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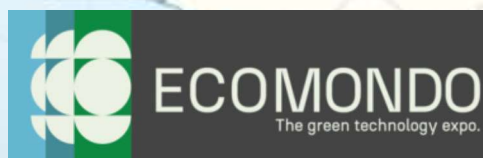
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Unravelling the petroleum hydrocarbon-triggered shift in root exudation of sunflower (*Helianthus annuus*)

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ABSTRACT

Background information. Plant-bacteria interactions are essential to maintain the holobiont fitness under environmental stress and to improve contaminant removal from polluted soils through phyto-rhizoremediation strategies. Soil pollution triggers phytotoxic responses in plants, causing a shift in the root exudation pattern, the so-called ‘cry-for-help’, to recruit a contaminant-degrading bacterial community able to release the plant from stress. This study aims to investigate the sunflower ‘cry-for-help’ triggered by petroleum hydrocarbon (PHC) contamination using 1) an *in vitro* hydroponic system where the plant was exposed to xylene, one of the most phytotoxic aromatic hydrocarbons and 2) a soil matrix collected from a historically polluted site in northern Italy, showing a complex profile of PHC pollution.

Methods. A novel experimental set-up was developed in this study to simulate HC stress *in vitro* by exposing sunflower plants to xylene. For the two aims of the project, root exudates (REs) were collected from plantlets cultivated both *in vitro* and in pots filled with contaminated soil. Untargeted metabolomics was performed to identify REs differentially released by plants in the two experimental conditions, characterized by a different degree of HC pollution.

Main results. In the developed *in vitro* set-up, xylene supplementation dramatically affected plant physiology, presumably causing oxidative stress that led to reduced chlorophyll A content, enhanced ROS production in leaves and increased expression of oxidative stress-related genes. Similarly, sunflower growth in presence of PHC polluted soil exhibited significantly lower fresh biomass compared to plants grown in non-contaminated soil as reference. Untargeted metabolomics permitted to clearly discriminate between REs released under stress and in control conditions for both experimental set-ups and allowed the annotation of 153 and 1482 metabolites for *in vitro* and soil experiments, respectively. In the *in vitro* experiment, molecules belonging to the classes of coumarins, terpenes and phenolic glycosides, often involved in plant stress response, were up regulated upon xylene exposure. The exudation profile in contaminated soil highlighted a higher presence of coumarins and aminoacids and decreased levels of steroid derivatives and indoles, while flavonoids and terpenes molecules resulted both up and down regulated. The classes of metabolites identified and their diverse abundances are ascribable to the substantial difference between the two experimental approaches, which in the case of soil involves a more complex HC contamination pattern. Selected key metabolites, that were reported as potential bioactive in plant-bacteria interactions, have been screened for their influence on bacterial features essential for fostering root colonization and improving phyto-rhizoremediation efficacy in PHC degrading strains, like bacterial growth, motility and biofilm formation.

Conclusions. Unravelling the “cry-for-help” response to PHCs is crucial to identify plant-derived compounds acting as biostimulants for degrading bacteria, improving beneficial bacterial recruitment and colonization and boosting plant fitness under stress, thus improving rhizoremediation strategies.

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