



Supplement of

Microbial methane formation in deep aquifers associated with the sediment burial history at a coastal site

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Supplementary Figures

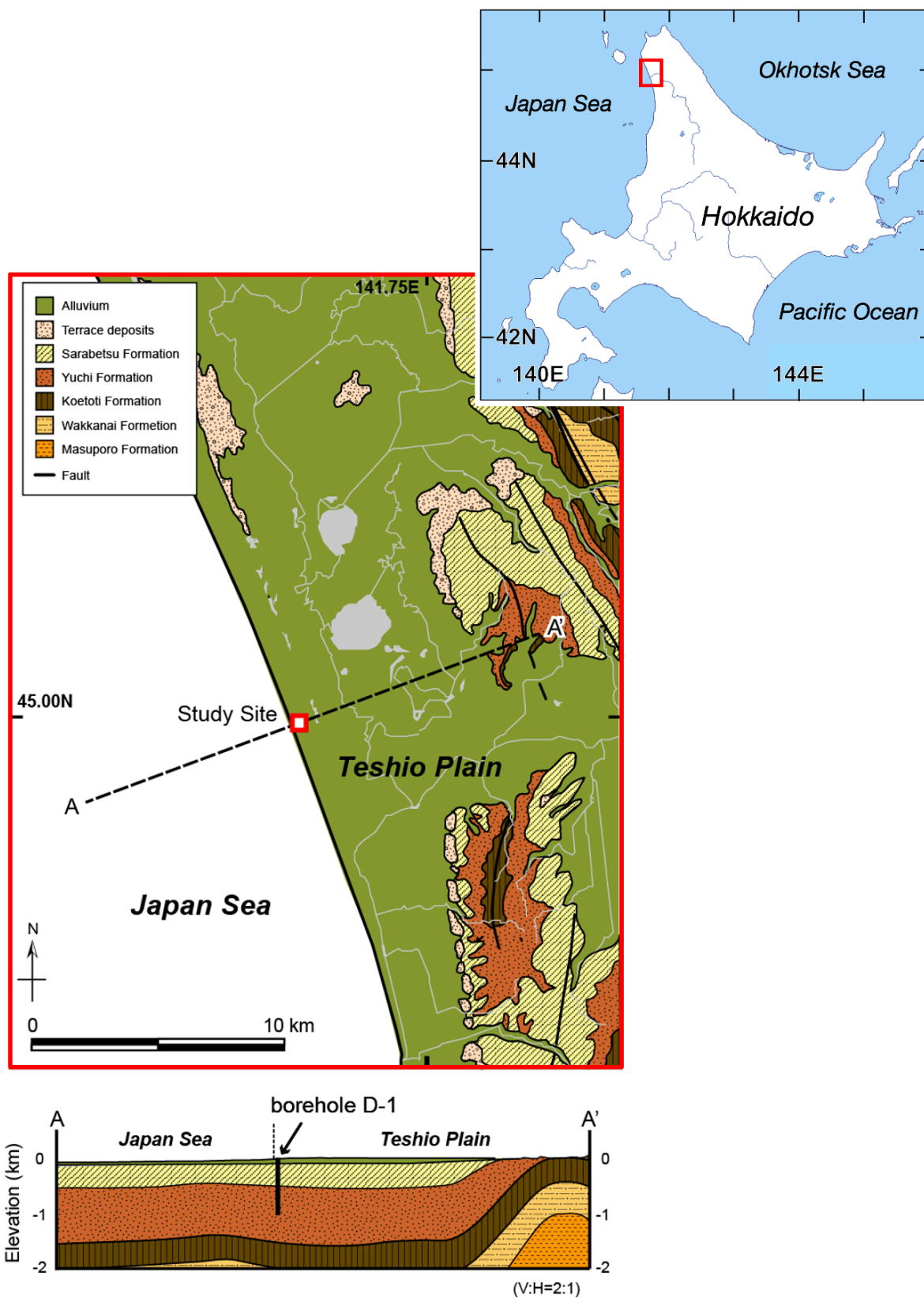


Fig. S1. Geological map of the study site (from Ikawa et al., 2014). The ratio of the vertical to horizontal scale of the cross section (A to A') is 2:1.

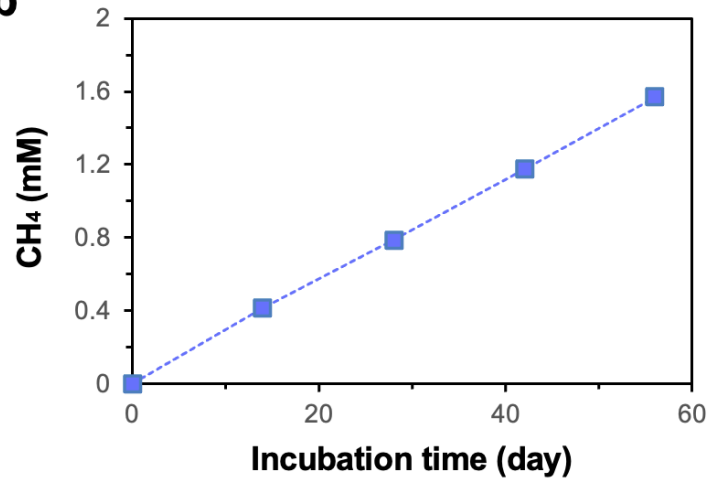
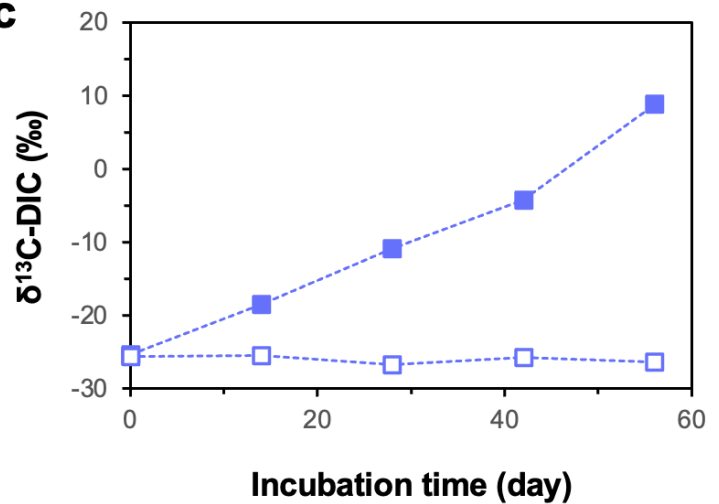
a**b****c**

Fig. S2. Culture experiments for microorganisms involved in syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis. **(a)** Photograph of the semi-continuous cultivation system used in this study. **(b)** Time course of CH₄ production and **(c)** the change in $\delta^{13}\text{C-DIC}$ in the cultures with [2-¹³C]-acetate (closed square) or non-labeled acetate (open square).

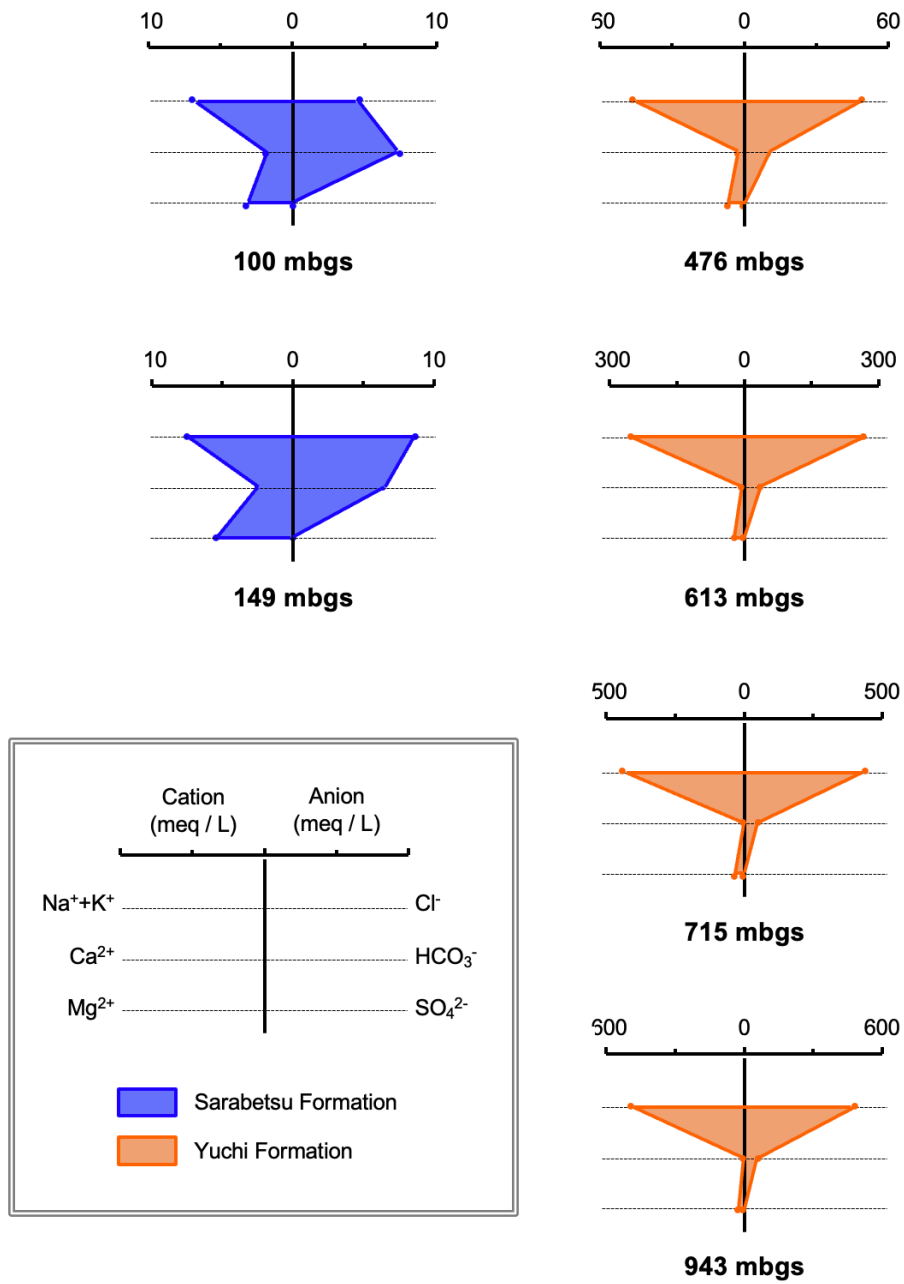


Fig. S3. Stiff diagrams showing the ionic strength of the major cation and anion concentrations in the collected water samples (Table 1).

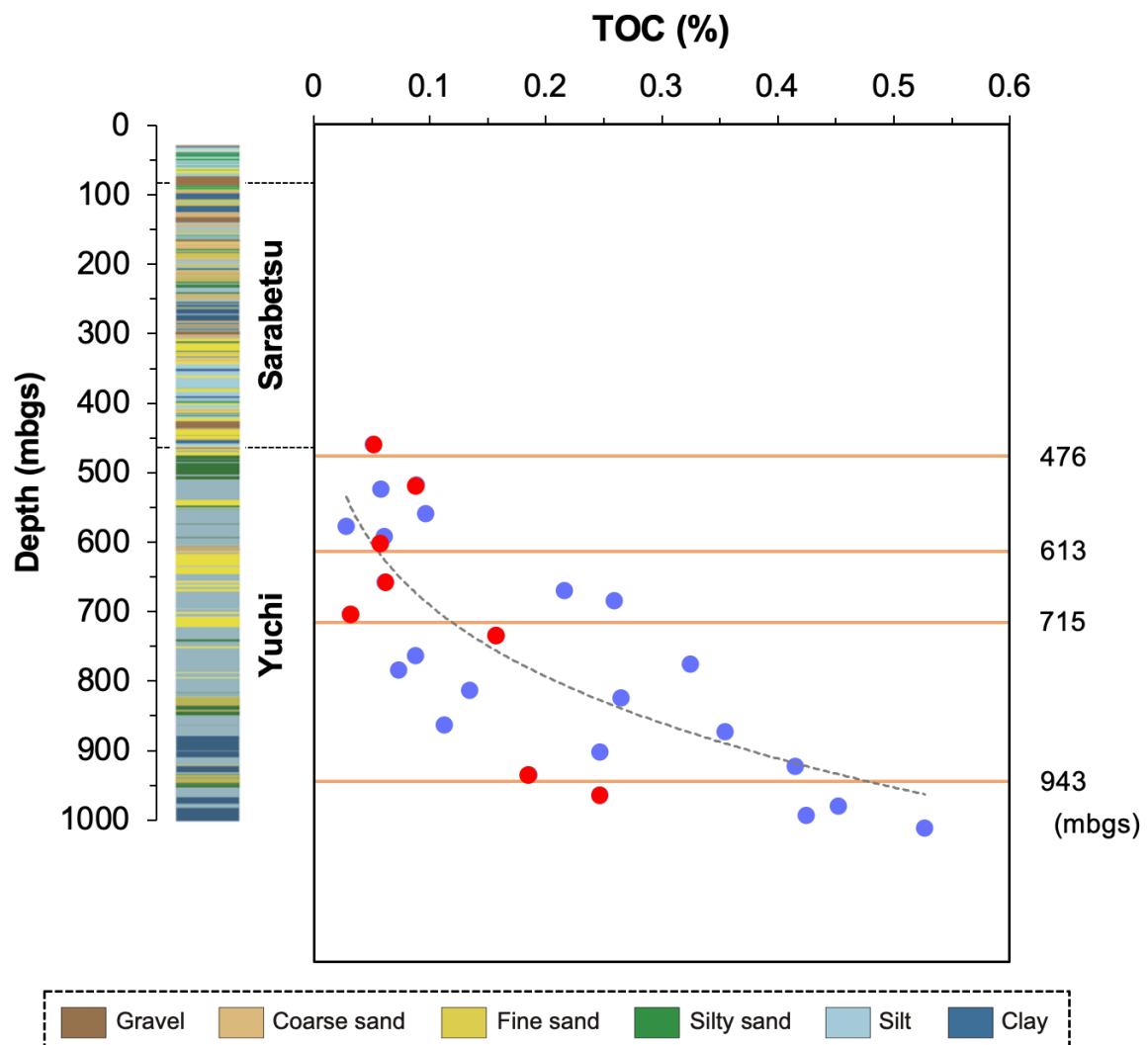


Fig. S4. Depth profiles of borehole D-1 showing the lithologies and TOC contents (blue circles) of the Yuchi sediment core samples (silt and clay). Horizontal orange lines indicate the depths of the sampled aquifers. The TOC contents in mud sediments adjacent to the sampled aquifers are indicated by red circles. The dashed line denotes the trend line fitted to the data points.

Supplementary Table

Table S1. Primers and probes used in this study.

Primer/probe	Sequence (5' to 3')	Target gene	Procedure	Reference
Bac1055YF	ATGGYTGTCGTCAGCT	bacterial 16S rRNA	real-time PCR	Ritalahti et al. 2006
Bac1392R	ACGGGCGGTGTGTAC	bacterial 16S rRNA	real-time PCR	Ritalahti et al. 2006
Bac1115Probe	CAACGAGCGCAACCC	bacterial 16S rRNA	real-time PCR	Ritalahti et al. 2006
Arc787F	ATTAGATACCCSBGTAGTCC	archaeal 16S rRNA	real-time PCR	Yu et al. 2005
Arc1059R	GCCATGCACCCWCCTCT	archaeal 16S rRNA	real-time PCR	Yu et al. 2005
Arc915Probe	AGGAATTGGCGGGGAGCAC	archaeal 16S rRNA	real-time PCR	Yu et al. 2005
ME1F	GCMATGCARATHGGWATGTC	<i>mcrA</i>	real-time PCR	Hales et al. 1996
ME2F	TCATKGCRTAGTTDGGRTAGT	<i>mcrA</i>	real-time PCR	Hales et al. 1996
Univ515F	GTGYCAGCMGCCGCGGTA	prokaryotic 16S rRNA	amplification	Ellis et al. 2013
Univ926R	CCGYCAATTCMTTTRAGTT	prokaryotic 16S rRNA	amplification	Ellis et al. 2013
MLf	GGTGGTGTMGGATTCACACARTAYGCWACAGC	<i>mcrA</i>	amplification	Luton et al. 2002
MLr	TTCATTGCRTAGTTWGGRTAGTT	<i>mcrA</i>	amplification	Luton et al. 2002

References

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