

Etude fondamentale et exploitation du microbiote du sol par l'approche metagénomique.

Pascal SIMONET

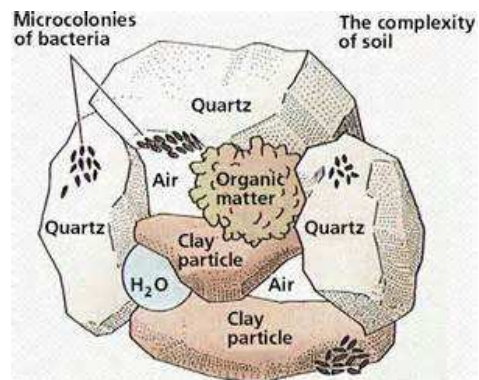


Environmental Microbial Genomics Group
Laboratoire Ampère . UMR CNRS 5005
Ecole Centrale de Lyon . Université de Lyon



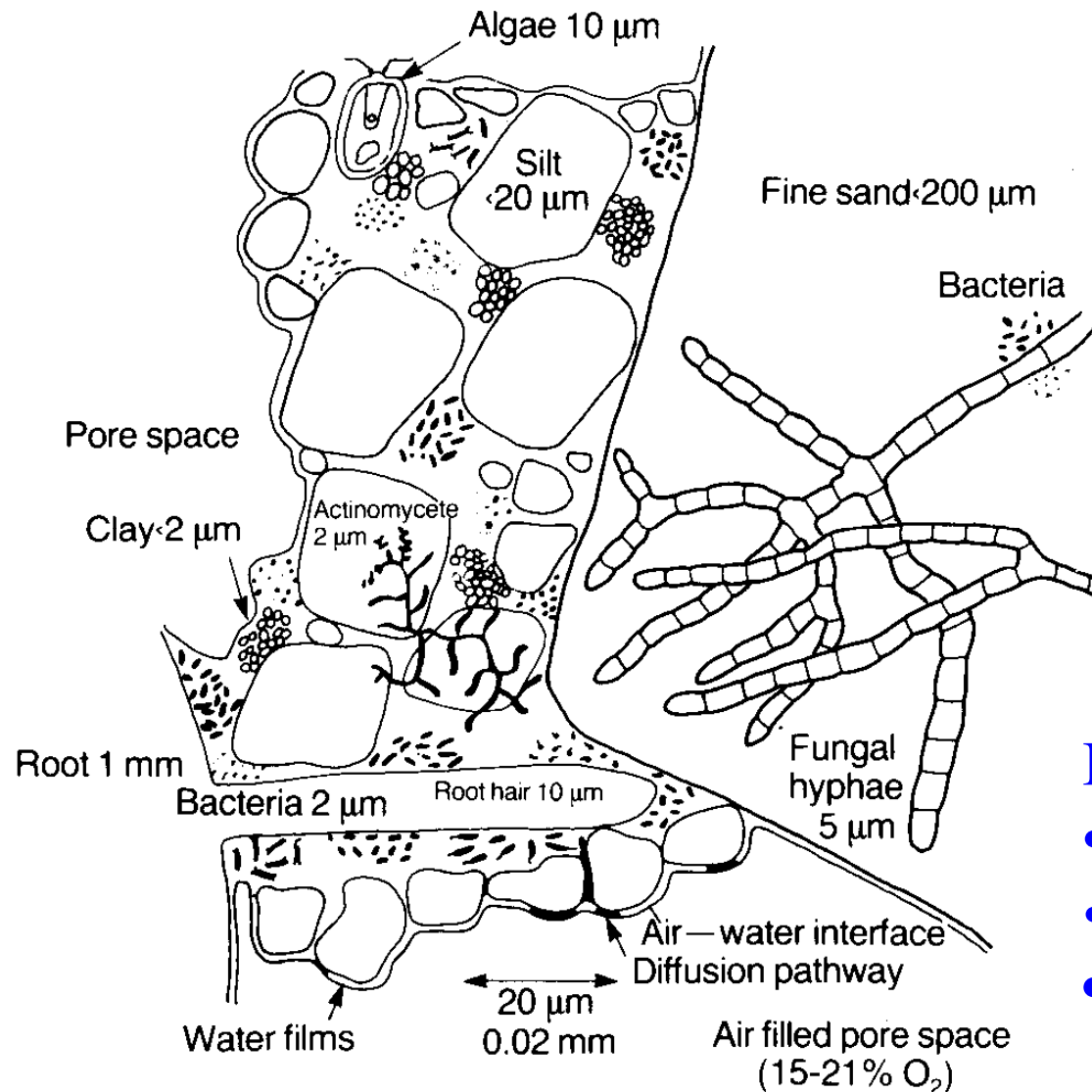
Launch of the Global Soil Biodiversity Atlas in France,
28th November 2016

SOIL





Soil, a Composite of Communities

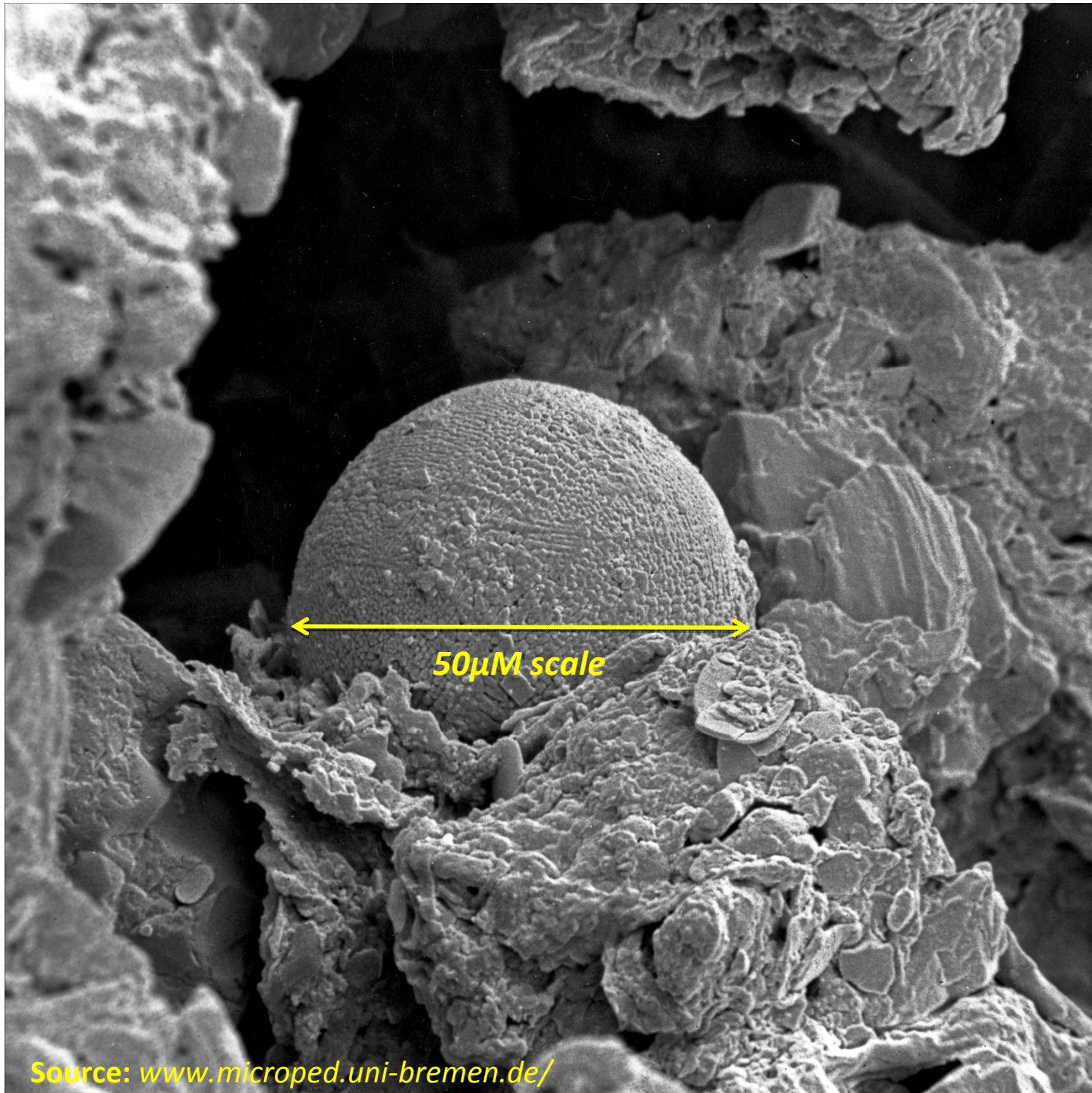


Its very diverse
Complex gradients
Unmixed

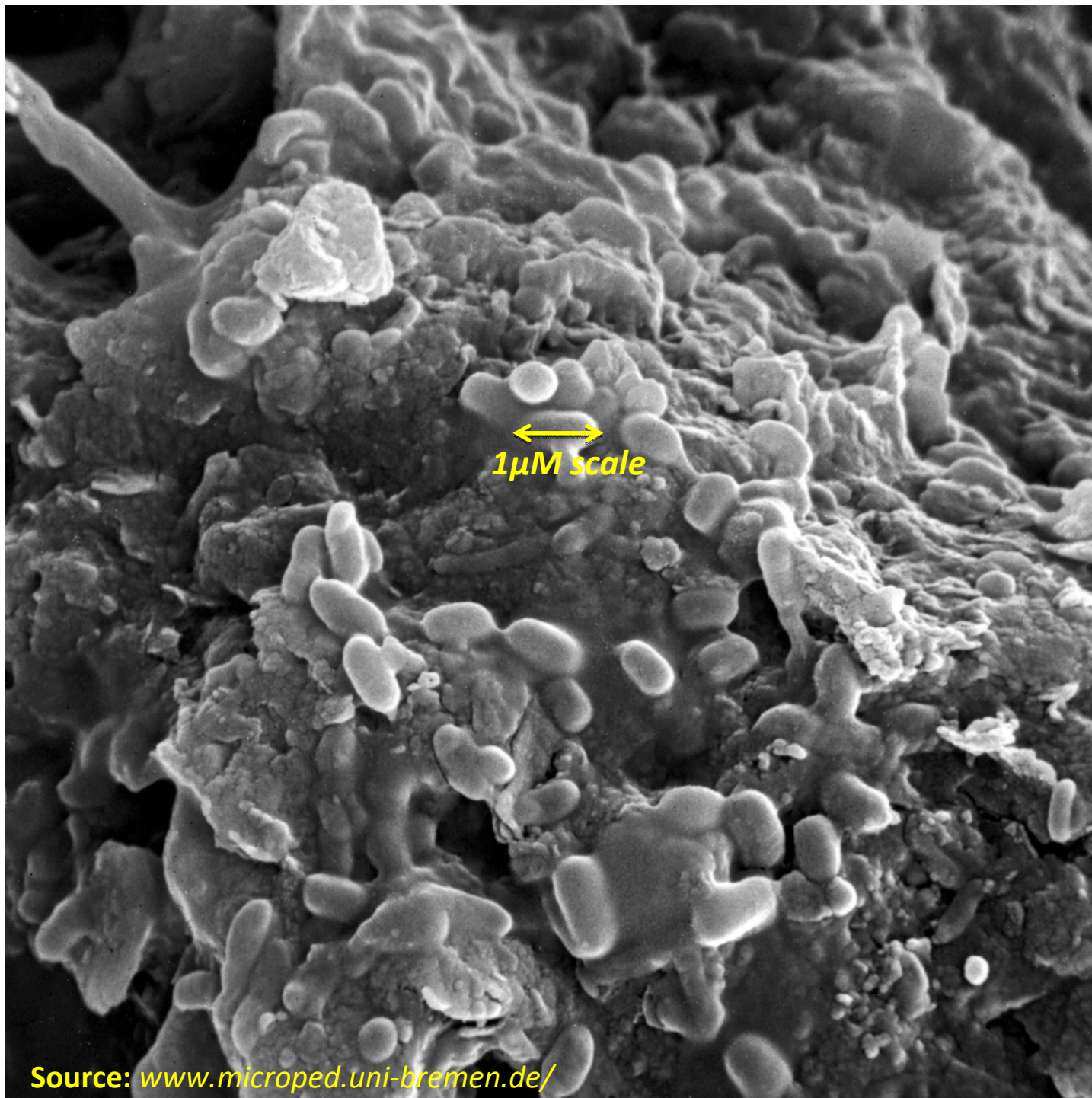
- microaggregates
- rhizosphere
- fungalsphere
- fauna
- pore surfaces
- OM coatings

Implications:

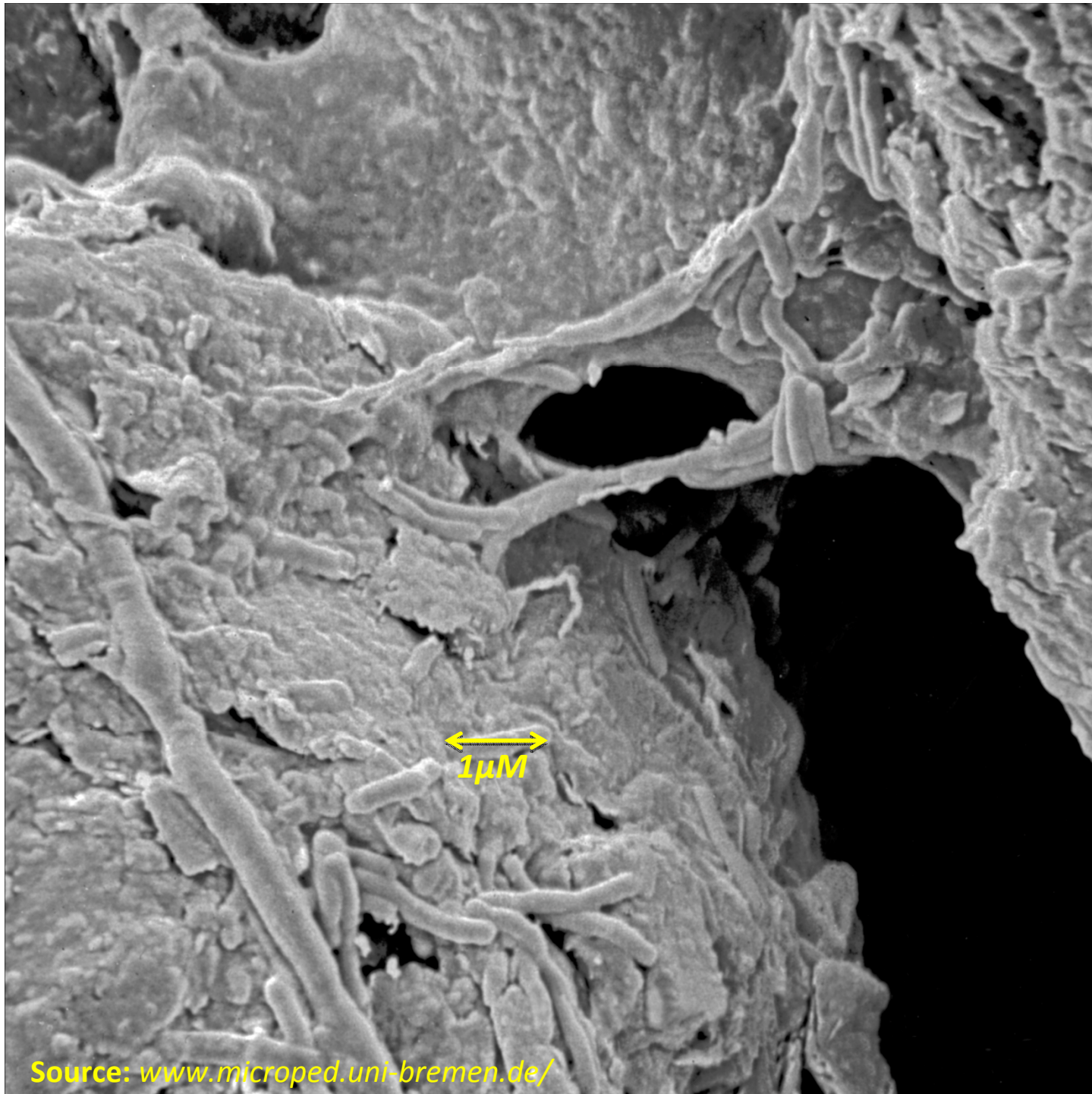
- *Spatial isolation*
- *Minimizes competition*
- *How to sample since multiple communities?*



Source: www.microped.uni-bremen.de/

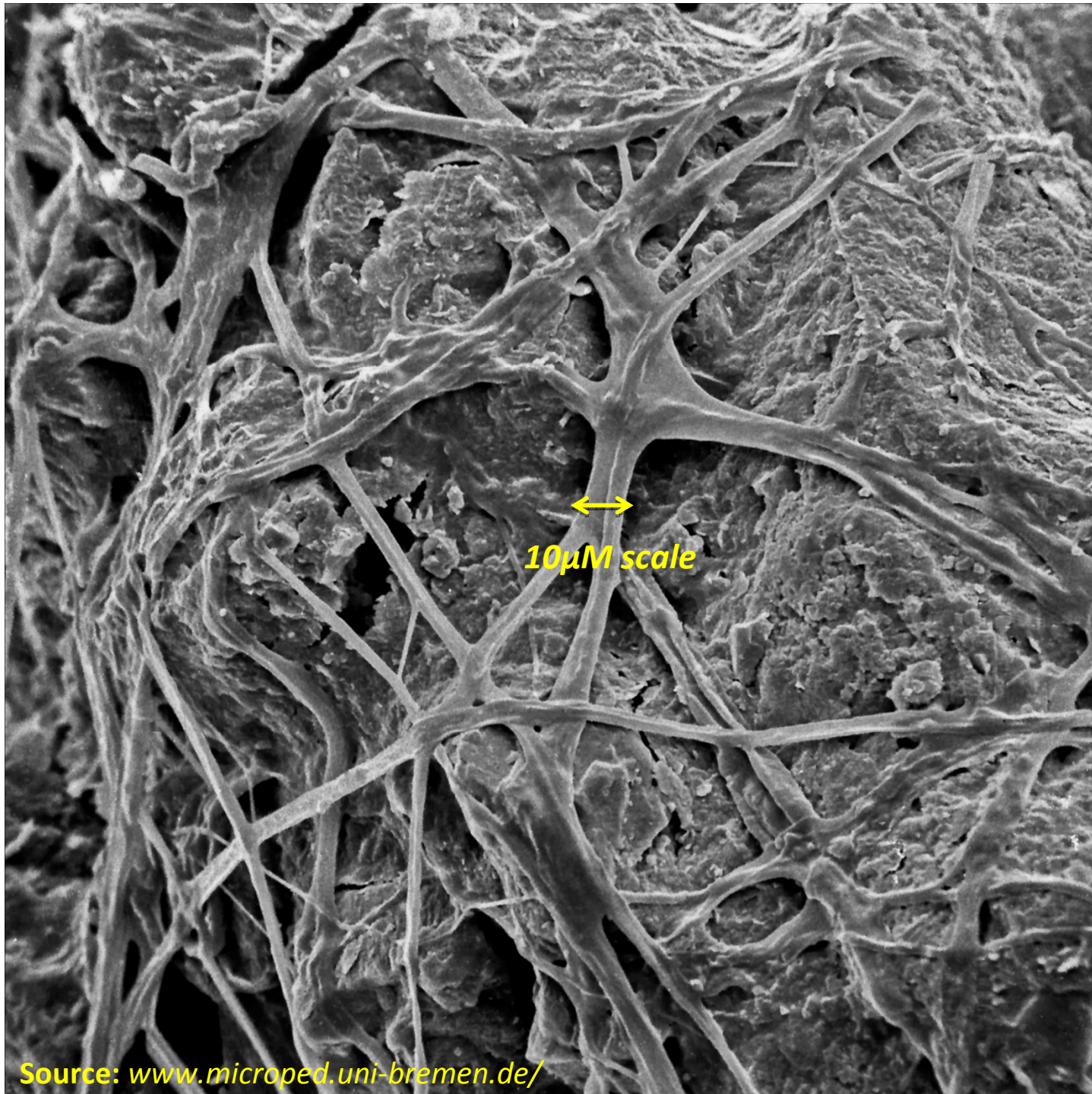


Source: www.microped.uni-bremen.de/



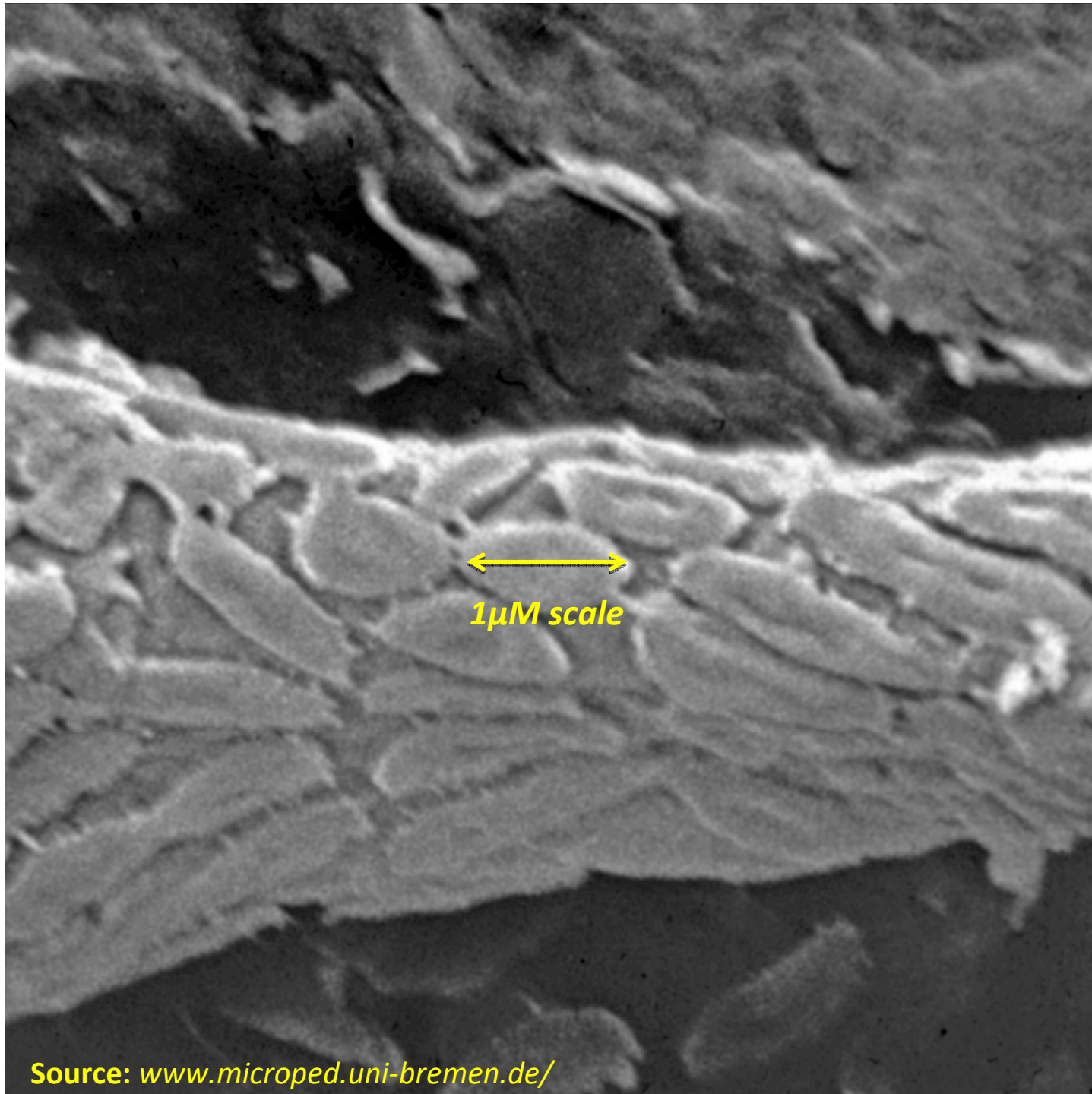
10 μm scale

Source: www.microped.uni-bremen.de/



Source: www.microped.uni-bremen.de/





Source: www.microped.uni-bremen.de/



1 g of soil

Number of bacterial cells : 1 000 000 000

Number of bacterial species:

10 000

Torsvik 2002 (Science)

Roesch 2007 (ISME J.)

>10 000 000

Gans 2005 (Science)

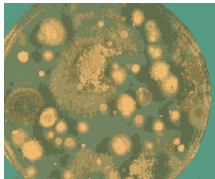


Soil

(meta-) genomics approach

«Environmental DNA»

in vitro
Culture



***in vitro* culture approach**

Cultivable bacteria? From 0,1 to 1%

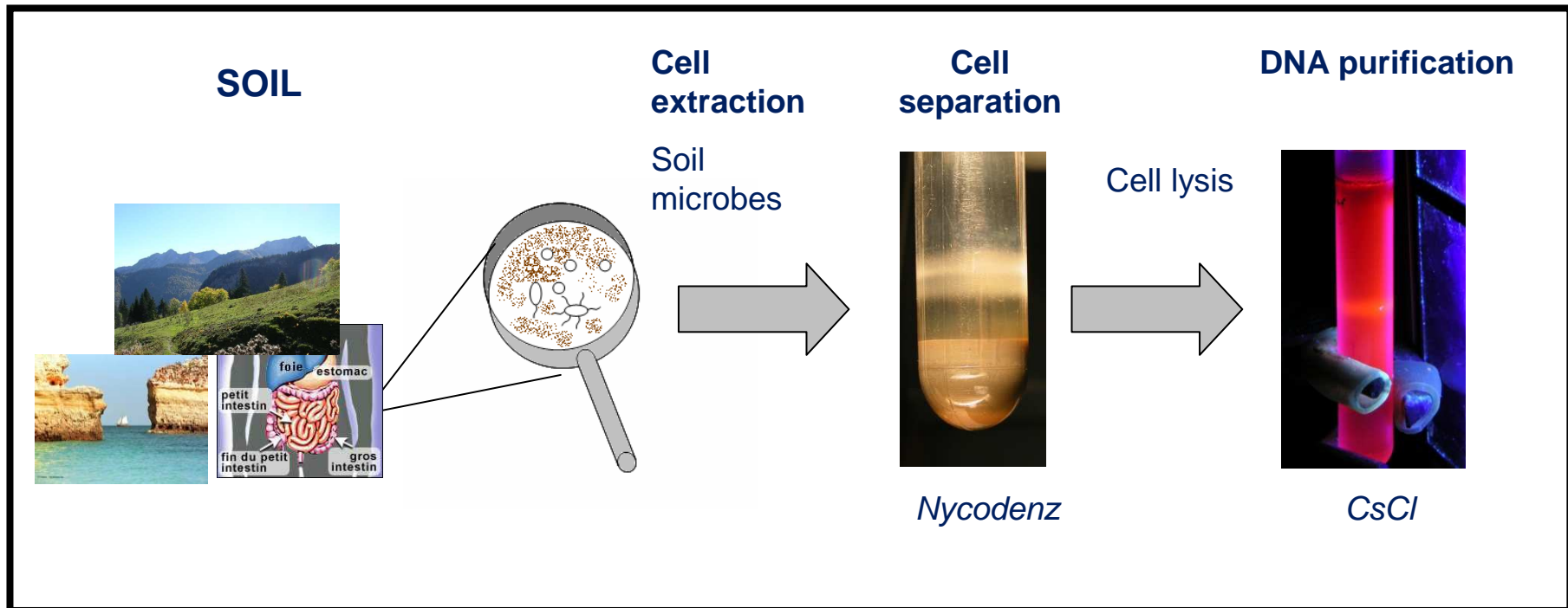


P.C.



Metagenome DNA extraction

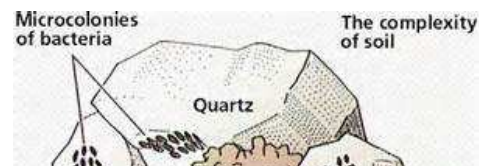
- *In situ* lysis and total DNA extraction
- Cell extraction and lysis





Who is Where doing What ?

**FUNDAMENTAL
QUESTIONS:**



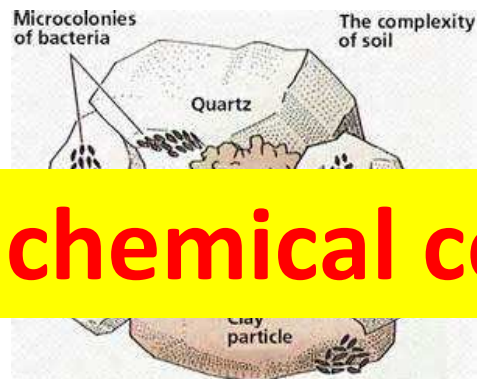
with Whom and Why ? And When?





Bacterial diversity: Huge reservoir of genetic resources

APPLICATIONS:



Enzymes, chemical compounds...

APPLICATIONS

It does not matter what you miss...
Exploit what you get.

Julian Davies, oral talk, many years ago

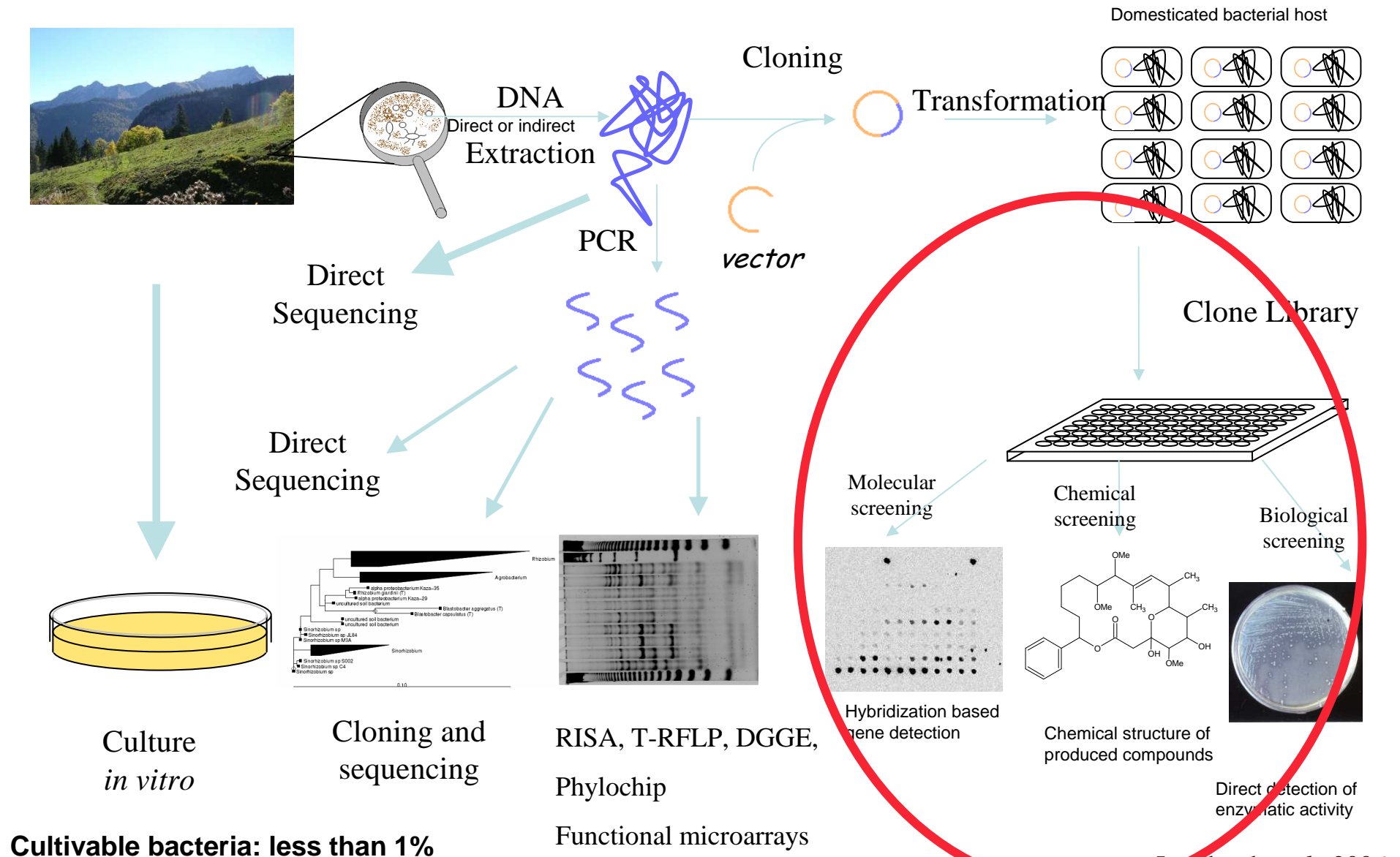
FUNDAMENTAL

Links taxonomy/functions Ecosystem functioning

It does matter what you miss...
Exploit what you get?



METAGENOME EXPLOITATION



FUNDAMENTAL

Links taxonomy/functions

Ecosystem functioning

It does matter what you miss...

Metagenomics:

Identification of a « rare biosphere ».

**Conditionally Rare Taxa (CRT) Disproportionately
Contribute to Temporal Changes in Microbial Diversity .
*Ashley Shade, Stuart E. Jones, J. Gregory Caporaso, et al.***

mBio 5(4): . 2014. doi:10.1128/mBio.01371-14.

“We discovered that CRT made up 1.5 to 28% of the community membership, represented a broad diversity of bacterial and archaeal lineages, and explained large amounts of temporal community dissimilarity (i.e., up to 97% of Bray-Curtis dissimilarity)”.

Role of the rare biosphere ?

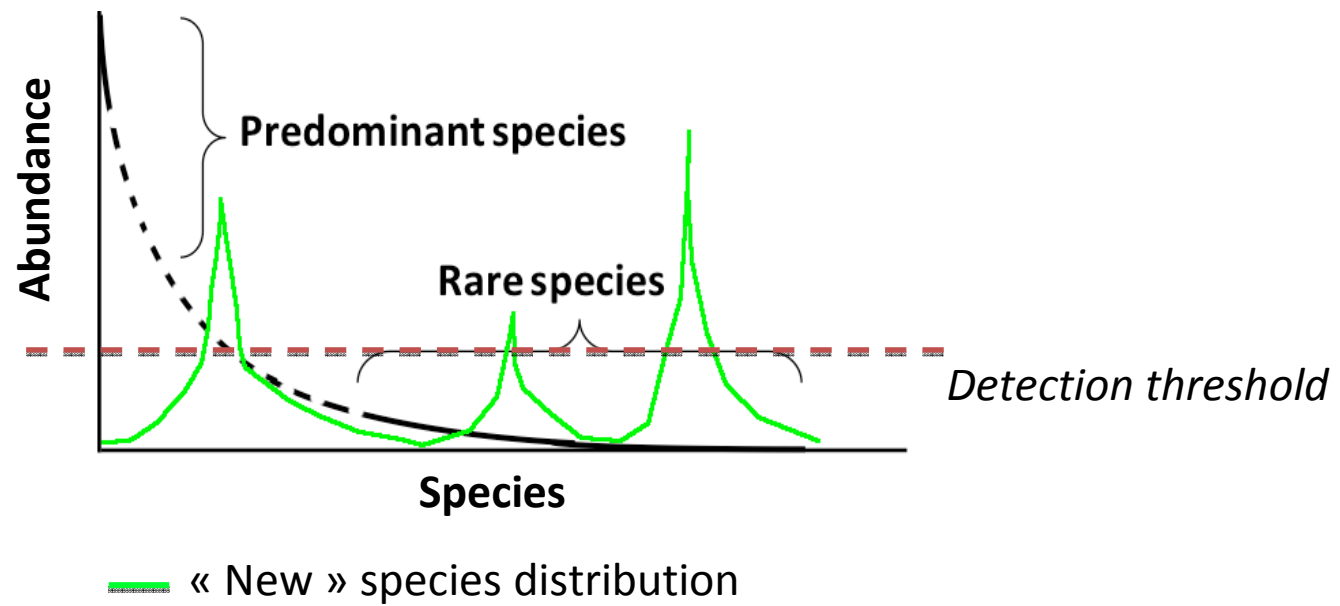
- Genes can be strongly expressed (numerous examples in the literature)
- Rare taxa can become dominant when environmental conditions change
- Rare taxa are a reservoir of transferable genetic information
- Rare taxa ?
- Inaccessible bacteria, unavailable DNA ?

Extent of the soil bacterial diversity?

How to get it?

- Genes can be strongly expressed (numerous examples in the literature)
- Rare (or unavailable) taxa can become dominant (or accessible) when environmental conditions change
- Rare taxa are a reservoir of transferable genetic information

Evenness modification : rare taxa become detectable.





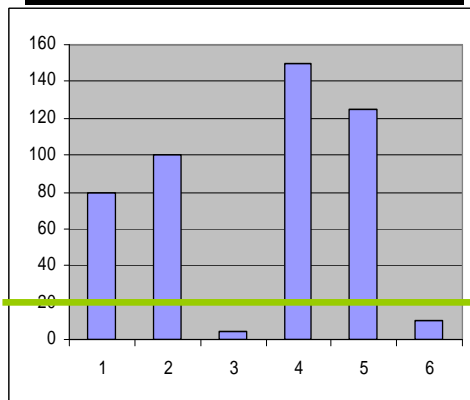
Aurélie Faugier

Conceptual approach:

Provide new developing conditions to soil bacterial communities

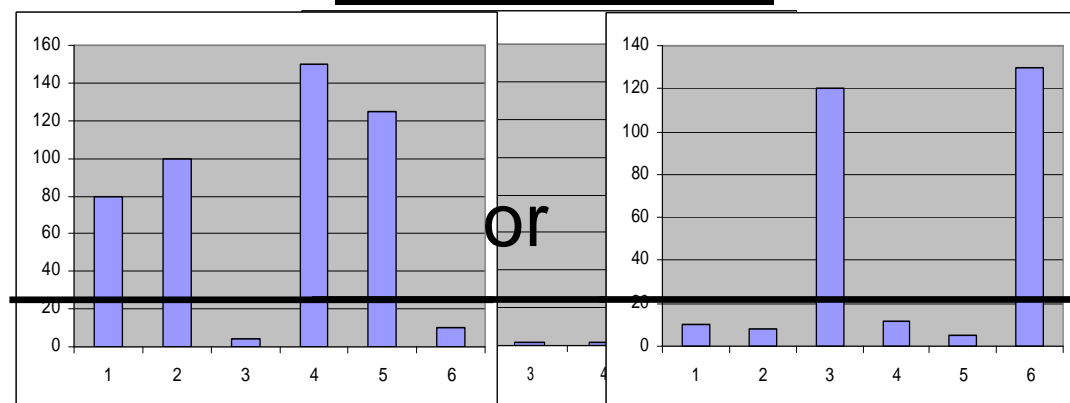
Bacterial community extracted from soil A

Soil A



Diversity in soil A

Sterilized Soil B





1. **Extraction** of the 9 bacterial communities

Nycodenz density gradient



2. **Inoculation** of each bacterial community into the nine sterilized soils

3. **Incubation** at RT for 1 day, 2 months, 6 months

4. **Monitoring** of bacterial community structure evolution (direct DNA extraction, PCR and phylochip)



Two questions:

- **Are new developing community structures different from the donor ones and from these of the recipient soils?**
- **Are new taxa detected?**



Are new taxa detected?

➤ A bacterial community inoculated into new (sterilized) soils reveals bacteria genera undetected in the original inoculum

➤ Each inoculated community: Extent of the diversity increases when considering the different recipient soils.

Genera detected in CS and not later

Genera detected at T0, T1, T2 and not in CS

Genera detected only at T1



Are new taxa detected?

➤ A bacterial community inoculated into new (sterilized) soils reveals bacteria genera undetected in the original inoculum

➤ Each inoculated community: Extent of the diversity increases when considering

the different recipient soils

the different incubation times

the different extraction techniques...

the different DNA analysis methods...

METAGENOMICS

- Inventory of bacteria

- Inventory of genes

- Genomics: the **exhaustive** study of genomes, more precisely of all genes, their location on chromosomes (or plasmids), their sequence, function, role.

- Metagenomics transcends the individual organism to the « **meta** » level.

... to understand soil microbial community diversity and function

The first but necessary step for unveiling cryptic microbial partnerships in nature, the interactions at various levels.

Reconstructing Genomes from the Soil Metagenome



Tom O Delmont, Laurie Maccario, Emmanuel Prestat, Eric Pelletier, Denis
LePaslier, Pascal Simonet and Timothy M Vogel

Environmental Microbial Genomics Group
Laboratoire Ampère . Ecole Centrale de Lyon . Université de Lyon



Metagenomic assembly



Acid mines



Cow rumens



Human feces



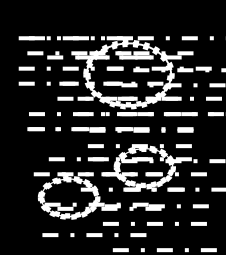
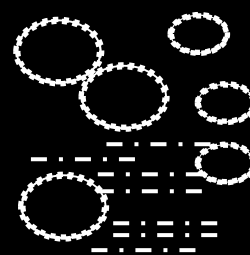
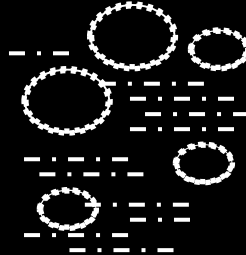
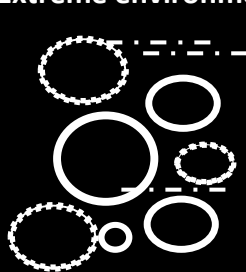
Oceans



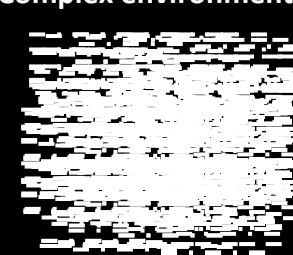
Soils



Extreme environments



Complex environments



Major difficulties:

- Balanced evenness
- Genomic similarity
- Length of generated sequences
- Computational limits

Solutions:

- Deeper sequencing efforts
- Improved assembly software
- Waiting for novel sequencing technologies
- Playing with other environments ...

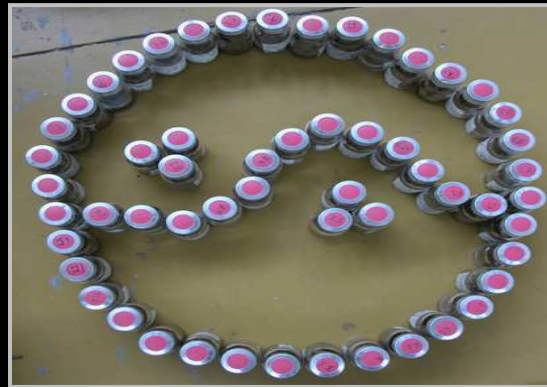
Experimental design



Pilot study: various environmental variations (diff. concentrations):

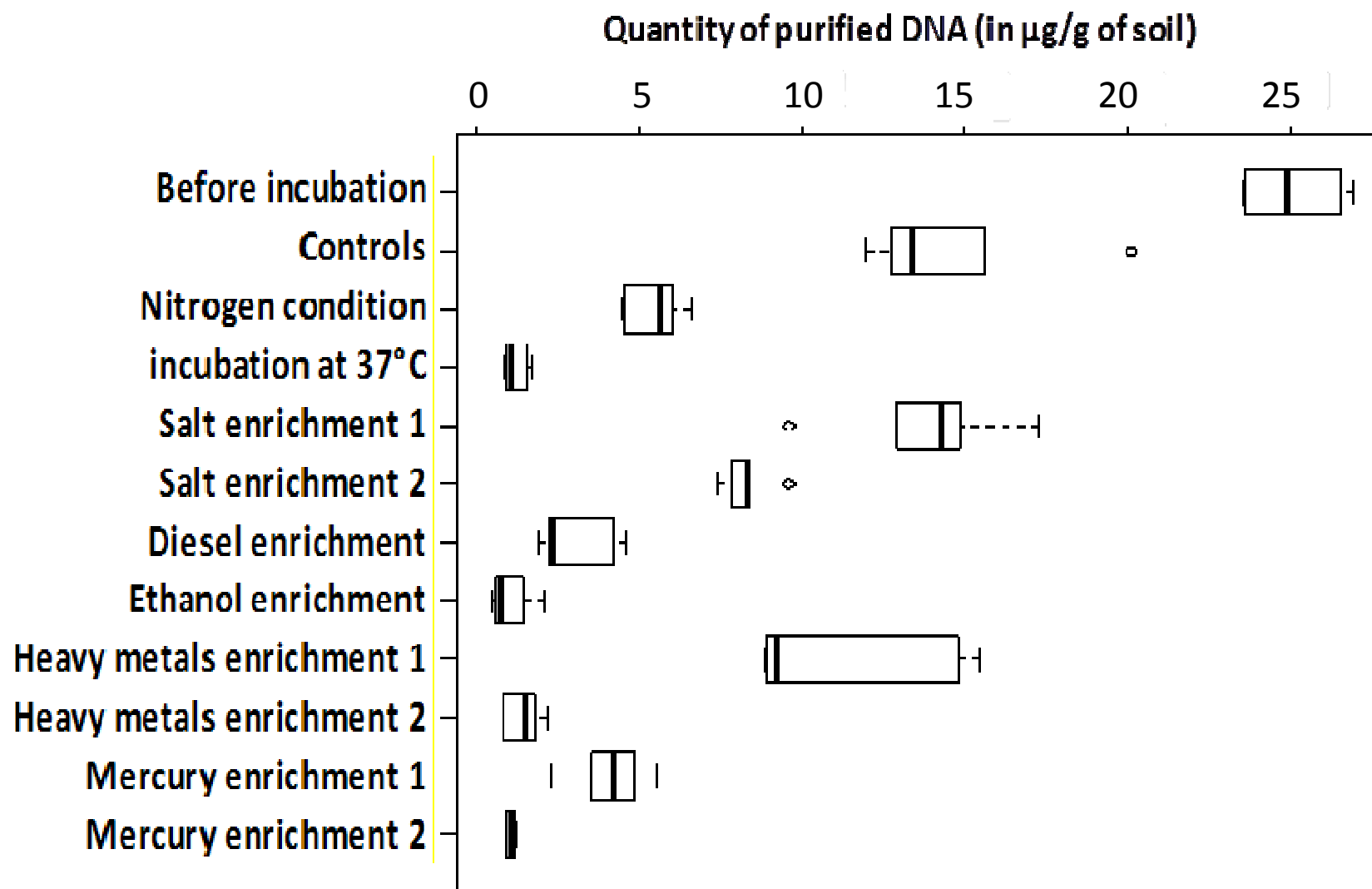
Heavy metals	Mercury	Diesel	Ethanol
No oxygen (only Nitrogen)	Salt	Temperature (37°C)	

Four month of incubation in biological replicates

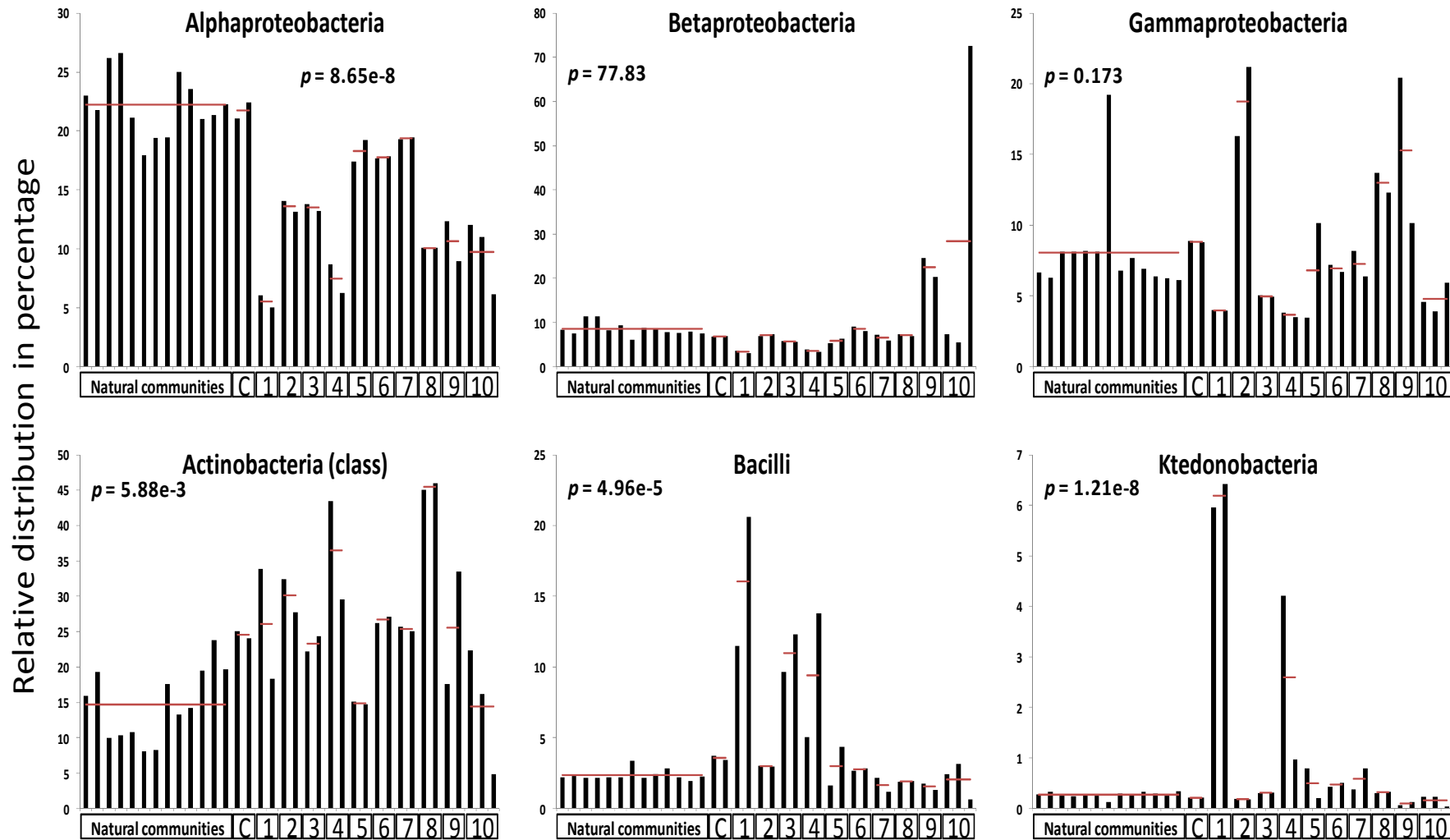


**Selected conditions sequenced in duplicates (Titanium pyrosequencing)
In triplicate for one condition (mercury enrichment)**

Population density estimation



Taxonomical composition



M5NR databases (E-value cut-off 10^{-5})

Reconstructed genetic structures



Condition	Assembled genetic structure	Related genus based on 16S rRNA
Heavy metals 8g / kg Microcosm 1	Bacterial chromosome	<i>Leifsonia</i>
	Bacterial chromosome	<i>Rhodanobacter</i>
	Plasmid IncP carrying metal resistance genes	Undefined
Heavy metals 8g / kg Microcosm 2	Bacterial chromosome	<i>Leifsonia</i>
	Bacterial chromosome	<i>Rhodanobacter</i>
	Plasmid IncP carrying metal resistance genes	Undefined
Ethanol 20% Microcosm 1	Bacterial chromosome	<i>Sporolactobacillus</i>
	Bacterial chromosome	<i>Ktedonobacter</i>
Ethanol 20% Microcosm 2	Bacterial chromosome	<i>Sporolactobacillus</i>
Mercury 0.02g / kg Microcosm 1	Bacterial chromosome	<i>Acidobacteria Gp1</i>
	Bacterial chromosome	<i>Rhodanobacter</i>
	Bacterial chromosomes	<i>Rhodanobacter</i>
	Bacterial chromosome	<i>Streptomyces</i>
	Undefined structure carrying one Mer operon	<i>Streptomyces</i>
	Probable phage infection	<i>Streptomyces</i>
Mercury 0.02g / kg Microcosm 2	Bacterial chromosome	<i>Acidobacteria Gp1</i>
	Bacterial chromosome	<i>Dyella</i>
	Bacterial chromosome	<i>Streptomyces</i>
	Bacterial chromosome	<i>Streptomyces</i>
	Undefined structure carrying one Mer operon	<i>Streptomyces</i>
	Probable phage infection	<i>Streptomyces</i>
	Probable phage infection	<i>Streptomyces</i>
Mercury 0.2g / kg Microcosm 3	Bacterial chromosome (replicon 1)	<i>Burkholderia</i>
	Bacterial chromosome (replicon 2)	
	Mega-plasmid incF carrying two Mer operons	
	Bacterial chromosome (replicon 1)	<i>Burkholderia</i>
	Bacterial chromosome (replicon 2)	
	Mega-plasmid incF carrying one Mer operon	

Reconstructed genetic structures



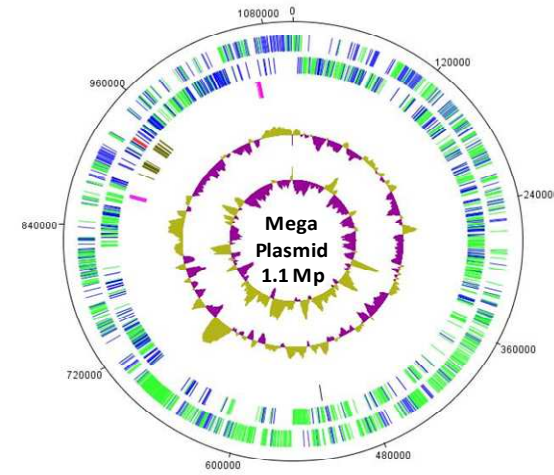
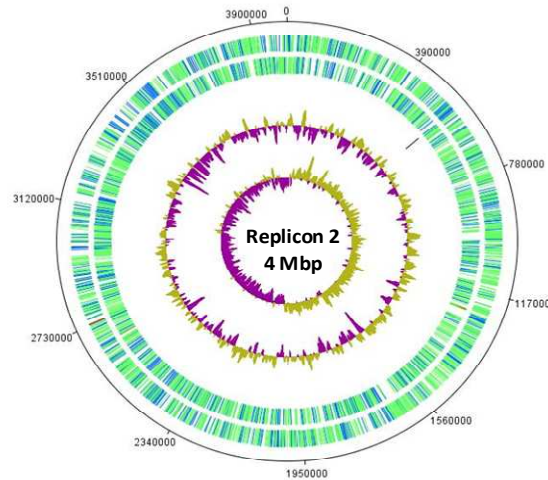
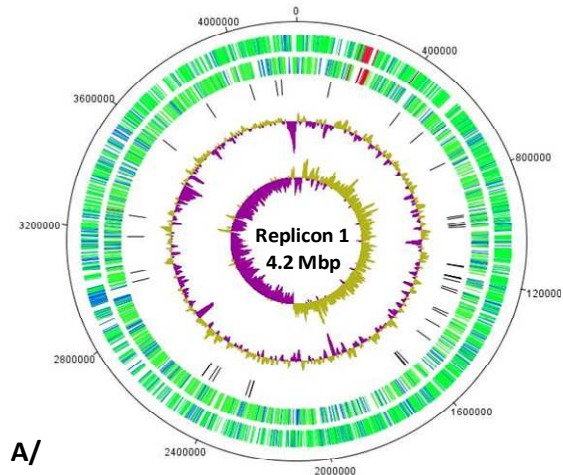
Condition	Assembled genetic structure	Related genus based on 16S rRNA	Microcosm relative proportion	Natural relative proportion
Heavy metals 8g / kg Microcosm 1	Bacterial chromosome	<i>Leifsonia</i>	7.77%	5.55E-04%
	Bacterial chromosome	<i>Rhodanobacter</i>	17.70%	1.17E-04%
	Plasmid IncP carrying metal resistance genes	Undefined	0.52%	< 1.E-04
Heavy metals 8g / kg Microcosm 2	Bacterial chromosome	<i>Leifsonia</i>	9.27%	7.58E-04%
	Bacterial chromosome	<i>Rhodanobacter</i>	11.22%	2.03E-04%
	Plasmid IncP carrying metal resistance genes	Undefined	0.47%	< 1.E-04
Ethanol 20% Microcosm 1	Bacterial chromosome	<i>Sporolactobacillus</i>	7.04%	< 1.E-04
	Bacterial chromosome	<i>Ktedonobacter</i>	16.51%	< 1.E-04
Ethanol 20% Microcosm 2	Bacterial chromosome	<i>Sporolactobacillus</i>	15.49%	< 1.E-04
Mercury 0.02g / kg Microcosm 1	Bacterial chromosome	<i>Acidobacteria Gp1</i>	4.52%	2.42E-04%
	Bacterial chromosome	<i>Rhodanobacter</i>	14.03%	1.92E-03%
	Bacterial chromosomes	<i>Rhodanobacter</i>	5.40%	7.89E-04%
	Bacterial chromosome	<i>Streptomyces</i>	9.30%	1.75E-03%
	Undefined structure carrying one Mer operon	<i>Streptomyces</i>	0.57%	< 1.E-04
	Probable phage infection	<i>Streptomyces</i>	1.33%	< 1.E-04
Mercury 0.02g / kg Microcosm 2	Bacterial chromosome	<i>Acidobacteria Gp1</i>	7.84%	2.58E-04%
	Bacterial chromosome	<i>Dyella</i>	12.08%	2.75E-03%
	Bacterial chromosome	<i>Streptomyces</i>	8.73%	2.43E-03%
	Bacterial chromosome	<i>Streptomyces</i>	4.39%	1.87E-02%
	Undefined structure carrying one Mer operon	<i>Streptomyces</i>	0.96%	< 1.E-04
	Probable phage infection	<i>Streptomyces</i>	7.68%	< 1.E-04
	Probable phage infection	<i>Streptomyces</i>	0.73%	< 1.E-04
Mercury 0.2g / kg Microcosm 3	Bacterial chromosome (replicon 1)	<i>Burkholderia</i>	26.32%	1.99E-03%
	Bacterial chromosome (replicon 2)		25.06%	5.63E-04%
	Mega-plasmid incF carrying two Mer operons		6.90%	2.34E-04%
	Bacterial chromosome (replicon 1)	<i>Burkholderia</i>	9.59%	1.28E-02%
	Bacterial chromosome (replicon 2)		7.13%	8.34E-03%
	Mega-plasmid incF carrying one Mer operon		2.45%	2.01E-03%

Reconstructed genetic structures

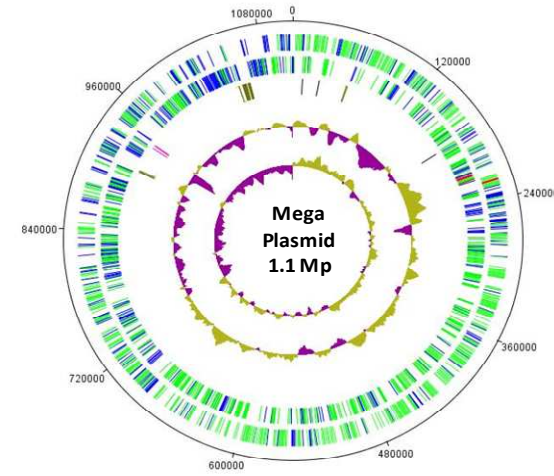
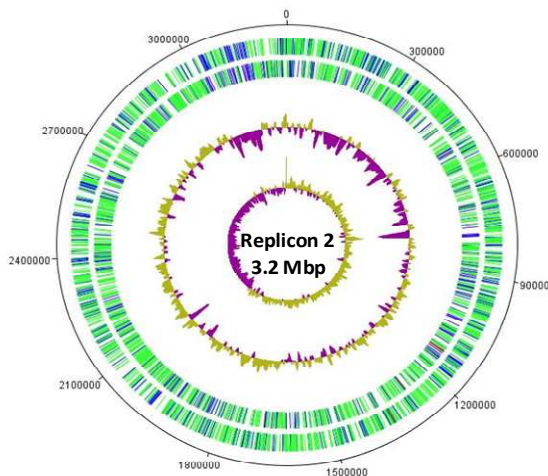
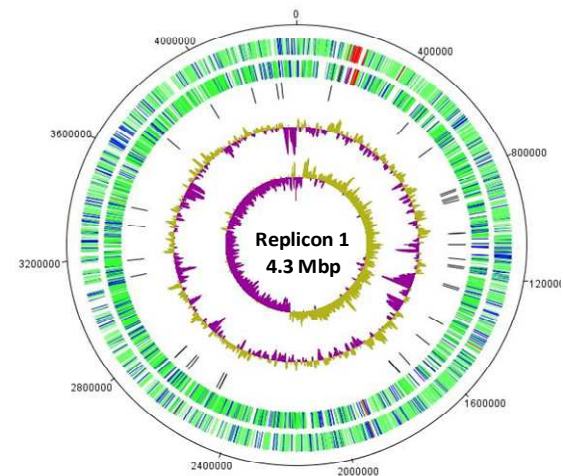


Condition	Assembled genetic structure	Related genus based on 16S rRNA	Microcosm relative proportion	Natural relative proportion	Number of contigs or scaffolds	Structure coverage in dataset	GC content	Genetic structure size
Heavy metals 8g / kg Microcosm 1	Bacterial chromosome	<i>Leifsonia</i>	7.77%	5.55E-04%	4 scaffolds	30X	67.8%	3,8 Mbp
	Bacterial chromosome	<i>Rhodanobacter</i>	17.70%	1.17E-04%	3 scaffolds	50X	68.0%	4,0 Mbp
	Plasmid IncP carrying metal resistance genes	Undefined	0.52%	< 1.E-04	1 scaffold	100X	63.4%	0.073 Mbp
Heavy metals 8g / kg Microcosm 2	Bacterial chromosome	<i>Leifsonia</i>	9.27%	7.58E-04%	5 scaffolds	30X	67.8%	3,7 Mbp
	Bacterial chromosome	<i>Rhodanobacter</i>	11.22%	2.03E-04%	30 scaffolds	40X	68.0%	4,0 Mbp
	Plasmid IncP carrying metal resistance genes	Undefined	0.47%	< 1.E-04	1 scaffold	100X	63.5%	0.072 Mbp
Ethanol 20% Microcosm 1	Bacterial chromosome	<i>Sporolactobacillus</i>	7.04%	< 1.E-04	49 contigs	25X	47.3%	3,4 Mbp
	Bacterial chromosome	<i>Ktedonobacter</i>	16.51%	< 1.E-04	39 contigs	60X	50.3%	3,3 Mbp
Ethanol 20% Microcosm 2	Bacterial chromosome	<i>Sporolactobacillus</i>	15.49%	< 1.E-04	42 contigs	70X	47.3%	3,3 Mbp
Mercury 0.02g / kg Microcosm 1	Bacterial chromosome	<i>Acidobacteria Gp1</i>	4.52%	2.42E-04%	19 scaffolds	15X	58.1%	5 Mbp
	Bacterial chromosome	<i>Rhodanobacter</i>	14.03%	1.92E-03%	14 scaffolds	60X	63.9%	5.4 Mbp
	Bacterial chromosomes	<i>Rhodanobacter</i>	5.40%	7.89E-04%	24 scaffolds	20X	64.9%	7.8 Mbp
	Bacterial chromosome	<i>Streptomyces</i>	9.30%	1.75E-03%	11 scaffolds	25X	71.0%	9 Mbp
	Undefined structure carrying one Mer operon	<i>Streptomyces</i>	0.57%	< 1.E-04	1 scaffold	50X	70.5%	0.309 Mbp
	Probable phage infection	<i>Streptomyces</i>	1.33%	< 1.E-04	1 scaffold	670X	65.1%	0.056 Mbp
Mercury 0.02g / kg Microcosm 2	Bacterial chromosome	<i>Acidobacteria Gp1</i>	7.84%	2.58E-04%	1 scaffold	25X	58.0%	4.8 Mbp
	Bacterial chromosome	<i>Dyella</i>	12.08%	2.75E-03%	6 scaffolds	60X	64.8%	4.6 Mbp
	Bacterial chromosome	<i>Streptomyces</i>	8.73%	2.43E-03%	46 scaffolds	25X	70.9%	9 Mbp
	Bacterial chromosome	<i>Streptomyces</i>	4.39%	1.87E-02%	169 scaffolds	15X	70.0%	7.8 Mbp
	Undefined structure carrying one Mer operon	<i>Streptomyces</i>	0.96%	< 1.E-04	1 scaffold	80X	70.5%	0.309 Mbp
	Probable phage infection	<i>Streptomyces</i>	7.68%	< 1.E-04	1 scaffold	1500X	64.4%	0.156 Mbp
	Probable phage infection	<i>Streptomyces</i>	0.73%	< 1.E-04	1 scaffold	180X	65.1%	0.142 Mbp
Mercury 0.2g / kg Microcosm 3	Bacterial chromosome (replicon 1)	<i>Burkholderia</i>	26.32%	1.99E-03%	6 scaffolds	75X	61.1%	4,2 Mbp
	Bacterial chromosome (replicon 2)		25.06%	5.63E-04%	4 scaffolds	75X	61.7%	4,0 Mbp
	Mega-plasmid incF carrying two Mer operons		6.90%	2.34E-04%	2 scaffolds	75X	59.9%	1,1 Mbp
	Bacterial chromosome (replicon 1)	<i>Burkholderia</i>	9.59%	1.28E-02%	7 scaffolds	25X	62.0%	4,3 Mbp
	Bacterial chromosome (replicon 2)		7.13%	8.34E-03%	6 scaffolds	25X	61.2%	3,2 Mbp
	Mega-plasmid incF carrying one Mer operon		2.45%	2.01E-03%	3 scaffolds	25X	60.3%	1,1 Mbp

Burkholderia species (mercury enrichment)

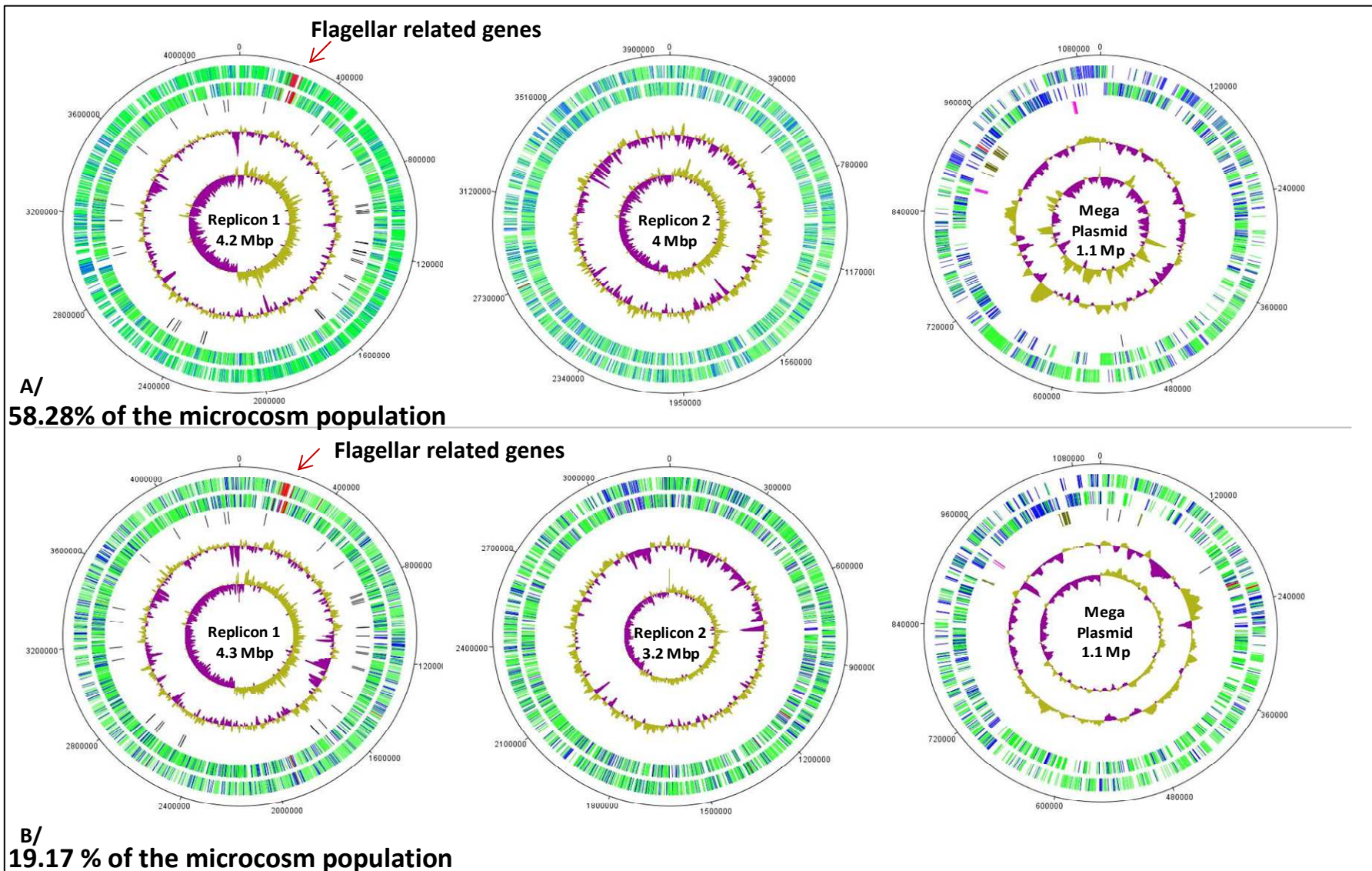


**A/
58.28% of the microcosm population**

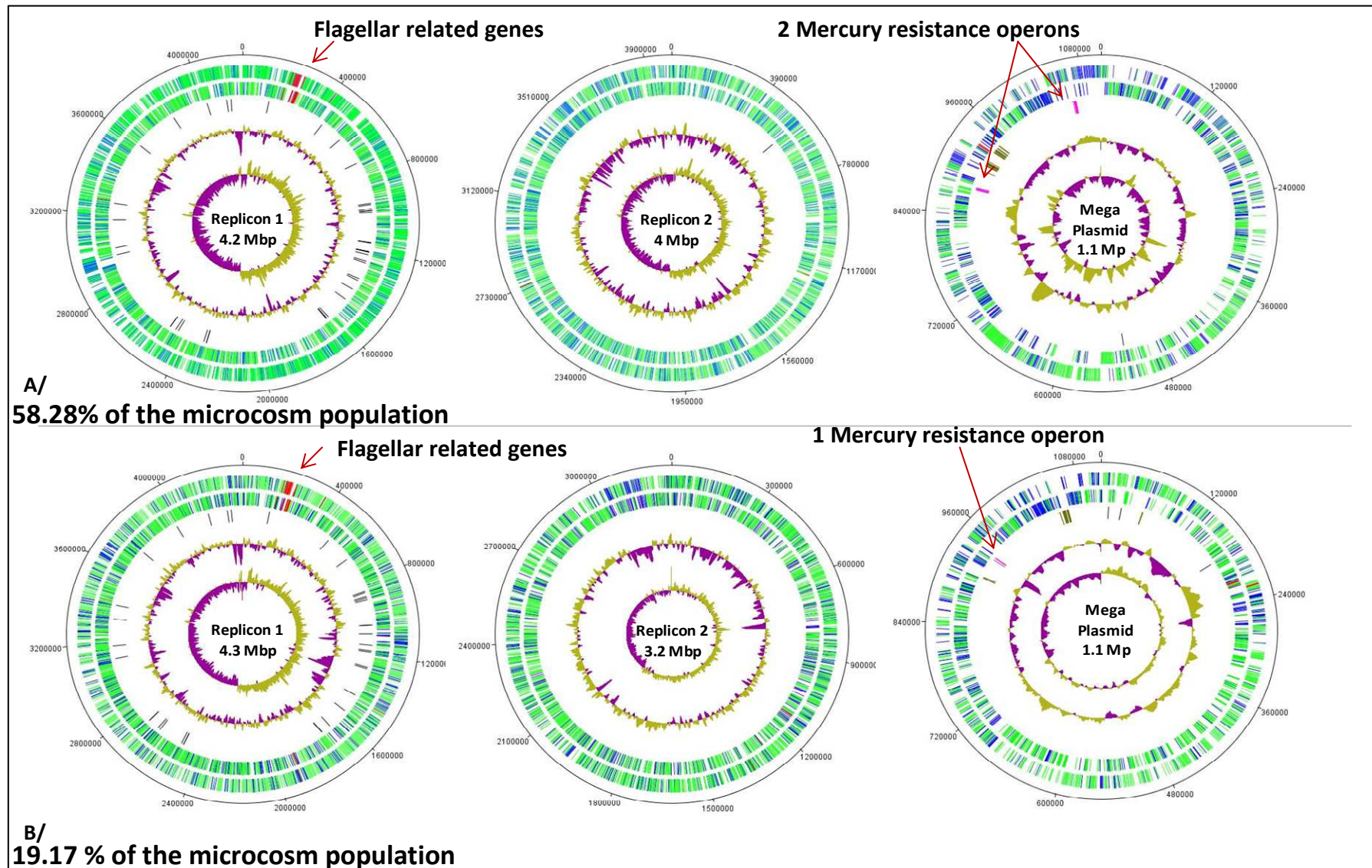


**B/
19.17 % of the microcosm population**

Burkholderia species (mercury enrichment)



Burkholderia species (mercury enrichment)



Global metagenomic comparisons

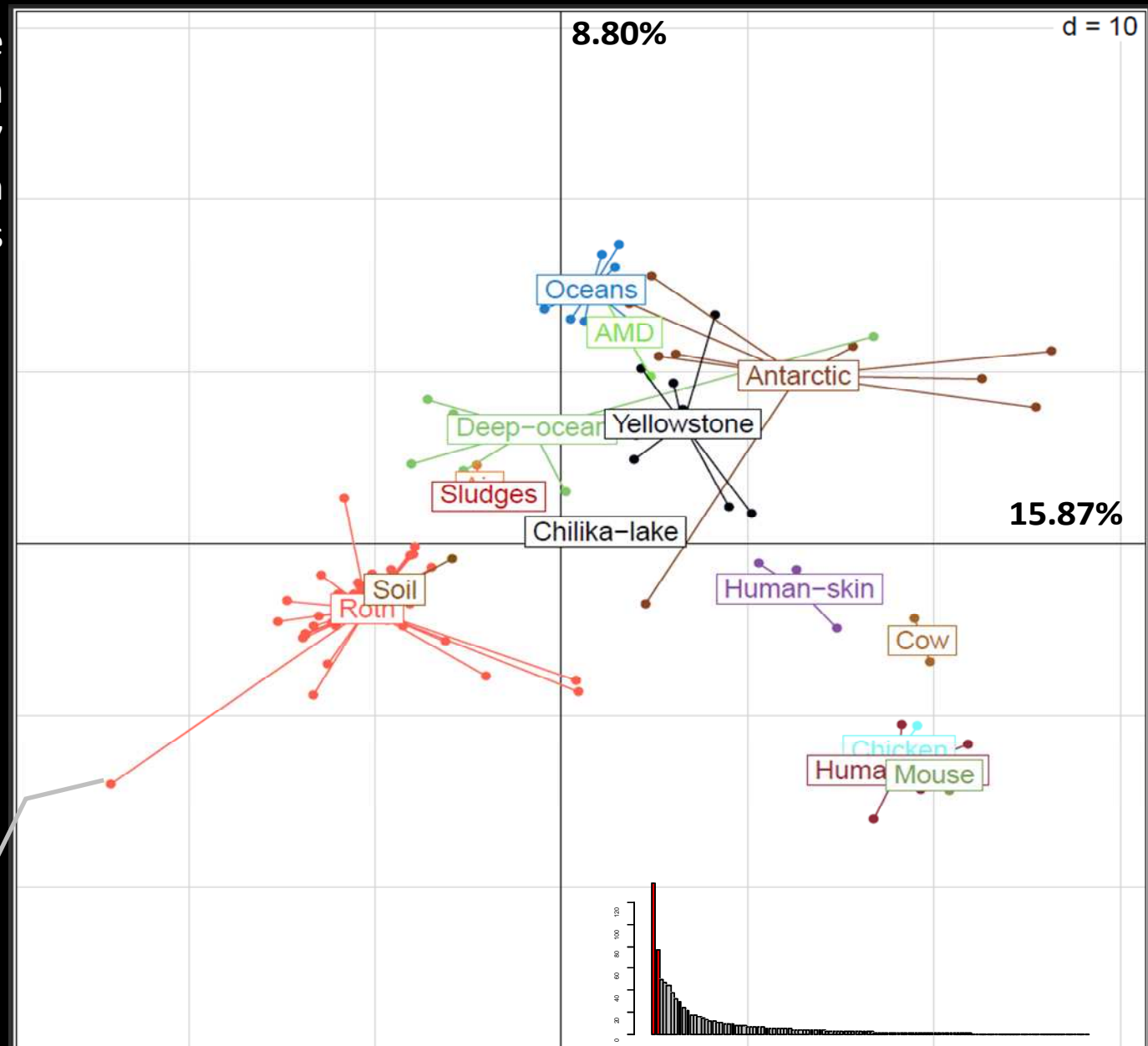


PCA based on the relative functional distribution (MG-RAST, level 3) of 117 metagenome from various environments

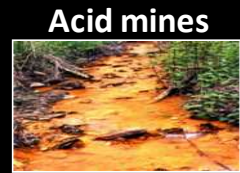
Pfam database (E-value cut-off 10^{-5})

Soil functional redundancy is high

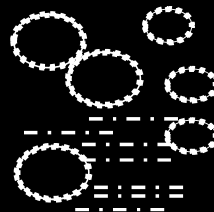
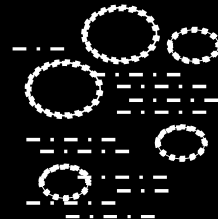
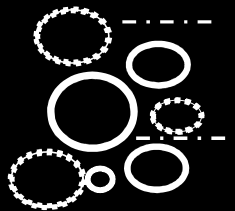
Mercury 0.2g/kg:
78% of *Burkholderia* species
Including two mega-plasmids



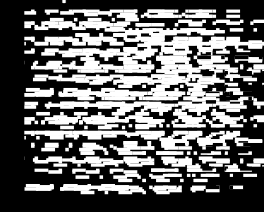
Strategy to assemble a soil metagenome



Extreme environments



Complex environments



Natural communities

Soft microcosm conditions



Nitrogen
Diesel

Stringent microcosm conditions



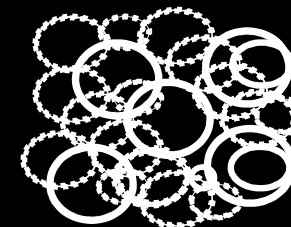
Salt
Temperature

Extreme microcosm conditions



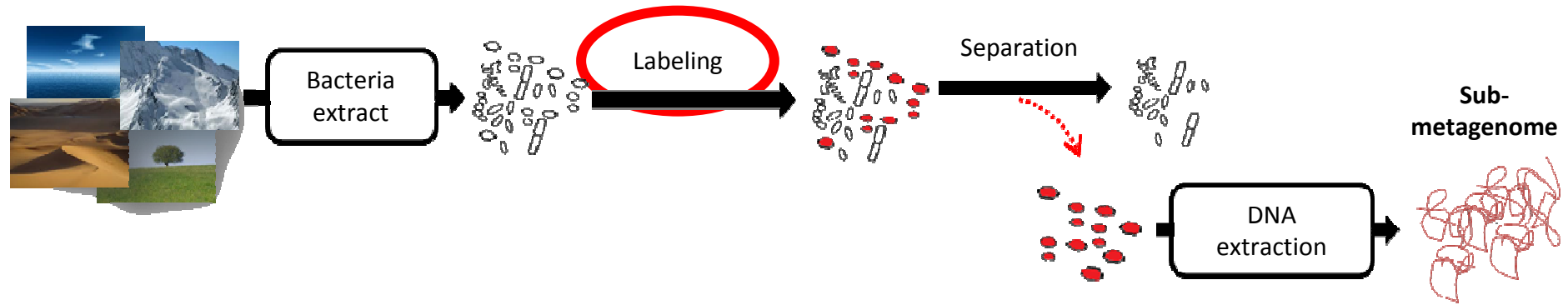
Ethanol
Mercury
Heavy metals

Direct sequencing
Pure culture
Single cell



Simplified communities

“Single population”



Labeling \longleftrightarrow Sub-Metagenome

Taxonomy



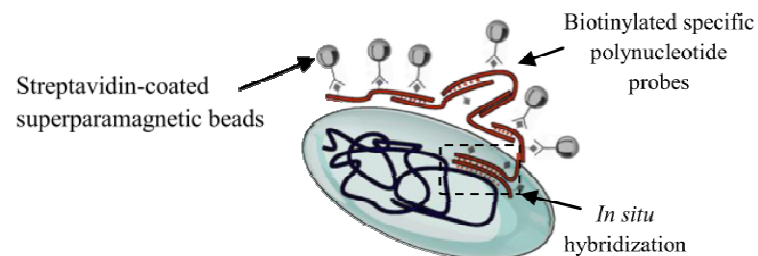
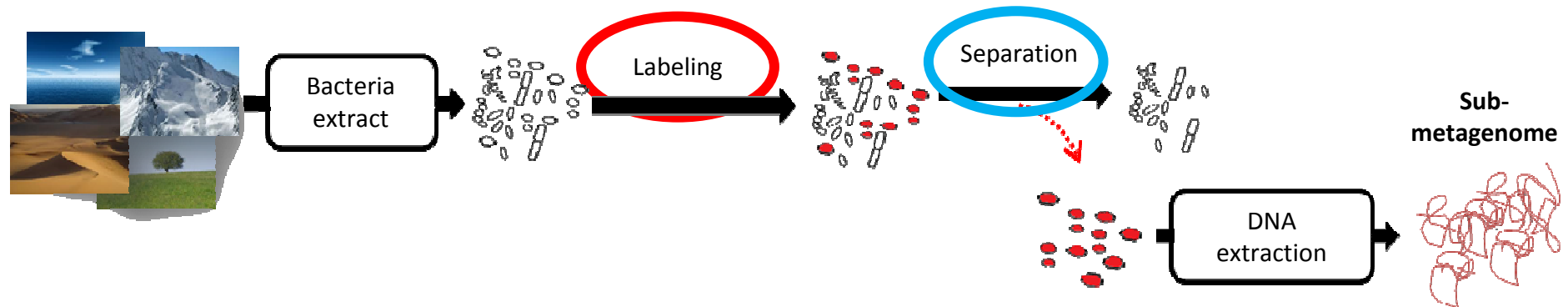
- Genetic potential of a specific population
- Genome reconstruction

Function



- Who's carrying the gene of interest ?

Stratégie de marquages et séparations (2)

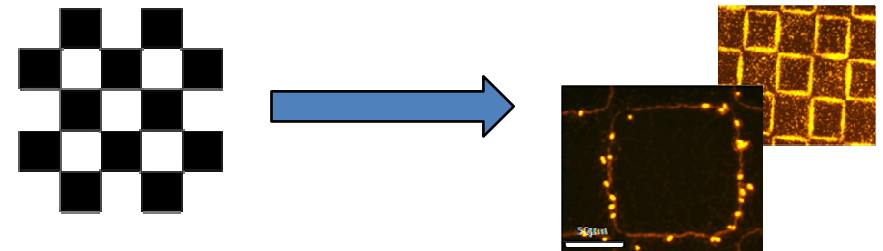
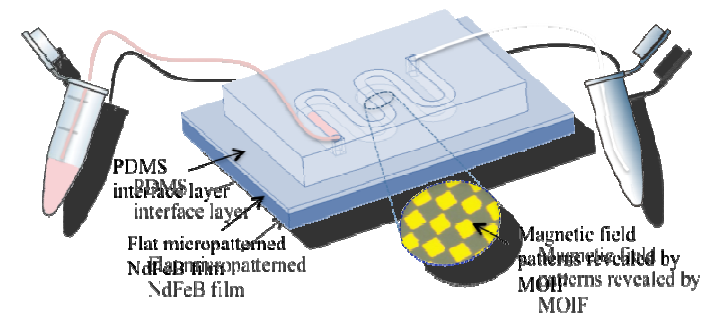


In situ Hybridization

-RING-FISH (23S)

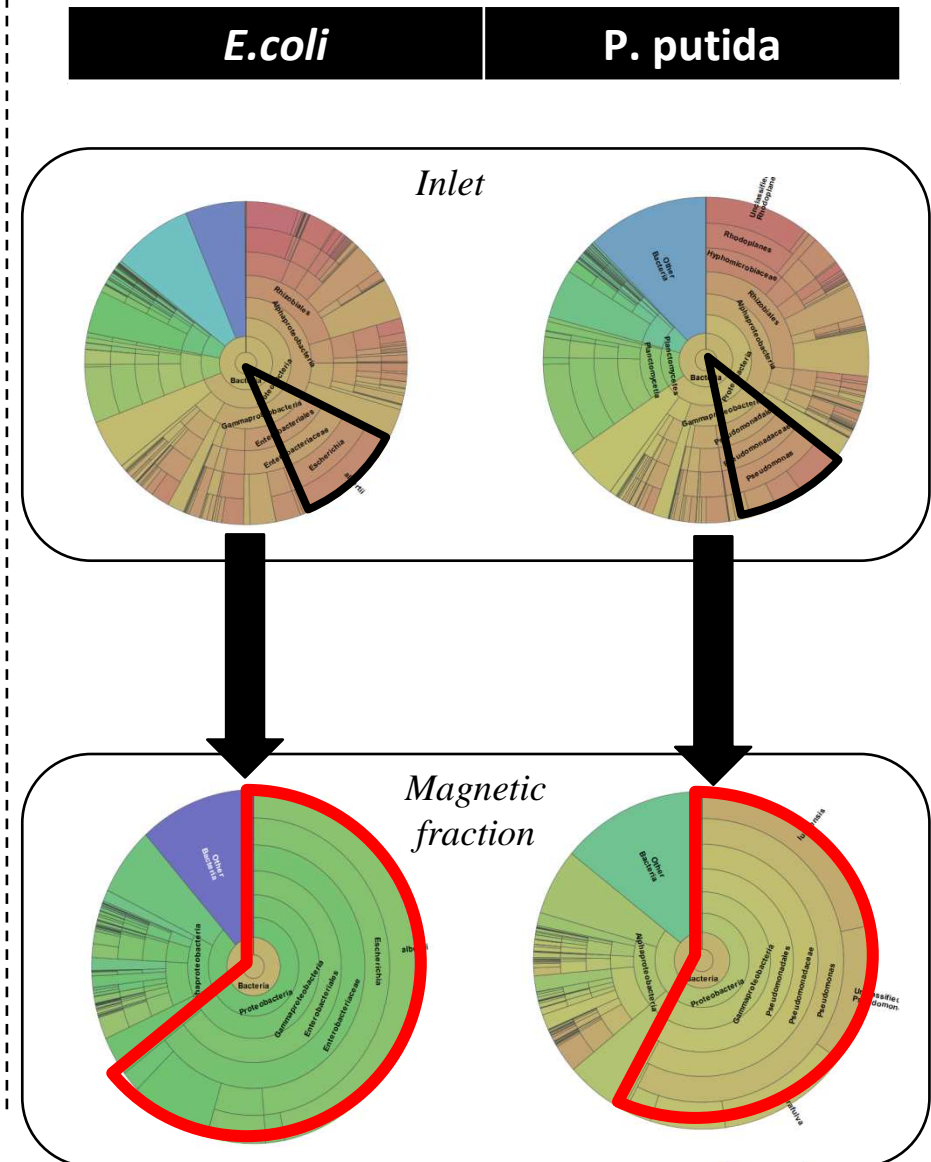
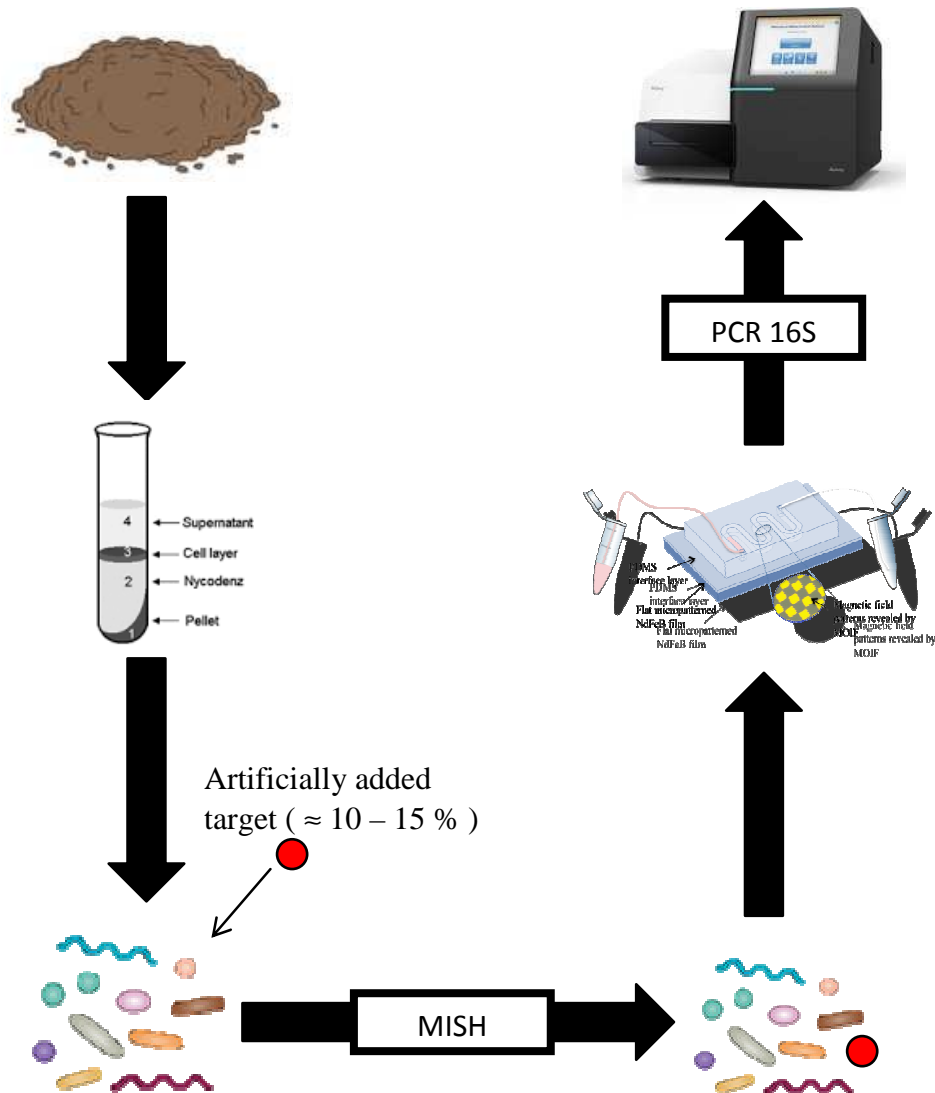
-Probe network formation

Polyribonucleotidic probes for extracellular binding to magnetic nanoparticles



Square patterned micro magnet included into microfluidic channel

Application to soil samples





CrossMark

Back to the Future of Soil Metagenomics

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