



Deciphering the response of canola plant microbiome to CeO₂ - based nanomaterials

Catherine Santaella

Lab Microbial Ecology of the Rhizosphere & Extreme Environments

UMR 7265 CNRS-CEA-Aix Marseille University

Biosciences and biotechnology Institute of Aix-Marseille (BIAM), St Paul lez Durance, France

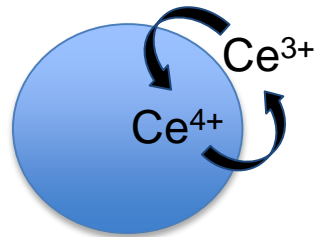
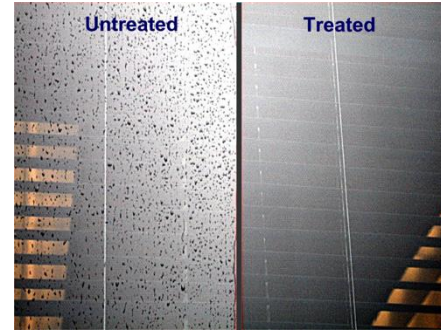
**International Consortium for the Environmental Implications of NanoTechnology (iCEINT),
Aix en Provence, France**

CEREGE UMR 7330, CNRS-AMU-IRD. Aix-en-Provence, France



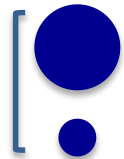
Cerium dioxide nanoparticles in this study

- Catalyst
- Fuel additive
- Wood coating
- Polishing agents...

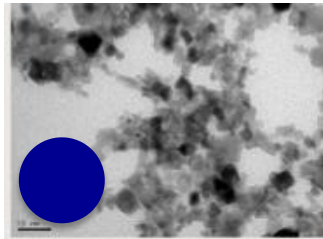
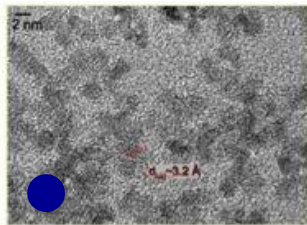


- Redox active
- Valence exchange between Ce^{4+} and Ce^{3+}

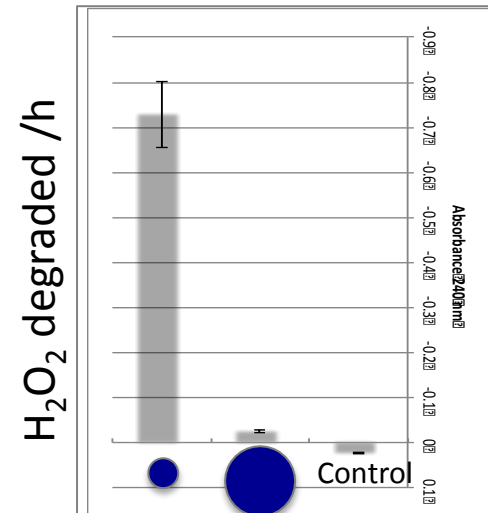
CeO_2
Pristine



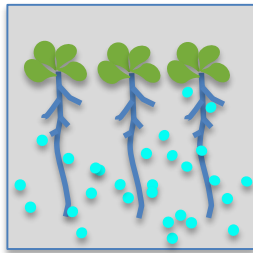
Particle size		SSA	Isoelectric point
TEM	DLS		
30 nm	170 nm	56 m^2g^{-1}	pl 7.3
4 nm	10 nm	271 m^2g^{-1}	pl 7.8



Catalase-Like Activity $2 H_2O_2 \rightarrow H_2O$



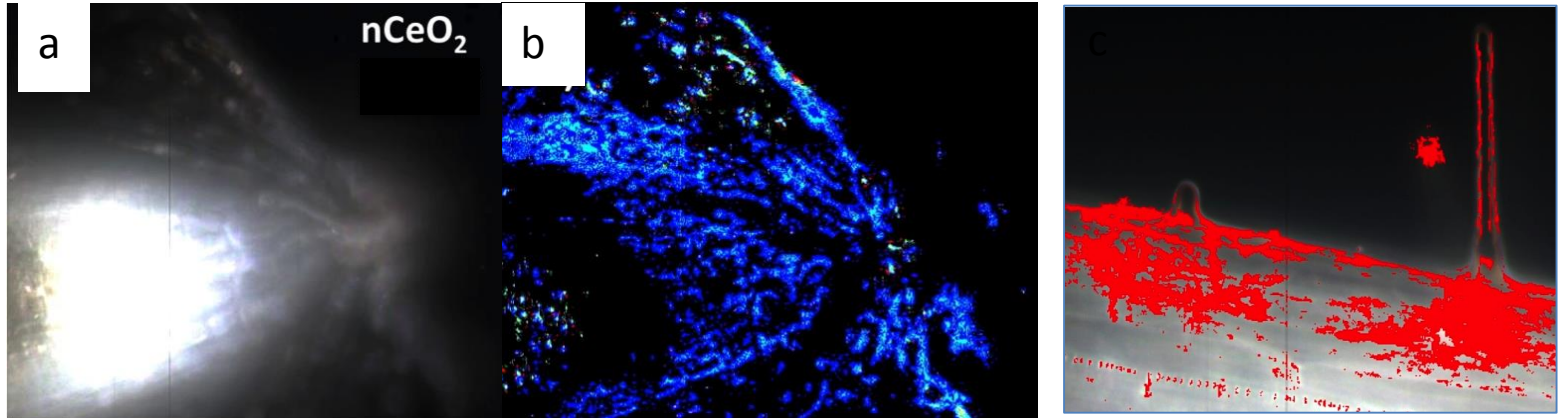
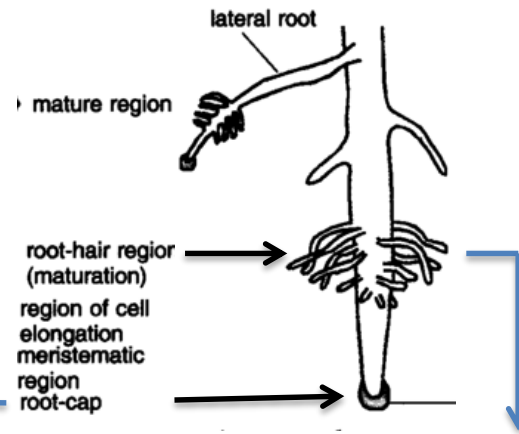
Interactions of CeO₂ NMs with *Arabidopsis thaliana*



CeO₂ 1 mg/L

Pristine

4 nm



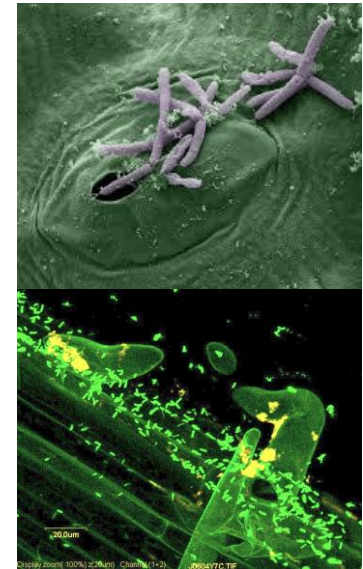
NanoCeO₂ has major interactions with root hairs, root cap and border cells
Bacteria also show tropism for these specific parts of the root
due to specific composition of these tissues and exudation of organic matter and ions

Bacteria and CeO₂ NMs compete for the same locations on a root system

Plant root bacteria interactions shape the bacterial microbiome in the rhizosphere



At the contact zone with soil, plants host a distinctive root-associated bacterial microbiome participating in plant nutrition, health and adaptation to the environment



“Plants wear their guts on the outside”

The bacterial root microbiome of plants is conceptually analogous to the gut microbiome of animals

Schlaeppli et al. PNAS 2014

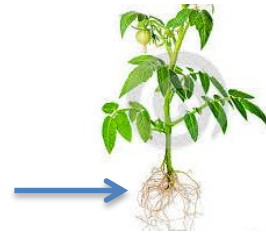
Rapid responses of soil microorganisms improve plant fitness in novel environments

Lau and Lennon, PNAS 2012

Multigenerational
exposure of plant
to drought

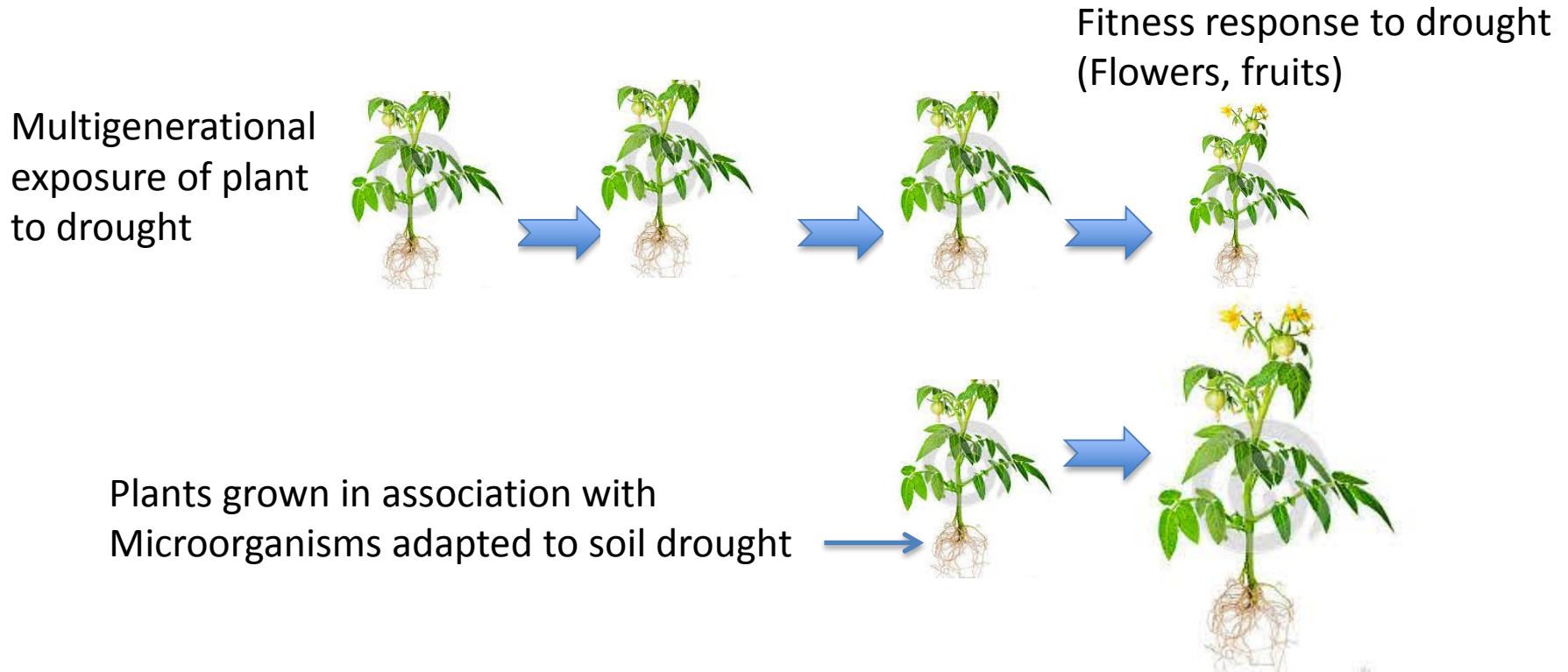


Plants grown in association with
Microorganisms adapted to drought



Rapid responses of soil microorganisms improve plant fitness in novel environments

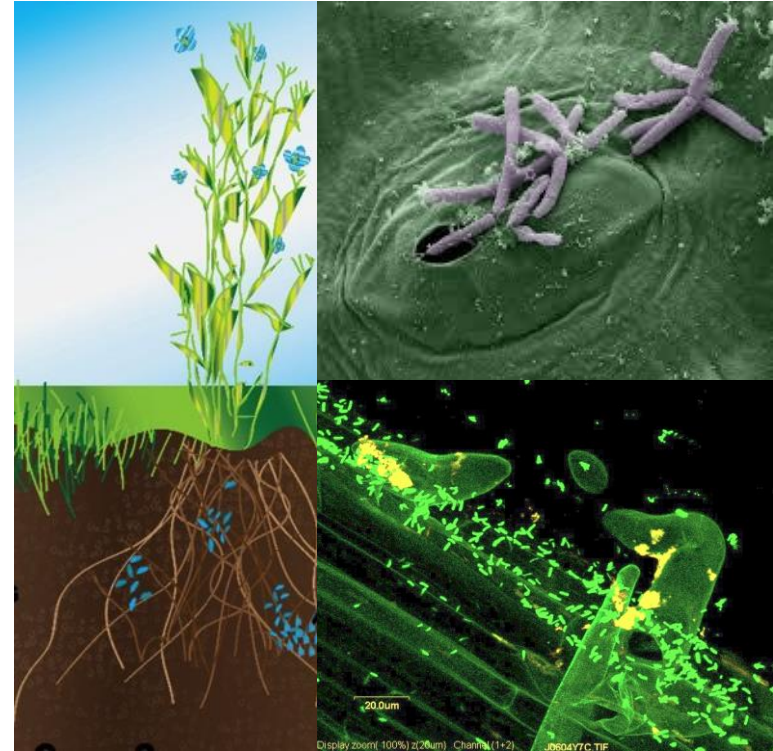
Lau and Lennon, PNAS 2012



**The adaptive plant response is driven primarily
by changes in the soil microbial community
rather than by genetic changes in the plants themselves**

Plant root bacteria interactions shape the bacterial microbiota in the rhizosphere

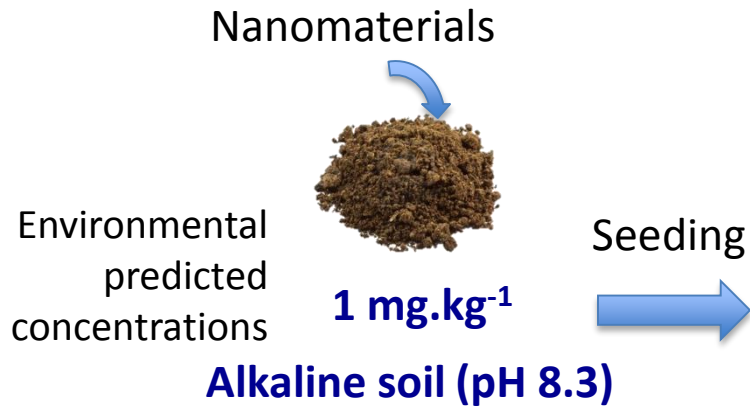
- At the contact zone with soil, plants host a distinctive root-associated bacterial microbiome participating in plant nutrition, health and adaption to the environment
- “Plants wear their guts on the outside”
The bacterial root microbiome of plants is conceptually analogous to the gut microbiome of animals
Schlaeppli et al. PNAS 2014



Selection on soil microbiomes reveals reproducible impacts on plant function
Beneficial communities in agricultural soils to promote plant health and nutrition

Any factor altering these interactions may alter plant growth and health

Simplified model of a terrestrial ecosystem



Alkaline soils account for 30% of the world's arable soils
Guerinot Nature Biotechnology 2001

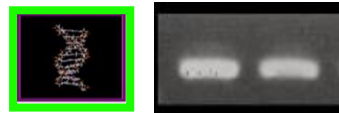


Phytotrons (CEA-IBEB-GRAP)
Atmospheric gaseous composition
Temperature, Irradiance levels and light periods, Watering

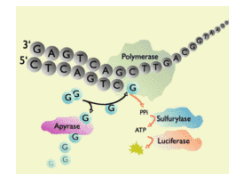
Root-associated microbes: DNA-based sequencing of 16S rRNA genes



Root tissue

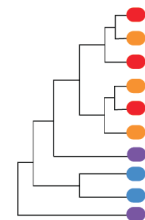


Amplification of 16S rRNA gene



High throughput sequencing

```
>AGTGAGAGAAGCAGGGTCGTAATGTT . . .
>AGTGCGATGCGTAGGGTCGTAATGCG . . .
>AGTGCGATGCGTAGGGTCGTAATGTA . . .
>AGTGGATGCTCTAGGGTCGTAATGCA . . .
>AGTGTACGGTCAGGGTCGTAATGGG . . .
>AGTGGATGCTCTAGGGTCGTAATGTT . . .
>AGTGTACGGTCAGGGTCGTAATGCC . . .
>AGTGAGAGAAGCAGGGTCGTAATCAC . . .
```



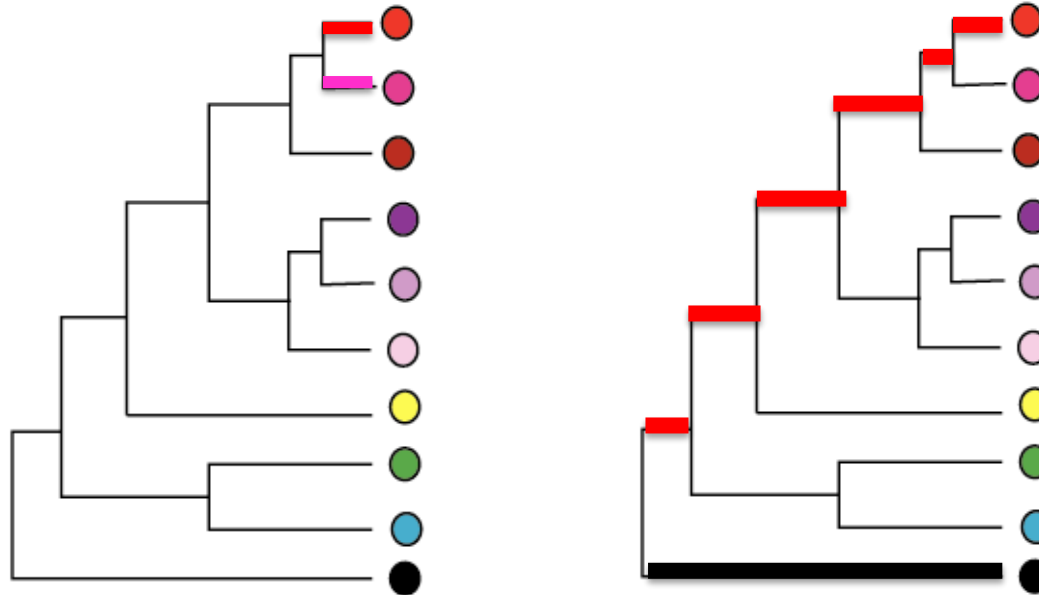
Impacts of CeO₂ NMs on root-associated bacterial community



Phylogeny-based beta diversity

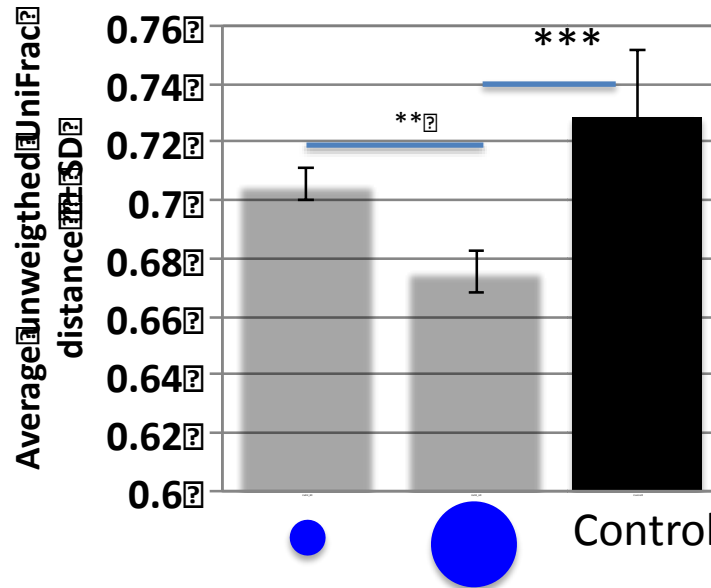
Do two samples contain significant different microbial communities?

Based on Average unweighted UniFrac distance



Impacts of CeO₂ NMs on root-associated bacterial community


Phylogeny-based beta diversity



The microbiome associated to the root systems of plants grown in the soil spiked with

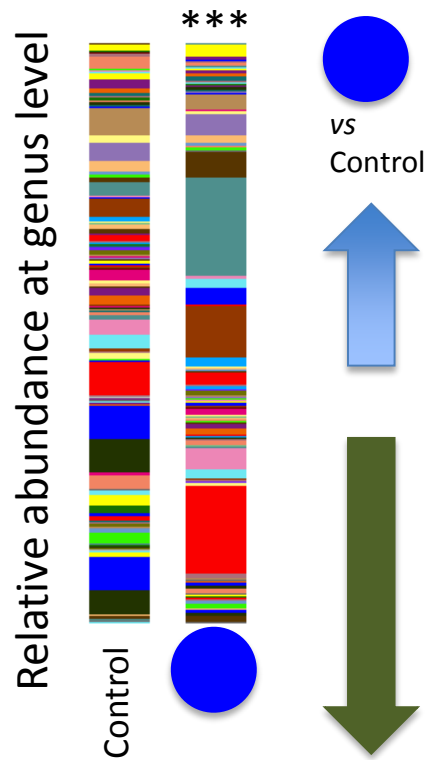


The microbiome associated to the root systems of control plants $p < 0.001$

The microbiome associated to the root systems of plants grown in the soil spiked with  $p < 0.01$

Hamidat et al. ES&T 2016

Impacts of CeO₂ NMs on root-associated bacterial community



Taxa related to		
Class	Family	Genus
<i>Betaproteobacteria</i>	<i>Comamonadaceae</i>	<i>Acidovorax</i>
		<i>Methylibium</i>
		<i>Rhizobacter</i>
		<i>Pelomonas</i>
<i>Actinobacteria</i>	<i>Streptomycetaceae</i>	<i>Streptomyces</i>
	<i>Nocardoidaceae</i>	<i>Kribbella</i>
	<i>Micrococcaceae</i>	<i>Arthrobacter</i>
	<i>Pseudonocardiaceae</i>	<i>Lentzea</i>
<i>Proteobacteria</i>	<i>Xanthomonadaceae</i>	<i>Pseudoxanthomonas</i>
	<i>Oxalobacteracea</i>	<i>Janthinobacterium</i>

Taxa relatives to bacteria associated to heavy metal and hydrocarbon contaminated environments

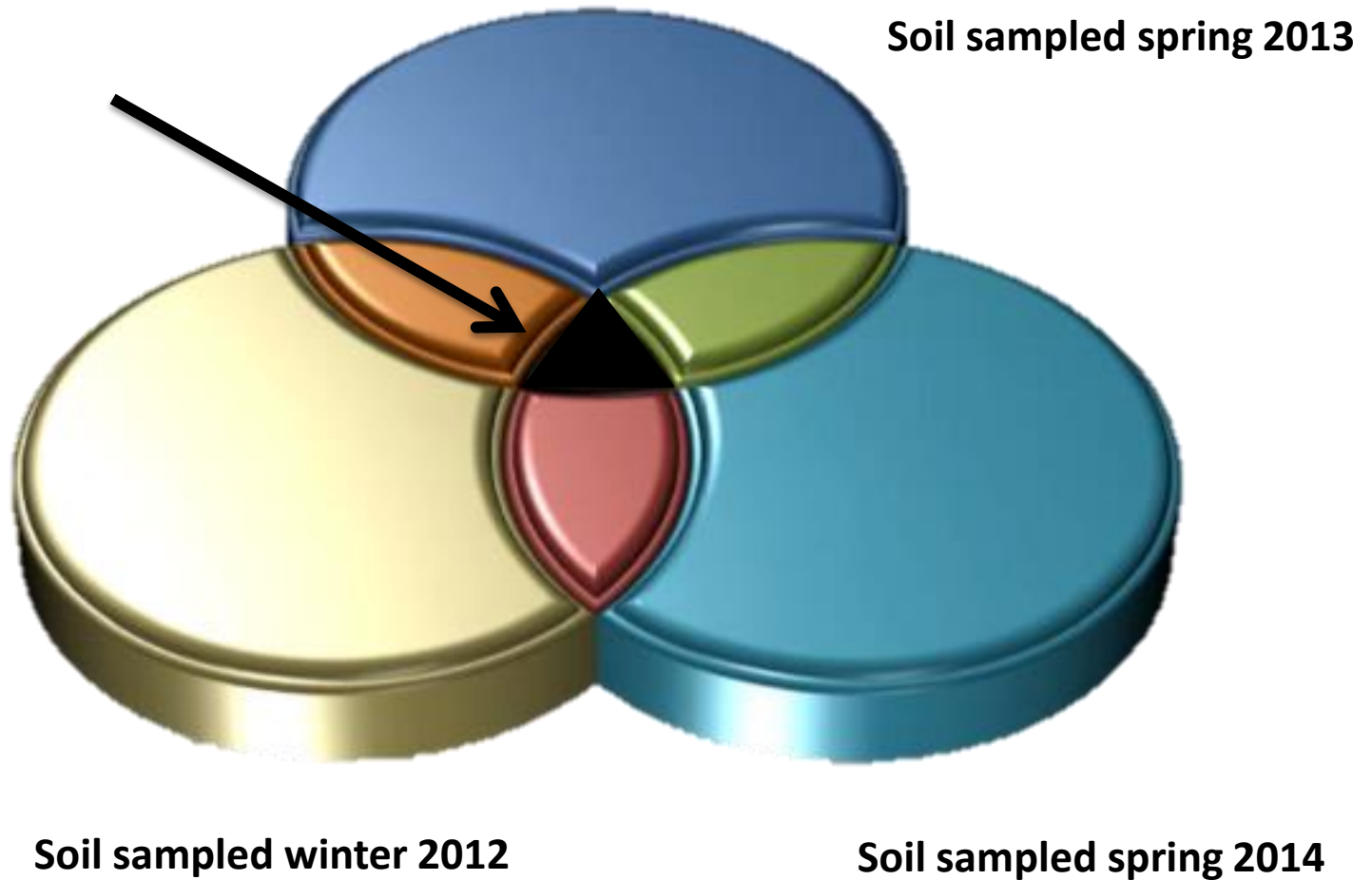
Likely properties: Polycyclic Aromatic Hydrocarbon degradation, resistance to metals and antibiotics
(Jechalke et al., 2012; Huang et al., 2012; Bodour et al. 2003, Lima et al. 2012)

Taxa correlated to soil disease suppressiveness and to plant health

(Cretoiu et al. 2014)

Core microbiome of canola roots

Taxa that were present in at least 90% of the samples from soil, rhizosphere and roots in control plants from three different biological experiments



Core microbiome of canola roots

Class	Order	Family	Genus
<i>Actinobacteria</i>	<i>Actinomycetales</i>	<i>Microbacteriaceae</i>	<i>Agromyces</i>
		<i>Micrococcaceae</i>	<i>Arthrobacter</i>
		<i>Nocardioideaceae</i>	<i>Nocardioides & Kribbella</i>
		<i>Propionibacteriaceae</i>	<i>Microlunatus</i>
		<i>Streptomycetaceae</i>	<i>Streptomyces</i>
<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Balneimonas</i>
		<i>Methylobacteriaceae</i>	<i>Microvirga</i>
		<i>Bradyrhizobiaceae</i>	<i>Bradyrhizobium</i>
		<i>Phyllobacteriaceae</i>	<i>Mesorhizobium & Nitratisreductor</i>
		<i>Rhizobiaceae</i>	<i>Rhizobium & Neorhizobium</i>
	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Reyranella & Dongia</i>
<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Methylibium, Ramlibacter, Variovorax</i>
		<i>Oxalobacteraceae</i>	<i>Massilia & Janthinobacterium</i>
<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>	<i>Sinobacteraceae</i>	<i>Steroidobacter</i>
<i>Verrucomicrobia</i>	<i>Opitutaes</i>	<i>Opitutaceae</i>	<i>Opitutus</i>

Core microbiome of canola roots is affected by CeO₂ NMs

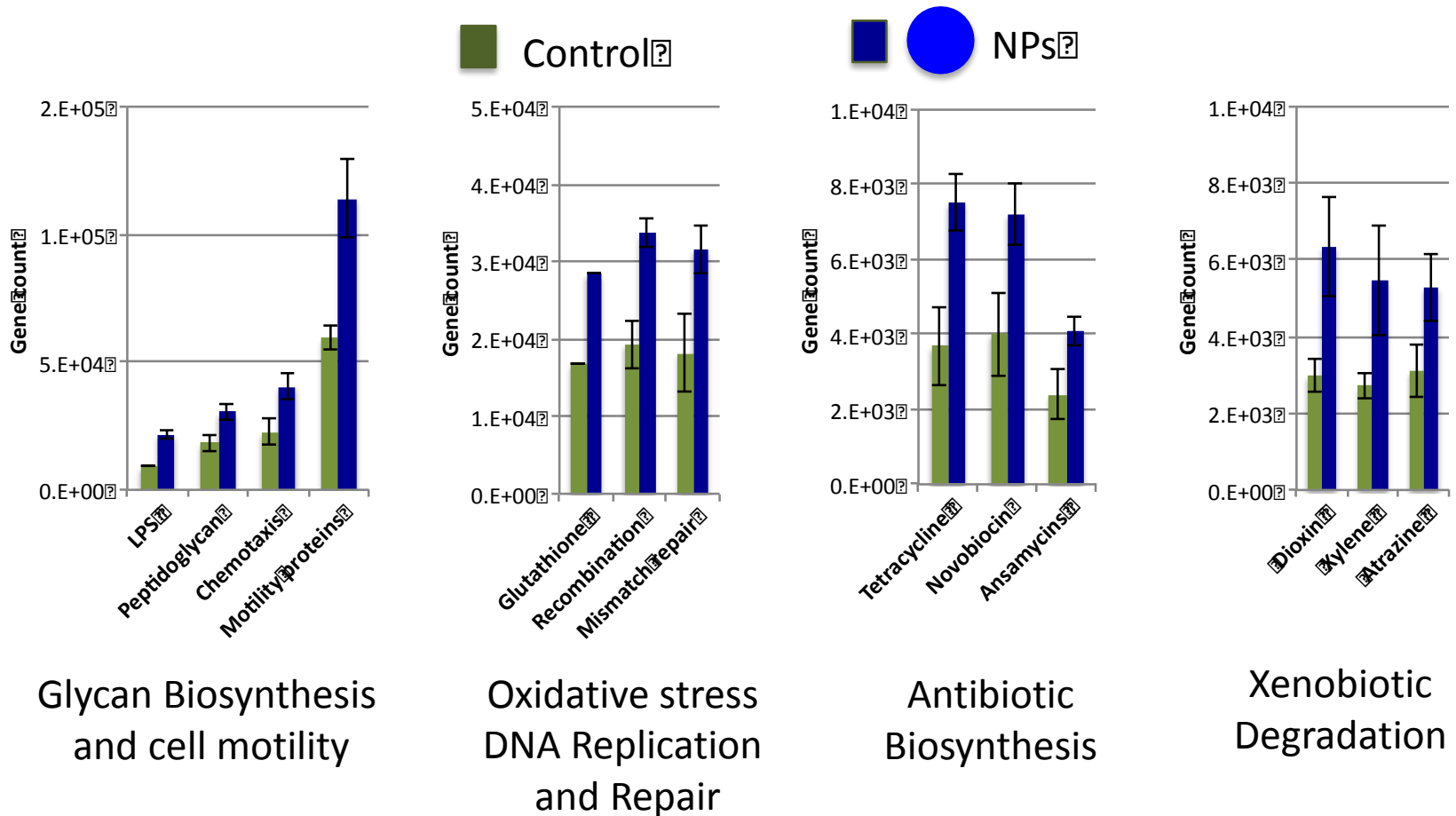
Class	Order	Family	Genus
<i>Actinobacteria</i>	<i>Actinomycetales</i>	<i>Microbacteriaceae</i>	<i>Agromyces</i>
		<i>Micrococcaceae</i>	<i>Arthrobacter</i>
		<i>Nocardioideaceae</i>	<i>Nocardioides & Kribbella</i>
		<i>Propionibacteriaceae</i>	<i>Microlunatus</i>
		<i>Streptomycetaceae</i>	<i>Streptomyces</i>
<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Balneimonas</i>
		<i>Methylobacteriaceae</i>	<i>Microvirga</i>
		<i>Bradyrhizobiaceae</i>	<i>Bradyrhizobium</i>
		<i>Phyllobacteriaceae</i>	<i>Mesorhizobium & Nitratisreductor</i>
		<i>Rhizobiaceae</i>	<i>Rhizobium & Neorhizobium</i>
	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Reyranella & Dongia</i>
<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Methylibium</i>, <i>Ramlibacter</i>, & <i>Variovorax</i>
		<i>Oxalobacteraceae</i>	<i>Massilia</i> & <i>Janthinobacterium</i>
<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>	<i>Sinobacteraceae</i>	<i>Steroidobacter</i>
<i>Verrucomicrobia</i>	<i>Opitutales</i>	<i>Opitutaceae</i>	<i>Opitutus</i>

Taxa in blue are significantly altered by CeO₂ NMs treatment

CeO₂ NMs affect the predictive functional profiling of microbial communities

PICRUSt tool: Phylogeny and function are sufficiently linked to allow predicting metagenomic

45 genes are predictively increased (1.7 to 2.4 fold) in CeO₂-NPs treated roots vs Control

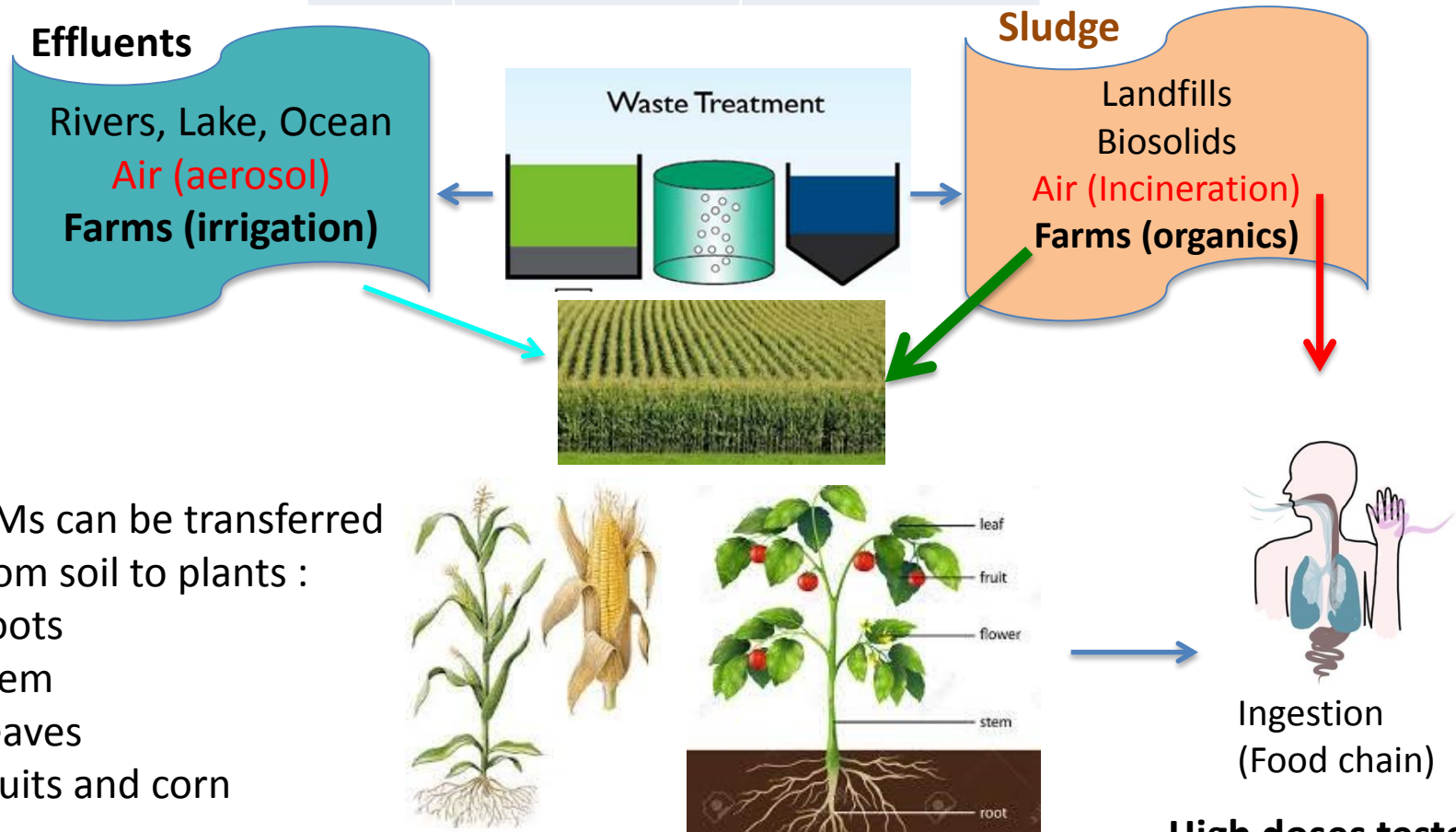


Nanomaterials in Waste Streams

Current Knowledge on Risks and Impacts (OCDE report 2016)

	Predicted Environmental Concentration	
	WWTP Effluent	Sludge
TiO ₂ NM	1- 100 µg/L	100- 2,000 mg/kg

Maurer-Jones *et al.* 2013



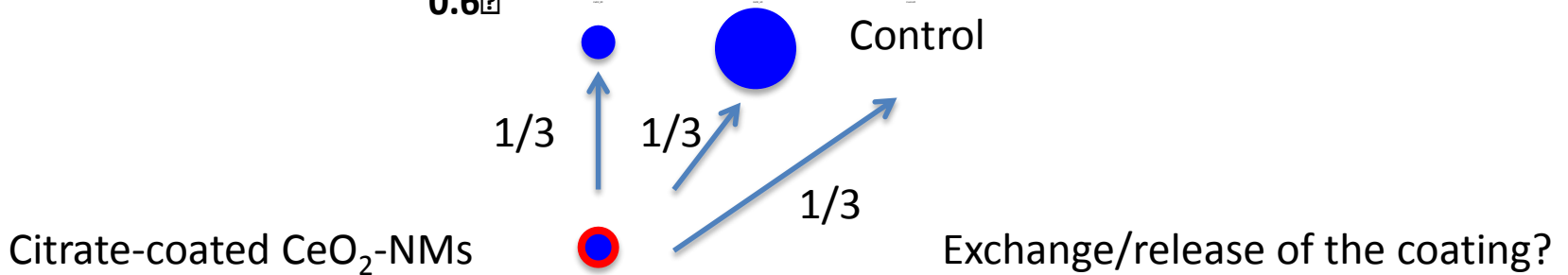
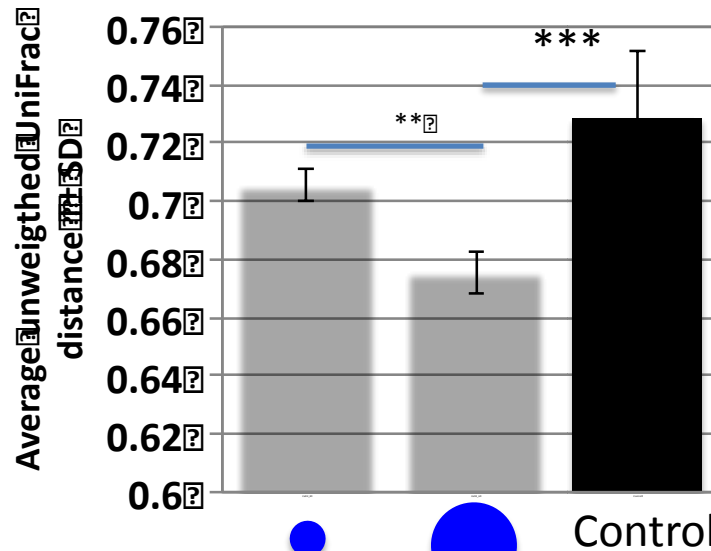
High doses tested!

Zhao *et al.*, Environ Sci Technol. 2015

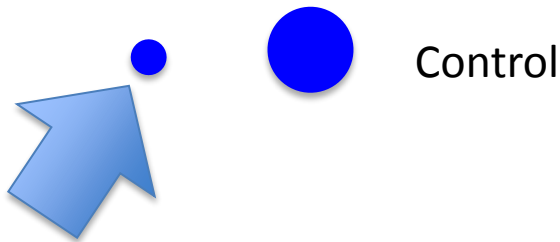
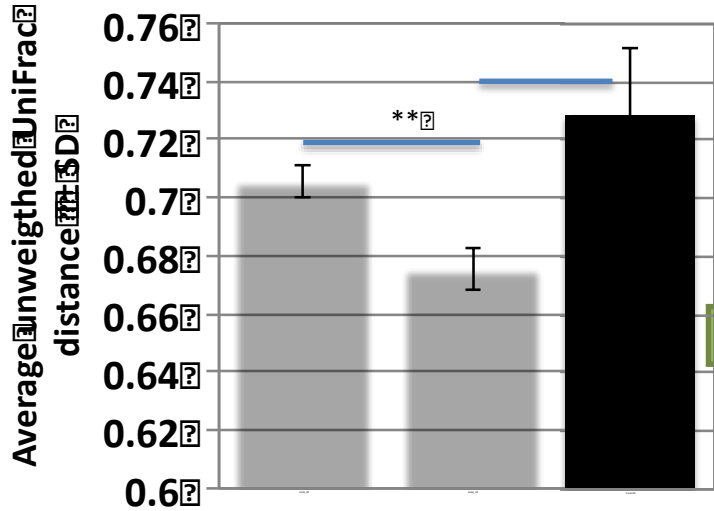
Raliya, *et al.*, Metallomics 2015

Impacts of CeO₂ NMs on root-associated bacterial community

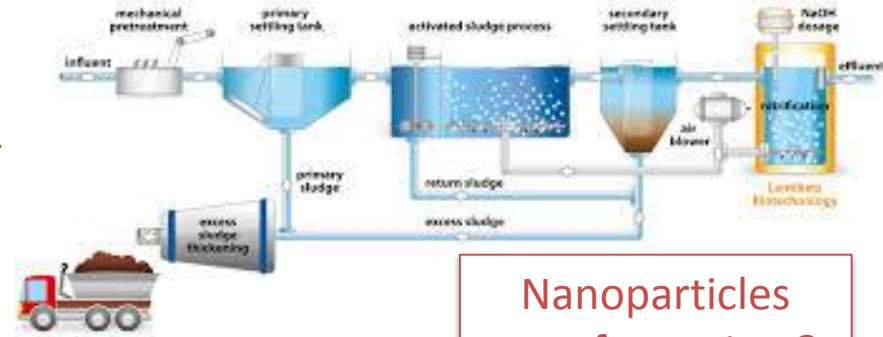
Phylogeny-based beta diversity



Indirect NMs release scenario



Waste water treatment scenario



Nanoparticles transformation ?

Indirect exposure through sludge




S5.2-P6 Impact of a CeO₂ nanoparticles enriched biosolid on a soil-plant-bacteria system

Blanche Collin, Mohamed. Barakat, Philippe. Ortet, Emmanuel Doelsch, Nicolas Roche, Mélanie Auffan, Catherine Santaella



Indirect release scenario

Cerium dioxide nanoparticles


CeO₂ NPs
4 nm

+



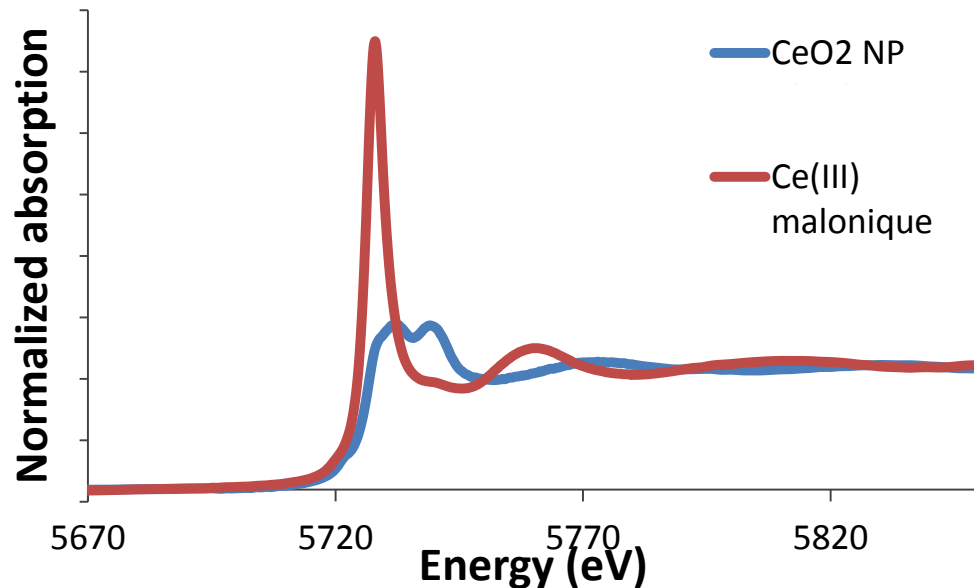
Activated sludge
Aix en Provence



Laboratory-scale
activated sludge reactors




5 weeks



X ray absorption spectroscopy XANES Ce L₃ edge – Synchrotron Soleil

Indirect release scenario

Cerium dioxide nanoparticles


CeO₂ NPs
4 nm

+



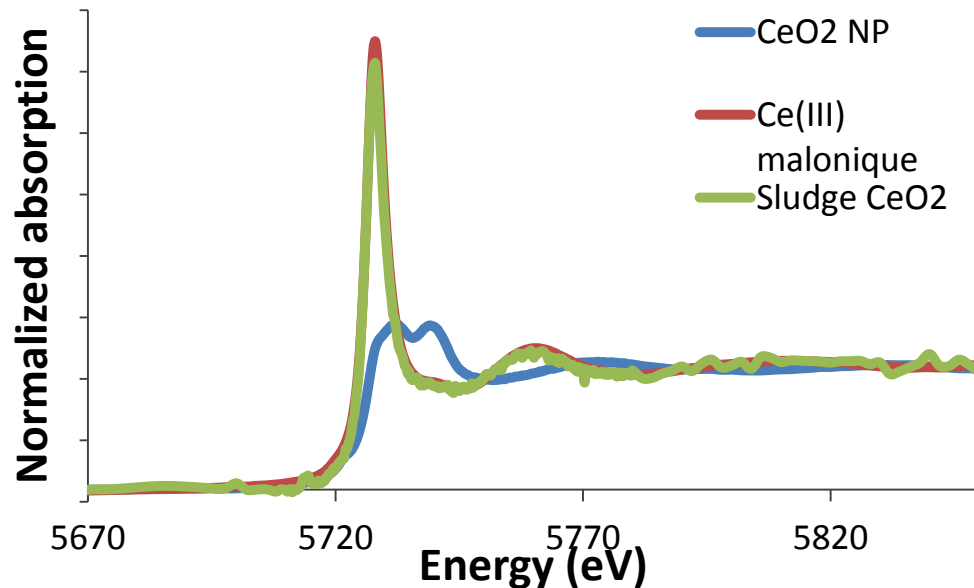
Activated sludge
Aix en Provence



Laboratory-scale
activated sludge reactors



5 weeks



CeO₂ NPs are transformed
as Ce(III) complexes
in the biosolid

Barton et al. 2014

X ray absorption spectroscopy XANES Ce L₃ edge – Synchrotron Soleil

Materials and methods



Couhins,
Sandy soil
pH 5.5

INRA's Couhins experimental farm in Bordeaux (SOERE PRO)

Long-term sewage sludge field experiment

ETM	Cd	Ni	Pb	Ce
mg.kg ⁻¹ DW	2.8	15	48.6	7.3

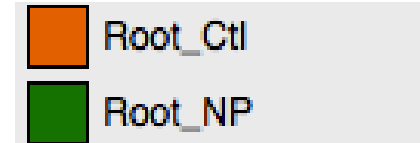
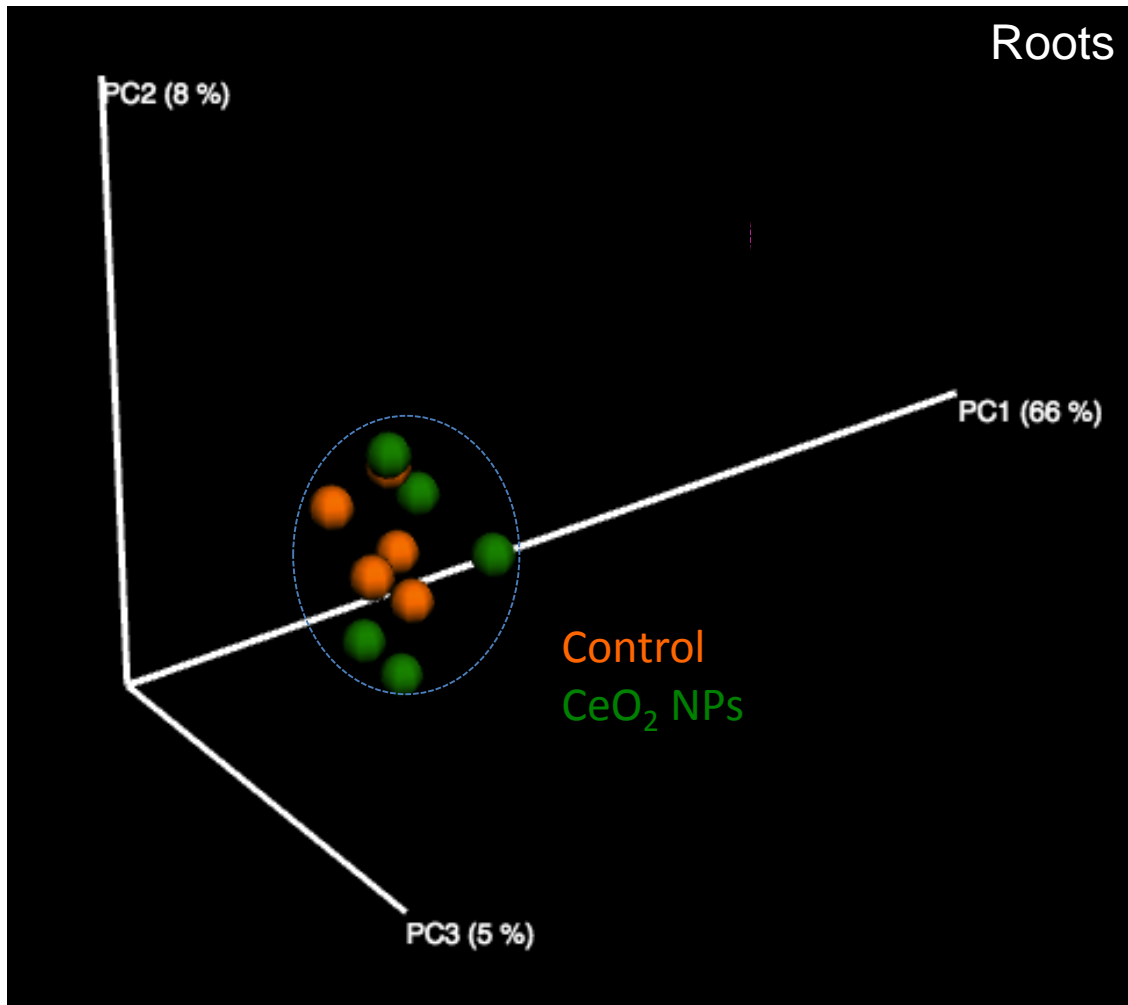


4 treatments :

- Soil Couhins
- Soil + native CeO₂ NP (1 mg.kg⁻¹)
- Soil + control sludge
- Soil + sludge containing CeO₂ NPs (1 mg.kg⁻¹)
-

Results : bacterial community structure

- Impact of CeO₂ NPs and sludge on the bacterial community structure in roots

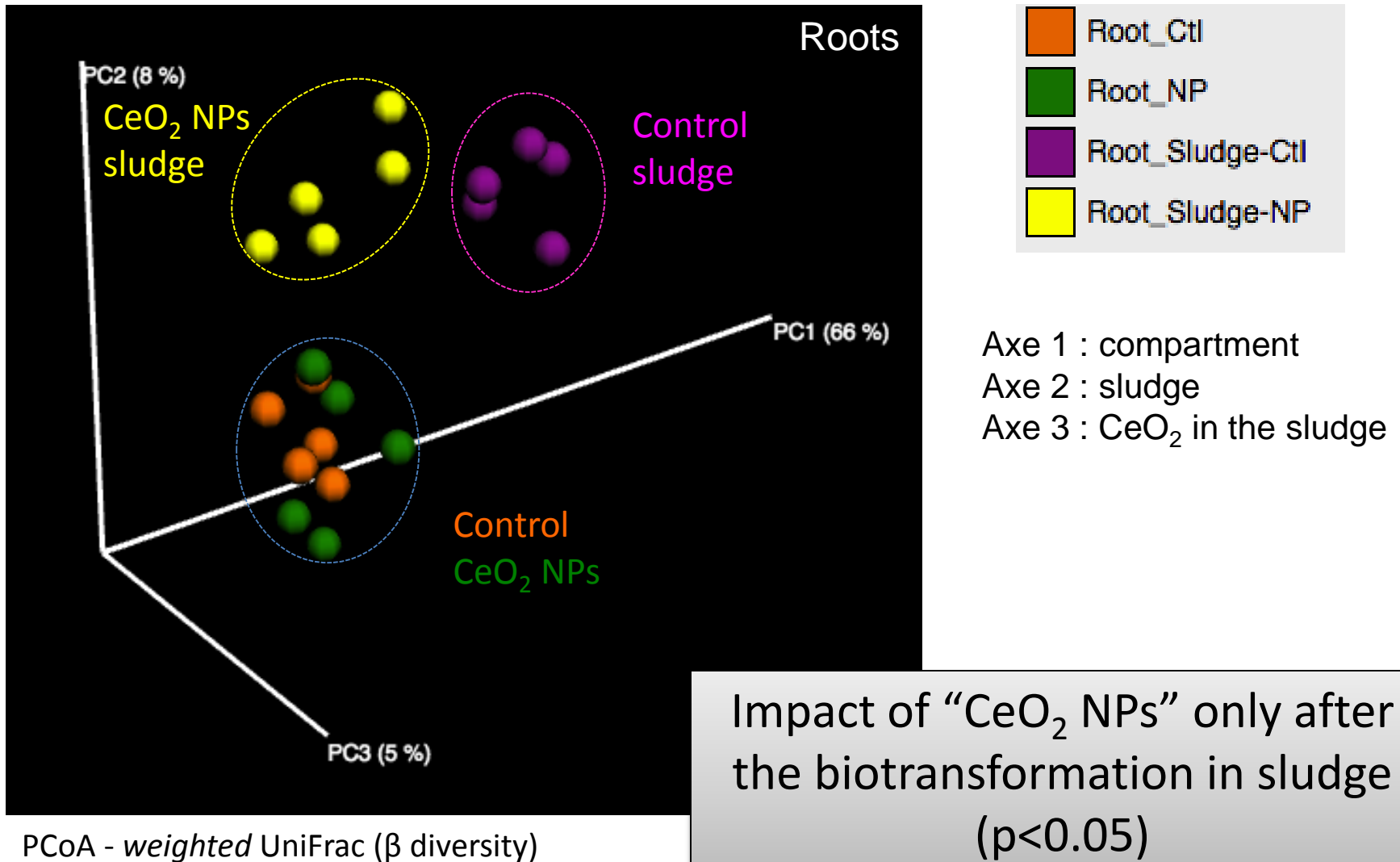


Axe 1 : compartment
Axe 2 : sludge
Axe 3 : CeO₂ in the sludge

PCoA - *weighted* UniFrac (β diversity)

Results : bacterial community structure

- Impact of CeO₂ NPs and sludge on the bacterial community structure in roots



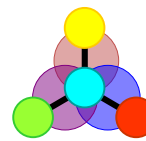
Take-home messages



- ✓ **CeO₂ NMs modulate root microbiome of canola and the predictive functional profile**
 - **NMs with high particle size and well crystallized faces produce the highest effects**
 - **The characteristics and properties of NMs can help to minimize these effects validating the safer by design concept**
- ✓ **The biotransformation of CeO₂ NPs in WWT can alter the bacterial microbiome associated to canola roots**

Thanks!

Mohamed Hamidat
Cécile Simonet
Philippe Ortet
Mohamed Barakat
Blanche Collin
Anais Cuny
Wafa Achouak
Thierry Heulin



Serenade

Mélanie Auffan
Jean Yves Bottero
Jérôme Rose
Emmanuel Doelsch
Clément Layet



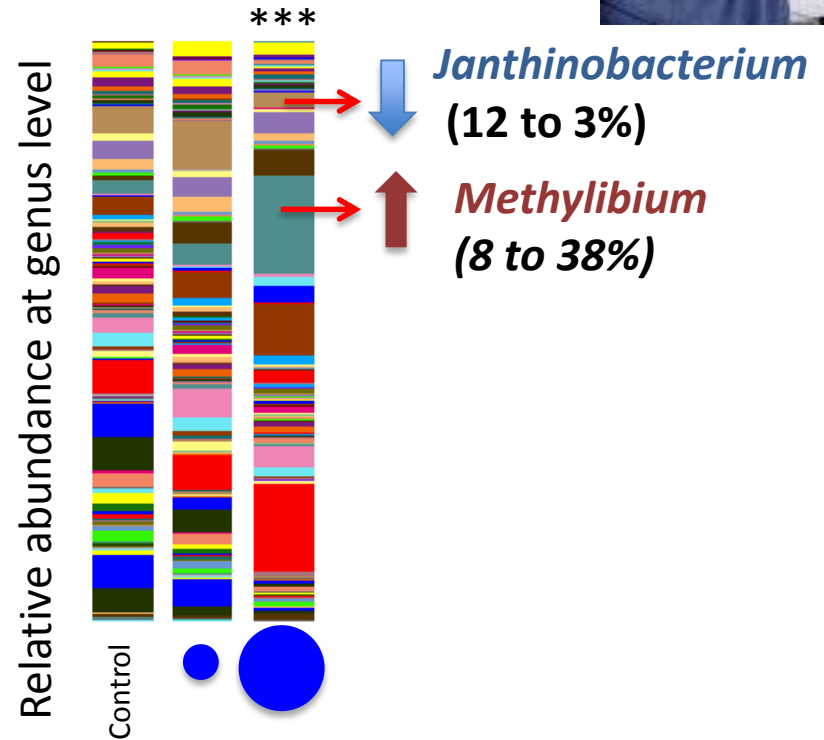
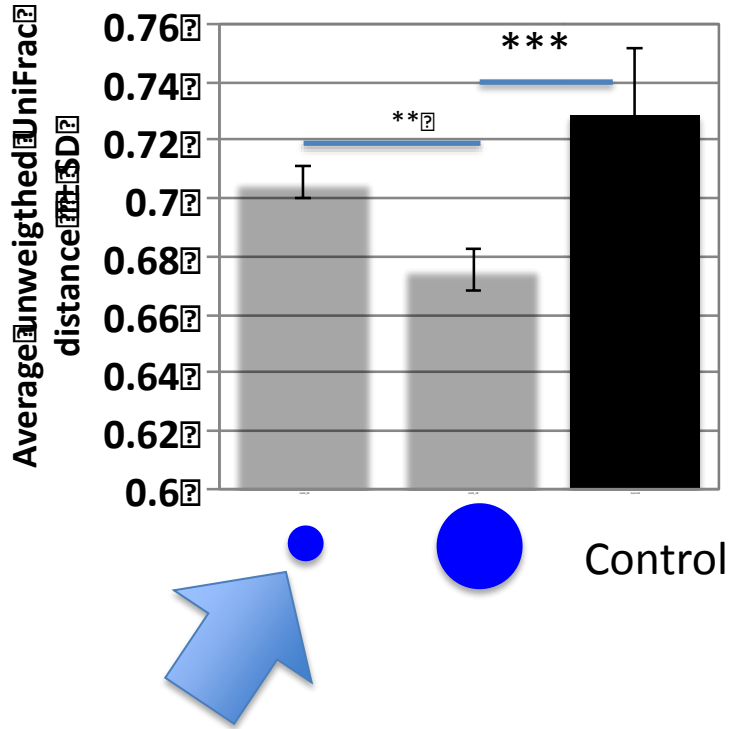
ANR MESONNET
directed by Jean Yves Bottero



Gautier Landrot (Synchrotron Soleil)
Nicolas Roche (M2P2, AMU)
Valérie Sappin Didier (INRA Bordeaux)



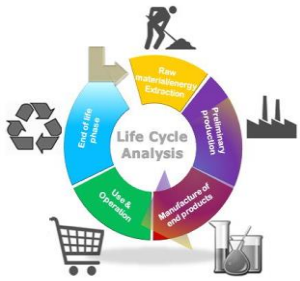
Impacts of CeO₂ NMs on root-associated bacterial community



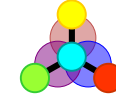
No significant impact on root-associated bacterial community



Next: NPs from use to WWTP and soil



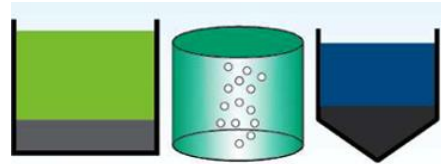
EcoSun (J. Labille...)



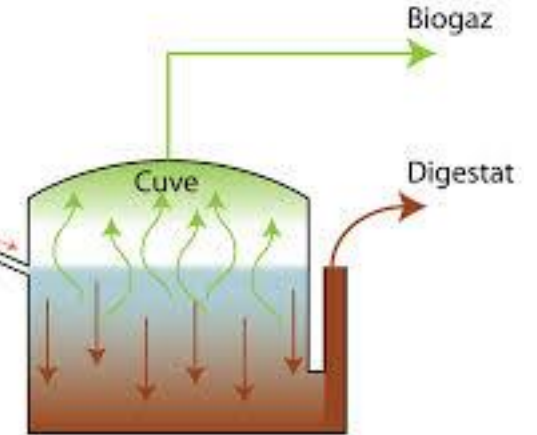
Serenade

NPs use, aging

Waste water treatment process



Valorization pathways



NanoSTEP

(M. Auffan, C. Santaella, E. Doelsch, N. Roche, Karim Kabore, Anais Cuny)

Soil-based system



Predicted Environmental Concentration of nanoCeO₂

Park et al., 2008

Emission source CeO₂-based diesel additive (Envirox)

PEC CeO₂ in soil in 2020 on 20 meters either side of a UK road: 0.32 - 1.12 mg.kg⁻¹

Johnson and Park, 2012

PEC CeO₂ 0.016 mg.kg⁻¹ in 2012 and 0.0390 mg.kg⁻¹ in the next five years.

	Unit	CeO ₂	
		Mode	Range
Technical compartments			
Sewage treatment effluent	ng/L	9.3	1.1–60
Sewage treatment sludge	µg/kg	350	44–2300
Waste mass incinerated	µg/kg	180	21–930
Bottom ash	µg/kg	360	50–2500
Fly ash	µg/kg	2200	240–12,000
Natural compartments			
Surface water (fresh water)	pg/L	4	0.6–100
Sea water	pg/L	0.3	0.03–2
Sediments (fresh water)	µg/kg	1.6	0.2–45
Sediments (sea water)	µg/kg	0.3	0.04–2
Agricultural soils	ng/kg	76	10–530
Natural soils	ng/kg	170	24–1500
Urban soils	ng/kg	300	39–2100
Sludge treated soils	ng/kg	1500	94–5100
Air	ng/m ³	0.1	0.01–0.6

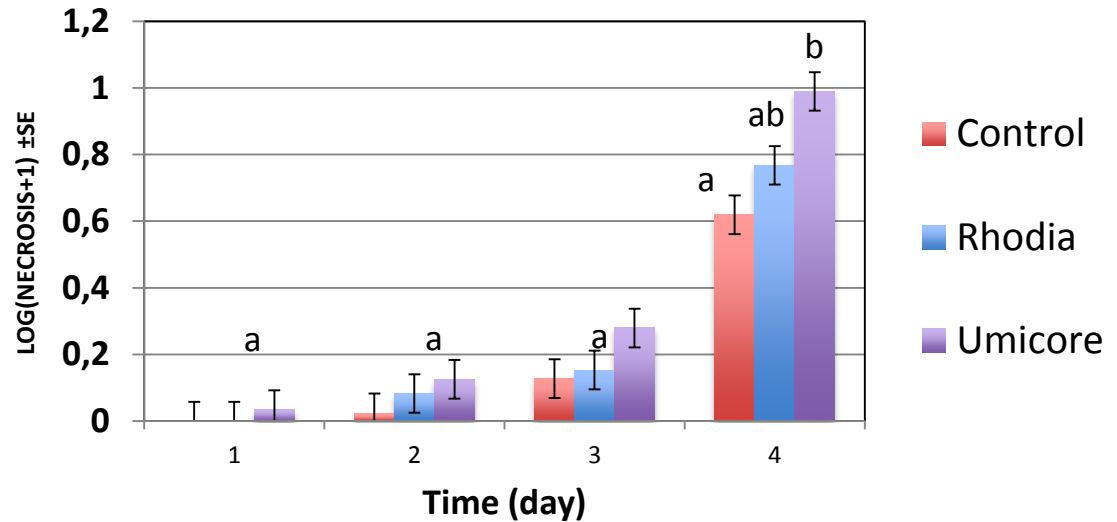
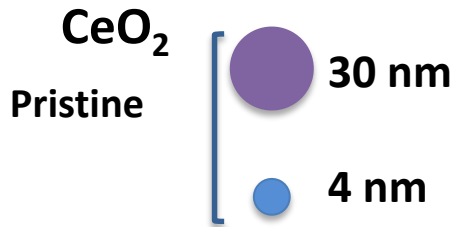
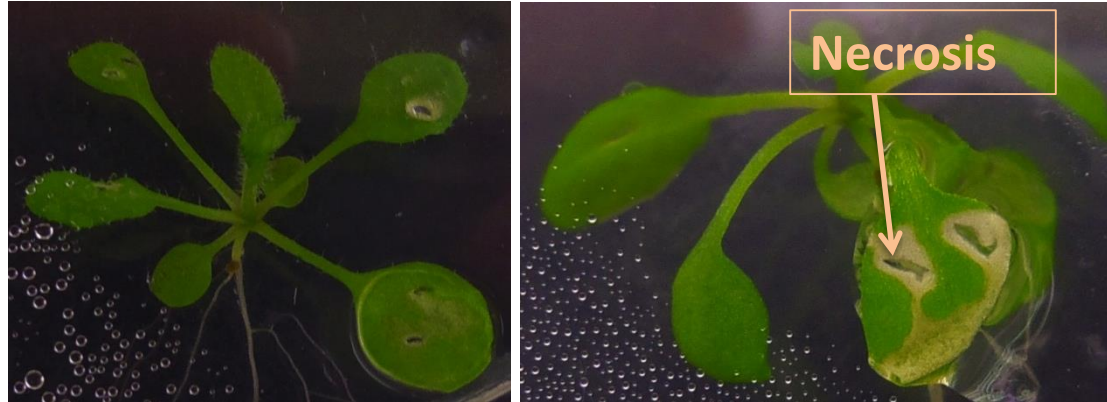
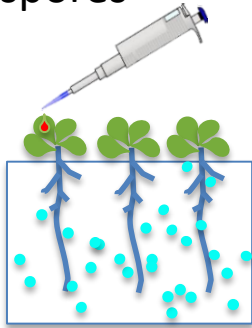
Modeling Flows and Concentrations of Nine Engineered Nanomaterials in the Danish Environment
Fadri Gottschalk , Carsten Lassen , Jesper Kjoelholm , Frans Christensen and Bernd Nowack

Int. J. Environ. Res. Public Health **2015**, *12*, 5581-5602

CeO₂ NMs can sensitize plants to pathogens



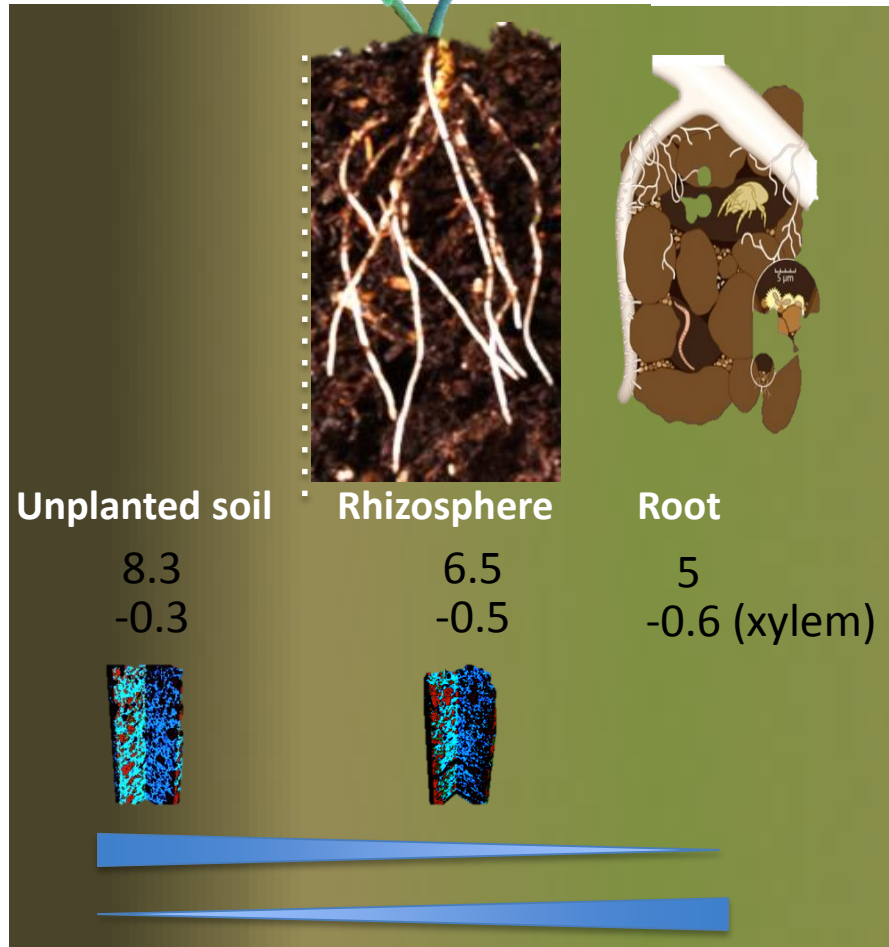
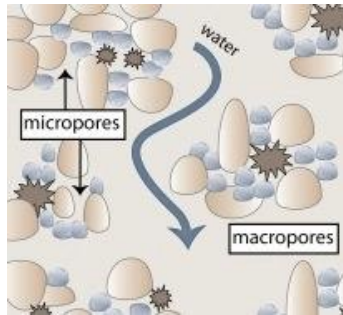
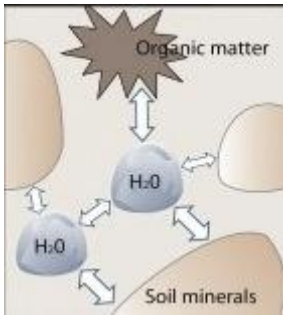
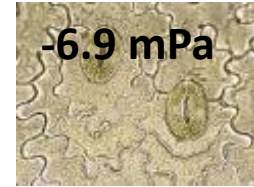
+ Pathogen spores



CeO₂ NMs can increase the infection speed of *Botrytis cinerea* (p < 0.05)

From unplanted soil to the plant roots: A changing environment

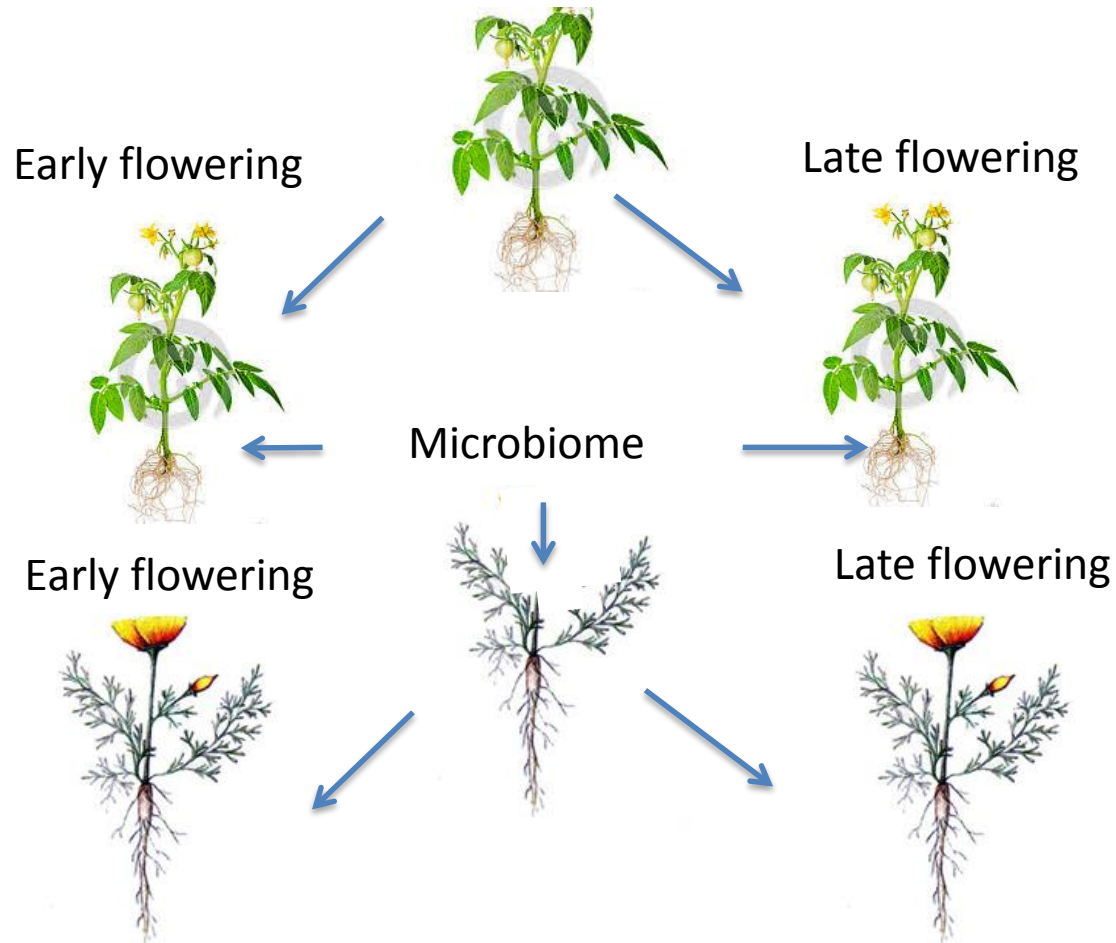
Water Potential



pH
Water Potential (mPa)
■ **Air filled spaces -75 kPa**
In an alkaline calcaerous soil
Redox potential (E_H)
Reducing capacity

Selection on soil microbiomes reveals reproducible impacts on plant function

Panke-Buisse, ISME J 2015



CeO₂ NM affect the predictive functional profiling of microbial communities

PICRUSt tool: Phylogeny and function are sufficiently linked to allow predicting metagenomic

45 genes are predictively increased (1.7 to 2.4 fold) in CeO₂-NPs treated roots vs Control

