Supplementing information for

The highest methane concentrations in an Arctic river are linked to local terrestrial inputs.

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Content

S.1. Methodology

S.1.1. Correction of underway *T* and DO due to travel distance

Despite the short travel distance of the sampled water from 1 m to the on-board bucket, two main effects could have been influenced this water due to its travel through the pipe: 1) warming, 2) consumption or production of DO. To quantify these potential effects, the in-situ water temperature and DO was measured with an independent handheld probe (YSI Professional Plus; previously calibrated on site) that was lowered to 1 m depth and also used at the bucket next to EXO2 probe, during five selected sites distributed during the campaign. The comparison of results between the YSI and EXO2 probe in these sites showed a consistent warming effect of the sampled water by 0.6 °C gained through the travel time in the pipe. Also, the DO was 1.2 mg L^{-1} consistently higher at the bucket compared to the in-situ value at 1 m depth. This slight increase in DO in the bucket water does not seem to have a relation to changes in solubility of the gas in response to changes in temperature, salinity and pressure (i.e., an increase of 0.6 °C under the same salinity and pressure conditions results in a decrease of DO by 0.15 mg L^{-1}), but rather it is due to the potential addition of DO in response to small turbulent flow generated at the outlet in the bucket.

S.1.3. DNA Isolation, amplicon sequencing, and 16S quantification

Triplicate samples for microbial community analysis were generated from 500 mL of water collected from the Niskin bottle. The water was homogenized and each of the three replicates was immediately passed through a sterile 0.2 µm Supor® filter using a hand-pump. The filter was suspended in a DNA/RNA shield (Zymo research) to preserve the genetic integrity of the samples and stored at room temperature for transport to the laboratory in Germany.

The DNA/RNA shield was removed following a 10 min centrifugation at 12,000 G and DNA was extracted from the filter and any evident pellet using a phenol-chloroform protocol as has previously been described (Taubert et al., 2018). The V4/V5 region of the 16S rRNA gene was amplified using the modified Earth Microbiome Project primerset 515F (Parada) / 926R (Quince) (Parada et al., 2016; Quince et al., 2011) and sequenced on an inhouse Illumina MiSeq with a v3 kit (2x300 read length). Adapter and primer sequences were removed using cutadapt v2.1 (Martin, 2011). Amplicon sequence variants (ASVs) and likely chimeric reads were determined using DADA2 following the recommended settings (Callahan et al., 2016). Due to sequencing-run specific differences in ASVs from replicated samples, operational taxonomic units (OTUs) were subsequently defined using vsearch v2.9.1 at 97 % sequence identity that were then classified using the RDP classifier implemented in DADA2 with the Silva database v132 (Quast et al., 2012; Wang et al., 2007). OTUs classified as belonging to

chloroplasts were removed. Analysis was carried out in Rstudio®, with R v3.5.2 (R Core Team, 2018), the Tidyverse package set (Wickham et al., 2019), and vegan v2.4-2 (Oksanen et al., 2007). Shifts in microbial community composition were visualized using Bray-Curtis dissimilarity values calculated from normalized OTU counts as determined with the metagenomeSeq package in R (Paulson et al., 2013). Relationships between Bray-Curtis dissimilatiry values and relevant environmental patterns were tested using the adonis2 function within the vegan package, and due to collinearity between temperature and specific conductivity, marginal effects of each term were determined. Color schemes were based on previously published values form the ggsci package v2.9 (Xiao, 2018).

Abundances of 16S genes were quantified using qPCR with Brilliant II SYBR Green qPCR Mastermix (Agilent Technologies). Bacterial 16S genes were quantified using the primer pair Bac308Fmod/Bac338R (Daims et al., 1999; Loy et al., 2002) and the archaeal 16S genes with the primer set Arch808F/Arch958R (DeLong, 1992; Takai and Horikoshi, 2000) with previously described cycling conditions (Herrmann et al., 2012). Standard curves exhibited efficiencies of 85-100% and $r^2 > 0.99$ in all cases. Pseudo-absolute abundances were calcuated from the summed bacterial and archaeal 16S gene counts.

The relative and pseudo-absolute abundances of putative methanogens and methanotrophs were based on the references provided by Kwon et al., 2017 and Dedysh and Knief, 2018; respectively.

S.2. Supplemental Tables

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Table S1 – Water properties measured at 20 stations sampled in Kolyma River during 15-16 June 2019 (upstream transect). Values in columns 4-9 are one minute averages from the continuous underway measurements.

Station	Longitude (East)	Latitude (North)	T_w (°C)	pH	DO (mg L^{-1})	κ (µS cm^{-1})	*f*DOM (QSU)	pCH_4 (µatm)	FCH_4 (mmol $m^{-2}d^{-1}$)	DOC (mg L^{-1})
PP05	161° 18' 16.628"	68° 45' 5.062"	14.4	6.6	10.3	58.6	81.1	-	0.216	11.9
PP06	161° 17' 22.459"	68° 42' 49.999"	13.8	6.9	11.2	82.4	60.6	25.3	0.204	8.2
PP07	161° 19' 50.030"	68° 40' 15.999"	14.2	7.0	11.2	75.2	65.1	26.7	0.195	10.4
PP08	161° 12' 32.288"	68° 37' 5.638"	13.4	7.2	11.4	111.6	60.0	17.3	0.192	8.6
PP09	161° 10' 6.239"	68° 34' 11.729"	13.4	7.2	11.4	110.4	666.3	19.3	-	9.2
PP10	161° 9' 46.630"	68° 32' 15.241"	15.0	6.7	11.0	48.0	98.8	24.5	0.020	10.1
PP11	160° 57' 19.450"	68° 30' 24.052"	14.9	6.8	11.0	52.1	77.4	39.8	0.021	9.0
PP12	160° 48' 28.771"	68° 31' 36.001"	13.6	7.2	11.6	97.4	190.0	22.3	0.013	8.6
PP13	160° 39' 25.520"	68° 31' 25.819"	13.9	7.1	11.5	82.5	104.7	27.5	0.015	8.6
PP14	160° 36' 0.359"	68° 29' 14.999"	13.9	7.1	12.0	86.2	64.7	24.6	0.019	8.5
PP15	160° 31' 36.818"	68° 28' 21.421"	13.5	7.2	11.6	112.6	43.2	23.0	0.033	8.4
PP16	160° 22' 46.441"	68° 30' 54.259"	14.0	7.1	11.5	75.7	26.7	27.9	0.017	9.4
PP17	160° 12' 44.290"	68° 31' 40.551"	13.4	7.2	11.6	116.0	39.1	21.7	0.021	8.9
PP18	160° 6' 5.068"	68° 34' 7.709"	14.8	7.1	11.4	112.4	52.0	-	0.019	10.2
PP19	160° 5' 6.219"	68° 33' 4.189"	14.3	7.1	11.5	113.4	47.3	41.2	0.018	9.4
PP20	159° 54' 7.538"	68° 33' 20.649"	13.8	7.2	11.6	109.9	58.5	20.2	0.022	8.8
PP21	159° 46' 4.519"	68° 33' 18.389"	13.9	7.2	11.6	105.7	51.1	20.9	0.016	9.5
PP22	159° 38' 0.629"	68° 34' 15.121"	13.8	7.2	11.6	116.1	56.8	21.0	-	7.9
PP23	159° 23' 12.869"	68° 35' 55.258"	15.2	7.2	11.4	66.8	41.6	40.4	0.033	8.8
PP24	159° 5' 25.404"	68° 38' 12.821"	14.2	7.2	11.6	106.2	57.5	23.6	0.015	8.1
PP25	161° 18' 16.628"	68° 45' 5.062"	13.9	7.2	10.7	101.3	61.8	32.6	0.016	7.5

S.3. Supplemental Figures

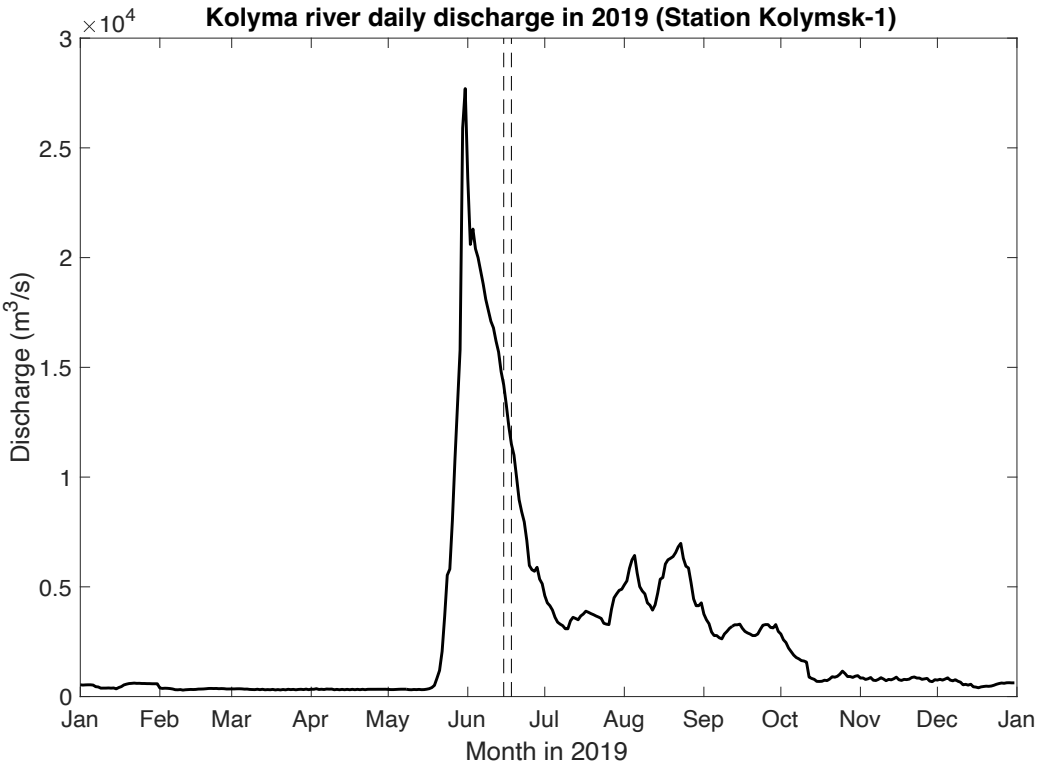

Figure S1 - Water discharge curve in Kolyma River in 2019, data from Arctic Great Rivers Observatory (Shiklomanov et al., 2020). Discharge curve during freshet period from 20 May (840 m^3 s^{-1}) to 10 July (3090 m^3 s^{-1}). Peak of the freshet: 31 May (27,700 m^3 s^{-1}). Vertical dashed lines indicate the sampling dates during the upstream transect (left line, 15-16 June 2019) and the downstream transect (16-17 June 2019).

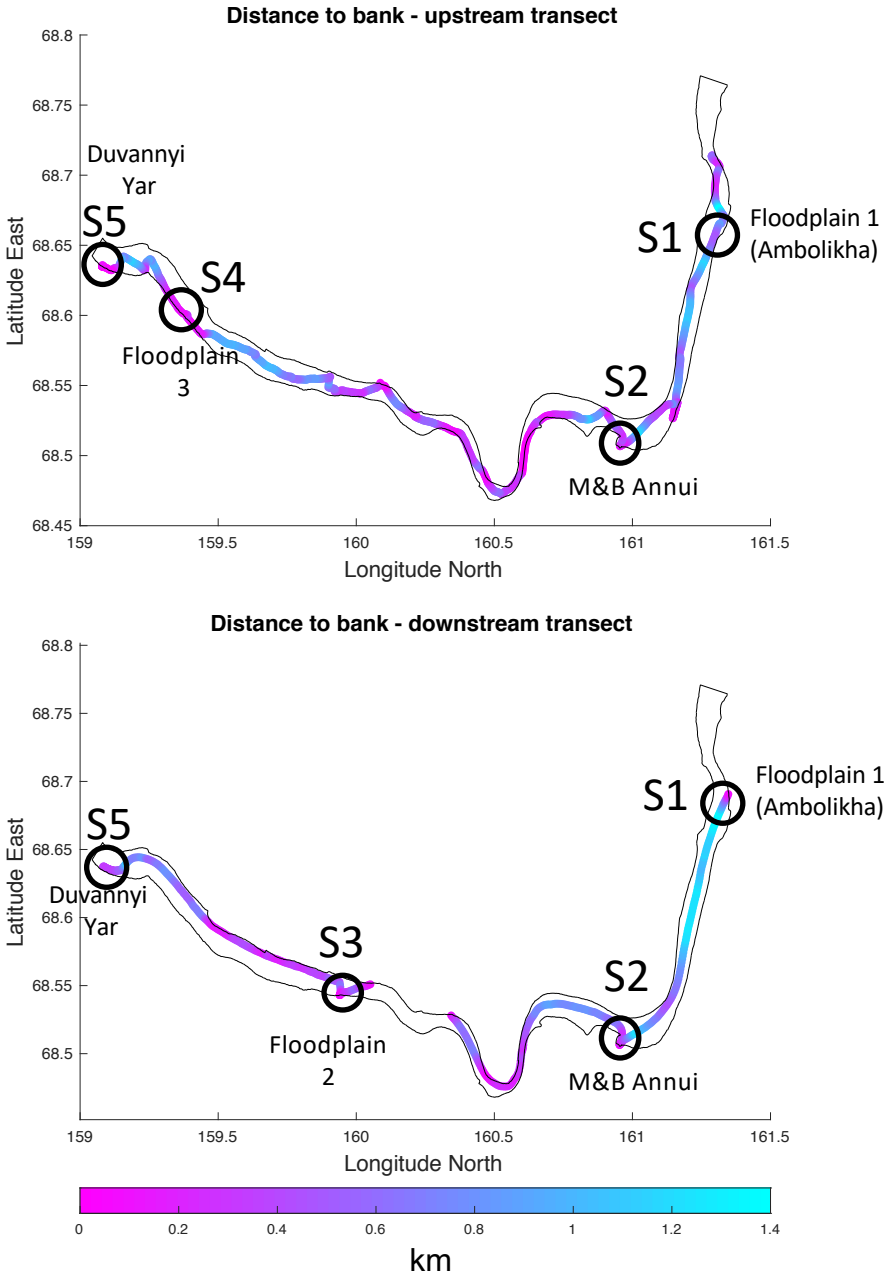

Figure S2 – Calculated distance from the transect to the bank

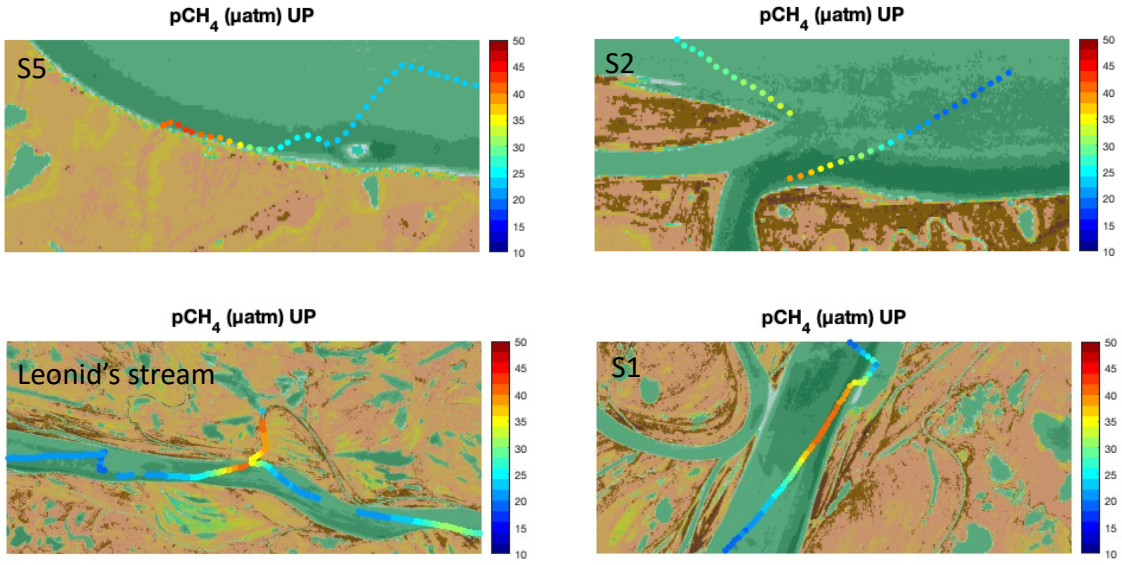

Figure S3 – Closer view to the measured surface pCH_4 in key sites and at Leonid's stream, where larger methane concentrations were observed compared to the surrounding areas.

Key sites

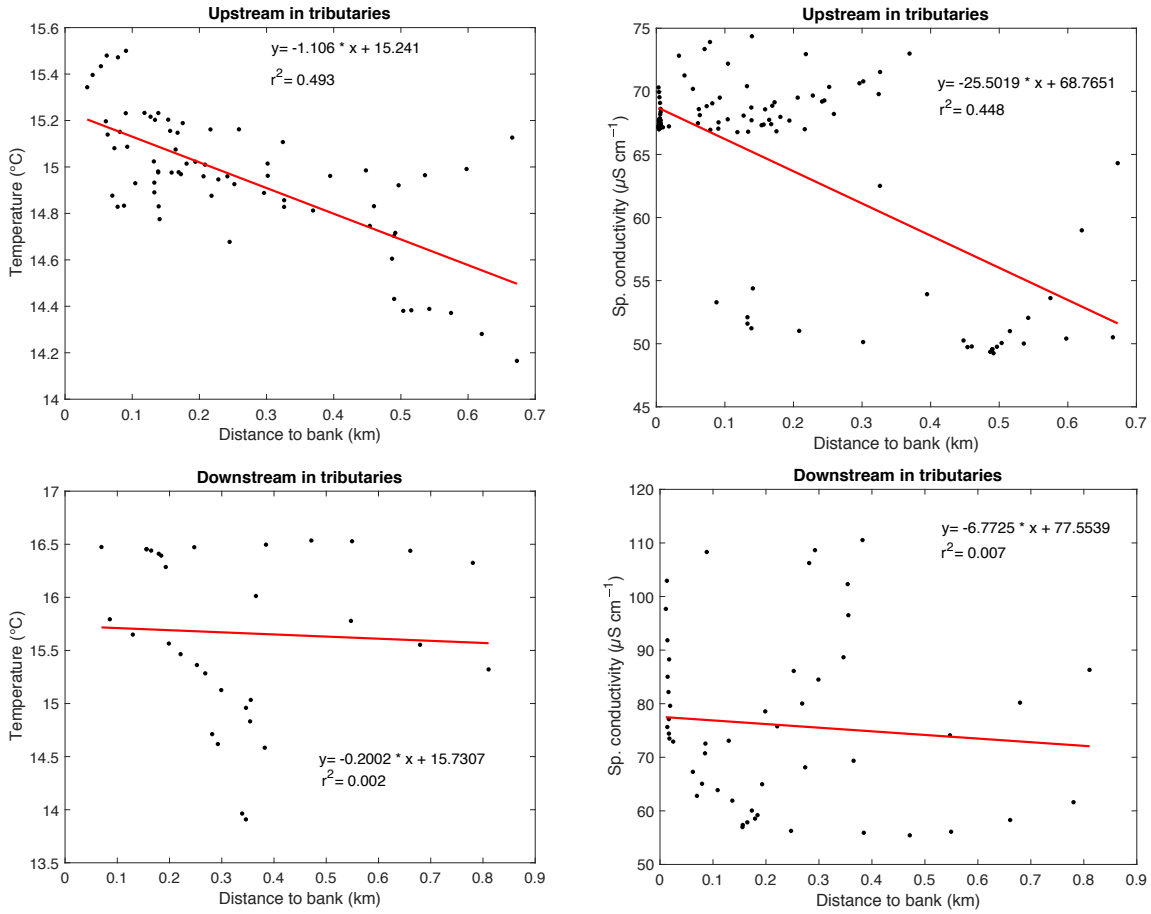

Figure S4 – Linear correlation analysis between the conservative properties temperature and specific conductivity against the distance to bank *z* during the UP and DOWN transects for key sites.

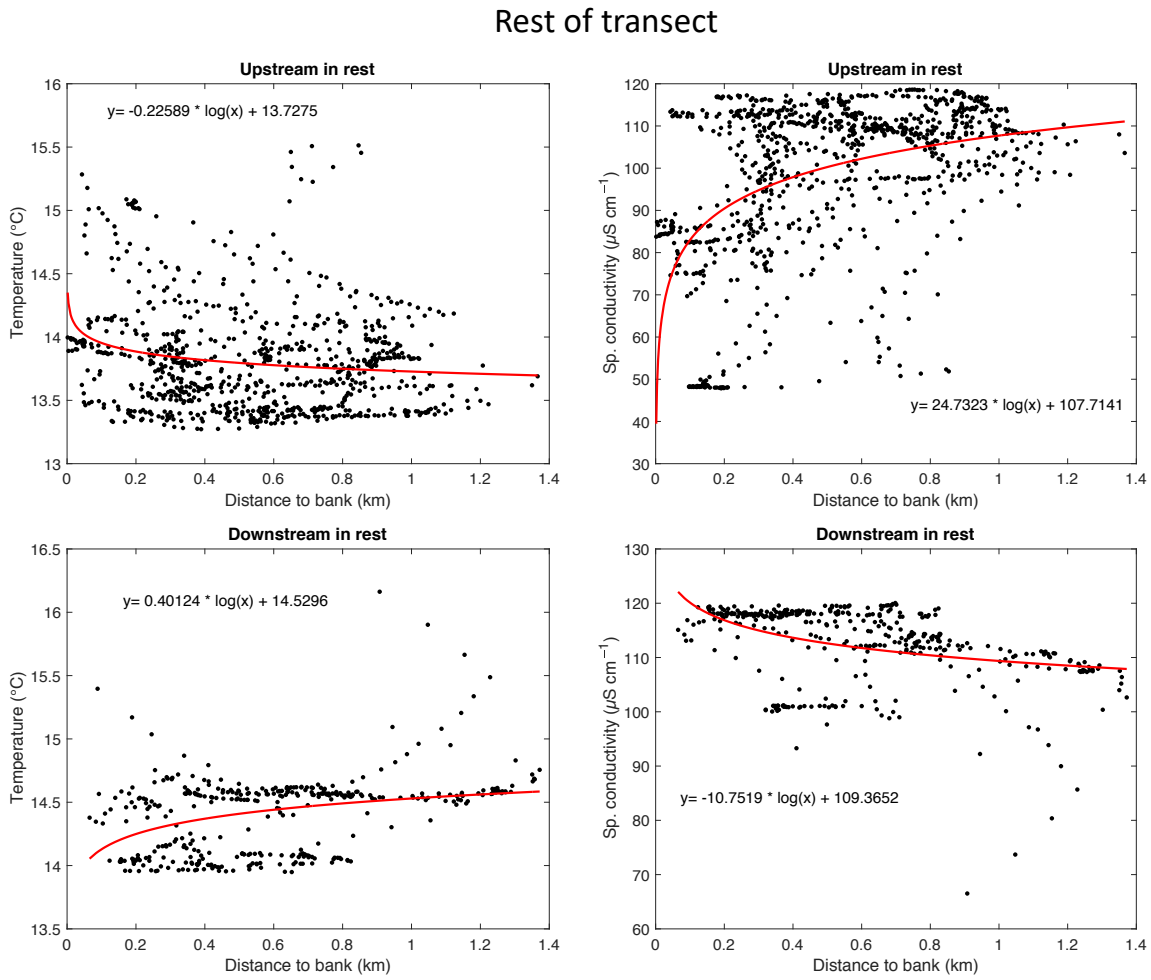

Figure S5 – Linear correlation analysis between the conservative properties temperature and specific conductivity against the distance to bank *z* during the UP and DOWN transects for data in the rest of the transect.

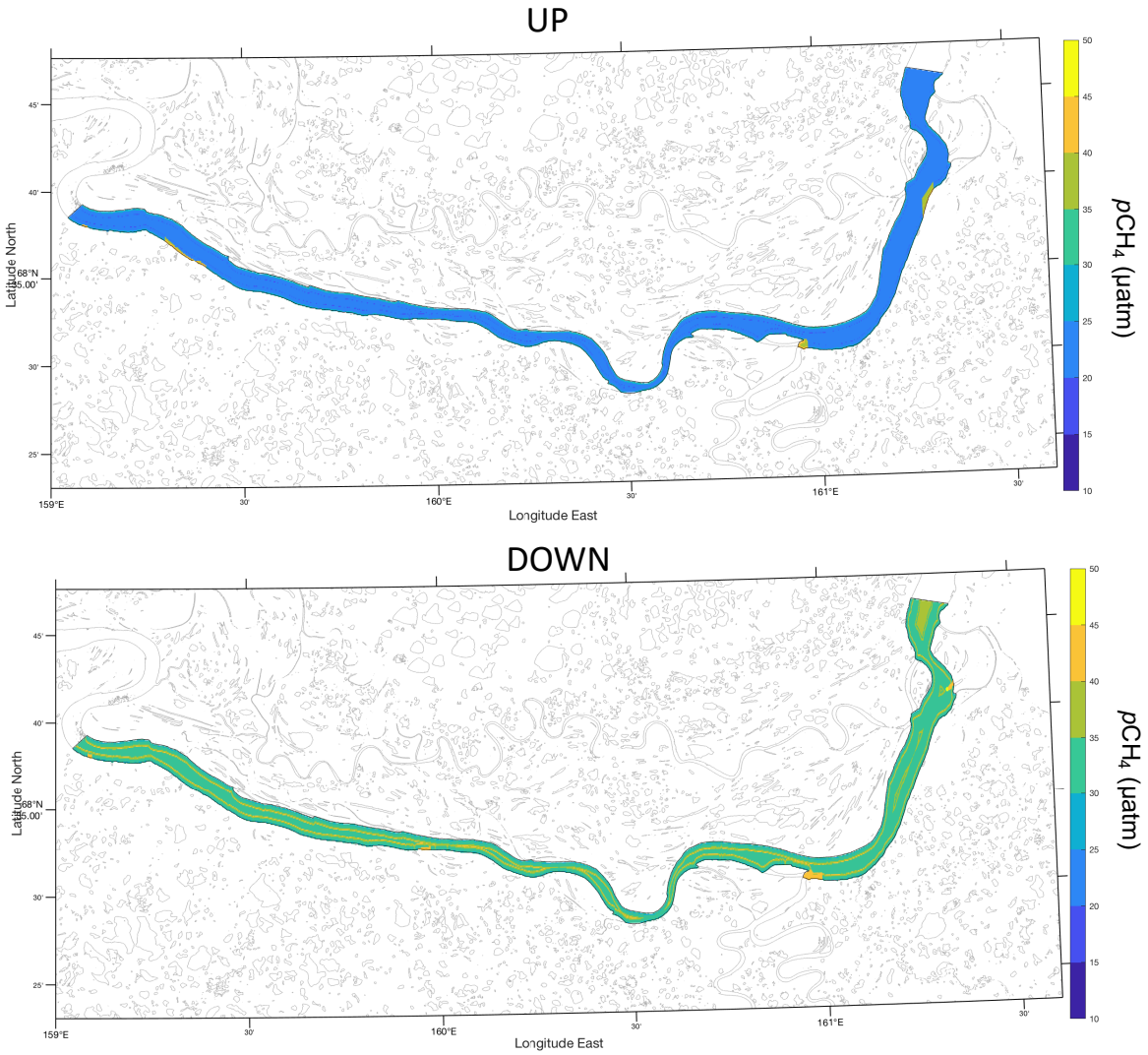

Figure S6 – pCH_4 obtained for the entire gridded polygon for the UP (top) and DOWN (bottom) transects after applying the random forest models as function of T and κ.

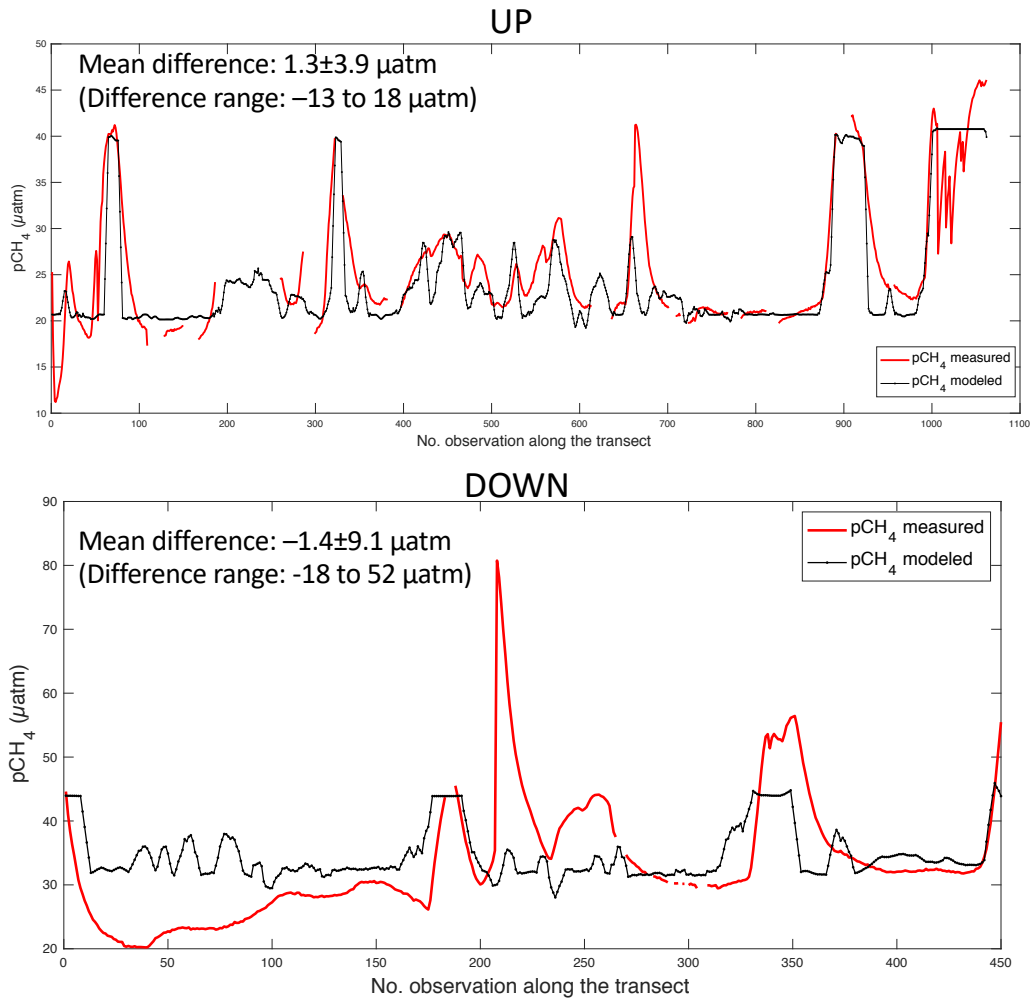

Figure S7 – Comparison between modeled pCH_4 from random forest regression and measured pCH_4 along the UP (top) and DOWN (bottom) transects.

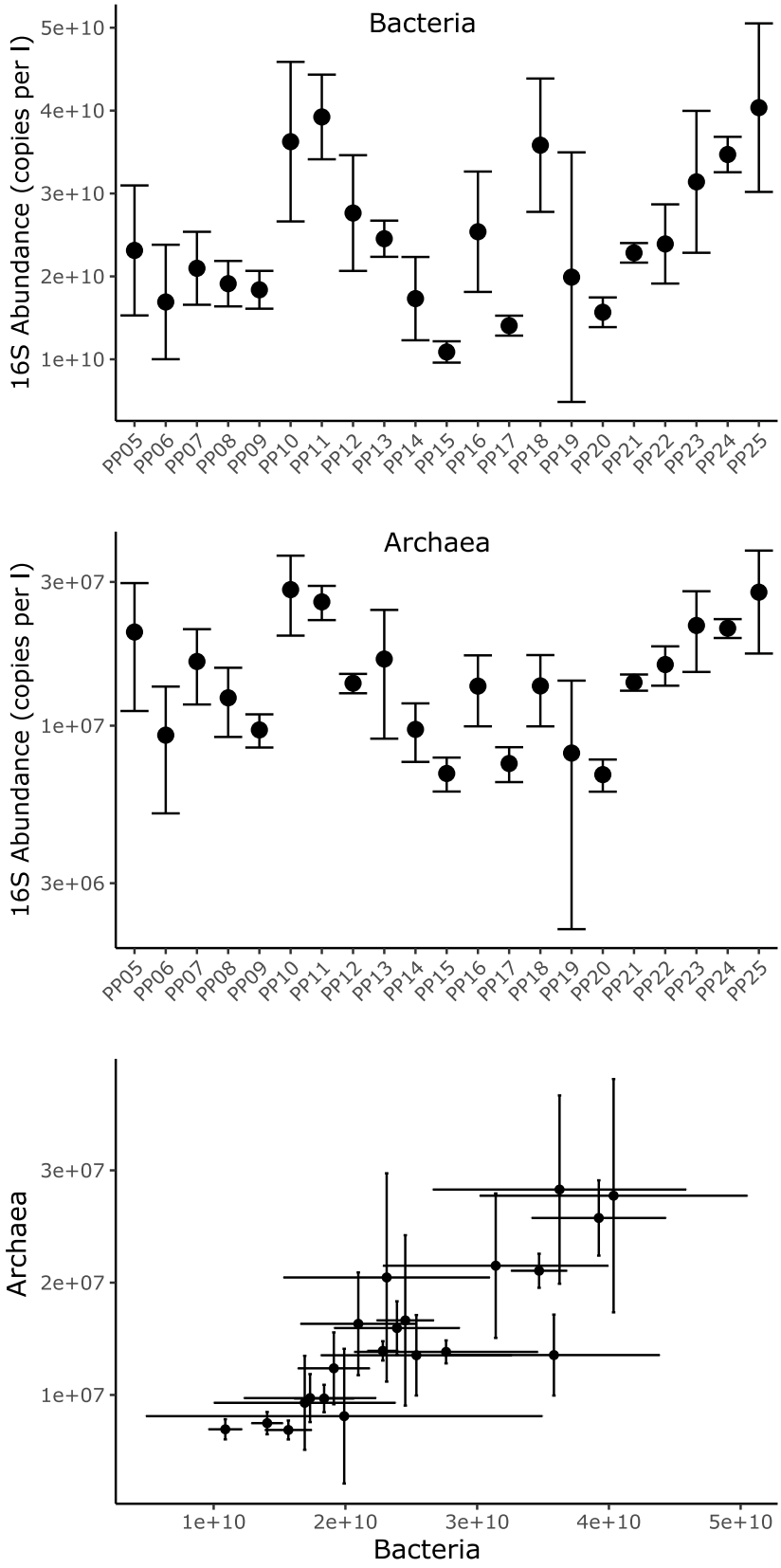

Figure S8 – qPCR graph showing the 16S abundance of bacteria (top) and archaea (middle) in samples from Kolyma River. Despite bacteria is three orders of magnitude more abundant than archaea, there is a positive linear correlation between them (bottom).

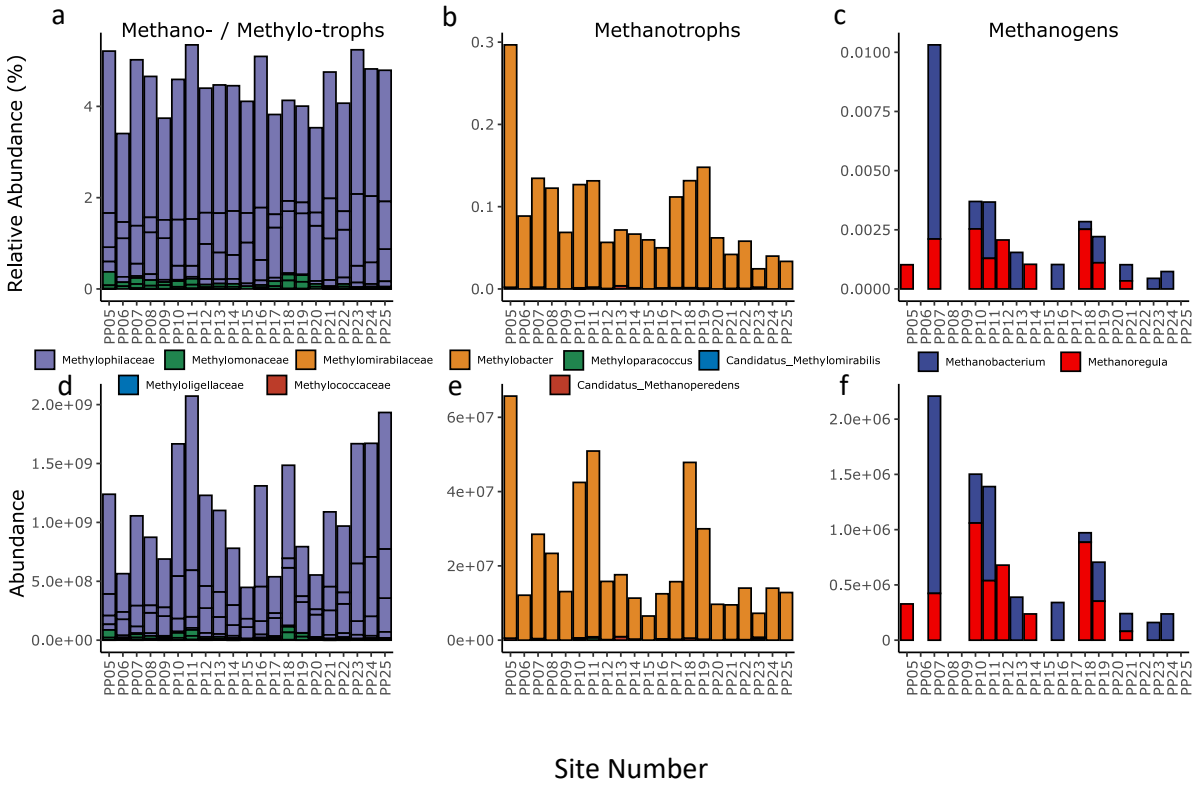

Figure S9 - Relative (top) and total abundances (bottom) of archaeal microbial communities in water samples from Kolyma River distributed across 21 sampling stations. Methanotrophs (left) and methanogens (right).

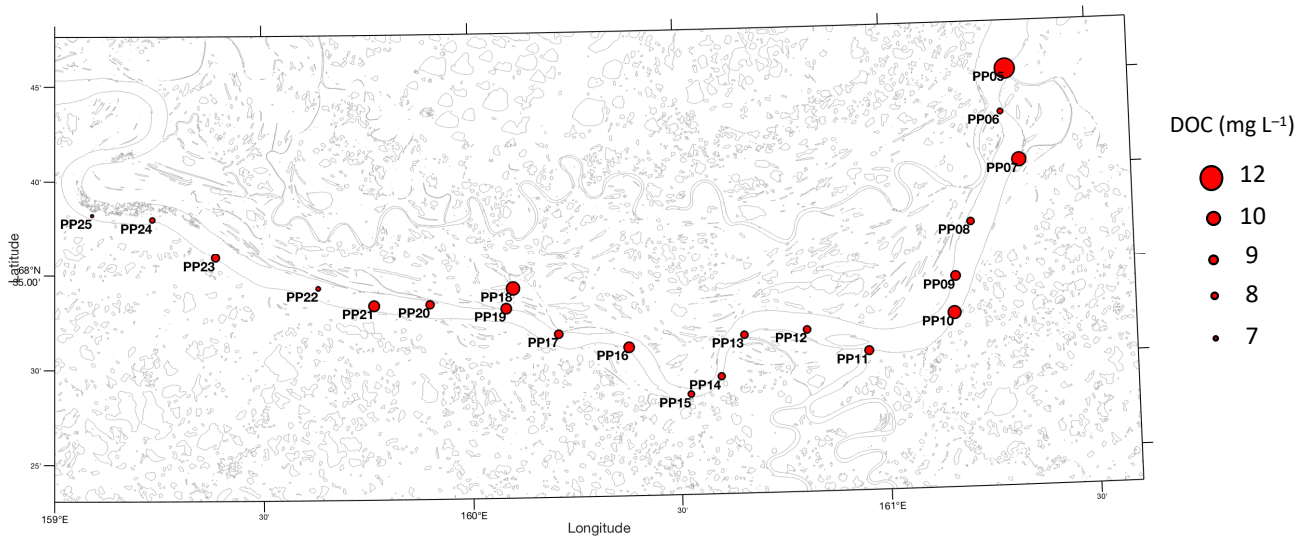

Figure S10 – Dissolved Organic Carbon concentration in discrete samples collected in 21 stations distributed along UP transect.

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