Supporting Information for Bioremediation of Uranium Contaminated Sites Through the Formation of U(VI) Phosphate (Bio)minerals

Supporting information for manuscript, including geochemical modelling, aqueous geochemical data for Calder River water experiment, 16S rRNA sequencing, XAS data and XRD analysis of uranyl orthophosphate standard.

Section S1. Geochemical Modelling Parameters and Results

Geochemical modelling was conducted using experimental aqueous data to determine the aqueous U and Ca speciation in the synthetic groundwater Ca-citrate/Na-phosphate, glycerol phosphate amended systems and sorption control. Additionally, the saturation index of U(VI) phosphates and amorphous calcium phosphates within amended experiments was also modelled. All calculations employed the PHREEQC version 3 geochemical modelling program,(1) using aqueous data from experimental systems with the ThermoChimie (V10a) database.(2) Table S1 gives the parameters used for modelling calculations alongside the background synthetic groundwater composition, taken from past work.(3) Solubility constants for U(VI) phosphates (chernikovite and autunite) (4) and complexation constants for U(VI)-glycerol phosphate were also modelled using relevant data from the literature.(5)

Table S1 Synthetic groundwater water composition and geochemical modelling parameters used for PHREECQ models of experiment systems.

Ion	ppm	mM
Ca^{2+}	27.73	0.69
Mg^{2+}	5.82	0.24
K^{+}	2.87	0.07
Na^{+}	35.19	1.53
HCO_3^{-}	60.03	0.98
Cl^{-}	53.79	1.51
NO_3^{-}	19.98	0.32
SO_4^{2-}	25.18	0.26

Experiment Model	Geochemical Data Used						Result
Synthetic Groundwater Ca-citrate/Na-phosphate			mM				Figure S1 (a)
	Days	pH	Ca	PO4	U	Citrate	
	0	6.48	1.72	9.1	0.053	2.2	
	1	7.61	1.64	8.65	0.009	2.2	
	3	7.68	1.67	8.34	0.009	1.8	
	7	8.38	0.56	7.45	0.001	0	
	14	8.36	0.44	7.03	0.001	0	
	21	8.63	0.37	7.06	0.001	0	
	31	8.64	0.33	7.74	0.001	0	
Synthetic Groundwater glycerol phosphate			mM				Figure S1 (b)
	Days	pH	Ca	PO4	U	GlyPO4	
	0	6.48	0.75	0	0.053	11.2	
	1	7.78	0.85	0.02	0.024	11.1	
	3	7.67	1.03	0.42	0.032	10.6	
	7	7.7	0.92	0.96	0.032	10.6	
	14	7.54	0.9	0.6	0.028	11.2	
	21	7.76	0.91	1.32	0.005	8.3	
	31	7.86	0.94	1.17	0.0013	7.8	
Synthetic Sediment Only Control		mM					Figure S1 (c)
	Time / Days	pH	Ca	U			
	0	6.48	0.87	0.053			
	1	6.94	0.95	0.009			
	3	6.64	0.98	0.006			
	7	6.63	0.89	0.005			
	14	6.69	1.06	0.003			
	21	6.67	0.96	0.003			
	31	6.93	1.08	0.003			

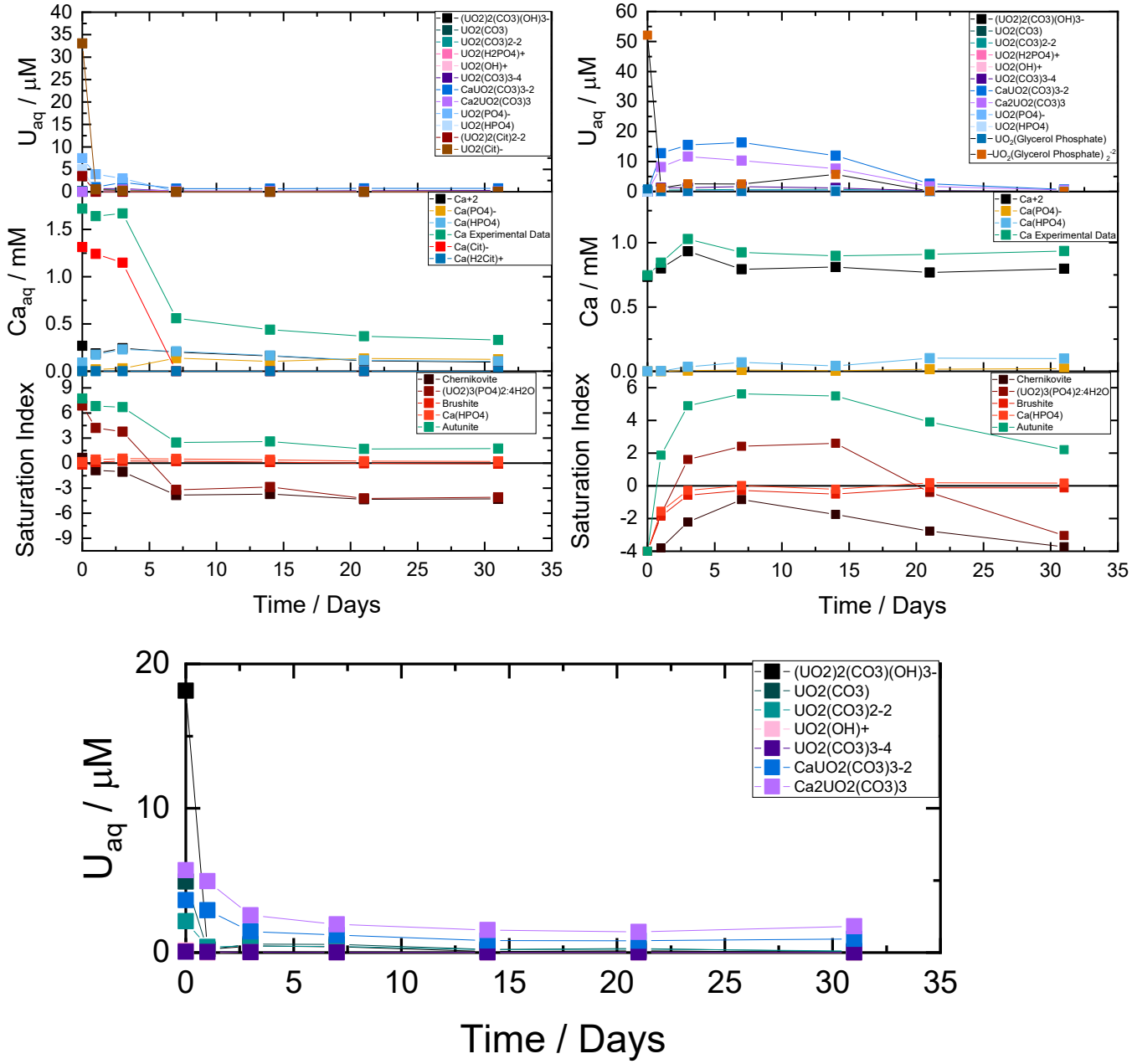
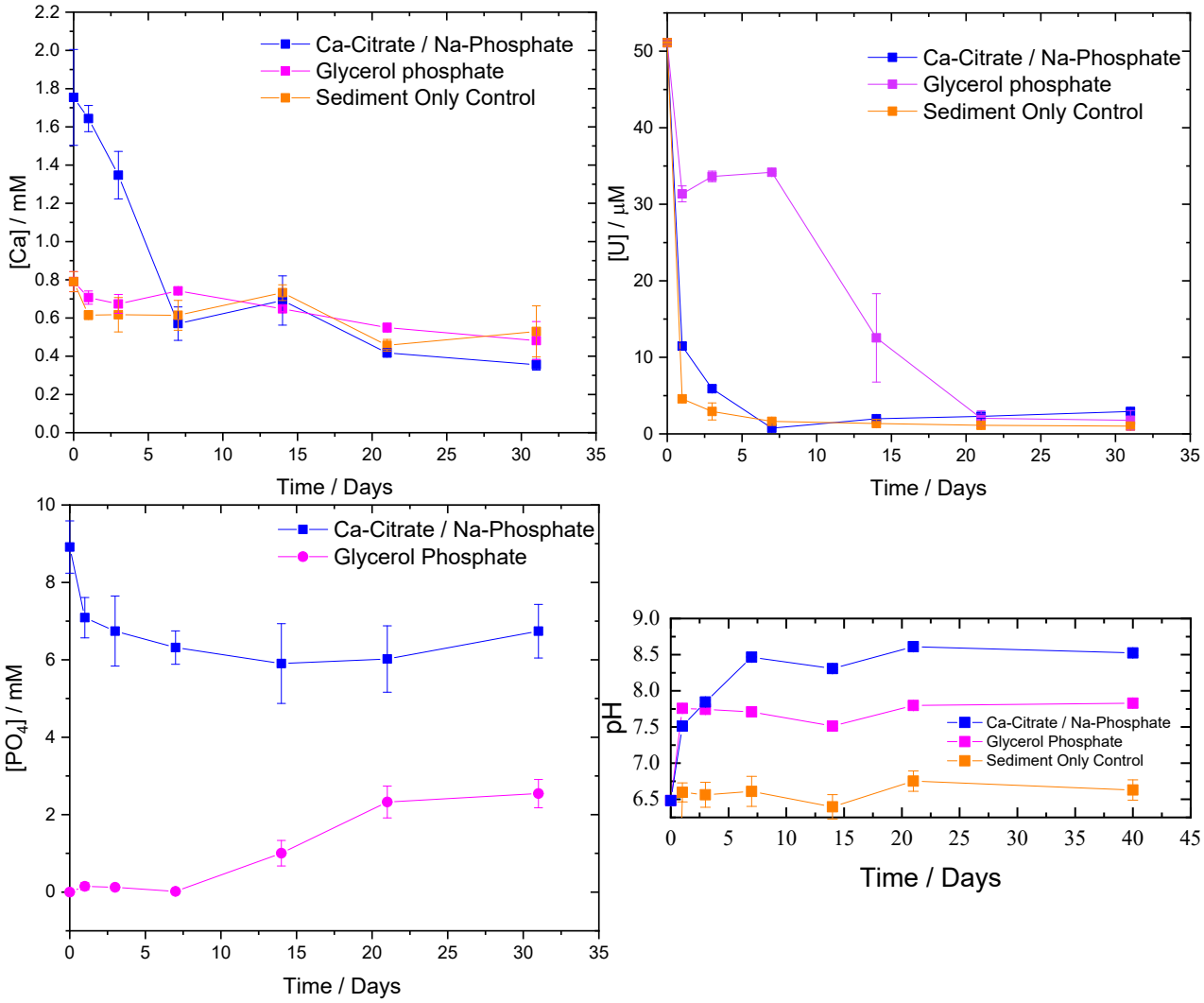


Figure S1 Geochemical model of the Ca and U speciation and saturation index for U(VI) phosphate and amorphous Ca-phosphate phase using aqueous data from synthetic groundwater (a) 1mM Ca^{2+}, 2.5 mM citrate and 10 mM phosphate, (b) 10 mM glycerol phosphate. U speciation in (c) synthetic groundwater sediment only control.



Section S.2: Aqueous Geochemical Data for Calder River Water Experiment

Figure S2 Aqueous geochemical data from Calder River water microcosms amended with 1 mM Ca^{2+}, 2.5 mM Citrate with 10 mM phosphate, 10 mM glycerol phosphate and a sediment only sorption control. Microcosms were run in triplicate with error bars representing ± one standard deviation.

Section S3: 16S rRNA Microbial Community Analysis

Synthetic Groundwater Experiments

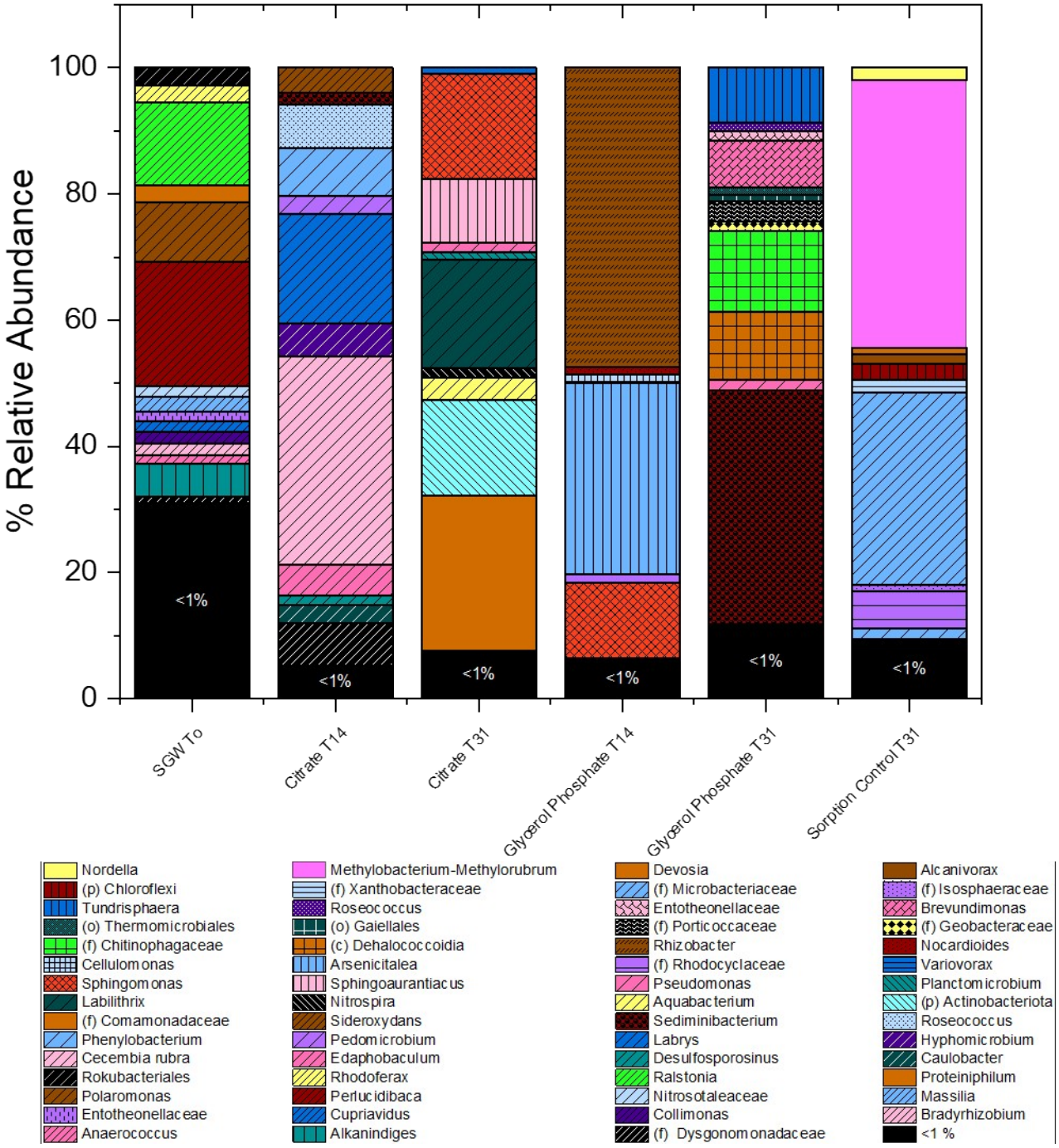


Figure S3 Data showing the microbial community structure at Genus level (>1% relative abundance) for synthetic groundwater sediment end points after 14 and 31 days of treatment with 1 mM Ca^{2+}, 2.5 mM citrate and 10 mM phosphate, 10 mM glycerol phosphate and sediment only control (sorption control). The initial starting peel place quarry (PPQ) sediment is also shown. Data is presented to the genus level, where the genus cannot be identified the family (f), order (o), phylum (p) or class (c) is given.

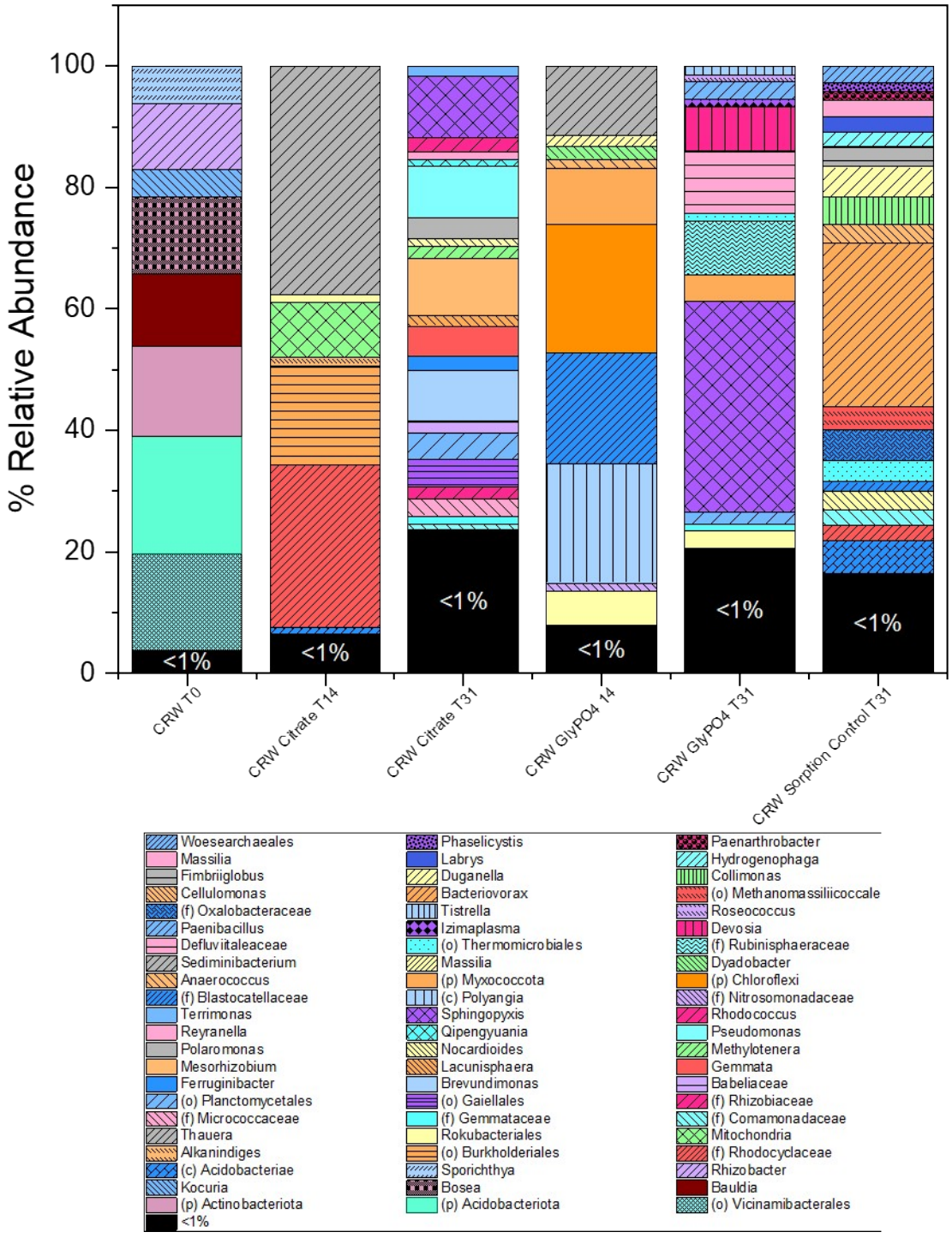


Figure S4 Data showing the microbial community structure at Genus level (>1% relative abundance) for Calder River water sediment end points after 14 and 31 days of treatment with 1 mM Ca^{2+}, 2.5 mM citrate and 10 mM phosphate, 10 mM glycerol phosphate and sediment only control (sorption control). The initial starting peel place quarry (PPQ) sediment is also shown. Data is presented to the genus level, where the genus cannot be identified the family (f), order (o), phylum (p) or class (c) is given.

Calder River water Experiments

Table S2 Shannon Diversity Index (H) of microbial communities from sediment samples and corresponding DNA yield in extracts prior to PCR amplification (measured using Qubit). Calder River water (CRW). Time (T) given in days. Synthetic Groundwater (SGW).

Sample	Shannon Diversity Index (H)	Extract Conc ng/ul
Synthetic Groundwater (SGW) T0	3.7	0.02
SGW Citrate T14	2.3	0.52
SHW Citrate T31	2.3	3.04
SGW Glycerol Phosphate T14	1.6	0.17
SGW Glycerol Phosphate T31	2.4	2.58
SGW Sediment Only Control T31	3.3	0.01
CRW T0	2.3	Below L.O.D
CRW Citrate T14	1.8	7.38
CRW Citrate T31	3.8	0.498
CRW GlyPO4 T14	1.6	0.169
CRW GlyPO4 T31	2.8	1.07
CRW Sediment Only Control T31	3.3	0.0114

Section S4: XAS Results and Fitting Table

U L_{III} EXAFS Fitting parameters for the sediment endpoints from sediment only control, Ca-citrate/Na-phosphate (1 mM Ca^{2+}, 2.5 mM citrate and 10 mM phosphate) and glycerol phosphate treatments. Additional EXFAS analysis for solution sample 14 days after amendment glycerol phosphate and uranyl orthophosphate standard. N is shell occupancy, R(Å) is interatomic distance, σ^2 ($Å^2$) is the Debye-Waller factor, S_O^2 amplitude factor, R (least squared residual) goodness of fit factor, ΔE_0 denotes the energy shift (calculated) from the fermi level and α denotes the statistical significance of each shell from the F-test, determined from whether the fit was significantly worsened on removal of an individual shell.(6)

Table S3 U L_{III} EXAFS Fitting parameters for the sediment microcosms sediment only control, Ca-citrate/Na-phosphate (1 mM Ca^{2+}, 2.5 mM citrate and 10 mM phosphate), glycerol phosphate amended, uranyl orthophosphate standard and glycerol phosphate solution phase at 14 day.

Treatment	**Scattering Path**	**N**	**R (Å)**	**σ^2 ($Å^2$)**	**S_O^2**	**R-factor**	**ΔE_o**	**F-Test (α) %**
Sediment Only Control	U-Oax	2	1.81± 0.01	0.004 ± 0.001	0.90	0.012	8.42	100
	$U\text{-}O_{eq}$	3	2.29 ± 0.03	0.006 ± 0.002	0.90			100
	$U\text{-}O_{eq}$	3	2.47 ± 0.03	0.005 ± 0.002	0.90			100
	U-C	1.7	2.95 ± 0.05	0.010 ± 0.009	0.90			89
	U-Fe	0.5	3.45 ± 0.05	0.008 ± 0.005	0.90			92
Ca-citrate/Na- phosphate	U-Oax	2	1.81 ± 0.006	0.003 ± 0.0006	1.0	0.016	10.4	100
	$U\text{-}O_{eq}$	2.3	2.30 ± 0.014	0.0011 ± 0.001	1.0			100
	$U\text{-}O_{eq}$	2.7	2.44 ± 0.010	0.003 ± 0.0015	1.0			100

	U-P	1.0	3.13 ± 0.018	0.003 ± 0.0020	1.0			100
	U-P	2.0	3.66 ± 0.023	0.005 ± 0.0025	1.0			100
	U-U	1.3	4.00 ± 0.036	0.007 ± 0.0038	1.0			93
Glycerol Phosphate	U-Oax	2	1.80 ± 0.010	0.003 ± 0.0009	1.0	0.019	9.9	100
	$U\text{-}O_{eq}$	2.7	2.32 ± 0.015	0.002 ± 0.0014	1.0			100
	$U\text{-}O_{eq}$	2.3	2.47 ± 0.025	0.004 ± 0.0027	1.0			100
	U-P	1.0	3.14 ± 0.023	0.003 ± 0.0026	1.0			99
	U-P	2.0	3.71 ± 0.048	0.009 ± 0.0057	1.0			88
	U-U	1.3	4.00 ± 0.053	0.007 ± 0.0055	1.0			70
Uranyl Orthophosphate Standard	U-Oax	2.0	1.78 ± 0.05	0.0018 ± 0.0004	0.9	0.0080	9.5	
	$U\text{-}O_{eq}$	8/3	2.33 ± 0.010	0.0024 ± 0.0010	0.9			
	$U\text{-}O_{eq}$	7/3	2.48 ± 0.013	0.0022 ± 0.0013	0.9			
	U-P	1.0	3.18 ± 0.021	0.0039 ± 0.0024	0.9			
	U-P	1.2	3.59 ± 0.039	0.0047 ± 0.0038	0.9			
	U-P	2	3.82 ± 0.033	0.0058 ± 0.0034	0.9			
	U-U	4/3	4.02 ± 0.010	0.0016 ± 0.0008	0.9			
Glycerol Phosphate Solution Phase 14 Days	U-Oax	2	1.84 ± 0.080	0.0049 ± 0.001	0.9	0.0079	13.5	100
	$U\text{-}O_{eq}$	6	2.47 ± 0.010	0.0089 ± 0.001	0.9			100
	U-C	3	2.92 ± 0.013	0.0027 ± 0.002	0.9			100
	U-O-U-O MS	2	3.67	0.0099	0.9			
	U-O-O MS	2	3.73	0.0099	0.9			

	U-P	1	3.27 ± 0.02	0.0030 ± 0.0026	0.9			99
	U-Ca	2	4.05 ± 0.02	0.0061 ± 0.003	0.9			99

XAS Spectra and EXAFS Fitting Glycerol Phosphate Aqueous Phase (T_{14})

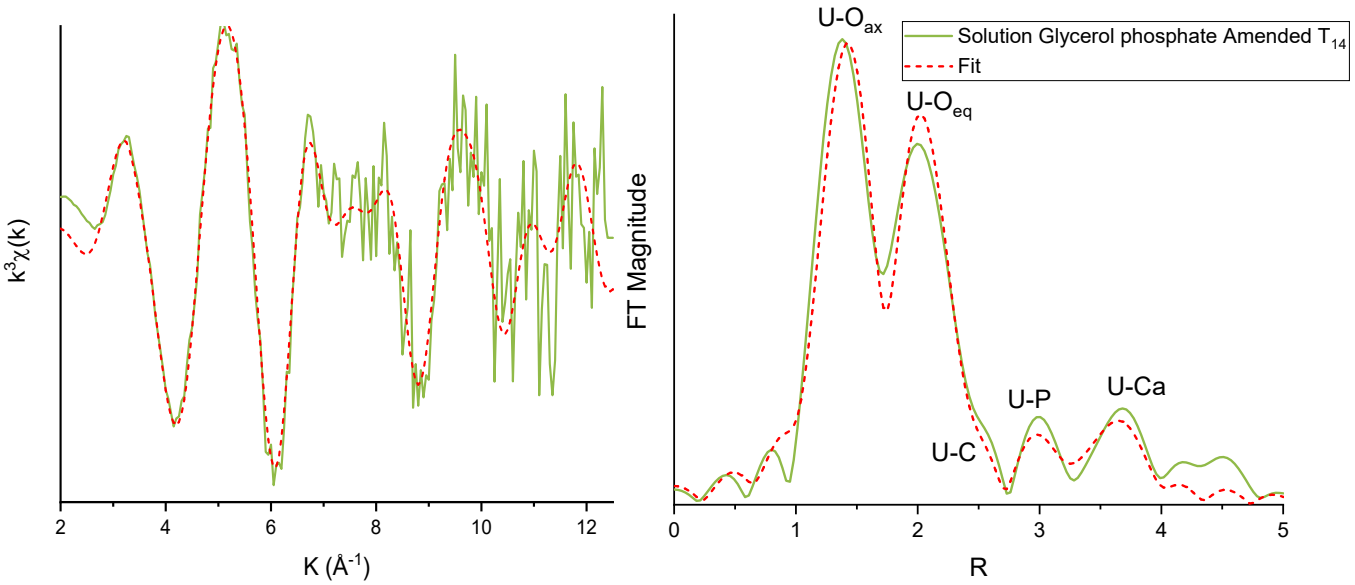


Figure S5 U L_{III} EXAFS synthetic groundwater solution sample 14 day post treatment with 10 mM glycerol phosphate.

XANES Spectra from microcosm experiments

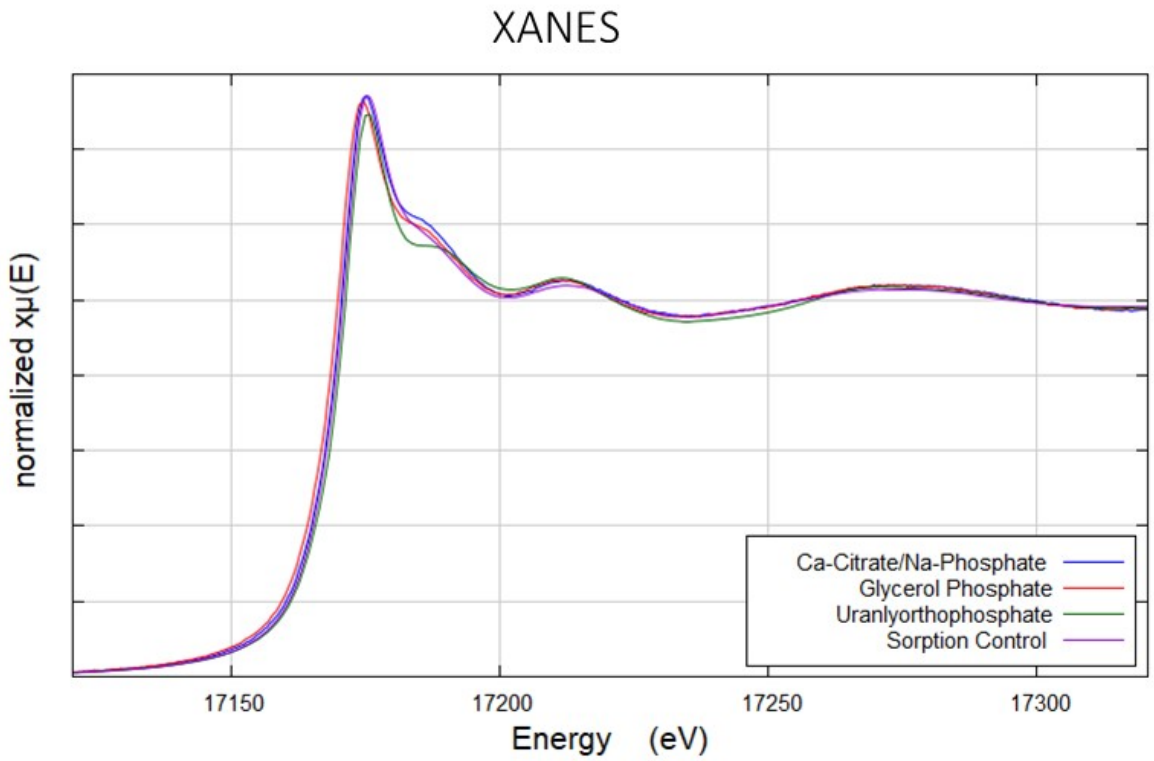


Figure S6 XANES spectra for uranyl orthophosphate standard and sediments after 31 days of treatment with Ca-citrate / Na-phosphate, glycerol phosphate and sediment only control (sorption control).

Section S5 XRD Analysis of Uranyl Orthophosphate Standard

Uranyl orthophosphate was synthesised following from the method of S. Yagoubi et al (7) briefly, 0.375 g of uranyl nitrate were dissolved in 5 ml of DIW, before the addition of 1.9 ml of phosphoric acid (0.17 M). The solution was stirred at 70°C until complete evaporation and formation of a yellow solid. After, serval washes with DIW the solid was ground to a fine powder with a pestle and mortar, before drying overnight. The dried powder was suspended in isopropanol, before being transferred to XRD sample holder. XRD analysis was conducted using a Bruker D2 PHASER. The synthesised mineral was confirmed as uranyl orthophosphate ($(UO_2)_3(PO_4)_2(H_2O)_4$) by comparison to previous XRD spectra from the literature generated using VESTA software and CIF file from Locock et al 2002.(8)

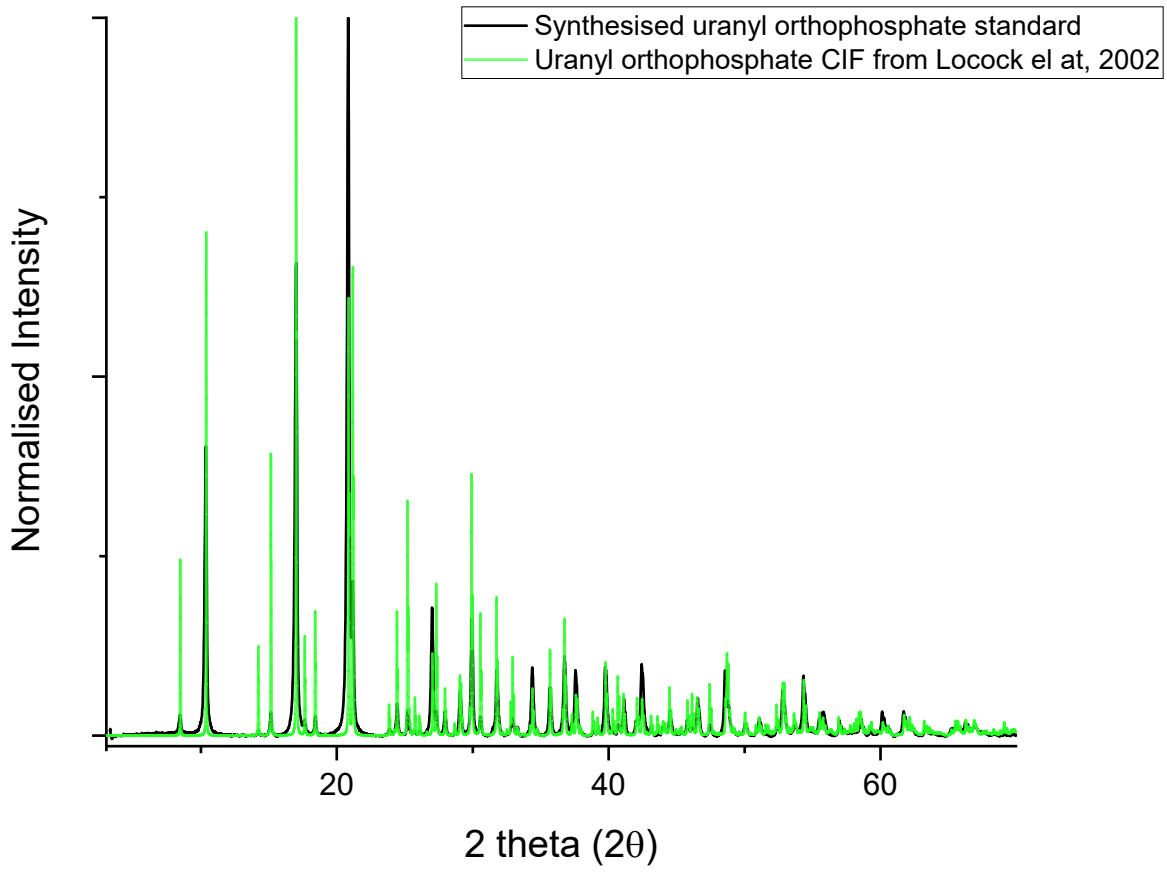


Figure S7 XRD from uranyl orthophosphate standard synthesised from the method of S. Yagoubi et al, 2013 and CIF file from Locock et al , 2002.

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Section S6: Supporting Information References

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