Plants are would not evolve and survive on the changing environment without a symbiosis with diverse microbial communities interacting on multiple levels. A core component of plant microbiome are arbuscular mycorrhizal fungi (AMF). AMF abundantly colonize plant roots and are crucial for proper nutrition, physiology and survival of plants under stress. Most of the studies performed do far were focused on the bilateral functioning of AMF and plants; however, recent findings have shown that arbuscular mycorrhiza (AM) is a very complexed multi-player symbiosis where AMF interact with plants and other soil microorganisms like mycorrhiza helper bacteria (MHB) that improve the development, viability and activity of AMF. Besides, AMF frequently coexist in the roots with other fungi, including dark septate endophytes (DSE). The character of DSE relationship with plants and other microbes is unclear; however, recent studies have revealed their high potential in plant growth promotion (PGP) in stressful environments.

The research problems of this project are to verify if: (1) AMF, MHB and DSE, as microorganisms of different lifestyles, but living in the same environmental niche, support each other in promoting the growth of plants exposed to abiotic stress; (2) the effects of AMF, MHB and DSE on plants might be mediated by other members of microbial communities associated with plant roots.

The main aims of the project are: (1) isolation of DSE and MHB from the polynuclear aromatic hydrocarbons (PAHs)-contaminated site, (2) the assessment of their potential to promote plant growth, degrade PAHs, and support AMF in improving the plants fitness in PAH-contaminated pots; (3) the evaluation of the effect of AMF-MHB-DSE associations on the biodiversity of native microbial communities living in the polluted site, focusing on their potential to degrade PAHs, PGP features and metabolic activities.

DSE and MHB isolated from the PAH-contaminated site will be selected considering their PGP features, abilities to: tolerate and degrade PAHs and different sources of carbon, phosphorous and nitrogen, colonize roots (DSE) and improve the development of AMF (MHB) in roots cultured *in vitro*. Selected DSE and MHB will be added together with AMF or singly to pot cultures planted with perennial ryegrass and clover, contaminated with PAHs (0, 20, 60, 120 mg kg^{-1}). AMF used in the experiment will be *Funneliformis caledonium*, a species selected in a previous research project as supporting the fitness of plants exposed to PAHs.

After the experiment, the plant growth, oxidative stress, activity of plant defense mechanisms and nutrient uptake will be assessed by measuring: root and shoot biomass, leaf pigment content, the level of lipid and DNA oxidative damage, the activity of antioxidative enzymes and the concentrations of antioxidative compounds, the activity of genes encoding antioxidative enzymes, nutrient transporters and involved in the induction of plant systemic resistance. The development and viability of AMF, DSE and MHB in the rhizosphere will be evaluated by assessing the AMF/DSE root colonization and the quantification and identification of genetic markers specific for AMF, bacteria and fungi. The effect of AMF, DSE and MHB on PAH degradation in the growth substrate will be assessed by analyzing PAH concentration and the activity of fungal and bacterial genes involved in hydrocarbon degradation.

Microorganisms of the highest effectiveness in PGP and PAH degradation will be used to set up starting pot cultures with perennial ryegrass and clover. The cultures will be introduced to pots containing plants and soil collected from the polluted site to study the effect of AMF, DSE and/or MHB on the growth of native plants, and the biodiversity of native AMF, fungal and bacterial communities by: the next generation sequencing, metabolic profiling, the assessment of soil enzymatic activity and the activity of genes encoding enzymes responsible for hydrocarbon degradation in soil.

The outputs of these project will contribute to better understanding of a deeply understudied biology and ecology of AMF, MHB and DSE and their direct, synergetic and environment-mediated potential in PGP. The project will provide the new knowledge on the influence of PAHs on MHB and DSE, that currently, is very scarce. This knowledge might be useful in improving the effectiveness of phytoremediation involving the cooperation between plants and microorganisms in the clean-up of hydrocarbon contaminated environments.