



Supplement of

High-resolution vertical biogeochemical profiles in the hyporheic zone reveal insights into microbial methane cycling

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Supplement 1: Hydrology, surface water chemistry, sampling details and sediment characteristics

Table S1 shows the surface water chemistry of the Moosach river. The water is of calcium-magnesium-bicarbonate type with elevated chloride concentrations.

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Table S1: Surface water chemistry. Concentrations represent mean values of data recorded between 2010-2018. Data retrieved from the Bavarian State Office of the Environment (2022).

Component	Concentration (mg L ⁻¹)
Na ⁺	30.9
Ca ²⁺	100
Mg ²⁺	20.7
Cl ⁻	54
NO ₃ ⁻	20.4
SO ₄ ²⁻	30.4
HCO ₃ ⁻	340
Dissolved O ₂	8.7
TOC	3.5
DOC	2.8

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Table S2 summarizes information on sampling intervals and measured basic chemical parameters of the surface water as measured on the days of sampling. Further, average discharge and temperature during equilibration period are given. Stream discharge and surface water temperature during the sampling periods is shown in Fig. S1.

Table S2: Background information on the five sampling periods, basic chemical parameters of the surface water on the days of sampling and mean discharge and surface water temperature during the sampling period.

Profile	Placement	Sampling	Days	Basic chemical parameters of the surface water on the days of sampling				Mean discharge & temperature during equilibration*	
				T _{sw} (° C)	O ₂ (mg L ⁻¹)	pH	Conductivity (µS cm ⁻¹)	Q _M (m ³ s ⁻¹)	T _M (° C)
A	02.03.2021	22.03.2021	36	7.0		no measurements		2.33	7.5
B	04.05.2021	26.05.2021	22	11.3	9.9	7.9	819	2.51	12.0
C	16.06.2021	06.07.2021	20	15.3	10.5	8.1	806	2.93	16.6
D	15.07.2020	20.08.2020	20	16.2	10.2	7.6	756	1.46	17.1
E	21.07.2021	18.08.2021	28	14.5	10.9	8.1	797	2.48	15.8

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*Data retrieved from the Bavarian State Office of the Environment (2022).

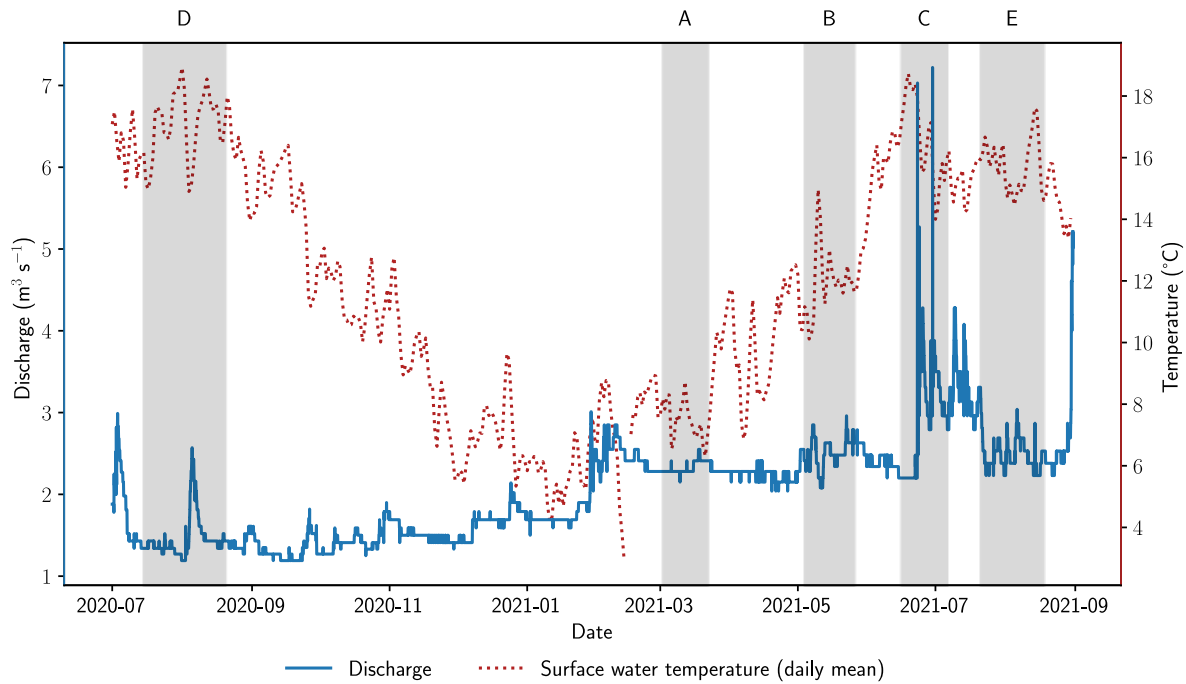


Figure S1: Stream water temperature (daily mean) und discharge. Data was recorded at gauging station Freising Moosach (river chainage: 9.45 km, 4.5 km downstream of the sampling sites), as retrieved from the Bavarian State Office of the Environment.

- Sediment cores were taken at each sampling site by manually pushing a coring tube (inner diameter 42 mm) into the sediment.
- 20 In the laboratory, each core was divided into homogeneous layers. Sieve-slurry analyses were performed to obtain sediment grain size distributions according to DIN EN ISO 17892-4. Sedimentation experiments failed for location B (11-22 cm) due to the high content of organic matter which induced coagulation at an unexpectedly high rate. Sedimentation experiments were not performed for location E 0-7 cm. The grain size distribution curves for each sampling site are displayed in Fig. S2 and characteristic values listed in Tab. S3.
- 25 Porosity φ was calculated as a function of the median grain diameter d_{50} as suggested by Wu and Wang (2006) who modified the formula for initial porosity of sediment deposits (less than one year after deposition) proposed by Komura and Colby (1963). Values for d_{50} and φ are also given in Tab. S3. For location B (11-22 cm), the given d_{50} is an estimation based exclusively on the sieving analysis.

$$30 \quad \varphi = 0.13 + \frac{0.21}{(d_{50} + 0.002)^{0.21}} \quad (\text{S1})$$

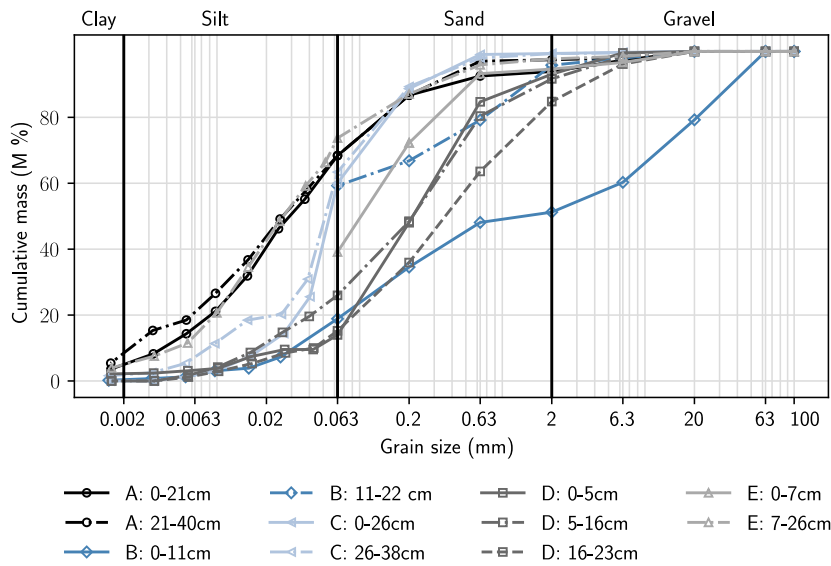


Figure S2: Grain size distribution curves

Table S3: Sediment characteristics and calculated porosity ϕ

Profile	Sampling date	Depth (cm)	Silt/Clay (%)	Sand (%)	Gravel (%)	d_{50} (mm)	ϕ
A	22.03.2021	0-21	65	29	6	0.030	0.56
		21-40	68	29	3	0.026	0.57
B	26.05.2021	0-11	19	32	49	1.46	0.32
		11-22	59	37	4	0.040	0.54
C	06.07.2021	0-26	60	39	1	0.030	0.51
		26-38	63	36	1	0.019	0.51
D	20.08.2020	0-5	14	79	7	0.22	0.42
		5-16	26	66	8	0.22	0.42
		16-23	15	70	15	0.42	0.38
E	18.08.2021	0-7	39	56	5	0.11	0.46
		7-26	74	24	2	0.027	0.57

Hydraulic conductivity K was roughly estimated using the formula introduced by Beyer (1964) (Eq. S2).

$$K = \beta \frac{g}{\nu} \log \left(\frac{500}{C_U} \right) d_{10}^2 \quad (\text{S2})$$

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with the coefficient $\beta = 1.30 \cdot 10^{-5}$ as recommended by Rosas et al. (2014) for river sediments, the gravitational constant $g = 9.81 \text{ m s}^{-2}$, the kinematic viscosity $\nu = 1.307 \text{ mm}^2 \text{ s}^{-1}$ for $10 \text{ }^\circ\text{C}$ (Kestin et al., 1978), the uniformity coefficient $C_U = d_{60}/d_{10}$ and the grain diameters d_{10} and d_{60} at 10 % and 60 % of the cumulative grain size distribution curve, respectively. For location B (11-22 cm) and location E (0-7 cm) the d_{10} was estimated only based on the sieving analysis. Results are given in Tab. S4.

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Table S4: Hydraulic conductivities estimated using the Beyer equation.

Profile	Sampling date	Depth (cm)	d_{10} (mm)	d_{60} (mm)	C_U	K (m s^{-1})
A	22.03.2021	0-21	0.0039	0.047	12.0	$2.4 \cdot 10^{-6}$
		21-40	0.0023	0.043	18.7	$7.4 \cdot 10^{-7}$
B	26.05.2021	0-11	0.041	6.2	150.5	$8.6 \cdot 10^{-5}$
		11-22	0.010	0.076	7.6	$1.8 \cdot 10^{-5}$
C	06.07.2021	0-26	0.0019	0.063	33.2	$4.1 \cdot 10^{-7}$
		26-38	0.008	0.062	7.8	$1.1 \cdot 10^{-5}$
D	20.08.2020	0-5	0.048	0.34	7.1	$4.2 \cdot 10^{-4}$
		5-16	0.018	0.36	20.0	$4.4 \cdot 10^{-5}$
		16-23	0.043	0.57	13.3	$2.8 \cdot 10^{-4}$
E	18.08.2021	0-7	0.020	0.15	7.5	$7.1 \cdot 10^{-5}$
		7-26	0.0047	0.039	8.3	$3.8 \cdot 10^{-6}$

Supplement 2: Determination of a cut-off threshold concentration for isotope

50 measurements

Measurements of $\delta^{13}\text{C}\text{-CH}_4$ at low headspace CH_4 concentrations in the sample vials showed large standard deviations between repeated measurements. Thus, an experiment was conducted to find an appropriate cut-off value above which reliable isotopic data could be obtained. Two standards with -21.1‰ and -69.0‰ were diluted to obtain different concentrations and measured repeatedly. A cut-off value of 30 ppm was chosen based on the results displayed in Fig. S3.

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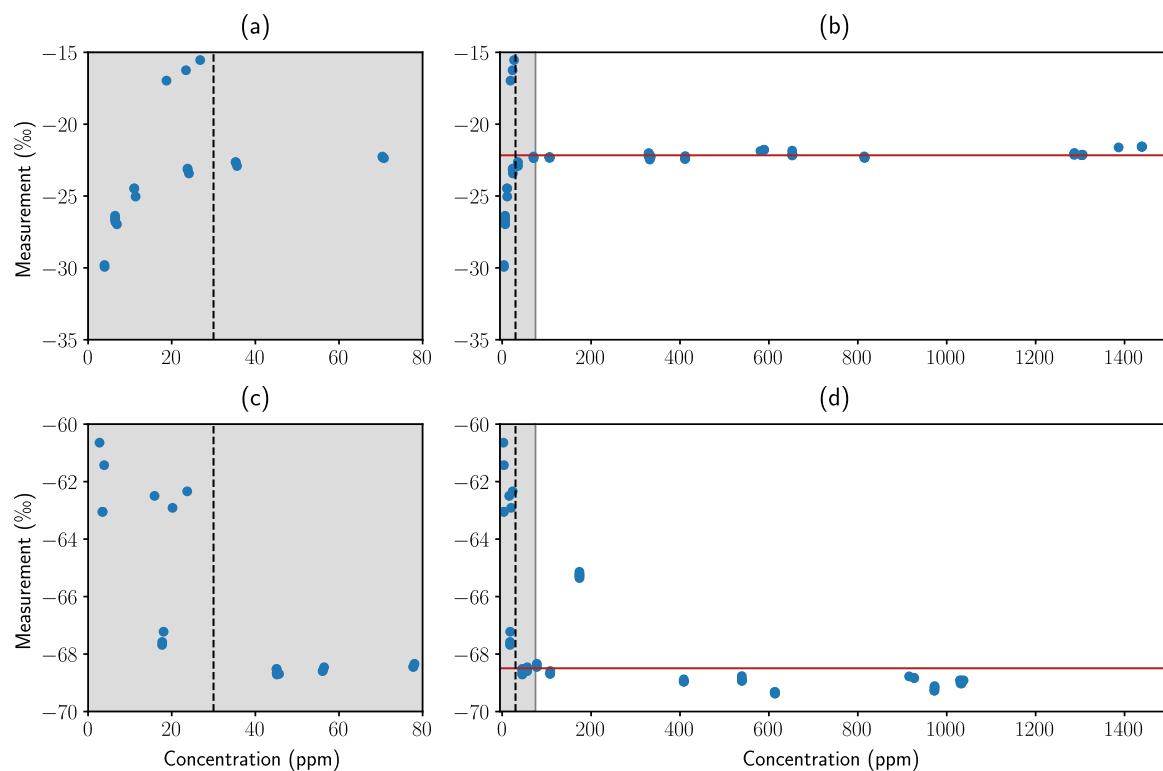


Figure S3: Repeated measurements of standards with $\delta^{13}\text{C}\text{-CH}_4$ values of -21.1‰ (panels (a) and (b)) and -69.0‰ (panels (c) and (d)). The red line in panels (b) and (d) represents the average value of all measurements above the cut-off threshold.

60 Supplement 3: Calculation of sediment diffusion coefficients

Diffusion coefficients were calculated based on Boudreau (1997). Equations S3 and S4 have been used for the diffusion coefficients in water D^0 of gases and ions, respectively. The mean surface water temperature during the equilibration period of the peeper T_M (Tab. S2) was used for temperatures in Eq. S3 and S4.

$$65 \quad D^0 = 4.72 \cdot 10^{-9} \frac{T}{\mu V_b^{0.6}} \quad (S3)$$

where μ is the dynamic viscosity of water in units of poise, T the absolute temperature [$^{\circ}\text{K}$] and V_b the molar volume of the solute. Values for V_b are given in Tab. S5.

70 **Table S5: Parameters for the calculation of D^0 in for relevant gases**

Species	V_b
O ₂	27.9
CH ₄	37.7

$$D^0 = (m_0 + m_1 t) \cdot 10^{-6} \quad (S4)$$

where m_0 and m_1 are parameters listed in Tab. S6 and t is temperature in [$^{\circ}\text{C}$].

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Table S6: Parameters for the calculation of D^0 for relevant ions

Ion	m_0	m_1
NO ₃ ⁻	9.50	0.388
SO ₄ ²⁻	4.88	0.232

Table S7 shows diffusion coefficient for the different solutes and sampling dates in water and Tab. S8 the calculated sediment diffusion coefficients based on the Eq. S5 (Iversen and Jørgensen, 1993).

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$$D_S = \frac{D^0}{1+3(1-\varphi)} \quad (S5)$$

where D_S is the diffusion coefficient in the sediment, D^0 the temperature-dependent diffusion coefficient in water and φ the porosity.

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Table S7: Calculated values for D^0 for mean surface water temperature during the sampling period T_M

Profile	Sampling date	T_M (°C)	D^0_{CH4} (x 10 ⁻⁵ cm ² s ⁻¹)	D^0_{O2} (x 10 ⁻⁵ cm ² s ⁻¹)	D^0_{NO3} (x 10 ⁻⁵ cm ² s ⁻¹)	D^0_{SO4} (x 10 ⁻⁶ cm ² s ⁻¹)
A	22.03.2021	7.4	1.04	1.25	1.22	6.50
B	26.05.2021	11.3	1.20	1.44	1.39	7.50
C	06.07.2021	15.3	1.36	1.63	1.54	8.43
D	20.08.2020	16.2	1.40	1.67	1.58	8.64
E	18.08.2021	14.5	1.33	1.59	1.51	8.24

90 **Table S8: Calculated values for D_s for sampling days and sedimentary layers**

Profile	Sampling date	Sediment depth (cm)	ϕ	$D_{s,CH4}$ (x 10 ⁻⁶ cm ² s ⁻¹)	$D_{s,O2}$ (x 10 ⁻⁶ cm ² s ⁻¹)	$D_{s,NO3}$ (x 10 ⁻⁶ cm ² s ⁻¹)	$D_{s,SO4}$ (x 10 ⁻⁶ cm ² s ⁻¹)
A	22.03.2021	0-21	0.56	4.50	5.39	5.27	2.80
		21-40	0.57	4.56	5.46	5.33	2.84
B	26.05.2021	0-11	0.32	3.99	4.75	4.57	2.47
		11-22	0.54	5.09	6.06	5.83	3.15
C	06.07.2021	0-26	0.51	5.53	6.62	6.25	3.41
D	20.08.2020	0-16	0.42	5.10	6.12	5.76	3.15
		16-23	0.38	4.89	5.86	5.52	3.02
E	18.08.2021	0-7	0.46	5.08	6.09	5.77	3.15
		7-26	0.57	5.81	6.97	6.61	3.60

Supplement 4: Correlation between CH₄ and NH₄⁺ data

95 Figure S4 shows the correlation between CH₄ and NH₄⁺ concentrations. In profile A, no NH₄⁺ could be detected, therefore only data for profiles B-E is displayed. A clear positive linear correlation can be observed for profiles C and D. In profile B, NH₄⁺ was mostly below the detection limit of 0.005 mmol L⁻¹. Higher concentrations were only found between 6-14 cm depth, above the zone where CH₄ concentrations peaked (increased CH₄ concentrations between 5-23 cm depth with a peak at 15 cm depth). A negative correlation between CH₄ and NH₄⁺ concentrations appears to exist between 6-14 cm depth. In profile E, no correlation can be observed, NH₄⁺ concentrations are generally very low compared to the other profiles.

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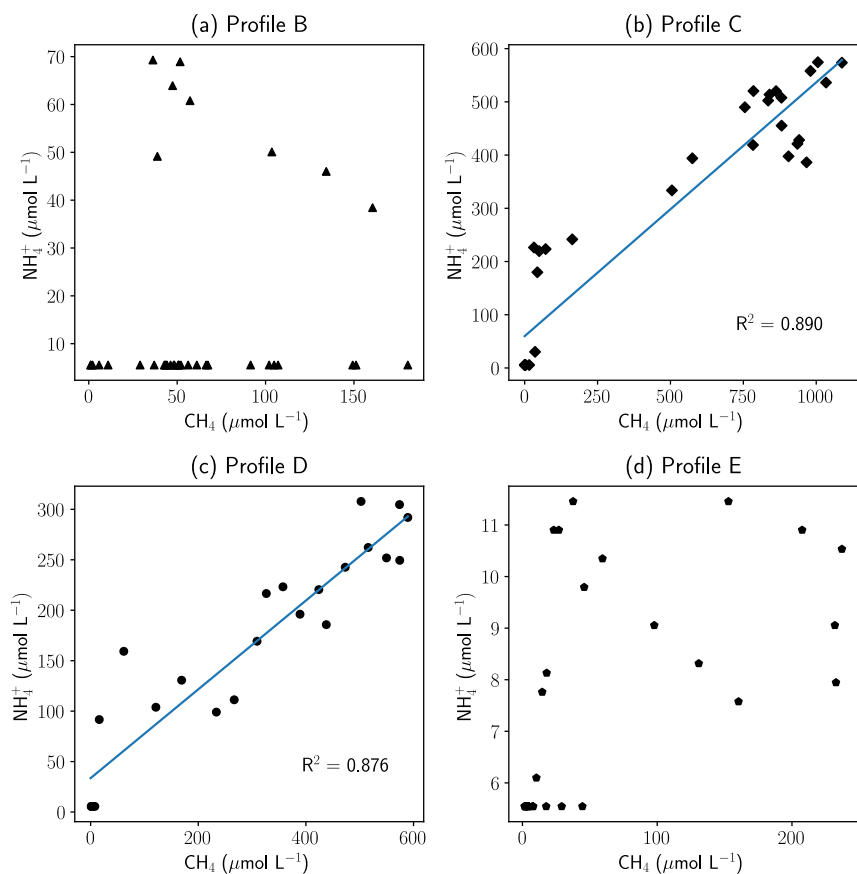


Figure S4: Correlation between CH₄ and NH₄⁺. Each panel corresponds to one measured profile. Axes are scaled in the range of the data. For profiles C and D, a linear regression was performed and R² values are given in the respective plots. Panels (a) to (d) correspond to profiles B-E, respectively.

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