Evaluation of the effect of different soil amendment

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Strategies for improvement the soil quality and increase 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

**Sewage sludge - Biosolids**
+ high quantities, rich on nutrients, soil structure, ‘3R’s’ principles
- pathogenic MO, antibiotic resistance genes, pollutants

**Manure**
+ high quantities, rich on nutrients, soil structure, ‘3R’s’ principles
- pathogenic MO, antibiotic resistance genes

- ‘3R’s’ - reduce, recycle, reuse
- Precautions pollution

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

**Mineral fertilizers NPK**
+ define composition
- excess of nitrogen (contamination of water supplies), no effect on soil structure, Haber process, P

**Experiment no. 1**

**Experiment no. 2**
Experiment no. 1 - impact of manure, sewage sludge and mineral fertilizers

- Experimental fields
  1. Chernozem (Praque - Suchdol)
  2. Luvisol (Hněvčevc)
  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 😊
- Occurrence of pathogenic microorganisms
- Presence of pollutants
- Antibiotic resistance genes
Did fertilization influence the presence of human pathogens?

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

<table>
<thead>
<tr>
<th>Soil samples</th>
<th>Potato tuber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces anulatus</td>
<td>Streptomyces anulatus</td>
</tr>
<tr>
<td>Mycobacterium phlei</td>
<td>Mycobacterium phlei</td>
</tr>
<tr>
<td>Streptomyces somaliensis</td>
<td>Streptomyces somaliensis</td>
</tr>
<tr>
<td>Nocardia brasiliensis</td>
<td>Nocardia brasiliensis</td>
</tr>
<tr>
<td>Aeromonas veronii / hydrophila / cavae</td>
<td>Aeromonas veronii / hydrophila / cavae</td>
</tr>
<tr>
<td>Yersinia pseudotuberculosis / pestis / enterocolitica</td>
<td>Yersinia pseudotuberculosis / pestis / enterocolitica</td>
</tr>
<tr>
<td>Sphingobacterium multivorum</td>
<td>Sphingobacterium multivorum</td>
</tr>
<tr>
<td>Bacillus cereus / anthracis</td>
<td>Bacillus cereus / anthracis</td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus</td>
<td>Acinetobacter calcoaceticus</td>
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<tr>
<td>Serratia marcescens</td>
<td>Serratia marcescens</td>
</tr>
<tr>
<td>Klebsiella oxytoca / Enterobacter aerogenes</td>
<td>Klebsiella oxytoca / Enterobacter aerogenes</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>Stenotrophomonas maltophilia</td>
</tr>
<tr>
<td>Staphylococcus lugdunensis / aureus</td>
<td>Staphylococcus lugdunensis / aureus</td>
</tr>
<tr>
<td>Brucella suis / canis / abortus / melitensis</td>
<td>Brucella suis / canis / abortus / melitensis</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>Enterobacter aerogenes</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>Klebsiella oxytoca</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>Burkholderia cepacia</td>
</tr>
<tr>
<td>Burkholderia pseudomallei</td>
<td>Burkholderia pseudomallei</td>
</tr>
<tr>
<td>Mycobacterium fortuitum</td>
<td>Mycobacterium fortuitum</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis / kansasil</td>
<td>Mycobacterium tuberculosis / kansasil</td>
</tr>
</tbody>
</table>

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 occurrence of ARGs

From the last fertilization

3 years | 0.5 year | 1 year

Sul1

<table>
<thead>
<tr>
<th>September 2015</th>
<th>March 2016</th>
<th>September 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF MF SF SF3</td>
<td>CF MF SF SF3</td>
<td>CF MF SF SF3</td>
</tr>
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TetW

<table>
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<tr>
<th>September 2015</th>
<th>March 2016</th>
<th>September 2016</th>
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</thead>
<tbody>
<tr>
<td>CF MF SF SF3</td>
<td>CF MF SF SF3</td>
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</tr>
</tbody>
</table>

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
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 influenced 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

**Sewage sludge**
- high quantities, rich on nutrients, soil structure, ‘3R’s’ principles
- pathogenic MO, antibiotic resistance genes, pollutants

**Manure**
- high quantities, rich on nutrients, soil structure, ‘3R’s’ principles
- pathogenic MO, antibiotic resistance genes

**Biochar**
- affect soil pH, increase macro and micronutrients, soil structure, water holding capacity, ‘3R’s’ principles
- various properties, pollutants?

**Mineral fertilizers**
- define composition
  - excess of nitrogen (contamination of water supplies), no effect on soil structure

**Experiment no. 1**

**Experiment no. 2**
Experimental design

- 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

- 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).

Wood chips - 300°C
- Macroporous structure
- Sizes from 2 µm to 30 µm
- Specific surface area 1 m²/g
- Total volume of pores 1.3 cm³/g

Wood chips - 500°C
- Microporous structure
- Sizes from 0.2 µm to 30 µm
- Specific surface area 198 m²/g
- Total volume of pores 1.6 cm³/g

Bone (waste after meat separation) - 300°C
- Mezoporous structure
- Sizes from 0.2 µm to 30 µm
- Specific surface area 126 m²/g
- Total volume of pores 0.6 cm³/g

Bone - 500°C
- Macroporous structure
- Sizes from 2 µm to 30 µm
- Specific surface area 1 m²/g
- Total volume of pores 1.3 cm³/g
### Increase of:

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

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**Biochar - Impact on soil properties and microbial communities**

<table>
<thead>
<tr>
<th></th>
<th>Days</th>
<th>Control</th>
<th>Bone char</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>300 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 %</td>
<td>5 %</td>
</tr>
<tr>
<td>Ca</td>
<td>7</td>
<td>2924</td>
<td>3145</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>3257</td>
<td>3800</td>
</tr>
<tr>
<td>K</td>
<td>7</td>
<td>243</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>281</td>
<td>345</td>
</tr>
<tr>
<td>Mg</td>
<td>7</td>
<td>302</td>
<td>297</td>
</tr>
<tr>
<td></td>
<td>360</td>
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<tr>
<td>P</td>
<td>7</td>
<td>93.7</td>
<td>284</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>110</td>
<td>471</td>
</tr>
<tr>
<td>S</td>
<td>7</td>
<td>13.2</td>
<td>19.8</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>29.6</td>
<td>52.4</td>
</tr>
</tbody>
</table>

Biochar:
- BWC - beach wood chips
- WMSM - bone char

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 and 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 affect the bacterial diversity, ANOVA, $p < 0.001$
- The differences between the individual treatments (Tukey-HSD test)

Kracmarova et al., manuscript in preparation