## Soil microbial community during PAH degradation

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Polycyclic aromatic hydrocarbons (PAHs) are mainly produced by hydrocarbon fuel combustion and in general by organic matter incomplete combustion. Soil PAH contamination represents a risk for humans and ecosystems, especially considering their carcinogenic nature (IARC, 2017) and their persistence. Since microbial degradation is the main responsible for PAH removal from soil, the understanding of the process is important for the effective bio-remediation of contaminated areas. Techniques aimed at promoting microbial community degradation of organic compounds, like biowaste compost amendment or mycoremediation (inoculum with fungal species), seem especially promising in this context by accelerating the natural process (Harms et al, 2011). Biowaste compost, in particular, is both a trophic resource for microbial communities and a source of allochthonous microorganisms to the soil, possibly able to metabolize PAHs (Balachandran et al., 2012).

The goal of this research was to investigate soil anthracene and benzo[a]pyrene degradation in relation to the composition of soil microbial community, either in control soils or in soils added with biowaste compost or fungal consortium. To evaluate structural changes of the microbial communities along time, phospholipid fatty acids (PLFAs) analysis was employed. PLFA profile represents an useful tool to investigate microbial community composition and structure (Schweitzer et al., 2011; Wei et al., 2014).

Soil (70 Kg) sampled from the top layer (0-10 cm) of an industrial area (40°56'38" N, 14°49'34" E) in southern Italy was homogenized and divided into three aliquots subjected to different treatments: 1) addition of biowaste compost, 0.6% w/w (CMP); 2) addition of fungal species (Armillaria mellea, Pleurotus ostreatus, Pleurotus eryngii and Stropharia ferii), 0.4% w/w of mycelium (FNG); 3) no addition (UNT). For each treatment, soil was spiked with benzo[a]pyrene and anthracene (150 mg/Kg, each). A mesocosm study was established by incubating 12 containers (4 for each treatment) in a dark room at controlled temperature (24 °C) and relative humidity (70%) for 274 days. Soil samples were collected at an initial time T0 after 24 hours of exposure to PAHs, T1 after 15, T2 after 107, and T3 after 274 days. At each sampling time, organic matter content was analysed by loss on ignition (550 °C for 4 h) and pH by potentiometric method in a water suspension (1.0:2.5 = w/v = soil:water). The total lipids from the soil were extracted according to Frostegård et al. (1993). The phospholipid fraction was transesterified, methylated and analysed by GC-FID. For PAH analyses, soil samples (15 g) were extracted by sonications in 150 mL of a mix dichloromethane-acetone (1:1) and the final extracts reduced in volume and analyzed by GC-MS (Baldantoni et al., 2017). The differences in PLFA concentrations among treatments and sampling times were evaluated through a two-way MANOVA, whereas the differences in anthracene and benzo[a]pyrene, related to the same factors, were evaluated through two-way ANOVAs. As post hoc tests following the MANOVA and the ANOVAs, Canonical Variates Analysis and Tukey HSD test were employed, respectively. In order to evaluate the importance of each PLFA in determining the degradation of anthracene and benzo[a]pyrene, random forests based on 10000 trees, with random splitting and out-of-bag prediction errors were trained separately on the data from the three soil treatments. Variable importance was derived through the Breiman-Cutler permutation algorithm, whereas variable selection was performed through the minimal depth algorithm. All of the analyses were performed using the "stats", "candisc" and "randomForestSRC" packages within the R 3.3.2 programming environment.

Along 274 days of incubation, a higher (P < 0.001) degradation of anthracene in the early stage of incubation was recorded in the mesocosms treated with fungal consortium (FNG) and compost (CMP). Already after 15 days, a decrease of approximately 15% was observed for treated mesocosms compared to a decrease of 2% in untreated mesocosms (UNT). At the end of the incubation anthracene reached a concentration equal to 5% of the initial value, both in untreated and treated mesocosms. Also benzo[a]pyrene content significantly (P < 0.001) decreased along 274 days of incubation, halving at the end of experiment in UNT and FNG mesocosms, and reaching 56% in CMP. The slower benzo[a]pyrene degradation may be due to its lower water solubility and higher sorption to soil organic fraction, making it is scantly available for microbial community (Baldantoni et al., 2017). The faster PAH degradation in compost amended soil than untreated soil, also reported by Ros et al. (2014), is likely related to the biomass and activity of compost microbial community, paramount for a successful bioremediation (Chen et al., 2015). Fungal inoculum promoted the degradation of anthracene, but not of benzo[a]pyrene, probably in relation to its use as carbon source favoring fungal activity (Qasemian et al., 2012). Most of the 29 PLFAs detected in the three soils were relatively more abundant in CMP and UNT soils, with the exception of 18:0, 10Me17:0 and i14:0 which clearly differentiated FNG treatment from the others. UNT and CMP treatments, instead, were primarily differentiated by the abundance of i16:0 in the latter. The minimum depth algorithm selected 11 variables from the random forests based on CMP and FNG data as the optimal set of predictors for both anthracene and benzo[a]pyrene, whereas no variable was selected from the random forests based on UNT data. Most of the PLFAs selected showed positive relationships with PAH concentrations, although a few of them showed an inverse relationship. Among these, 18:0 and 10Me17:0 were selected for both the PAHs in both CMP and FNG soils, whereas the others were related specifically to one of the treatments: i17:0, br18:0, cy19:0 for CMP and 17:0, 10Me18:0, 14:0, i14:0 for FNG. Conversely, the selected PLFAs were similar, within the same soil, for the prediction of anthracene and benzo[a]pyrene, with usually only one predictor, among the minor ones, varying between the sets associated to the two PAHs. Gram + bacteria (i17:0 and br18:0) contributed to the degradation of the two investigated PAHs in CMP mesocosms. Gram-positive bacteria were hypothesized to be the dominant degraders of recalcitrant compounds such as PAH (Leys et al., 2005). In FNG mesocosms, actinomycetes (10Me18:0) also contributed to PAH degradation, as also observed in previous researches (Chaudhary et al., 2011, Balachandran et al., 2012). Methanotrophic bacteria (18:0), instead, were involved in the degradation of PAHs in both CMP and FNG mesocosms, and appeared to be the principal functional group contributing to the process. Indeed, methanotrophic bacteria, because of the low specificity of some methane-monooxygenase, are able to use aromatic compounds in methane deficiency conditions (Pandey et al., 2014).

The enhanced PAH degradation by the microbial community inoculated with compost, suggests biowaste compost amendment as eco-friendly solution in soil PAH bioremediation.

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