

Table S1 Overview on sampling and on physiochemical parameters measured in sampled wells

Well	Date of sampling	Type of sampling	temperature (°C)	redox potential (mV)	pH	Conductivity (mS/cm)
LO-A	12.6.2018	subsurface	21.9	-201.8	7.85	4.56
LO-B	12.10.2018	subsurface	12.6	-208	7.62	3.79
LO-C	12.6.2018	overflow	25.8	-138.5	7.83	3.9
	29.10.2018	overflow	26.3	-460	7.61	3.89
	11.11.2019	overflow	25.6	-250	7.54	3.85
LO-D	29.10.2018	overflow	26.2	-370	7.7	3.9
	11.11.2019	overflow	25.5	-293	7.82	3.57
LO-E	12.11.2019	overflow	24.9	-245	7.5	3.73
LO-F	17.5.2018	overflow	28.4	-169.7	7.9	3.7
	11.11.2019	overflow	27.0	-280.0	7.8	3.7
LO-G	31.10.2018	overflow	15,5	-188	8,7	6,7
LO-G	12.11.2019	subsurface	20.8	-188.7	8.6	6.6
LO-H	12.11.2019	overflow	19.6	-170.1	8.4	6.4
LO-H	31.10.2018	subsurface	14.5	-174.5	8.4	6.3
LO-I	7.3.2019	overflow		-319	9.3	3
DUN-A	22.10.2018	subsurface	43.4		8.9	77.4
DUN-B	19.12.2018	subsurface	40.4		8.2	
DUN-C	23.10.2018	subsurface	33.9		8.3	38.5
TVR-A	4.3.2019	subsurface	46		8	
TVR-B	5.4.2019	subsurface	17	-130	8.1	19.2

Table S2 Table of gas composition measured in sampled wells

Well	date of sampling	methane	carbon dioxide	oxygen	nitrogen	helium	hydrogen	ethane	propane	i-butane	n-butane	neopentane
		(vol. %)	(Vol. %)	(vol. %)	(vol. %)	(vol. %)	(vol. %)	(vol. %)	(vol. %)	(vol. %)	(vol. %)	(vol. %)
LO-A	12.6.2018	92.92	2.958	<0.005	2.47	0.018	< 0.001	1.348	0.226	0.024	0.025	< 0.001
LO-B	12.6.2018	55.86	0.069	<0.005	43.99	0.069	< 0.001	0.003	< 0.001	< 0.001	< 0.001	< 0.001
LO-C	12.6.2018	60.93	4.204	<0.005	34.48	0.382	< 0.001	0.003	< 0.001	< 0.001	< 0.001	< 0.001
	29.10.2018	60.33	4.146	<0.005	35.14	0.367	< 0.001	0.003	< 0.001	< 0.001	< 0.001	< 0.001
	11.11.2019	62.23	4.481	<0.005	32.98	0.311	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001
LO-D	29.10.2018	70.58	1.568	<0.005	27.5	0.182	0.001	0.16	0.013	< 0.001	< 0.001	< 0.001
	11.11.2019	77.55	1.397	<0.005	20.6	0.195	< 0.001	0.253	0.018	0.001	< 0.001	< 0.001
LO-E	12.11.2019	65.31	2.98	<0.005	31.36	0.351	< 0.001	0.003	< 0.001	< 0.001	< 0.001	< 0.001
LO-F	17.5.2018	63.5	1.624	<0.005	34.53	0.342	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001
	11.11.2019	69.1	1.608	<0.005	28.93	0.362	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001
LO-I	7.3.2019	95.67	0.014	<0.005	2.85	0.02	< 0.001	1.089	0.257	0.042	0.042	< 0.001
DUN-A	22.10.2018	99.5	0.003	<0.005	0.25	0.016	0.001	0.2	0.019	0.004	0.002	<0.005
DUN-B	19.10.2018	99.35	0.009	<0.005	0.14	0.013	<0.001	0.434	0.049	0.007	0.004	<0.006
DUN-C	23.10.2018	99.64	0.007	<0.005	0.17	0.013	0.001	0.158	0.008	0.001	<0.001	<0.003
DUN-D	18.10.2018	98.64	0.021	<0.005	1.14	0.016	0.003	0.145	0.024	0.003	0.003	<0.004

TVR-A	4.3.2019	99.68	0.072	<0.005	0.1	0.004	< 0.001	0.146	0.001	< 0.001	< 0.001	< 0.001
TVR-B	5.3.2019	99.54	0.18	<0.005	0.07	0.004	< 0.001	0.173	0.005	0.003	0.005	< 0.001

Table S3 Primer sequences for Illumina analysis

hypotFW	AATGATACGGCGACCACCGAGATCTACACGCTGxxxxxxxTATGGTAA TTGTGTGYCAGCMGCCGCGGTAA
REV	CAAGCAGAAGACGGCATACGAGATTCxxxxxxxGTCAGCCAGCCGGA CTACNVGGGTWTCTAAT
Seq1	TATGGTAATTGTGTGYCAGCMGCCGCGGTAA
Seq2	AGTCAGCCAGCCGGACTACNVGGGTWTCTAAT
Index1	ATTAGAWACCCBNGTAGTCCGGCTGGCTGACT
Index2	TTACCGCGGCKGCTGRCACACAATTACCATA

Table S4 Results of isotopic analysis of degas water and injected gas.

well	Date of sampling	$\delta^{13}\text{C}$ in CH_4 (‰)	δD in CH_4 (‰) measured	δD in H_2O (‰)	δD (CH_4) (‰) corrected
TVR-A	4.3.2019	-57.9	-194.6	-32.3	-162.3
TVR-B	5.3.2019	-53.6	-188.4	-24.9	-163.5
DUN-A	22.10.2018	-62.7	-212.3	-26.1	-186.1
DUN-B	18.10.2018	-62	-202.2	-33.8	-168.4
LO-A	12.6.2018	-74.2	-263.1	-77.4	-185.7
LO-B	29.10.2018	-77.8	-237.1	-76.3	-160.8
Injected gas	8.7.2020	-43.6	-229.8		

Table S5 Absolute quantification of mcr-A gene via qPCR (gene copies per ml).

well no. m/y	mcrA gcp	well no. m/y	mcrA gcp	well no. m/y	mcrA gcp	well no. m/y	mcrA gcp
LO-A 6/18	4.40E+04	LO-D 10/18	2.03E+07	LO-G 10/18	6.55E+03	DUN-A 10/18	1.7E+05
LO-B 6/18	2.77E+04	LO-D 11/19	1.7E+03	LO-G 11/19	1.33E+04	DUN-B 12/18	-
LO-C 6/18	9.89E+03	LO-E 11/19	4.57E+04	LO-H 10/18	-	DUN-C 10/18	1.03E+06
LO-C 10/18	2.4E+07	LO-F 5/18	2.00E+02	LO-H 11/19	-	TVR-A 3/19	1.86E+04
LO-C 11/19	2.78E+03	LO-F 11/19	6.13E+06	LO-I 3/19	-	TVR-B 4/19	1.42E+06

Table S6 Table of Illumina reads for DADA2 analysis

	sample	input	filtered	denoisedF	denoisedR	merged	nonchim
1	LO-A 6/18	134872	130780	129796	130078	127292	123206
2	LO-B 10/18	176721	171244	170204	170523	166085	159352
3	LO-C 10/18	136802	132175	131797	131931	129928	128992
4	LO-D 11/19	190299	184398	182755	183250	175668	171798
6	LO-F 11/19	134772	131044	130330	130573	129531	129272
7	LO-G 11/19	175037	170306	169181	169492	164117	158386
8	LO-H 11/19	167434	162934	162119	162405	158156	150821
9	DUN-A 10/18	119984	116781	116417	116461	115645	115050
10	DUN-B 12/18	151156	146909	146422	146548	143378	135907
11	DUN-C 10/18	240131	233716	232843	233179	227040	204513
12	TVR-A 3/19	264170	258140	257010	257504	255069	250471
14	TVR-B 4/19	121949	118540	118281	118340	117193	116084
17	LO-D 10/18	130538	125959	124539	125006	117653	109756
18	LO-E 11/19	153548	147855	147167	147517	143246	135229
19	LO-F 5/18	189416	184085	182962	183220	178740	171122
20	LO-G 10/18	245591	240339	239812	239849	235933	195883
21	LO-H 10/18	130287	126456	125793	126034	123906	114428
22	LO-I 3/19	107968	104975	104615	104659	103129	94616
23	LO-C 6/18	224348	218448	217340	217808	212573	203747
24	LO-C 11/19	151509	147686	146804	147137	143931	142458

Figure S1 Piper and Durov diagrams for groundwaters sampled at UGS Lobodice, UGS Tvrdonice, and UGS Dunajovice

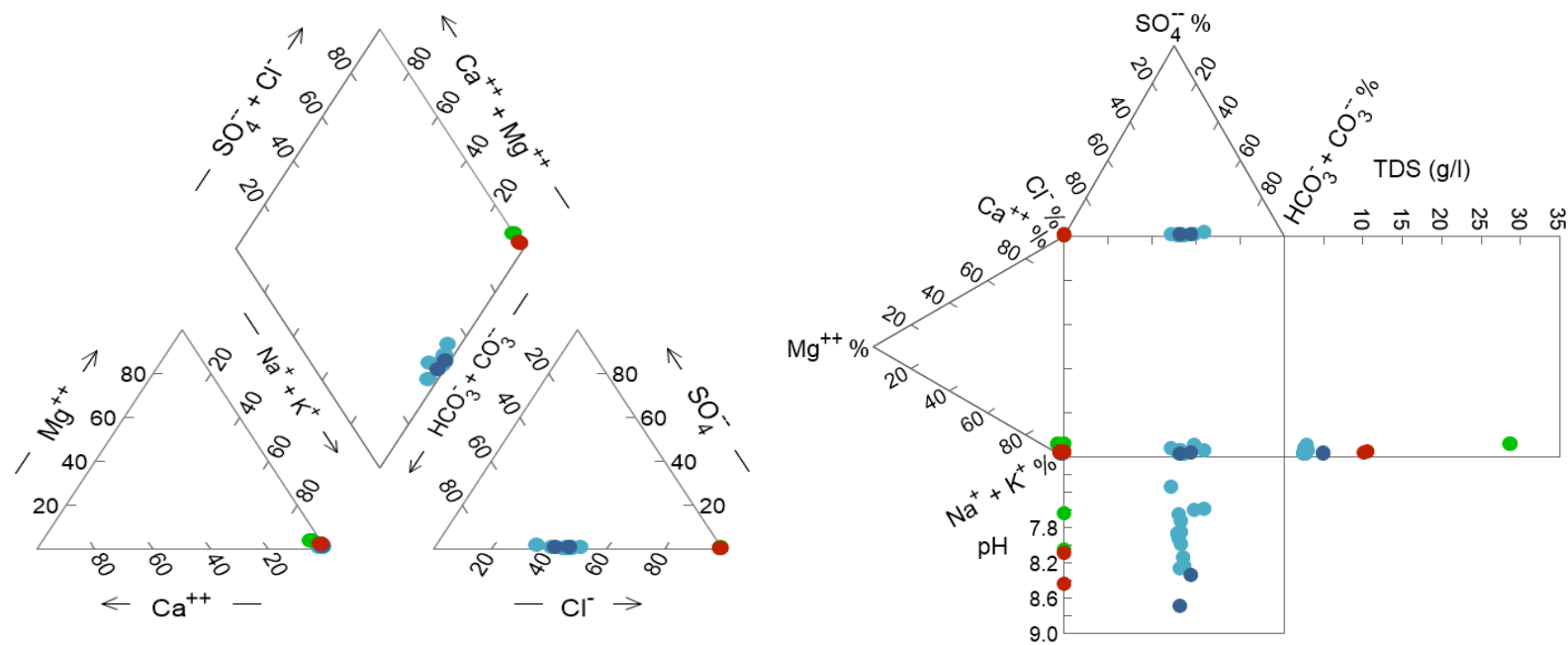
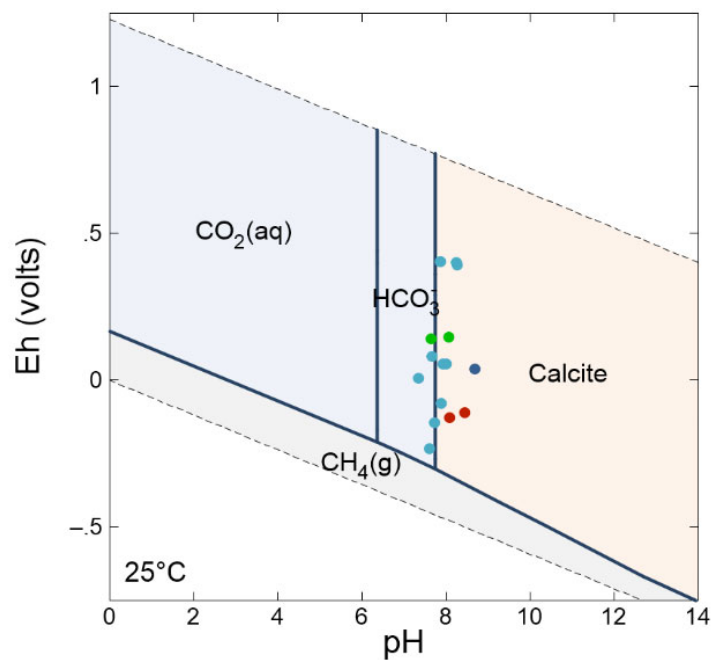
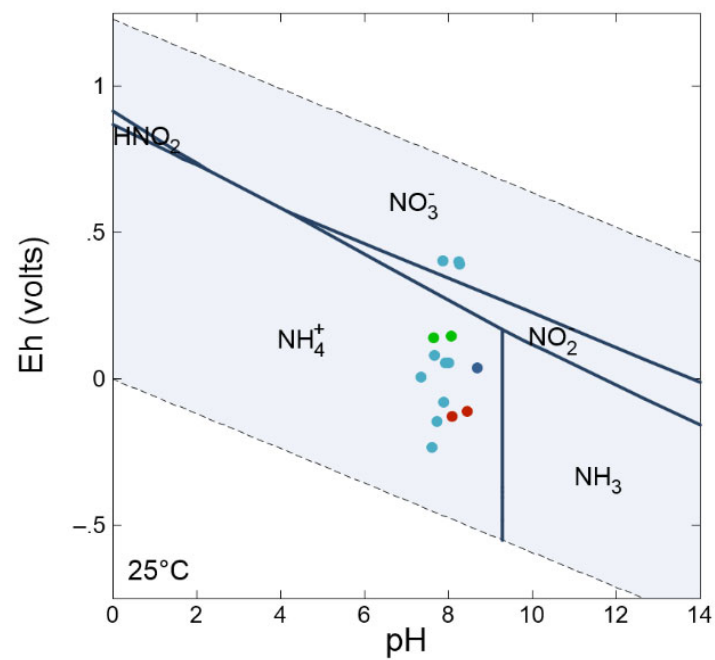


Figure S2 Stability pH-Eh diagrams for carbon (a), nitrogen (b), and sulfur (c) dissolved components. Diagrams were prepared for UGS Lobodice groundwater conditions. for conditions of UGS Tvrdonice and UGS Dunajovice are not significantly different. (d) Speciation of carbon dissolved species in dependence on redox potential.

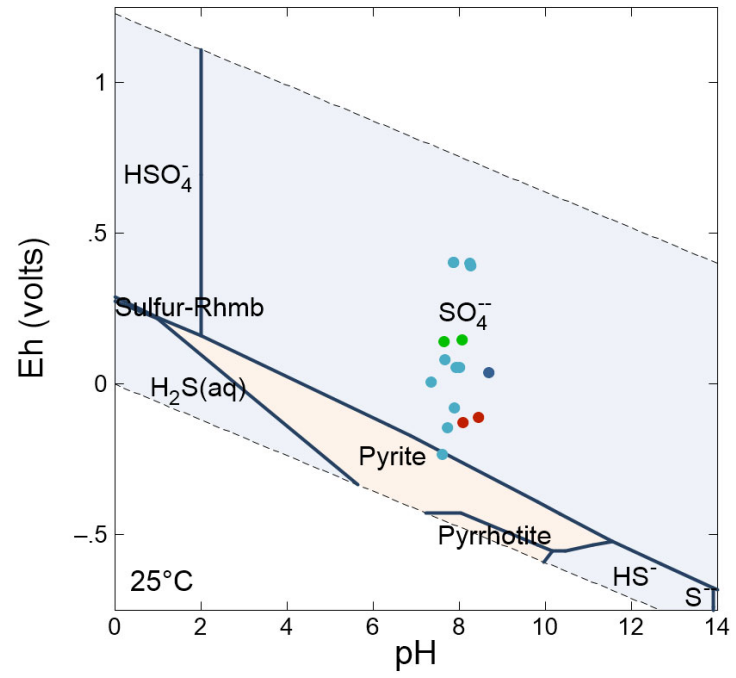
a



b



c



d

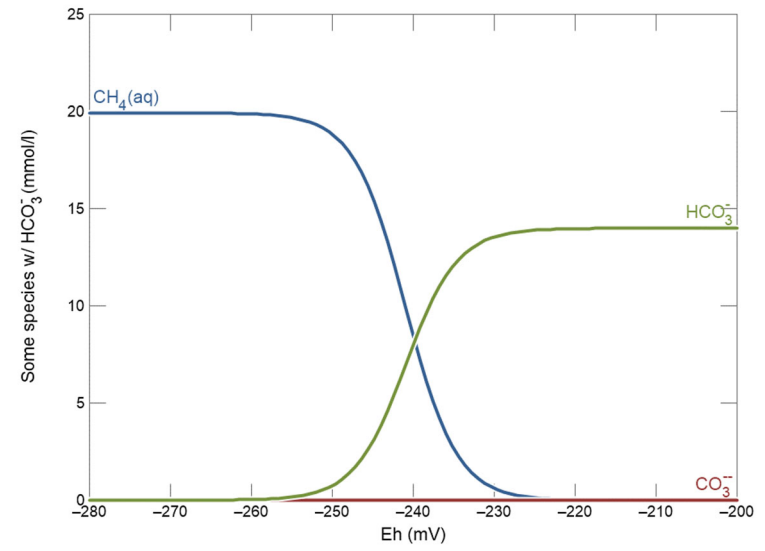


Figure S3 Rarefaction curve

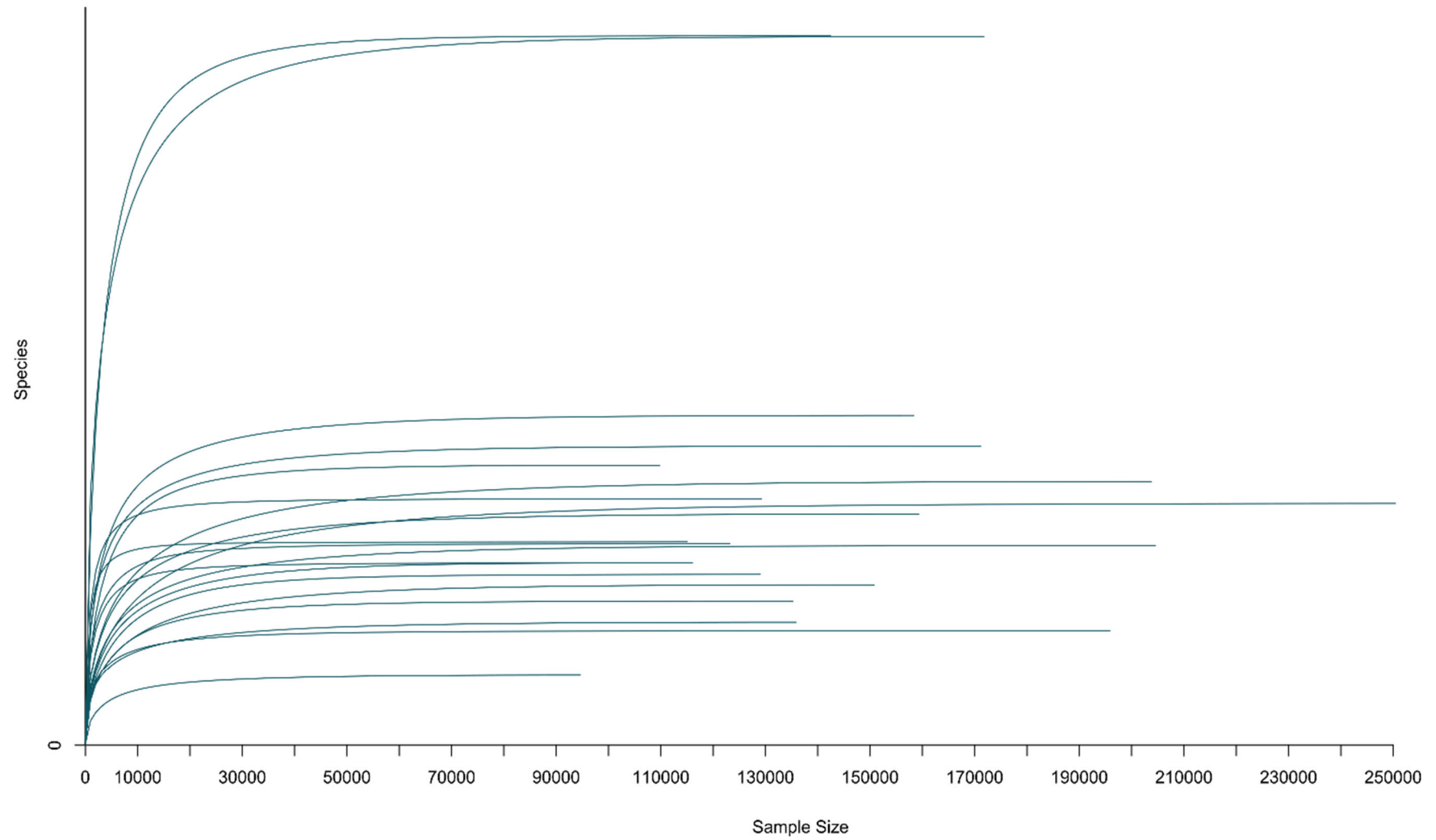


Figure S4 Physical-chemistry well water properties in different sites and composition of archaeal community.

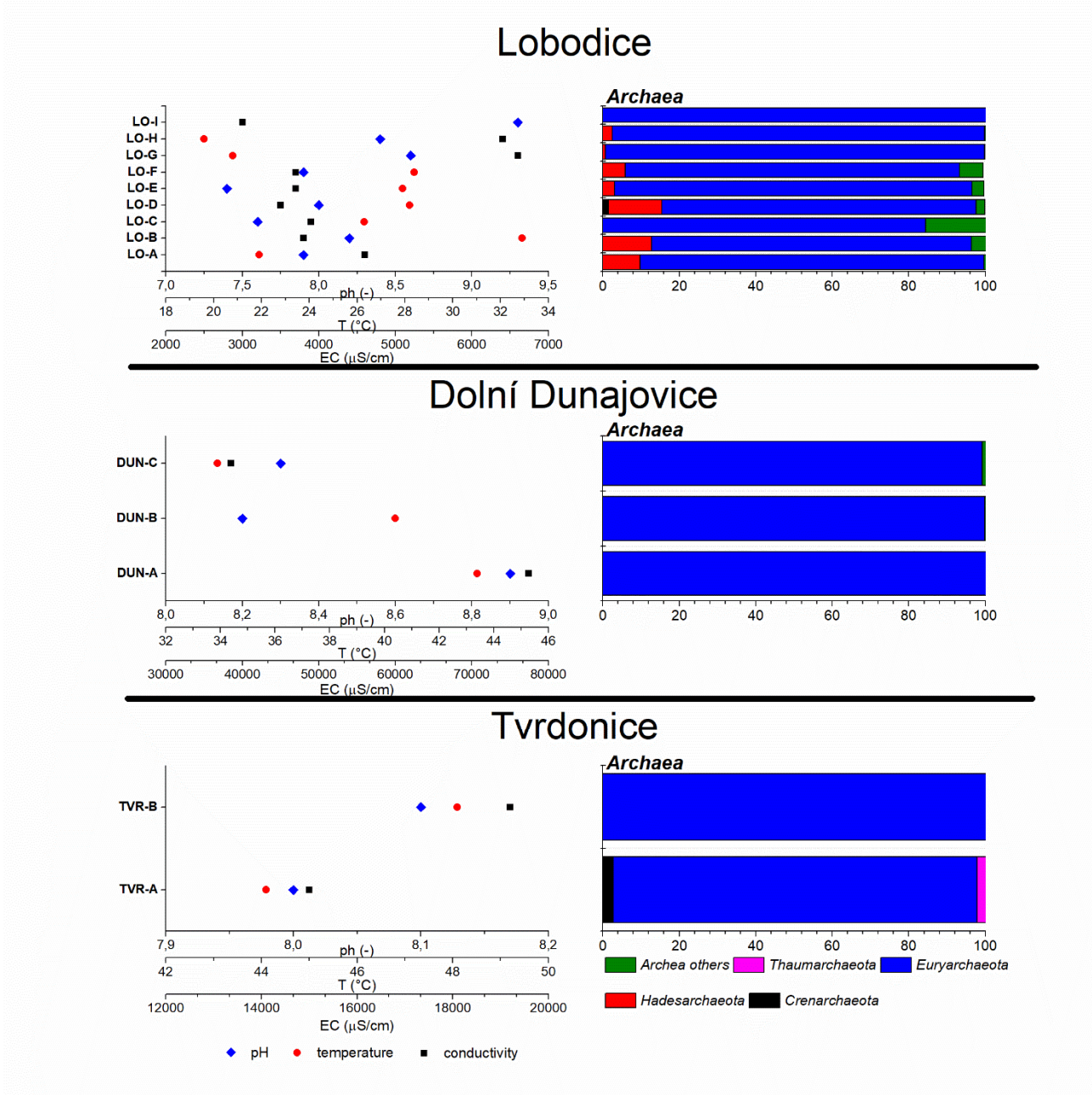


Figure S5 The Alpha biodiversity index (Shannon) of sampled wells

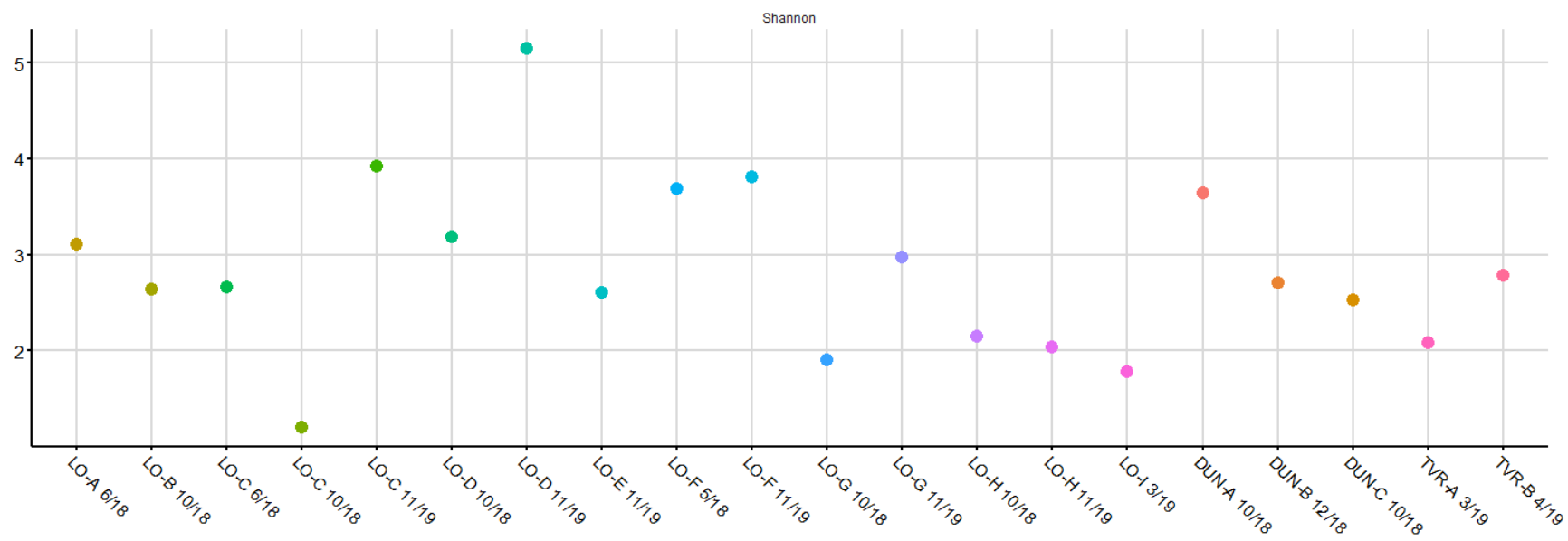


Figure S6 The Beta biodiversity index (unweighted UniFrac) of sampled wells

