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Supplement of

Composition and niche-specific characteristics of microbial consortia colonizing Marsberg copper mine in the Rhenish Massif

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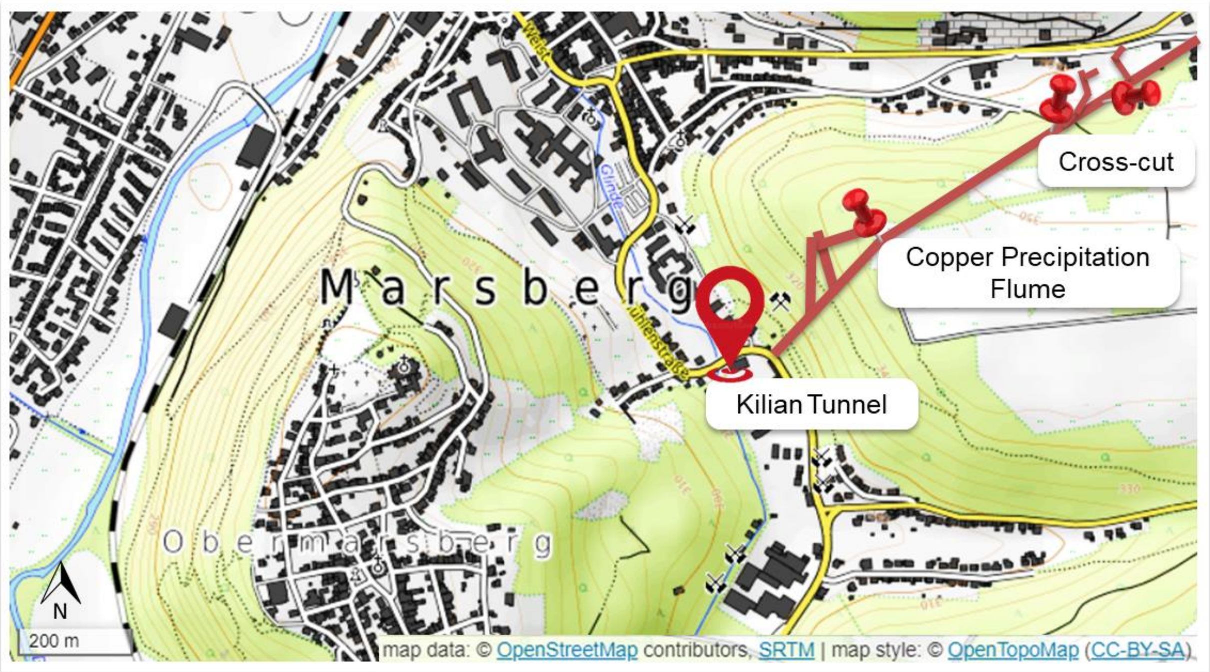

Figure S1. Map of sampling site (Kilianstollen, Marsberg) in the Rhenish Massif Mountain range. Reproduced with modifications from OpenTopoMap (https://opentopomap.org; CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0/legalcode). Adits (simplified course) are indicated by red lines. Sampling sites are marked by red pushpins.

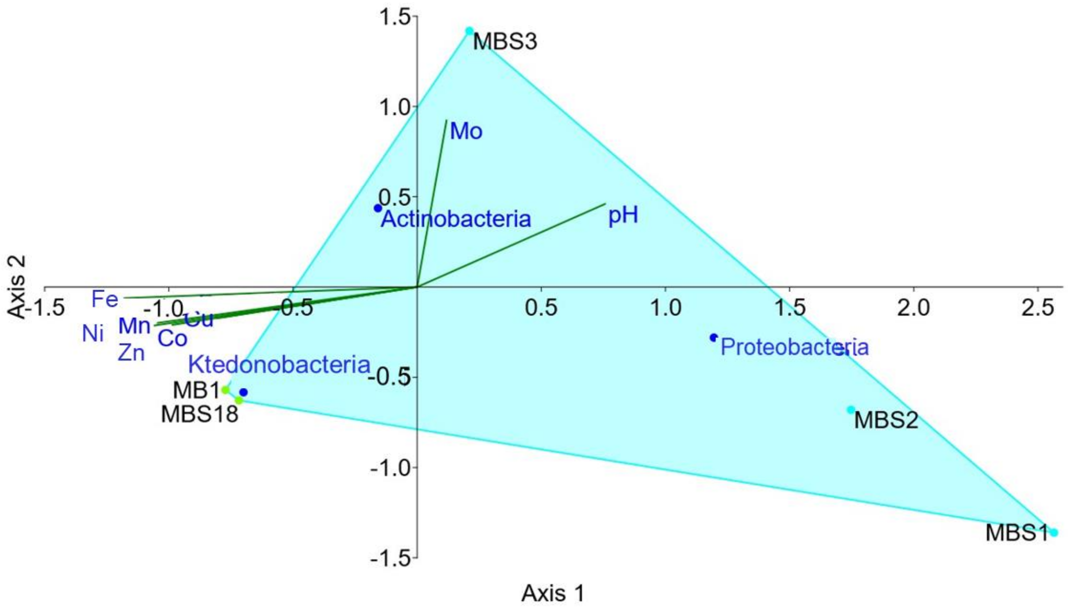

Figure S2. Canonical correspondence analysis of the biological samples collected at the leachate and spring water site. The heavy metals concentrations were taken as the abiotic factors against the abundance of *Proteobacteria*, *Actinobacteria*, and *Ktedonobacteria* for the representative of leachate (MB1, MBS18) and spring water (MBS1, MBS2, MBS3) groups.

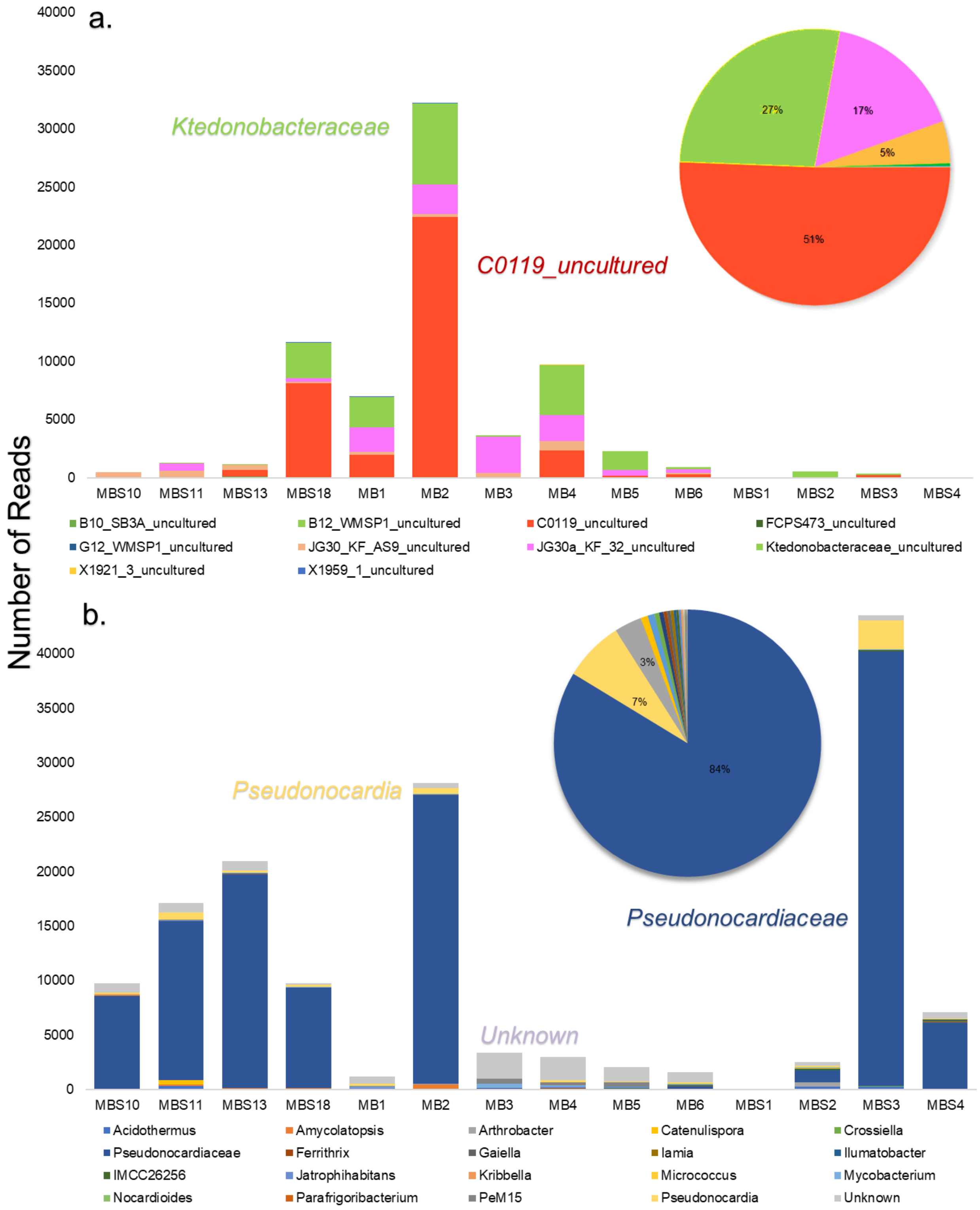

Figure S3. Actual abundance of *Ktedonobacteria* and *Actinobacteria* OTUs at genus level. The actual abundance of OTUs belonging to *Ktedonobacteria* (a) and *Actinobacteria* (b) class were observed at genus level, without any normalization, to analyse the low abundant OTUs. The pie chart insert shows the cumulative abundance of the major genus OTUs for all samples based on actual number of reads.

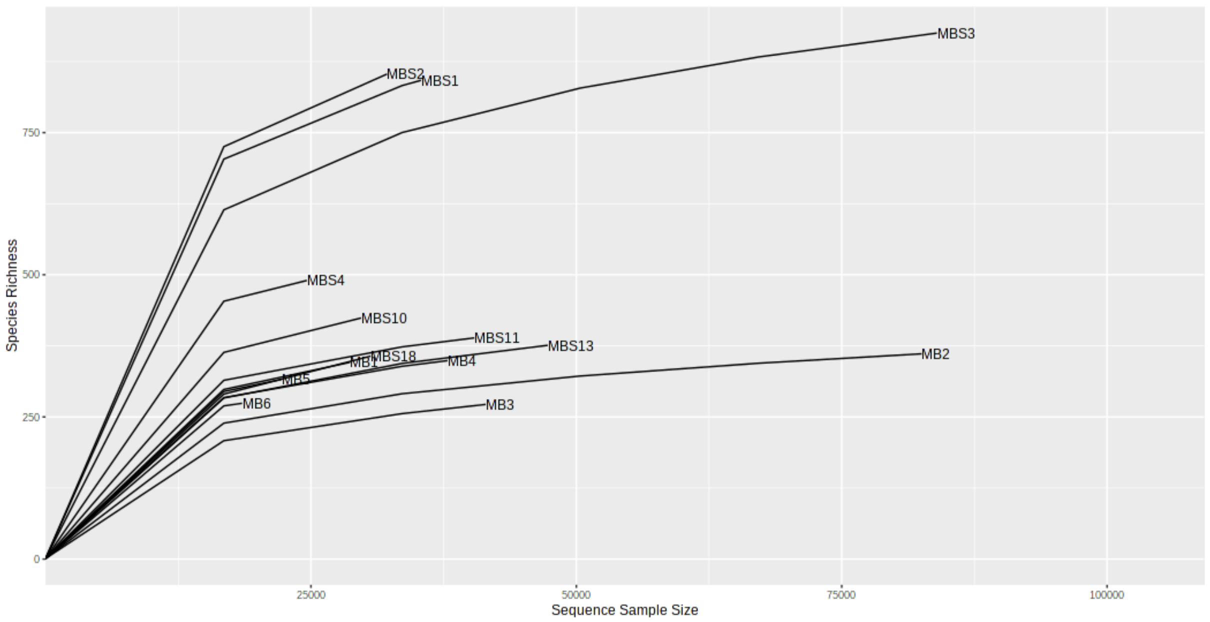

Figure S4. Rarefaction Curve Analysis. The samples are plotted in terms of OTUs observed (species richness) and sampled reads (sequence sample size).

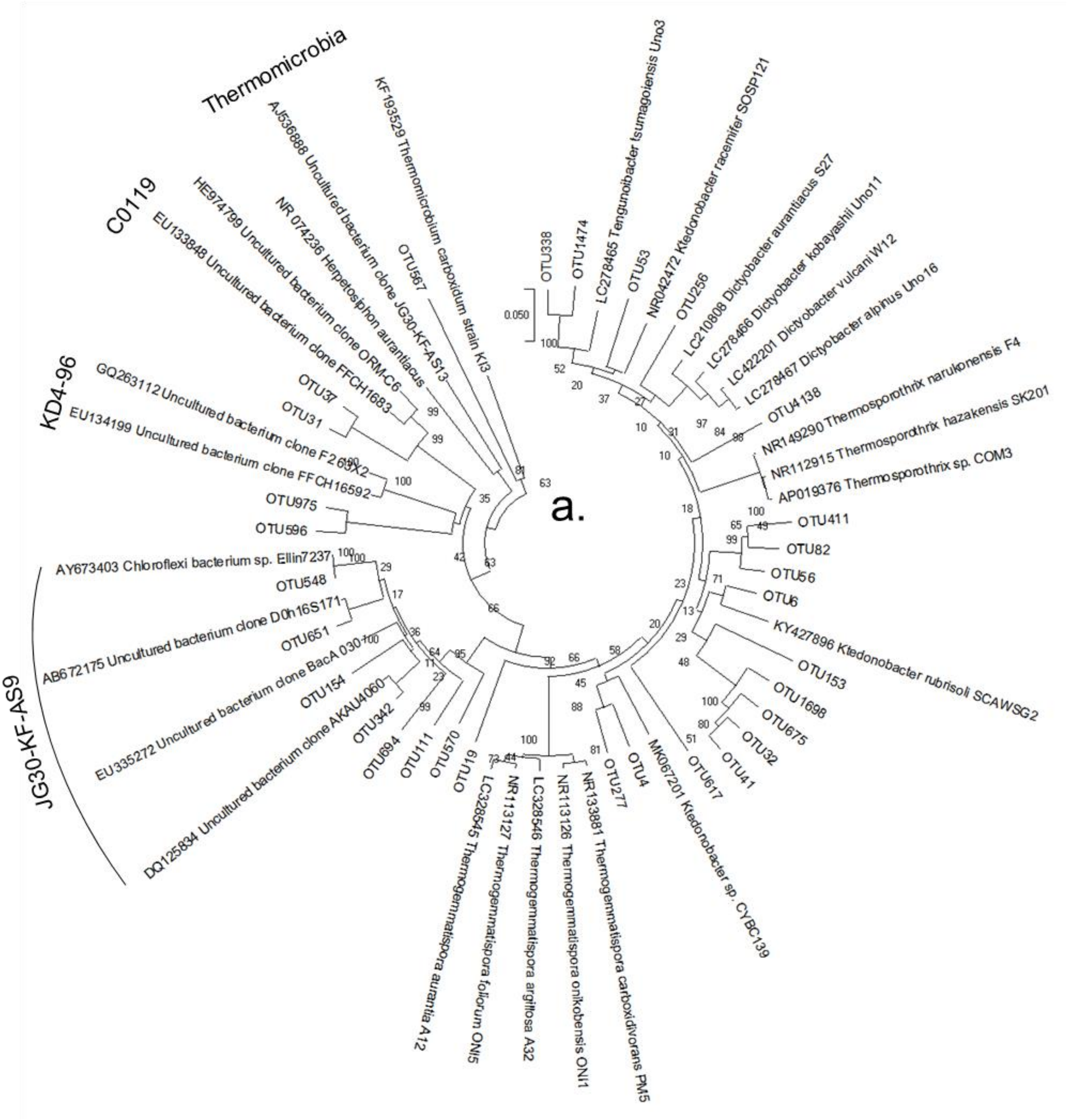
a.
0.050
Thermomicrobia
C0119
KD4-96
JG30-KF-AS9
OTU338
OTU1474
LC278465 Tengunoibacter tsumagoiensis Uno3
OTU53
NR042472 Ktedonobacter racemifer SOSP121
OTU256
LC210808 Dictyobacter aurantiacus S27
LC278466 Dictyobacter kobayashii Uno11
LC422201 Dictyobacter vulcani W12
LC278467 Dictyobacter alpinus Uno16
OTU4138
NR149290 Thermosporothrix narukonensis F4
NR112915 Thermosporothrix hazakensis SK201
AP019376 Thermosporothrix sp. COM3
OTU411
OTU82
OTU56
OTU6
KY427896 Ktedonobacter rubrisoli SCAWSG2
OTU153
OTU1698
OTU675
OTU32
OTU41
OTU617
MK067201 Ktedonobacter sp. CYBC139
OTU4
OTU277
NR133881 Thermogemmatispora carboxidivorans PM5
NR113126 Thermogemmatispora onikobensis ONI1
LC328546 Thermogemmatispora argillosa A32
NR113127 Thermogemmatispora foliorum ONI5
LC328545 Thermogemmatispora aurantia A12
OTU19
OTU570
OTU111
OTU694
OTU342
DQ125834 Uncultured bacterium clone AKAU4060
OTU154
EU335272 Uncultured bacterium clone BacA 030
OTU651
AB672175 Uncultured bacterium clone D0h16S171
OTU548
AY673403 Chloroflexi bacterium sp. Ellin7237
OTU596
OTU975
EU134199 Uncultured bacterium clone FFCH16592
GQ263112 Uncultured bacterium clone F26X2
OTU31
OTU37
EU133848 Uncultured bacterium clone FFCH1683
HE974799 Uncultured bacterium clone ORM-C6
NR 074236 Herpetosiphon aurantiacus
AJ536888 Uncultured bacterium clone JG30-KF-AS13
OTU567
KF193529 Thermomicrobium carboxidum strain KI3

Figure S5. Phylogenetic tree of the small subunit SSU ribosomal RNA genes of OTUs. *Ktedonobacteria* (a) and *Actinobacteria* (b). Maximum-likelihood phylogenetic tree constructed based on V3-V4 amplicon sequences OTUs of *Ktedonobacteria* 16S rRNA gene (a) with the validly published *Ktedonobacteria* species. For the *Actinobacteria* class OTUs (b), the highest blast hits sequences were used. Bootstrap values are given at the branching points.

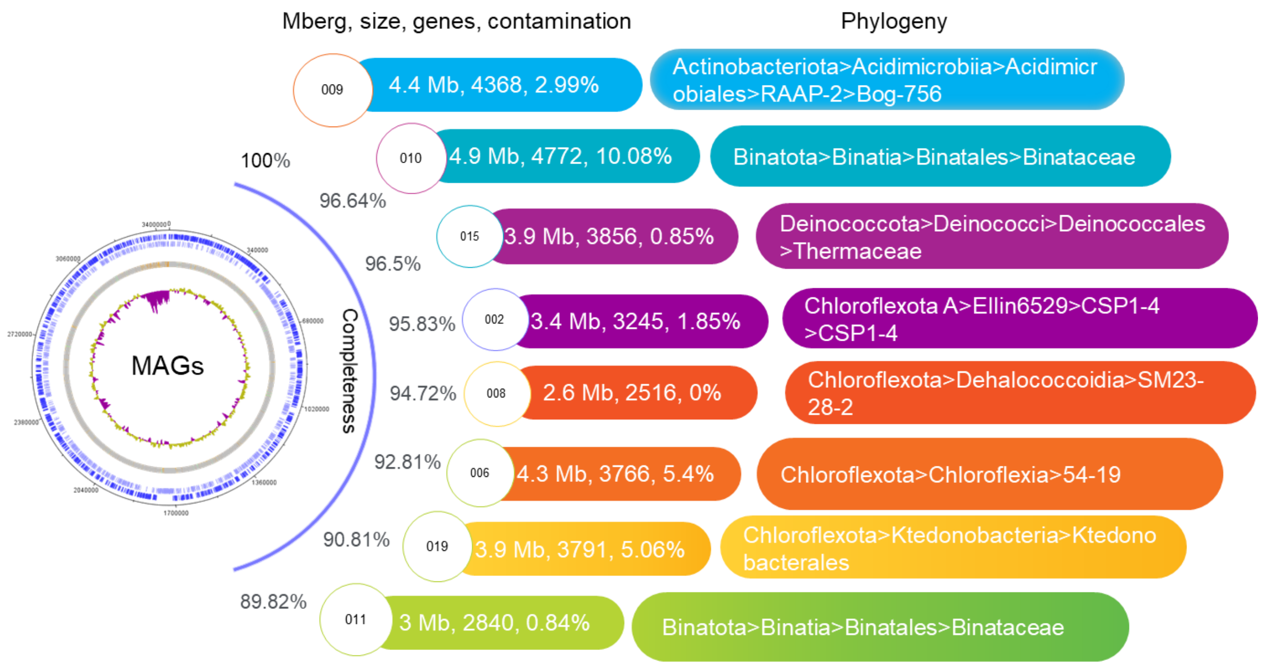

Figure S6. Overview of metagenome-assembled genomes and their key features

Table S1. Alpha diversity indexes of the Marsberg environmental samples

	p-value: [ANOVA]	**F-value**
Chao1	0.00030265	18.499
ACE	0.0002466	19.409
Shannon p-value	0.025179	5.242
Samples	**Shannon**	**Chao1**
MBS10	3.510842108	560.25
MBS11	3.192731872	463.375
MBS13	3.035107497	452.1091
MBS18	2.63632027	503.8723
MBS1	3.819791427	1013.383
MBS2	4.526088138	1002.609
MBS3	3.207834475	1095.5
MBS4	4.2432666	569.4727
MB1	3.165745015	437.0227
MB2	2.643642847	461.6364
MB3	2.616643974	374.7
MB4	3.276870267	438.3846
MB5	3.312069095	397.2778
MB6	3.709287532	317.24

Table S2. Analysis of molecular variance and unifrac analysis of the Marsberg samples

A. Unifrac.weighted

Tree	Groups	UWScore	UWSig
1	L-M	0.953519	0.0970
1	L-W	0.980299	0.0340
1	M-W	0.981865	0.1450

L Leachate M unconsolidated rocks W Spring water

B. AMOVA (Analysis of Molecular Variance)

L-M-W	**Among**	**Within**	**Total**
SS	1.37542	2.93434	4.30976
df	2	11	13
MS	0.687712	0.266758	

Fs: 2.57803

p-value: <0.001*

L-M	**Among**	**Within**	**Total**
SS	0.331903	1.82319	2.15509
df	1	8	9
MS	0.331903	0.227898	

Fs: 1.45637

p-value: 0.077

L-W	**Among**	**Within**	**Total**
SS	0.979114	2.52913	3.50824
df	1	9	10
MS	0.979114	0.281014	

Fs: 3.48422

p-value: 0.002*

M-W	**Among**	**Within**	**Total**
SS	0.738092	1.51637	2.25446
df	1	5	6
MS	0.738092	0.303274	

Fs: 2.43375

p-value: 0.024

Table S3. DESeq2 analysis of leachate, spring water and unconsolidated rocks at Phylum level

	log2FC	**lfcSE**	**Pvalues**	**FDR**
Cyanobacteria	-6.6952	0.95446	2.31E-12	1.13E-10
Deinococcus_Thermus	-25.413	3.84	3.64E-11	8.92E-10
Epsilonbacteraeota	22.588	3.7255	1.34E-09	2.18E-08
Chloroflexi	-4.8682	0.95591	3.53E-07	4.32E-06
Dependentiae	-4.4792	0.99165	6.28E-06	6.15E-05
Firmicutes	5.0962	1.1976	2.09E-05	0.00017
Omnitrophicaeota	7.1101	2.4263	0.003385	0.023694
Crenarchaeota	3.6381	1.3612	0.007525	0.04609
Spirochaetes	2.6811	1.2503	0.032007	0.17426
Verrucomicrobia	3.2298	1.7224	0.060765	0.29775
Chlamydiae	-1.7982	1.0282	0.080303	0.35772
Dadabacteria	5.7891	3.5245	0.10048	0.37546
Patescibacteria	-1.4265	0.87188	0.1018	0.37546
Elusimicrobia	3.5064	2.1809	0.10788	0.37546
Acidobacteria	-1.2842	0.81462	0.11494	0.37546
Proteobacteria	-0.62999	0.40834	0.12288	0.37631