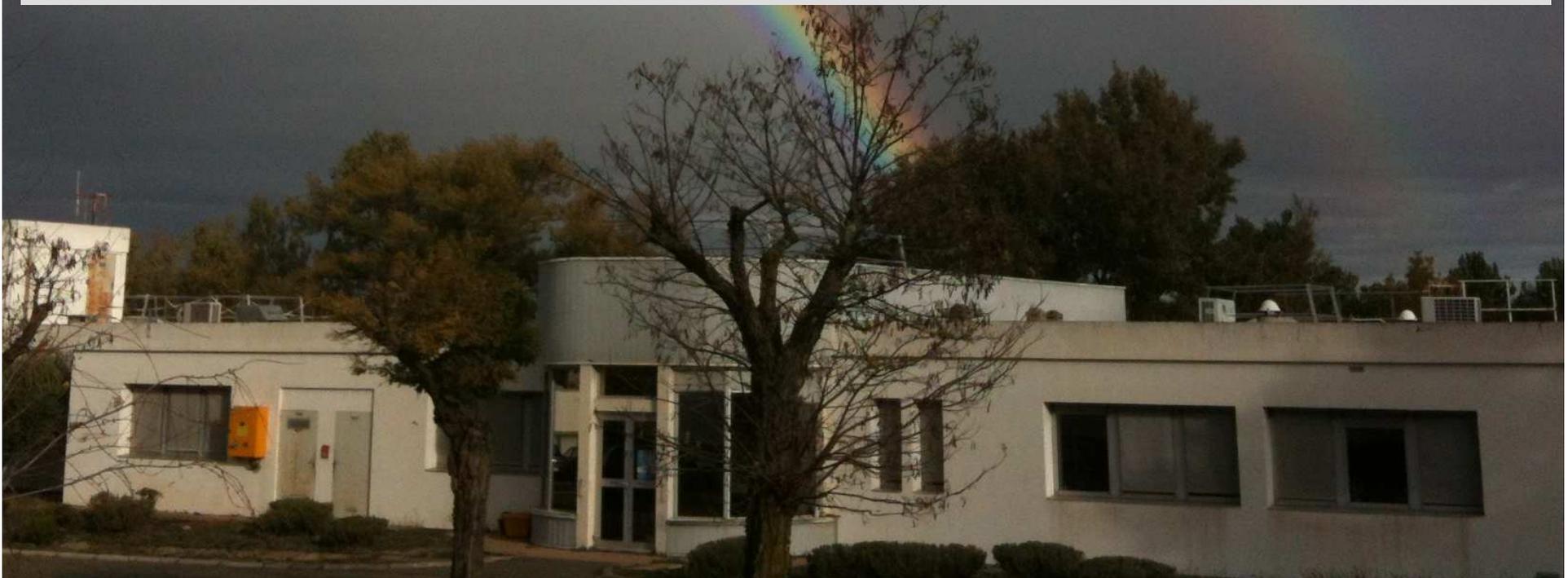


Virginie Chapon

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UMR 7265 - CEA Cadarache - France

Les communautés bactériennes de sols uranifères naturels : impact de l'uranium sur la diversité



A toxic element widespread in the environment

Anthropogenic uranium is widely disseminated in the environment (nuclear industry, mining activities, use of phosphate fertilizers).



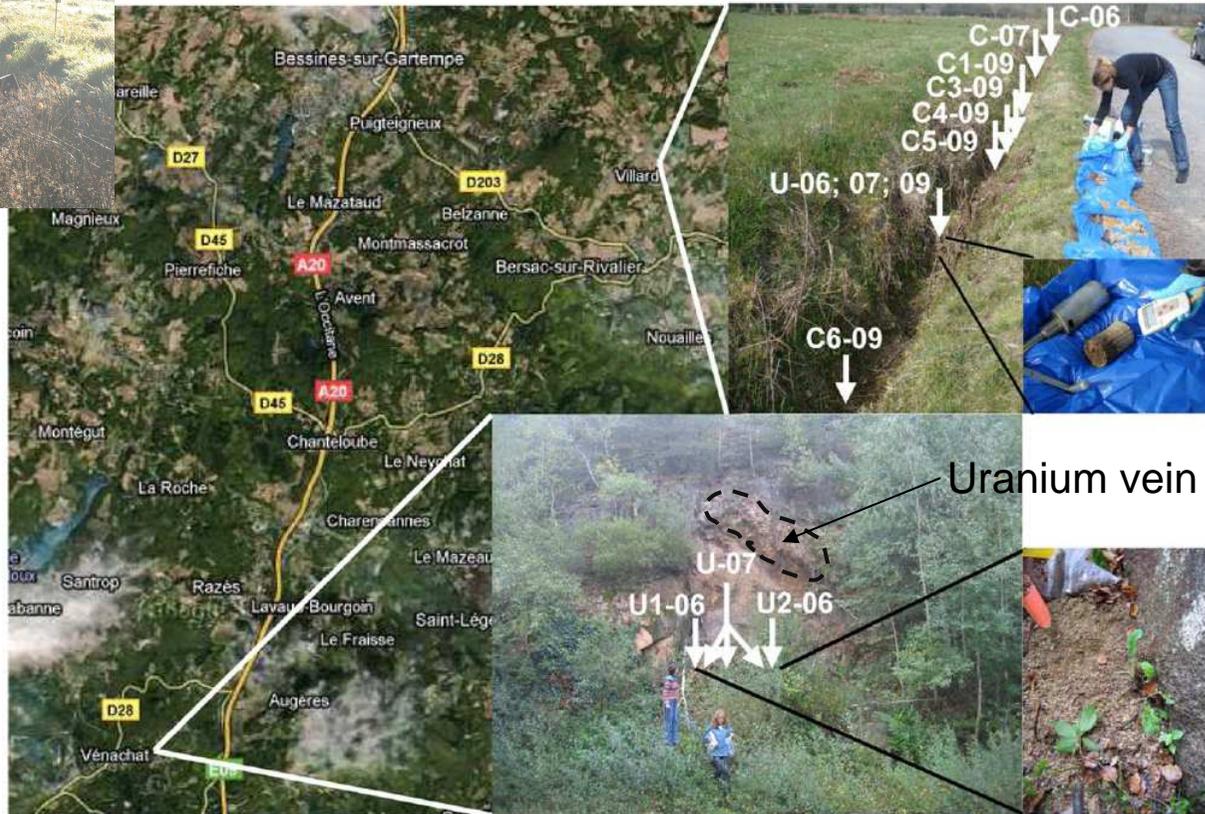
Occurs naturally in some minerals (uranium ores)



Bacterial diversity in natural uranium ores ?
Impact of uranium on soil bacterial communities in
natural conditions ?
Toxic effect ?

Sampling sites : Bessines (Limousin)

natural uranium ores



Villard

Clays
ditch along a field

Vénachat

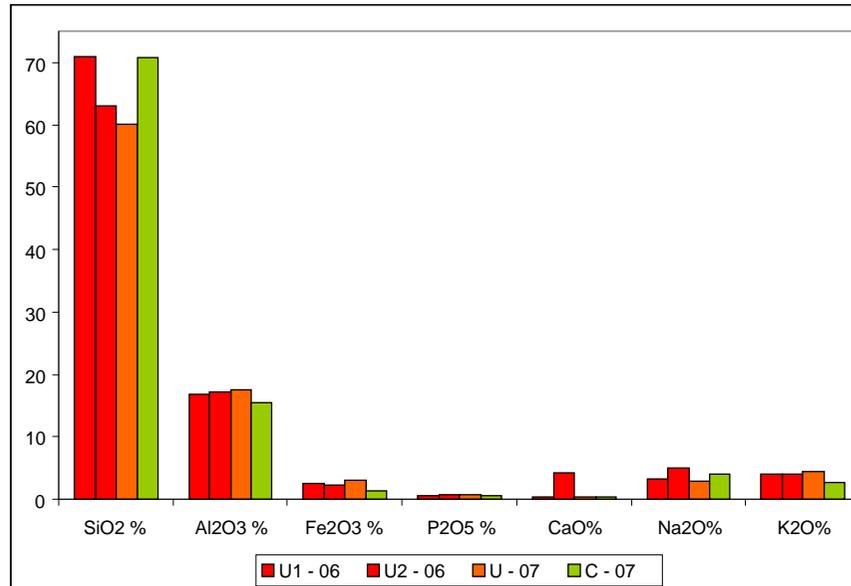
Granitic sand

Collection of radioactive samples

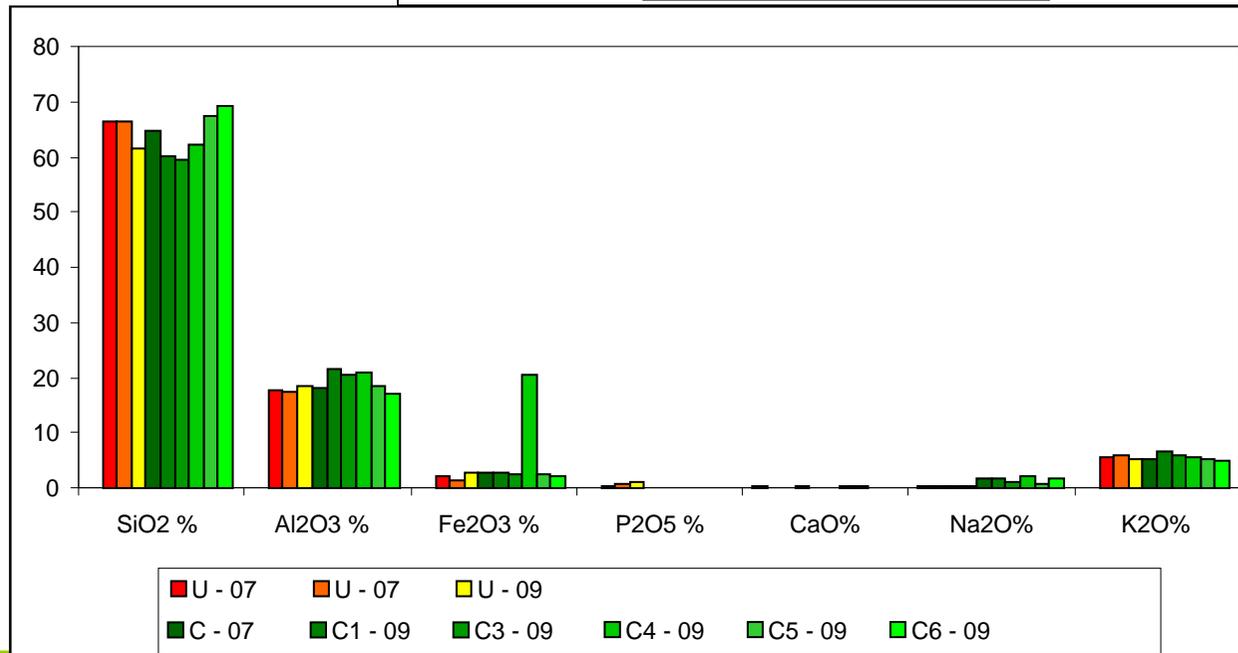
Collection of non-radioactive controls in close vicinity <5 m

Mondani *et al.* (2011) PLoS ONE

Physicochemical characteristics of the soil samples



Vénachat



Villard

Physicochemical characteristics of the soil samples

Sol	TOC %	pH	CFU/g soil (x10 ⁵)
C - 06	0.56	-	2.25
U1 - 06	-	-	4.18
U2 - 06	0.52	-	2.2
C - 07	1.35	4.8	3.8
U - 07	1.38	4.4	12
Sol	TOC %	pH	CFU/g soil (x10 ⁵)
C - 06	0.61	4.7	-
U - 06	0.43	4.7	-
C - 07	0.29	3.8	5
U - 07	0.17	3.8	6.8
C1 - 09	0.12	5.5	11.7
C3 - 09	0.25	5.5	15
C4 - 09	0.45	5.8	16.9
C5 - 09	0.13	5.8	20.7
C6 - 09	0.15	5.8	21.9
U - 09	0.48	5	9

Vénachat

Villard

✓ no major differences between radioactive and control samples.

Physicochemical characteristics of the soil samples

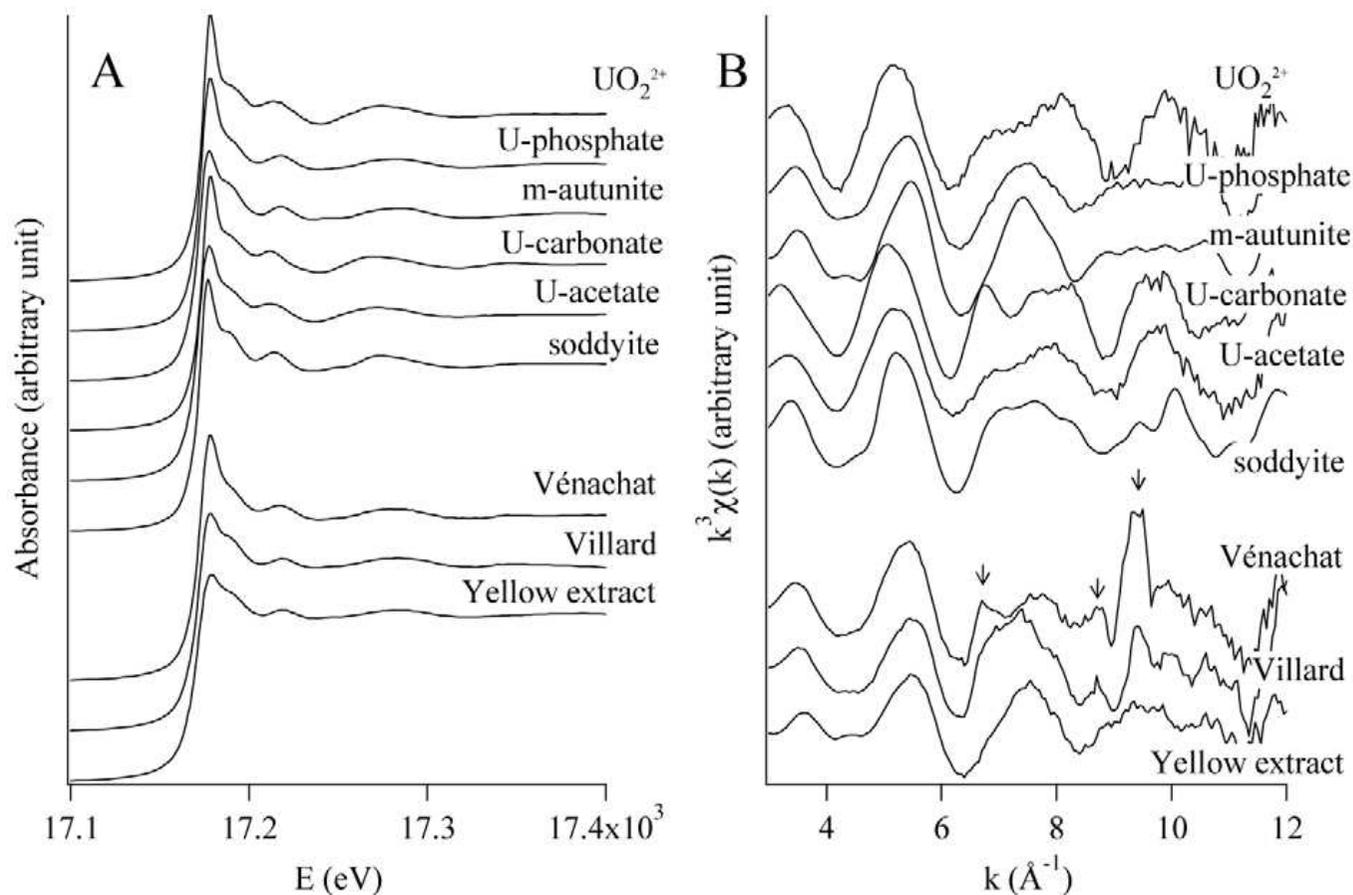
Sol	Uranium total (ppm)
C - 06	30
U1 - 06	2,617
U2 - 06	2,140
C - 07	71
U - 07	4,718
Sol	Uranium total (ppm)
C - 06	27
U - 06	37,768
C - 07	74,5
U - 07	14,860
C1 - 09	<30
C3 - 09	<30
C4 - 09	<30
C5 - 09	124
C6 - 09	<30
U - 09	255,000

Vénachat

✓ contrasted uranium concentrations between U and C samples

Villard

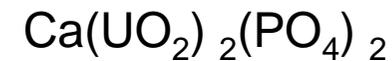
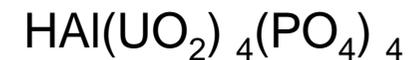
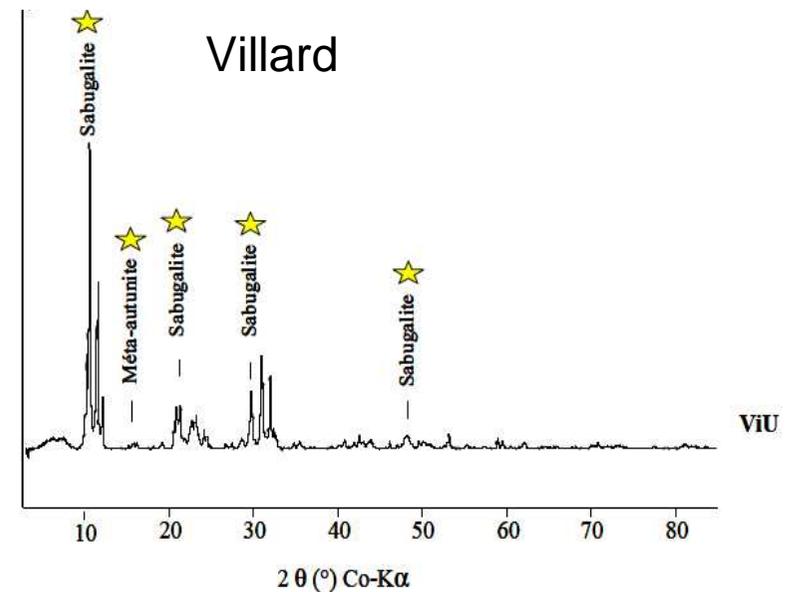
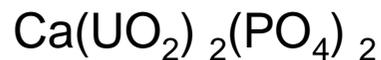
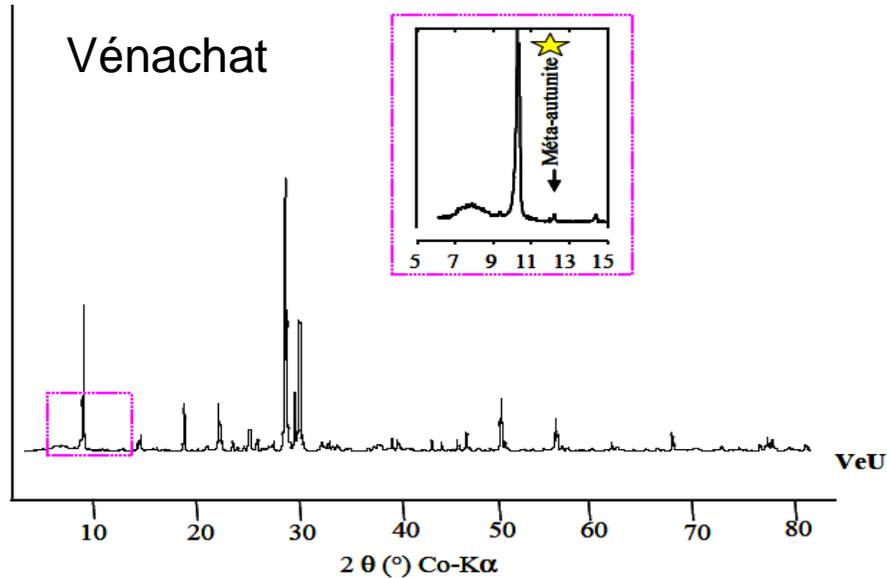
Uranium speciation in soils



✓XANES region: U(VI)

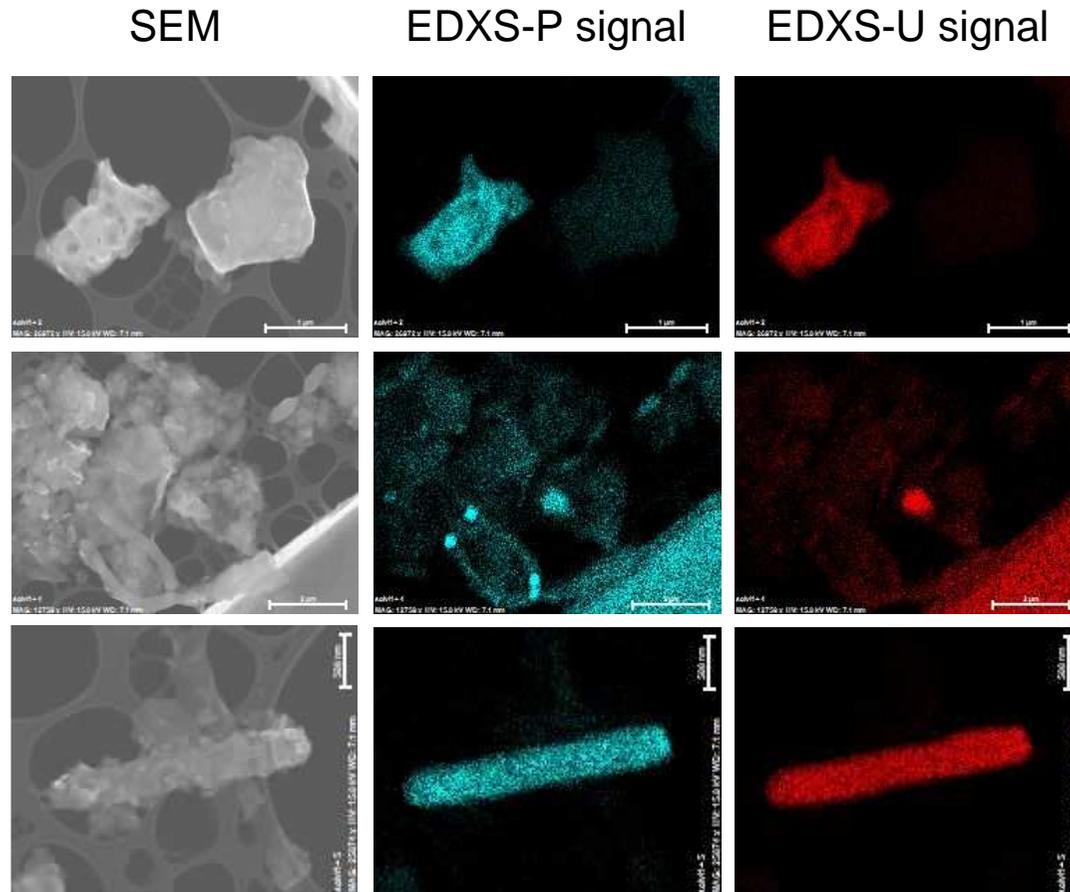
✓EXAFS: U-phosphate and non-identified species

Mineralogical analysis : XRD



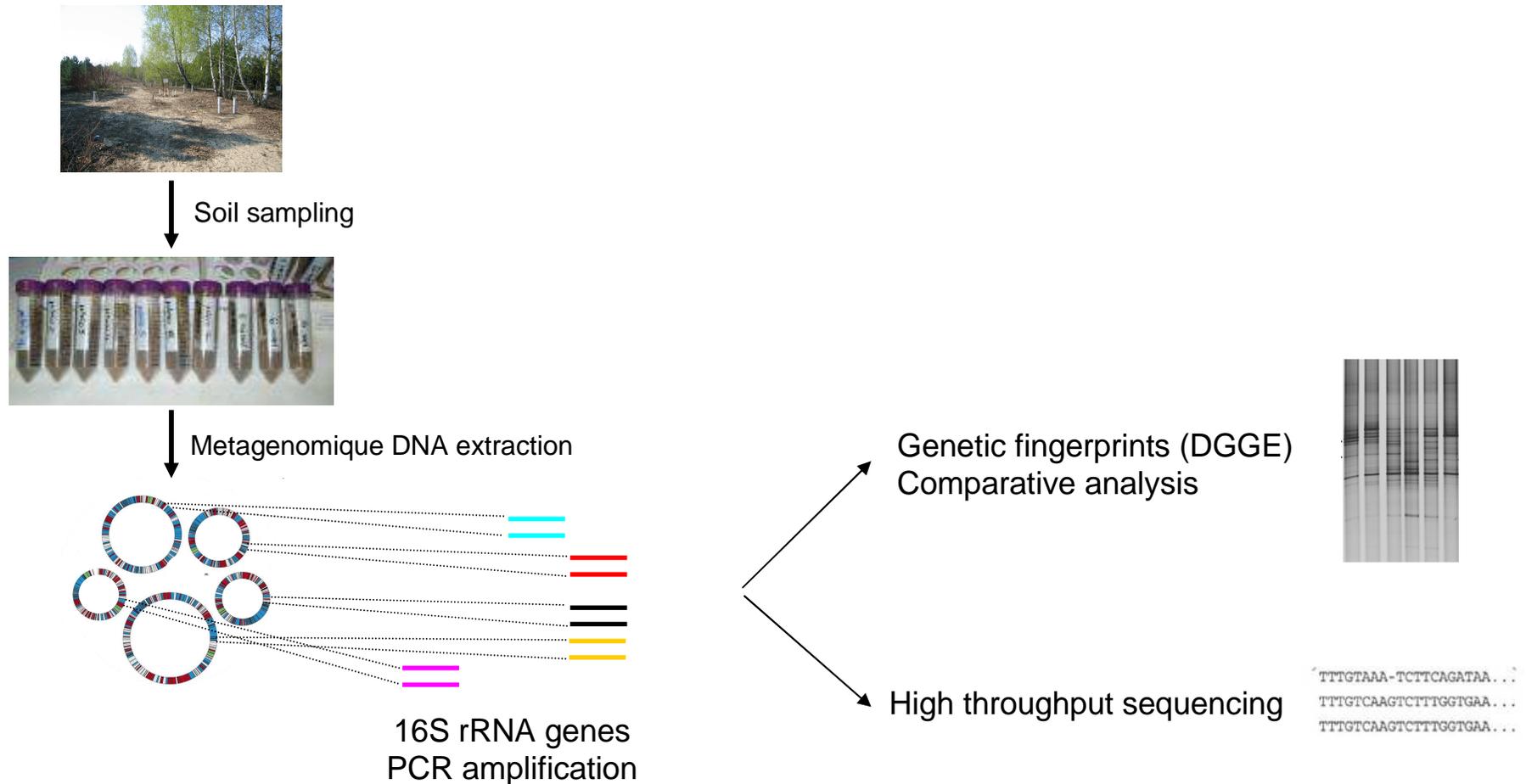
- ✓ meta-autunite and sabugalite belong to the mineral family of uranium-phosphate
- ✓ bioavailability of uranium in soils ?

Microscopic observations of Villard soil particles

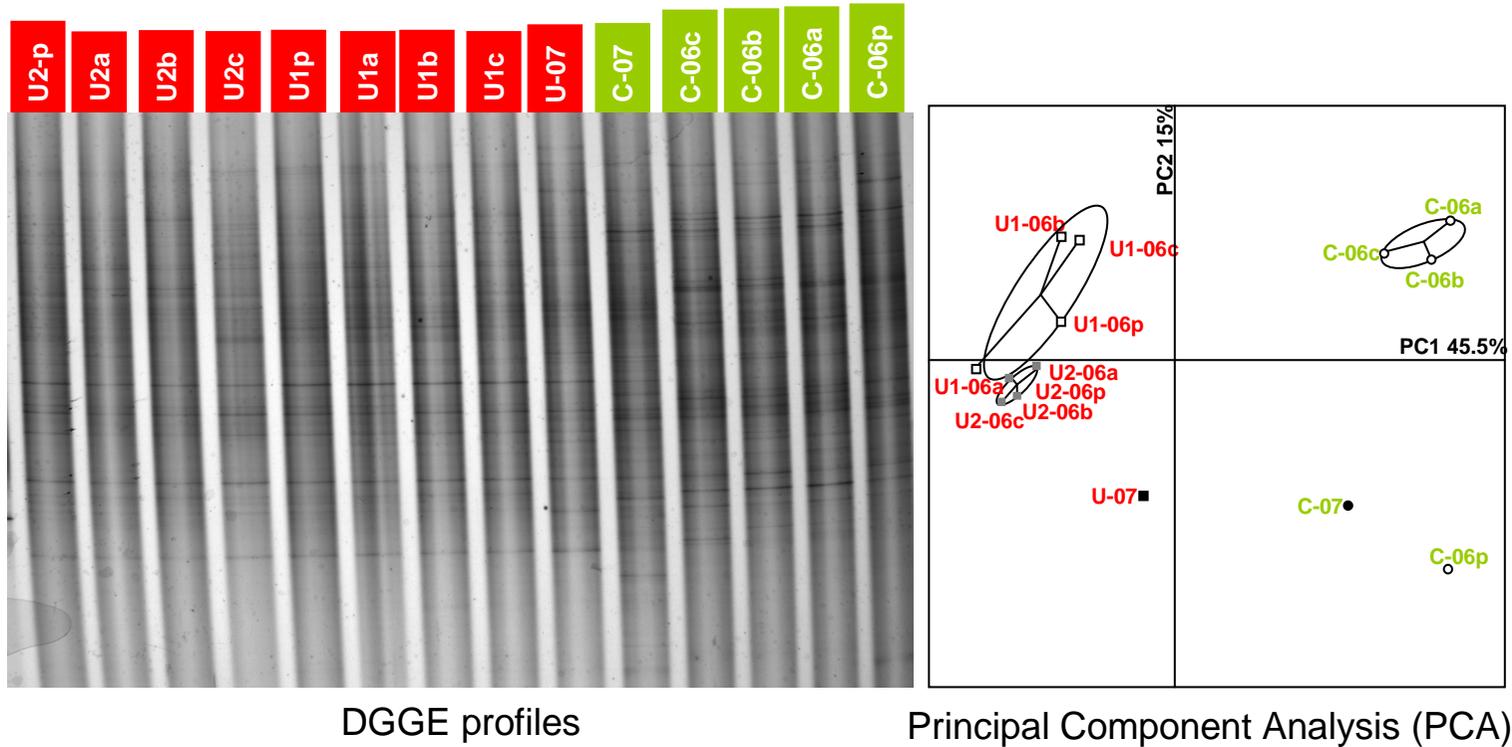


✓ bacteria are exposed to uranium *in situ*

Molecular approaches to study bacterial communities

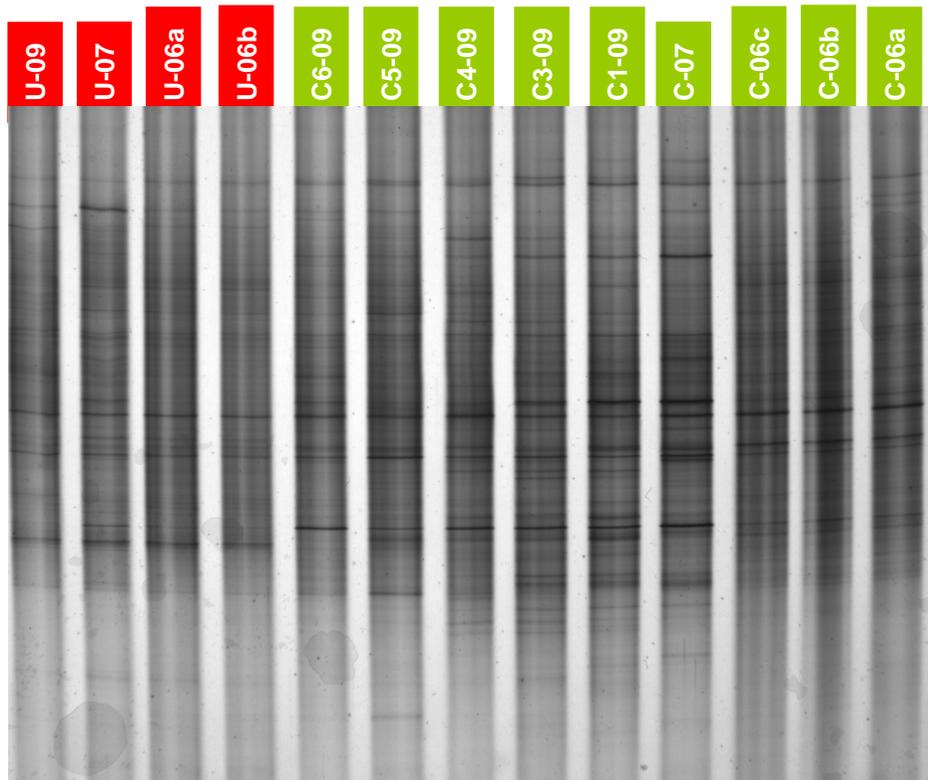


Bacterial community analysis (Vénachat)

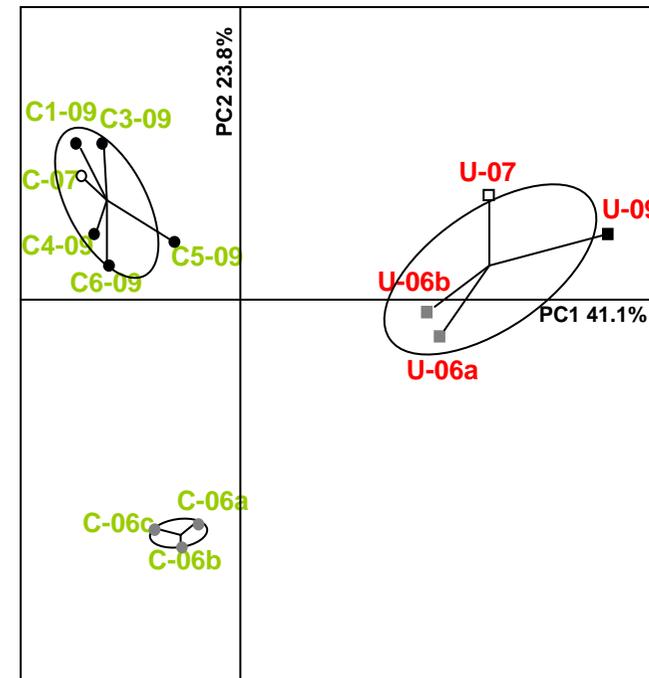


- ✓ high diversity in all samples
- ✓ U and C profiles clearly separated on the PCA

Bacterial community analysis (Villard)



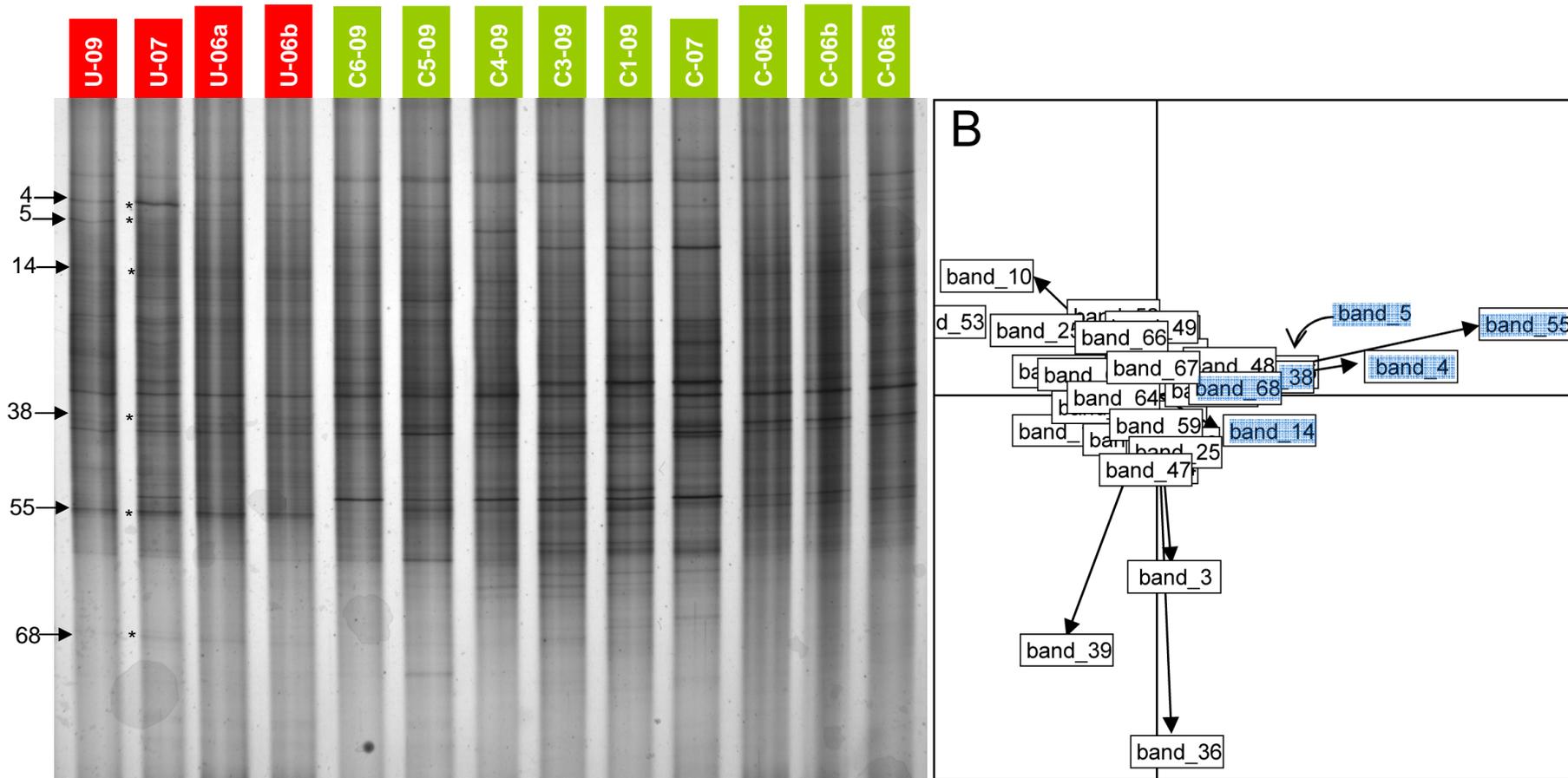
DGGE profiles



Principal Component Analysis (PCA)

- ✓ U-specific bacterial community
- ✓ Highly stable

Identification of uranium-specific bacteria



Identification of some uranium-specific bacteria

DGGE band	Closest relatives (Silva 104)		
	similarity (%)	Taxonomy	
B4_U09_02; 16	99.1-98.2	Acidobacteria	Geothrix Geobacteraceae
B14_U07_16	98.7	Acidobacteria	
B5_U07_12	91.3	Deltaproteobacteria	
B14_U07_05	91.3	Deltaproteobacteria	
B4_U09_13	97.8	Deltaproteobacteria	
B5_U07_09	98.7	Deltaproteobacteria	
B55_U07_18	94.7	Acidobacteria	DA023
B55_U09_02	99.5	Acidobacteria	DA023
B55_U07_12	99.5	Acidobacteria	DA023
B55_U09_13; 20	92.7	Acidobacteria	DA023
B5_U07_03	96.1	Acidobacteria	DA023
B38_U07_13	98.5	Acidobacteria	DA023
B55_U07_13; B4_U09_03	98.9-98.7	Acidobacteria	DA052
B55_U07_02; 04; 07; 08; 11; 14	99.8-99.1	Acidobacteria	DA052
B55_U09_07; 10; 11	99.6-99.1	Acidobacteria	DA052
B55_U06_04; 07	99.6	Acidobacteria	DA052
B55_U09_01	98.3	Acidobacteria	DA052
B55_U07_01	99.1	Acidobacteria	DA052
B55_U07_16	98.9	Acidobacteria	DA052
B55_U07_19	99.6	Acidobacteria	DA052
B14_U07_03	97.5	Betaproteobacteria	Sideroxydans
B4_U07_E11	98.9	Betaproteobacteria	
B4_U09_08	99.8	Betaproteobacteria	Gallionellaceae
B38_U07_05	94.4	Betaproteobacteria	
B14_U07_04	96.2	Betaproteobacteria	
B4_U07_E16	93.3	Betaproteobacteria	

✓ a redox cycle of uranium in the soil ?

High throughput pyrosequencing (454)



ADN génomique

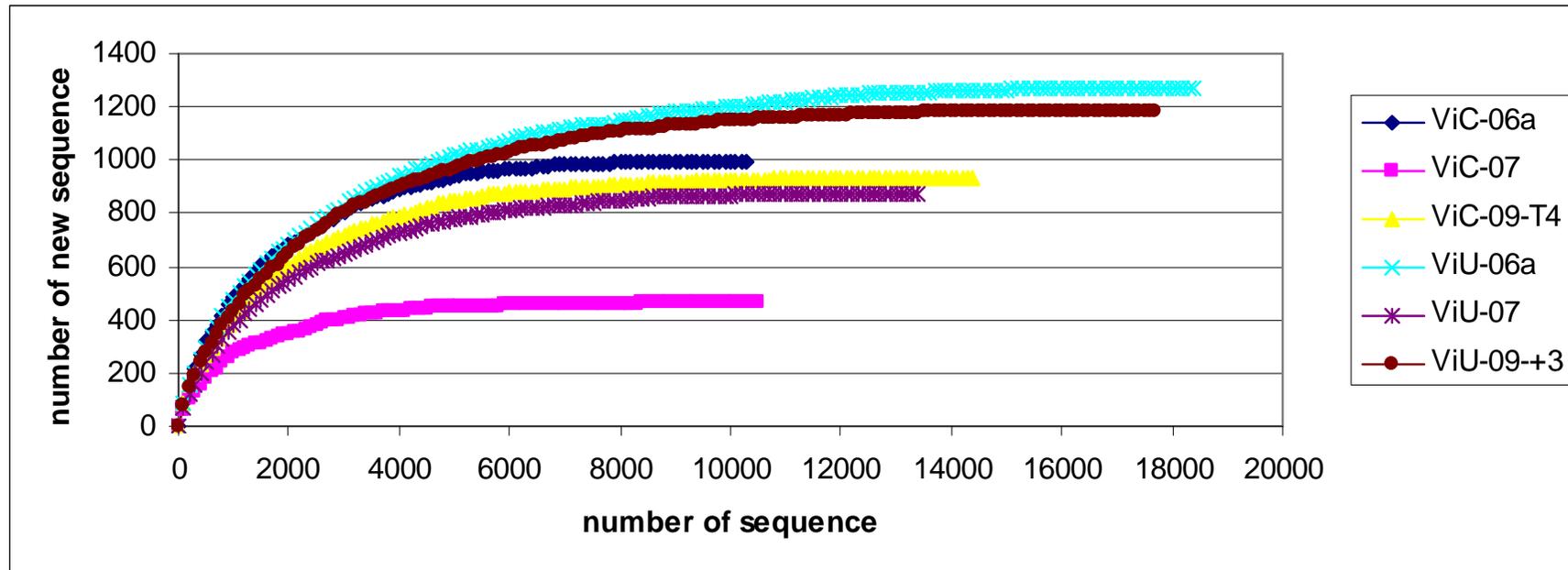


ADNr 16S



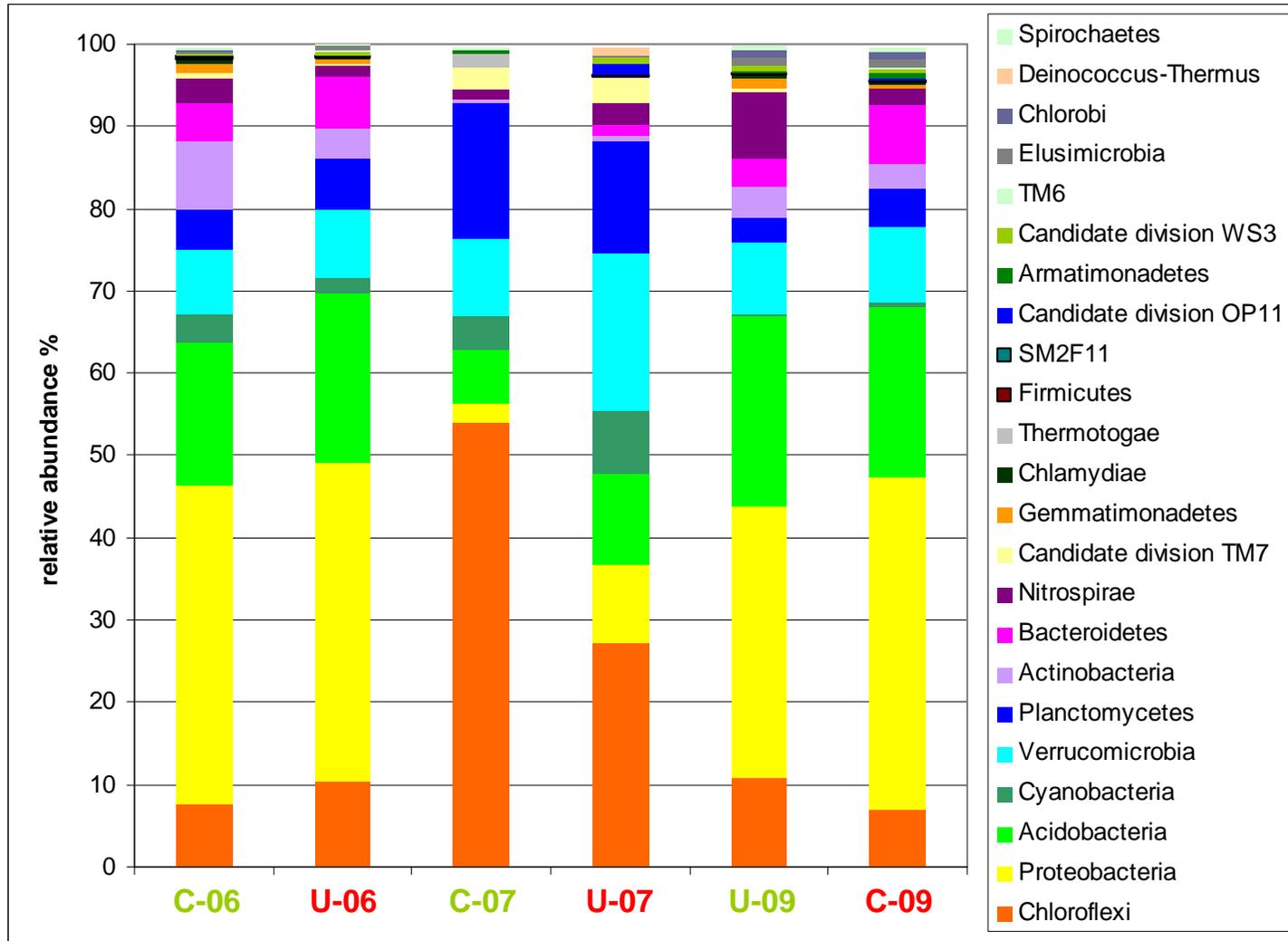
An average of 16,000 sequences per sample

An in-depth analysis of bacterial communities



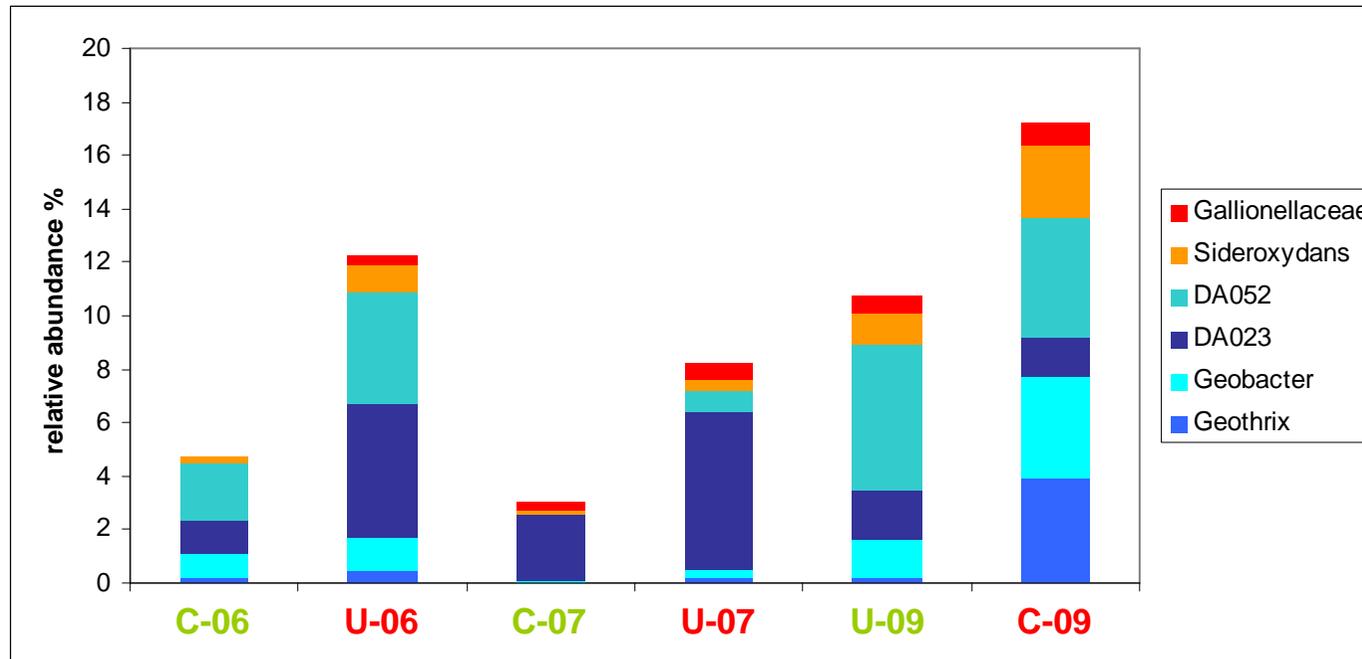
✓ the diversity is saturated

Relative abundance of the 23 phyla detected in the soils



✓ no obvious differences between radioactive and control samples.

Focus on iron-reducing and -oxidizing bacteria



✓ Enrichment in iron-reducing and oxidizing species in the presence of high uranium content

Iron-reducing bacteria are active in the soil samples



iron(III) culture media
10 days incubation

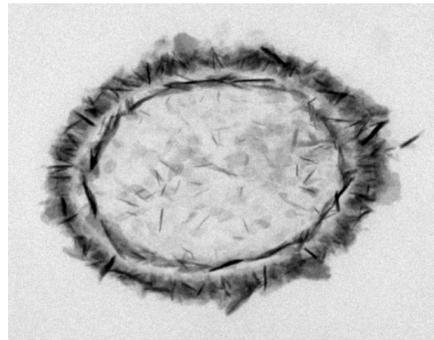
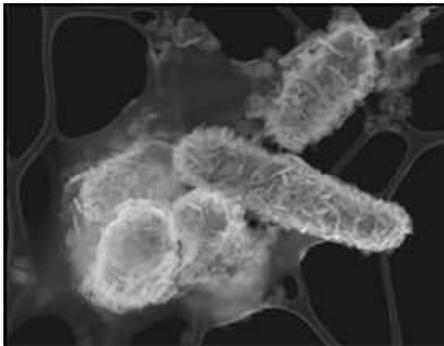
iron(III) culture media
+
uraniferous soil
10 days incubation

Conclusions

- bacterial diversity is not significantly reduced in meta-autunite and sabugalite rich soils,
- enrichment in iron-reducing and iron-oxidizing species is detected in the presence of uranium ,
- these species represent a significant fraction of the total population in uranium rich samples and are active in the soil samples.

Ongoing studies...

A collection of uranium-resistant isolates:
Microbacterium, *Arthrobacter*,
Stenotrophomonas, *Bacillus*...



Biochemical analyses
Comparative genomics



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