



Supplemental Material

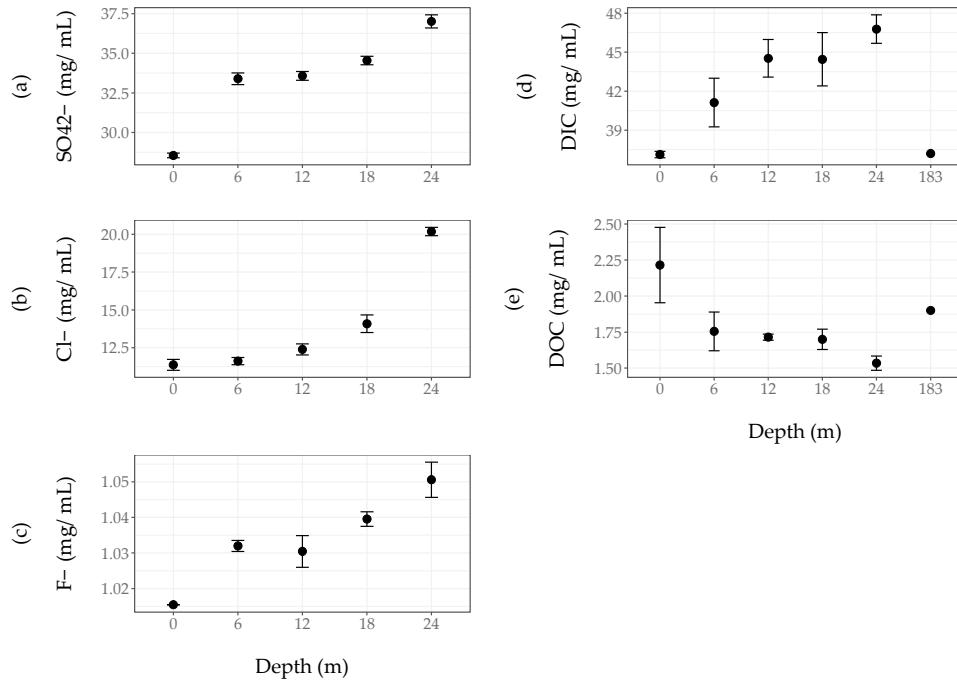


Figure S1: Physicochemical profile of mine water along the depth gradient, SO_4^{2-} (a), Cl^- (b), F^- (c), DIC (d), DOC (e), $n = 2$.

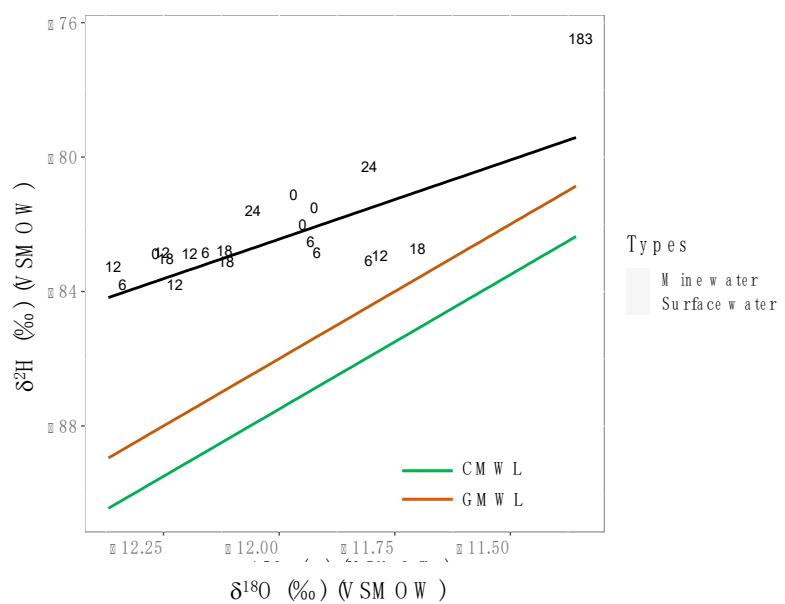


Figure S2. Isotopic composition of Forsyth mine water ($n = 21$). The Global meteoric water line (GMWL) and Canadian meteoric water line (CMWL) derived from the data are also shown. Isotopic values are reported in permil units (‰) against the Vienna Standard Mean Oc. Values are depth in meters.

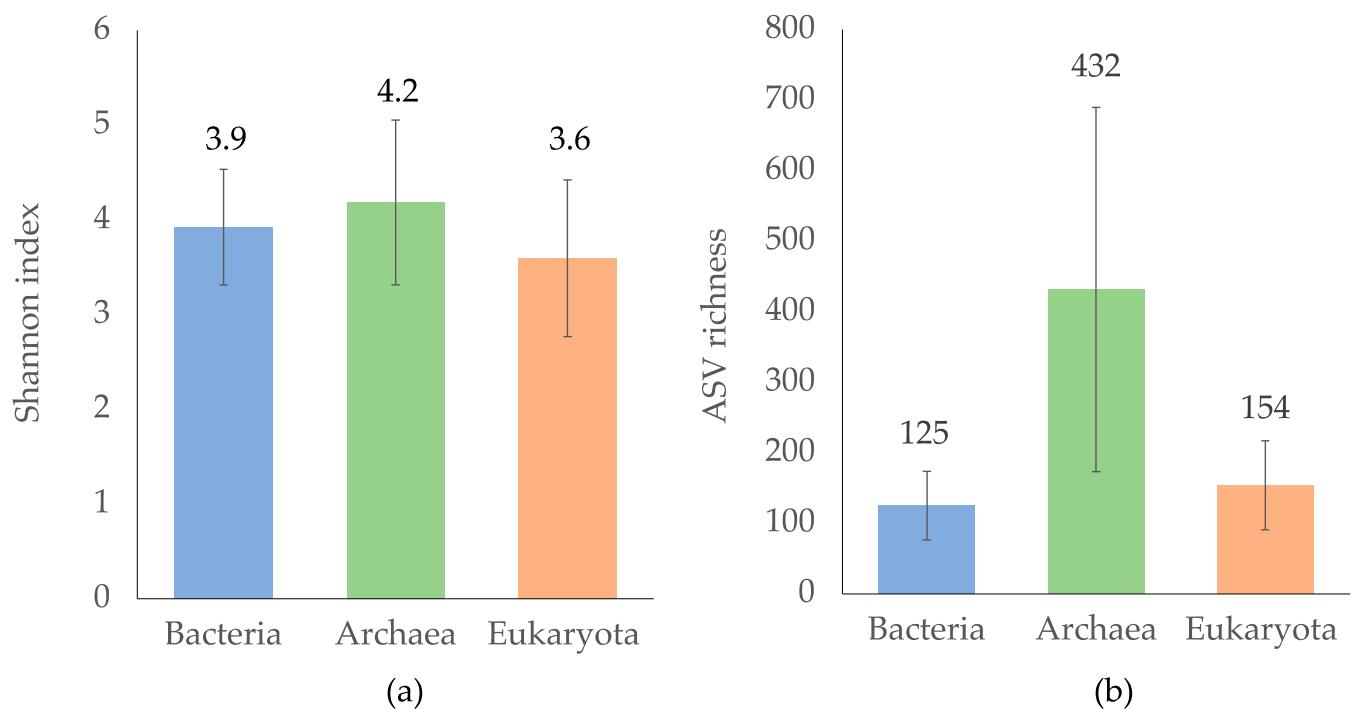


Figure S3. Shannon diversity indices (a), and ASV richness (b) for the three domains (mean \pm std, n= 37, n = 34, n = 40, respectively).

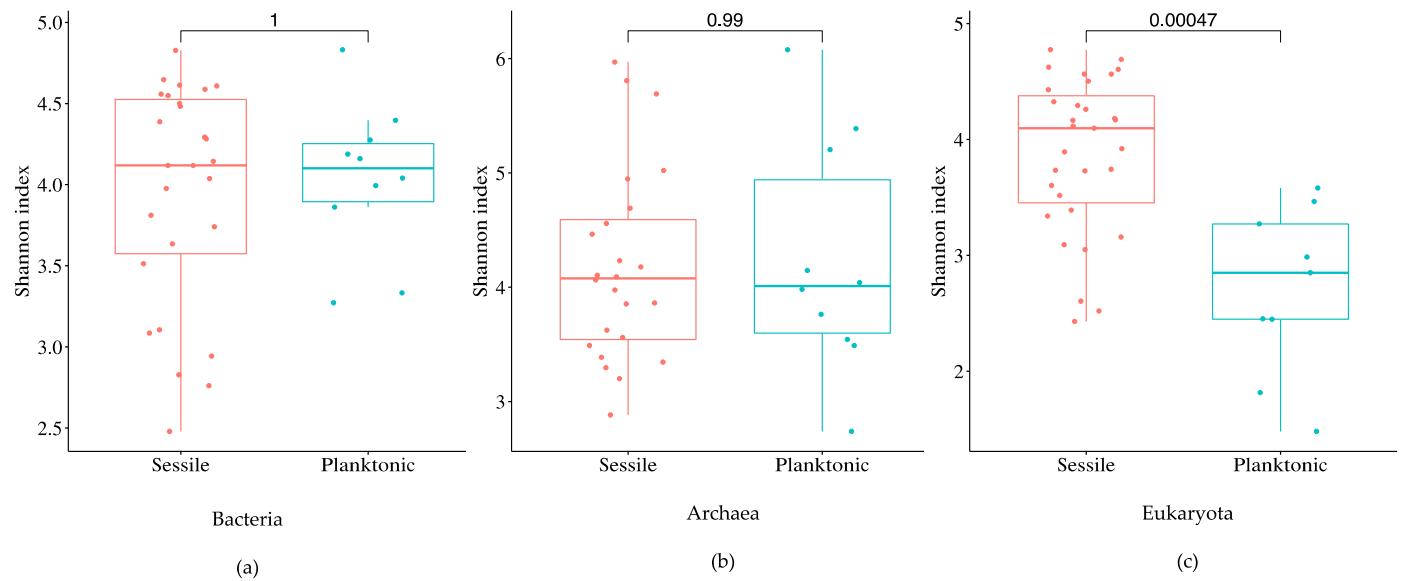


Figure S4. Boxplots showing Shannon index differences between lifestyles for bacteria (a), archaea (b) and eukaryote (c) communities, $n = 37$, $n = 34$, $n = 40$, respectively. Significant differences $p < 0.05$.

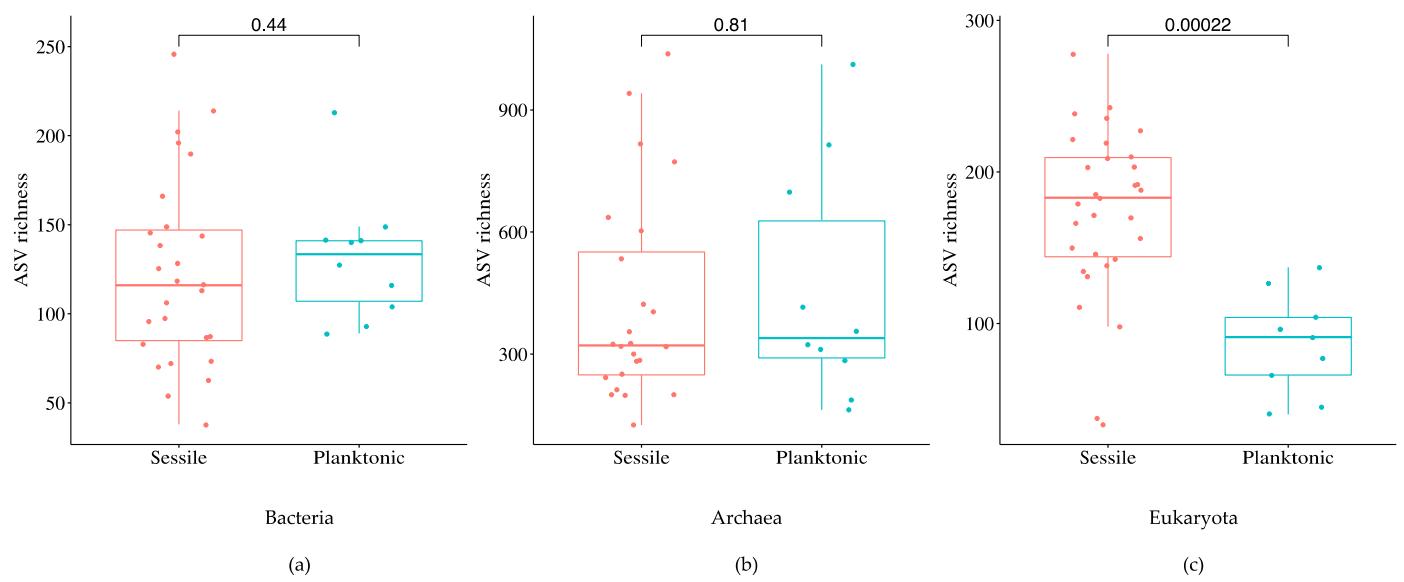


Figure S5. Boxplots showing ASV richness differences between lifestyles for bacteria (a), archaea (b) and eukaryote (c) communities, $n = 37$, $n = 34$, $n = 40$, respectively. Significant differences $p < 0.05$.

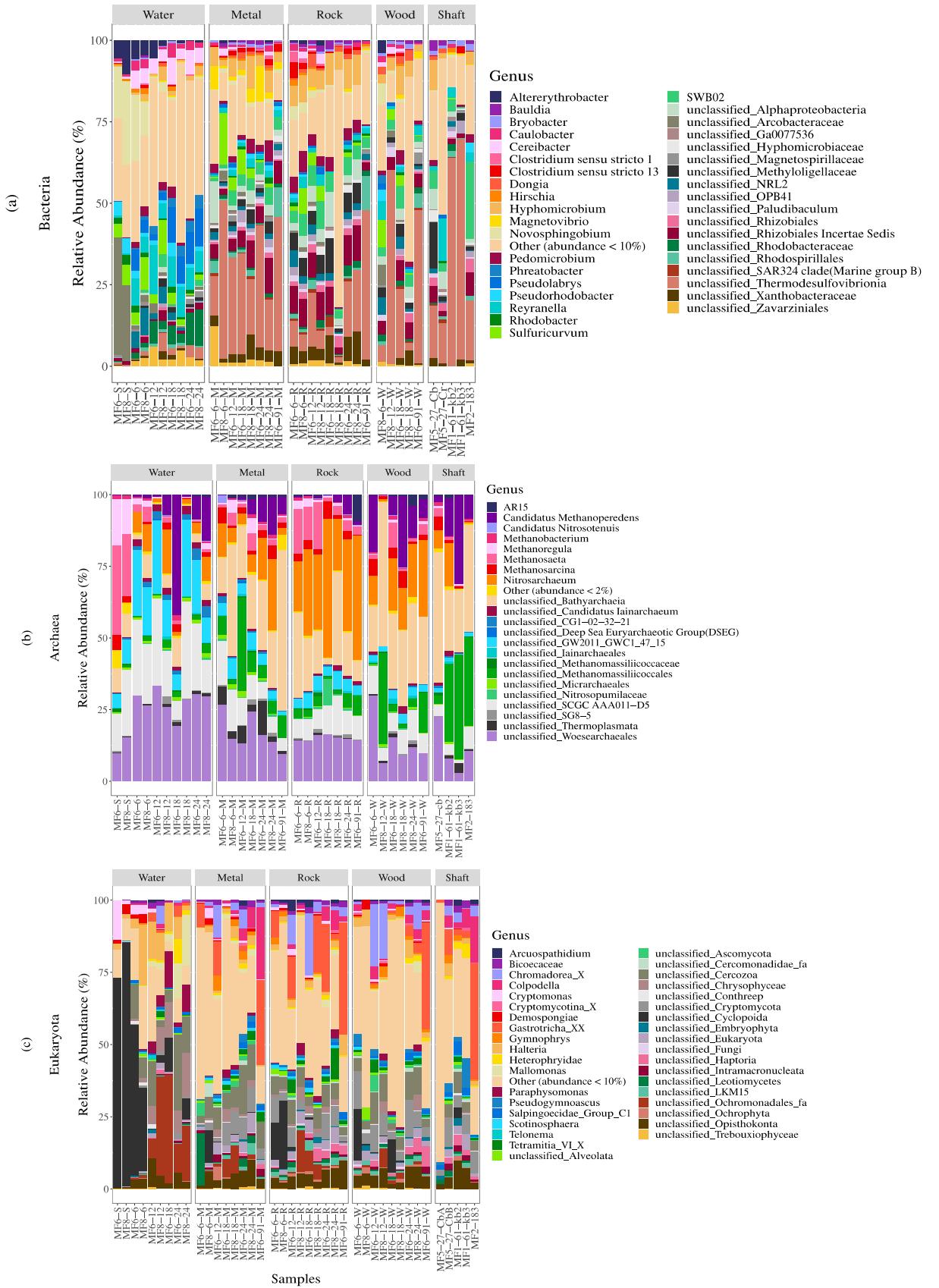


Figure S6. Relative abundance (% of total reads) for bacteria (a), archaea (b) and eukaryote (c) communities, at the genus level.

Table S1. PCR conditions for the amplification of the 16S/18S rRNA genes.

	Amorces	Tm °C	nº cycles
Bacteria	B341F B785R	57	35
Archaea	A340F A915R	67	35
Eukaryota	E960F E1438R	61	35

Table S2. Spearman correlation between depth and all physicochemical parameters (n = 2).

Environmental variables	Depth	
	r	p
DOC	0.036	0.964
DIC	0.214	0.661
SO ₄ ²⁻	1	0.017
Cl ⁻	1	0.017
F ⁻	0.900	0.083

Table S3. Spearman correlation between alpha diversity indices and depth or all physicochemical parameters, for bacterial, archaeal, and eukaryote communities.

Environmental variables	Bacteria				Archaea				Eukaryota			
	Shannon index		ASV richness		Shannon index		ASV richness		Shannon index		ASV richness	
	r	p	r	p	r	p	r	p	r	p	r	p
Depth	0.143	0.676	-0.713	0.014	0.119	0.727	0.440	0.1752	0.423	0.223	0.534	0.112
DOC	-0.509	0.114	0.273	0.418	-0.173	0.614	-0.118	0.7343	-0.418	0.232	-0.249	0.492
DIC	0.409	0.214	-0.309	0.356	0.255	0.451	0.045	0.9029	0.273	0.448	0.042	0.919
SO ₄ ²⁻	0.430	0.218	-0.430	0.218	-0.164	0.657	-0.176	0.632	0.467	0.213	0.500	0.178
Cl ⁻	0.297	0.407	-0.479	0.166	-0.224	0.537	-0.309	0.3871	0.467	0.2123	0.333	0.385
F ⁻	0.467	0.178	-0.442	0.204	-0.079	0.838	-0.042	0.9186	0.350	0.359	0.550	0.133

Table S4. Permutation homogeneity tests between lifestyle, substrate type and depth gradient.

	Bacteria	Archaea	Eukaryota
	<i>p</i>		
Depth	0.001	0.001	0.001
Lifestyle	0.088	0.87	0.126
Substrates	8.341	0.454	0.756