Supplementary Material

Influence of microorganisms on uranium release from mining-impacted lake sediments under various oxygenation conditions

Marina Seder-Colomina[a], Arnaud Mangeret[a,*], Pascale Bauda[b], Jessica Brest[c], Lucie Stetten[a,c], Pauline Merrot[c], Anthony Julien[a], Olivier Diez[a], Evelyne Barker[a], Elise Billoir[b], Pascal Poupin[b], Antoine Thouvenot[d], Charlotte Cazala[a], Guillaume Morin[c]

[a]Institut de Radioprotection et de Sûreté Nucléaire, IRSN, PRP-DGE, 31 avenue de la Division Leclerc, 92262 Fontenay-aux-Roses, France.

[b] Université de Lorraine, CNRS, LIEC, F-57000 Metz, France

[c]Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie (IMPMC), UMR 7590 CNRS-UPMC-IRD-MNHN, case 115, 4 place Jussieu, 75252 Paris Cedex 5, France

[d]Université de Clermont Ferrand, Athos Environnement, 63171 Aubière, France

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*Corresponding author: arnaud.mangeret@irsn.fr

Tel +33 1 58 35 76 95

Fax +33 1 46 57 62 58

This file contains 18 pages, 5 figures and 6 tables. Table S7 showing the Spearman correlation matrix between prototypes and Table S8 listing the different OTUs identified associated to their respective prototype are inserted as external Excel© files.

- **Figure S1.** Sediment sampling site location
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- **Figure S3.** Total Organic Carbon
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- **Table S5** Calculation of aqueous U(IV) and U(VI) speciation and determination of major and minor U(IV) and U(VI) complexes at each set of experimental conditions with irradiated sediments.

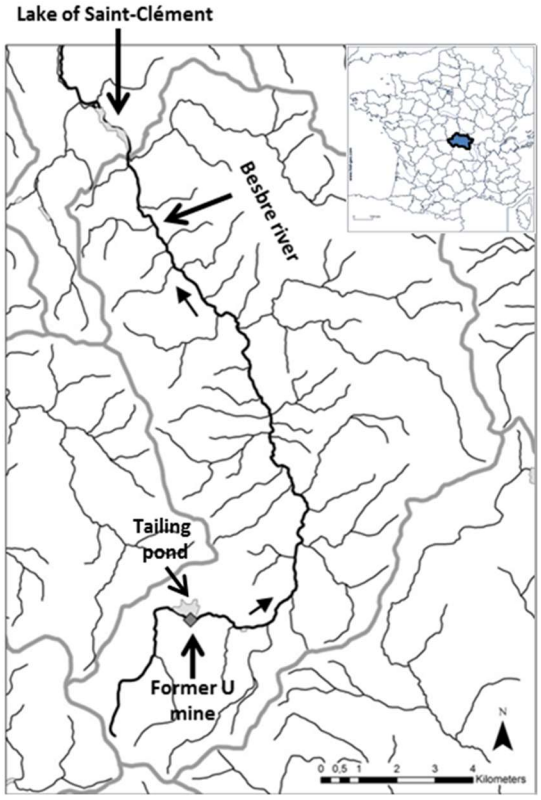

Figure S1. Sediment sampling site location. Lake Saint-Clément is located in Allier, Massif Central, France. The lake is supplied by the Besbre River that drains the discharges from the Bois-Noirs tailing pond treated water, located 20 km upstream from the lake.

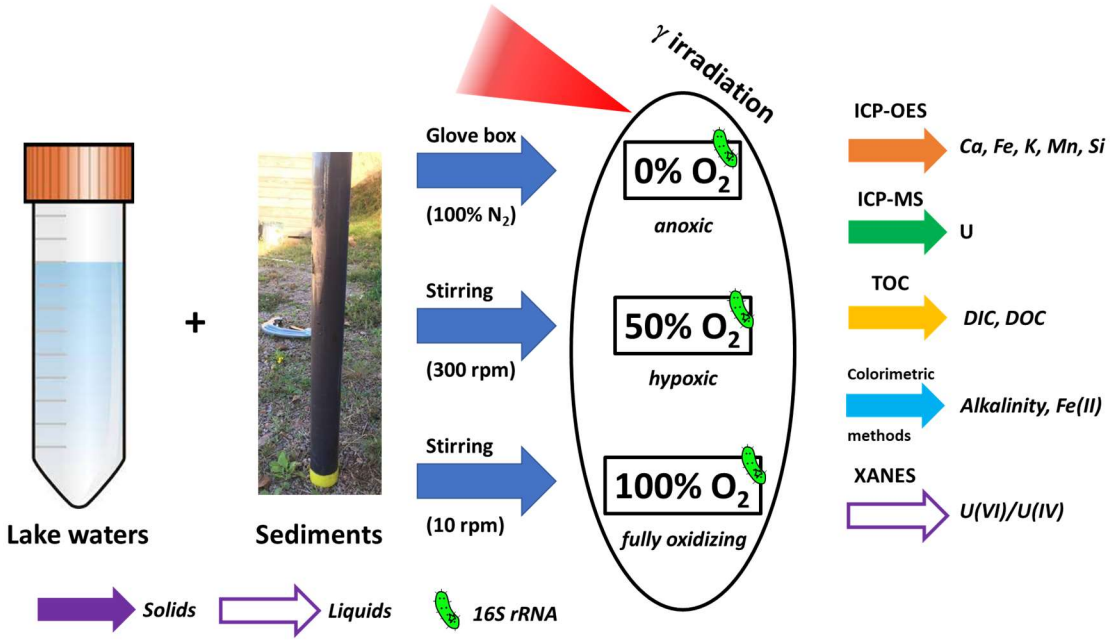

Figure S2. Description of the experimental and analytical procedures of the incubation experiments.

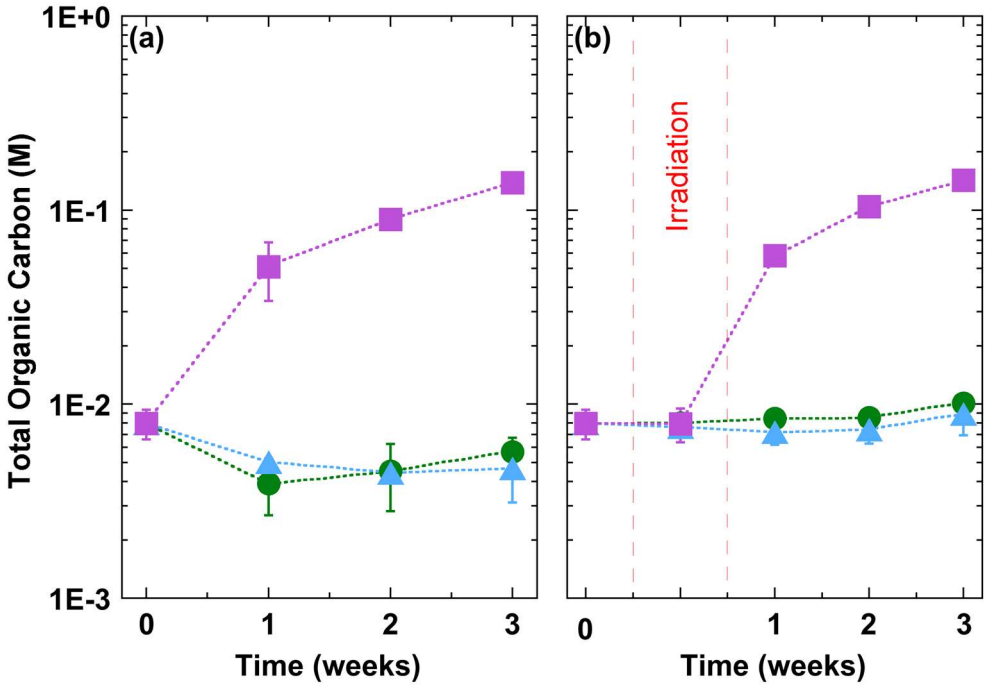

Figure S3. Total Organic Carbon in solution for (left) fresh and (right) irradiated sediments, throughout 3-week incubation under different O_2 conditions: green circles (●), *100% O_2* incubation; blue triangles (▲), *50% O_2* incubation and purple squares (■), *0% O_2* incubation. Error bars represent the standard deviation over the 3 replicates for each condition.

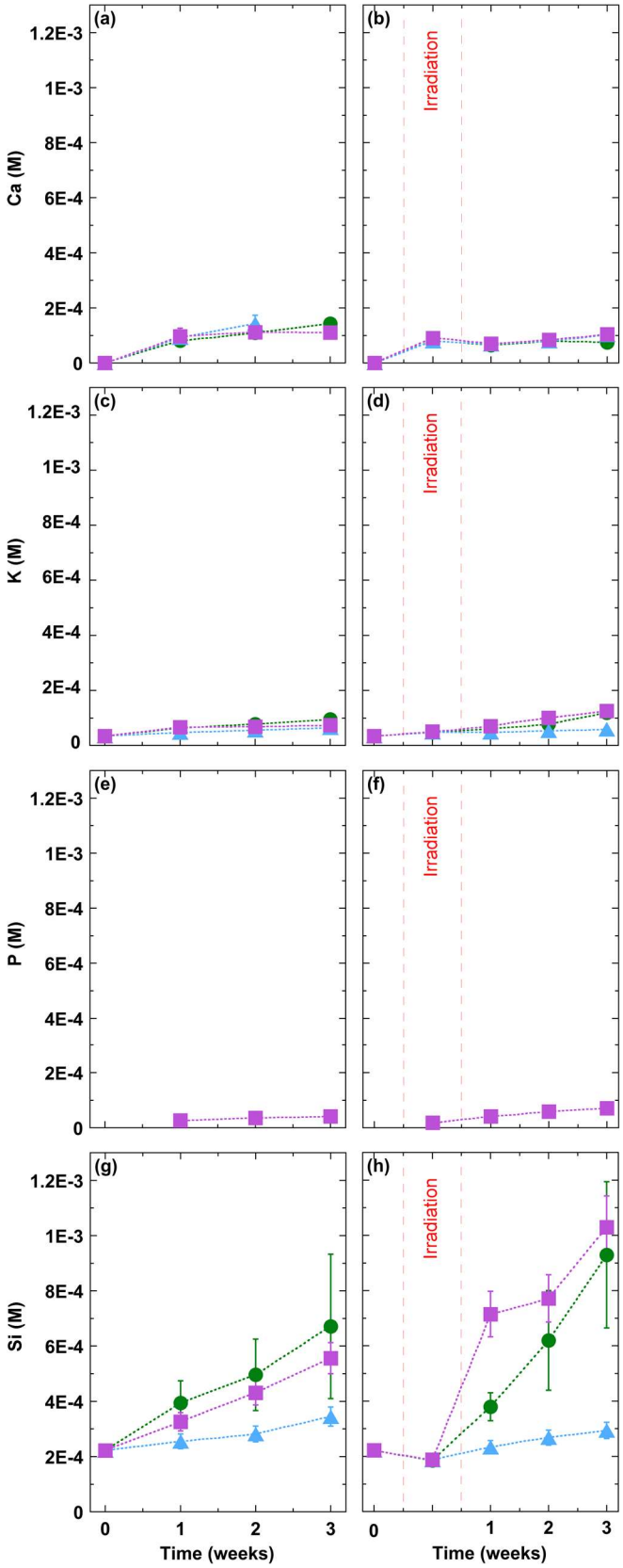

Figure S4. Ca, K, P and Si for (left) fresh and (right) irradiated sediments, throughout 3-week incubation under different O_2 conditions: green circles (●), *100% O_2* incubation; blue triangles (▲), *50% O_2* incubation and purple squares (■) *0% O_2* incubation. Error bars represent the standard deviation over the 3 replicates for each condition.

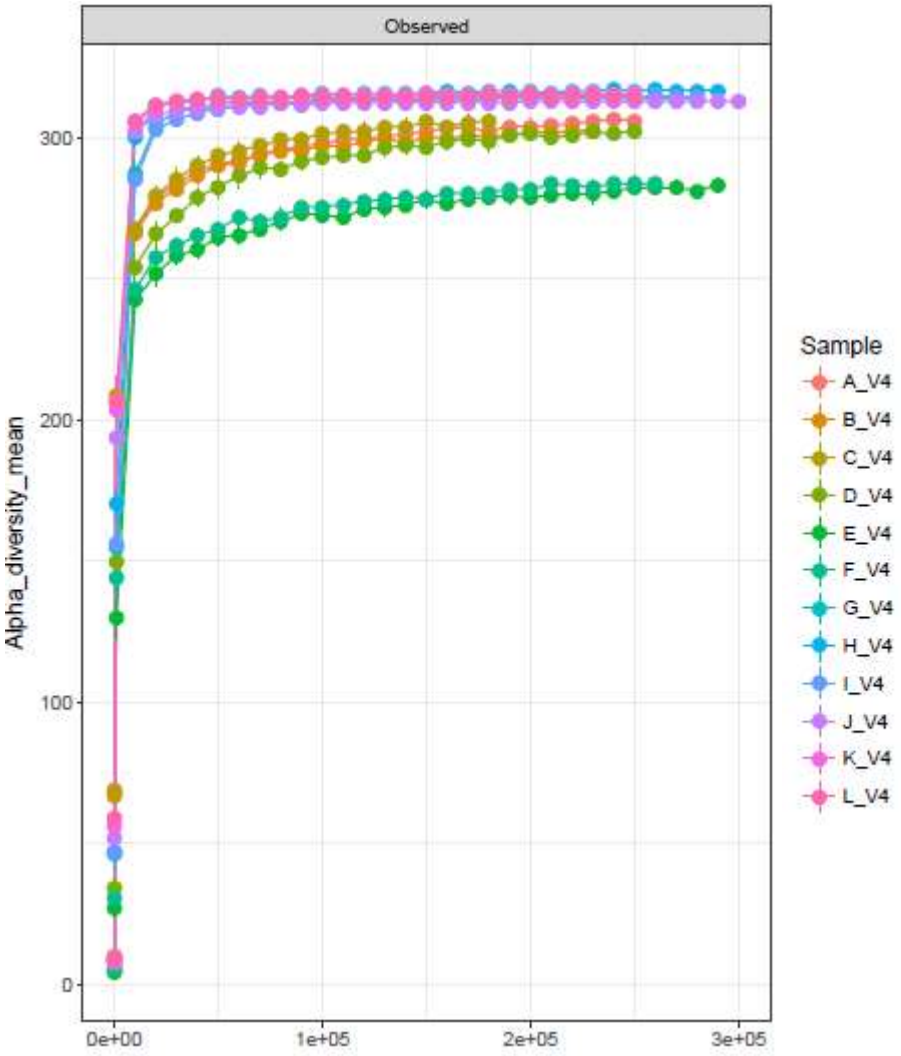

Figure S5. Rarefaction curves of observed OTU for analyzed samples (ABC initial sediment, DEF anoxic, GHI 100% oxic, JKL 50% oxic conditions).

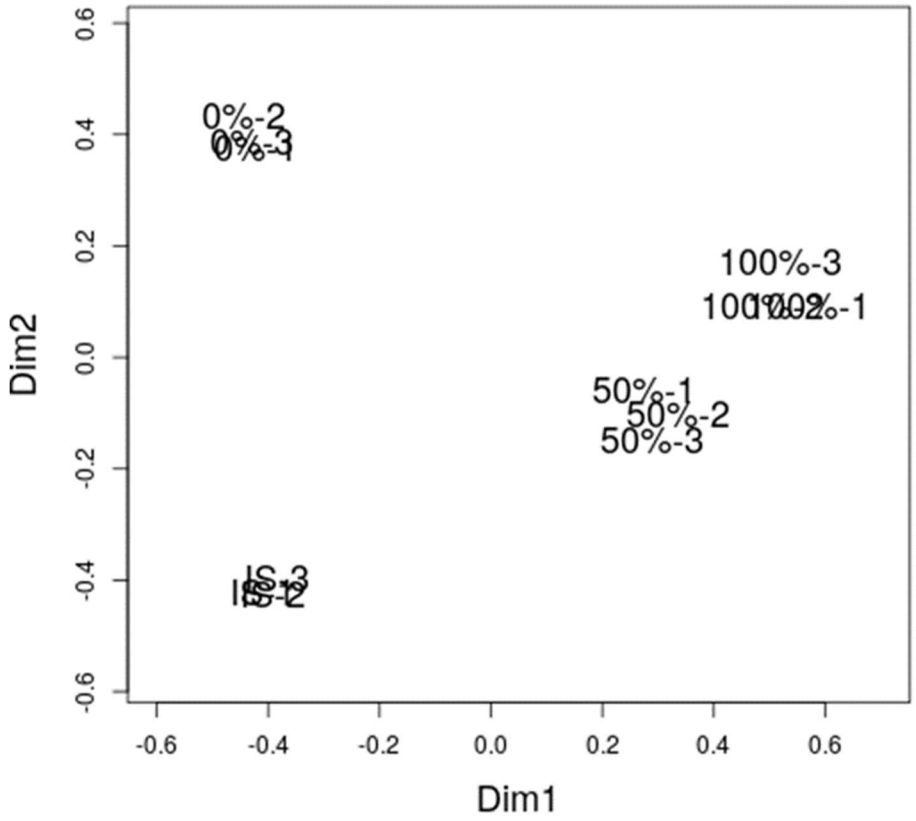

Figure S6. Non-parametric multidimensional scaling (NMDS) plot of prokaryotic communities associated for triplicate independent determinations for the Initial Sediments (IS) and after 3 weeks of incubation under different O_2 conditions (*100% O_2*, *50% O_2* and *0% O_2* samples). Performed using the isoMDS function of the Vegan R package [2]

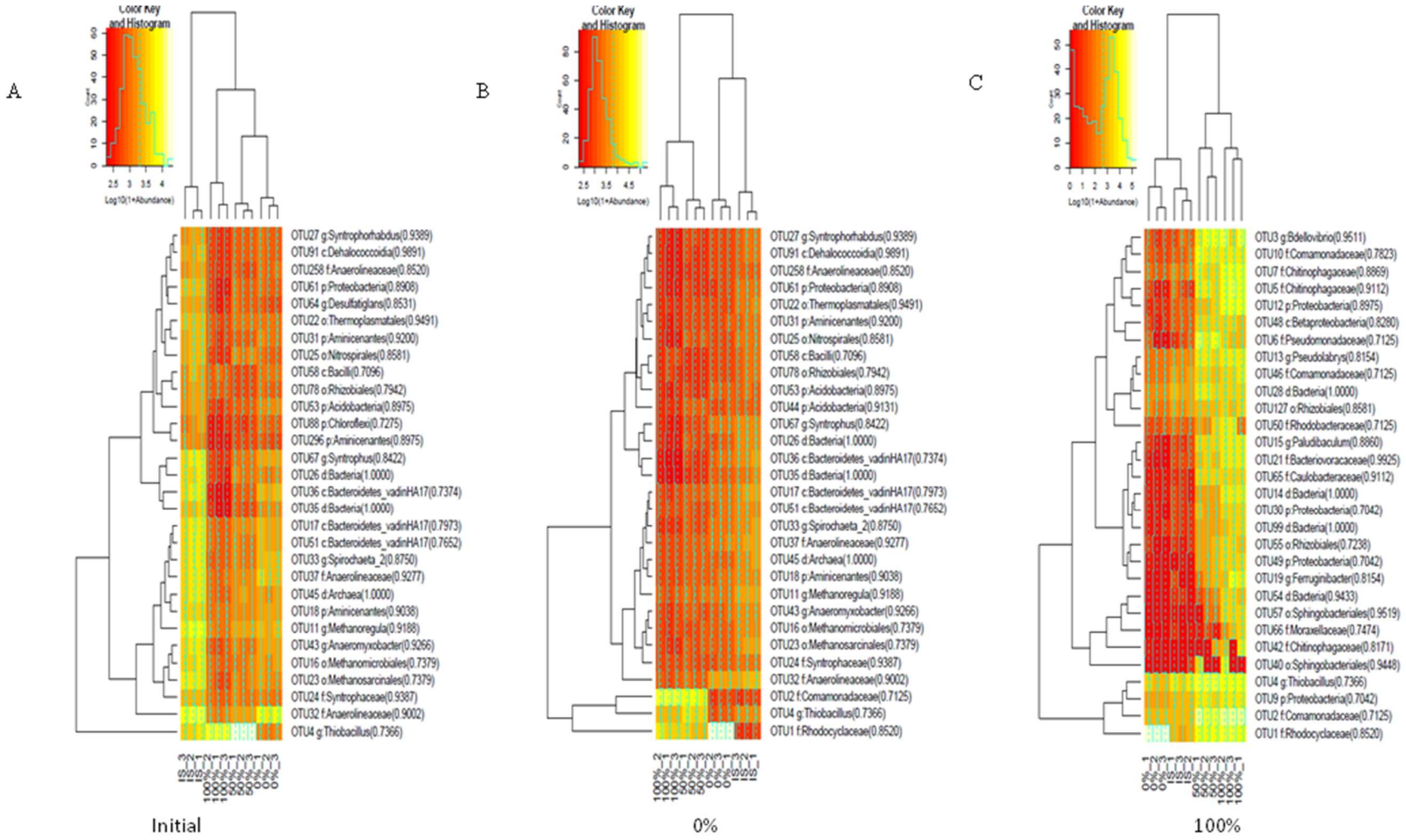

Figure S7: Heat map and hierarchical cluster analysis of log-transformed abundances of the 30 most abundant OTUs (based on raw abundances in treatment) for the initial sediments (A) and after 3 weeks of incubation under 0% O_2 (B), and 100% O_2 (C). The dendrogram represents complete linkage clustering. Assignments after OTUs numbers give the lowest taxonomic level associated with the OTU using the silva database k: kingdom, p: phylum, o: order, f: family, s: genus_species. Values in brackets indicate sequence similarity.

Table S1. Richness and diversity indices of the initial and exposed prockaryotic communities for the initial sediments and after 3 weeks of incubation under different O_2 conditions (*100% O_2*, *50% O_2* and *0% O_2* samples). All diversity statistics were calculated using a 97% sequence similarity outOTU threshold. Richness was calculated using the number of OTUs and Chao1 estimators. Diversity was estimated from Shannon index.

Condition	initial			anoxia			50% oxique			100% oxique		
number of sequence per sample	449757	417808	334196	358108	388020	361316	404349	354278	356962	365926	382832	361921
Observed OTUs	310	306	311	308	288	290	313	316	315	314	317	315
chao1 estimation	316	320	322	311	291	299	313	316	315	314	317	315
Shannon index	5.00	4.99	4.99	2.77	2.17	2.58	4.40	4.48	4.60	4.04	4.06	4.00

Table S2. Oxidation state of U in the solid determined by LC-LS fit of XANES data at the U L_{III}-edge. The uncertainties on the measured values are given under brackets and refer to the last digit (see text).

Sample	U(IV) (%)	U(VI) (%)	Sum (%)	R-factor	Chi^2 R
Fresh sediment T0	86(21)	14(21)	100	6.88E-5	2.29E-2
Fresh sediment 0% O_2 - T3 weeks	90(24)	11(24)	101	8.79E-5	2.95E-2
Fresh sediment 50% O_2 - T3 weeks	100(0)	1(2)	101	1.31E-4	4.29E-2
Fresh sediment 100% O_2 - T3 weeks	100(0)	1(2)	101	1.96E-4	6.41E-2
Irradiated sediment T0	65(22)	35(22)	100	7.38E-5	2.46E-2
Irradiated sediment 0% O_2 - T3 weeks	51(21)	49(21)	100	6.98E-5	2.33E-2
Irradiated sediment 50% O_2 - T3 weeks	97(28)	3(28)	100	1.29E-4	4.20E-2
Irradiated sediments 100% O_2 - T3 weeks	98(24)	2(24)	100	8.75E-5	2.85E-2

Table S3. Formation constants of minerals and complexes and their relevant reactions used for this study.

Reaction	log K (25°C)	Ref.
Aqueous species		
$UO_{2(s)} + 4H^+ = U^{4+} + 2H_2O$	0.1	
$U^{4+} + 4CO_3^{2-} = U(CO_3)_4^{4-}$	32.9	
$U^{4+} + 5CO_3^{2-} = U(CO_3)_5^{6-}$	34.0	Wateq4f database
$U^{4+} + 3H_2O = U(OH)^{3+} + 3H^+$	-4.935	
$U^{4+} + 2H_2O = U(OH)_2^{2+} + 2H^+$	-2.27	
$U^{4+} + 4H_2O = U(OH)_4^{(aq)} + 4H+$	-8.498	
$U^{4+} + HHumic^- = Uhumic^{2+} + H^+$	18.1 (pH = 5.50) 19.4 (pH = 5.90) 19.6 (pH = 5.98) 19.8 (pH = 6.02) 23.0 (pH = 7.00) 24.3 (pH = 7.40)	3,4
$UO_2^{2+} + 4H^+ + 2e- = U^{4+} + 2H_2O$	9.04	
$UO_2^{2+} + CO_3^{2-} = UO_2(CO_3)^{(aq)}$	9.63	
$UO_2^{2+} + 2CO_3^{2-} = UO_2(CO_3)_2^{2-}$	17.00	
$UO_2^{2+} + 3CO_3^{2-} = UO_2(CO_3)_3^{4-}$	21.63	
$UO_2^{2+} + H_2O = UO_2(OH)^+ + H^+$	-5.2	
$UO_2^{2+} + 3H_2O = UO_2(OH)_3^- + 3H^+$	-19.2	Wateq4f database
$UO_2^{2+} + 4H_2O = UO_2(OH)_4^{2-} + 4H^+$	-33.0	
$2UO_2^{2+} + H_2O = (UO_2)_2(OH)_2^{3+} + H^+$	-2.7	
$3UO_2^{2+} + 6CO_3^{2-} = (UO_2)_3(CO_3)_6^{6-}$	54.00	
$3UO_2^{2+} + 4H_2O = (UO_2)_3(OH)_4^{2+} + 4H^+$	-11.90	
$3UO_2^{2+} + 5H_2O = (UO_2)_3(OH)_5^+ + 5H^+$	-15.55	
$3UO_2^{2+} + 7H_2O = (UO_2)_3(OH)_7^- + 7H^+$	-31.00	Wateq4f database
$4UO_2^{2+} + 7H_2O = (UO_2)_4(OH)_7^+ + 7H^+$	-21.90	
$Ca^{2+} + UO_2^{2+} + 3CO_3^{2-} = CaUO_2(CO_3)_3^{2-}$	27.18	1
$2Ca^{2+} + UO_2^{2+} + 3CO_3^{2-} = Ca_2UO_2(CO_3)_3^{(aq)}$	30.70	1
$UO_2^+ + 3CO_3^- + e- = UO_2(CO_3)_3^{5-}$	7.43	Wateq4f database
$UO_2^{2+} + e- = UO_2^+$	1.49	

$UO_2^{2+} + Hhumic^- = UO_2Humic^+ + H^+$	-0.5 (pH = 5.50) 0.1 (pH = 5.90) 0.2 (pH = 5.98) 0.3 (pH = 6.02) 1.7 (pH = 7.00) 2.4 (pH = 7.40)	3,4
Minerals		
Uraninite + $4H^+ = U^{4+} + 2H_2O$	-4.8	
$UO_2(a) + 4H+ = U^{4+} + 2H_2O$	0.1	
$Fe_{2+} = Fe^{3+} + e-$	-13.02	Wateq4f database
$Fe(OH)_3 + 3H^+ = Fe^{3+} + 3H_2O$	4.89	
Calcite = $Ca^{2+} + CO_3^{2-}$	-8.48	

Table S4. Calculation of aqueous U(IV) and U(VI) speciation and determination of major and minor U(IV) and U(VI) complexes at each set of experimental conditions with fresh sediments.

Dissolved species	Molality (M)	Percentage for each U(IV) and U(VI)-complex
	0% O_2[1]	
Total U	$6.7.10^{-8}$	100
Total $HHumic^-$	$8.3.10^{-4}$	-
Total Ca^{2+}	$1.1.10^{-4}$	-
Total HCO_3^-	$7.7.10^{-4}$	-
Total Fe	$9.3.10^{-5}$	
$CaUO_2(CO_3)^{2-}$	**$4.8.10^{-8}$**	**71.9**
$UHumic^{2+}$	$1.2.10^{-8}$	18.1
$UO_2(CO_3)_2^{2-}$	$5.2.10^{-9}$	7.7
	50% O_2[2]	
Total U	$1.6.10^{-8}$	100
Total $HHumic^-$	$2.8.10^{-5}$	-
Total Ca^{2+}	$1.2.10^{-4}$	-
Total HCO_3^-	$6.9.10^{-5}$	-
Total Fe	<	
UO_2CO_3	**$1.2.10^{-8}$**	**77.1**
$UO_2(OH)^+$	$1.5.10^{-9}$	9.6
UO_2^{2+}	$9.9.10^{-10}$	6.3
$UO_2(CO_3)_2^{2-}$	$9.5.10^{-10}$	6.0
	100% O_2[3]	
Total U	$4.7.10^{-8}$	100
Total $HHumic^-$	$3.4.10^{-5}$	-
Ca^{2+}	$1.4.10^{-4}$	-
HCO_3^-	$1.6.10^{-4}$	-
Total Fe	<	
UO_2CO_3	**$3.3.10^{-8}$**	**69.6**
$UO_2(CO_3)_3^{2-}$	$8.7.10^{-9}$	18.6
$UO_2(OH)^+$	$3.5.10^{-9}$	7.4
$CaUO_2(CO_3)_3^{2-}$	$8.1.10^{-10}$	1.7

Note : pH = [1]7.4, [2]5.5, [3]5.9 , < detection limit

Table S5. Calculation of aqueous U(IV) and U(VI) speciation and determination of major and minor U(IV) and U(VI) complexes at each set of experimental conditions with irradiated sediments.

Dissolved species	Molality (M)	Percentage for each U(IV) and U(VI)-complex
	0% O_2[1]	
Total U	$1.6.10^{-7}$	100
Total $HHumic^-$	$8.6.10^{-4}$	-
Total Ca^{2+}	$1.0.10^{-4}$	-
Total HCO_3^-	$1.0.10^{-3}$	-
Total Fe	$1.1.10^{-4}$	-
$CaUO_2(CO_3)_3^{2-}$	**$7.1.10^{-8}$**	**45.2**
$UO_2(CO_3)_2^{2-}$	$4.8.10^{-8}$	30.6
$UHumic^{2+}$	$1.8.10^{-8}$	11.4
UO_2CO_3	$9.7.10^{-9}$	6.1
UO_2Humate	$8.2.10^{-9}$	5.2
	50% O_2[2]	
Total U	$1.3.10^{-7}$	100
Total $HHumic^-$	$5.4.10^{-5}$	-
Total Ca^{2+}	$1.0.10^{-4}$	-
Total HCO_3^-	$6.2.10^{-4}$	-
Total Fe	$1.6.10^{-5}$	
UO_2CO_3	**$9.1.10^{-8}$**	**69.8**
$UO_2(OH)^+$	$1.6.10^{-8}$	12.0
$UO_2(CO_3)_2^{2-}$	$2.9.10^{-8}$	1.6
UO_2Humate	$4.4.10^{-9}$	3.4
	100% O_2[3]	
Total U	$1.6.10^{-7}$	100
Total $HHumic^-$	$6.1.10^{-5}$	-
Total Ca^{2+}	$7.5.10^{-5}$	-
Total HCO_3^-	$7.6.10^{-4}$	-
Total Fe	$2.2.10^{-5}$	
UO_2CO_3	**$1.1.10^{-7}$**	**68.8**
$UO_2(OH)^+$	$1.9.10^{-8}$	11.9
$UO_2(CO_3)_2^{2-}$	$1.9.10^{-8}$	11.8
UO2Humate	$7.5.10^{-9}$	4.7

Note : pH = [4]7.0 [5]5.98, [6]6.02 , < detection limit

References

1. W. Dong, S. C. Brooks, Determination of the formation constants of ternary complexes of uranyl and carbonate with alkaline earth metals (Mg2+, Ca2+, Sr2+, and Ba2+) using anion exchange method, *Environ. Sci. Technol.,* 2006, 40, 4689-95.
2. J. Oksanen, F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, P. R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs, H. Wagner, Vegan: Community Ecology Package. R package version 2.4-5, 2017, https://CRAN.R-project.org/package=vegan.
3. P. E. Reiller, N. D. M. Evans, G. Szabo, Complexation parameters for the actinides(IV)-humic acid system: a search for consistency and application to laboratory and field observations, 2008, *Radiochim. Acta,* **2008**, 96, 345-358.
4. P. Warwick, N. Evans, A. Hall, G. Walker, E. Steigleder, Stability constants of U(VI) and U(IV)-humic acid complexes, *J. Radioanal. Nucl. Chem.,* **2005**, 266, 179-190.