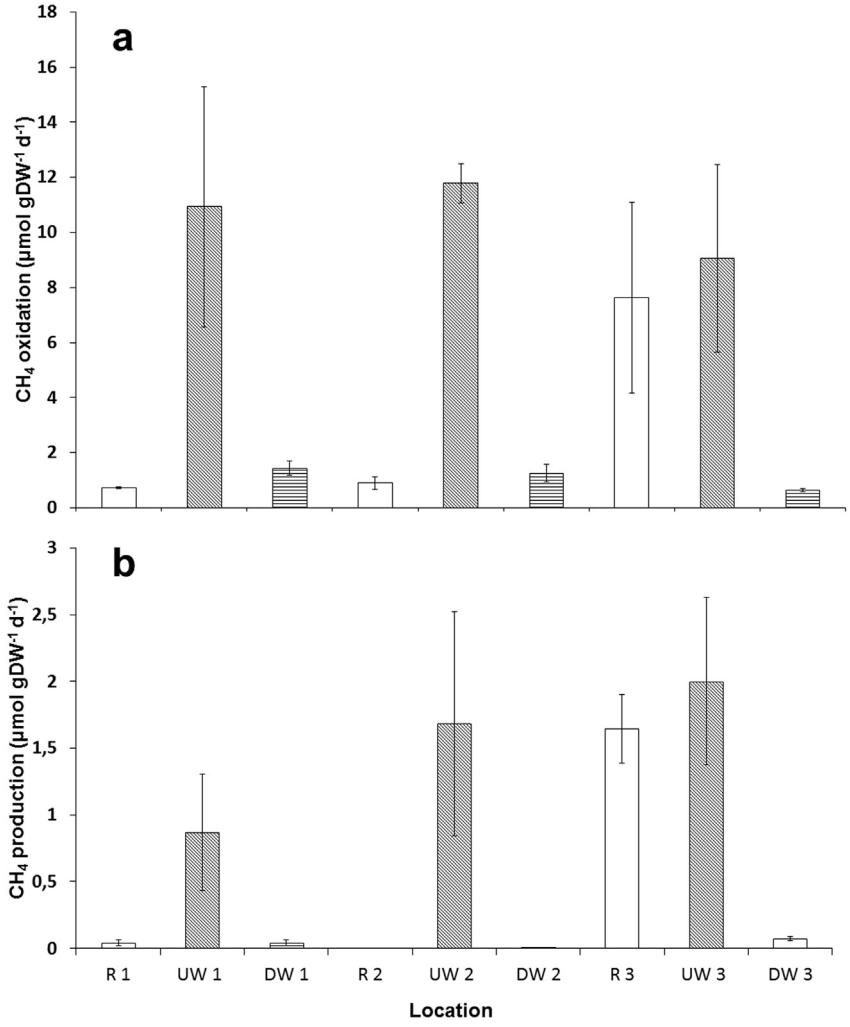

Fig. 2. Methane oxidation (A) and production potentials (B) of sediments (mean values ± SE) calculated from the incubation experiments (UW = upstream of the weir, DW = downstream of the weir, R = river section).

surface water and the atmosphere (Eq. (7)). All values concerning the determination of the CH_4 emissions are given in Table 4.

The CH_4 emissions determined by floating chamber method and by calculation using Eq. (7) were not significantly different in river reaches and downstream of the weirs except the site DW 2. However CH_4 emissions upstream of the weirs obtained by floating chamber method were considerably higher than calculated CH_4 diffusion, because the higher contribution of ebullition to the total CH_4 emissions. Hence we calculated the total CH_4 emission from CH_4 diffusive fluxes (based on *k* and concentration difference) and from directly measured ebullition by bubble traps.

All sampling sites, especially weirs, were strong sources of CH_4. Total CH_4 fluxes to the atmosphere (diffusion + ebullition) were in the range of 0.8 to 207.1 mmol CH_4 m^{-2} d^{-1} (Table 4). The CH_4 emissions were significantly higher upstream of the weirs (mean 68.5 ± 29.9 mmol CH_4 m^{-2} d^{-1}) followed by sites downstream of the weir (mean 3.4 ± 0.6 mmol CH_4 m^{-2} d^{-1}) and lowest in river sections (mean 1.6 ± 0.3 mmol CH_4 m^{-2} d^{-1}) (K-W test; $p < 0.001$).

Table 3
Results based on the ^{13}C values of parameters measured in the CH_4 producing sediment samples (mean values ± SE). (UW = upstream of the weir, DW = downstream of the weir, R = river section, n.m. = not measured).

Site	$\delta^{13}C$ of sediment carbon (‰)	Sediment incubation experiments				In situ samples (ebullition)	
		$\delta^{13}C$ of acetate (‰)[a]	α_{app}[b]	α_{mc}[c]	$\delta^{13}C$ of CH_4 (‰)[d]	α_{app}	$\delta^{13}C$ of CH_4 (‰)
UW 1	−28.5 ± 0.06	−20.9 ± 0.0	1.040 ± 0.00	1.068 ± 0.01	−63.9 ± 0.0	1.043 ± 0.01	−54.9 ± 6.0
UW 2	−28.3 ± 0.07	−17.7 ± 3.1	1.051 ± 0.01	1.079 ± 0.01	−60.4 ± 0.9	1.048 ± 0.00	−59.4 ± 0.7
UW 3	−28.2 ± 0.05	−14.3 ± 5.0	1.054 ± 0.00	1.078 ± 0.01	−60.2 ± 0.1	1.047 ± 0.00	−52.5 ± 2.9
DW 1	−27.2 ± 0.17	−29.8 ± 0.0	1.049 ± 0.00	1.081 ± 0.00	−63.8 ± 0.0	n.m.	n.m.
R 1	−26.2 ± 0.05	−29.0 ± 3.2	1.054 ± 0.00	1.074 ± 0.00	−59.8 ± 0.0	n.m.	n.m.
R 3	−28.3 ± 0.10	−24.9 ± 0.7	1.049 ± 0.00	1.081 ± 0.01	−61.6 ± 0.0	1.038 ± 0.01	−53.8 ± 10.2

[a] Measured in the samples inhibited by CH_3F.
[b] α_{app} calculated according to Eq. (2).
[c] α_{mc} calculated according to Eq. (3).
[d] End of the incubation of the uninhibited samples.

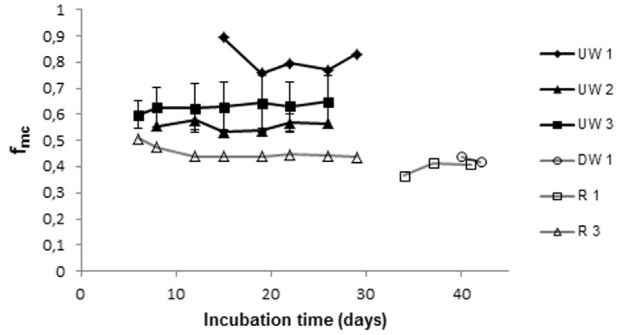

Fig. 3. Contribution of H_2/CO_2-dependent methanogenesis (f_{mc}) to total CH_4 production of sediments during incubation experiments. f_{mc} was calculated using Eq. (4) with measured values for δ_{CH4} (uninhibited incubations) and δ_{mc} (CH_3F inhibited incubations) and calculated values for δ_{ma} using Eq. (5) and $\alpha_{ma} = 1.009$.

Degassing at weirs (calculated using Eq. (11)) reached mean values of 3.2 ± 2.3, 4.5 ± 1.4 and 0 ± 0 mol CH_4 m^{-2} d^{-1} for site UW 1, UW 2 and UW 3, respectively. Bubble release was recorded for all locations with placed bubble traps (UW 1–3, R 3). Estimated average contribution of ebullition to total CH_4 emissions was 91, 98, 98 and 10% for site UW 1, UW 2, UW 3 and R 3, respectively, implying, that most of the CH_4 upstream of the weirs was transported through air-water interface by ebullition. Individual values of CH_4 content in trapped bubbles ranged from 16.2 to 61.1% and the $\delta^{13}C$ of CH_4 in bubbles ranged from −64 to −31‰ (average − 55 ± 9‰). We did not quantify the remaining composition of the bubbles.

4. Discussion

Our results show that dams affect several processes in the CH_4 dynamics of a river continuum. We summarized our findings in Fig. 5 and will discuss the individual processes. First we discuss the sediment samples, then the water samples, and finally the CH_4 emissions to the atmosphere.

However, we did not intend to calculate the CH_4 mass balance, because 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.

4.1. Effect of dams on the CH_4 dynamics in river sediments

We found that dams across a river resulted in several effects on the sediment processes including changes of the sediment characteristics, enhanced microbial activities in the sediment, ebullition of trace gases, and different contributions of hydrogenotrophic methanogens to the released CH_4.

In detail, we observed significant changes in the sediment characteristics between individual river reaches in this study: The highest

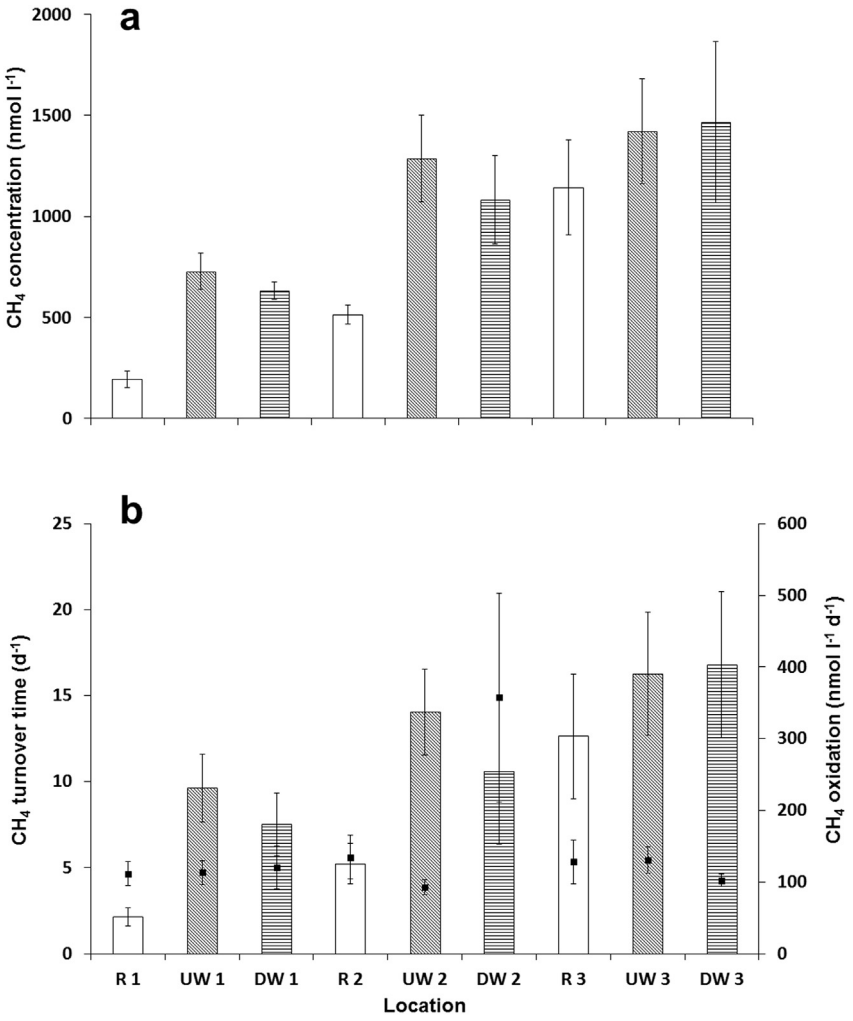

Fig. 4. CH_4 concentrations in surface water (A) (mean value of n = 4–8 ± SE) and aerobic CH_4 oxidation potential in surface water (B) expressed as potential CH_4 turnover time (black squares) and CH_4 oxidation rate (bars) (mean values of n = 6–12 ± SE) along the studied river reach (UW = upstream of the weir, DW = downstream of the weir, R = river section).

Table 4
The CH_4 fluxes from the surface water to the atmosphere and calculated gas transfer velocity k (mean values ± SE).

Site	Directly measured CH_4 emission[a] ($mmol\ m^{-2}\ d^{-1}$)	Calculated CH_4 diffusive fluxes[b] ($mmol\ m^{-2}\ d^{-1}$)	Total CH_4 emission[c] ($mmol\ m^{-2}\ d^{-1}$)	kCH_4[d] ($cm\ h^{-1}$)	k600[e] ($cm\ h^{-1}$)	kCH_4[f] ($cm\ h^{-1}$)
R 1	0.75 ± 0.18	0.83 ± 0.18	0.83 ± 0.27	19.0 ± 2.29	19.3 ± 2.32	18.1 ± 4.41
UW 1	63.4 ± 30.9	0.54 ± 0.15	8.15 ± 2.26	3.14 ± 0.06	3.18 ± 0.06	297.2 ± 225.3
DW 1	2.28 ± 0.32	2.78 ± 0.02	2.78 ± 0.02	18.4 ± 0.27	18.7 ± 0.28	15.2 ± 5.78
R 2	2.26 ± 0.59	2.01 ± 0.38	2.05 ± 0.29	16.4 ± 0.79	16.6 ± 0.80	17.6 ± 6.76
UW 2	176.5 ± 32.7	1.15 ± 0.55	66.1 ± 1.00	3.57 ± 0.53	3.61 ± 0.54	747.2 ± 489.6
DW 2	10.31 ± 1.47	3.67 ± 1.30	3.65 ± 1.25	14.1 ± 0.20	14.3 ± 0.20	46.0 ± 17.9
R 3	1.62 ± 0.60	1.92 ± 0.63	2.13 ± 0.71	7.05 ± 0.10	7.14 ± 0.10	7.69 ± 5.09
UW 3	392.1 ± 108.6	1.27 ± 0.55	131.9 ± 75.2	3.83 ± 0.19	3.89 ± 0.19	1820 ± 1398
DW 3	2.87 ± 0.55	3.81 ± 1.84	3.77 ± 1.77	10.8 ± 0.17	11.0 ± 0.18	13.1 ± 10.4

[a] Diffusion together with ebullition directly measured by chamber method.
[b] Calculated according to Eq. (7).
[c] The total CH_4 emissions estimated from calculated diffusive fluxes (Eq. (7)) and ebullition measured with bubble traps.
[d] Calculated according to Eq. (8).
[e] Calculated according to Eq. (10).
[f] Calculated according to Eq. (9).

sediment carbon content that was observed upstream of the weirs (5.5 ± 0.8%), was at the lower end of values reported from lake sediments (1–34%; Huttunen et al. (2006); Duc et al. (2010)). Carbon content was much lower (0.8 ± 0.6%) in sediments downstream of the weirs and in river reaches, which corresponds to values from other rivers (1.1% Trimmer et al. (2009), 0.2–0.8% Sollberger et al. (2014)).

Organically rich and fine sediments upstream of the weirs resulted in considerably higher values of potential CH_4 consumption and production compared to locations downstream of the weirs and river sections. Slow moving river sections with higher sedimentation rates have been previously shown to result in enhanced methanogenesis and ebullition (Sanders et al., 2007; Maeck et al., 2013).

The high methanogenic potential of sediments recorded upstream of the weirs is comparable to organically rich lake sediments (0.2–8.4 μmol $gDW^{-1}\ d^{-1}$; Conrad et al. (2009); Duc et al. (2010)), while the CH_4 formation in the other locations studied was lower, comparable to other rivers (Gebert et al., 2006; Sanders et al., 2007; Mach et al., 2015). The only exception was observed in location R 3, where high organic carbon content in accumulated sediment and incubation potentials were almost the same as upstream of the weirs, which could be given by local conditions of this river reach. Location R 3 could be partially affected by backwater region of third weir as indicated by reduced flow velocity and higher organic carbon content in sediment, although median grain size of sediment is high and more comparable to river reaches.

Anoxic conditions and high CH_4 concentrations together with increased contribution of ebullition are positively related to the proportion of fine sediments (Baulch et al., 2011). It is consistent with our results, where we observed significant amounts of CH_4 transported in bubbles from sites with the highest CH_4 production potential and the finest median grain size. The same positive relationship exists between organic matter content in sediments and CH_4 formation (Yang, 1998; Gebert et al., 2006).

Measurements of stable isotopic composition of CH_4 produced during inhibited and uninhibited incubation experiments showed prevailing hydrogenotrophic methanogenesis upstream of the weirs, whereas CH_4 was rather produced from acetate in river reaches. Again the methanogenic pathways upstream of the weirs corresponds more to lake sediments, while the river reaches in this study represent values previously observed in other rivers (see Introduction).

Conrad (1999) and Conrad et al. (2009) provided conceivable explanations for the prevalence of hydrogenotrophic methanogenesis in

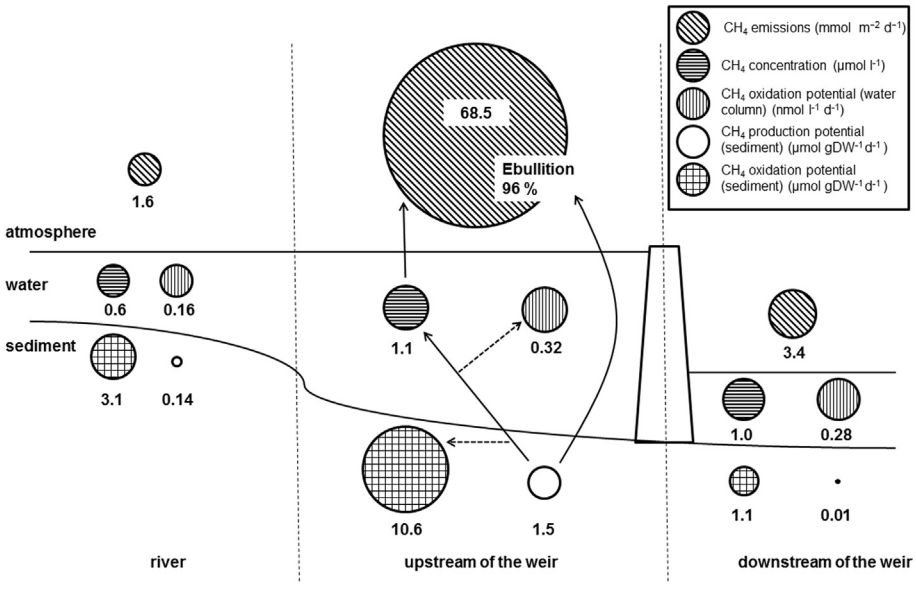

Fig. 5. Schematic illustration of measured parameters related with CH_4 budget in impounded river. Size of circles for each parameter corresponds to its mean value. Arrows suggest possible fate of CH_4 produced in sediments. Solid arrows show CH_4 diffusion to the surface water and to the atmosphere (or release directly to the atmosphere in bubbles). Dashed arrows indicates potential sink for CH_4 during its transport. Discrepancy between CH_4 production potential and CH_4 oxidation potential in sediments is given by substrate addition (CH_4) during incubation experiments (see 2.1.). Hence it serves mainly for comparison of microbial activity between locations and not for calculation of net CH_4 emissions from the sediments.

freshwater sediments. It may be due to: non-steady-state conditions (different pace of acetate and H_2 consumption), additional sink of acetate (syntrophic acetate-consuming microbial consortia) or incomplete degradation of organic matter. Generally, the additional sink of acetate in natural habitats is caused by competition of methanogens with ferric iron reducers or sulfate reducing bacteria, which inhibit methanogenesis until depletion of their electron acceptors (Zinder, 1993). The next potential sink is acetate turnover by syntrophic acetate oxidation, which is characterised by a small isotopic effect. Hence, it may easily be overlooked and thus be more widespread than presently assumed (Conrad and Klose, 2011). Nevertheless, syntrophic acetate oxidation takes place mainly in sediments, where the acetoclastic *Methanosaeta* is absent, which is not a usual situation in freshwater sediments (Karakashev et al., 2006). Moreover, we can probably exclude the contribution of homoacetogenesis as additional source of acetate or sink of H_2 and CO_2, because we observed $\delta^{13}C$ of acetate values enriched (~5‰) compared to $\delta^{13}C$ of organic matter, while the $\delta^{13}C$ of acetate is strongly depleted compared to $\delta^{13}C$ of organic matter, when the homoacetogens contribute to acetate production together with fermentation of organic matter. These factors together can theoretically lead to an increased contribution of hydrogenotrophic methanogenesis in our examined sediments; however only partial mineralization of organic matter, which may result in CH_4 production from H_2 and CO_2, seems to be the most plausible explanation for the dominance of CH_4 production by CO_2 reduction in these sediments as well as in lake sediments (Conrad et al., 2009). This finding together with the high CH_4 production potentials and other sediment characteristics (Table 2) shows that CH_4 related processes upstream of the weirs are more comparable with lake sediments than river sediments.

4.2. Effect of dams on the CH_4 dynamics of river water

One would expect that the impacts of dams on the microbial processes in the sediment described above might also shape other differences, which we find in the water column. Neither the CH_4 concentration nor the methanotrophic activity in the water column, however were dramatically impacted by the the weirs.

Our CH_4 surface concentrations observed in this study (0.1–2.2 μmol l^{-1}) were in the range of values reported from other streams and rivers: 0.01–1.2 μmol l^{-1} (Lilley et al., 1995), 0.01–2.2 μmol l^{-1} (Wilcock and Sorrel, 2008), and 0.04–6.8 μmol l^{-1} (Yvon-Durocher et al., 2011).

The increase of CH_4 concentrations downstream is a usual trend in rivers, when CH_4 sources contribute gradually to the overall CH_4 concentration in surface water (Wang et al., 2009; Anthony et al., 2012; Striegl et al., 2012). Downstream increase of CH_4 concentrations observed in this study is relatively high for such a short river reach, pointing to important CH_4 sources. An even greater increase of surface CH_4 concentration along an 800 m long pool resulting from ponding behind a spillway has been recorded by Lilley et al. (1995), where the concentration changed from 7.7 to 244 nM.

CH_4 concentrations were strongly affected by microbial CH_4 oxidation in the water column without difference between types of locations (i.e. upstream of the weirs, downstream of the weirs, river sections). The potential CH_4 turnover time was about six days, which corresponds to high methanotrophic activity in a temperate river (De Angelis and Scranton, 1993). Undetectable microbial CH_4 oxidation was observed by Anthony et al. (2012) in the Willamette River and by Bussmann (2013) in the Lena River. However, there may be considerable differences between rivers or between separate sites of the same river. For instance Lilley et al. (1995) recognized microbial CH_4 oxidation responsible for 25% of methane sink in the Columbia River, but it was negligible in the Wenatchee River. Dzyuban (2011) observed significantly higher rates of bacterial CH_4 oxidation in his examined group of rivers, which were subject to stronger anthropogenic impact, compared with streams without input of organic pollutants.

Considering the high methanotrophic activity in surface water of the Morava River, the occurrence of ebullition is probably more important because CH_4 release in bubbles directly to the atmosphere is not exposed to CH_4 oxidizing bacteria in the water column of shallow impoundments (McGinnis et al., 2006).

4.3. Effects of dams on CH_4 emission

Our values were measured during the summer and over a short term. Thus, they may correspond to an upper maximum of seasonal fluxes and they are not useful for annual estimates of CH_4 emissions (Ortiz-Llorente and Alvarez-Cobelas, 2012; Mach et al., 2016). Although the lower end of this range derived from river reaches (R 1–R 3) is consistent with published values from rivers (Ortiz-Llorente and Alvarez-Cobelas, 2012), the upper limits derived from locations upstream of the weirs (UW 1–UW 3) are considerably larger (mean 68.5 ± 29.9 mmol CH_4 m^{-2} d^{-1}). These values are more comparable to surface CH_4 evasion estimates reported from reservoirs (0.2–131 mmol CH_4 m^{-2} d^{-1}, DelSontro et al. (2011) or lakes (0.3–312 mmol CH_4 m^{-2} d^{-1}, Gonzalez-Valencia et al. (2013).

Moreover, high CH_4 concentrations upstream of the weirs probably influenced CH_4 emissions downstream of the weirs, where emissions were significantly higher than from unaffected river sites. This effect of dams has also been observed by Guerin et al. (2006), where CH_4 concentrations were significantly higher in river sections just below the dam with a decreasing trend downstream of the dams and CH_4 fluxes from downstream rivers were considerably higher than fluxes from the reservoir surface. Although methane loss mediated by degassing at weirs can reach high efficiencies, up to 80% of CH_4 contained in surface water, river downstream of the weirs still remained affected by increased CH_4 concentration (Abril et al., 2005; Richard et al., 2005). The CH_4 concentration decrease between surface water upstream of the weir and downstream of the weir observed in our study was on average 5.5% ranging from 0 to 32%. Consequently, downstream emissions together with degassing at weirs can represent a dominant part of the total emissions (Li and Zhang, 2014) and therefore, it is important to consider all these components when studying the effect of impoundment on CH_4 emissions. Likewise it is clear, that small impoundments significantly increase the importance of rivers as a substantial source of CH_4 to atmosphere.

It has been shown that estimation of CH_4 ebullition and diffusion through the air-water interface is more accurate in case of long term measurements for days or weeks (Joyce and Jewell, 2003; Maeck et al., 2014), because afternoon fluxes could be approximately twice as high as fluxes near sunset and sunrise (Bastviken et al., 2010; Yang et al., 2012). During warmer periods ebullition becomes more important because higher water temperatures, higher CH_4 concentrations and lower gas solubility (Crill, 1996). Ebullition accounted for 74.5% of total CH_4 release to the atmosphere in this study, while wide range of ebullition contribution to total emissions have been reported for rivers (10–80%; Wilcock and Sorrel, 2008; Baulch et al., 2011; Sawakuchi et al., 2014) and lakes (28–98%; Casper et al., 2000; Bastviken et al., 2004; Bastviken et al., 2010).

The CH_4 content in bubbles, which ranged from 16.2 to 61.1%, was comparable to existing literature values from various freshwater ecosystems: streams 26% (Baulch et al., 2011), lakes 44–88% (Casper et al., 2000), and reservoirs 0.001–69% (Deshmukh et al., 2014). Remaining bubble content is usually formed by N_2 and CO_2 (Casper et al., 2000). Ebullition probably contributed to considerable difference in total CH_4 emissions observed between sites upstream of the weirs and river reaches just below the weirs (see above). The CH_4 released in bubbles does not dissolve to surface water in large extent in a shallow water column (McGinnis et al., 2006). Therefore, river sites with similar surface CH_4 concentrations could release significantly different amounts of CH_4 to the atmosphere.

5. Conclusions

The CH_4 dynamics of a river continuum are dramatically impacted by the introduction of weir (compare Fig. 5). In general we see increased microbial activities in the weir sediments affecting methanogenic as well as methanotrophic potentials, resulting in increased emissions at the weir (via diffusion and especially ebullition) and downstream river reaches. Many parameters found for weirs resemble observations for lake systems (reduced water velocity, fine sediment fraction, higher sediment carbon content, high CH_4 production potentials, ebullition of CH_4, high contribution of hydrogenotrophic methanogenesis, high methanotrophic potentials in the sediment as well as in the water). In contrast CH_4 production in the sediments of the other river sections was an order of magnitude lower and characterised by a higher contribution of acetoclastic methanogenesis. The CH_4 emissions within the 16 km river stretch varied over four orders of magnitude, with a peak for the weirs and lower emissions from the other river sections.

Altogether our results confirm that the CH_4 dynamics in a river system show a high local variability and that multiple measurements are needed to characterize the sources and fates of the CH_4. Overall, CH_4 emissions from average weir impoundment can reach up to 42 times higher values than from the river section of an equal area. Therefore, sampling carried out regardless of occurrence of small impoundments can considerably underestimate CH_4 emissions to atmosphere from lotic ecosystems and the importance of these barriers for river CH_4 dynamic.

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7.3. Methane formation and consumption by sediments in the cross-channel profile of the small river impoundment

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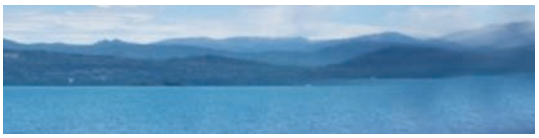

Methane dynamics in aquatic sediments

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_4) into the atmosphere and may contribute significantly to total CH_4 emissions. Even though the details of sources of CH_4 in rivers are not fully understood, weirs have been recognized as a hotspot of CH_4 emissions. In this study, we investigated CH_4 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_4 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^{-1} DW d^{-1}). The CH_4 production was predominated by H_2/CO_2 dependent methanogenesis in the surface sediment layer (0-10 cm),

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while the proportion of acetoclastic and hydrogenotrophic methanogenesis in the deeper sediment layer (10-20 cm) was balanced. The CH_4 oxidation potential of sediments showed the same spatial pattern as observed for the CH_4 production. Our results showed high spatial variability of sediment CH_4 production and oxidation in the cross-channel profile upstream of the weir, whereas the highest CH_4 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_4) into the atmosphere (Bastviken *et al.*, 2011; IPCC, 2013; Deemer *et al.*, 2016). Recent studies show that impounded river zones are CH_4 emission "hotspots", significantly enhancing our estimations of CH_4 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_4 emitted from these sites is mostly derived from sediment CH_4 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_4 production and oxidation in these environments is poorly understood, as is the contribution of individual methanogenic pathways to the total CH_4 production.

In principal, CH_4 is produced during anaerobic degradation of organic matter in freshwater sediments (Zinder, 1993). Anaerobic degradation of carbohydrates results in two dominant intermediates (H_2/CO_2 and acetate), which are further processed by two different metabolic pathways of CH_4 production - hydrogenotrophic methanogenesis (using H_2/CO_2) 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 $^{13}C/^{12}C$ fractionation during conversion of CO_2 and acetate methyl to CH_4 (Conrad, 2005). Contribution of these methanogenic pathways to total CH_4 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_4 production in the sediments of lakes, reservoirs and rivers 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_4 production than profundal sediments, and further served as a source of dissolved CH_4 in lakes together with tributary rivers. Increased CH_4 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_4 production rates (Bussmann, 2005; Hofmann *et al.,* 2010).

Similarly, there is an evident spatial distribution of surface water CH_4 concentration in large rivers. Richey *et al.* (1988) and Anthony *et al.* (2012) have shown CH_4 cross-channel gradients with increased CH_4 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_4 concentrations and fluxes. However, the cross-channel variability of CH_4 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_4 in the sediments of rivers report higher CH_4 concentration in pore water of nearshore and riparian habitats, while the hyporheic sediments in mid-channel have usually lower CH_4 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 (i.e. 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_4 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_4-related processes in a river impoundment. For this purpose, we examined 1) CH_4 production and oxidation rates of the sediments; 2) relationships between environmental variables; and 3) contribution of the methanogenic pathways to total CH_4 production using stable carbon isotopes, all in a cross-channel profile and two different sediment depths of a small river impoundment in Central Europe.

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^3 s^{-1} 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 (version 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) by the Analytical Chemical Laboratory of the University of Marburg, Germany. The dry weight of the sample was determined gravimetrically.

For determination of CH_4 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_2, closed with butyl rubber stoppers and incubated at 25 °C in a dark room. At the start of the incubation (before flushing with N_2), 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 $\delta^{13}C$ of acetate. The gas headspace of half of the bottles was supplemented with 3% CH_3F 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_4, CO_2, and $\delta^{13}C$ of CH_4 and CO_2. The CH_4 concentration was analyzed by gas chromatography (GC) using a flame ionization detector (Shimadzu, Kyoto, Japan) and CO_2 concentration was analyzed after conversion to CH_4 with a methanizer (Ni-catalystat 350 °C, Chrompack, Middelburg, the Netherlands).

Twenty grams (wet weight) of sediment samples for determination of the CH_4 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_4 to give a final concentration of 10 000 ppm. The incubation was performed at 25 °C in a dark room. The concentration of CH_4 in the headspace of each bottle was measured at 0 h and then ten times over 190 h. The CH_4 production and oxidation potentials were calculated from the slope of CH_4 concentration change over time.

Isotopic analyses and calculations

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 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, 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:

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

where f_{mc} is the fraction of hydrogenotrophic methanogenesis; δ_{CH4} is the directly measured isotopic signature of the carbon in CH_4; δ_{mc} is the carbon isotopic signature of CH_4 solely produced from CO_2 (directly measured from assays inhibited by methylfluoride) and δ_{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 α_{ma} is the fractionation factor for acetoclastic methanogenesis (α_{ma} = 1.009; Goevert and Conrad, 2009) and δ_{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 ± 0.95 mm) compared to deeper sediments (10-20 cm; mean 6.5 ± 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 ± 0.6 %) compared to deeper sediments (mean 1.1 ± 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 ± 0.05 %) compared to deeper sediments (mean 0.11 ± 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 ± 0.1 (Tab. 1).

Methane production and oxidation by sediments

Mean CH_4 production potential ranged between 0 and 2.4 µmol gDW^{-1} d^{-1} (Fig. 3A). The methane production of nearshore sediments (0-4 m; mean 1.3 ± 0.3 µmol gDW^{-1} d^{-1}) was significantly higher compared to the rest of the samples (6-14 m; mean 0.2 ± 0.1 µmol gDW^{-1} d^{-1}) ($P< 0.05$). In total, the surface sediment layer (0-10 cm; mean 0.9 ± 0.2 µmol gDW^{-1} d^{-1}) had significantly higher CH_4 production than the deeper layer (10-20 cm; mean 0.3 ± 0.2 µmol gDW^{-1} d^{-1}) ($P< 0.05$). Mean CH_4 oxidation potential of sediments ranged from 0.5 to 13.3 µmol gDW^{-1} d^{-1} and showed the same spatial pattern as observed for the CH_4 production potential (Fig. 3B). The nearshore sediments (0-4 m; mean 7.4 ± 1.1 µmol gDW^{-1} d^{-1}) had higher CH_4 oxidation potential compared to rest of the sampled sediments (6-14 m; mean 1.7 ± 0.6 µmol gDW^{-1} d^{-1}) ($P< 0.05$). In addition, the surface sediment layer (0-10 cm; mean 5.6 ± 1.1 µmol gDW^{-1} d^{-1}) reached significantly higher CH_4 oxidation than deeper sediments (10-20 cm; mean 2.0 ± 0.7 µmol gDW^{-1} d^{-1}) ($P< 0.05$).

Samples with the highest CH_4 production rates were characterized by immediate and linear CH_4 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_4 concentration increase (Fig. 4B). The CH_4 concentration in the headspace of the vials incubated for determination of the CH_4 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_4 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 $\delta^{13}C$ of organic matter in sediments was -27.9 ± 0.4 ‰ VPDB (n = 48) (Tab. 1). The $\delta^{13}C$ of acetate accumulated in inhibited samples (without acetoclastic methanogenesis) was on average very comparable with ^{13}C of the organic matter with mean -26 ± 1.2 ‰ VPDB (n = 25). Acetate did not accumulate in uninhibited samples. The stable carbon isotopic composition of CH_4 ($\delta^{13}C$-CH_4) produced at the end of the uninhibited incubation was on average -69.0 ± 1.6 ‰ VPDB.

The contribution of hydrogenotrophic methanogenesis (f_{mc}) to CH_4 production was stable during the whole incubation time and ranged from 41 to 75 % for individual samples (Fig. 6). The CH_4 production was predominated by the H_2/CO_2-dependent methanogenic pathway in the surface sediment layer (0-10 cm) with mean f_{mc} = 56 ± 0.02 % at the end of the incubation (Figure 6A). However, the contribution of acetoclastic and hydrogenotrophic methanogenesis to the total CH_4 production in the deeper sediment layer (10-20 cm) was balanced with mean f_{mc} = 51 ± 0.05 % and prevalence of 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 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 (0-4m), we observed high potential 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 overlaying river water is lower and alternative electron acceptors such as dissolved Fe^{2+}, NO_3^-, 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 CH_4 production, since a strong positive correlation between CH_4 production potential and organic carbon content exists ($r = 0.94$). High 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 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 CH_4 formation rate is in the sediments with lower C/N ratios (<10), while the sediments with higher C/N ratios (~20) are characterized by lower 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 (~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 CH_4 oxidation potential compared to the CH_4 production potential of all incubated sediment samples (Fig. 3), sediments represent a source of CH_4 into the surface water and the atmosphere. As suggested by Bednařík *et al.,* 2017, increased CH_4 concentrations were observed in surface water, together with high contribution of ebullition to the total CH_4 emission upstream of the weirs. The discrepancy between CH_4 production potential and CH_4 oxidation potential in sediments is given by substrate addition (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 CH_4 flux from the sediments. It would be necessary to measure in-situ 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 CH_4 (Sansone *et al.,* 1998; Bednařík *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, stay rates and pathways of CH_4 production remain similar despite changes in the microbial community composition.

Methanogenic pathways

Generally, CH_4 production consists of three distinct phases: 1) 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_2 is produced; 2) the methanogenic phase, characterized by strong CH_4 formation which maximally depends on the sediment characteristics; 3) in the third phase (the steady state phase), CH_4 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_4 production was rapid. An absence of lag phase at the start of the CH_4 production could be explained either by 1) overlap of the reduction and methanogenic phases (i.e. CH_4 production started at a relatively high redox potential before the full depletion of inorganic electron acceptors; *Yao et al.,* 1999); or 2) 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_4 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_4 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_4 production in surface sediments was the consistent reduction of H_2 and CO_2 (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_2/CO_2; 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_4 production from acetate, this can be easily explained by homoacetogenesis (reduction of CO_2 with H_2 via the acetyl-CoA) (Mach *et al.,* 2015), while the higher contribution of the hydrogenotrophic pathway to total CH_4 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_3F). Syntrophic acetate oxidation is exceptional in freshwater sediments and unlikely to explain the major part of CH_4 production. The most probable explanation remains incomplete degradation of organic matter, i.e. an additional source of H_2, 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_4 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 methanogenetic 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_4 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.

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_4 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_4 production potential upstream of weirs, studies focusing on quantification of direct CH_4 fluxes from sediments to surface water and conducted in-situ are necessary.

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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 ^{13}C of organic matter
			0-10 cm		
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
			10-20 cm		
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

Tab. 2 Values of the correlation coefficients (r) expressing the relationship between the examined variables. All correlations shown in the table are significant at $P<0.05$ (MPP = CH_4 production potential; MOP = CH_4 oxidation potential).

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

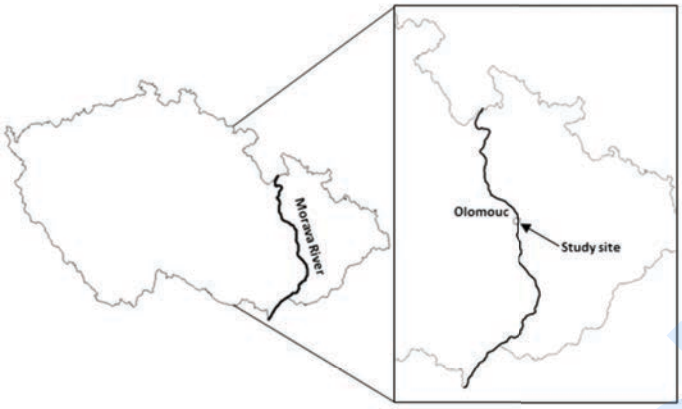

Fig. 1. Location of the Morava River in the Czech Republic and position of the study site in the river.

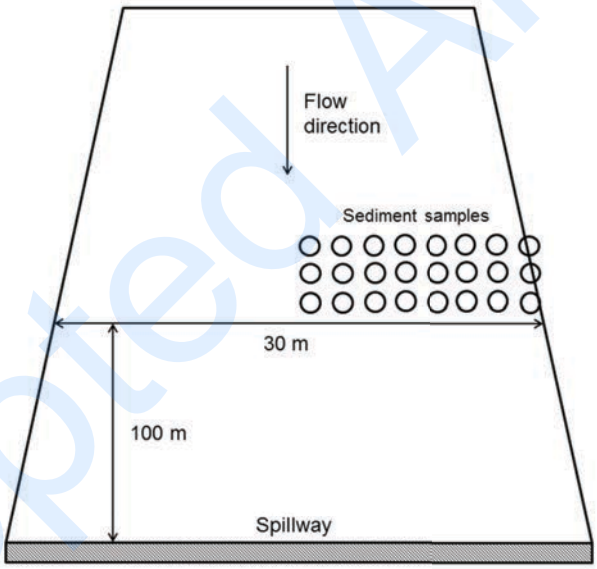

Fig. 2. The schematic of the sediment sampling in the cross-channel profile of a small river impoundment. Triplicates were taken from eight different distances from the bank line to the mid-channel (0 m, 2 m, 4 m, 6 m, 8 m, 10 m, 12 m, 14 m).

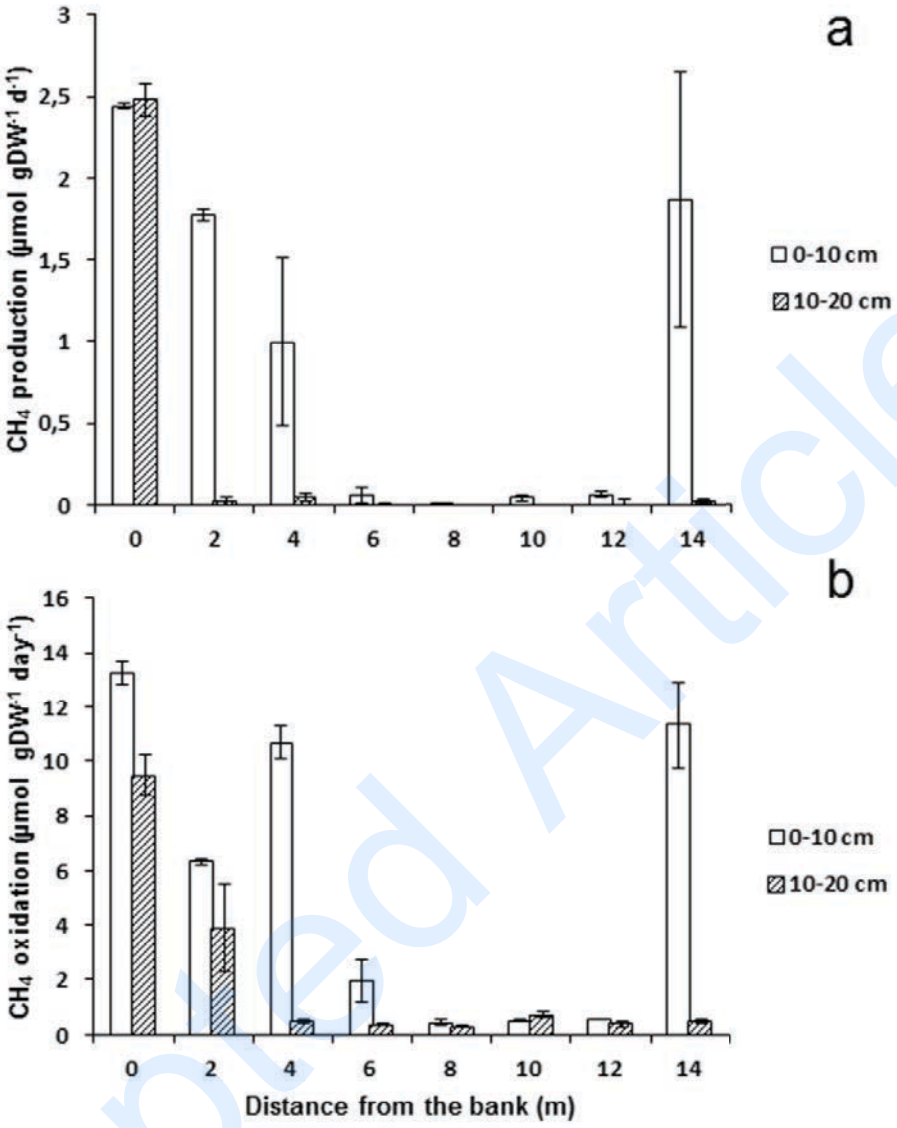

Fig. 3. The CH_4 production (a) and oxidation (b) potentials of sediments (mean values ± SE).

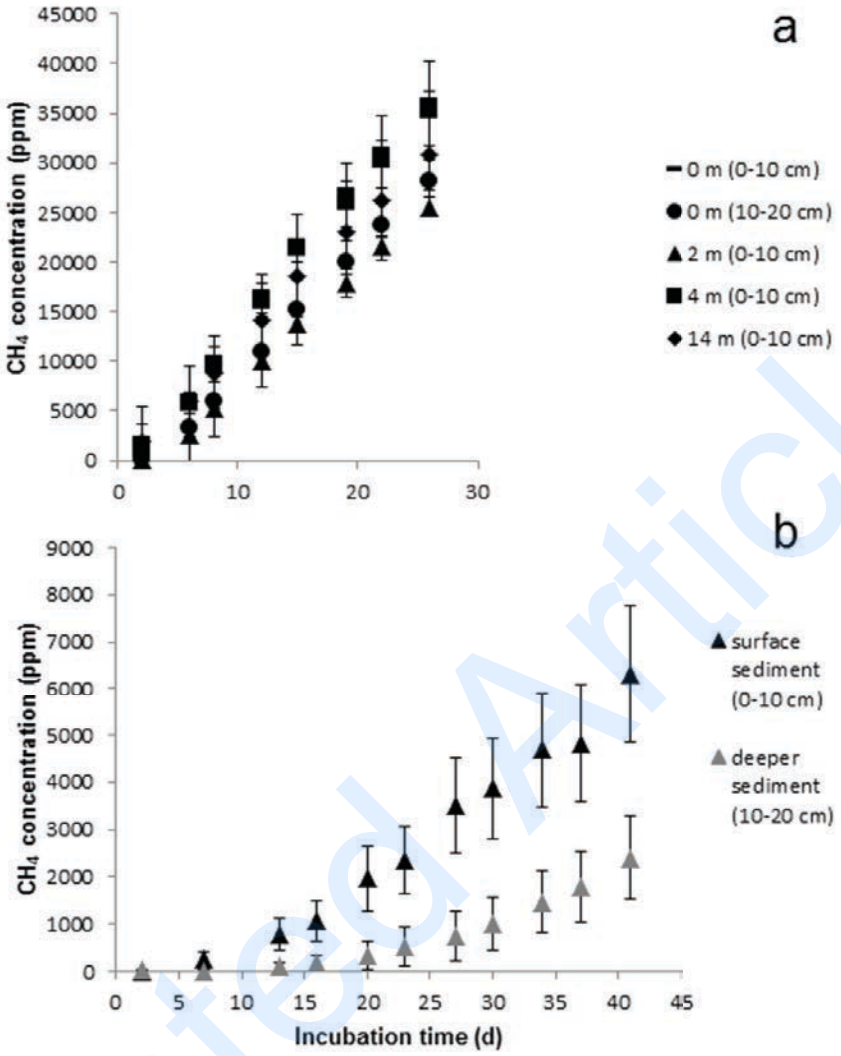

Fig. 4. Dynamics of CH_4 formation during the incubation of (a) the most productive sediment samples (mean values ± SE), and (b) remaining (i.e. less productive samples characterized by a lag phase lasting more than one week after the start of the incubation) surface (6-12 m; black triangles) and deeper (2-14 m; gray triangles) sediment samples in anoxic conditions (mean values ± SE).

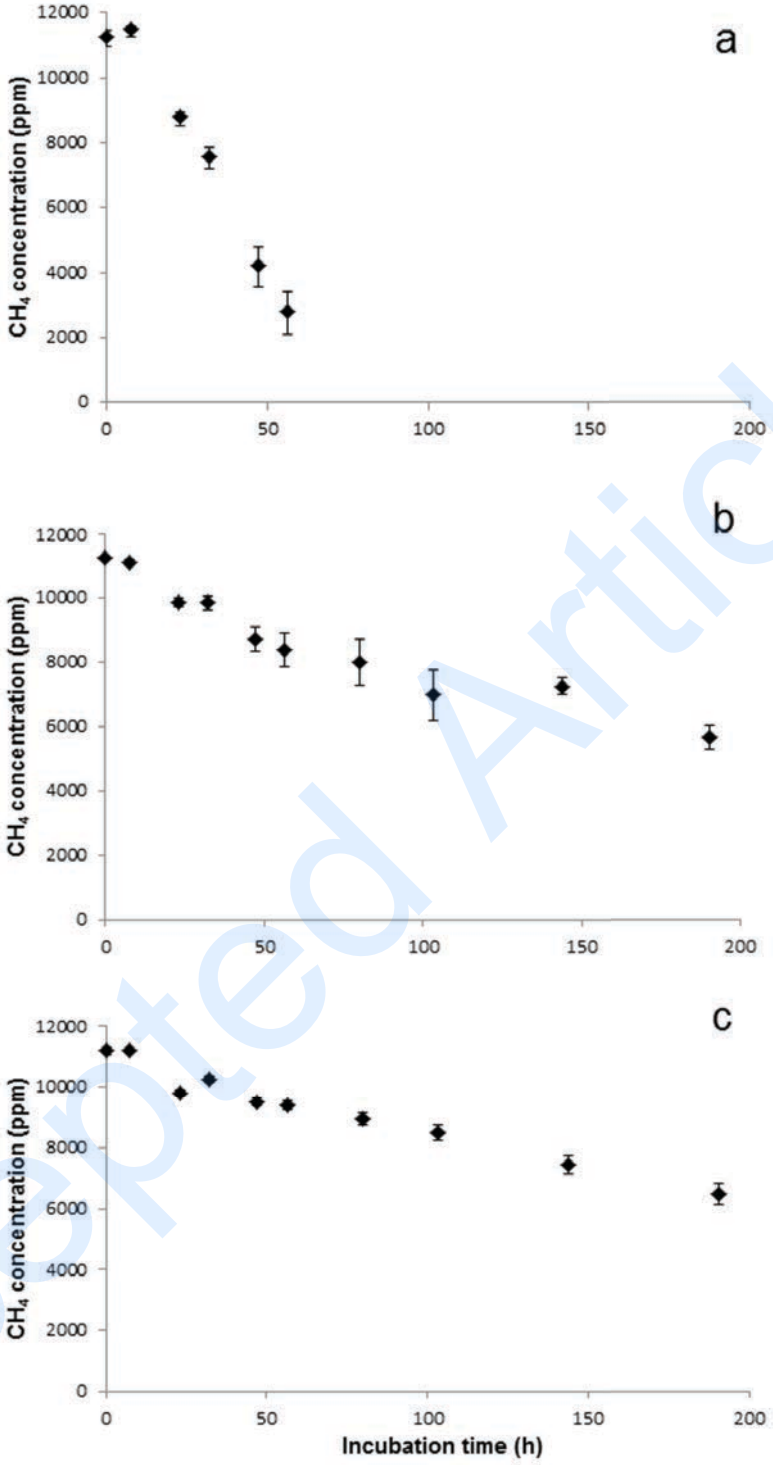

Fig. 5. Methane concentration decrease during the incubation of (a) the most active sediment samples (0 m, 2 m, 4 m, 14 m), (b) remaining surface (0-10 cm) sediments (6-12 m), and (c) remaining deeper (10-20 cm) sediments (6-12 m) in oxic conditions (mean values ± SE) .

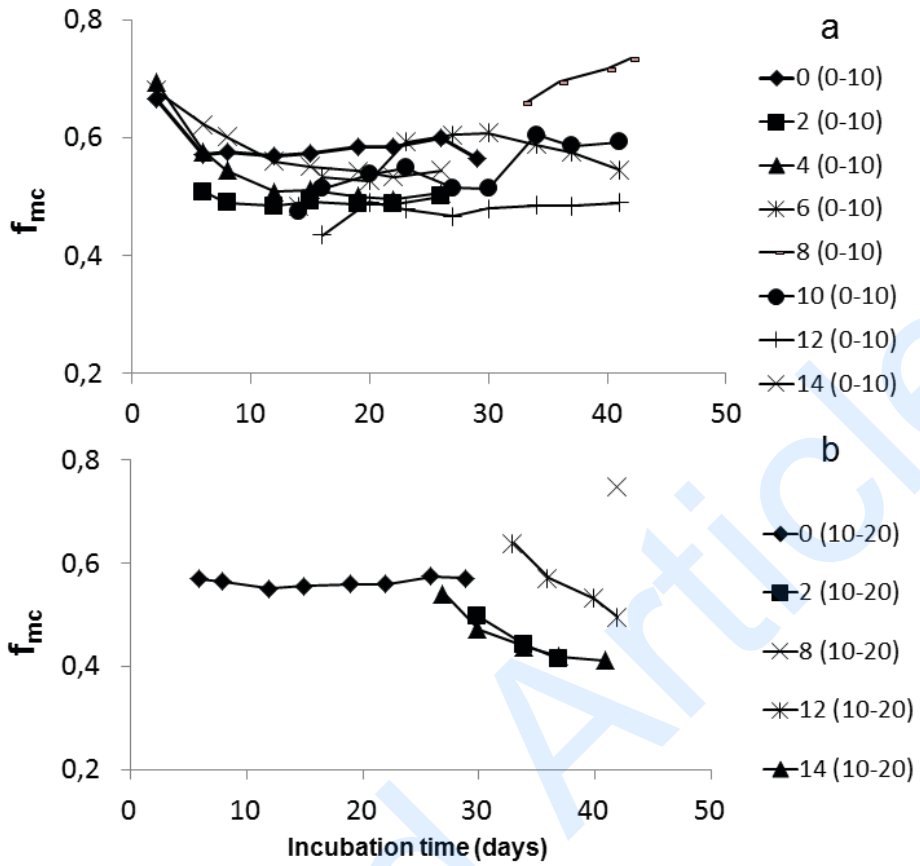

Fig. 6. The contribution of hydrogenotrophic methanogenesis (f_{mc}) to total CH_4 production in the samples of (a) the surface sediments (0-10 cm) and (b) the deeper sediment layers (10-20 cm). The missing time points are given by insufficient CH_4 production rate for stable carbon isotopes measurement of the incubated sample.

8. Curriculum vitae

Name:	Adam Bednařík
Born:	March 27, 1989 in Zlín

Education

2013 to present	PhD study – Ecology Faculty of Science, Palacký University, Olomouc
2011 − 2013	Master study – Hydrobiology Faculty of Science, Palacký University, Olomouc
2008 − 2011	Bachelor study – Protection and creation of the environment, Faculty of Science, Palacký University, Olomouc

Work experience

2017 to present	The Krkonoše Mountains National Park Administration (hydrobiologist)
2014 – 2015	Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University, Olomouc (pedagogical worker)

Cooperation on research projects

2015 – 2016	Sources of methane in the river (Endowment Fund of Palacký University, Olomouc) - principal researcher of the project
2016 – 2017	EuroRun – Assessing CO_2 Fluxes from European Running Waters - participant

Abroad stay

01/2015 – 02/2015	Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
02/2014 – 05/2014	Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
01/2014	Universiti Brunei Darussalam – Kuala Belalong Field Studies Centre (KBFSC), Brunei

Membership

2013 to present	Czech Limnological Society

Publications in peer-reviewed journals with IF

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Bednařík A, Rulík M (2017): Benthic methane fluxes through the sediment-water interface of a lowland river. 10th Symposium for European Freshwater Sciences, Olomouc, Czech Republic – poster

Bednařík A, Blaser M, Matoušů A, Rulík M (2016): Effect of weirs on methane budget in river. In: Klimaszyk P, Marszelewski W, Rzymski P (eds.): International konference "Lakes, Reservoirs & Ponds: Impacts – Threats – Conservation", Ilawa, Poland – poster

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Importance of stable carbon isotopes for studying dynamics of methanogenesis in rivers

Summary of the Ph.D. Thesis

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The oral defence will take place on ..... at ..... in the room ..... at the Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, Olomouc.

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Abstract

Methane is one of the most important greenhouse gases. Despite of recent studies pointing out important contribution of running waters to natural methane emissions to the atmosphere, data concerning the methane sources in rivers are very scarce. This thesis deals with methane dynamic in river ecosystems with special emphasis on an effect of river impoundments on methane related processes. Beside the methane concentrations, oxidation, production and emission to the atmosphere, the changes in contribution of two main methanogenic pathways (hydrogenotrophic and acetoclastic) to the total methane production were determined using the stable carbon isotopes analysis. We found hotspots of the sediment methane production in a river continuum, which are connected with the local driving factors including mainly the existence of artificial barriers as weirs. Changes in rate of individual components of river methane dynamic were further examined in cascade of three weirs and river reaches between them. We found that river impoundments affect the sediment processes in several ways, including changes of the sediment characteristics (fine sediment fraction, higher sediment carbon content), enhanced microbial activities in the sediment (methane production and oxidation), ebullition of methane, and different contributions of hydrogenotrophic methanogens to the released methane. Thus, many parameters found for weir impoundments resemble observations for lake systems. Moreover, remarkable spatial variability in sediment methane production was demonstrated in cross-section profile of the one studied impoundment. Presented studies point to only the part of the samples could be activated for methane production despite of presence of methanogens (most probably due to substrate limitation). This suggest that the observed variability of the microbial activities as well as the resulting methane concentrations in the water column are only indirectly linked to the presence of different microbial guilds, but rather affected by their activity. Altogether our results confirm that the methane dynamics in a river system show a high local variability and that multiple measurements are needed to characterize the sources and fates of the methane. Obtained results might be further used for better estimates of importance of rivers in a global methane budget.

Contents

1. Introduction

Methane (CH_4) is one of the most potent greenhouse gases with a global warming potential ~28 times higher than carbon dioxide (CO_2) over time horizon of 100 years and represents about 15 % of the anthropogenic greenhouse effect (IPCC 2013). From the mid-Holocene to about 300 years ago atmospheric methane concentration rose steadily by about 25 % (Brook et al. 2000). With human population increase and industrialization, methane concentration is now about 250 % higher than it was in the preindustrial age (Etheridge et al. 1998). Current atmospheric methane concentration is 1858 ppb (Dlugokencky 2018).

While the sinks of the methane in the environment are quite clear (reaction with hydroxyl radicals in the troposphere or oxidation by methanotrophic bacteria), the sources of methane are much more diverse. Among main atmospheric methane sources are included wetlands, ruminants, termites, oceans, freshwater sediments, landfills, biomass burning and fossil methane released during fossil fuel extraction (Wuebbles and Hayhoe, 2002). The most of the methane is produced microbiologically, while contribution of freshwater habitats (wetlands, rice fields) creates ~33 % of the annual atmospheric methane flux (Conrad 2009). Moreover, recent studies estimate that annual methane emission from fluvial ecosystems is equivalent to 20-50 % of lake or wetland effluxes (Stanley et al. 2016). However, compared to other natural ecosystems, very scarce data were available for estimation of their global significance and thus importance of lotic ecosystems contribution to global methane emissions from natural environments were overlooked in the past (Saarnio et al. 2009, Bastviken et al. 2011).

Methane dynamic in lotic ecosystems consists of (1) production of the methane within hyporheic sediments, which are place of anaerobic metabolism and formation of methane, (2) subsequent diffusion of methane to the surface water, where the methane is (3) transported downstream or (4) emitted to the atmosphere. The methane is also a subject of significant (5) oxidation by methane oxidizing bacteria during its transport in lotic ecosystems. Methanotrophs are often found at the anoxic/oxic interface of various habitats including freshwater sediments, where they consume the methane arising from methanogenesis and are thus able to reduce the most of the potential methane flux to surface water (Segers 1998, Trimmer et al. 2010). Last but not least, methane has been recently recognised as a potentially important carbon and energy source for freshwater food webs due to conversion of methane to microbial biomass by methane oxidation bacteria, which can be highly productive (Jones and Grey 2011). In rivers, grazing

methane-oxidizing bacteria could provide the caddis larvae (genuses *Agapetus* and *Silo*) up to 30 % of their carbon (Trimmer et al. 2009).

Basically, there are three main sources of methane to surface water of fluvial ecosystems: drainage of surrounding methane rich habitats, groundwater input and river sediments, while exact role of each of these sources is not yet quantified in the overall river C budget. Moreover, water inflows enriched in methane from wastewater treatment plants are significant source of methane in the human influenced rivers (Alshboul et al. 2016). The largest and most ecologically significant pathways of methane efflux from natural environments to the atmosphere are diffusion, ebullition (escape of methane in gas bubbles directly from the sediments) and passage through vascular plants. In natural streams and rivers both methane diffusion and ebullition were recognized to contribute significantly to the total methane emissions from these ecosystems. Moreover, ebullition bypasses the importance of oxidation by methanotrophic bacteria and hence, ebullition of sediment gas bubbles is an important transport process accounting up to 60 % of the total methane emissions from the fluvial ecosystem (Wilcock and Sorrel 2008).

In man-altered rivers, important elements modifying the methane dynamics in lotic ecosystems are artificial impoundments, which reduced flow water velocity and thus increase water residence time that allows organic matter sedimentation and development of anoxic conditions suitable for methane production (e. g. Maeck et al. 2013, Wilkinson et al. 2015, Crawford et al. 2016). Moreover, it was found that smaller impoundments have greater sediment accumulation rates per unit area than the large ones, while small impoundments create a significant part of the total area of impoundments (Downing et al. 2006, Downing et al. 2008). Existence of these artificial barriers play important role in resulted contribution of different methane evasion pathways. Molecular diffusion is usually dominant pathway in rivers (with exceptions described above), while ebullitive emissions are the dominant way for methane emissions from the surface of tropical reservoirs, and it is less significant way for methane emission at the air–water interface in the temperate reservoirs, where the diffusive fluxes are prevailing (Yang et al. 2014).

Generally, the most of the methane produced in nature originates from acetate, however, the relative amounts of methane produced from the methyl group of acetate or reduction of CO_2 can vary depending on the presence of other metabolic groups of anaerobes and the environment (Ferry 1993). Hydrogen should theoretically account for

33 % of total methanogenesis when carbohydrates or similar forms of organic matter are degraded (Conrad 1999). Many methanogenic environments show both much lower and much higher contributions of H_2 to methane production than is considered normal. The relative contribution of the two main methanogenic pathways to total methane production can be calculated due to the sufficient difference in isotopic fractionation during both the hydrogenotrophic and acetoclastic methanogenesis. However, experimental determination of fractionation factors in environmental samples is difficult, since either the hydrogenotrophic or the acetoclastic methanogenic pathway must be suppressed in order to determine the isotope fractionation by one of the two pathways specifically. For that reason, it was important milestone, when the methyl fluoride (CH_3F) was reported to be a specific inhibitor of acetoclastic methanogens, while hydrogenotrophic methanogens were not affected (Janssen and Frenzel 1997).

2. Aims of dissertation thesis

The aims of this thesis were:

1) To characterize the methane production and oxidation potential of the river sediments in longitudinal profile of the Elbe River and to reveal the contribution of individual methanogenic pathways to the total methane production using the stable carbon isotope analysis.
2) To compare the rate of the methane related processes (methane production, oxidation, emissions to the atmosphere) between weir impoundments and free flowing river sections of Morava River. Part of it was also quantification of methane ebullition and extent of methane degassing in the spillways.
3) To determine the proportion of methane production pathways in the sediments of the examined weir impoundments and river sections.
4) To characterize the spatial variability of methane production and consumption by the river sediments including the proportion of the methanogenic pathways in the cross-section profile of weir impoundment.

3. Material and methods

The samples collection and field measurements have taken place at the 11 locations along the Elbe River from river km 8 to river km 948 and in the cascade of three weirs in the 16 km long stretch of Morava River. More detailed characterization of studied sites is included in the Paper I and Paper II, respectively. Determination of individual components of methane dynamic in river demands involvement of many different methods, which are individually described in detail in the attached papers.

Briefly, methane production and oxidation potentials of sediments were measured during incubation experiments in the laboratory. Sediment for measurement of methane production potential was incubated in anoxic conditions (headspace of bottle was flushed with nitrogen) approximately one months. Gas samples from headspace were taken repeatedly during the course of incubation (4-6 weeks) and analysed for concentrations of methane. The rate of methane production was calculated from the slope of the linear regression given by the graph of methane concentration increase over time. Sediment for measurement of methane oxidation potential was incubated under the oxic conditions (ambient air in a headspace) with addition of methane. Potential methane oxidation rates were obtained from the slope of the methane concentration decrease over time.

Determination of the contribution of individual methanogenetic pathways to total methane production was carried out using the stable carbon isotope measurements. It is based on effect of methylfluorid (CH_3F), which completely inhibits acetate-dependent methanogenesis. Methane was then exclusively produced by hydrogenotrophic methanogenesis and thus allowed determination of the fractionation factors specific for this methanogenic pathway.

Methane emissions from the surface water to the atmosphere were detected by three different methods fully described in Paper II. First, methane emissions across the air-water interface were directly measured by a floating chamber method. Second, methane diffusion fluxes (i.e. without contribution of the ebullition) to the atmosphere were determined using calculations derived from recent studies and based on the gas transfer velocity and the methane concentration gradient between the river water and the atmosphere (Striegl et al. 2012, McGinnis et al. 2014, Borges et al. 2015, Bodmer et al. 2016). Third, ebullition measurements were carried out using submerged gas funnel traps. Moreover, degassing at weirs was estimated on the basis of methane concentration differences and water discharge. From comparison of first and second

method for measurement of methane emissions in our conditions followed, that we did not observe the increased methane emissions caused by the additional induced turbulence arising from application of anchored chambers described by Lorke et al. (2015). Diffusive fluxes calculated from gas transfer velocity and directly measured emissions by chambers were not different in sites with the marginal contribution of the ebullition.

4. Main results

4.1. Sediment methane dynamics along the Elbe River

The methane production was detected in six sediment samples (from 11 of total) along the Elbe River, while the methanotrophy was found in all examined sediment samples. The methane production and oxidation differed considerably in the river longitudinal profile without any clear trend and without any correlation with other studied environmental parameters. Moreover, the mcr-A and pmo-A gene copy numbers (genes showing the presence of methanogens and methanotrophs, respectively) were similar and quite stable among the sediment samples. It follows that while it was found hotspots of the measured methane processes, the molecular data showed no spatial characteristics. Incubation experiments with isotopic analyses of CO_2 and methane revealed that the hydrogenotrophic pathway of methane formation (CO_2 reduction) was dominant for all examined methane productive sites accounting for 52 to 78 % of total methane release, thus implying the most probably the incomplete degradation of organic matter.

4.2. Effect of weir impoundments on methane dynamics in a river

We found that river methane dynamics might be highly influenced by weirs, especially by increased methane production and consumption by sediments, followed by increasing methane emissions to the atmosphere. Both methane production and oxidation potential of sediments were higher upstream of the weirs compared to downstream of the weirs or usual river reaches. The total methane emissions to the atmosphere reached the highest values upstream of the weirs, while the ebullition accounted for ~96 % of the total methane emissions. Methane consumption in the sediments together with the microbial methane oxidation in the water column substantially contributed to the methane removal from surface water. Thus, the contribution of the ebullition to the methane emissions in these shallow impoundments was enhanced by bypassing microbial methane oxidation, compared to relatively slow diffusion fluxes. Overall, methane emissions from average weir impoundment can reach up to 42 times higher values than from the river section of an equal area. In spite of such high emission fluxes including further methane release by degassing in the spillways of the weirs and high methane oxidation, considerable 7.5 times increased of methane concentration in the surface water was observed in the 16 km long examined section, pointing to important methane sources in such a short river reach.

The contribution of H_2/CO_2-dependent methanogenesis to total produced methane tended to be higher for sediments upstream of the weirs, compared to the sediments from river sections or downstream of the weirs. More precisely, hydrogenotrophic methanogenesis contributed 37 to 89 % of the total methane production and was dominant (more than 50 %) in sediments upstream of the weirs, while acetoclastic methanogenesis was probably prevailing in remaining sediments.

4.3. Methane formation and consumption by sediments in the cross-channel profile of the impoundment

We hypothesized that more uniform sedimentation in cross-channel profiles compared to river due to the overall decrease of the flow velocity upstream of the weir will result in more identical rates of methane-related processes through the transect. Instead, we found frequently observed pattern in lakes and rivers, that littoral zones are main sites of the methanogenic activity. In our study, sediments near the bank zones and in the mid-channel were characterised by the highest organic carbon content (6.9 %) as well the highest methanogenic activity (2.5 mmol g^{-1} DW d^{-1}). Samples from the surface sediment layer (0-10 cm) reached higher methane production than sediments from the deeper layer (10-20 cm) during the incubation experiments. The methane oxidation potential of sediments showed the same spatial pattern as observed for the methane production.

Stable carbon isotopes analysis, used for determination of individual methanogenic pathways, confirms our previous findings that the methane production is dominated by H_2/CO_2 dependent methanogenesis upstream of the weir. However, it was more evident in the surface sediment layer (0-10 cm), while the proportion of acetoclastic and hydrogenotrophic methanogenesis in deeper sediment layer (10-20 cm) was more balanced. This slight shift in the contribution of methanogenic pathways was not caused by the lability or availability of organic substrate because the similarity of these parameters between examined sediment layers.

5. Conclusions

Altogether results presented in this study confirm that river sediments are important place of anaerobic degradation of organic matter with methane as a final product. Moreover, the methane dynamics in a river system show a high local variability, indicating that multiple measurements are needed to characterize the sources and fates of the methane. The methane dynamics along the river continuum is dramatically impacted by the building of small impoundments, which contribute significantly to the total methane production and its subsequent emission disproportionately to their area. Therefore, sampling carried out regardless of occurrence of small impoundments can considerably underestimate methane emissions to atmosphere from lotic ecosystems and the importance of these barriers for river methane dynamic. We found out 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. Modifications of methane related processes in impounded river zones are reflected also by different contribution of individual metabolic pathways of methane production compared to usual river sections.

Recent studies clearly show that rivers may emit considerable amount of methane to the atmosphere. Hence, rivers should be included into the future estimations and models of a global methane budget. Namely, high spatial variability of methane related processes is remarkable in rivers compared to other ecosystems and deserves to be considered in greater details in the further studies. In any case, it is necessary to consider the sampling location of particular water habitats, because sediment samples taken only in the particular zones of water habitats can significantly misrepresent the further extrapolation of obtained results. Considering the considerable sediment methane production upstream of the weirs, more studies focusing on quantification of direct methane fluxes from sediments to surface water and conducted in-situ would also be of great importance.

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7. List of author´s papers

The thesis is based on the following papers:

Paper I:

Bednařík A, Blaser M, Matoušů A, Tušer M, Chaudhary PP, Šimek K, Rulík M (2019): Sediment methane dynamics along the Elbe River. Limnologica (*in review*)

Paper II:

Bednařík A, Blaser M, Matoušů A, Hekera P, Rulík M (2017): Effect of weir impoundments on methane dynamics in a river. Science of the Total Environment, 584–585: 164–174. http://dx.doi.org/10.1016/j.scitotenv.2017.01.163

Paper III:

Bednařík A, Blaser M, Rulík M (2019): Methane formation and consumption by sediments in the cross-channel profile of the small river impoundment. Journal of Limnology (*in press*). https://doi.org/10.4081/jlimnol.2019.1898

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Rulík M, **Bednařík A**, Mach V, Brablcová L, Buriánková I, Badurová P, Gratzová K (2013): Methanogenic system of a small lowland stream Sitka, Czech Republic. In: Matovic MD (ed.): Biomass now – cultivation and utilization (chapter 17), InTech, Rijeka, 395−426 pp.

8. Souhrn [Summary, in Czech]

Metan je spolu s oxidem uhličitým a oxidem dusným řazen mezi nejvýznamnější skleníkové plyny. Navzdory mnoha současným studiím vyzdvihujícím význam říčních ekosystémů jako přírodního zdroje emisí metanu do atmosféry, jsou poznatky týkající se zdrojů metanu v řekách nedostatečné. Předložená práce se zabývá dynamikou metanu v říčních ekosystémech se zvláštním důrazem na vliv umělých příčných bariér (jezů) na procesy spojené s koloběhem metanu. Kromě koncentrací metanu, jeho oxidace, produkce a emisí do atmosféry byl determinován příspěvek dvou hlavních metabolických cest vzniku metanu do jeho celkové produkce, a to s využitím analýzy obsahu stabilních izotopů uhlíku. Na základě provedených měření v říčním kontinuu řeky Labe byly detekovány místa s nezvykle vysokou produkcí metanu v sedimentech, které odpovídaly změnám v lokálních faktorech prostředí spojených především s existencí příčných bariér na vodním toku. Další práce se proto blíže zaměřila na porovnání změn v jednotlivých složkách dynamiky metanu v kaskádě tří jezů a říčních úsecích mezi nimi. Bylo zjištěno, že jezy vyvolávají řadu změn v sedimentačních procesech (usazování jemnější frakce, vyšší obsah organického uhlíku), které se následně projevují v procesech spojených s koloběhem metanu, jako je zvýšená mikrobiální aktivita (produkce a oxidace metanu v sedimentech), vysoký podíl ebulice na celkové emisi do atmosféry (uvolňování metanu ve formě bublin) a rozdílný poměr metabolických cest vzniku metanu. To spolu s ostatními charakteristikami nadjezí vypovídá o tom, že mnoho procesů probíhajících v sedimentech jezových zdrží včetně tvorby bublin a metabolismu uhlíku je lépe srovnatelných s prostředím sedimentů jezer než s říčními sedimenty. Mimo to byla zjištěna také značná variabilita v produkci metanu uvnitř vybraného nadjezí. Další výsledky ukázali, že jen část vzorků inkubovaných v anoxických podmínkách produkuje metan, přestože v nich bylo detekováno srovnatelné množství metanogenních archaea jako v aktivních vzorcích, což bylo s největší pravděpodobností dáno nedostatkem vhodného substrátu. Toto zjištění naznačuje, že pozorovaná variabilita v mikrobiální aktivitě stejně jako výsledné koncentrace metanu ve vodě jsou jen nepřímo řízeny přítomností určitého mikrobiálního společenstva, ale jsou spíše ovlivněny jeho aktivitou. Naše studie tak potvrzuje, že dynamika metanu v říčních ekosystémech vykazuje vysokou prostorovou variabilitu a z toho důvodu lze charakterizovat zdroje metanu a jeho další osud v ekosystému jen s využitím velmi komplexních měření. Získaná data mohou mimo jiné posloužit i jako

cenný údaj pro zpřesnění odhadů významu říčních systémů v bilanci metanu v rámci vnitrozemských vod a v kontextu globální dynamiky metanu.