Technical seminar – WP2 – HMGU

Bacterial communities response to heavy metals in rhizocompartments of *Miscanthus* x *giganteus*

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PHYTO2ENERGY

Phytoremediation driven energy crops production on heavy metal degraded areas as local energy carrier

Comparative Microbiome Analysis

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 610797.

Research objectives

Evaluating the impact of metal contamination on the bacterial community of biomass producing plant in controlled conditions

1. In which rhizocompartments of *M. giganteus* microbial communities will respond to metal treatment?
2. Who are the main microbial responders to metal treatment?
3. Do the responders possess known PGPR properties?

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Experimental setup

- *Miscanthus* x *giganteus* rhizomes acclimatized in non-contaminated agricultural soil for 3 months

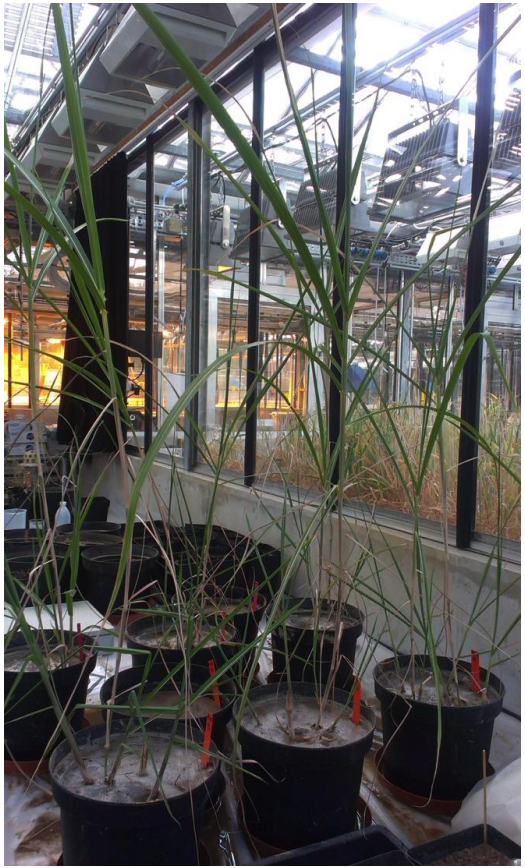
- Application of two different concentrations of Pb, Zn, Cd:

10x dil.:
Pb: 54,7 mg/kg
Zn: 217,5 mg/kg
Cd: 2,1 mg/kg

100x dil.:
Pb: 5,5 mg/kg
Zn: 21,8 mg/kg
Cd: 0,2 mg/kg

- Sampling after 3 months:
  - Bulk soil (BUL),
  - Rhizosphere (RH),
  - Rhizoplane (PL),
  - Endosphere (END) – surface sterilized.

- Analyses:
  - Total and bioavailable metal concentrations in soil and roots
  - Pedological parameters
  - qPCR based assessment of bacterial abundance
  - Preparation of Bacterial 16S rRNA sequencing libraries



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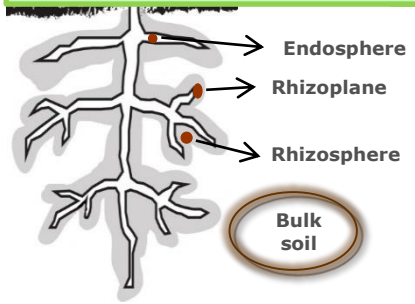
Workflow

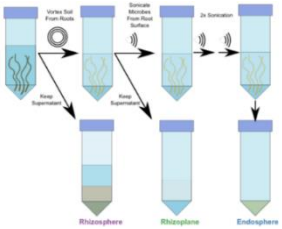
Sampling

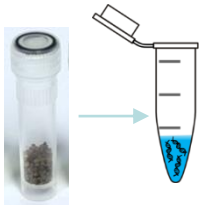
Sample processing

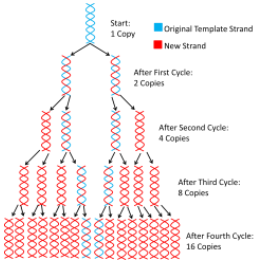
DNA extraction
MoBio PowerSoil DNA Isolation Kit

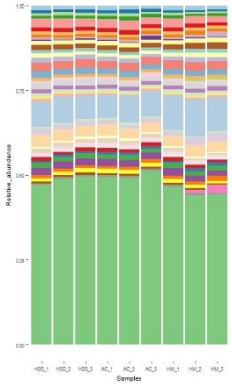
PCR
Bacterial 16S rRNA gene, region V3-V4







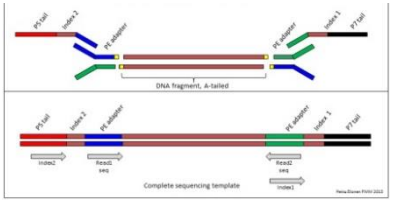




Data analysis

Sequencing
MiSeq, Illumina

Library preparation



Quantitative Insights Into Microbial Ecology

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Leggend and color coding

H_2O ... H_2O treatment
AN ... Acetate (acetic acid) and nitrate (ammonium nitrate) treatment
HM ... Heavy metal (Pb, Zn, Cd) treatment

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Pedological parameters

Silt loam
texture

Sand 2 mm-63 µm	Silt 63 µm-2 µm	Clay <2 µm
18,9	58,6	22,5

Treatment	DOC mg/g of dry soil	TNb mg/g of dry soil	Ammonium µg/L of dry soil	Nitrat mg/L of dry soil	Nitrit µg/L of dry soil	pH
H2O	**a** 0.12 ± 0.01	**b** 0.01 ± 0.00	**a** 0.44 ± 0.09	**b** 0.00 ± 0.00	LOQ	**b** 7,08 ± 0,01
AC10x	**b** 0.04 ± 0.00	**a** 0.02 ± 0.00	**b** 0.31 ± 0.04	**a** 0.02 ± 0.00	0.06 ± 0.01	**a** 7,13 ± 0,02
HM10X	**b** 0.06 ± 0.00	**a** 0.02 ± 0.01	**b** 0.26 ± 0.03	**a** 0.02 ± 0.01	LOQ	**c** 7,03 ± 0,01

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Total and bioavailable metal concentrations in soil

ICP-AES
Prof. Michalke, BGC

AVERAGE depth by replicates (3x)

		TOTAL conc.			BIOAVAILABLE conc.		
		Pb (mg/kg)	Zn (mg/kg)	Cd (µg/kg)	Pb (µg/g dw)	Zn (µg/g dw)	Cd (ng/g dw)
depth 1	H2O	17.5±0.3	68±7.7	651.7±30.7	LOQ	0.01±0	LOQ
depth 2	H2O	17.4±0.4	59.1±7	652.3±29	LOQ	0.01±0	LOQ
depth 3	H2O	17.8±0.5	61.4±4.1	638.7±44.2	0.03±0.01	LOQ	LOQ
bulk 1	H2O	17.0	56.4	617	LOQ	0.01	LOQ
bulk 2	H2O	17.0	56.6	602	0.02	LOQ	LOQ
bulk 3	H2O	17.1	55.8	611	LOQ	LOQ	LOQ
depth 1	Ac-Nit 10x	17.2±0.2	70.1±15.2	654.5±38.9	LOQ	LOQ	LOQ
depth 2	Ac-Nit 10x	17.1±0.0	60.1±2.5	652±26.9	LOQ	LOQ	LOQ
depth 3	Ac-Nit 10x	16.5±0.4	58.2±3.1	626.5±2.1	LOQ	LOQ	LOQ
bulk 1	Ac-Nit 10x	16.6	57.1	562	LOQ	LOQ	LOQ
bulk 2	Ac-Nit 10x	16.5	55.4	592	0.03	LOQ	LOQ
bulk 3	Ac-Nit 10x	15.7	52.7	577	LOQ	LOQ	LOQ
depth 1	HM 10x	59.2±7.4	444.3±214.6	3153.3±1276.6	LOQ	0.15±0.18	13.26±11.81
depth 2	HM 10x	22.8±3.1	101.2±25.2	866.3±175.2	LOQ	LOQ	LOQ
depth 3	HM 10x	21.2±0.7	76.6±11.8	703±80.7	LOQ	LOQ	LOQ
bulk 1	HM 10x	138	952	7000	LOQ	1.33	7.73
bulk 2	HM 10x	18.8	65	600	LOQ	LOQ	LOQ
bulk 3	HM 10x	18.7	64.5	602	LOQ	0.00	LOQ

10x HM treatment
HM added to soil:
- Pb: 54.7 mg/kg
- Zn: 217.5 mg/kg
- Cd: 2.1 mg/kg

LOQ … Limit of quantification: 6,2 µg Pb/L; 1,2 µg Zn/L; 545 ng Cd/L

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Roots accumulated metals

AVERAGE by replicates (3x)

	TOTAL conc. in ROOTS		
	Pb (mg/kg)	**Zn** (mg/kg)	**Cd** (µg/kg)
H2O	2.3±1.0	17.9±3.4	284.3±78.7
AN 10x	3.2±0.8	20.7±4.1	350.3±87.0
HM 10x	10.6±6	62.9±34.0	1862.7±1206.0
10x HM treatment HM added to soil: Pb: 54.7 mg/kg Zn: 217.5 mg/kg Cd: 2.1 mg/kg	19,4 %	28,9 %	88,7 % of added HM

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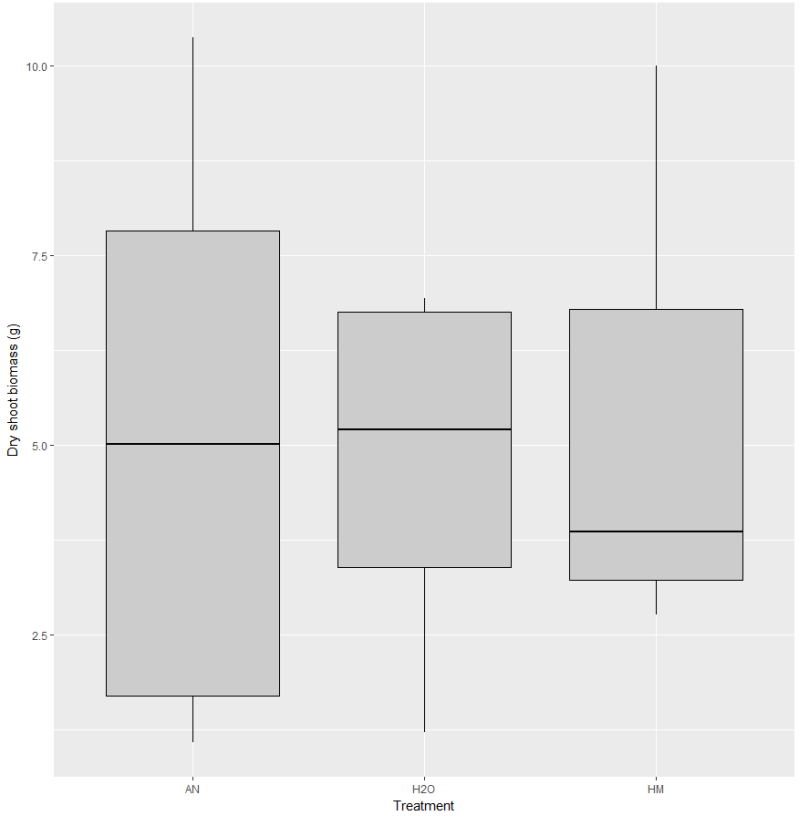
Biomass of *M. giganteus* under metals stress did not change

Treatment	p (dry shoots)
H_2O-AN	0.9854233
HM-AN	0.9945335
HM-H_2O	0.9626671



n = 7

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Bacterial abundance

Bacterial 16S rRNA gene copy number/ng DNA

By rhizocompartments

Significance	Soil comp.	Mean
a	Rhizoplane	389.1
a	Rhizosphere	343.7
b	Bulk	260.4

By treatment

Significance	Treatment	Mean
a	AN10x	384.6
a	HM10x	362.7
a	H2O	328.2

a, b, c … Statistically different samples, Duncan´s test

Alpha diversity: lower in rhizoplane and the lowest in endosphere

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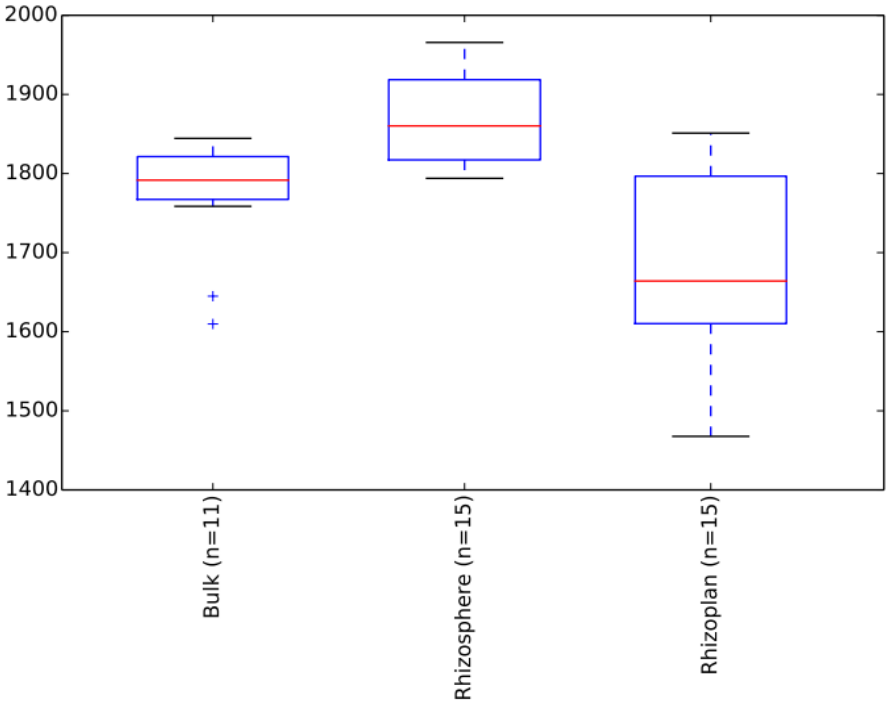
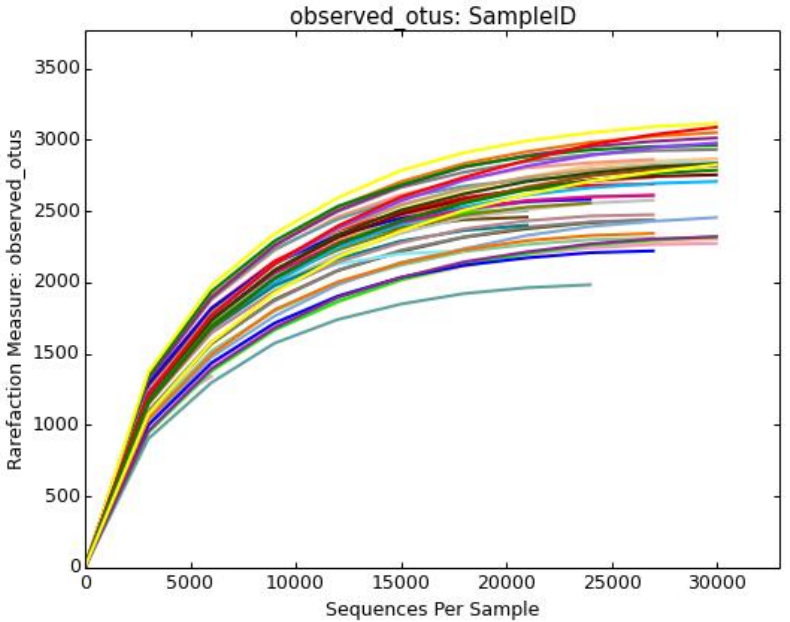
α-diversity and sequencing effort





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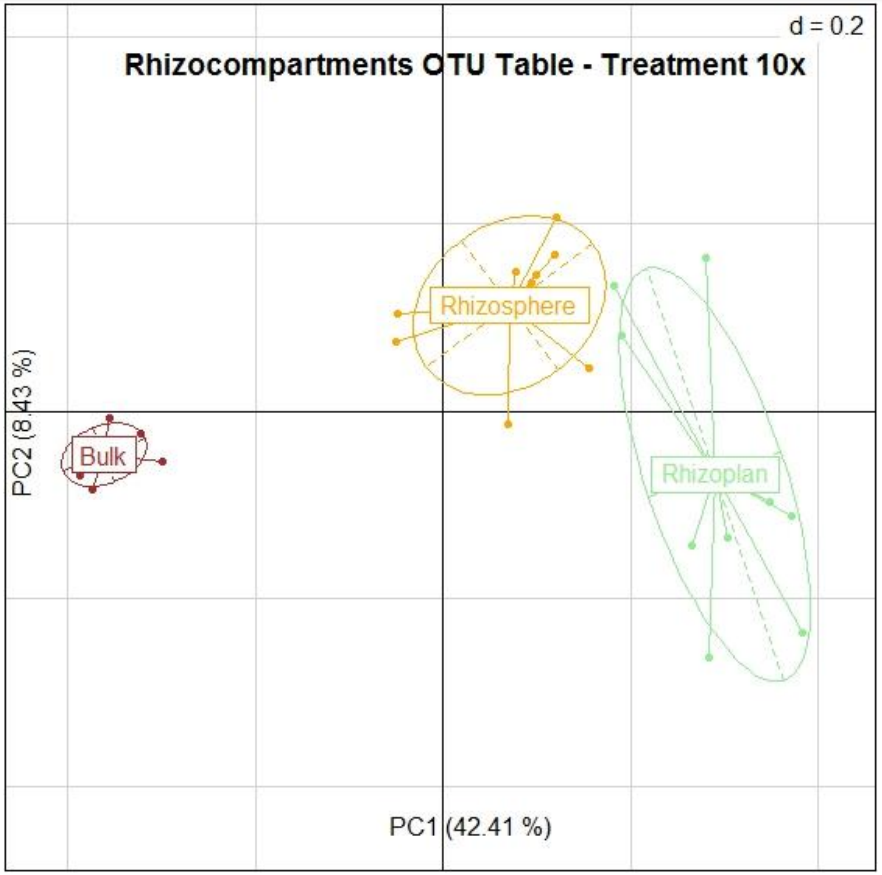
Bacterial communities in three rhizocompartments are different



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Bacterial genera in **bulk** soil

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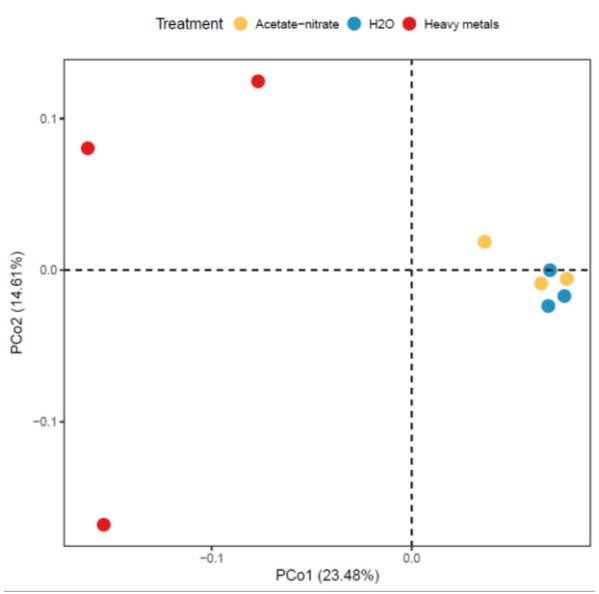
Bacterial structure in **bulk soil**

OTU 97 %, 19802 reads

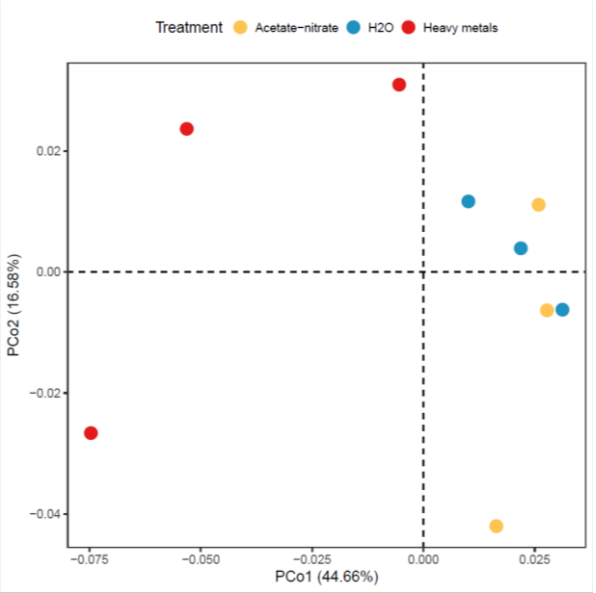
Unweighted Unifrac (Adonis)

- BUL: p = 0,003
- H_2O-HM: **p = 0,0019**
- AN-HM: **p = 0,0014**
- H_2O-AN: p = 0,2



Weighted Unifrac (Adonis)

- BUL: p = 0,006
- H_2O-HM: **p = 0,0014**
- AN-HM: **p = 0,0014**
- H_2O-AN: p = 0,2



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Bacterial genera in **rhizosphere**

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Bacterial structure in **rhizosphere**

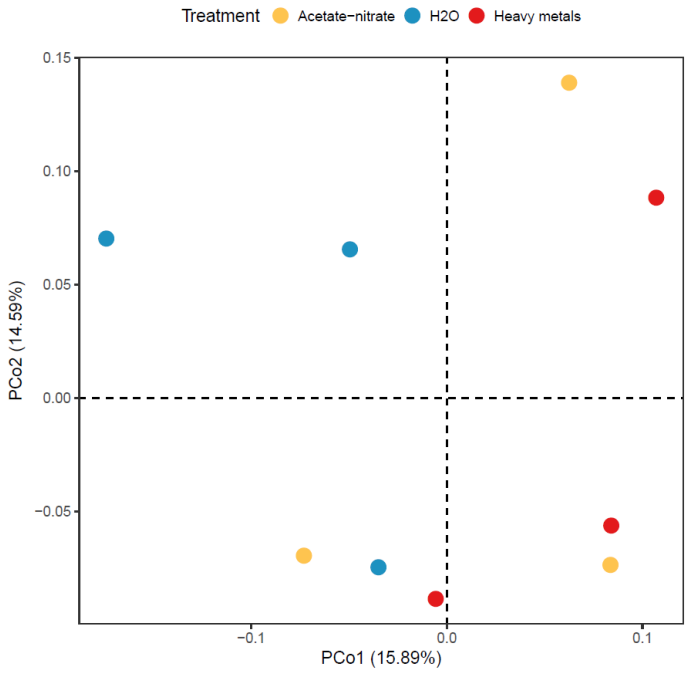
OTU 97 %, 19802 reads

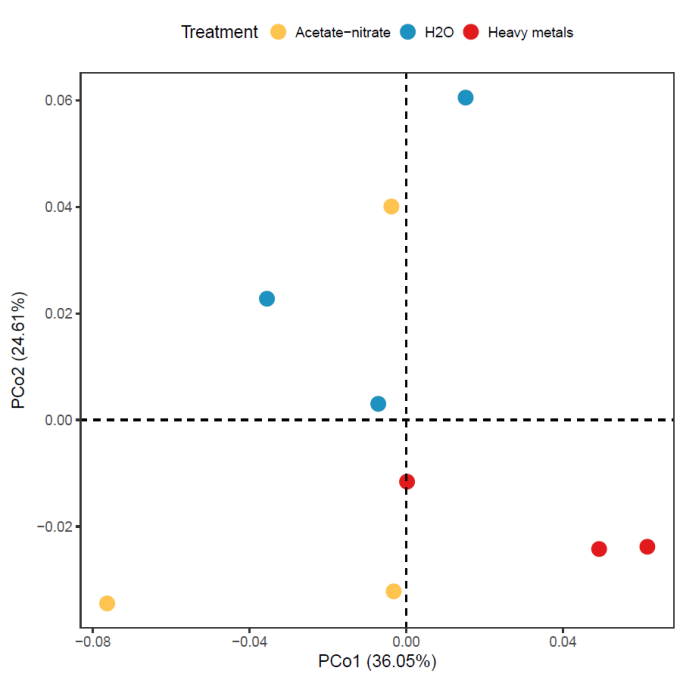
Unweighted Unifrac (Adonis):

- RH: p = 0,027
- H_2O-HM: **p = 0,0014**
- AN-HM: p = 0,4
- H_2O-AN: p = 0,1

Weighted Unifrac (Adonis):

- RH: p = 0,019
- H_2O-HM: p = 0,1
- AN-HM: p = 0,1
- H_2O-AN: p = 0,301





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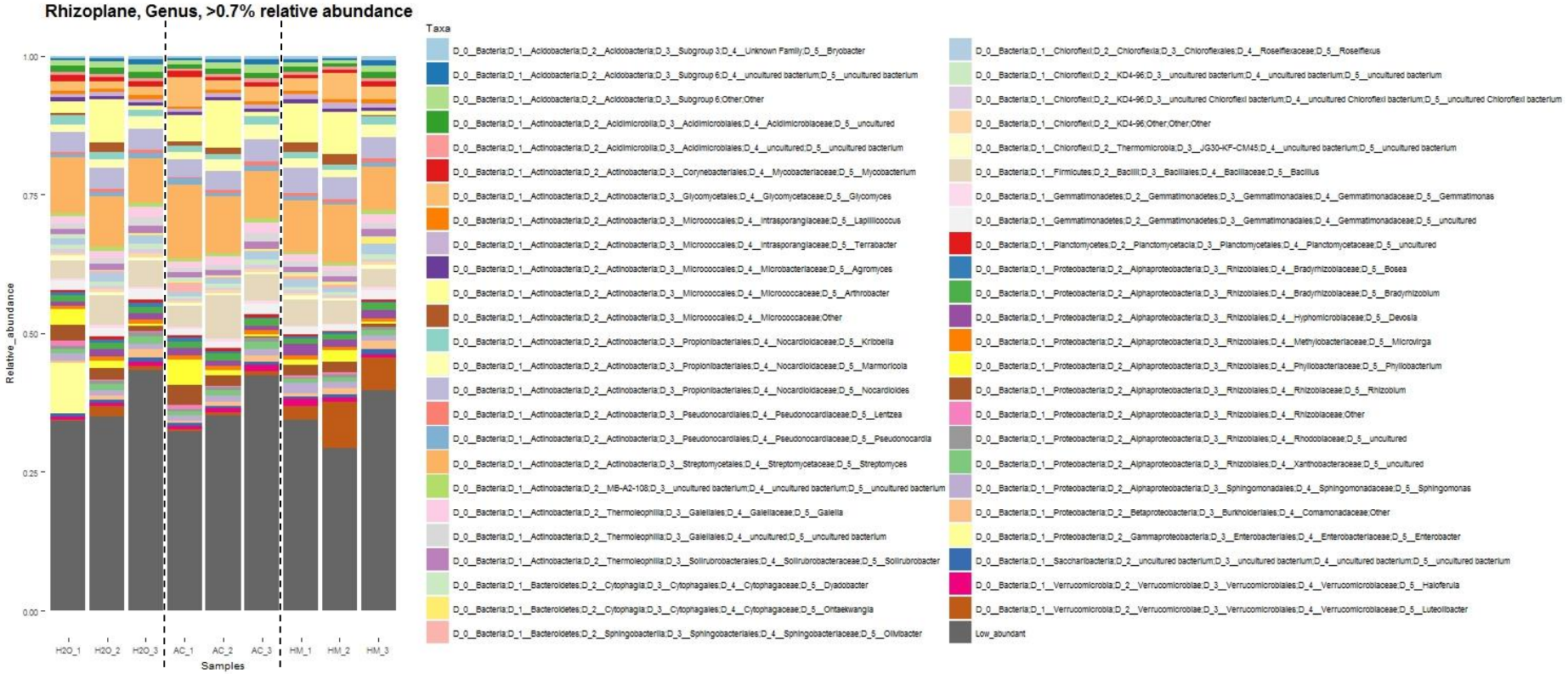
Bacterial genus in **rhizoplane**



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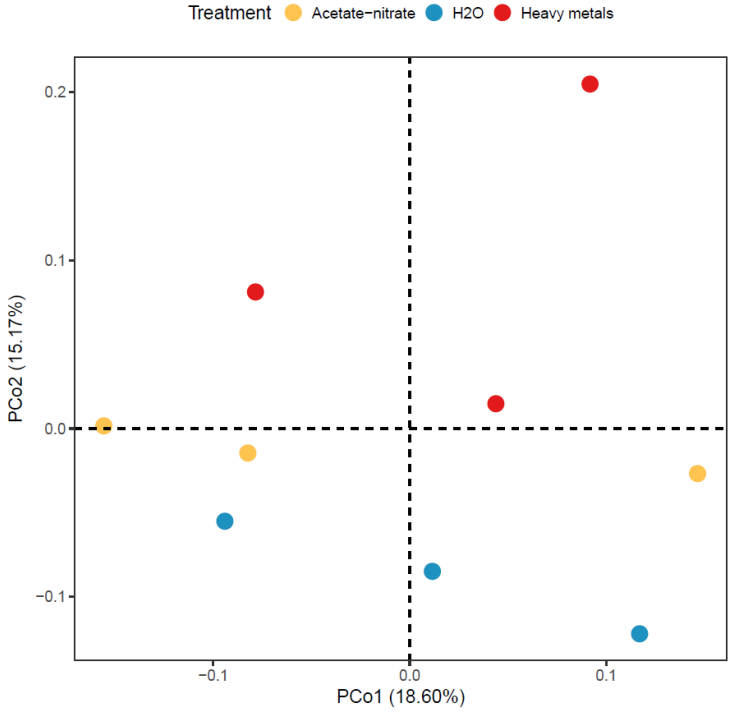
Bacterial structure in **rhizoplane**

OTU 97 %, 19802 reads

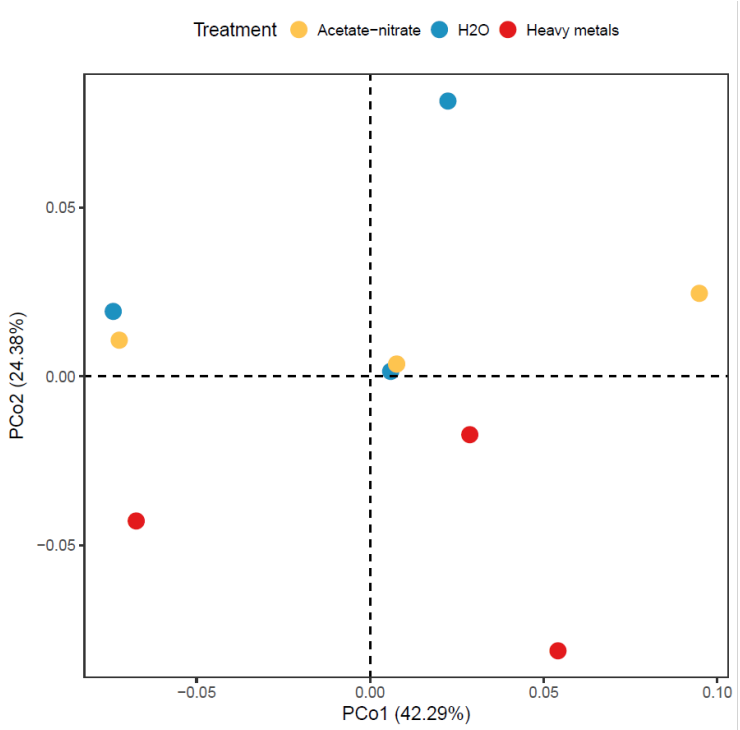
Unweighted Unifrac (Adonis):

PL: p = 0,097



Weighted Unifrac (Adonis):

PL: p = 0,453



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Bacterial genera in **endosphere**

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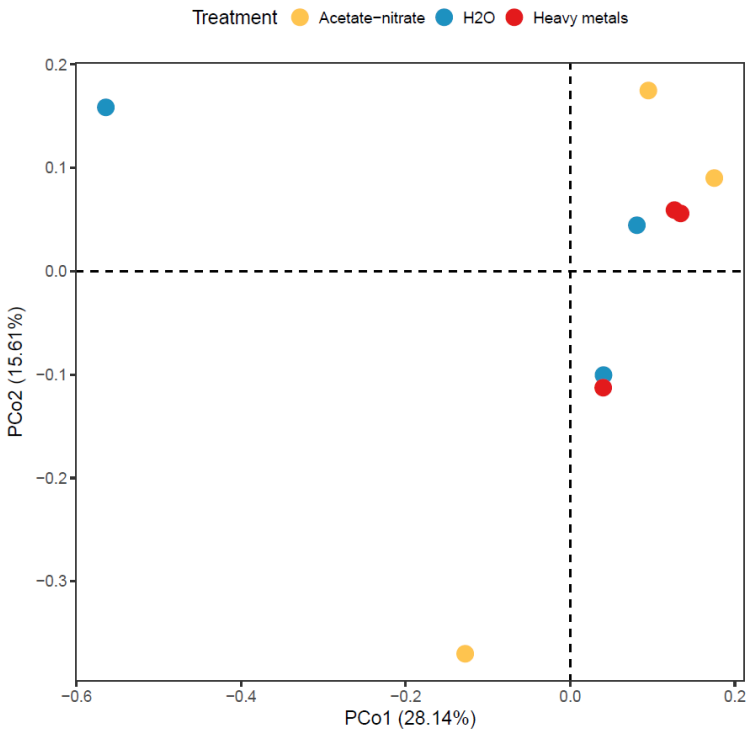
Bacterial structure in **endosphere**

OTU 97 %, 11283 reads

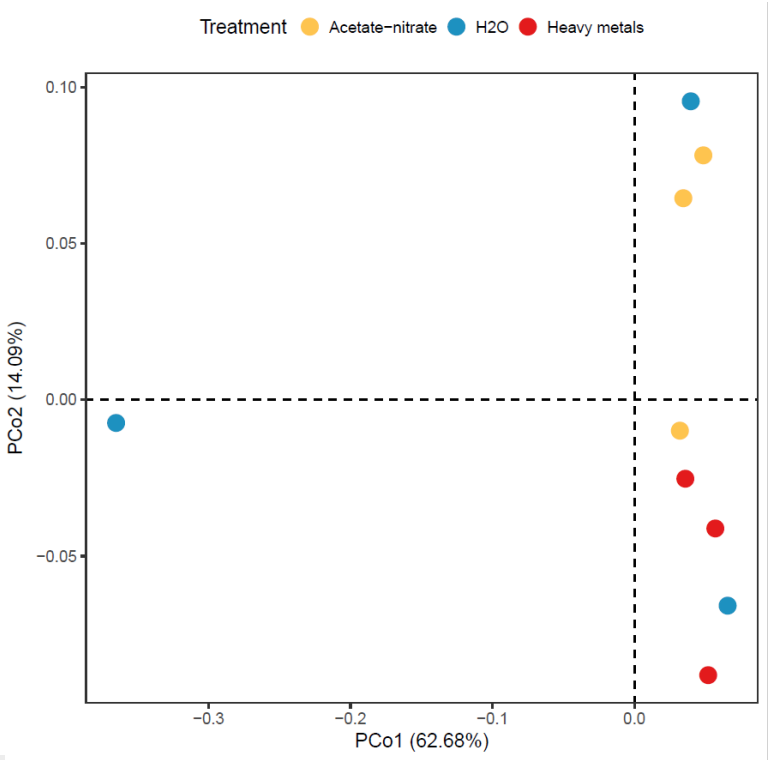
Unweighted Unifrac (Adonis):

END: p = 0,485



Weighted Unifrac (Adonis):

END: p = 0,482



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Bacterial responders to metal stress

Bulk soil	Rhizosphere	Rhizoplane	Root endosphere
Solirubrobacter	*Luteolibacter*	*Luteolibacter*	*Streptomyces*
Roseiflexus	*Roseiflexus*	*Roseiflexus*	*Glycomyces*
Pir4 lineage	*Flavisolibacter*	*Flavisolibacter*	*Kibdelosporangium*
Xanthomonas	*Nocardioides*	*Nocardioides*	*Devosia*
Bacillus	*Pseudoxanthomonas*	*Bacillus*	*Rhizobium*
43 responders in total	**26** responders in total	**20** responders in total	**23** responders in total

Uncorrected IndVal index for presented OTUs: 1.00-0.93 ($p < 0.05$) (De Cáceres and Legendre, 2009)

Metal responders will be further analysed for known PGPR properties.

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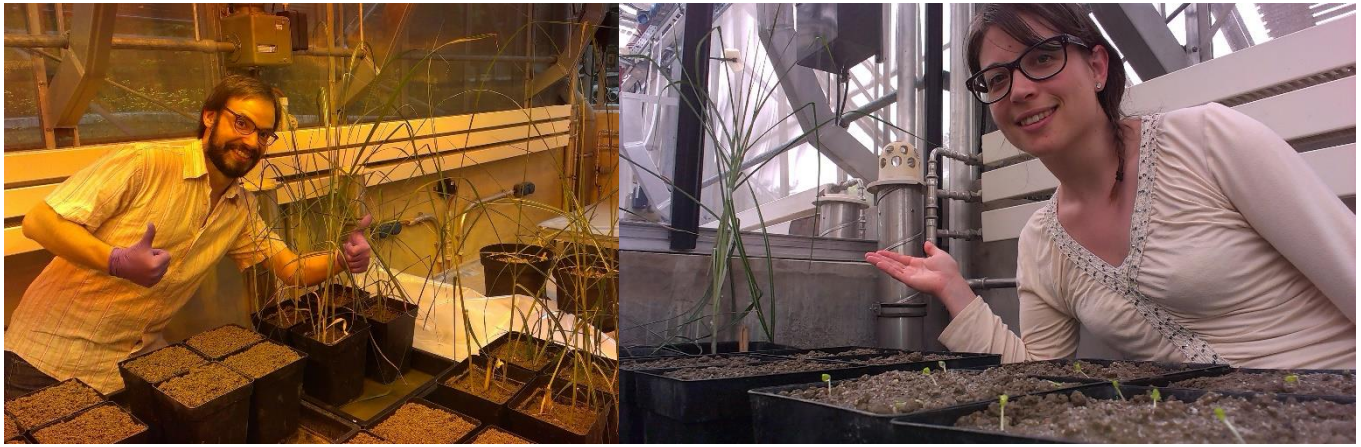
Thank you for your attention



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