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

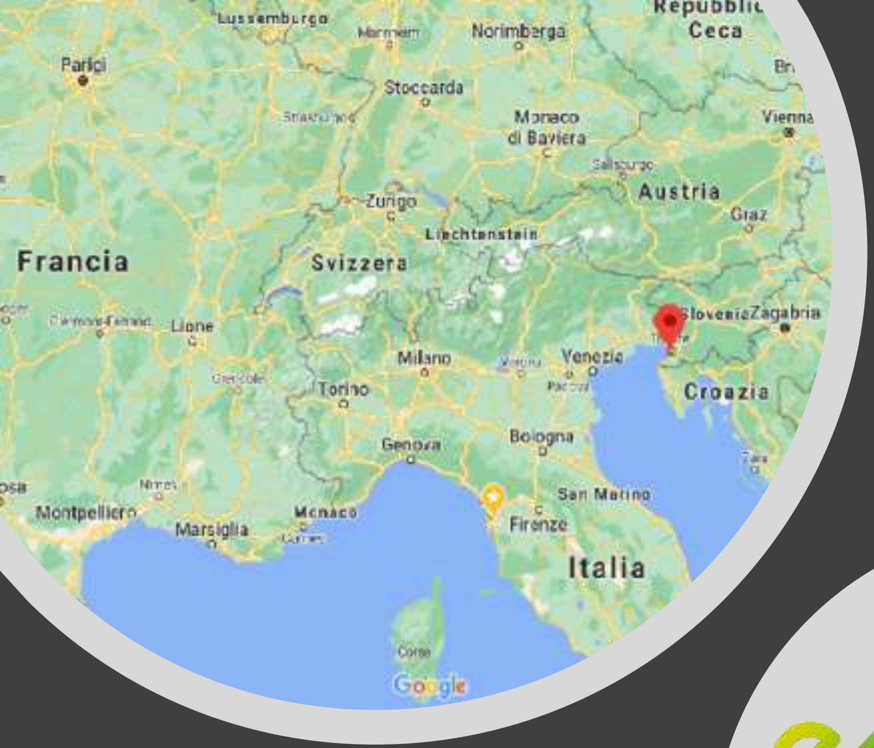
- However, their functional potential in disturbed soils WHERE THEY ARE DOMINANT, is poorly characterized
- Some of them already described as capable of degradation of recalcitrant compounds

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

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IN SUPREMÆ DIGNITATIS

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DoE

Ciboria sp. growth on diesel oil

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

***Ciboria* sp.**

DoE

- *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.

DoE

Biostimulation
C/N/P 100:10:1

Bioaugmentation
1% *Ciboria* sp. fw/fw

Bioaugmentation
7% *Ciboria* sp. fw/fw

WHAT WE MEASURE

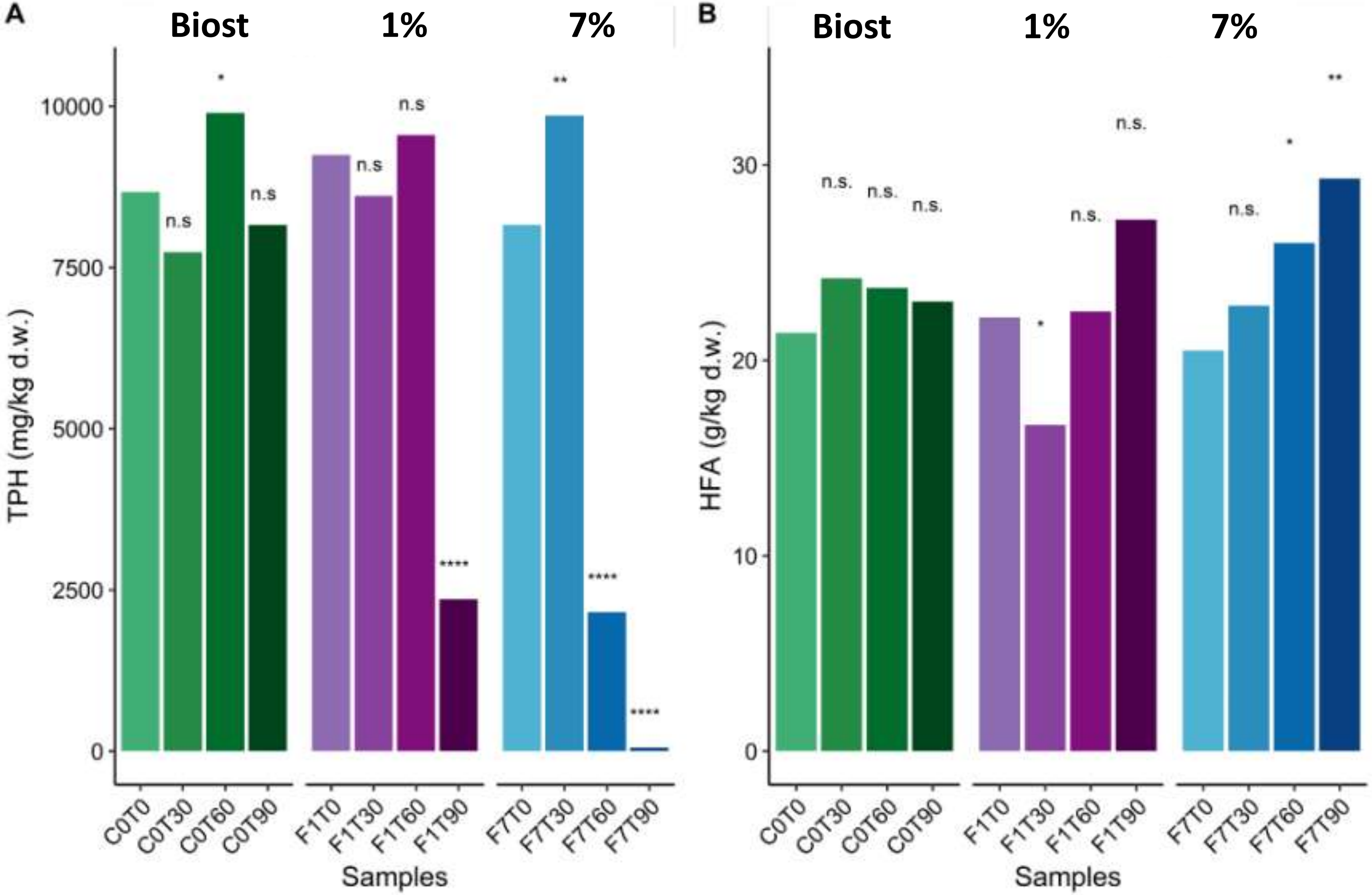
1. TPH content

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%
TPH (mg/kg d.w.)
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n.s
n.s
n.s
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C0T0
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C0T60
C0T90
F1T0
F1T30
F1T60
F1T90
F7T0
F7T30
F7T60
F7T90
Samples
B
Biost
1%
7%
HFA (g/kg d.w.)
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C0T0
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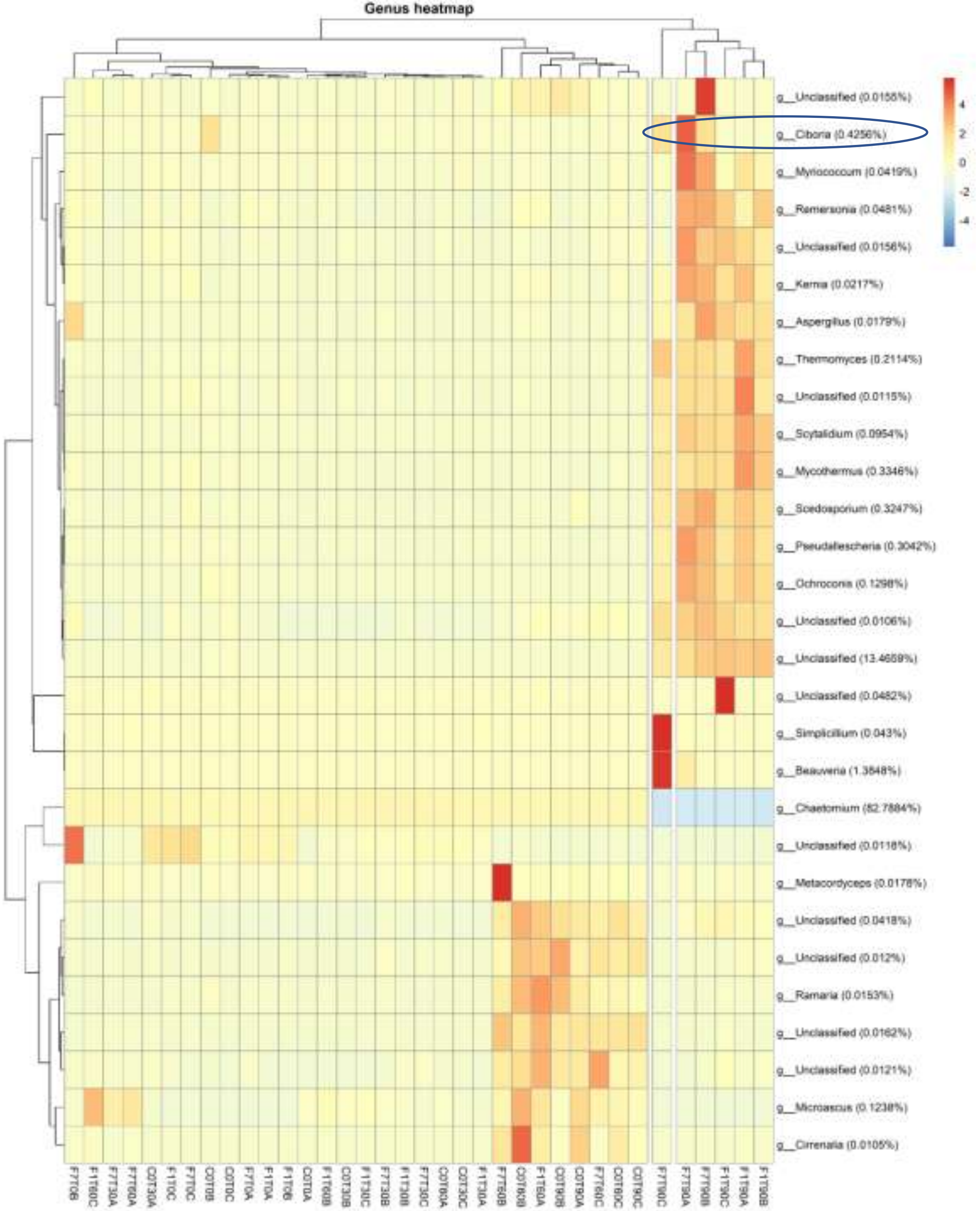
F1T90
F7T0
F7T30
F7T60
F7T90
Samples


Genus heatmap
g__Unclassified (0.0156%)
g__Ciboria (0.4256%)
g__Myriococcum (0.0419%)
g__Remersonia (0.0481%)
g__Unclassified (0.0156%)
g__Kernia (0.0217%)
g__Aspergillus (0.0179%)
g__Thermomyces (0.2114%)
g__Unclassified (0.0115%)
g__Scytalidium (0.0954%)
g__Mycothermus (0.3346%)
g__Scedosporium (0.3247%)
g__Pseudallescheria (0.3042%)
g__Ochroconis (0.1298%)
g__Unclassified (0.0106%)
g__Unclassified (13.4659%)
g__Unclassified (0.0482%)
g__Simplicillium (0.043%)
g__Beauveria (1.3848%)
g__Chaetomium (82.7884%)
g__Unclassified (0.0118%)
g__Metacordyceps (0.0178%)
g__Unclassified (0.0418%)
g__Unclassified (0.012%)
g__Ramaria (0.0153%)
g__Unclassified (0.0162%)
g__Unclassified (0.0121%)
g__Microascus (0.1238%)
g__Cirrenalia (0.0105%)
4
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F7T0B
F1T60C
F7T30A
F7T60A
C0T30A
F1T0C
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C0T0B
C0T0C
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F1T0A
F1T0B
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F1T60B
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F7T30B
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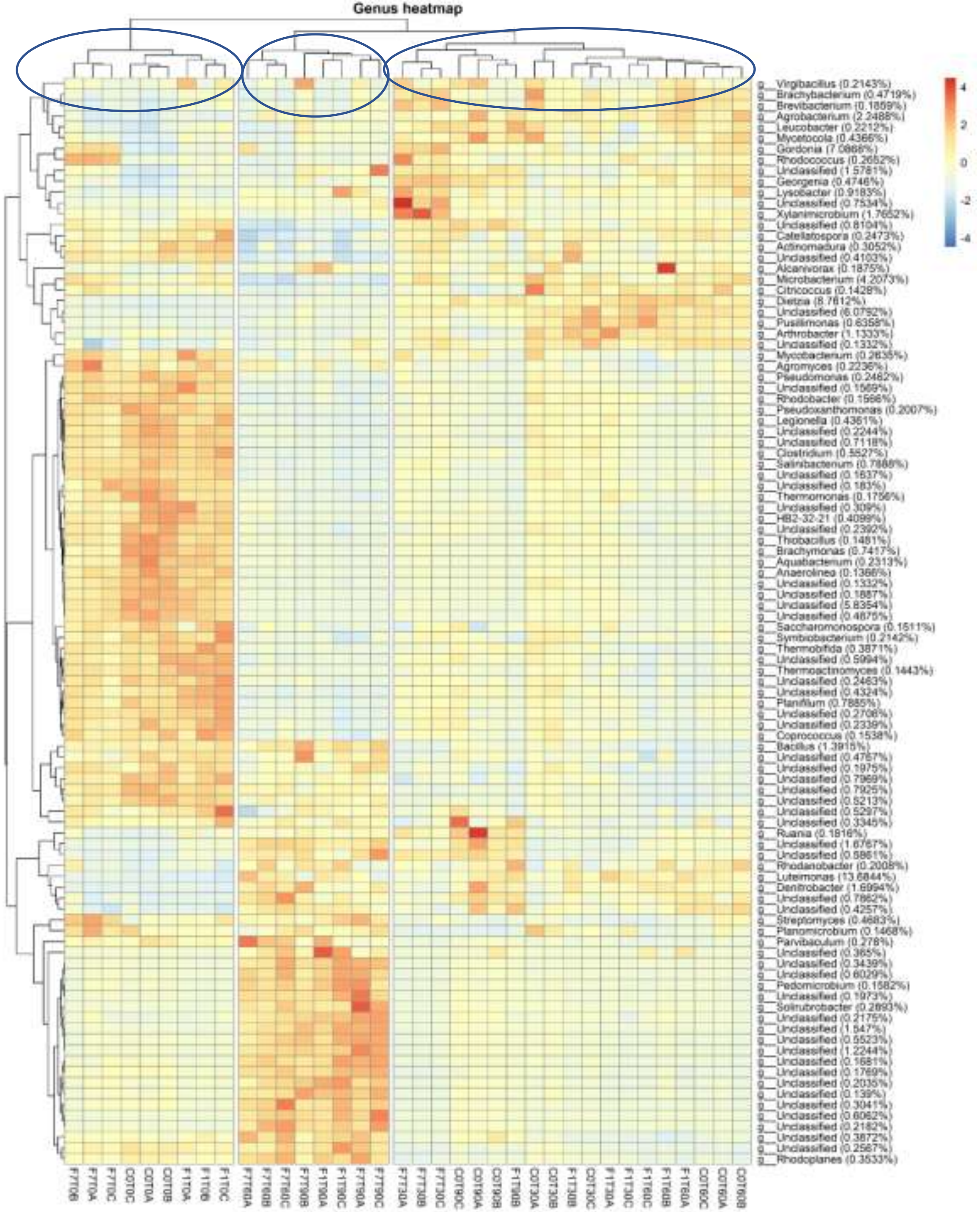
Genus heatmap
g__Virgibacillus (0.2143%)
g__Brachybacterium (0.4719%)
g__Brevibacterium (0.1859%)
g__Agrobacterium (2.2488%)
g__Leucobacter (0.2212%)
g__Mycetocola (0.4366%)
g__Gordonia (7.0868%)
g__Rhodococcus (0.2652%)
g__Unclassified (1.5781%)
g__Georgenia (0.4746%)
g__Lysobacter (0.9183%)
g__Unclassified (0.7534%)
g__Xylanimicrobium (1.7652%)
g__Unclassified (0.8104%)
g__Catellatospora (0.2473%)
g__Actinomadura (0.3052%)
g__Unclassified (0.4103%)
g__Alcanivorax (0.1875%)
g__Microbacterium (4.2073%)
g__Citricoccus (0.1428%)
g__Dietzia (8.7612%)
g__Unclassified (6.0792%)
g__Pusillimonas (0.6358%)
g__Arthrobacter (1.1333%)
g__Unclassified (0.1332%)
g__Mycobacterium (0.2835%)
g__Agromyces (0.2236%)
g__Pseudomonas (0.2482%)
g__Unclassified (0.1569%)
g__Rhodobacter (0.1566%)
g__Pseudoxanthomonas (0.2007%)
g__Legionella (0.4361%)
g__Unclassified (0.2244%)
g__Unclassified (0.7118%)
g__Clostridium (0.5527%)
g__Salinibacterium (0.7888%)
g__Unclassified (0.1637%)
g__Unclassified (0.183%)
g__Thermomonas (0.1756%)
g__Unclassified (0.309%)
g__HB2-32-21 (0.4099%)
g__Unclassified (0.2392%)
g__Thiobacillus (0.1481%)
g__Brachymonas (0.7417%)
g__Aquabacterium (0.2313%)
g__Anaerolinea (0.1366%)
g__Unclassified (0.1332%)
g__Unclassified (0.1887%)
g__Unclassified (5.8354%)
g__Unclassified (0.4875%)
g__Saccharomonospora (0.1511%)
g__Symbiobacterium (0.2142%)
g__Thermobifida (0.3871%)
g__Unclassified (0.5994%)
g__Thermoactinomyces (0.1443%)
g__Unclassified (0.2463%)
g__Unclassified (0.4324%)
g__Planifilum (0.7885%)
g__Unclassified (0.2706%)
g__Unclassified (0.2339%)
g__Coprococcus (0.1538%)
g__Bacillus (1.3915%)
g__Unclassified (0.4767%)
g__Unclassified (0.1975%)
g__Unclassified (0.7969%)
g__Unclassified (0.7925%)
g__Unclassified (0.5213%)
g__Unclassified (0.5297%)
g__Unclassified (0.3345%)
g__Ruania (0.1816%)
g__Unclassified (1.6767%)
g__Unclassified (0.5861%)
g__Rhodanobacter (0.2008%)
g__Luteimonas (13.6844%)
g__Denitrobacter (1.6994%)
g__Unclassified (0.7862%)
g__Unclassified (0.4257%)
g__Streptomyces (0.4683%)
g__Planomicrobium (0.1468%)
g__Parvibaculum (0.278%)
g__Unclassified (0.365%)
g__Unclassified (0.3439%)
g__Unclassified (0.6029%)
g__Pedomicrobium (0.1582%)
g__Unclassified (0.1973%)
g__Solirubrobacter (0.2893%)
g__Unclassified (0.2175%)
g__Unclassified (1.547%)
g__Unclassified (0.5523%)
g__Unclassified (1.2244%)
g__Unclassified (0.1681%)
g__Unclassified (0.1769%)
g__Unclassified (0.2035%)
g__Unclassified (0.139%)
g__Unclassified (0.3041%)
g__Unclassified (0.6062%)
g__Unclassified (0.2182%)
g__Unclassified (0.3872%)
g__Unclassified (0.2567%)
g__Rhodoplanes (0.3533%)
4
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F7T0B
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F7T0C
C0T0C
C0T0A
C0T0B
F1T0A
F1T0B
F1T0C
F7T60A
F7T60B
F7T60C
F7T90B
F1T90A
F1T90C
F7T90A
F7T90C
F7T30A
F7T30B
F7T30C
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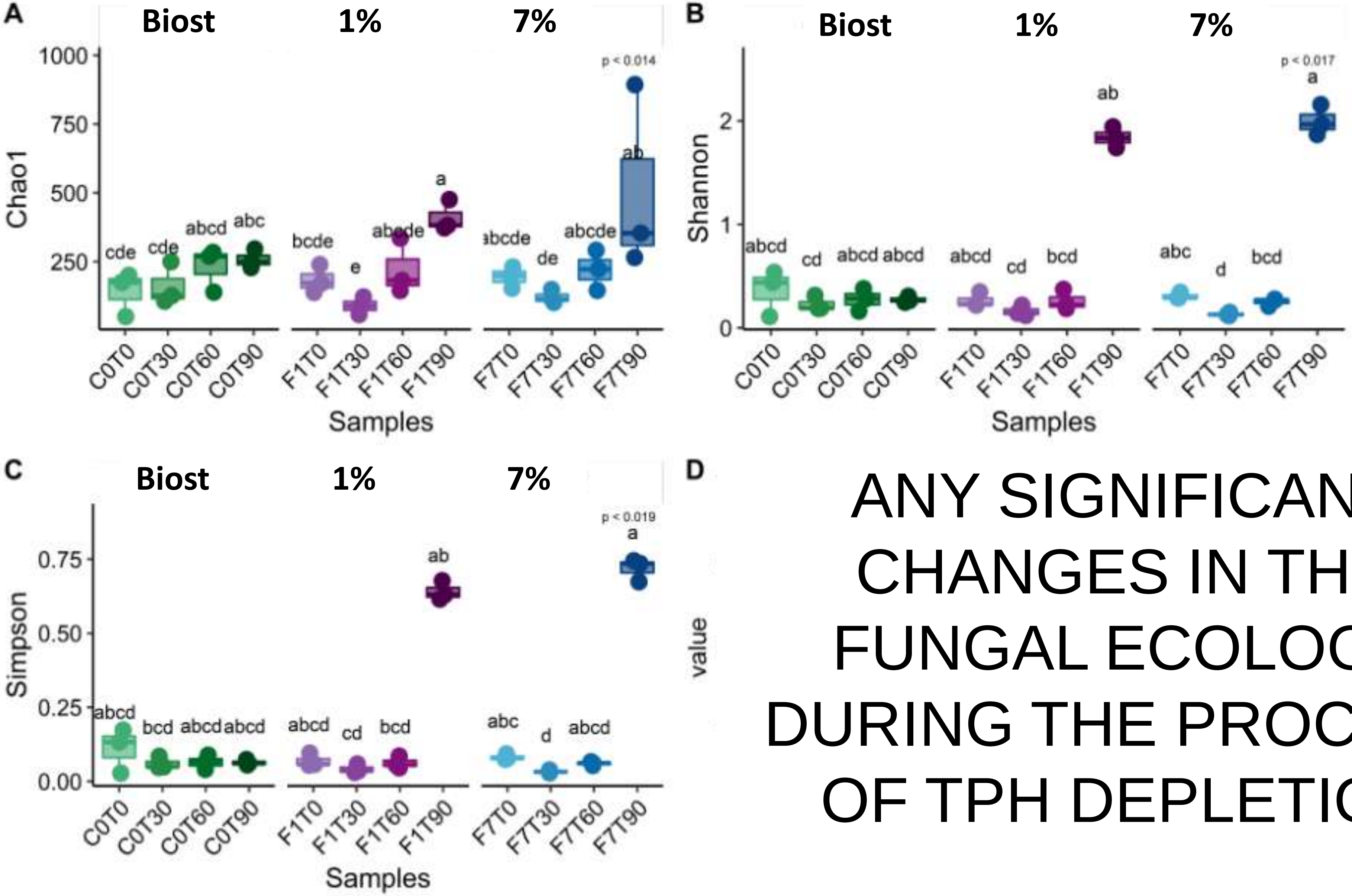
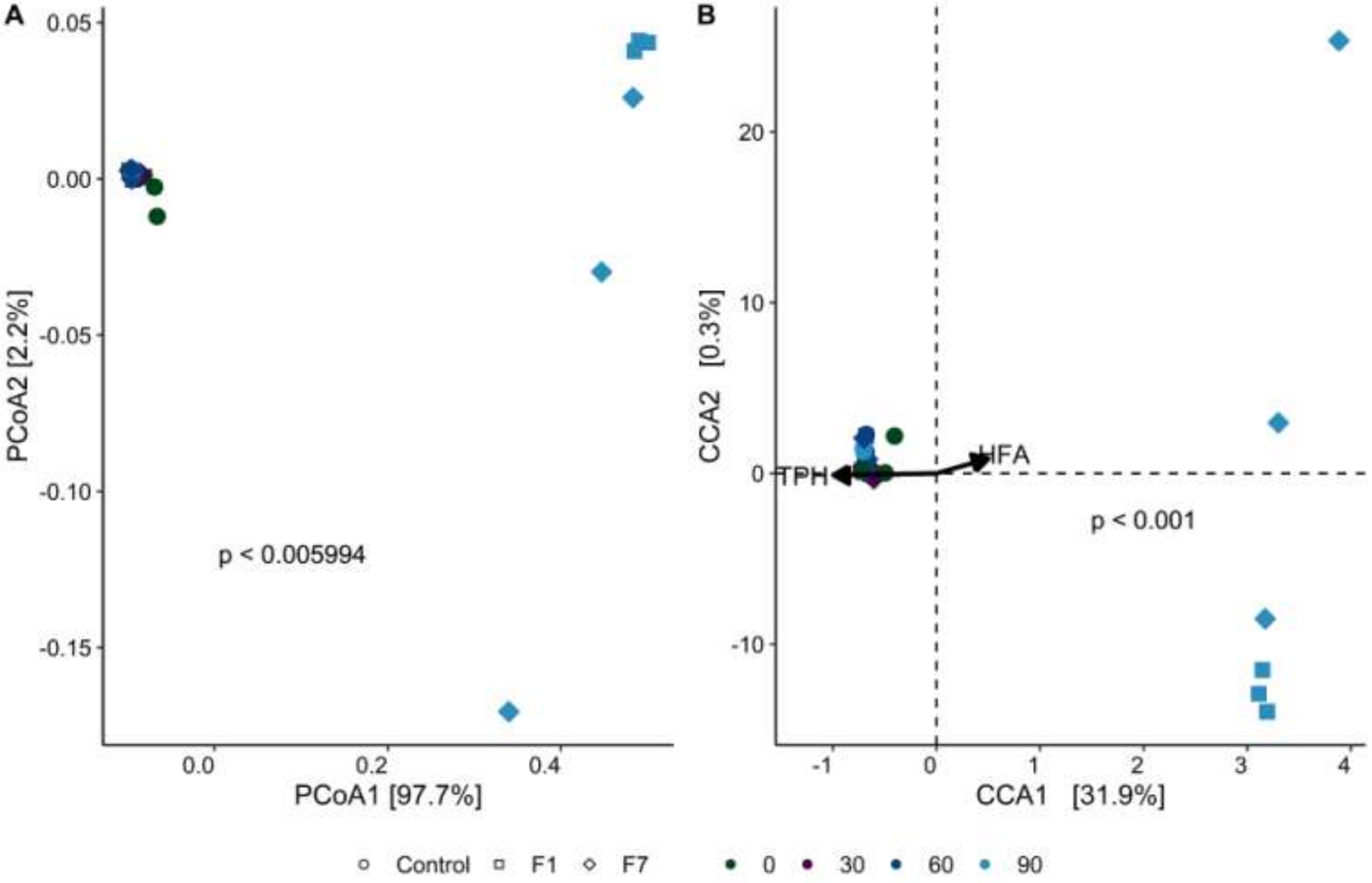
Results



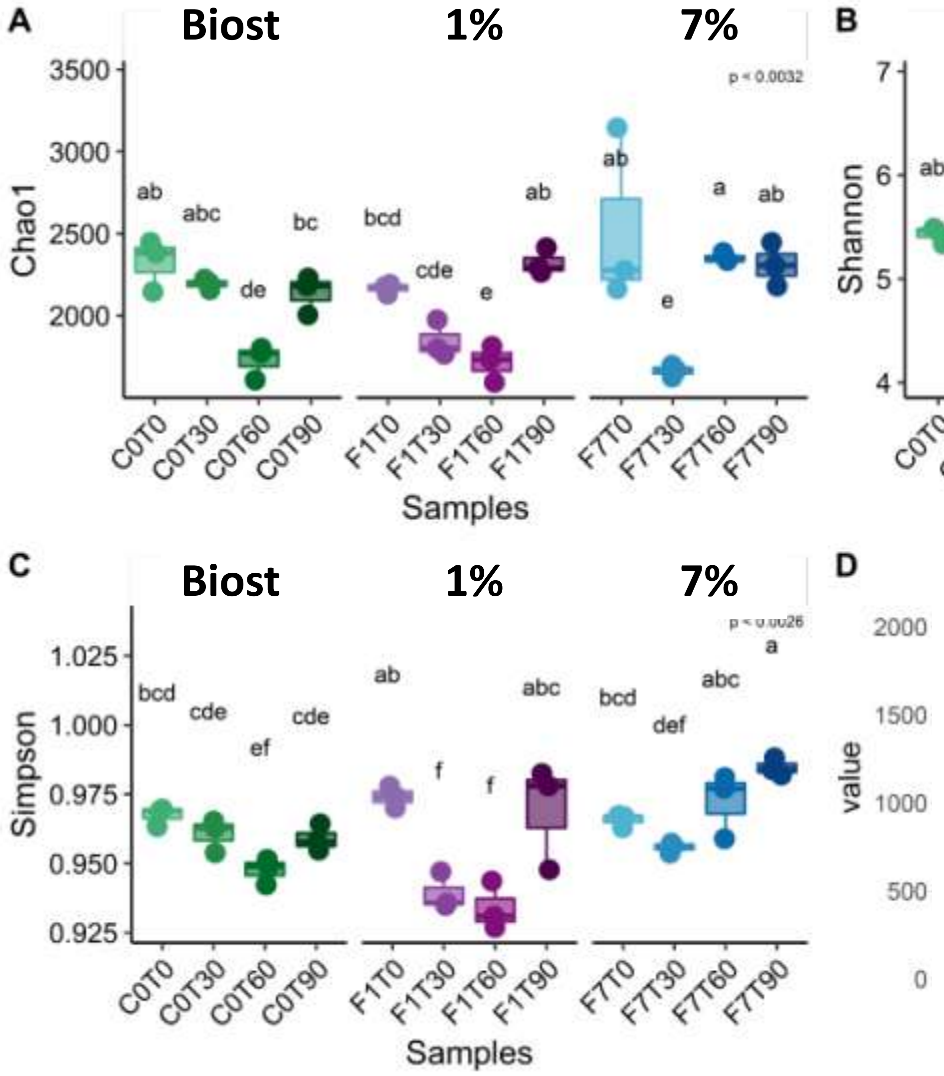
ANY SIGNIFICANT CHANGES IN THE FUNGAL ECOLOGY DURING THE PROCESS OF TPH DEPLETION

Results



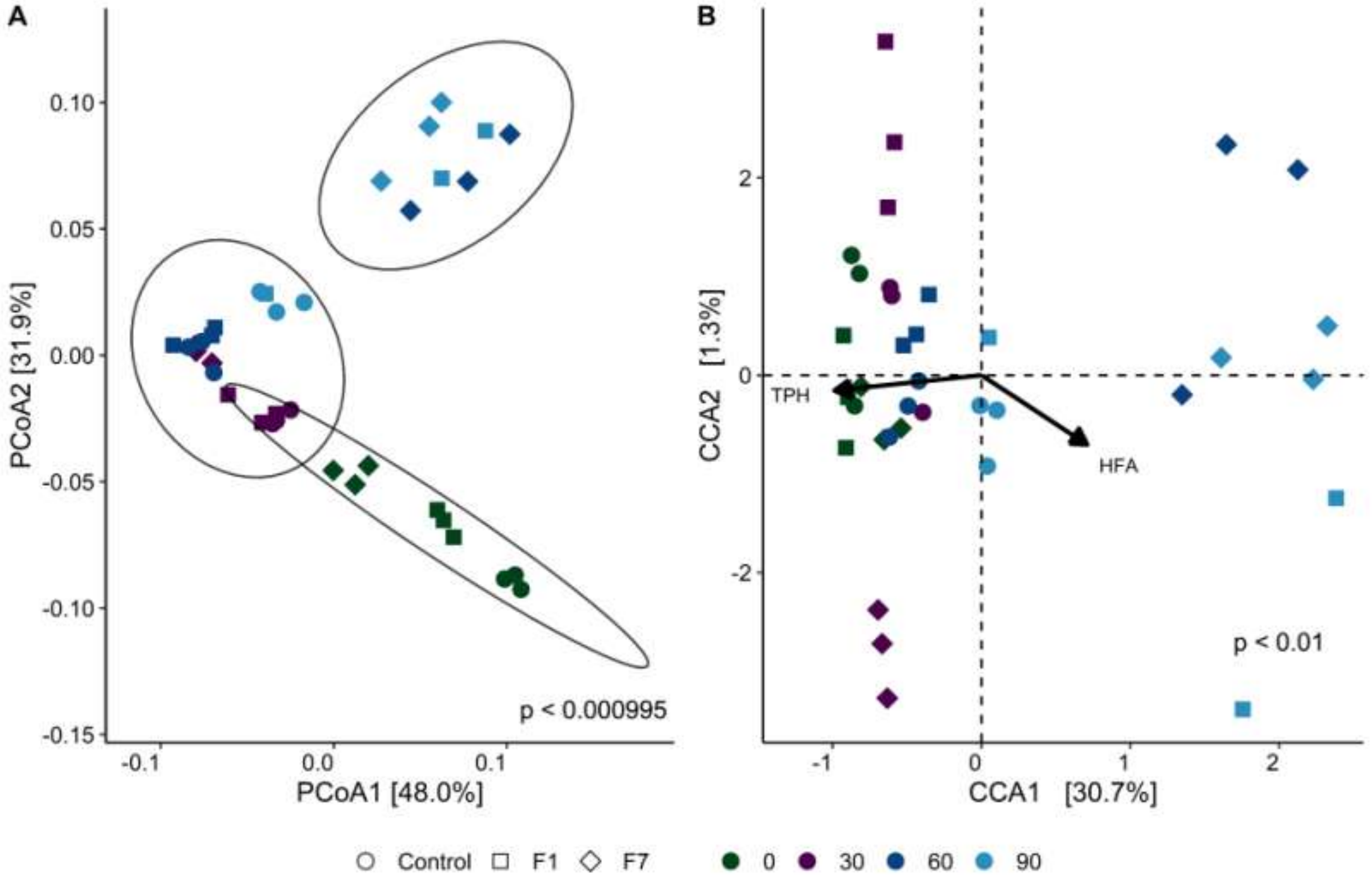
ANY SIGNIFICANT CORRELATION WITH TPH DEPLETION AND HFA INCREMENT

Results



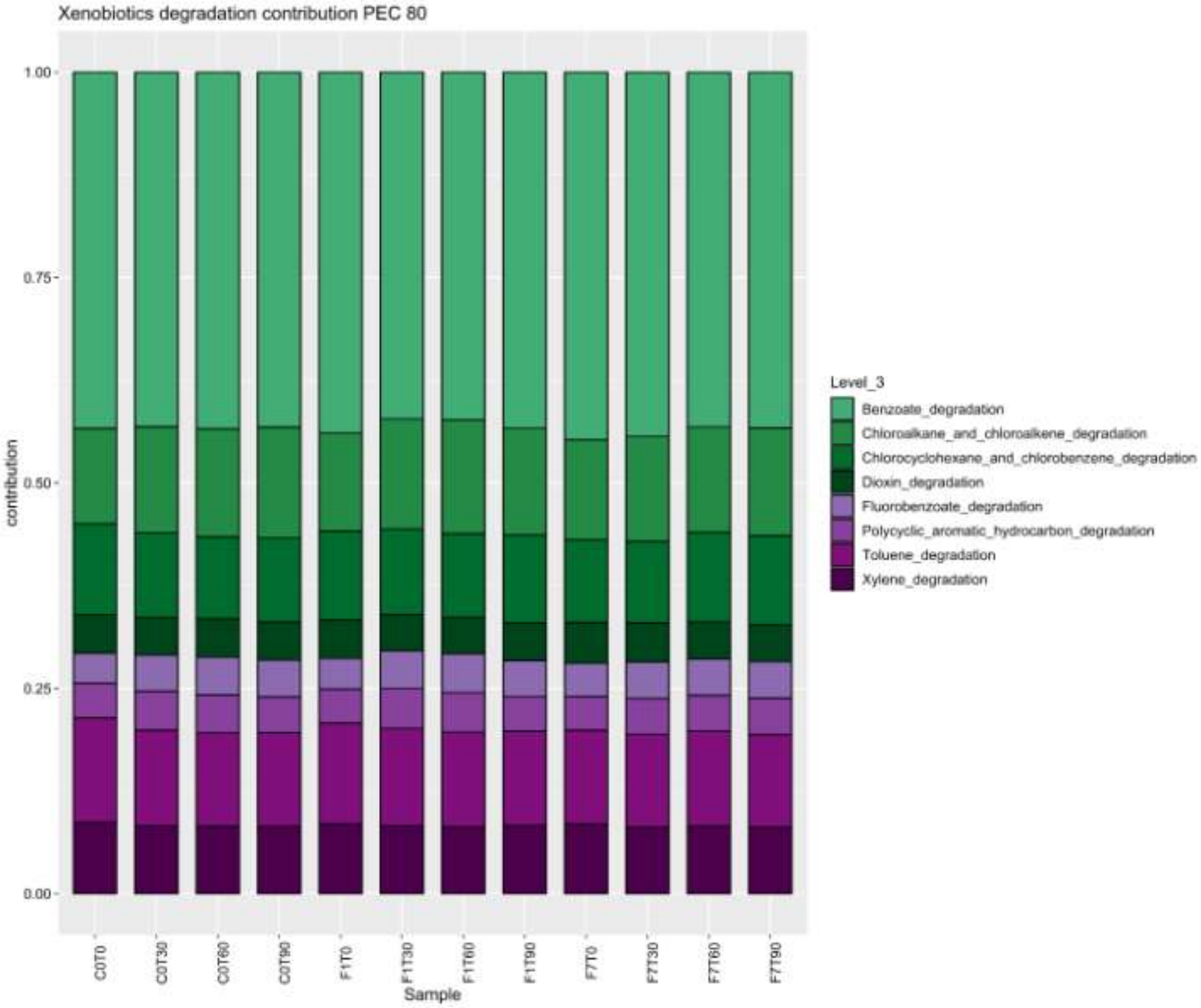
SIGNIFICANT CHANGES IN THE BACTERIAL ECOLOGY DURING THE PROCESS OF TPH DEPLETION

Results



SIGNIFICANT CORRELATION WITH TPH DEPLETION AND HFA INCREMENT

Results: predictive functional metagenomic analysis


Xenobiotics degradation contribution PEC 80

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.

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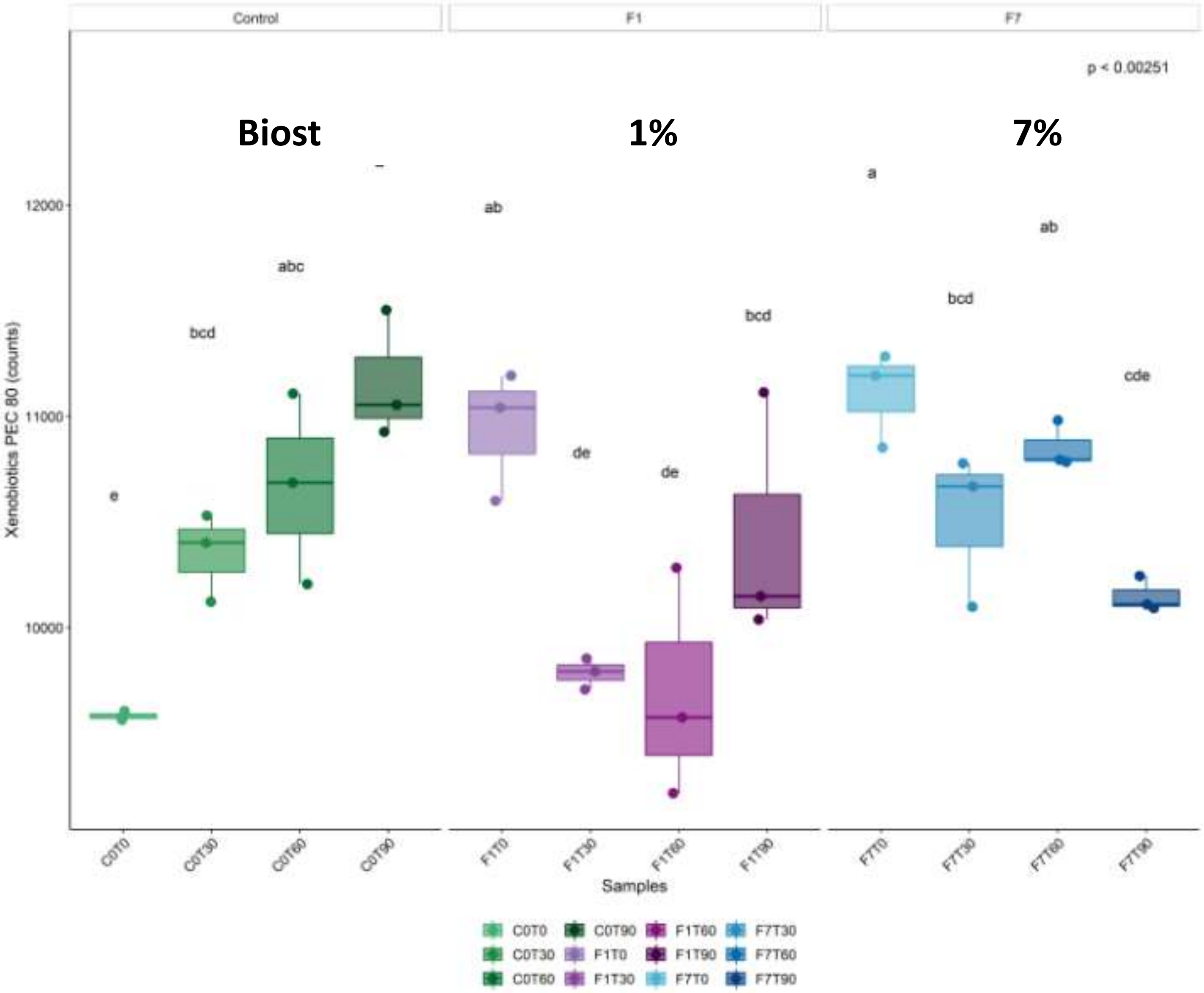
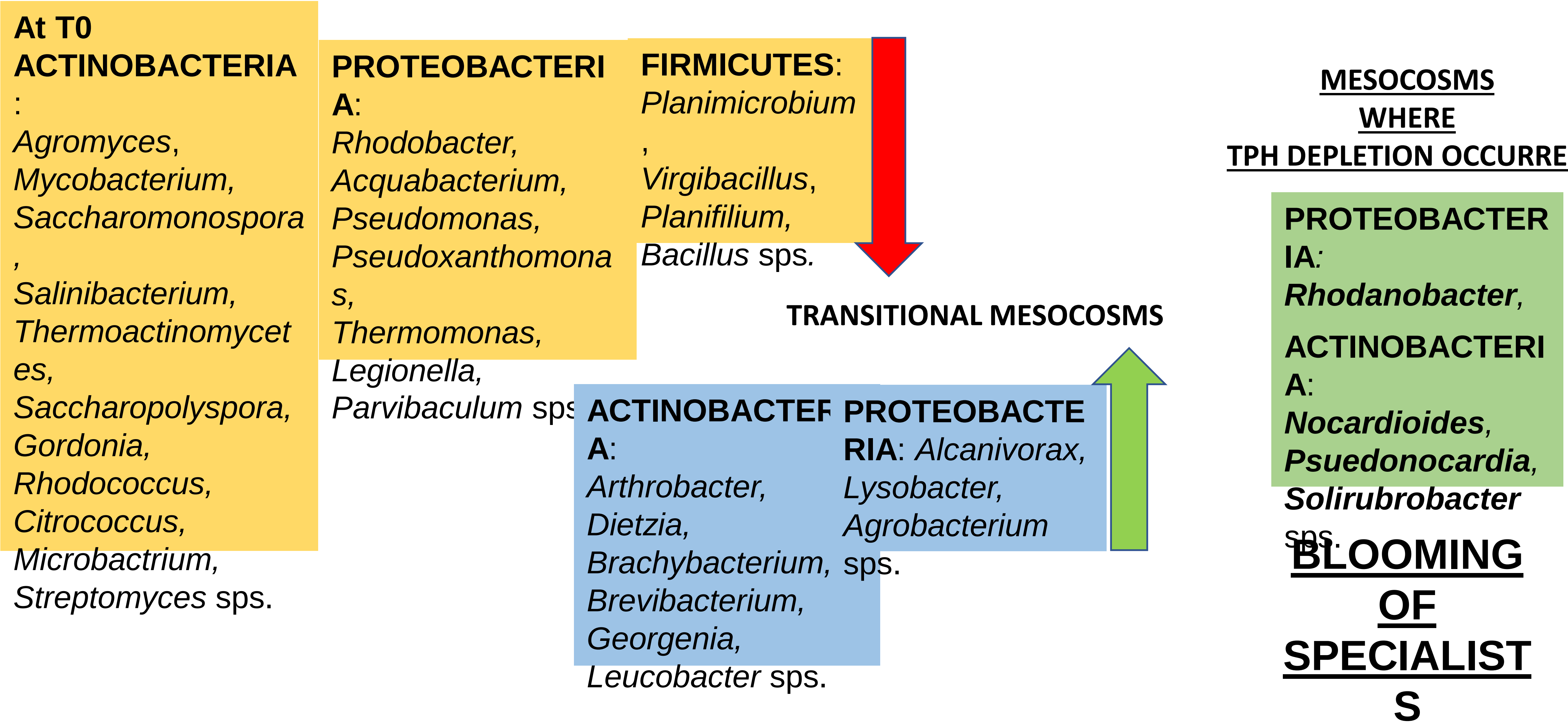
Results



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* sps.

PROTEOBACTERIA: *Rhodobacter*, *Acquabacterium*, *Pseudomonas*, *Pseudoxanthomonas*, *Thermomonas*, *Legionella*, *Parvibaculum* sps

FIRMICUTES: *Planimicrobium*, *Virgibacillus*, *Planifilium*, *Bacillus* sps.

TRANSITIONAL MESOCOSMS

ACTINOBACTER A: *Arthrobacter*, *Dietzia*, *Brachybacterium*, *Brevibacterium*, *Georgenia*, *Leucobacter* sps.

PROTEOBACTERIA: *Alcanivorax*, *Lysobacter*, *Agrobacterium* sps.

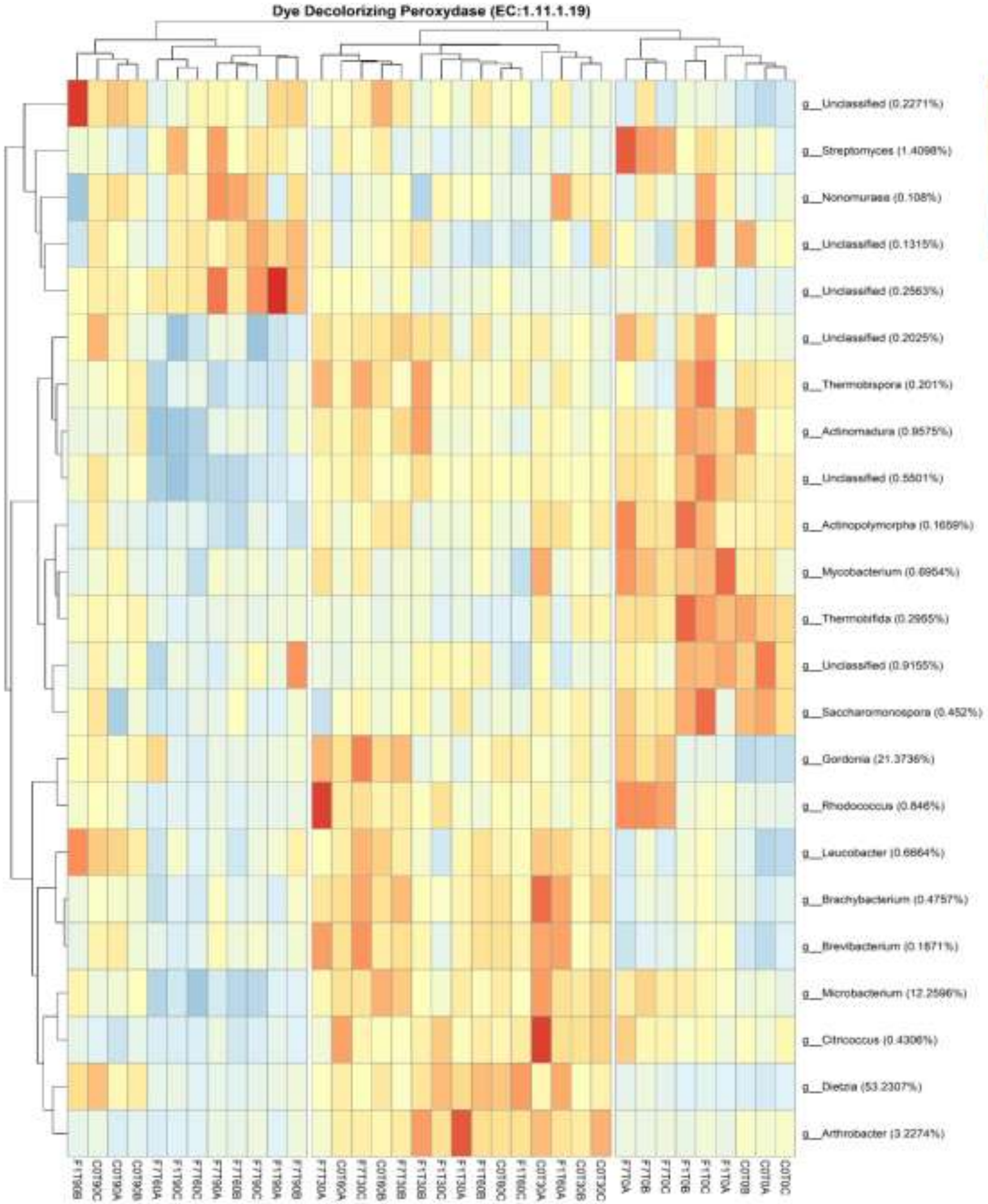
MESOCOSMS WHERE TPH DEPLETION OCCURRED

PROTEOBACTERIA: ***Rhodanobacter***,

ACTINOBACTERIA: ***Nocardioides***, ***Psuedonocardia***, ***Solirubrobacter*** sps.

BLOOMING OF SPECIALISTS

Results

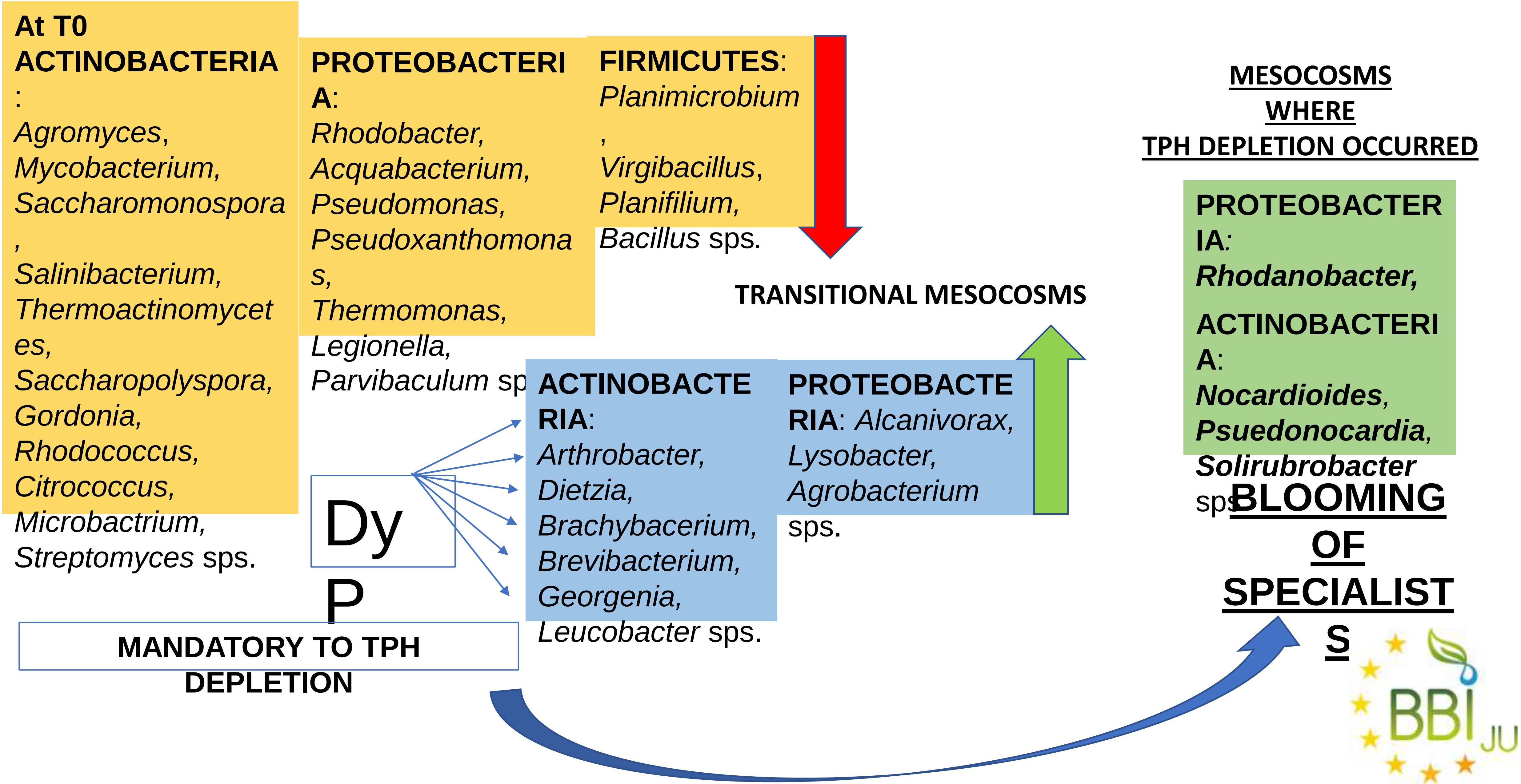


the **Dye decolorising peroxidases**.

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*, *Pseudoxanthomonas*, *Thermomonas*, *Legionella*, *Parvibaculum* sp

FIRMICUTES: *Planimicrobium*, *Virgibacillus*, *Planifilium*, *Bacillus* sps.

TRANSITIONAL MESOCOSMS

DyP

ACTINOBACTERIA: *Arthrobacter*, *Dietzia*, *Brachybacerium*, *Brevibacterium*, *Georgenia*, *Leucobacter* sps.

PROTEOBACTERIA: *Alcanivorax*, *Lysobacter*, *Agrobacterium* sps.

MANDATORY TO TPH DEPLETION

<u>MESOCOSMS WHERE TPH DEPLETION OCCURRED</u>

PROTEOBACTERIA: ***Rhodanobacter***,

ACTINOBACTERIA: ***Nocardioides***, ***Psuedonocardia***, ***Solirubrobacter*** sps

<u>BLOOMING OF SPECIALISTS</u>

Results

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

LOW CORRELATION

	q	TPH/EC:1.11.1.19
Biostimulated	**Control**	0.18
1%	**F1**	0.32
7%	**F7**	0.88

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 of 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

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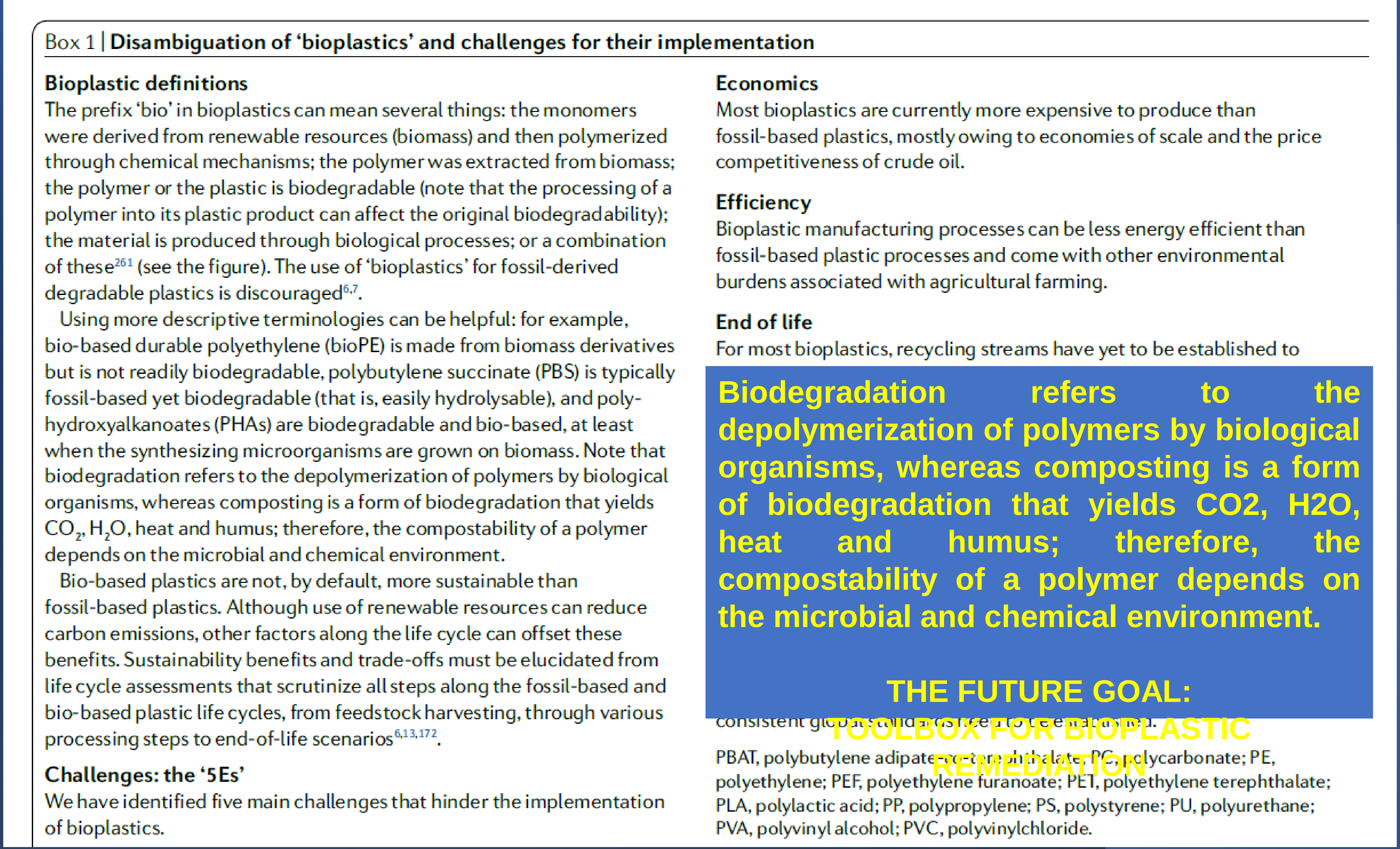

Box 1 | Disambiguation of 'bioplastics' and challenges for their implementation

Bioplastic definitions

The prefix 'bio' in bioplastics can mean several things: the monomers were derived from renewable resources (biomass) and then polymerized through chemical mechanisms; the polymer was extracted from biomass; the polymer or the plastic is biodegradable (note that the processing of a polymer into its plastic product can affect the original biodegradability); the material is produced through biological processes; or a combination of these[261] (see the figure). The use of 'bioplastics' for fossil-derived degradable plastics is discouraged[6,7].

Using more descriptive terminologies can be helpful: for example, bio-based durable polyethylene (bioPE) is made from biomass derivatives but is not readily biodegradable, polybutylene succinate (PBS) is typically fossil-based yet biodegradable (that is, easily hydrolysable), and polyhydroxyalkanoates (PHAs) are biodegradable and bio-based, at least when the synthesizing microorganisms are grown on biomass. Note that biodegradation refers to the depolymerization of polymers by biological organisms, whereas composting is a form of biodegradation that yields CO_2, H_2O, heat and humus; therefore, the compostability of a polymer depends on the microbial and chemical environment.

Bio-based plastics are not, by default, more sustainable than fossil-based plastics. Although use of renewable resources can reduce carbon emissions, other factors along the life cycle can offset these benefits. Sustainability benefits and trade-offs must be elucidated from life cycle assessments that scrutinize all steps along the fossil-based and bio-based plastic life cycles, from feedstock harvesting, through various processing steps to end-of-life scenarios[6,13,172].

Challenges: the '5Es'

We have identified five main challenges that hinder the implementation of bioplastics.

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

consistent global standards need to be established.

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

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.

THE FUTURE GOAL:
TOOLBOX FOR BIOPLASTIC REMEDIATION