Evaluation of the effect of different soil amendment

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Strategies for improvement the soil quality and increasement crop yields

- Crop rotation
- Breeding new cultivars
- Usage of biofertilizers - microbial inoculant of rhizobium, plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi
- Usage of fertilizers
  - Mineral or synthetic
  - Organic – stabilized sewage sludge (biosolids), manure – waste material
  - Biochar – carbon rich material made by pyrolysis from organic waste

Impact on soil quality

Framework Directive on Waste (2018/851/EC)
- '3R's' – reduce, recycle, reuse
- Precautions pollution

Sewage sludge- Biosolids

+ high quantities, rich on nutrients, soil structure, '3R's' principles
- pathogenic MO, antibiotic resistence genes, pollutants

Manure

+ high quantities, rich on nutrients, soil structure,'3R's' principles
- pathogenic MO, antibiotic resistence genes

Experiment no.1

Experiment no. 2

Biochar

+ affect soil pH, carbon sequestration, soil structure, water holding capacity,
'3R's' principles
- various properties, pollutants?

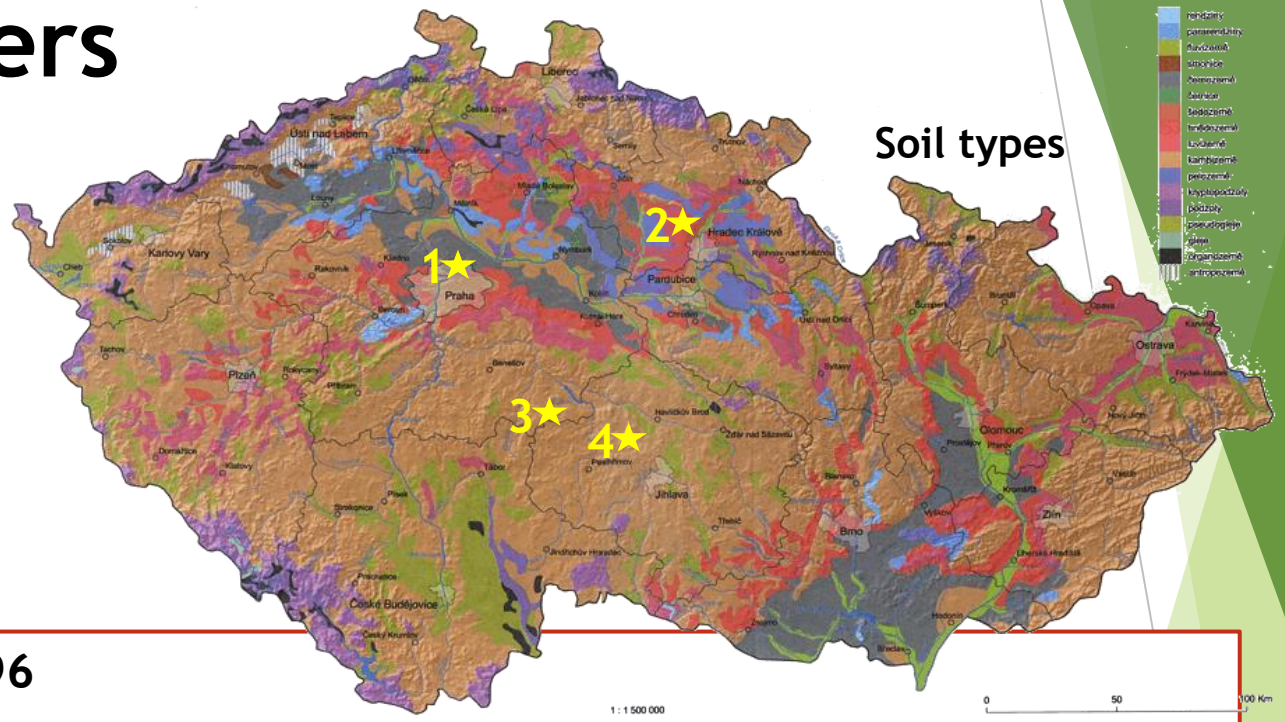
Mineral fertilizers NPK

+ define composition
- excces of nitrogen (contamination of water suplies), no effect on soil structure, Haber process, P

Experiment no. 1 - impact of manure, sewage sludge and mineral fertilizers

- **Experimental fields**

1. Chernozem (Praque –Suchdol)
2. Luvisol (Hněvčevec)
3. Cambisol (Lukavec)
4. Cambisol (Humpolec)



- **Experimental design - started in 1996**

A) Amendments

1. CF- control soil
2. MF- manure - 330 kg N/ha
3. SF - sewage sludge (biosolids) - 330 kg N/ha
4. SF3x -sewage sludge (biosolids) - 990 kg N/ha
5. NPK – NPK 330-90-330 kg/ha

B) Crop rotation

1. potatoes (*Solanum tuberosum* L.)
2. winter wheat (*Triticum aestivum* L.)
3. spring barley (*Hordeum vulgare* L.)

Manure and sewage sludge (biosolids) – thermophilic stabilization

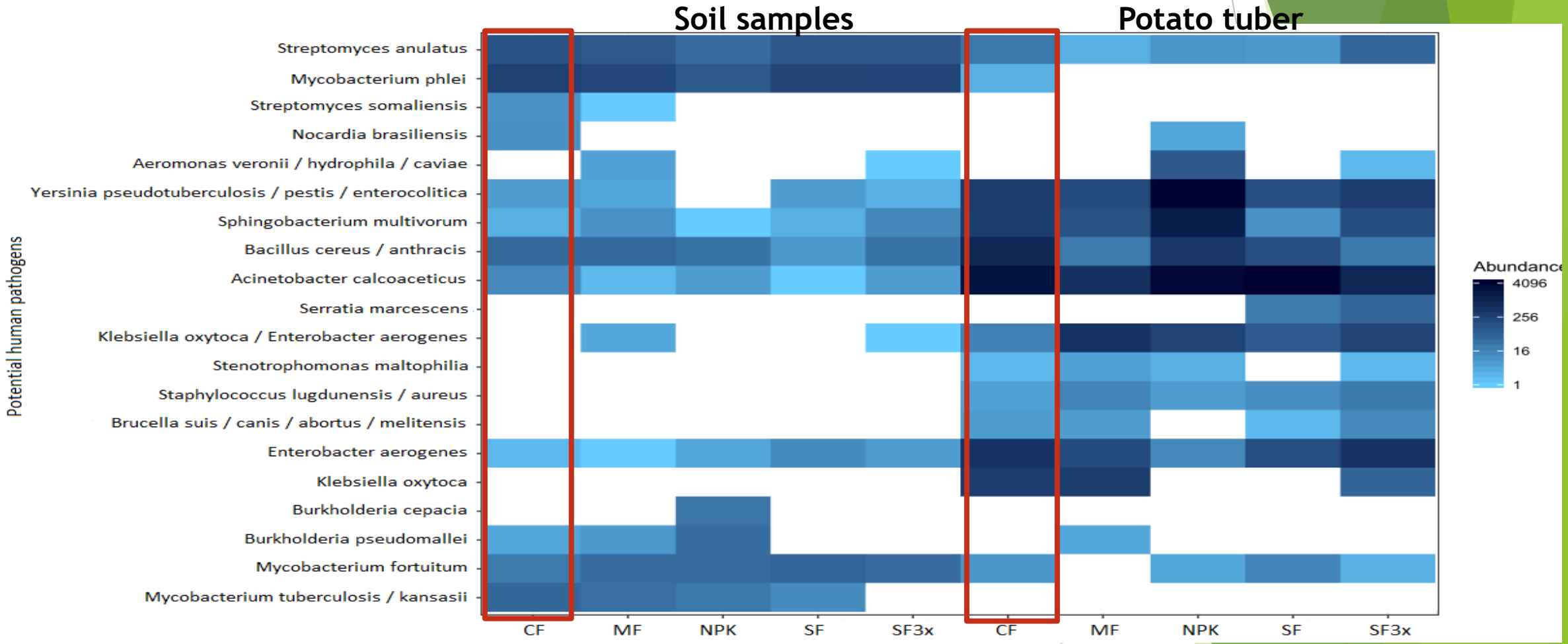
Impact of long-term fertilizers on:

- Soil chemistry ☺
- Soil microbial communities ☺
- Enzyme soil activities ☺
- Endophytic microorganisms ☺

- Occurence of pathogenic microorganisms
- Presence of pollutants
- Antibiotic resistance genes

Did fertilization influence the presence of human pathogens?

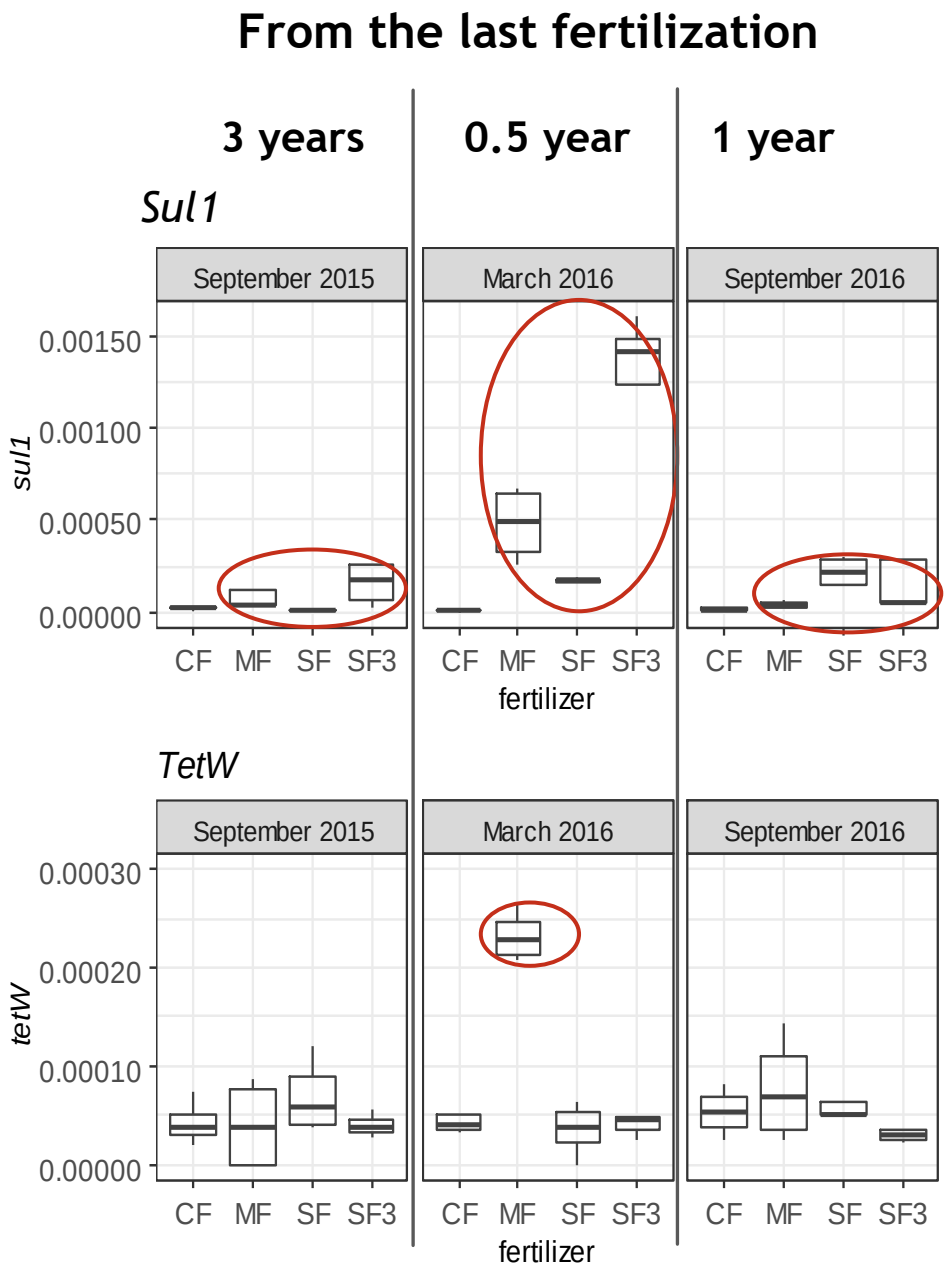
- 19 potencial pathogenic bacteria (soils, potatoe tubers) – base on 99% identity of 16S rRNA ASV and sequence of pathogenic type strain
- Majority ubiquitous in control samples



CF- control soil
MF – manure fertilization
NPK – mineral fertilizers
SF – sewage fertilization, SF3x triple dose sewage sludge

Kracmarova et al., *Microorganisms* 2020, 8(9), 1377

The impact of fertilization on the occurence of ARGs



- **Chernozem Prague-Suchdol**
- **Selected ARGs:**
  - *tetA* and ***tetW*** - tetracycline
  - ***sul1*** and *sul2* - sulphonamide
  - *ermB* – erythromycin
  - *vanA* - vancomycin
  - *intI1* - integron genetic element

Fertilizers temporarily increased some ARGs.

Fertilization type:
1. CF - control soil
2. MF - manure - 330 kg N/ha
3. SF - sewage sludge - 330 kg N/ha
4. SF3 - sewage sludge - 990 kg N/ha

Stiborova et al., *Agronomy-Basel* 2021, 11 (7)

Does fertilization increase the contamination?

- Analyzed samples - from four locations, all fertilization regimes
- **Sewage Sludge Directive 86/278/EEC – heavy metal concentrations**
- **EU countries have implemented stricter values for selected contaminants, Czech Republic – metals, PCBs and AOX (absorbable organic halogens)**
- Monitored organic pollutants – PCBs, **PAHs, organohalogenated pesticides** (**DDT** and metabolites, HCH, HCB), **polyfluoroalkylated substances (PFASs)**, **brominated flame retardants** (**PBDEs** and **HBCD**), **musk pollutants**, antibiotics (tetracyclines, sulfonamides, fluorochinons)
- Monitored heavy metals - Cu, Pb, Zn

Soils were repeatedly fertilized from 1996.
Fertilization did not increase the concentration of the pollutants on environmental risk levels.

Pulkrabova et al., J. Soil Sed., 2018; Kracmarova et al., *Environmental Microbiome* 2022, 17, 13

Conclusion - I. part

Usage of manure and sewage sludge (biosolids) in agriculture can be safe

- Fertilization infuenced the soil chemistry without the negative impact on the presence of organic pollutants and metals
- The connections among human pathogenic microorganism occurrence and fertilization regimes were not verified

- Fertilization by manure and sludge increased ARGs (*sul1 and tetW)*
- **Manure and sludge were stabilized under thermophilic conditions**

Spreding of ARGs is emergent problem - some indicator ARGs should be included in the Sewage Sludge Directive 86/278/EEC

Impact on soil quality

Experiment no. 2

Sewage sludge

+ high quantities, rich on nutrients, soil structure, '3R's' principles
- pathogenic MO, antibiotic resistence genes, pollutants

Biochar

+ affect soil pH, increase macro and micronutrients, soil structure, water holding capacity, '3R's' principles
- various properties, pollutants?

Manure

+ high quantities, rich on nutrients, soil structure, '3R's' principles
- pathogenic MO, antibiotic resistence genes

Experiment no.1

Mineral fertilizers NPK

+ define composition
- excces of nitrogen (contamination of water suplies), no effect on soil structure

Experimental design

Beech wood chips

Waste after mechanical meat separation

300°C | 500°C | 300°C | 500°C

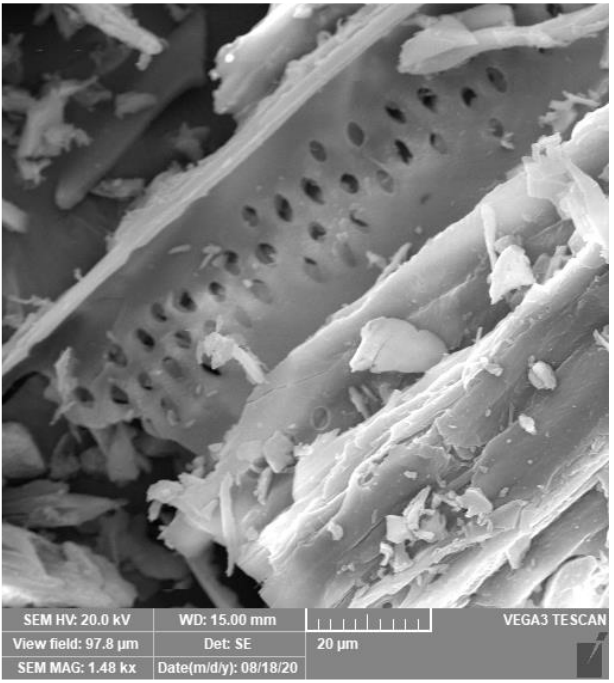
Cambisol | Luvisol | Cambisol | Luvisol | Cambisol | Luvisol | Cambisol | Luvisol

5 % | 2 % | 5 % | 2 % | 5 % | 2 % | 5 % | 2 % | 5 % | 2 % | 5 % | 2 % | 5 % | 2 % | 5 % | 2 %

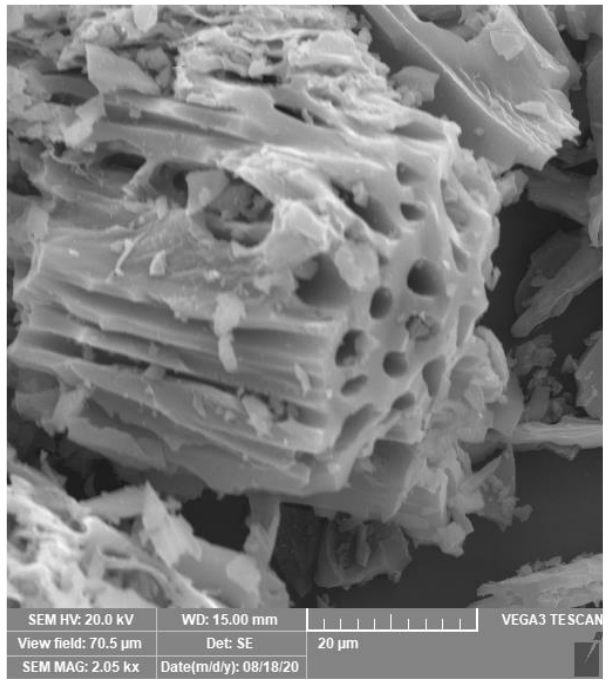
- Each variant: 3 biological replicates; controls: 6 biological replicates
- Sampling points: 3 days, 2 weeks, 1 months, 6 months, 12 months

=> Altogether 300 samples

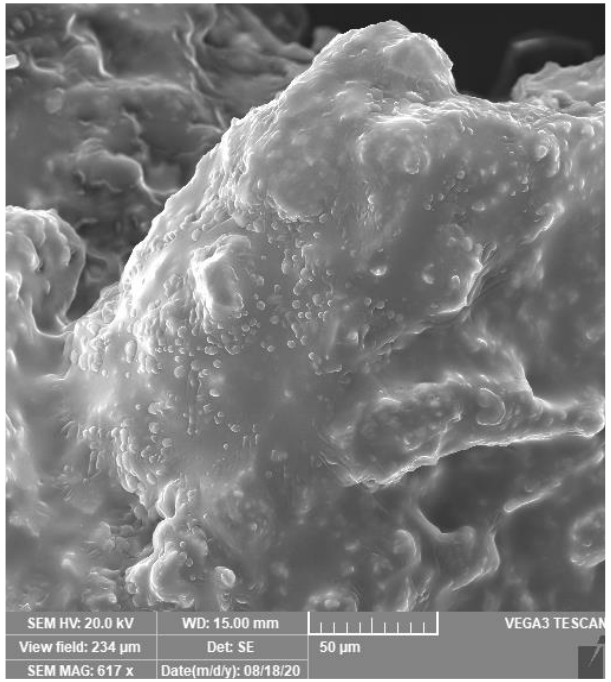
Characterization of biochars



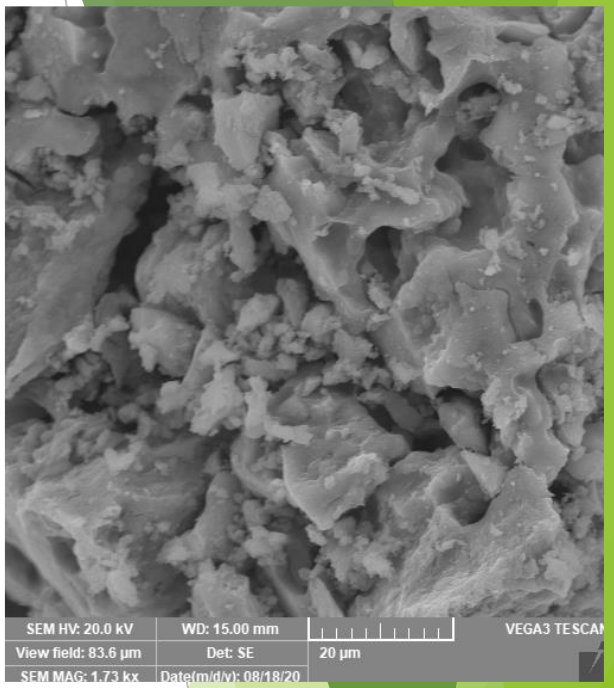
Wood chips - 300°C
Macroporous structure
Sizes from 2 µm to 30 µm
Specific surface area 1 m^2/g
Total volume of pores
1.3 cm^3/g



Wood chips - 500°C
Microporous structure
Sizes from 0.2 µm to 30 µm
Specific surface area 198 m^2/g
Total volume of pores
1.6 cm^3/g



Bone (waste after meat separation) - 300°C

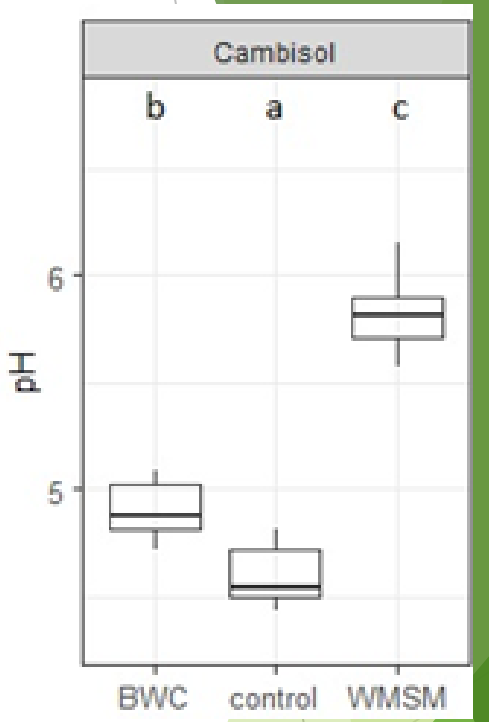


Bone - 500°C
Mezoporous structure
Sizes from 0.2 µm to 30 µm
Specific surface area 126 m^2/g
Total volume of pores
0.6 cm^3/g

- The origin of feedstock and temperature of pyrolysis affected the structure of biochar
- Biochar - from waste after meat separation (**bone char**) **had higher content of macronutrients (Ca, K, P, S, Mg)**

Biochar – Impact on soil properties and microbial communities

		Days	Control	Bone char			
				300 °C		500 °C	
				2 %	5 %	2 %	5 %
Ca	mg/kg	7	2924	3145	3535	3922	4882
		360	**3257**	**3800**	**4407**	**4160**	**5893**
K	mg/kg	7	243	282	339	365	531
		360	**281**	**345**	**465**	**368**	**623**
Mg	mg/kg	7	302	297	309	362	442
		360	**309**	**325**	**376**	**350**	**485**
P	mg/kg	7	93.7	284	482	643	1262
		360	**110**	**471**	**829**	**788**	**1703**
S	mg/kg	7	13.2	19.8	36.1	39.2	71.7
		360	**29.6**	**52.4**	**82.7**	**60.6**	**117**



Biochar:
BWC – beach wood chips
WMSM – bone char

Increase of:

- **Phosphate solubilizing activity**
- **Nitrogen fixators**
- **Bone char 300°C decreased diversity of soil microorganisms**

Szakova et al., JCTB, 2023
doi.org/10.1002/jctb.7421

Conclusion - Biochar

- **Origin of feedstock and pyrolysis temperature** influence the structure and composition of biochars
- Their effect on soils chemistry an soil microbial communities are different
- **Higher temperature (500 °C)** for pyrolysis of both organic wastes, WMSM (waste after mechanically separated meat) and BWC (beech wood chips), was found to address changes in soil microbial community that **reflected better soil quality.**
- **These changes were even more visible in cambisol**, than in luvisol

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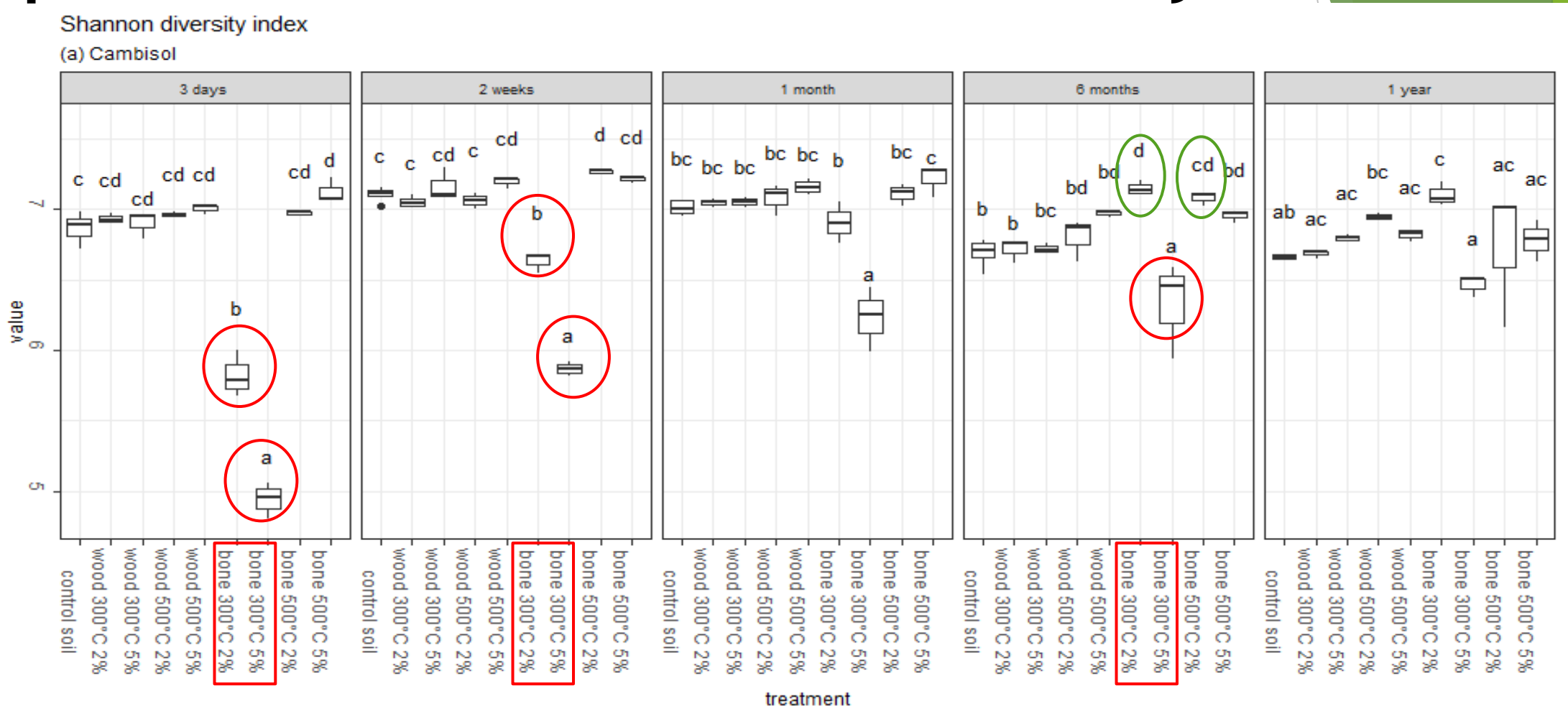
Impact of soil biochars on soil diversity



Wood – biochar from beech wood chips, bone – biochar from waste after meat separation, aplication rate 2% and 5% (w/w)

- **treatment**, **time** and **interaction** of these two factors afect the bacterial diversity, ANOVA, $p < 0{,}001$
- The diferences between the individual treatments (**Tukey-HSD test**)

Kracmarova et al., manuscript in preparation