Composition and Niche-Specific Characteristics of Microbial Consortia colonizing a Copper Mine in the Rhenish Massif

Supplementary Information

Sania Arif1, Heiko Nacke2, Elias Schliekmann1, Andreas Reimer3, Gernot Arp3, and Michael Hoppert 1

1Department of General Microbiology, Institute of Microbiology and Genetics,

2Department of Genomic and Applied Microbiology, Institute of Microbiology and Genetics,

3Geoscience Centre, Department of Geobiology, Georg-August-Universität Göttingen, Germany

*Correspondence to sarif@gwdg.de

Figure S1, Figure S2, Figure S3, Figure S4, Figure S5, Figure S6, Figure S7, Table S1, Table S2

Supplementary Information

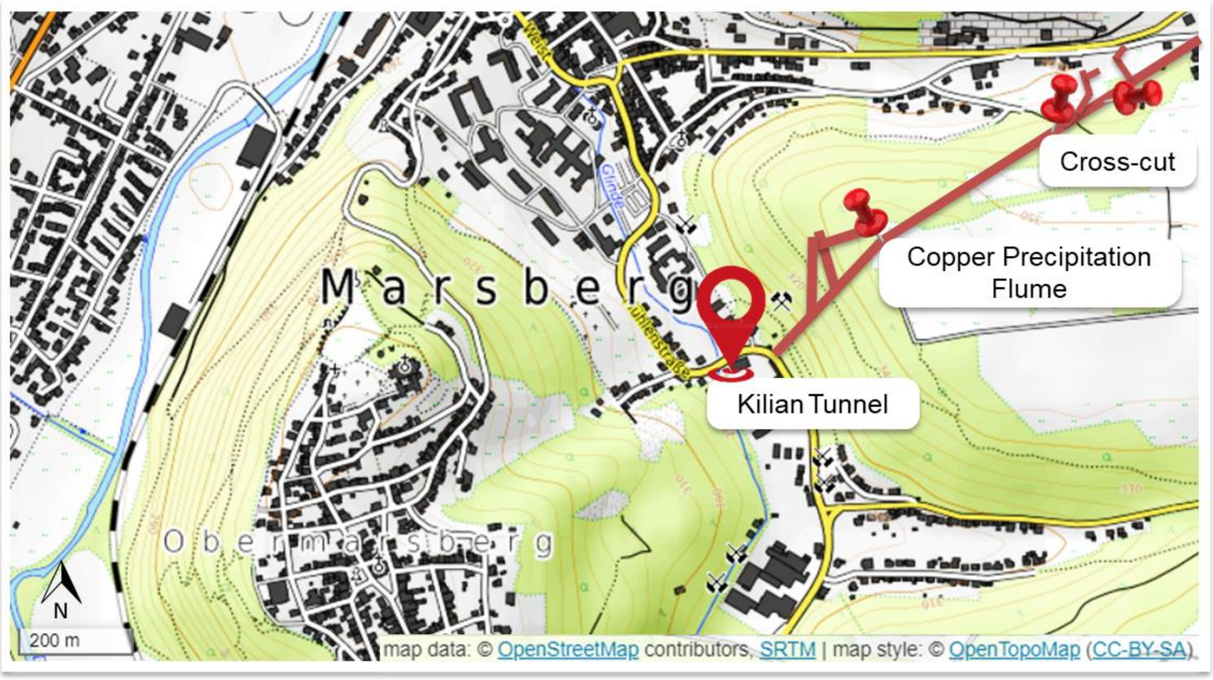

Figure S1. Map of sampling site (Kilianstollen, Marsberg) in the Rhenish Massif mountain range. Reproduced from OpenTopoMap (https://opentopomap.org), under creative common license CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0/legalcode), the map was subsequently modified with the sampling sites (red pushpins) and simplified underground Kilian copper mine adit.

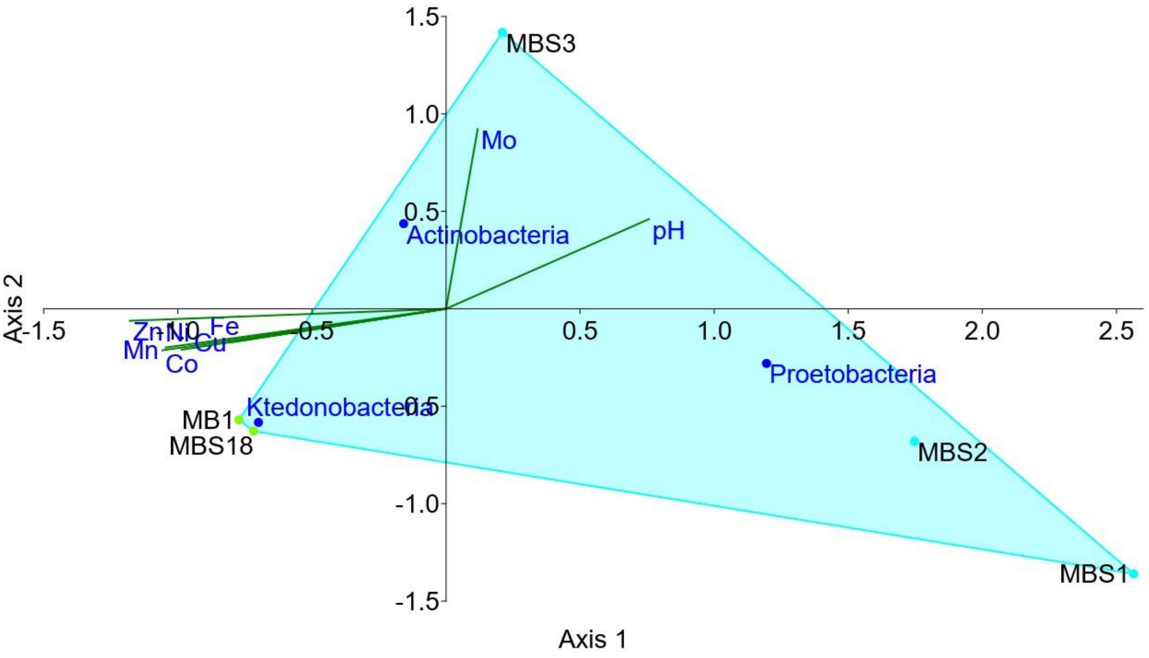

Figure S2. Canonical correspondence analysis of the biological samples collected at the leachate and spring water site. The heavy metals concentrations were taken as the abiotic factors against the abundance of *Proteobacteria*, *Actinobacteria*, and *Ktedonobacteria* for the representative of leachate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

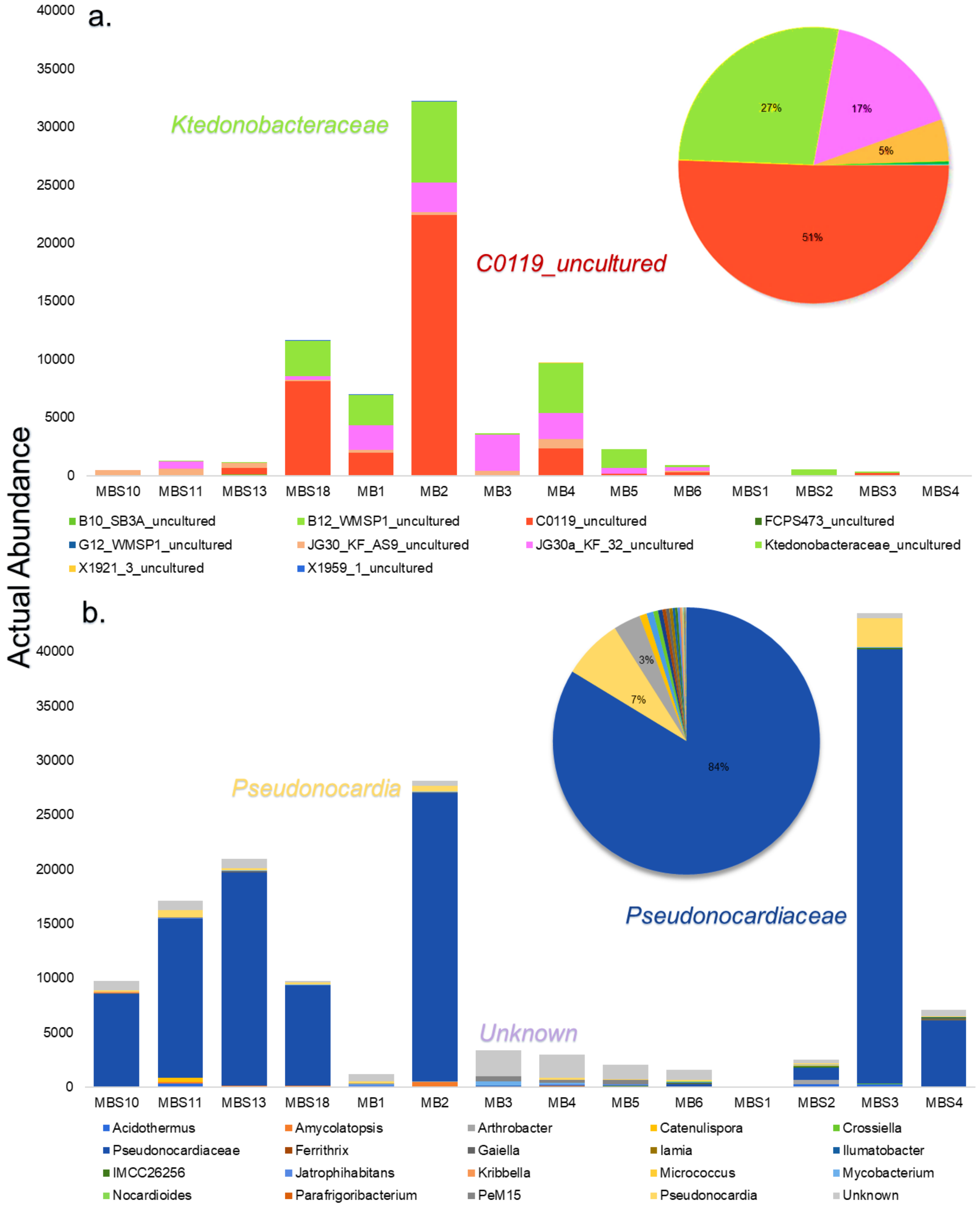

Figure S3. Actual abundance of *Ktedonobacteria* and *Actinobacteria* OTUs at genus level. The actual abundance of OTUs belonging to *Ktedonobacteria* (a) and *Actinobacteria* (b) class were observed at genus level, without any normalization, to analyse the low abundant OTUs. The pie chart insert shows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

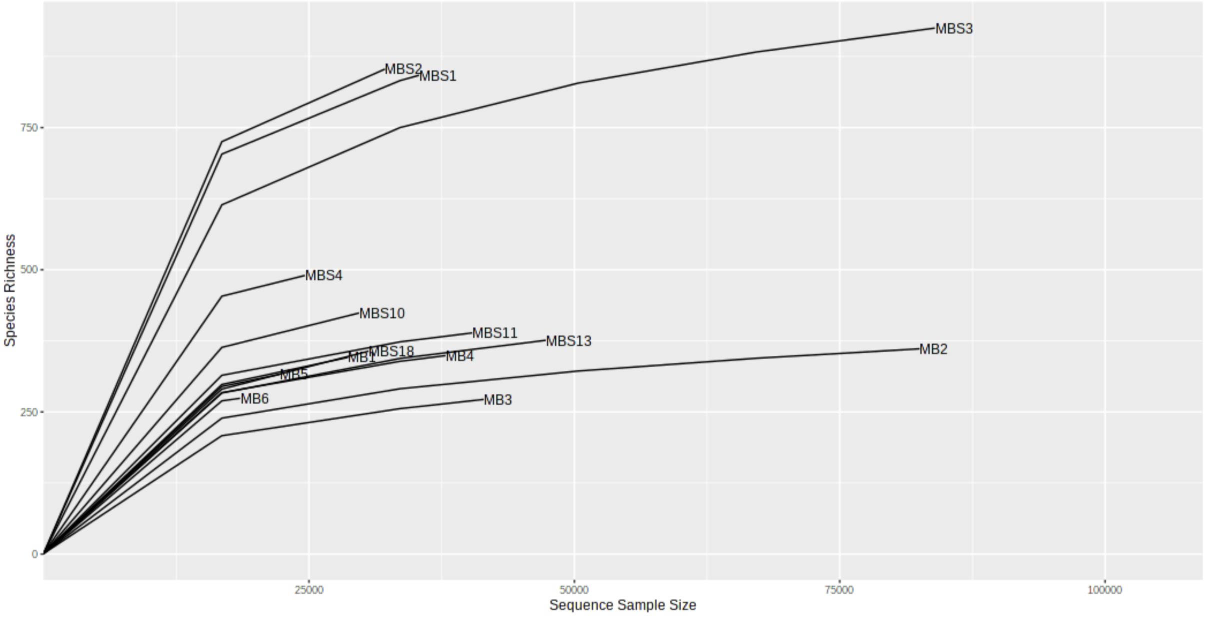

Figure S4. Rarefaction Curve Analysis. The samples are plotted in terms of OTUs observed (species richness) and sampled reads (sequence sample size).

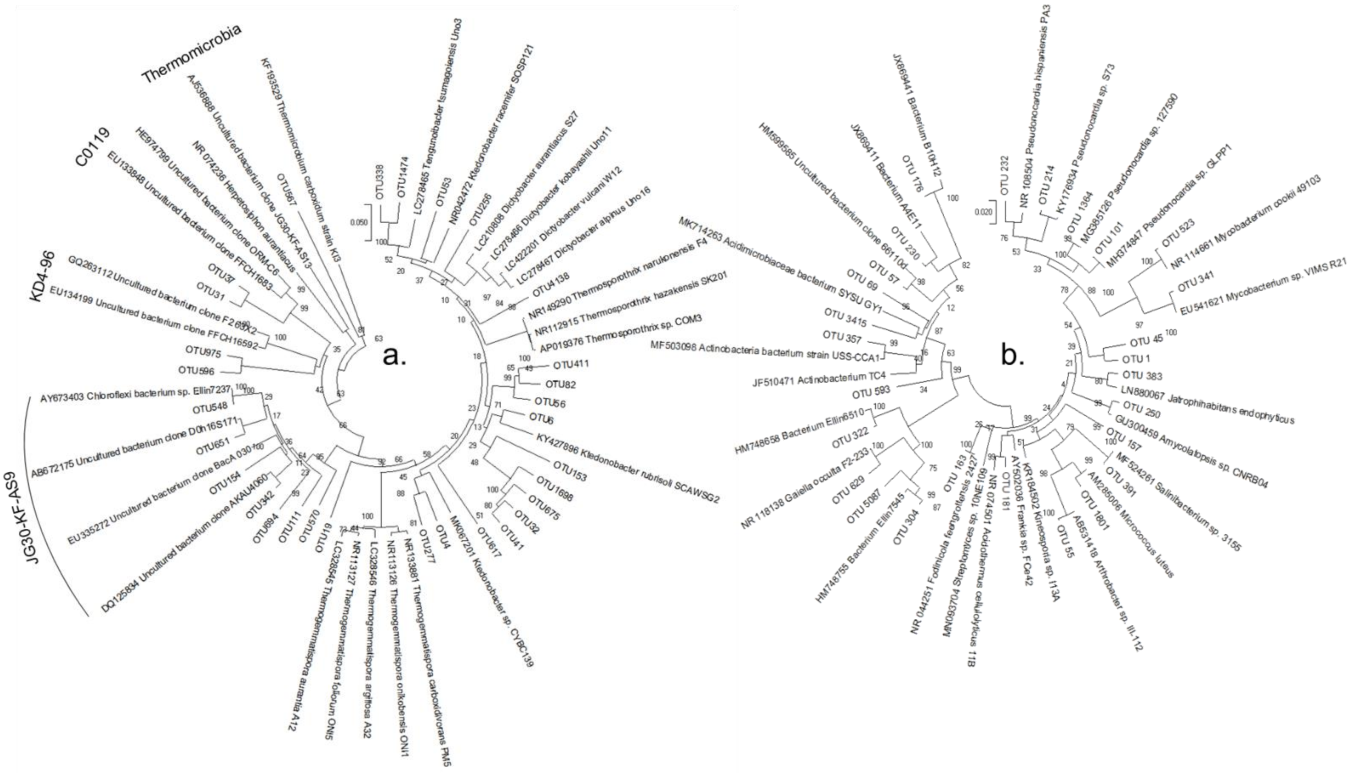

Figure S5. Phylogenetic tree of the small subunit SSU ribosomal RNA genes of OTUs. *Ktedonobacteria* (a) and *Actinobacteria* (b). Maximum-likelihood phylogenetic tree constructed based on V3-V4 amplicon sequences OTUs of *Ktedonobacteria* 16S rRNA gene (a) with the validly published *Ktedonobacteria*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

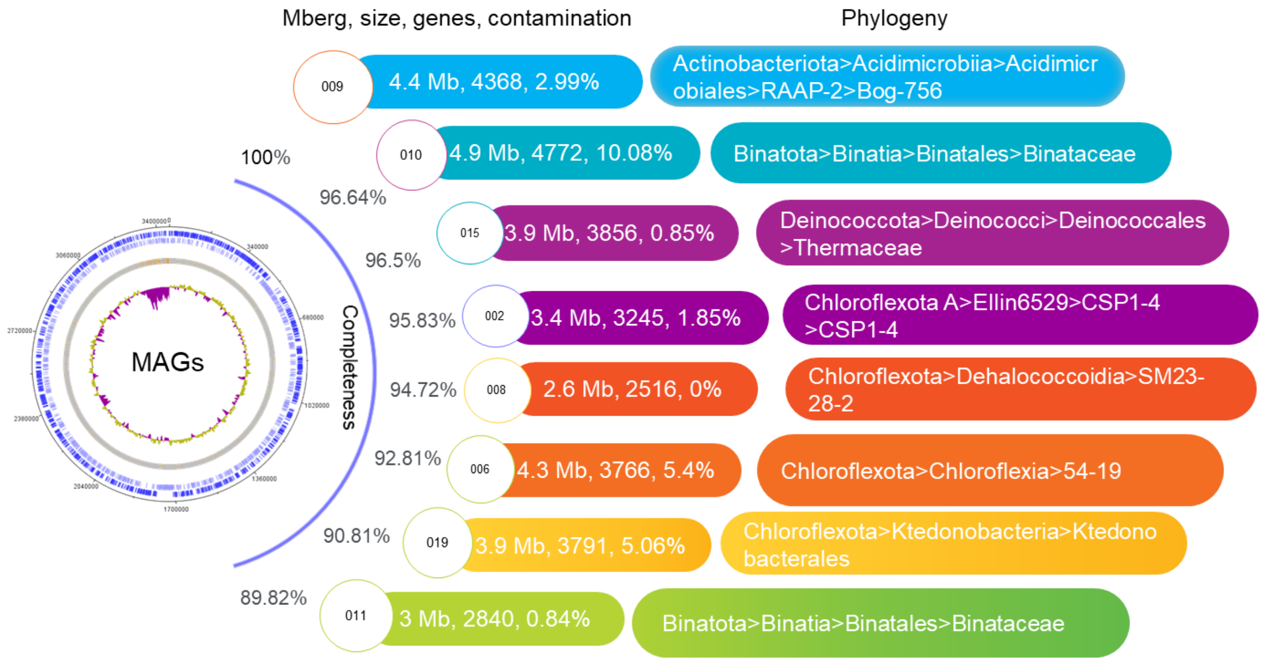

Figure S6. Overview of metagenome-assembled genomes and their key features

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

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

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

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

L Leachate M unconsolidated rocks W Spring water