Supplement of

Beyond saturation zones: localized hydrobiogeochemical heterogeneity shapes microbial functional profiles across an arid critical zone

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Table S1. Total borehole depth, mean depth to the groundwater table (2019–2024), number of substrate samples analysed per borehole, and sampling depths. Substrate samples were generally collected at 5 m intervals, except for the deepest samples from boreholes 1 and 4. DTGW values are presented as mean ± SD.

Borehole	Perforation depth (m)	Water table (m) (Mean ± SD)	Number of samples	Sampling depth (m) by each 5 m
1	27	2.2 ± 0.1	7	1 - 27
2	33	7.2 ± 0.1	4	1 - 15
3	30	8.6 ± 0.1	6	1 - 25
4	33	11.5 ± 0.1	7	1 - 27
5	36	13.9 ± 0.1	8	1 - 35
6	38	19.2 ± 0.1	8	1 - 35
7	51	24.9 ± 0.1	9	1 - 40
8	117	25.2 ± 0.1	9	1 - 40

Table S2. Classification of the 31 Biolog EcoPlate™ carbon sources into the six functional categories used in the carbon-source-group analyses. Letters (A–H) denote plate rows and numbers (1–12) denote plate columns. Columns are arranged into three technical replicate blocks (1–4, 5–8, and 9–12). Wells A1, A5, and A9 correspond to the water controls. Each substrate appears once in each block, providing three technical replicates per compound within the same microplate.

Carbon source	Functional category	EcoPlate well position
Pyruvic Acid Methyl Ester	Carboxylic acids	A2 / A6 / A10
D-Malic Acid	Carboxylic acids	A3 / A7 / A11
Itaconic Acid	Carboxylic acids	A4 / A8 / A12
γ-Hydroxybutyric Acid	Carboxylic acids	B1 / B5 / B9
α-Ketobutyric Acid	Carboxylic acids	B2 / B6 / B10
D-Galactonic Acid γ-Lactone	Carboxylic acids	B3 / B7 / B11
D-Malic Acid	Carboxylic acids	B4 / B8 / B12
2-Hydroxy Benzoic Acid	Phenolic compounds	C1 / C5 / C9
4-Hydroxy Benzoic Acid	Phenolic compounds	C2 / C6 / C10
α-Cyclodextrin	Polymers	C3 / C7 / C11
Glycogen	Polymers	C4 / C8 / C12
Tween 40	Polymers	D1 / D5 / D9
Tween 80	Polymers	D2 / D6 / D10
D-Cellobiose	Carbohydrates	D3 / D7 / D11
α-D-Lactose	Carbohydrates	D4 / D8 / D12
β-Methyl-D-Glucoside	Carbohydrates	E1 / E5 / E9
D-Xylose	Carbohydrates	E2 / E6 / E10
i-Erythritol	Carbohydrates	E3 / E7 / E11
D-Mannitol	Carbohydrates	E4 / E8 / E12
N-Acetyl-D-Glucosamine	Carbohydrates	F1 / F5 / F9
D-Glucosaminic Acid	Carbohydrates	F2 / F6 / F10
Glucose-1-Phosphate	Carbohydrates	F3 / F7 / F11
D,L-α-Glycerol Phosphate	Carbohydrates	F4 / F8 / F12
L-Arginine	Amino acids	G1 / G5 / G9
L-Asparagine	Amino acids	G2 / G6 / G10
L-Phenylalanine	Amino acids	G3 / G7 / G11
L-Serine	Amino acids	G4 / G8 / G12
L-Threonine	Amino acids	H1 / H5 / H9
Glycyl-L-Glutamic Acid	Amino acids	H2 / H6 / H10
Phenylethyl-amine	Amines	H3 / H7 / H11
Putrescine	Amines	H4 / H8 / H12

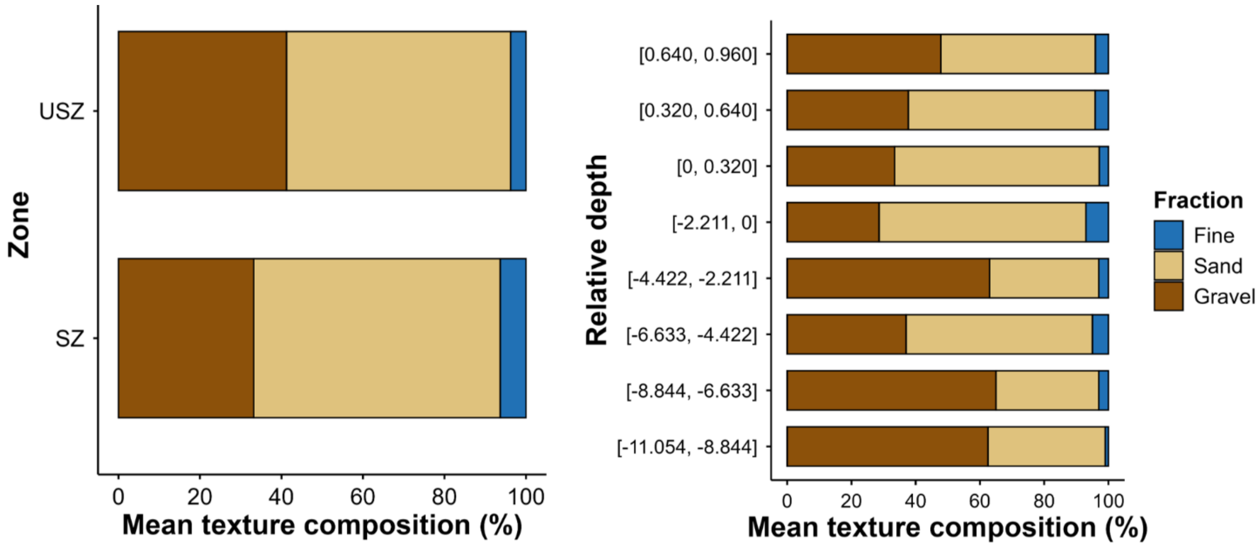

Figure S1. Mean particle-size composition of subsurface materials in the unsaturated (USZ) and saturated (SZ) zones and across relative-depth intervals referenced to the groundwater table.

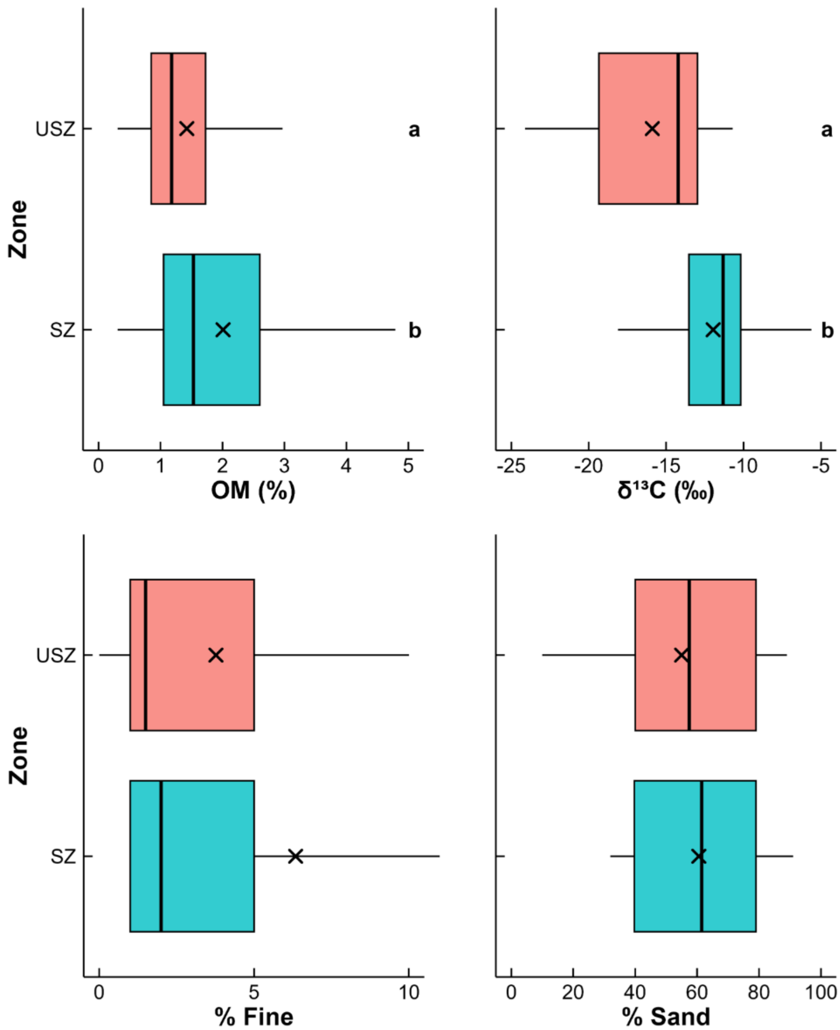

Figure S2. Box-and-whisker plots of geological substrate properties, including organic matter content (OM, %), $\delta^{13}C$ values (‰), and sand and fine fractions (%Sand and %Fine), in the unsaturated (USZ) and saturated (SZ) zones. Boxes represent the interquartile range (IQR), with the median shown by the central line, the mean marked by a cross, and whiskers extending to 1.5 × IQR. Different letters, where present, denote significant differences between hydrological zones according to Wilcoxon rank-sum tests ($p < 0.05$).

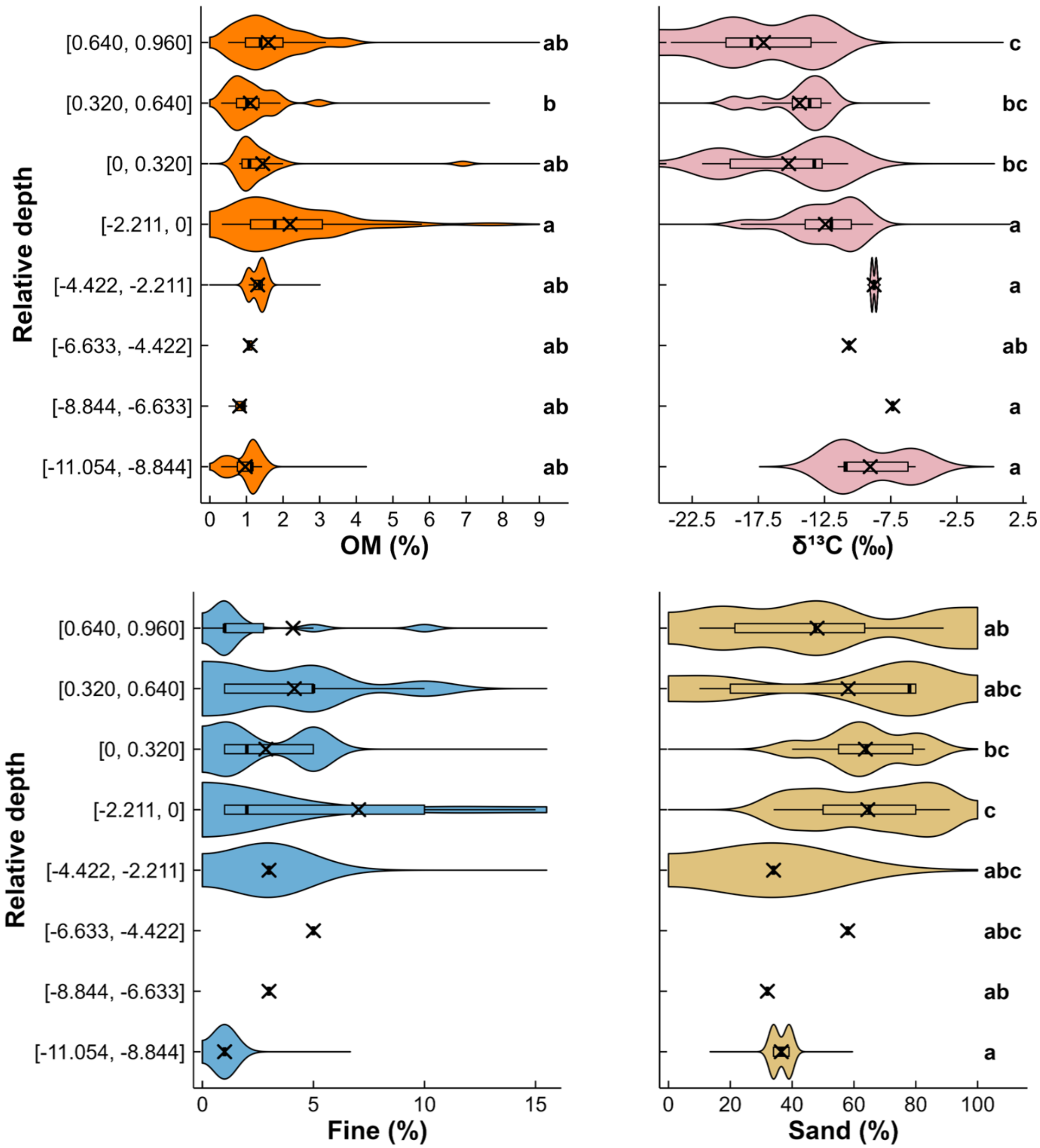

Figure S3. Violin plots showing the distribution of geological substrate properties, including organic matter content (OM, %), $\delta^{13}C$ values (‰), and sand and fine fractions (%Sand and %Fine), across depth intervals. Violin widths reflect the kernel density distribution of the data. Overlaid boxplots represent the interquartile range (IQR), with the median shown by the central line, the mean marked by a cross, and whiskers extending to 1.5 × IQR. Different letters, where present, denote significant differences among depth intervals according to Kruskal-Wallis tests followed by Dunn's *post hoc* tests ($p < 0.05$).

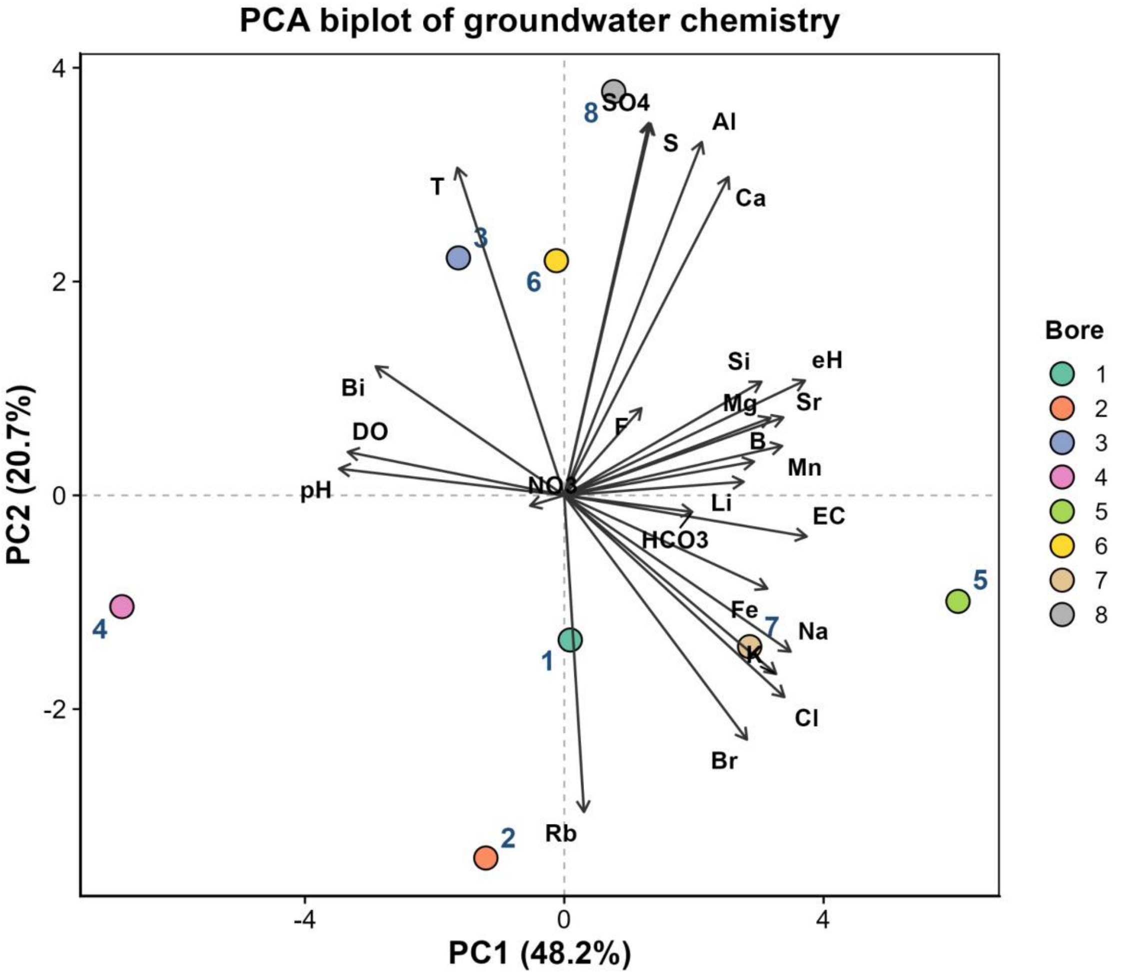

Figure S4. Principal component analysis (PCA) of groundwater physicochemical variables measured in the eight boreholes included in the study. Points represent boreholes, and arrows indicate the direction and relative contribution of each variable to the ordination. PCA1 and PCA2 explained 48.2% and 20.7% of the total variance, respectively (68.9% cumulative variance explained). PCA1 mainly represented a gradient of groundwater mineralization and hydrogeochemical evolution, whereas PCA2 reflected secondary variation associated primarily with sulphate chemistry and groundwater temperature.