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Is the methanogenic community reflecting the methane emissions of river sediments? – Comparison of two study sites

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29 **Abstract**

30 Studies on methanogenesis from freshwater sediments have so far primarily focused on lake
31 sediments. To expand our knowledge on the community composition of methanogenic archaea in
32 river sediments we studied the abundance and diversity of methanogenic archaea at two localities
33 along a vertical profile (top 50 cm) obtained from sediment samples from Sitka stream (Czech
34 Republic). In this study we contrast two sites which previously have been shown to have a
35 tenfold different methane emission. Archaeal and methanogen abundance were analyzed by real-
36 time PCR and T-RFLP. Our results show that the absolute numbers for the methanogenic
37 community (qPCR) are relatively stable along a vertical profile as well as for both study sites.
38 This was also true for the archaeal community and for the three major methanogenic orders in
39 our samples (*Methanosarcinales*, *Methanomicrobiales* and *Methanobacteriales*). However the
40 underlying community structure (T-RFLP) reveals different community compositions of the
41 methanogens for both locations as well as for different depth layers and over different sampling
42 times. In general our data confirm that *Methanosarcinales* together with *Methanomicrobiales* are
43 the two dominant methanogenic orders in river sediments, while members of *Methanobacteriales*
44 contribute a smaller community and *Methanocellales* are only rarely present in this sediment.
45 Our results show that the previously observed tenfold difference in methane emission of the two
46 sites could not be explained by molecular methods alone.

47 **Introduction**

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49 River sediments are an example of a unique type of ecosystem which is structured longitudinally
50 as well as vertically and is affected by the fluctuating availability of decayed organic matter
51 coming mostly from the surrounding terrestrial environment. Depending on the local conditions
52 the decaying organic matter can either be oxidized to CO_2 if oxygen is present or it can be
53 anaerobically fermented to CO_2 and methane if other electron acceptors like nitrate, iron, and
54 manganese are depleted. Current data suggest that rivers contribute about 3% of the total release
55 of methane into the atmosphere ( ) or 15-40 % of the efflux of wetland and
56 lakes (Stanley *et al.*, 2016). The majority of this methane is produced in anoxic environments by
57 methanogenic archaea (Wuebbles & Hayhoe, 2002, Bastviken *et al.*, 2004, Ciais & Jones, 2014).
58 Generally, the mineralization of the organic matter under anaerobe conditions is carried out by
59 several microbial organisms: Initially the organic matter is depolymerized and then the
60 monomers are fermented to CO_2 and short chain fatty acids alcohols and other substances, which
61 in turn can be further degraded by syntrophic organisms to finally H2, CO_2 and acetate (Schink,
62 1997). In the absence of other electron acceptors like nitrate, iron, manganese etc. the terminal
63 step of the anaerobic organic matter mineralization results in the release of methane and CO_2
64 (, Schink, 1997).

65 Methanogens are considered to be of prime importance because they are responsible for the final
66 step of mineralization of organic carbon to methane (CH_4) (Capone & Kiene, 1988, Delong,
67 1992). Methane is one of the most potent greenhouse gases with a global warming potential 25
68 times higher than carbon dioxide. A significant contribution to the annual atmospheric methane
69 flux (40-50%) comes from freshwater sediments like lakes, wetlands and rice paddy fields
70 (Cicerone & Oremland, 1988, Conrad, 2009, Rulik *et al.*, 2013). As the sediment depth increases
71 there is also a shift in the physical and chemical conditions, such as redox potential and dissolved
72 oxygen, an increase in temperature and nutrient gradients, which constitutively provides a unique
73 environment for the growth of metabolically diverse microorganisms (Chunleuchanon *et al.*,
74 2003, Newberry *et al.*, 2004, Orphan *et al.*, 2008).

75 In a previous study we already evaluated the methane emissions as well as the methanogenic
76 potential of several sites of River Sitka (Rulik *et al.*, 2013). In the present study we focused on
77 the methanogenic community composition of river sediment samples and compare the

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78 community composition of a low emitting site (Location I: 2.39 mg CH_4 m^{-2} water day^{-1}) with
79 that of a high emitting site (Location IV: 32.1 mg CH_4 m^{-2} water day^{-1}) (Rulik *et al.*, 2013).

80 Currently there are seven orders of methanogenic archaea described in literature (Borrel *et al.*,
81 2013, Borrel *et al.*, 2014, Lang *et al.*, 2015). However, our previous study conducted on the Sitka
82 stream (Location IV) revealed only three major methanogenic groups using molecular techniques
83 (denaturing gradient gel electrophoresis and cloning): *Methanosarcinales*, *Methanomicrobiales*
84 and *Methanobacteriales* (Buriankova *et al.*, 2013, Brablcova *et al.*, 2014, Chaudhary *et al.*,
85 2014). Hence we focused our attempts to verify these results with molecular fingerprinting and
86 qPCR to cover these three groups in addition we want to expand our knowledge by comparing
87 two different sites and two sampling occasions. .

88 In the Sitka stream, previous studies showed that methanogenic archaea are almost ubiquitous
89 along the longitudinal profile of the stream (Buriankova *et al.*, 2012, Brablcova *et al.*, 2014) and
90 their density tends to be stable with increasing sediment depth (Location IV) (Buriankova *et al.*,
91 2012). However, quantification of total methanogens was made using Fluorescence In-Situ
92 Hybridization (FISH) (Buriankova *et al.*, 2012) which is suitable for aqueous systems but may
93 lack precision in sediment samples due to high background fluorescence.

94 The present study aimed to analyze the vertical distribution of methanogens in the top 50 cm of
95 river Sitka sediment cores from one high and one lower methane producing localities and to
96 quantify the methanogenic communities using a combination of terminal Restriction-Fragment-
97 Length-Polymorphism (T-RFLP) and qPCR. We expected that especially the quantification with
98 qPCR not only for total archaea but likewise for the three dominant methanogenic orders would
99 help to increase our understanding on the different methane emissions of the two sites. The group
100 specific qPCR has so far not been applied to many environmental systems. Since the *mcrA*
101 primers are highly degenerated to cover a broad community we hoped to improve our
102 understanding of the system by using group specific qPCR. Likewise our new dataset provided
103 us to contrast our T-RFLP results with previous work on Location IV (Mach *et al.*, 2015) and
104 demonstrate the development of the methanogenic community over one and a half years.

105 **Material and Methods**

106

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107 **Ethics statement**

108 For the collection of sediment samples from the specific sites no specific permits were required.
109 The locations were not privately owned, nor were they in restricted or protected areas. Moreover,
110 no activities involving endangered or protected species were untaken during the collection of
111 samples.

112

113 **Study site**

114 Sitka stream is considered to be an undisturbed, 35 km long, lowland, third-order stream
115 originating in the Hrubý Jeseník Mountains, 650 m above sea level. Of the two localities studied,
116 one (Location I) was situated in an upper forested area, whereas the second location (Location
117 IV) was situated in agricultural landscape (further description of the sampling sites has been
118 provided earlier (Hlavacova *et al.*, 2005, Buriankova *et al.*, 2013, Rulik *et al.*, 2013, Brablcova *et*
119 *al.*, 2014). These two sites were selected on the basis of the different amount of methane
120 production and methanogenic potential on the basis of earlier studies (Buriankova *et al.*, 2012,
121 Buriankova *et al.*, 2013). Location IV was studied previously in more detail because of
122 maximum methane production and methanogenic potential (Buriankova *et al.*, 2013, Mach *et al.*,
123 2015). Sediment sampling for studying the vertical distribution of methanogens was performed
124 in July 2013. Three sediment cores (50 cm deep) were taken randomly at each Location I and
125 Location IV, along Sitka stream flowing through Olomouc province in Czech Republic. The
126 focus of the present study was to compare depth profiles of both locations using community
127 profiling (T-RFLP) as well as quantification of the methanogenic community not only using the
128 commonly used *mcrA* marker gene but to use group specific primers to quantify the three
129 dominant methanogenic orders.

130

131 **Collection and processing of sediment sample**

132 Hyporheic sediment samples were collected using the liquid N_2 freeze-core method (Bretschko
133 & Klemens, 1986). A total of three cores were gathered and taken for subsequent analyses. After
134 sampling, five layers (i.e., 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm) were

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135 immediately separated for subsequent molecular analysis and stored at low temperature during
136 transport to the laboratory. Samples were then thawed and wet sediment from each layer was
137 sieved and only particles < 1 mm were considered for DNA isolation since most of the
138 microorganisms would be attached to them (Leichtfried, 1988, Ramakrishnan *et al.*, 2000).
139 Fifteen subsamples (three from each depth) were used for DNA extraction. Dry weight of the
140 samples was determined by drying 1 g of the samples at 60 °C over night.
141
142 **DNA extraction and Terminal restriction fragment length polymorphism (T-RFLP)**
143 **analysis**
144 For genomic DNA extraction, 1 g wet weight of sediment sample was processed using the
145 PowerSoil DNA Isolation Kit (MO-BIO, USA), according to the manufacturer's instructions.
146 Extracted DNA was checked for quality and concentration using a Nanodrop spectrophotometer
147 (Nano-Drop Technologies, Wilmington). Terminal restriction fragment length polymorphism (T-
148 RFLP) analysis of the methanogenic *mcrA* genes was carried out as described previously
149 (Lueders & Friedrich, 2003), using the primer pairs MCRf and MCRr, with the forward primer
150 labelled with FAM (Table 1). The PCR products were purified using the QIAquick PCR
151 purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.
152 Aliquots of the purified amplicons (200 ng) were digested with *Sau*96I (Fermentas). After the
153 digestion, the DNA samples were precipitated in 200 µl of 75% isopropanol for 30 min at room
154 temperature, followed by centrifugation at 14,000 x g for 30 min at 4°C. The DNA pellets were
155 washed with 70% ethanol, air-dried, and resuspended in 20 µl of purified water. The
156 fluorescently labelled T-RF were size-separated on the automatic sequencer ABI 3100 Avant
157 Genetic Analyzer (Applied Biosystems) equipped with POP6 polymer-filled capillary under
158 denaturing condition. The T-RFLP electropherograms were analysed by peak area integration of
159 the T-RF using the GeneScan analysing software (Applied Biosystems). The lengths of the T-RF
160 were determined by comparison to an internal standard (GeneScan-1000-ROX size standard;
161 Applied Biosystems). The relative abundance of a single T-RFLP was represented by the
162 percentage fluorescence intensity calculated relative to the total fluorescence intensity of all
163 well-resolved peaks with area over 1000 or > 2% of the maximum peak of an electropherogram.
164 The possible phylogenetic affiliations were determined by comparison of the T-RFLP length of
165 clones of the sediment samples (Mach *et al.*, 2015) to the theoretical T-RFLP lengths generated

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166 from the sequences deposited in GeneBank database using Ribosomal Database Project T-RFLP
167 online analysis.

168

169 **qPCR analysis**

170 In order to quantify the microbial community we used a set of different primers targeting the
171 total archaea (16S *rRNA* genes), methanogenic archaea (*mcrA* gene), and three major
172 methanogenic orders *Methanobacteriales* (MBT-set), *Methanomicrobiales* (MMB-set), or
173 *Methanosarcinales* (MSL-set) (Ovreas *et al.*, 1997, Luton *et al.*, 2002, Yu *et al.*, 2005) (Table 1).
174 qPCR was performed using the BioRad CFX Connect™ qPCR Detection System (BioRad,
175 USA). The 25µL real-time PCR mixture was prepared using the Brilliant II SYBR master mix
176 (Agilent Technologies, USA) 12.5 µL of 2x reaction solution, 0.25 µL of each primer (final
177 concentration 0.25 µM), 5 µL of template DNA, and 7 µL of PCR-grade water. The two-step
178 amplification protocol was as follows: initial denaturation for 5 min at 94 ºC followed by 45
179 cycles of 30 s at 94 ºC and combined annealing and extension for 30 s at XºC (X values are given
180 in Table 1). The fluorescent signal was measured at the end of each annealing/extension step.
181 DNA samples were analyzed in triplicate at each point.

182 In order to generate standard curves target genes were amplified with PCR. The PCR products
183 were cloned into the pGEM-T Easy vector (Promega, Madison, WI). The plasmids were
184 extracted, serially diluted, and used as templates in qPCR for generating standard curves.

185

186 **Results**

187

188 **Quantification (qPCR) of archaeal, *mcrA* gene copies and three orders of methanogens**

189

190 The measurements were made for all five depths of the two localities I & IV (i.e., 0-10, 10-20,
191 20-30, 30-40 and 40-50 cm of depth) (Figure 1), an overview of the q-PCR results for the
192 individual locations can be found in the supplementary as Figure S1). Archaeal densities were
193 found to be in the range of 10^8 copies/g dry weight with a slight increase in density as the depth

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194 increases (Figure 1a). The copy numbers of the *mcrA* gene characteristic for the methanogens,
195 remained stable at around 10^7 copies/g dry weight at all depths for Location I and IV (Figure 1b).
196 A slight increase in the copy numbers at 20 and 30 cm depths can be seen from the samples at
197 locality I (Figure 1b), followed by a decrease at 40 and 50 cm of depth. However, for Location
198 IV *mcrA* gene numbers were slightly greater at 50 cm depth as compared to 40 cm depth.
199 The highest copy numbers for the analyzed methanogenic orders belonged to the order
200 *Methanomicrobiales* (Figure 1c). Here $3.6*10^6$ to $5.8*10^7$ copies/g dry weight could be reported.
201 While the average copy numbers slightly decreased with depth in Location I; they slightly
202 increased in Location IV. Gene copy numbers of methanogens belonging to the order
203 *Methanosarcinales* were in a similar range covering $3.6*10^6$ to $2.7*10^7$ copies/g dry weight
204 (Figure 1d). In Location I again a slight decrease with depth could be observed; while in
205 Location IV a maximum at 20-30 cm was observed. Methanogens belonging to the order
206 *Methanobacteriales* were found with roughly two orders of magnitude lower copy numbers
207 ranging from $1.4*10^4$ to $3.6*10^5$ copies/g dry weight (Figure 1e). Again a decrease was observed
208 over the different depth at Location I while a slight increase was reported for Location IV.
209 Irrespective of the tested methanogenic order all three primer-sets revealed a decrease over depth
210 in methanogenic copy numbers per gram dry weight for Location I (Figure S1) while all three
211 sets gave consistently low copy numbers for the 10-20 cm depth samples at Location IV.
212

213 **Terminal restriction length polymorphism of *mcrA* genes**

214 The methanogenic community composition was determined by analysis of the terminal
215 restriction fragment length polymorphism (T-RFLP) of the *mcrA* gene in both localities (I &IV),
216 at the five different depths (Figure 2). The T-RFLP-profiles show 8-13 different TRF's (Figure
217 S2). The relative contribution of the order *Methanosarcinales* to total methanogenic TRF's was
218 almost always dominant contributing 48% to 84% of the total TRF's. While the relative
219 contribution of *Methanosarcinales* decreased with sediment depths at Location IV, it had a
220 maximum at 40 cm for the samples taken at Location I. A closer look on the six TRF's assigned
221 to the *Methanosarcinales* (252-3 bp, 390-1 bp, 415-7 bp, 423-427 bp, and 491-2 bp, 504-6 bp)
222 revealed that the top sediments at Location IV was dominated by a single TRF (491-2 bp); while
223 Location I showed a different dominating TRF (504-6 bp) for the 30-40 cm depth layer (Figure
224 S2).

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225 The relative contribution of methanogens belonging to the order *Methanobacteriales* increased
226 with sediment depth reaching 11 to 17% in Location IV; at Location I their values decreased
227 from 26 to 8% over the sediment depth. Only one TRF (400-3) could be assigned to
228 *Methanobacteriales*.
229 The relative abundance of the third methanogenic order *Methanomicrobiales* ranged from 5 to
230 23% and did not show a clear trend over the different depth of the sediment profile. Four TRF's
231 (324-5 bp, 405-406 bp, 410 bp and 472-4 bp) could be attributed to this order.
232 While most of the TRF's found in Location IV could be attributed to the three dominant
233 methanogenic orders, up to 28% of the TRF's in Location I (mainly TRF 366 bp) could not be
234 assigned to any known methanogen.
235 Rivers are very dynamic systems, hence we wanted to compare the temporal changes of the
236 methanogenic community at the high methane emitting site. A comparison of cores taken at
237 Location IV in April 2012 and July 2013 reveals that the community profiles are rather stable
238 over the different depth layers (Figure 3). However the relative contribution of individual TRF's
239 is quite different over time. For example the 491-2 bp TRF which contributes 54-58% to the
240 community of the top twenty centimeter in July 2013 represents only 10-18% in the earlier
241 samples. Likewise several minor TRF's which have been reported for the top layer of the
242 samples taken in July 2013 (TRF 131, 199, 278, 342) have not been found in the samples taken
243 in April 2012.
244 **Discussion**
245

246 Although methanogenesis is one of the main processes responsible for terminal anaerobic
247 organic matter mineralization in the river hyporheic sediments (Hlavacova *et al.*, 2005), very
248 little is known about the methanogens involved in this process. One would expect that the
249 diversity of the methanogenic community should to some extent reflect the level of
250 methanogenic production. However, microbial diversity and how it correlates with the function
251 in the sediments is not trivial. Moreover, the diversity and composition of the methanogenic
252 community might change along the longitudinal profile, as well as along the vertical profile of
253 the stream (Brablcova *et al.*, 2014).

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255 ***Contribution of methanogenic archaea to total microorganisms /archaea in freshwater***
256 ***sediments.***

257 In lake sediments, archaea account from less than 1% (Schwarz *et al.*, 2007) to 96.9% (Ye *et al.*,
258 2009) of the prokaryotic community when comparing qPCR results of the archaeal 16S rRNA
259 gen to the bacterial counterpart. Our previous data from a vertical profile of the Sitka sediments
260 indicated a relative contribution of 13.8 to 14.7% of archaea to the overall microbial community
261 (Buriankova *et al.*, 2012).

262 While the archaeal abundance has been reported to either decrease (Chan *et al.*, 2005) or increase
263 with depth of sediments (Kotsyurbenko *et al.*, 2004) it was rather constant in our study. The
264 methanogenic (*mcrA* copy numbers) contribution to the archaeal community was roughly 10%
265 (ranging from 2.5% to 14.8% in Location I and 4.6 to 18.2% in Location IV).

266 ***Methanogenic community in river sediments analyzed by different molecular techniques***

267 The methanogenic community based on T-RFLP of *mcrA* has so far primarily been described for
268 rice field soils (Lueders *et al.*, 2001, Ramakrishnan *et al.*, 2001, Chin *et al.*, 2004, Kemnitz *et al.*,
269 2004, Conrad *et al.*, 2008). While our previous studies of river Sitka sediments using T-RFLP
270 (Mach *et al.*, 2015) already show that the community pattern changes over the depth profile we
271 wanted to confirm these results for two locations and further support them using order specific q-
272 PCR. However, the results can not directly be compared since T-RFLP is based on the highly
273 degenerated *mcrA* primers and only gives relative abundances, while the order specific primers
274 for qPCR gives absolute numbers for the respective methanogenic order according to the
275 standards used. In addition, the primers used for T-RFLP target a different region of the *mcrA*
276 gen than the ones used for qPCR of *mcrA*. Both primer-sets are wobbled to allow a broad
277 coverage. The group specific primers are much more precise and hence the sum of the copy
278 numbers obtained for the three groups is up to 1.6 times higher than the results obtained by the
279 general *mcrA* primer-set making a relative quantification of the qPCR results difficult. While
280 both methods are consistently showing a dominance of *Methanosarcinales*; *Methanomicrobiales*
281 likewise have high copy numbers and contribute between 5 and 23% of the TRF's (and 15 to
282 50% of the qPCR). The *Methanobacteriales* have two orders of magnitude lower copy numbers

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283 (Figure S1) and contribute only one TRF. However this TRF (400-3 bp) accounts for up to 26%
284 of the methanogenic community shown for the top sediment of Location I (Figure S2).

285 Our previous study conducted on the Sitka stream also revealed phylotypes from the orders
286 *Methanosarcinales*, *Methanomicrobiales* and *Methanobacteriales* (Buriankova *et al.*, 2013,
287 Brablcova *et al.*, 2014, Chaudhary *et al.*, 2014). A community profiling using denaturing
288 gradient gel electrophoresis DGGE presented by Brablcova et al. (Brablcova *et al.*, 2014)
289 showed 9 bands for *Methanosarcinales*, one band for *Methanomicrobiales* and one band for
290 *Methanocellaceae*. It is interesting to note the one clone obtained for *Methanocellaceae*
291 (Brablcova *et al.*, 2014) originates from Location I and only for this location we could assign one
292 TRF (238 bp) to *Methanocellaceae* for the 40-50 cm depth confirming the presence of this
293 microbial order in the sediments of Location I. A microscopic study using Fluorescence insitu
294 hybridization (FISH) of *Methanosarcinaceae*, *Methanosaetaceae* as well as
295 *Methanobacteriaceae*, not only revealed the presence of these three groups with each
296 contributing roughly 10% to the total cell counts (DAPI counts) (Rulik *et al.*, 2013), but also
297 showed that the vertical distribution is quite stable.

298 The currently available two clone libraries for the Sitka river sediments (Buriankova *et al.*, 2013,
299 Mach *et al.*, 2015) show both a dominance of Methanosarcinales (47 to 56 % of the clones), the
300 second equally important group was *Methanomicrobiales* covering 40 to 42 % of the clones; a
301 less frequently found order was *Methanobacteriales* with 4 to 10% of the clones. Together these
302 data demonstrate that *Methanosarcinales* are the dominant order in the Sitka River sediments
303 followed by *Methanomicrobiales* and *Methanobacteriales*. A smaller clone library (Brablcova et
304 al., 2014) confirmed the dominant contribution of *Methanosarcinales* (6 out of 11 clones).

305 Likewise in other environmental samples Methanosarcinales and Methanomicrobials have been
306 described as dominant methanogenic members using various archaea/methanogen-specific
307 primers, e.g. from river freshwater and estuarine sediment (Munson *et al.*, 1997, Purdy *et al.*,
308 2002, Buriankova *et al.*, 2013, Brablcova *et al.*, 2014), as well as from peat bog sites (Galand *et*
309 *al.*, 2005), freshwater lake sediments (Falz *et al.*, 1999, Koizumi *et al.*, 2004), Florida Everglades
310 wetland soils (Castro *et al.*, 2004), hydrocarbon-contaminated aquifer (Kleikemper *et al.*, 2005)
311 and deep-sea hydrothermal sediments (Dhillon *et al.*, 2005).

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312 In general our results are in good agreement with reported methanogenic community profiles of
313 other freshwater habitats (e.g. lakes) which usually are also dominated by *Methanomicrobiales*
314 and *Methanosarcinales* (Banning *et al.*, 2005, Castro *et al.*, 2005, Barreto *et al.*, 2014, Conrad *et*
315 *al.*, 2014). In contrast the T-RFLP profiles of rice field soil are more diverse and contain
316 additional methanogenic orders (Lueders *et al.*, 2001, Ramakrishnan *et al.*, 2001, Chin *et al.*,
317 2004, Kemnitz *et al.*, 2004, Conrad *et al.*, 2008).

318

319 ***Comparison of the vertical distribution and composition of the methanogenic community***

320 The different depth profiles show that the major methanogenic orders are relatively stable over
321 the analyzed top 50 cm of the sediment (Figure 2). This is in agreement with the previously
322 published T-RFLP profile for Location IV (sampled at a different year) (Mach *et al.*, 2015). Only
323 a finer resolution of the different TRF's shows that the members of the different orders vary for
324 different depth as well as for the two sampled locations (Figure S2). A recent study on the
325 methanogenic community of the Yangtze River estuary using 454 pyrosequencing also shows
326 that in this river sediment *Methanosarcinales* as well as *Methanomicrobiales* are the dominant
327 members of the methanogenic community (Zeleke *et al.*, 2013). In this study they also analyzed
328 the *mcrA* copy numbers / g dry weight and confirm the overall picture of relatively stable 10^7 to
329 10^8 copies for the top 50 cm. Only at deeper sediment depth they found an increase in *mcrA*
330 copies (Zeleke *et al.*, 2013), which is in agreement with our results. In addition we could show
331 that even for the three tested methanogenic orders we generally find quite stable copy numbers
332 for both locations as well as over the different depth (Figure 1).

333 If we compare both locations we see that the overall *mcrA* copy numbers (as well as the group
334 specific copy numbers) are relatively stable along the depth profiles. Astonishingly the lower
335 methane emitting site (Location I) has on average higher cell counts for all tested methanogenic
336 groups when compared to the higher methane emitting site (Location IV). This suggest that the
337 activity of the methanogenic community is rather controlled by other factors (e.g. substrate
338 supply) than by size of the community.

339 The detailed methanogenic community profile (Figure S2) is different for both locations and
340 changes over the depth profile of the sediment cores. While a core set of seven TRF's was

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341 reported for both locations, individual TRF's were only present in one of the two sampling sites
342 (e.g. TRF 366 bp (others) Location I, 410 bp (*Methanomicrobia)* Location I, 491/2 bp
343 (*Methanosarcina)* Location IV) (Figure S2).

344 Likewise we could report a change in the community profile comparing samples from April 2012
345 and July 2013. Currently it can not be excluded that these differences are due to seasonal
346 variations.

347 Looking at the relative stable copy numbers and the methanogenic community profile one may
348 assume that the different depth as well as the different locations will show similar methanogenic
349 potentials. Our previous studies however show that the methanogenic potential for Location IV
350 showed two distinct activity peaks (for the top sediment as well as the 40-50 cm depth) (Mach *et*
351 *al.*, 2015); likewise the methane emissions for both locations is quite distinct providing evidence
352 that Location IV is a ten times stronger methane emitting site (Rulik *et al.*, 2013). This suggest
353 that the methanogenic potential is not limited by the presence of the different methanogens but
354 more likely regulated by environmental factors (e.g. substrate supply) as well as the activity of
355 certain members of the methanogenic community. Hence fine resolved studies like the presented
356 T-RFLP profiles or next generation sequencing data are needed to fully resolve the complex
357 processes involved in the methane release from river sediments.

358

359 ***Conclusions***

360

361 Data obtained in this study validated our previous measurements for Location IV on the
362 composition and diversity of the methanogenic archaea within the hyporheic sediments of the
363 Sitka stream and contrasted these results to a lower methane emitting site (Location I).
364 Generally, this study confirms that, methanogens are ubiquitous members of the microbial
365 community within river hyporheic sediments. The richness of the methanogenic community is
366 less diverse in river sediments compared to those from wetlands or rice paddies.

367 Our results show that the methanogenic community in methane emitting river sediments is
368 relatively stable in absolute numbers along a vertical profile and for both study sites (irrespective

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369 of the reported methane emissions) not only on the level of total archaea and total methanogens
370 but likewise on the level of the three dominant methanogenic orders. Especially the
371 quantification of different methanogenic orders has so far not been applied to river sediment
372 samples and provides additional evidence for the quantification of the individual methanogens.
373 However, the underlying community structure reveals different community compositions of the
374 methanogens for both locations as well as for different depth layers and different sampling times.
375 In general our data confirm that *Methanosarcinales* together with *Methanomicrobiales* are the
376 two dominant methanogenic orders in river sediments, while members of *Methanobacteriales*
377 contribute a smaller community and *Methanocellales* are only rarely present in this sediment.

378

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380

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387

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Name	Target Group	Sequence (5´- 3´)	Annealing Temperature (°C)	Amplicon size (bp)	Reference

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539

540

541 **Table 1:** Characteristics of primer sets used in Quantitative PCR and T-RFLP

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PARCH340-F **PARCH519-R**	Archaea (qPCR)	CCC TAC GGG GYG CAS CAG TTA CCG CGG CKG CTG	58.3	152	(Ovreas *et al.*, 1997)
MCRA-F **MCRAR- R**	Methanogens (qPCR)	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 (qPCR)	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 (qPCR)	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 (qPCR)	GTA AAC GAT RYT CGC TAG GT GGT CCC CAC AGW GTA CC	52.7	354	(Yu *et al.*, 2005)
mcrA-F(FAM Labelled) **mcrA-R**	Methanogens (T-RFLP)	TAY GAY CAR ATH TGG YT ACR TTC ATN GCR TAR TT	50	516	(Springer *et al.*, 1995)

542

543 **Figure 1.** qPCR results given as copy numbers per gram dry weight of A) total archaea (*16S*
544 *RNA*), B) total methanogens (*mcrA*), C) *Methanomicrobiales*, D) *Methanosarcinales*, E)
545 *Methanobacteriales*. For different depth (10 = 0-10 cm, 20 = 10-20 cm, 30 = 20-30 cm, 40 = 30-
546 40 cm, 50 = 40-50 cm) for Location I and Location IV of Sitka river sediments. Comparison of
547 different genes for the two locations can be found in the supplementary as Figure S1.

548

549 **Figure 2** Community profile using T-RFLP of *mcrA* for both locations. Results are given on the
550 order level, details for individual TRF's can be found in the supplementary as Figure S2.

551

552 **Figure 3** Comparison of the community profile (T-RFLP of *mcrA* gen) for the depth profile of
553 two sediment cores from different sampling time points of the high methane emitting site
554 (Location IV). The samples from April 2012 have been previously evaluated in a different
555 context (Mach *et al.*, 2015).

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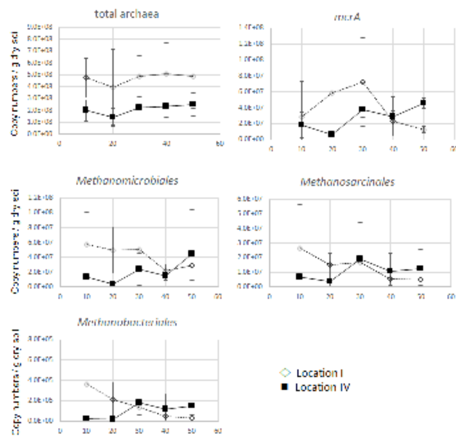

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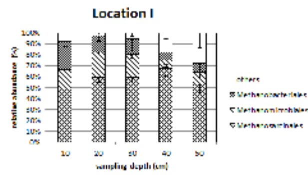

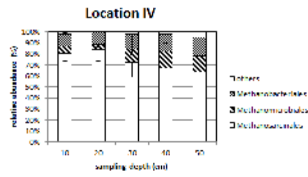

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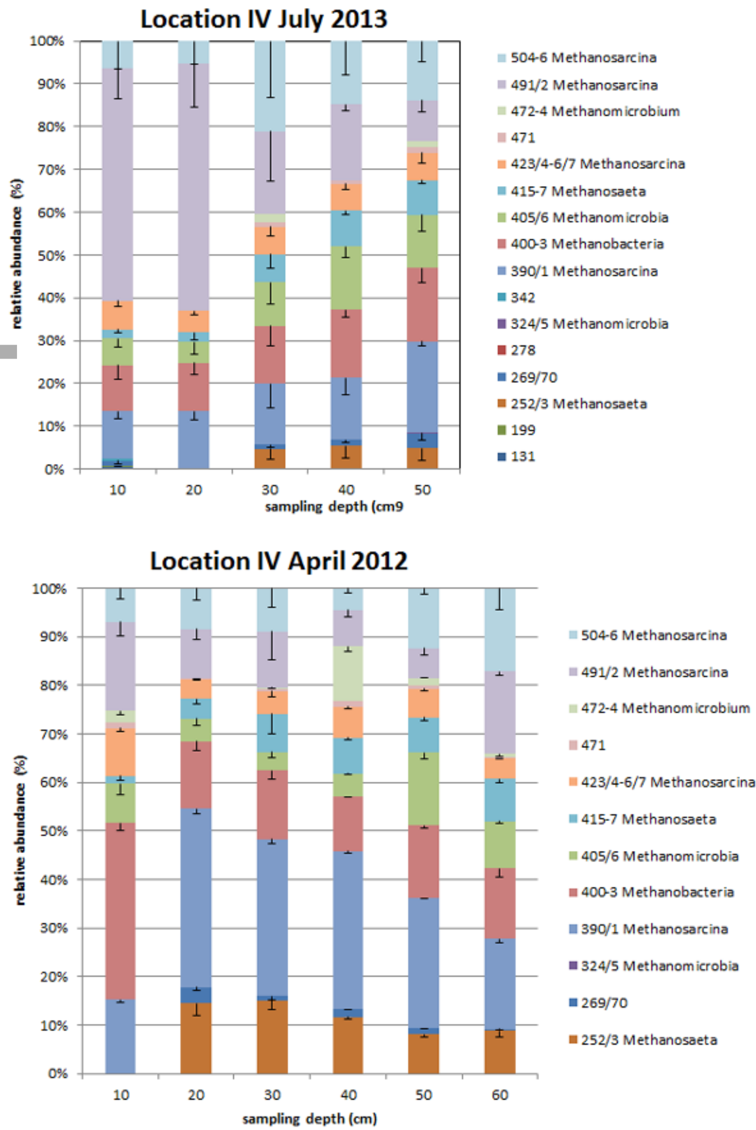

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