Defining functional metabolic diversity of microbiota during bioremediation of hydrocarbon polluted soil with Biolog® EcoPlates™

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INTRODUCTION

Petroleum products are, because of its ubiquitous use, one of most common pollutants found in soil. According to EU Soil Strategy for 2023[1], in 2018, more than 530 million tonnes of excavated soils were generated and reported as waste, of which only two thirds were recovered. The treatment of contaminated soil with the approach of excavation and removal is not in accordance with the zero-waste initiative, and if we want to take the latter into account, it is necessary to choose a different approach to the treatment of soil contamination. So, in that context, in 2022, the company Petrol d.d. agreed to remediate excavated soil, which was a product of construction activity in the petroleum products storage facility in Lendava, Slovenia.

Excavated soil was contaminated with petroleum products that were a remnants of petroleum storage activity in that area. A total of 120m^3 of soil, was relocated within storage facility and arranged in a landfarming unit for utilization of phytoremediation technique procedures. The landfarming unit was divided into four experimental plots, with two plots seeded with a combination of grasses and two plots seeded with the combination of forbs. Functional metabolic diversity of microbiota was evaluated with Biolog® EcoPlates™ (Biolog Inc., Hayward, CA, USA).

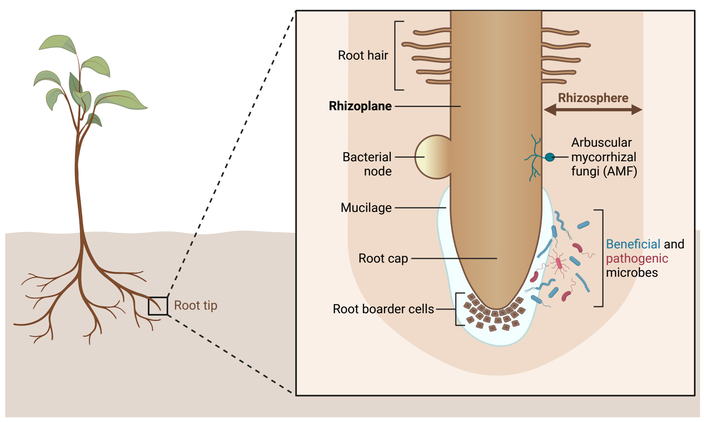
METHODS

Soil in landfarming unit was sampled and analysed by three different approaches:

- **Bulk soil**
- **Rhizosphere soil**
- **Rhizoplane soil**

Bulk soil was sampled in accordance with ISO 18400-203: 2018 Investigation of potentially contaminated sites, as a composite sample for each experimental plot. For Rhizosphere and rhizoplane soil sampling, individual plant species were collected on site, relocated to IKEMA d.o.o. laboratory and prepared within laboratory. Rhizosphere and rhizoplane soil was prepared as suggested in a study by Barillot et al. (2013)[2].

All soil samples were collected and treated with aseptic techniques.



Rhizosphere is segment of the soil under the direct influence of the roots of higher plants whereas the rhizoplane encompasses the root surface and its adhering soil [3].

CONCLUSION

- Significant differences for Polymers and Amino acids compounds in case of functional metabolic diversity between forbs and grasses
- Lowest functional metabolic diversity at rhizosphere soil with *Matricaria chamomilla* species
- Highest functional metabolic diversity at rhizoplane soil with *Phacelia tanacetifolia* Benth.

RESULTS

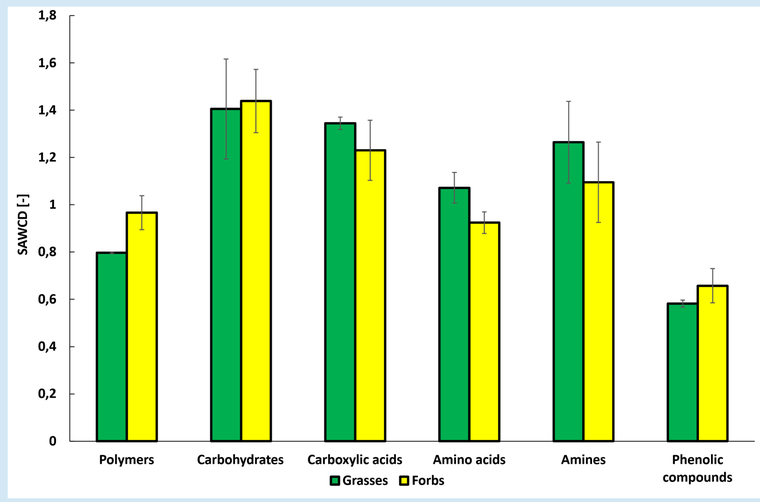
The Biolog EcoPlate™ test method with community-level physiological profiling (CLPP) was utilized to determine the effect of different plant species on the microbial communities, during full-scale bioremediation process representing bulk, rhizosphere and rhizoplane soil.

AWCD (Average Well Colour Development)[4] presents the general potential metabolic activity and indicates the total bioactivity of the Biolog® EcoPlate™

$$AWCD = \frac{\sum_{i=1}^{N} ODi}{N}$$

SAWCD (Substrate Average Well Colour Development)[4] presents the colour development of biochemical categories of the Biolog EcoPlate™.

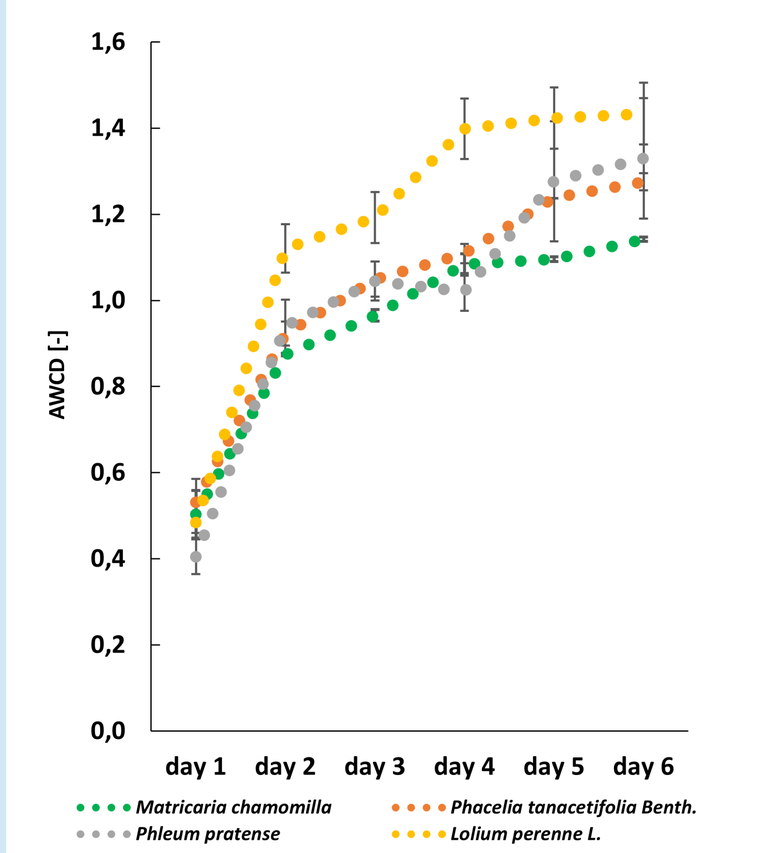
$$SAWCD = \frac{\sum_{i=1}^{N} ODi}{N}$$



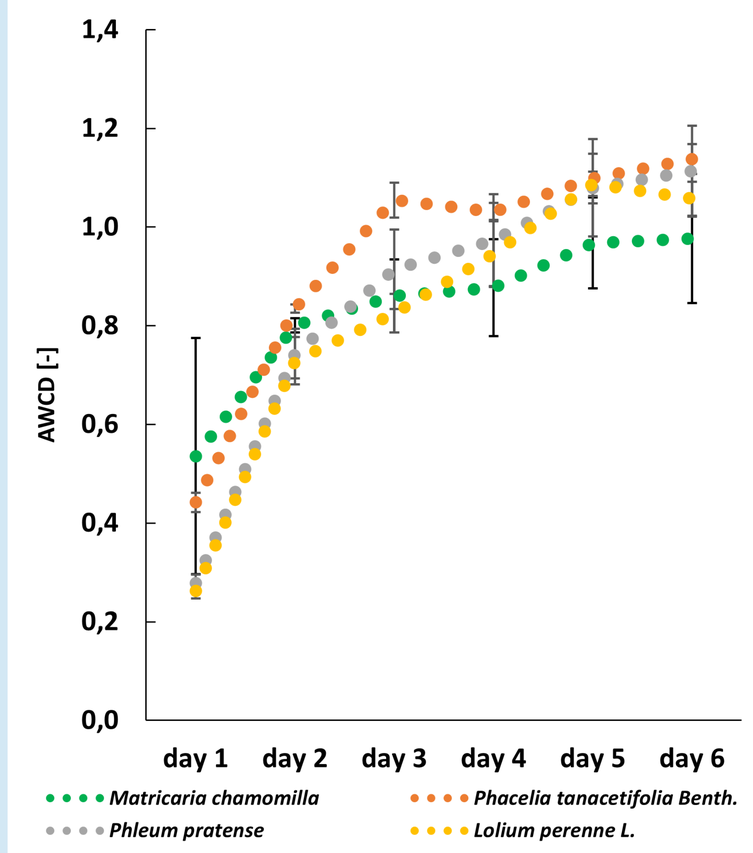
↑ Defining functional metabolic diversity with SAWCD between forbs and grasses (bulk soil analysis)

OD = optical density
ODi = corrected OD value of each substrate-containing well
N = number of substrates

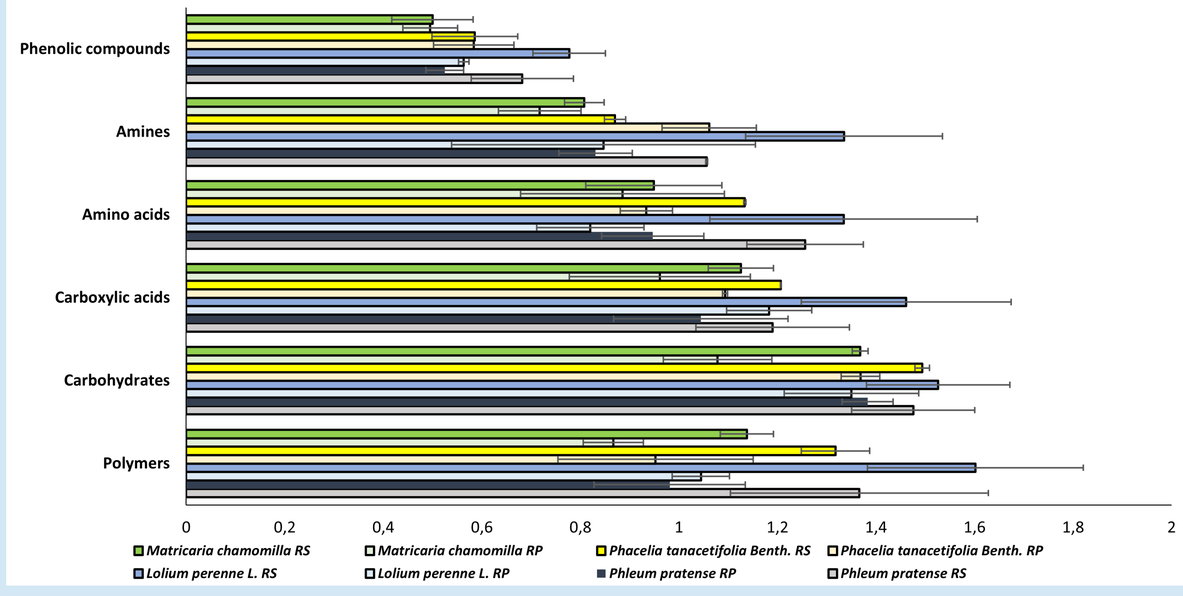
Rhizoplane soil analysis for Biolog® Ecoplate™ application were diluted in ratio 1:1000.



↑ Defining functional metabolic diversity with AWCD of different plant species in rhizosphere soil



↑ Defining functional metabolic diversity with AWCD of different plant species in rhizoplane soil



↑ Defining functional metabolic diversity with SAWCD between different plant species
RS = Rhizosphere RP = Rhizoplane

REFERENCES

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