Team work among *Ciboria* sp. and bacteria for the depletion of TPH in historically polluted soil.

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Background

The dominant fungi in contaminated environments are members of the saprophytic Ascomycota phylum.

- However, their functional potential in disturbed soils WHERE THEY ARE DOMINANT, is poorly characterized.

- Some of them already described as capable of degradation of recalcitrant compounds.
Ascomycota are reported as responsible for the synthesis of the organic matter in soils and for its smooth mobilisation.

Ascomycota produce polyphenol oxidases and laccases (Saprophytes) responsible for:

- Partial depolimerisation of the organic matter in soil
- Generation of radical cation intermediates: polycondensation reactions

Polycondensation of organic compounds is actually responsible for the synthesis of the organic matter in soils.
Developing bio-based approaches for treating soils polluted with Total Petroleum Hydrocarbons (TPHs), 8538 mg/Kg at a decommissioned oil refinery in Trieste, Italy (45° 36' 16.9" N; 13° 47' 56.4" E).
Objectives

• Isolation & identification of hydrocarbon-degrading Ascomycetes

• Design a Mycoremediation approach based on

• Exploitation of both the Carbon mobilising & stabilising activity of fungi

• Study synergisms between fungi and bacteria
Ciboria sp. growth on diesel oil

Basal Salt Medium
1%v/v diesel oil and antibiotics mix

Ciboria sp.
• *Ciboria* sp., is quite rare in the environment.

• Recent research led to the isolation of a *Ciboria* sp. strain capable to transform polymeric Azo-dyes and aryl amines.
Biostimulation
C/N/P 100:10:1

Bioaugmentation
1% Ciboria sp. fw/fw

Bioaugmentation
7% Ciboria sp. fw/fw

WHAT WE MEASURE

1. TPH content

2. Stabilised Carbon in soils: Humic and fulvic acids content

3. Metagenomic DNA Metabarcoding
   V4–V5 hypervariable regions of the bacterial 16S rRNA
   18S rDNA Internal Spacer region of fungi

4. Predictive functional metagenomics
Results

A

Biost

1%

7%

B

Biost

1%

7%

TPH (mg/kg d.w.)

HFA (g/kg d.w.)
ANY SIGNIFICANT CHANGES IN THE FUNGAL ECOLOGY DURING THE PROCESS OF TPH DEPLETION
Results

ANY SIGNIFICANT CORRELATION WITH TPH DEPLETION AND HFA INCREMENT
Results

SIGNIFICANT CHANGES IN THE BACTERIAL ECOLOGY DURING THE PROCESS OF TPH DEPLETION
Results

SIGNIFICANT CORRELATION WITH TPH DEPLETION AND HFA INCREMENT
To better evaluate the metabolic potential of the different bacterial taxa during the process of TPH degradation:

the contribution of the different taxa to abundances of functional features of interest was evaluated

Predicted proteins were classified by their Enzymatic Commission number (EC), resulting in the identification of 2313 ECs, and by KEGG orthology resulting in the identification of 7374 KOs, across all samples.
Xenobiotic Biodegradation and Metabolism Module (KEGG Pathway 1.11) and related maps resulted to be among the top modules recovered across all samples.
Results: limited to mycoaugmented mesocosms

At T0
**ACTINOBACTERIA**:
- Agromyces
- Mycobacterium
- Saccharomonospora
- Salinibacterium
- Thermoactinomycetes
- Saccharopolyspora
- Gordonia
- Rhodococcus
- Citrococcus
- Microbactrium
- Streptomyces spp.

**PROTEOBACTERIA**:
- Rhodobacter
- Acquabacterium
- Pseudomonas
- Pseudoxanthomonas
- Thermomonas
- Legionella
- Parvibaculum spp.

**FIRMICUTES**:
- Planimicrobium
- Virgibacillus
- Planilillum
- Bacillus spp.

**TRANSITIONAL MESOCOSMS**

**ACTINOBACTERIA**:
- Arthrobacter
- Dietzia
- Brachybacterium spp.
- Brevibacterium
- Georgenia
- Leucobacter spp.

**PROTEOBACTERIA**:
- Alcanivorax
- Lysobacter
- Agro bacterium

**MESOCOSMS WHERE TPH DEPLETION OCCURRED**

**PROTEOBACTERIA**:
- Rhodanobacter
- Nocardioides
- Psuedonocardia
- Solirubrobacter

**ACTINOBACTERIA**:
- Alcanivorax
- Lysobacter
- Agro bacterium
the Dye decolorising peroxidases.

Bacterial DyP have a quite high redox potential that allows both the oxidation of phenolic and non-phenolic lignin model compounds.

The Actinobacteria showing functional predominance in the transitional mesocosms, besides harbouring functional features for the transformation of the contamination, harboured also other functional feature that might be of interest for the process of TPH depletion.
Results: limited to mycoaugmented mesocosms

At T0
ACTINOBACTERIA:
Agromyces, Mycobacterium, Saccharomonospora, Salinibacterium, Thermoactinomycetes, Saccharopolyspora, Gordonia, Rhodococcus, Citrococcus, Microbactrium, Streptomyces sps.

PROTEOBACTERIA:
Rhodobacter, Acquabacterium, Pseudomonas, Pseuodoxanthomona, Thermomonas, Legionella, Parvibaculum sps.

FIRMICUTES:
Planimicrobium, Virgibacillus, Planifilium, Bacillus sps.

TRANSITIONAL MESOCOSMS

ACTINOBACTERIA:
Arthrobacter, Dietzia, Brachybacterium, Brevibacterium, Georgenia, Leucobacter sps.

PROTEOBACTERIA:
Alcanivorax, Lysobacter, Agrobacterium sps.

MESOCOSMS WHERE TPH DEPLETION OCCURRED

PROTEOBACTERIA:
Rhodanobacter, Nocardioides, Psuedonocardia, Solirubrobacter sps.

BLOOMING OF SPECIALISTS

MANDATORY TO TPH DEPLETION
Results

Spearman’s rank correlation coefficient for combination of DyP total counts per sample and relative TPH concentrations:
LOW CORRELATION

<table>
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<th>Biostimulated</th>
<th>q</th>
<th>TPH/EC:1.11.1.19</th>
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<tr>
<td></td>
<td>Control</td>
<td>0.18</td>
</tr>
<tr>
<td>1%</td>
<td>F1</td>
<td>0.32</td>
</tr>
<tr>
<td>7%</td>
<td>F7</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Conclusion: DyP is not necessarily involved in the depletion of the contamination but it is associated to the saprophytic metabolisms of Actinobacteria,
• Saprophytic Actinobacteria and *Ciboria* sp. are mandatory for TPH depletion

• Saprophitic Actinobacteria are mandatory for the establishment of SPECIALIST bacterial species

The saprophytic metabolism is associated to microbial GENERALIST species in the environment: organisms able to utilize a broad variety of carbon sources in low concentration in the environment utilizing extracellular oxidizing enzymes (Ascomycota)

• The saprophytic GENERALIST result to be pivotal to prime the actual degradation of contaminants and the blooming SPECIALISTS
TEAM WORK BETWEEN

Generalists ↔ Specialists

historical contamination = oligotrophic environment

Smoothly providing C source

ISOLATION OF GENERALISTS MIGHT BE FUNDAMENTAL

MIBIREM 2022-2027
Innovation Action
Toolbox for Microbiome based Remediation

AT BE IT NL FR DE
Box 1 | Disambiguation of ‘bioplastics’ and challenges for their implementation

Biodegradation refers to the depolymerization of polymers by biological organisms, whereas composting is a form of biodegradation that yields CO₂, H₂O, heat and humus; therefore, the compostability of a polymer depends on the microbial and chemical environment.

Economics
Most bioplastics are currently more expensive to produce than fossil-based plastics, mostly owing to economies of scale and the price competitiveness of crude oil.

Efficiency
Bioplastic manufacturing processes can be less energy efficient than fossil-based plastic processes and come with other environmental burdens associated with agricultural farming.

End of life
For most bioplastics, recycling streams have yet to be established to

THE FUTURE GOAL: TOOLBOX FOR BIOPLASTIC REMEDIATION

Consistent global recycling of bioplastics.

PBAT, polybutylene adipate-co-terephthalate; PC, polycarbonate; PE, polyethylene; PEF, polyethylene furanoate; PET, polyethylene terephthalate; PLA, polyactic acid; PP, polypropylene; PS, polystyrene; PU, polyurethane; PVA, polyvinyl alcohol; PVC, polyvinyl chloride.