Supplementary Material

Stability of floodplain subsurface microbial communities through seasonal hydrological and geochemical cycles

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1 Supplementary Data

2 Supplementary Figures and Tables

2.1 Supplementary Tables

Table S1. Summary of sample data collected at the Riverton, WY, site during the 2017 field season. See included Excel file: "TableS1_Sample_Data.xlsx".

Table S2. Summary of taxonomic data, including OTUs, from sequencing of sediment samples collected during 2017. See included Excel file: "TableS2_OTU_Data.xlsx".

Table S3. Analysis of molecular variance (AMOVA) and homogeneity of molecular variance (HOMOVA) of microbial communities grouped by Soil Horizon (Depth)

AMOVA (Community Difference) - Soil Horizon

Topsoil	April	May	July 7	July 25	Aug	Sept
April						
May	0.221					
July 7	0.393	1				
July 25	0.183	1	1			
Aug	0.584	1	0.66	1		
Sept	0.264	1	1	1	0.67	

Evaporite	April	May	July 7	July 25	Aug	Sept
April						
May	0.128					
July 7	0.108	0.597				
July 25	0.18	0.84	0.532			
Aug	0.202	0.48	0.524	0.707		
Sept	0.09	0.229	0.517	0.41	0.71	

Sand	April	May	July 7	July 25	Aug	Sept
April						
May	0.069					
July 7	0.205	0.347				
July 25	0.194	0.31	1			
Aug	0.133	0.103	0.243	0.274		
Sept	0.137	0.318	0.343	0.341	0.389	

EvapClay	April	May	July 7	July 25	Aug	Sept
April						
May	0.066					
July 7	0.091	0.364				
July 25	0.017	0.2	0.264			
Aug	0.015	0.18	0.194	0.276		
Sept	0.079	0.323	0.358	0.208	0.621	

TRZ	April	May	July 7	July 25	Aug	Sept
April						
May	0.05					
July 7	0.012	0.081				
July 25	0.012	0.338	0.332			
Aug	0.01	0.002	0.042	0.012		
Sept	0.002	0.153	0.047	0.256	0.079	

Clay	April	May	July 7	July 25	Aug	Sept
April						
May	0.245					
July 7						
July 25	0.237	1				
Aug	0.231	1		1		
Sept	0.249	1		1	1	

Aquifer	April	May	July 7	July 25	Aug	Sept
April						
May	0.789					
July 7	0.217	1				
July 25	0.803	1	1			
Aug	0.113	0.201	0.179	0.414		
Sept	0.046	0.58	1	0.215	0.407	

HOMOVA (Community Stability) - Soil Horizon

Topsoil	April	May	July 7	July 25	Aug	Sept
April						
May	<0.001					
July 7	0.412	<0.001				
July 25	<0.001	<0.001	<0.001			
Aug	<0.001	<0.001	<0.001	<0.001		
Sept	0.469	<0.001	1	<0.001	<0.001	

Evaporite	April	May	July 7	July 25	Aug	Sept
April						
May	0.236					
July 7	0.66	0.259				
July 25	0.611	0.036	0.673			
Aug	0.357	0.173	0.89	0.922		
Sept	0.873	0.731	0.437	0.731	0.609	

Sand	April	May	July 7	July 25	Aug	Sept
April						
May	0.134					
July 7	<0.001	<0.001				
July 25	<0.001	<0.001	<0.001			
Aug	0.937	0.091	<0.001	<0.001		
Sept	0.942	0.335	<0.001	<0.001	0.888	

EvapClay	April	May	July 7	July 25	Aug	Sept
April						
May	0.117					
July 7	<0.001	<0.001				
July 25	0.292	0.2	<0.001			
Aug	0.097	0.011	<0.001	0.919		
Sept	0.907	0.323	<0.001	0.3	0.609	

TRZ	April	May	July 7	July 25	Aug	Sept
April						
May	0.558					
July 7	0.966	0.656				
July 25	0.151	0.182	0.339			
Aug	0.025	0.01	0.755	0.231		
Sept	0.253	0.417	0.318	0.095	0.044	

Clay	April	May	July 7	July 25	Aug	Sept
April						
May	<0.001					
July 7						
July 25	<0.001	<0.001				
Aug	<0.001	<0.001		<0.001		
Sept	<0.001	<0.001		<0.001	<0.001	

Aquifer	April	May	July 7	July 25	Aug	Sept
April						
May	<0.001					
July 7	<0.001	<0.001				
July 25	<0.001	<0.001	<0.001			
Aug	0.028	<0.001	<0.001	<0.001		
Sept	0.605	<0.001	<0.001	<0.001	0.674	

$p < 0.01$	$p < 0.05$	$p > 0.05$	no data

Table S4. Analysis of molecular variance (AMOVA) and homogeneity of molecular variance (HOMOVA) of microbial communities grouped by Sample Month

AMOVA (Community Difference) - Sample Month

April	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	0.007						
Sand	<0.001	0.003					
EvapClay	<0.001	<0.001	0.001				
TRZ	0.002	0.001	0.001	<0.001			
Clay	0.003	0.008	0.028	0.007	0.021		
Aquifer	0.019	0.005	0.025	<0.001	0.003	0.178	

May	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	0.163						
Sand	0.353	0.076					
EvapClay	0.363	0.065	0.331				
TRZ	0.14	0.007	0.025	0.131			
Clay	1	0.192	0.324	0.35	0.143		
Aquifer	1	0.2	0.351	0.358	0.145	1	

July 7	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	0.337						
Sand	0.671	0.689					
EvapClay	0.337	0.32	1				
TRZ	0.325	0.312	0.312	0.678			
Clay							
Aquifer	0.325	0.333	1	1	0.348		

July 25	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	0.234						
Sand	1	0.189					
EvapClay	0.025	0.32	0.276				
TRZ	0.335	0.055	0.341	0.19			
Clay	1	0.236	1	0.47	0.354		
Aquifer	1	0.176	1	0.515	0.337	1	

Aug	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	0.045						
Sand	0.244	0.01					
EvapClay	0.176	0.016	0.043				
TRZ	0.03	0.002	0.005	0.01			
Clay	1	0.039	0.246	0.201	0.027		
Aquifer	0.202	0.022	0.032	0.023	0.004	0.197	

Sept	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	0.799						
Sand	0.333	0.183					
EvapClay	0.33	0.105	0.332				
TRZ	0.023	0.004	0.023	0.046			
Clay	0.323	0.238	0.347	0.334	0.109		
Aquifer	0.066	0.023	0.064	0.073	0.003	0.806	

HOMOVA (Community Stability) - Sample Month

April	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	0.923						
Sand	0.028	<0.001					
EvapClay	0.24	<0.001	0.031				
TRZ	0.684	0.161	0.013	0.117			
Clay	0.024	0.052	0.744	0.136	0.041		
Aquifer	0.031	0.097	0.002	0.042	0.118	0.03	

May	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	<0.001						
Sand	<0.001	0.013					
EvapClay	<0.001	0.117	0.36				
TRZ	<0.001	0.449	0.043	0.091			
Clay	<0.001	<0.001	<0.001	<0.001	<0.001		
Aquifer	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

July 7	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	1						
Sand	<0.001	<0.001					
EvapClay	<0.001	<0.001	<0.001				
TRZ	0.325	0.351	<0.001	<0.001			
Clay							
Aquifer	<0.001	<0.001	<0.001	<0.001	<0.001		

July 25	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	<0.001						
Sand	<0.001	<0.001					
EvapClay	<0.001	0.649	<0.001				
TRZ	<0.001	0.061	<0.001	0.331			
Clay	<0.001	<0.001	<0.001	<0.001	<0.001		
Aquifer	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Aug	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	<0.001						
Sand	<0.001	0.102					
EvapClay	<0.001	0.572	0.025				
TRZ	<0.001	0.173	0.229	0.078			
Clay	<0.001	<0.001	<0.001	<0.001	<0.001		
Aquifer	<0.001	0.024	0.025	0.023	0.023	<0.001	

Sept	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil							
Evaporite	0.582						
Sand	0.329	0.107					
EvapClay	0.33	0.182	0.332				
TRZ	0.77	0.558	0.107	0.32			
Clay	<0.001	<0.001	<0.001	<0.001	<0.001		
Aquifer	0.923	0.036	0.132	0.184	0.225	<0.001	

p < 0.01	p < 0.05	p > 0.05	no data

Table S5. Analysis of molecular variance (AMOVA) and homogeneity of molecular variance (HOMOVA) of microbial communities in July 7 versus July 25 samples – a period of rapid change in soil moisture at the site, particularly in the upper layers (post-flood into onset of summer drought).

	AMOVA: July 7 (rows) versus July 25 (columns)						
	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil	1	0.161	1	0.103	0.308	0.336	0.339
Evaporite	0.326	0.532	0.363	0.203	0.335	0.315	0.34
Sand	1	0.209	1	0.498	0.343	1	1
EvapClay	1	0.201	1	0.264	0.319	1	1
TRZ	0.323	0.062	0.293	0.179	0.332	0.342	0.346
Clay							
Aquifer	1	0.197	1	0.215	0.338	1	1

	HOMOVA: July 7 (rows) versus July 25 (columns)						
	Topsoil	Evaporite	Sand	EvapClay	TRZ	Clay	Aquifer
Topsoil	<0.001	0.661	<0.001	0.684	0.343	<0.001	<0.001
Evaporite	<0.001	0.673	<0.001	0.591	0.33	<0.001	<0.001
Sand	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EvapClay	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TRZ	<0.001	0.411	<0.001	0.789	0.339	<0.001	<0.001
Clay							
Aquifer	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

p < 0.01	p < 0.05	p > 0.05	no data

Table S6. Linear discriminant analysis (LDA) effect size (LEfSe) testing of significantly different OTUs between samples categorized by soil type or sample month. A significant p-value (<0.05) indicates an OTU with elevated abundance in the specified sample category. NS = not significant. Results for the Top 30 OTUs are shown for brevity; for the taxonomic information of each OTU, see Table S2.

OTU	Soil Layer	LDA	p-value	Month	LDA	p-value
Otu00001	TRZ	4.099	<0.0001	-	NS	NS
Otu00002	TRZ	4.161	<0.0001	-	NS	NS
Otu00003	aquifer	4.340	<0.0001	-	NS	NS
Otu00004	aquifer	4.135	<0.0001	-	NS	NS
Otu00005	aquifer	3.836	<0.0001	-	NS	NS
Otu00006	aquifer	3.614	0.022	-	NS	NS
Otu00007	TRZ	3.727	<0.0001	-	NS	NS
Otu00008	evaporite	4.145	<0.0001	April	3.767	0.005
Otu00009	sand	3.750	<0.0001	-	NS	NS
Otu00010	clay	3.766	<0.0001	August	3.659	0.042
Otu00011	evapclay	3.515	<0.0001	April	3.397	0.014
Otu00012	clay	3.717	<0.0001	-	NS	NS
Otu00013	evapclay	3.725	<0.0001	-	NS	NS
Otu00014	clay	3.712	<0.0001	-	NS	NS
Otu00015	evaporite	3.621	<0.0001	-	NS	NS
Otu00016	topsoil	3.923	0	-	NS	NS
Otu00017	topsoil	3.704	<0.0001	-	NS	NS
Otu00018	evaporite	3.968	<0.0001	-	NS	NS
Otu00019	evapclay	3.600	<0.0001	-	NS	NS
Otu00020	evaporite	3.579	<0.0001	April	3.376	0.028
Otu00021	evaporite	4.029	<0.0001	-	NS	NS
Otu00022	topsoil	3.227	<0.0001	-	NS	NS
Otu00023	clay	3.751	<0.0001	-	NS	NS
Otu00024	sand	3.768	<0.0001	-	NS	NS
Otu00025	clay	2.379	<0.0001	-	NS	NS
Otu00026	sand	3.379	<0.0001	-	NS	NS
Otu00027	evaporite	3.739	<0.0001	-	NS	NS
Otu00028	clay	3.191	<0.0001	-	NS	NS
Otu00029	evaporite	3.412	<0.0001	-	NS	NS
Otu00030	sand	3.538	<0.0001	May	3.740	0.001

2.2 Supplementary Figures

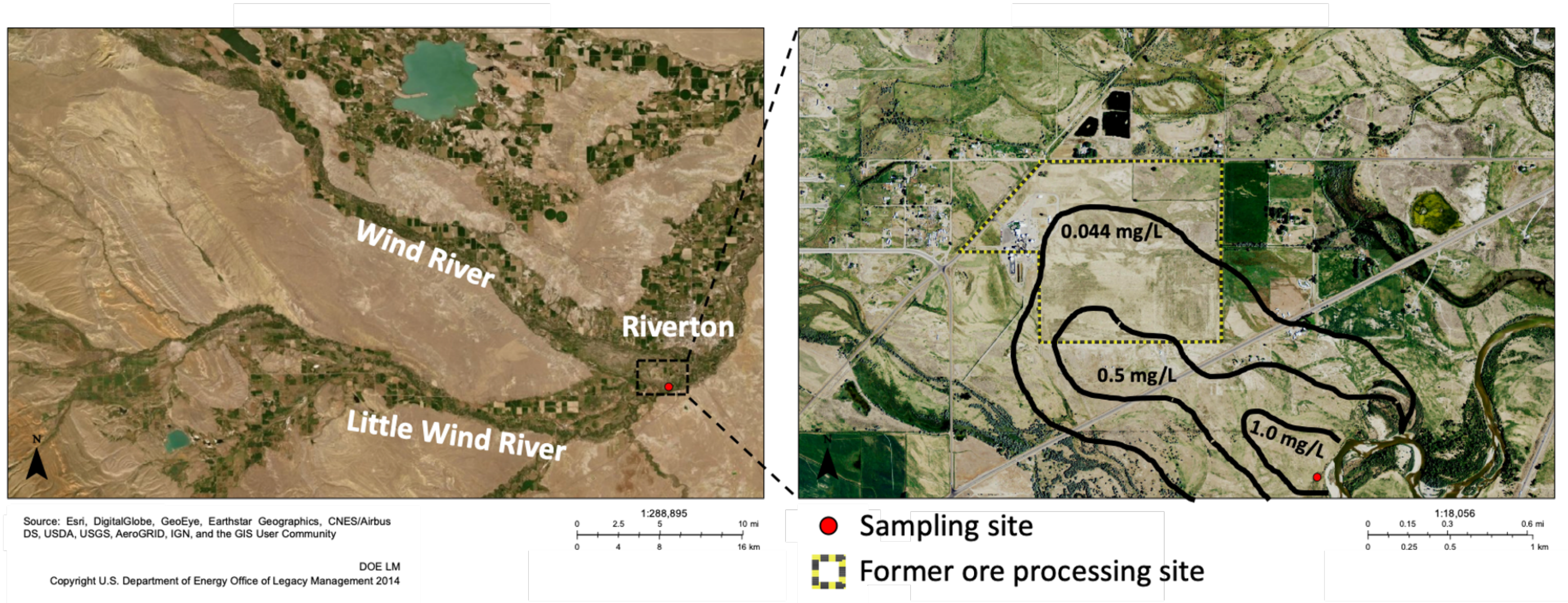
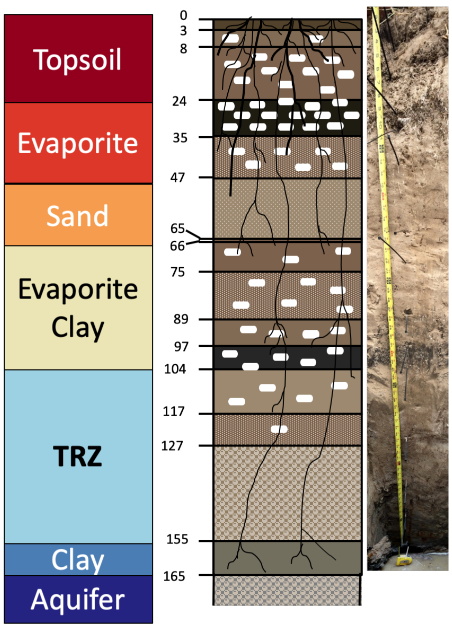


Figure S1. Riverton, WY, sample site (42° 59' 19.1" N, 108° 23' 58.6" W) in the Wind River-Little Wind River floodplain, including the former U ore processing site (inset) and resulting groundwater plume of U (indicated by black contour lines, with U concentration in mg/L).



Horizon	Depth (cm)	Color	Texture	Roots	Moisture	Structure	Evaporites (white)	Comments
O	0-3	na	na	mostly roots	dry	na	none	
A1	3-8	10YR 4/2 dark grayish brown	clay	very abundant	very slightly moist	granular	few	
A2	8-24	10YR 4/3 brown	clay	abundant fine, some coarse	slightly moist	subangular blocky	very abundant	
A3	24-35	10YR 2/1, black (btw evaporites)	clay	relatively abundant fine, few coarse	slightly moist	subangular blocky	extremely abundant	few large wood fragments
A4	35-47	10YR 6/3 pale brown (dry), 10YR 4/3 brown (wet)	silty clay (35% clay)	some fine, very few coarse	slightly moist	subangular blocky	abundant	
B1	47-65	10YR 5/3 dark brown	sandy loam	some fine, very few coarse	dry	massive	none	few iron oxides
B2	65-66	10YR 4/3 brown	sandy loam	very few	dry	massive	none	some iron oxides
B3	66-75		clay				some	
B4	75-89	10YR 4/3 brown	silty clay	very few	very slightly moist	massive	relatively abundant	relatively abundant iron oxides
B5	89-97	10YR 4/2 dark grayish brown	clay	some	moist		abundant	redoxymorphic features
B6	97-104	Gley1 2/5 black	clay	few very fine/fine	moist		abundant	char (not sulfide)
B7	104-117	10YR 5/3 dark brown	clay	very few	moist		relatively abundant	few iron oxides
B8	117-127	10YR 4/3 brown	silty clay	very few	very moist		very few	few iron oxides
B9	127-155	10YR 5/2 grayish brown	gravel w some clay	very few fine	wet			redoxymorphic features
B10	155-165	2.5Y 4/1 dark gray	sandy/ gravelly clay	relatively abundant fine	saturated			some iron oxides, few sulfides
C1	165-	na	gravel/sand					aquifer, groundwater level

Figure S2. Soil profile at the Riverton, WY, site determined in 2016 (center) with soil characteristics (right). Sediment layers categorized during the 2017 season (left) are included for comparison; depths for these layers from soil cores collected for this study were as follows: Topsoil (~0-30 cm), Evaporite (~30-55 cm), Sand (~55-75 cm), Evaporite-Clay (~75-100 cm), TRZ (~100-140 cm), Clay (~140-150 cm), and Aquifer (~150-170 cm).

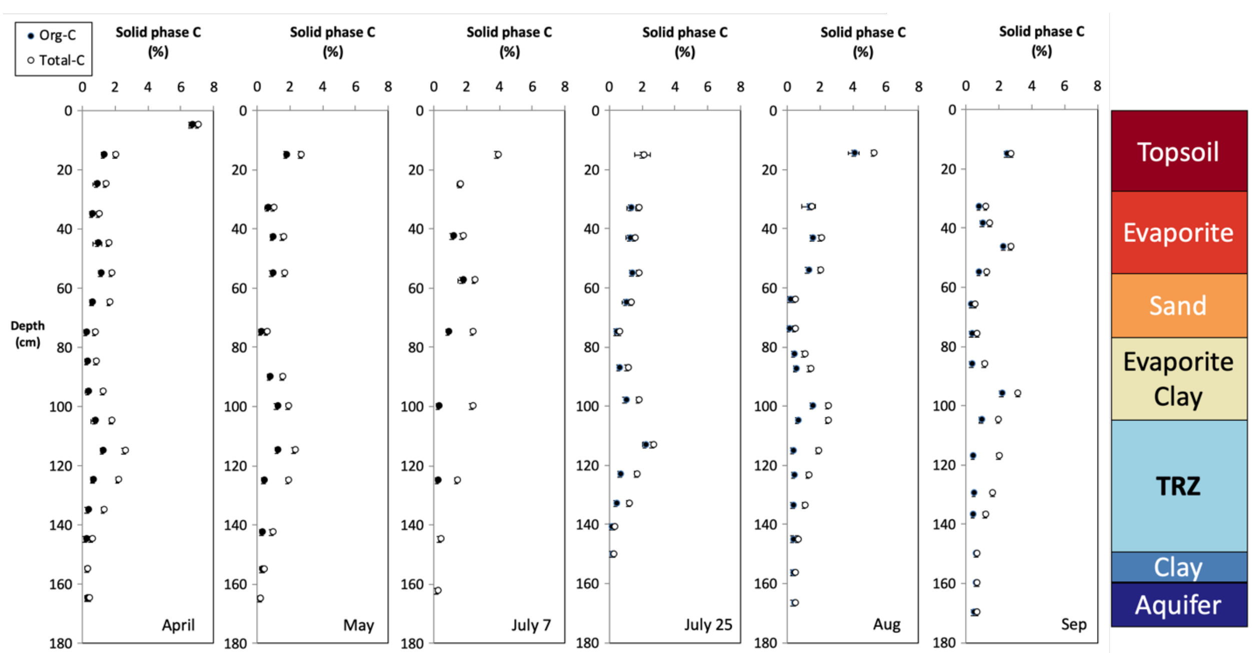


Figure S3. Depth profiles of total C (open circles) and total organic C (filled circles) from sediment collected during the 2017 season at the Riverton, WY, site. Error bars denote 1 standard deviation.

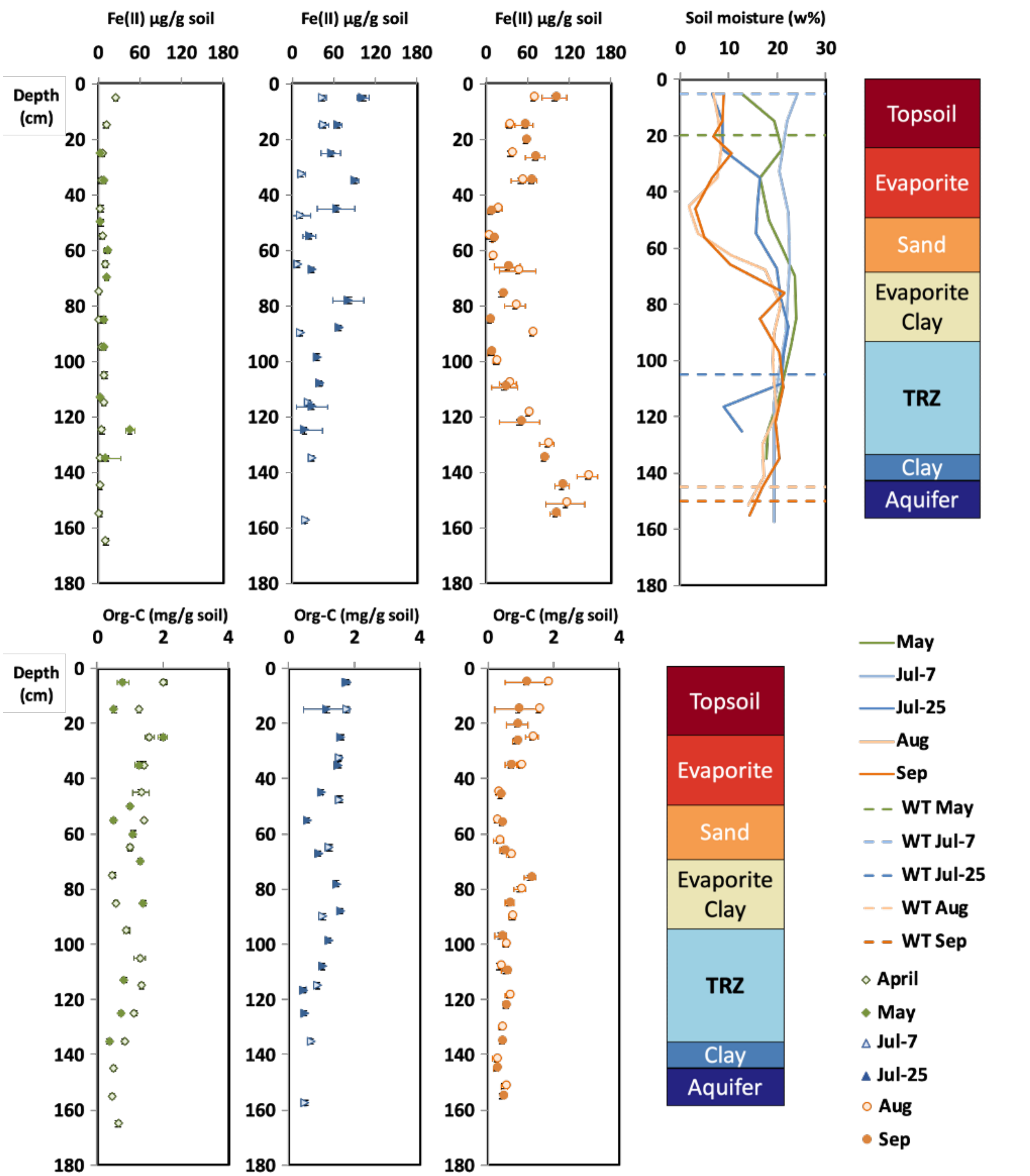


Figure S4. Depth profiles of HCl-extractable Fe(II), soil moisture, and water extractable organic C during the 2017 season at the Riverton, WY, site. Error bars denote 1 standard deviation. WT = water table depth for each month (plotted with soil moisture).

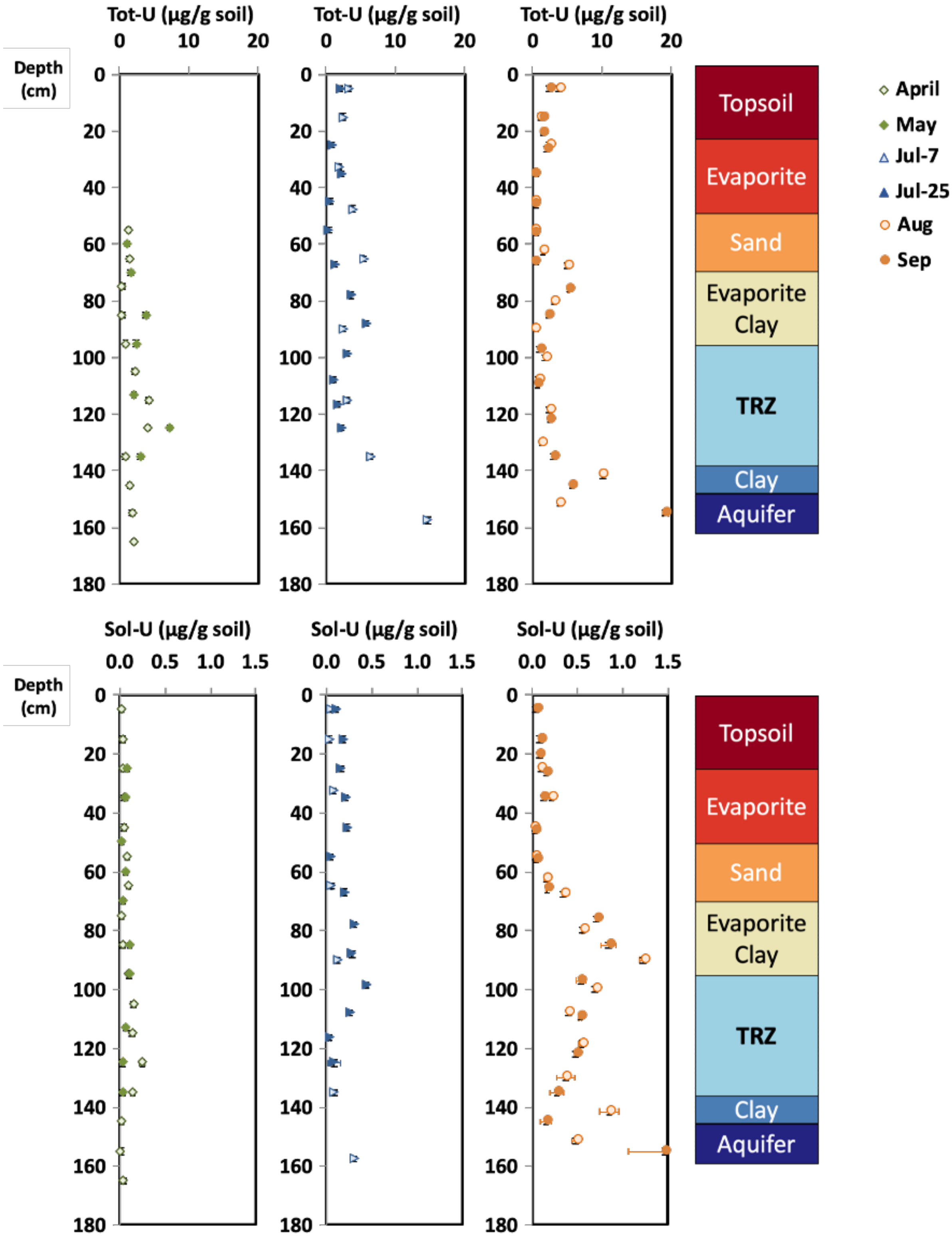


Figure S5. Depth profiles of total U (top) and water extractable U (bottom) from sediment collected during 2017 at Riverton. Error bars denote 1 standard deviation.

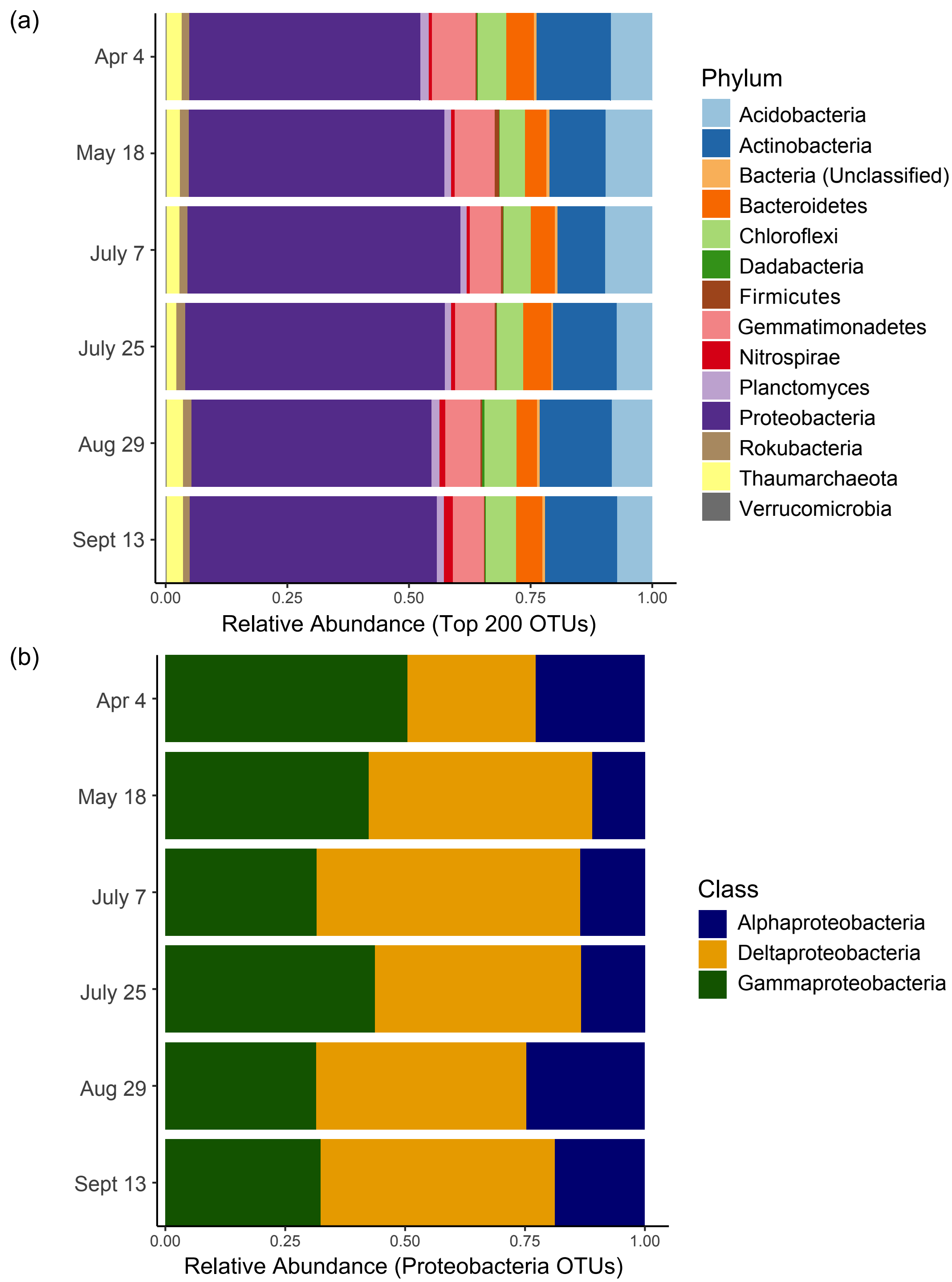


Figure S6a-b. Relative abundance of **(a)** phyla and **(b)** classes within Proteobacteria, grouped by sample time.

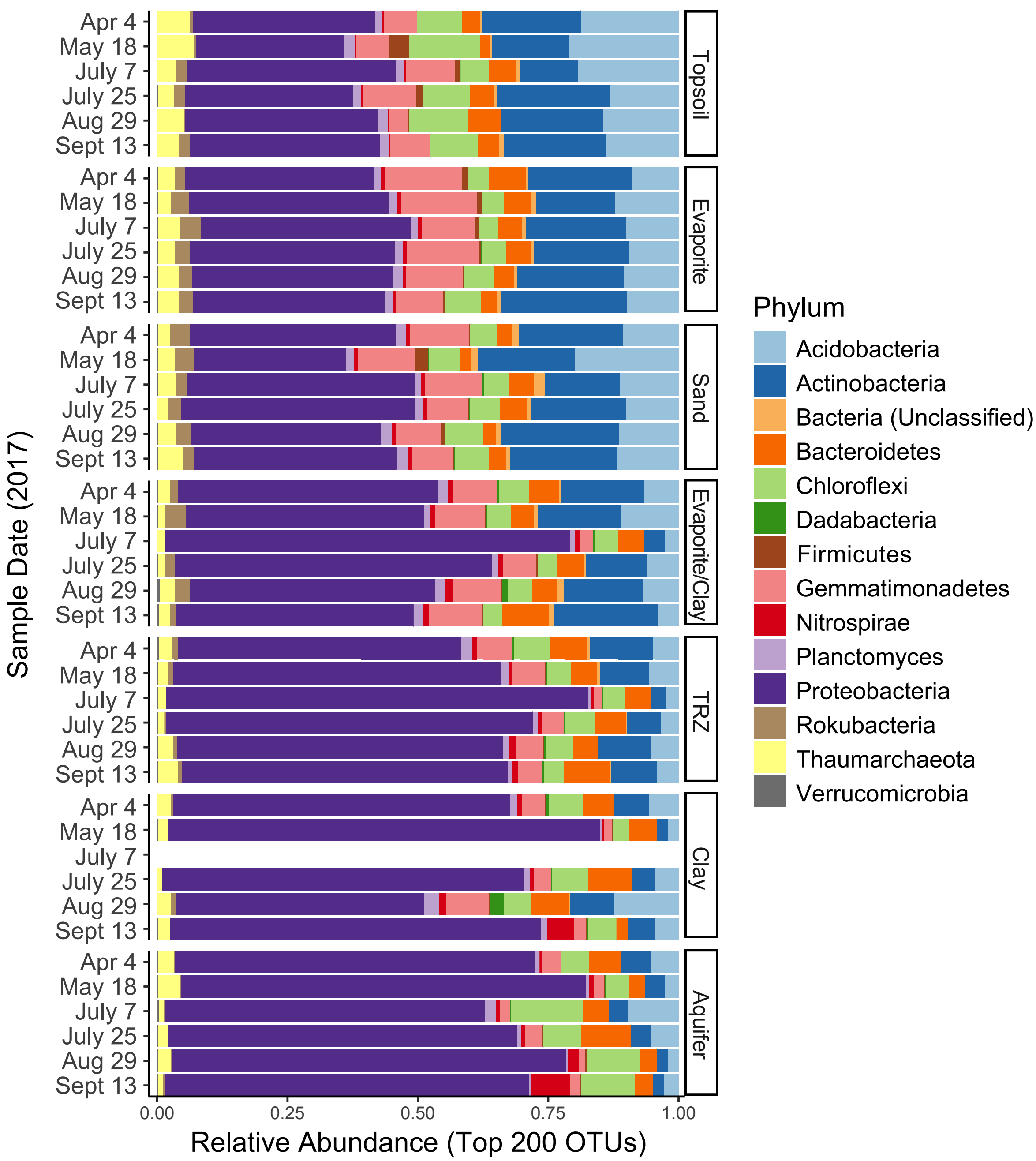


Figure S7. Relative abundance of Top 200 most abundant taxa (OTUs) in our dataset, classified at the Phylum level and grouped based on soil category (right). Within each soil layer, we have divided samples based on sample time (left).

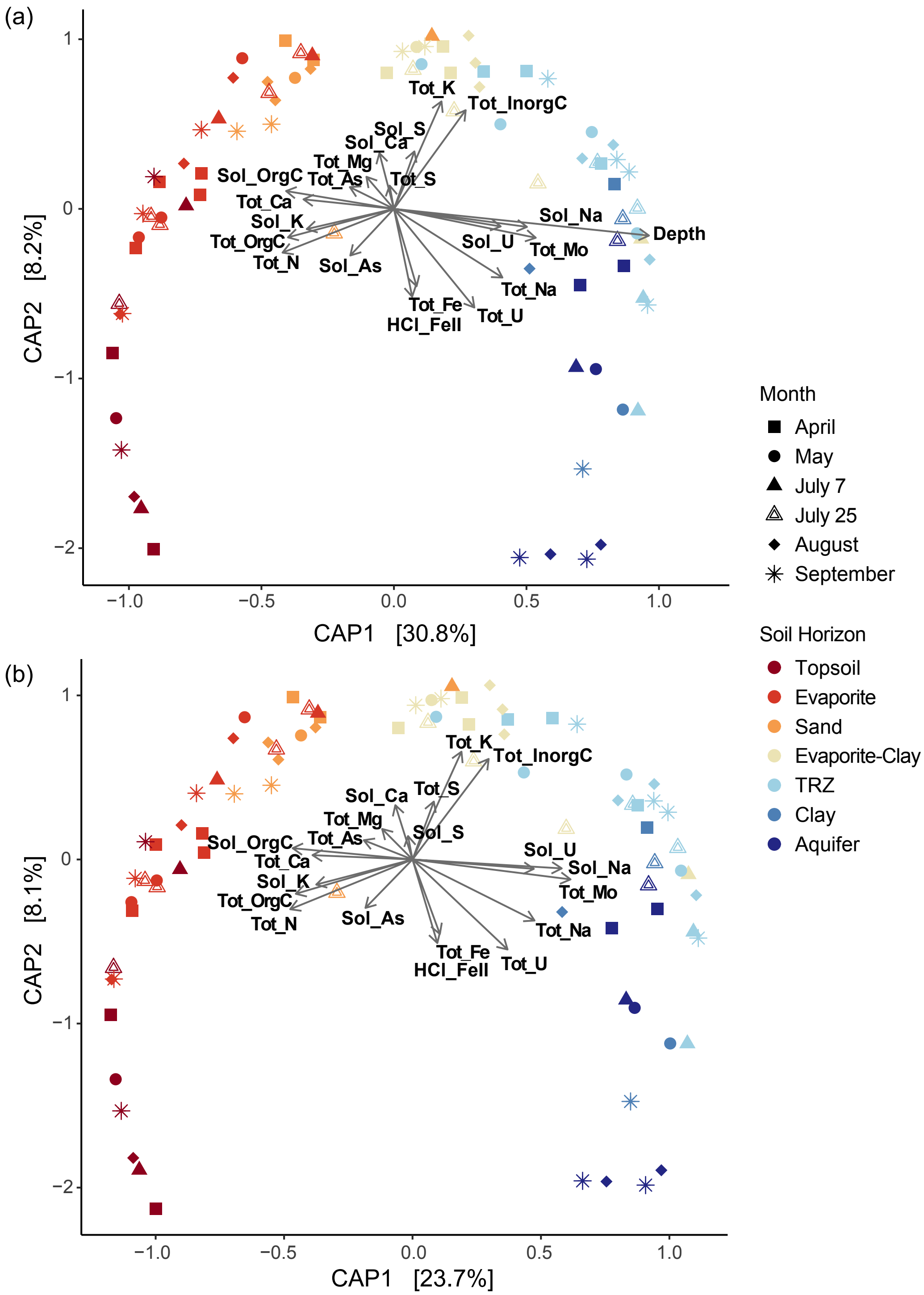


Figure S8a-b. Constrained Analysis of Principal Coordinates (CAP) showing sample distributions based on microbial data with environmental data overlain as vectors, using the **(a)** full dataset with all variables included or **(b)** full dataset with sample depth excluded. Tot = total, Sol = soluble; orgC = organic carbon, InorgC = inorganic (carbonate) carbon; HCl_FeII = acid-extracted Fe(II).

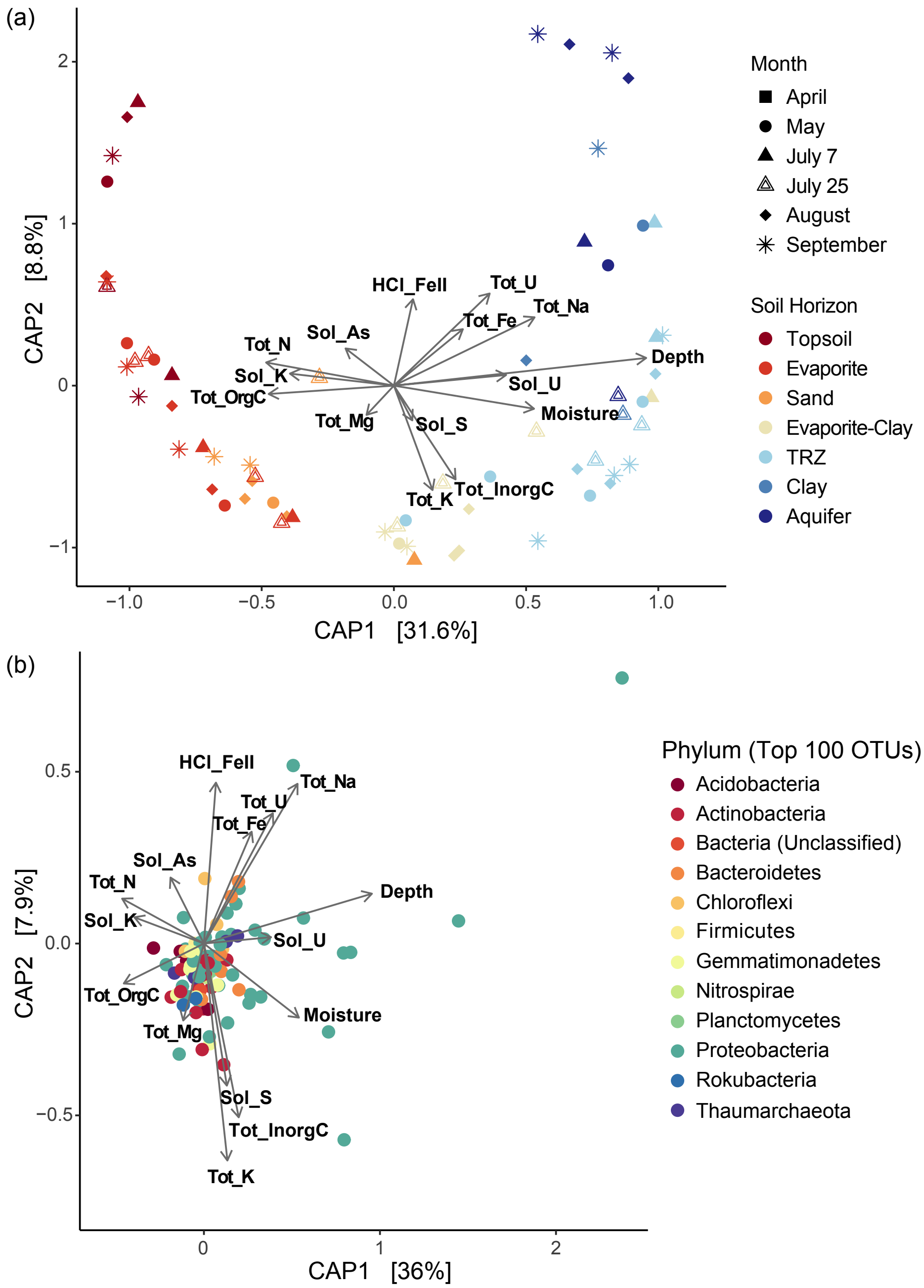


Figure S9a-b. Constrained Analysis of Principal Coordinates (CAP) showing sample distributions based on both microbial and environmental data using a reduced dataset (excluding April samples) to include soil moisture data. Plots generated to display **(a)** overall differences in diversity among samples or **(b)** differences between individual taxa (OTUs) from all samples. Environmental data used in CAP analysis is overlain on each plot, displayed as vectors. Tot = total, Sol = soluble; orgC = organic carbon, inorgC = inorganic (carbonate) carbon; HCl_FeII = acid-extracted Fe(II). Covarying environmental measurements were removed for CAP analysis: total C (represented by total organic and inorganic C); soluble Mg and Ca, total As (covaries with total Mg); total Ca and soluble organic C (total organic C); soluble Na, total and soluble Mo (depth and soluble U). Total S was also removed due to low significance.

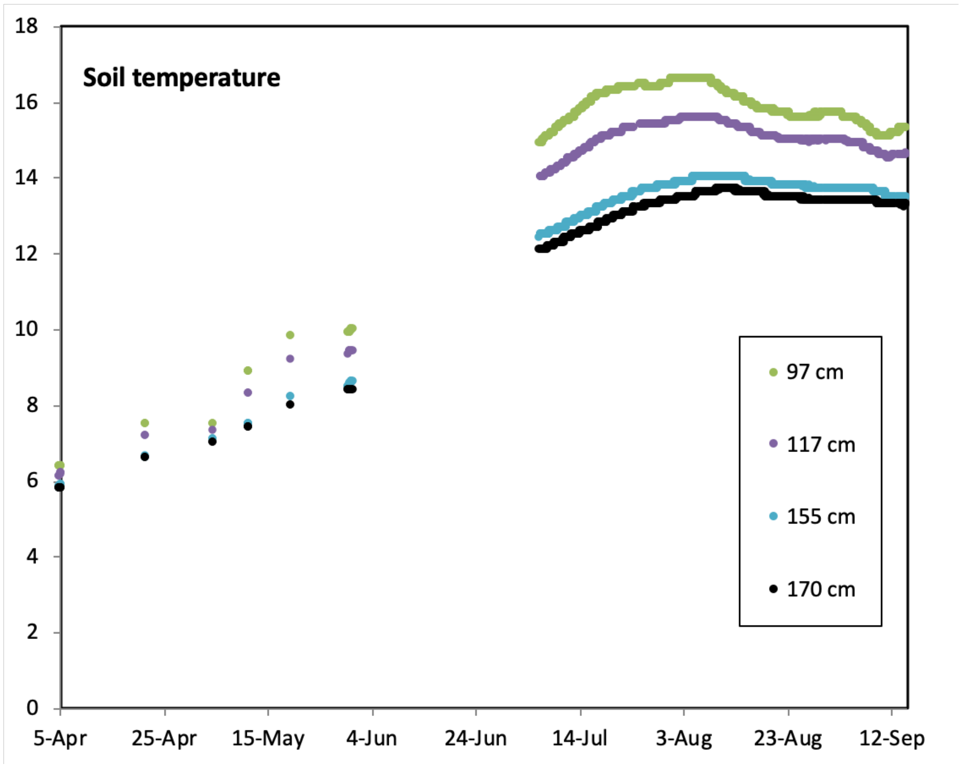


Figure S10. Soil temperature recorded by Acclima True Time Domain Reflectometer (TDR-15L) sensors installed the previous season (2016) in a location <5 m from the current study sampling area and in soil layers corresponding to Evaporite-Clay (green, 97 cm), TRZ (purple, 117 cm), Clay (blue, 155 cm), and Aquifer (black, 170 cm).