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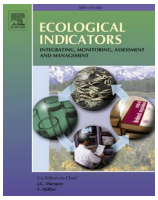

A synthetic quality index to evaluate the functional stability of soil microbial communities after perturbations

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ABSTRACT

Soil stability includes both resistance, the ability to withstand a perturbation or stress, and resilience, the ability to recover to pre perturbation levels. The functional stability of soil microbial communities is of paramount importance for the ecosystem functioning. We investigated the differences in the stability (resistance and resilience) of three enzyme activities (hydrolytic, laccase and peroxidase) in three different forest (holm oak, black pine and beech) soils after addition of PAHs (phenanthrene, pyrene and benzo[a]pyrene) with different molecular weights. Furthermore, we proposed a new soil quality index (MAI) based on the measured enzyme activity values, useful to quantify the ecological impact of soil perturbations (PAH exposure in our case).

The degradation rates of different PAHs follow their complexity, slowing with increasing of PAH molecular weight in all soil types. Moreover, we found higher microbial resistance to PAH perturbation in "broad scale" enzyme activity (hydrolase), in respect to the two "narrow-niche" enzyme activities (laccase and peroxidase). The results demonstrate a higher functional stability in soils with a higher content of recalcitrant organic matter (soil under pine), compared to soils with higher content of labile organic matter (soil under holm oak). In particular, laccase activity is less affected by phenanthrene and pyrene addition in soil under pine; peroxidase activity shows a higher resistance and resilience in soil under beech for all PAHs added. Resistance and/or resilience to PAH contamination, observed for hydrolytic functional stability in the three soils, is mainly due to the high diversity of enzymes expressing this type of catalytic activity.

1. Introduction

Soil is increasingly under anthropogenic pressures that induce significant perturbation and alter its capacity to provide essential ecosystem services. To protect soil and maintain its crucial functions, it is important to know how soil microorganisms respond to perturbation or environmental changes. Because of anthropogenic activities, soil pollution by both inorganic, such as heavy metals, and organic, such as polycyclic aromatic hydrocarbons (PAHs), xenobiotics is a widespread concern (Steffan et al., 2018).

Soil stability comprises both resistance and resilience (Griffiths et al., 2000). Whereas soil resistance is the ability to resist to a perturbation, soil resilience is the ability to recover to pre perturbation levels. It has been hypothesized that soil functional stability is primarily generated by the functional redundancy of the soil microbial taxa (Chaer et al., 2009; Degens et al., 2001; Griffiths et al., 2004; Van Bruggen and Semenov, 2000). The effects of stress or perturbation on the functional stability of soils depend on the specificity of the function. For example, broad-scale functions, such as decomposition of organic matter, are generally scantly affected by microbial diversity, whereas more specialized niche functions, such as degradation of xenobiotics, decrease as biodiversity decreases (Griffiths et al., 2000, 2005). Several soil physico-chemical factors affect both microbial diversity and its stability, as demonstrated for bacteria in laboratory culture (Johnston and Brown, 2002; Nickerson et al., 2003). Soil organic matter content, in particular, is positively correlated with resistance to a contaminant stress (Kuan et al., 2007), and also contributes to biological resilience, e.g. by adsorption of substances toxic to microorganisms (Hund-Rinke and Kördel, 2003). Also soil structure may protect soil microorganisms from perturbation, influencing their spatial niches (Young and Ritz, 2000). In particular, PAHs may be sorbed on soil organic matter or buried in pores inaccessible to microbes (Ren et al., 2018) affecting microbial responses to

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contamination stress.

Soil enzyme activities are often used as indicators of soil quality and the mathematical expressions to derive it have increased in recent years (Paz-Ferreiro and Fu, 2016). Soil quality indicators should meet several criteria (Dale et al., 2008): they should provide information about the function, composition and structure of the ecological system, integrating its complexity and providing an early warning signal of changes (Cairns et al., 1993). Since soil quality depends on several parameters (Bünemann et al., 2018), the recent trend is to assess soil quality on the basis of several biological factors, but the problem related to enzymatic activities (expressed with different units of measurement) is largely still unsolved (Wyszkowska et al., 2013).

In this study, soil microbial responsiveness to a perturbation event was monitored in experimental mesocosms along 360 days after spiking with phenanthrene, pyrene or benzo[a]pyrene. The functional stability of three soils under different vegetation covers (holm oak, black pine and beech) was evaluated by one "broad scale" soil enzyme (total hydrolase) and two "narrow-niche" soil enzymes (laccase and peroxidase). It was expected: 1) a decrease in resistance and resilience for more specialized microbial groups and then 2) a different recovery rate of the enzyme activities after the perturbation among the three soils in relation to the spiked PAHs. The objectives were to explore the responses of the three soil enzyme activities along the time after spiking by different molecular weight PAHs, in relation to their degradation dynamics. Because environmental perturbations may exert in long-term on ecosystems, it is important to identify early indicators of change and to monitor the ecosystem recover in order to protect it. For this purpose, here we also proposed a new and synthetic soil quality index (based on the enzyme activities measured in the study), useful to quantify the ecological impact of different soil perturbations. In relation to PAH spiking, in particular, several studies reported an overall inhibition in soil enzyme activities and metabolic diversity (Baldantoni et al., 2017; Picariello et al., 2020). Anyway, little is known about how soil microbial communities of natural systems globally respond to contamination stresses. This aspect could be crucial not only in relation to the importance of microbial communities in ecosystem functioning, but also for their successful use in bioremediation of soils contaminated by organic xenobiotics, such as PAHs.

2. Materials and methods

2.1. Soil sampling and mesocosms set-up

Eutrisilic Andosols on Matese Mountains (Apennines district, southern Italy) under three different vegetation covers, holm-oak (H, 41°15′26″ N, 14°29′43″ E), black pine (P, 41°23′56″ N, 14°25′58″ E) and beech (B, 41°23′44″ N, 14°27′26″ E) were chosen. At each site, surface (0–20 cm) soil was collected, after litter removing, to mesocosms set-up. In the laboratory, each soil was separated in three parts individually spiked with a solution in acetone of benzo[a]pyrene (1.3 g/L), pyrene (2 g/L) and phenanthrene (2 g/L). PAH solutions, whose concentrations have been chosen according to our previous experiments in which effects on several enzyme activities were observable (Baldantoni et al., 2017; Picariello et al., 2020), were added drop by drop to soil, with a view to obtaining a homogeneous contamination.

About 7 kg of each individually spiked soil was used to fill 2 mesocosms (20 × 30 × 10 cm), that were incubated in dark, under controlled conditions (T: 22 °C, R.H.: 88%) and weekly irrigated with distilled water (for details see Picariello et al., 2020). Soil samples were collected from each mesocosm type before (time 0) and after 4, 52, 108, 213 and 360 days from spiking. At each sampling, the fresh soil was sieved to 2 mm and divided in several aliquots for chemical (organic matter content, phenanthrene, pyrene and benzo[a]pyrene concentrations) and biological (total hydrolase, laccase and peroxidase activities) analyses. Details on chemical and biological characteristics of soils at time 0 are reported in SM1 and in Picariello et al. (2021).

2.2. Laboratory analyses

Soil organic matter (SOM) was determined on sieved (<2 mm) soil samples by calcination (550 °C for 4 h) in muffle (Nabertherm GmbH, Controller B 170). Phenanthrene, pyrene and benzo[a]pyrene concentrations were determined according to De Nicola et al. (2019).The fluorescein diacetate hydrolytic activity (FDAase) was determined by Schnurer and Rosswall (1982) method. The laccase activity was determined by Floch et al. (2007) method and the peroxidase activity by Bach et al. (2013) optimized method.

All analyses were carried out in triplicates per soil treatment and per each site, for a total of 162 samples analyzed for each chemical and biological parameter (3 treatments × 3 sites × 6 sampling times × 3 laboratory replicates).

2.3. Metabolic activity index

The proposed Metabolic Activity Index (MAI) of overall soil activity is based on the ratio of the enzyme activities in the spiked soil to those in the unperturbed control soil. The enzyme activities are normalized to the soil organic carbon content (SOC).

$$\mathrm{MAI} = \frac{1}{n}\sum_{i=1}^{n}\frac{Pij}{Pcij}$$

where:

$$P_{ij} = A_{ij}/Ref_j$$

$$Pc_{ij} = Ac_{ij}/Refc_j$$

and A_{ij} is each enzyme activity value in each sampling time; Ref_j is a reference parameter, in our case the SOC content; Ac_{ij} is each enzyme activity value in unperturbed control soil; $Refc_j$ is the reference parameter in unperturbed control soil.

SOC is calculated considering that the average carbon content in the SOM is ~ 58% (Pribyl, 2010). Consequently, the factor 1.724 can be used to transform the organic matter content into the corresponding g * kg^{-1} of organic carbon. We choose to use SOC content to normalize the enzyme activities, since the overall soil activity is mainly dependent on the content of carbon (Wyszkowska et al., 2013). In addition, we referred our enzyme activities in respect to the unperturbed control soil (before spiking, time 0) to highlight changes over time. The MAI values may range from + 1 to + ∞, with values that increase with increasing soil metabolic activity.

2.4. Data analysis

The dynamics of phenanthrene, pyrene and benzo[a]pyrene concentrations along the time were described using an exponential decay model:

$$y(t) = y_0 \cdot e^{-kt}$$

where: y(t) is each PAH concentration at time t; y_0 is the initial PAH value (100); k is the exponential decay constant.

Functional resistance (the ability of enzyme activity to avoid displacement during PAH spiking) of soils under the three different vegetation covers was evaluated in treated soils after 4 days, as the percentage of enzyme activity change relative to pre perturbation condition (before spiking, time 0). The functional resilience (the ability of enzyme activity to return to initial levels or recover from PAH spiking) of the soils was assessed by evaluating the shift in enzyme activities from the pre perturbation condition (time 0) and along 360 days after PAH perturbation, in order to detect when each activity is able to return to values comparable to the pre perturbation. Equations (Sousa, 1980) used to calculate enzyme activity changes relative to the unperturbed soil are:

$$Resistance = D_x/C_0 \times 100$$
$$Resilience = D_x/C_0 \times 100$$

where: C_0 is the variable measured in the unperturbed soil at time 0 (before spiking); D is the variable measured in perturbed soil at different times (x) after perturbation (4 days after perturbation for resistance).

Normality of the data was assessed using the Shapiro-Wilk test and homoscedasticity using the Brown-Forsythe test. Since the data were normal and homoscedastic, we proceeded with the analysis of variance; the significance of the differences in the dynamics of the three investigated PAHs and in the dynamics of the three enzyme activities were evaluated by two-way RM ANOVAs (two-way repeated measures analysis of variance), considering sampling time and forest system as fixed factors. The ANOVAs were followed by the Tukey *post hoc* tests (for $\alpha = 0.05$). All the statistical analyses were performed using the SigmaPlot 14.0 software (Systat Software, Inc.).

3. Results

Along 360 days of incubation, phenanthrene and pyrene concentrations significantly ($P < 0.001$) decreased (Fig. 1), with differences among soil types (Table 1). In H soil, phenanthrene and pyrene reached, after one year, about the 0.8 and 3.0% of the initial values, respectively, whereas in B soil the 4.0 and 3.5%, respectively. In P soil, instead, both PAHs decreased to a lesser extent, reaching at the end of incubation, 38% of the initial value for phenanthrene and 14% for pyrene. Phenanthrene already after 52 days almost completely degraded both in H (residual content 1.5%) and in B (residual content 9%); in P at the first sampling time it decreased, reaching 52% of the initial content going through a further decrease after 108 days (41% initial content), and then remaining constant until the end of incubation. For pyrene, a further degradation was observed at 108 days, maintained constant up to the end of incubation in all the investigated soils. Even if to a lesser extent than phenanthrene and pyrene, also benzo[a]pyrene content significantly ($P < 0.05$) decreased along 360 days of incubation, reaching after one year, the 33% of the initial values in H, 73% in P, and 44% in B. After 108 days, benzo[a]pyrene did not degrade with a great extent, but after 213 days of incubation it reached about the 67% of the initial value in H, 83% in P, and 76% in B.

The investigated PAHs showed different decay constants (k) in the soils under the three different vegetation covers (Table 2). Phenanthrene degraded faster in H, followed by B and P, whereas pyrene degradation was faster in B followed by H and P. The decay constant for benzo[a]pyrene in P was significantly lower in respect to H and B, whereas no significant difference was observed between the decay constants of H and B. In H soil, PAH degradation followed the order phenanthrene > pyrene > benzo[a]pyrene, whereas in B and P soils, the order of degradation was pyrene > phenanthrene = benzo[a]pyrene (Table 2).

Table 1
Two-way RM ANOVA F-values evaluated for the three investigated PAHs in soils under holm oak (H), black pine (P) and beech (B), along the time. The q coefficients of the *post hoc* tests of Tukey (for $\alpha = 0.05$) performed for the soil system factor are also shown. The asterisks indicate the P values (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

	Phenanthrene	Pyrene	Benzo[a]pyrene
Two-way RM ANOVA			
time	4952.74***	3040.43***	13.33*
soil system	439.41**	825.53***	6.27*
time × soil system	213.10***	316.38***	11.42***
Tukey (comparison for soil system factor)			
P - H	41.12**	57.21***	5.60*
P - B	27.63**	33.241**	2.578
B - H	13.49*	23.97**	3.292

Table 2
Decay constants for phenanthrene, pyrene and benzo[a]pyrene in soils under holm oak (H), black pine (P) and beech (B). Different lowercase letters indicate significant (for $\alpha = 0.05$) differences among PAH treatments in each forest system, and different uppercase letters indicate significant (for $\alpha = 0.05$) differences among forest systems.

	Phenanthrene	Pyrene	Benzo[a]pyrene
H	0.009 ± 0.001^{aA}	0.007 ± 0.001^{bB}	0.003 ± 0.001^{cA}
P	0.002 ± 0.001^{bC}	0.005 ± 0.0001^{aC}	0.001 ± 0.001^{cB}
B	0.006 ± 0.0001^{bB}	0.009 ± 0.0001^{aA}	0.002 ± 0.0001^{cA}

The three enzyme activities showed different dynamics along the time (SM2), and each of them showed significant differences both in relation to the time ($P < 0.001$) and among forest systems ($P <$ at least 0.05) (Table 3), except for laccase activity in benzo[a]pyrene treatment, with values generally higher in soil under black pine.

The three enzyme activities showed different dynamics along the time (SM2), and each of them showed significant differences in relation to the time ($P < 0.001$). Hydrolase and peroxidase activities showed differences among forest systems ($P < 0.001$) in all the treatments, whereas laccase activity only in phenanthrene ($P < 0.05$) and pyrene ($P < 0.01$) treatments (Table 3), with values generally higher in soil under black pine for all the enzymatic activities (SM2). Generally, lower values were observed in the pyrene and benzo[a]pyrene spiked soil mesocosms. Almost all the enzyme activities, after some fluctuations in the early stages of incubation, decreased after 108 days (SM2).

Resistances to all perturbation events (phenanthrene, pyrene and benzo[a]pyrene spiking) were observed for hydrolytic activity in soil under holm oak (with resistance values meanly ranging around 126% in respect to the unperturbed soil), while in soils under pine (81%) and

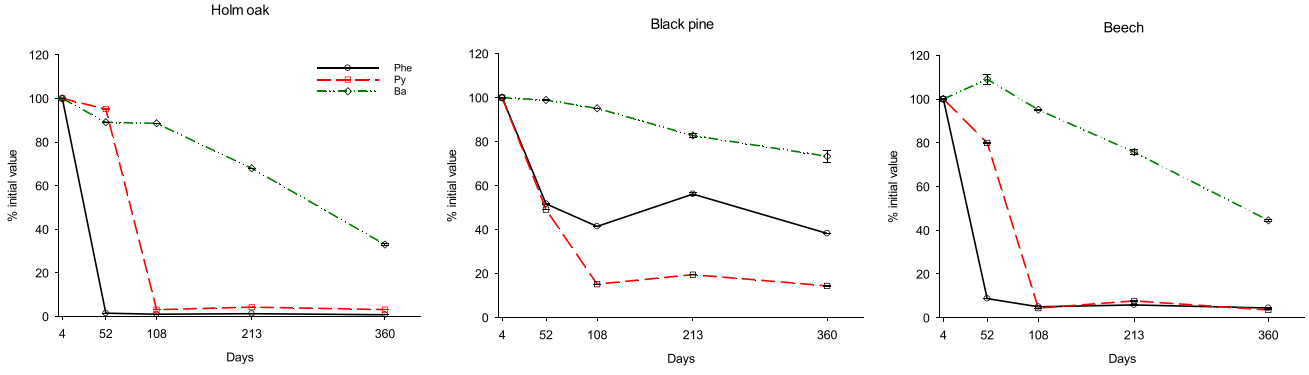

Fig. 1. Degradation dynamics (in percentage of the initial values) of phenanthrene (black), pyrene (red) and benzo[a]pyrene (green) in soil collected under holm oak, black pine and beech, during mesocosms incubation. Vertical bars represent standard errors of the means. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Two-way RM ANOVA F-values evaluated for the three enzyme activities in soils under holm oak, black pine and beech along the time. The asterisks indicate the P values (**≤0.01, ***≤0.001).

Two-way RM ANOVA	Phenanthrene	Pyrene	Benzo[a]pyrene
Hydrolytic activity			
soil system	317.93 ***	1518.81 ***	700.27***
time	1603.04 ***	700.89***	809.50***
soil system × time	28.48 ***	41.34***	223.396***
Laccase activity			
soil system	13.74*	38.10**	1.71
time	37.82***	63.16***	19.83 ***
soil system × time	4.80**	27.63***	2.57
Peroxidase activity			
soil system	98.54***	672.81***	104.87***
time	49.50***	40.77***	147.66***
soil system × time	16.40***	15.58***	42.11***

beech (78%) there was a slight decrease compared to the pre perturbation values (Table 4). Laccase activity showed a resistance after PAH addition in soil under pine (on average 89% of the pre unperturbed values), while peroxidase activity showed a higher resistance in soil under beech for all PAH spiking (≥100%).

Resilience to PAHs (Fig. 2) was observed in soil under black pine for hydrolytic activity, with a recover after 52 days. In all soil systems, at 108 days of incubation, the hydrolytic activity showed comparable or even higher values than pre perturbation condition, decreasing only at the end of incubation (after 213 and 360 days). Laccase activity recovered after 108 days following pyrene treatment, only in soil under black pine, decreasing after 213 days from spiking. Peroxidase activity in soil under beech showed values higher than pre perturbation yet after 4 days and along 360 days after spiking with all the three PAHs.

The MAI temporal dynamics (Fig. 3), considering the overall soil activity along the time, highlighted a decrease of the index values after the addition of the three PAHs in soils under holm oak and black pine. On the contrary, soil under beech usually showed MAI values > 1. To validate our results, MAI values were compared to values obtained (SM3) applying the index proposed by Lemanowicz (2019). Lemanowicz index values ranged from 1.75 to 2.43, with on average highest values for soil under black pine.

4. Discussion

In this mesocosm study, phenanthrene and pyrene, with 3- and 4-aromatic rings, respectively, showed at the end of one year of incubation from spiking almost complete degradation, mainly in soils under holm oak and beech, whereas benzo[a]pyrene, with 5-aromatic rings, reached about 50% of residual content. Moreover, phenanthrene and pyrene showed a fast degradation in the first months after spiking, reaching about 3% of residual content already after 108 days in soils under holm oak and beech, whereas benzo[a]pyrene, gradually degraded over the year of incubation. The degradation of the added PAHs well fitted exponential decay models, with k values lowest for benzo[a]pyrene, pinpointing that PAH degradation rate is negatively related to their molecular weight (Fernández-Luqueño et al., 2011; Ghosal et al., 2016). The fast degradation of PAHs, with the exception of the 5-rings benzo[a]pyrene, in the first months of incubation was also observed in a microcosm study carried out on a natural soil artificially contaminated with PAHs (Khomarbaghi et al., 2019). Anyway, Ge et al. (2018) demonstrated that the soil content of benzo[a]pyrene rapidly decreased in the early stage of degradation (14 days from incubation) and slowly decreased in the late stage of degradation (from 14 to 56 days).

Even if a quick and consistent loss of the three PAHs was observed during mesocosms incubation, their volatilization in the effective PAH removal from soils may be mostly ruled out. This consideration is based on the very low values of Henry's constant for the majority of these compounds (Cousins et al., 1999; Harmsen, 2004); however, empirical investigations indicated that losses by volatilization (mainly for low molecular weight compounds) cannot be definitively excluded in the disappearance of PAHs from wet soils (Park et al., 1990). The slower and lower degradation rate of benzo[a]pyrene in respect to lighter PAHs, confirming that reported in Baldantoni et al. (2017), can be attributable to a limited number of bacteria that use PAHs with five or more aromatic rings as energetic source. This observation is supported by studies carried out on bacteria in pure cultures containing high molecular weight (HMW) PAHs. To be degraded, PAHs must be made available for uptake and degradation by bacteria (Cerniglia, 2003; Fredslund et al., 2008), and an important factor regulating PAH bioavailability is their solubility. Since PAH solubility decreases with increasing molecular weight (Chen and Aitken, 1999; Thorsen et al., 2004), the high retention of these compounds by the soil solid phase results in very low mass transfer rates of HMW-PAHs to the bacterial cells to match the basic metabolic requirements cells.

All the studied soils, irrespective of vegetation covers, indicated a capability of the native microbial community to degrade PAHs yet after one year, although we know that freshly applied PAHs, rapidly desorbing from the soil (Uyttebroek et al., 2007), can be more rapidly degraded than PAHs in historically contaminated soil. Moreover, controlling moisture and temperature in experimental mesocosms can affect microbial activity during incubation, and in turn favor PAH biodegradation. In the case of PAH bioremediation processes, optimizing biodegradation conditions enhances the biotreatment efficiency (Namkoong et al., 2002; Sarkar et al., 2005). These observations are noteworthy, considering that the high recalcitrance of these compounds may determine soil accumulation, posing risks to human health linked to PAH carcinogenicity (De Nicola et al., 2019). In soil under pine, compared to soils under holm oak and beech, the degradation proceeds with a lesser extent for all the three PAHs. This effect is mainly related to the sequestration of organic compounds in soil rich in organic matter (Cornelissen et al., 2005), according to the higher content and stability of organic matter found in soil under pine (Picariello et al., 2021) that can protect PAHs from microbial degradation. Ultimately, the different PAH degradation rates among soil systems may be attributable to differences in plant-soil-microorganism interactions, being the plant microbiome dominated by different populations. Although several fungi have been identified as PAH-degrading microorganisms (Kadri et al., 2017), soil under pine showed the lowest degradation rate despite the highest fungal biomass (Picariello et al., 2021). This can be related to fungal species not directly involved in PAH degradation, or to biotic and/or abiotic factors limiting the process.

Enzyme activities, effective proxies of organic matter turnover in natural habitats (Nannipieri et al., 1990), may be differently involved in PAH degradation. Among the investigated activities, laccase and peroxidase are characteristic of ligninolytic fungi and are clearly involved in PAH degradation (Cerniglia and Sutherland, 2010; Li et al.,

Table 4
Enzyme activity resistance 4 days following PAH contamination of soils under holm oak (H), black pine (P) and beech (B).

	H	P	B
HYDROLYTIC ACTIVITY			
Phenanthrene	>100	79.7	71.1
Pyrene	>100	83.3	85.7
Benzo[a]pyrene	>100	81.1	76.5
LACCASE ACTIVITY			
Phenanthrene	59.2	99.2	31.8
Pyrene	63.2	94.8	32.3
Benzo[a]pyrene	52.5	71.8	28.1
PEROXIDASE ACTIVITY			
Phenanthrene	63.1	49.2	> 100
Pyrene	56.2	46.2	> 100
Benzo[a]pyrene	50.7	55.6	100

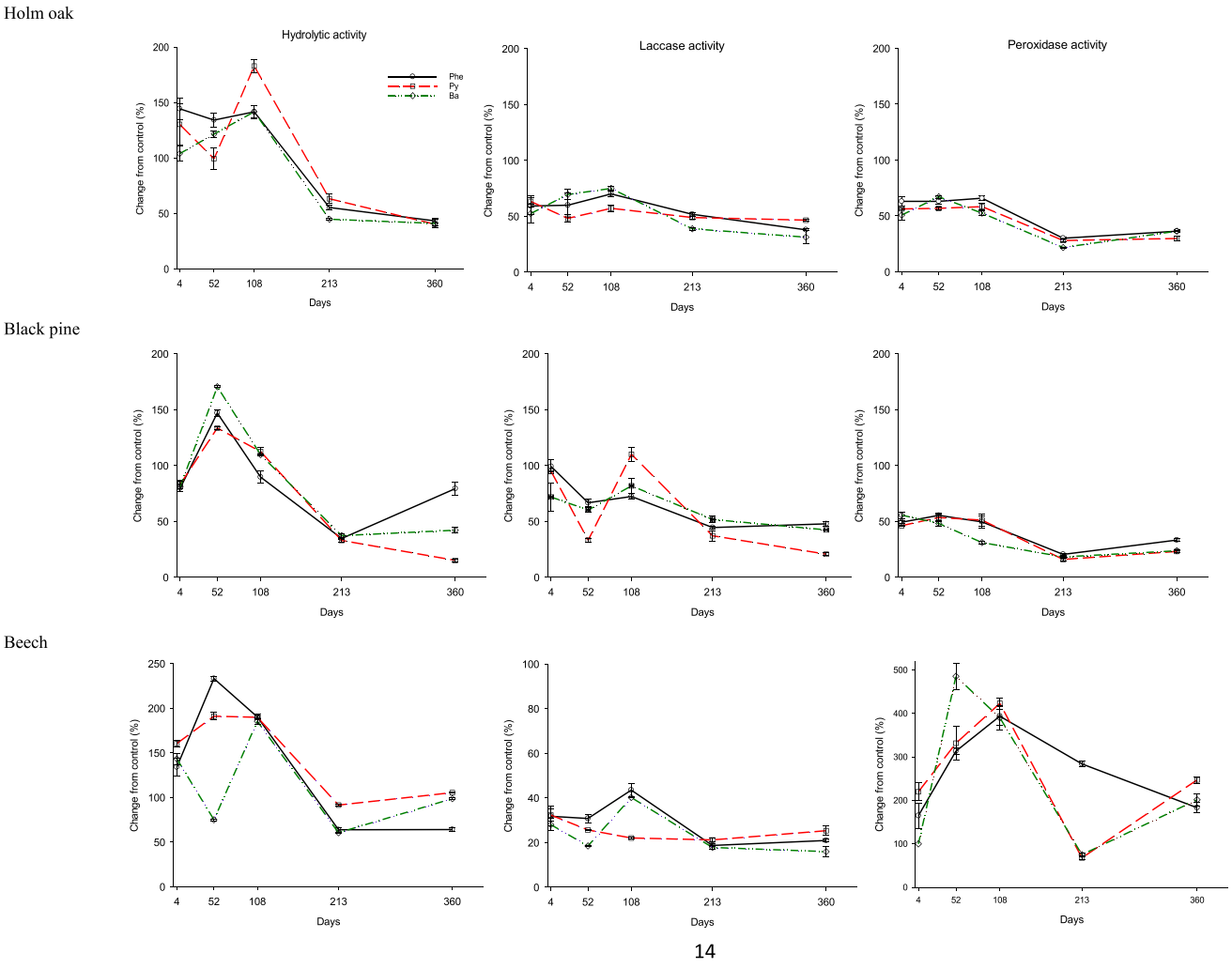

Fig. 2. Changes (in percentage of the relative control) in the rate of hydrolytic, laccase and peroxidase activities up to 360 days after phenanthrene (black), pyrene (red) and benzo[a]pyrene (green) spiking in soils collected under holm oak, black pine and beech. Vertical bars represent standard errors of the means. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

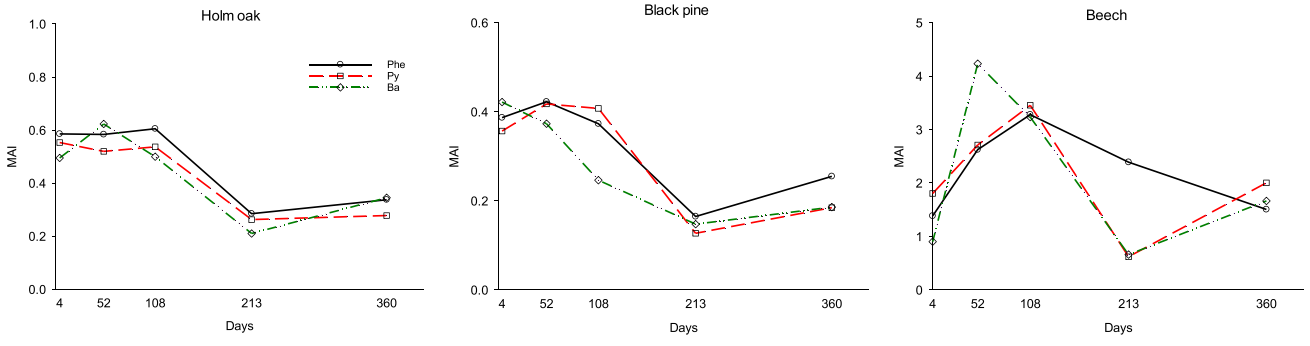

Fig. 3. MAI temporal dynamics based on the overall soil enzyme activity of each soil system (holm oak, black pine and beech), spiked with phenanthrene (black), pyrene (red) and benzo[a]pyrene (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2010). Conversely, hydrolase activity is carried out by metabolically active microbial cells and is a good proxy of the total microbial activity (Schnurer and Rosswall, 1982). During the rapid and strong PAH degradation, i.e. up to 52 or 108 days, hydrolytic activity showed high and constant values, highlighting a stability for a long time after spiking. Functional stability in the soils under three different vegetation covers is mainly associated to the resistance of hydrolytic activity, likely due to the high diversity of enzymes expressing this type of catalytic activity (Schnurer and Rosswall, 1982). Unlike an already spiked industrial soil, in which the presence of PAHs can select a community adapted to this kind of stress (Baldantoni et al., 2017), enzyme activities in the investigated soils showed a decrease over time after PAH spiking, with the exception of the resilient activities.

The resilience of functions related to the laccase activity in soil under

pine and of functions related to the peroxidase activity in soil under beech can be attributed to the high organic matter content in these soils. Indeed, high organic carbon concentrations contribute to alleviation of stress and increase the stability of soil microbial communities (Wardle, 1998). On the contrary, Jiang et al. (2020) found great functional stability under chemical stresses in soils poorer in organic matter. Nevertheless, different soil enzymes, as urease, sucrase and dehydrogenase, are reported to be rapidly inhibited in the early stages after PAH contaminations and stimulated during the rest of the incubation (Ge et al., 2018). Since perturbations determine shifts in the composition and function of soil microbial communities from their original state, potentially rendering them more vulnerable to abrupt transitions to alternative taxonomic and functional states in response to the perturbation (Bardgett and Caruso, 2020), it is important to study this phenomenon after PAHs spiking. The differences in resistance and resilience among the soil under the three different vegetation covers can be then related to the different microbial community composition and properties (Griffiths et al., 2008, 2001). In fact, the pre perturbation microbial community composition varies among the three soils, with fungi dominating soil under black pine, actinomycetes characterizing the soil under holm oak, and soil under beech that mainly sustains bacteria (Picariello et al., 2021). In the functional resilient systems, redundant species were likely present, enabling the community to produce process rates similar to the original community, after the perturbation condition occurred. Plant community composition can also contribute to different soil microbial stabilities (Bardgett and Caruso, 2020). Plant diversity exerts a control on soil microbial community structure and function, by increasing the diversity of litter and rhizodeposition products (Bartelt-Ryser et al., 2005), as well as the number of mycorrhizal hosts (DeBellis et al., 2006; Kernaghan et al., 2003). Different soil physico-chemical properties might also affect microbial resistance and resilience, as demonstrated for bacteria in laboratory culture (Griffiths et al., 2008; Johnston and Brown, 2002; Nickerson et al., 2003). The reduction in enzyme activity values, detected at the end of incubation, is likely linked to a natural process, since no resources were added during incubation, and almost all PAHs have been degraded.

Even if the role of single enzymes (affected by several environmental factors, both natural and anthropogenic) involved in soil ecosystem functioning is important, measuring an individual soil parameter is generally insufficient, since the soil has a number of functions (De la Rosa, 2005). Similarly, a correct interpretation of the overall soil activity is difficult to obtain when several parameters are considered. For these reasons, the use of a robust synthetic index of the overall soil activity may be of paramount importance in assessing soil quality. On the one hand, the use of a metabolic index based on a minimum set of parameters easy-to-measure, is an effective approach. On the other hand, more complex indices may better reflect the state of the studied ecosystem, but they are very time consuming (Wyszkowska et al., 2013). MAI is a particularly advantageous index, based on the determination of few or several enzyme activities (or other soil metabolic characteristics), especially useful when data from different parameters, expressed with different units of measurement, are difficult to manage. Analyzing the values of the proposed MAI, soils spiked with phenanthrene and pyrene, with 3 and 4 aromatic rings, respectively, showed similar trends, different from soils spiked with benzo[a]pyrene, with 5 aromatic rings. Generally, the trends of MAI values followed the PAH degradation rates, with a similar behavior for phenanthrene and pyrene. Thus, the developed index was able in effectively discriminating among different PAH spiking in the soils under the three different vegetation covers. The lowest MAI values were observed in soils spiked with benzo[a]pyrene, sampled both under holm oak and black pine, showing a negative effect of this organic contaminant on soil ecological functions, in agreement with Ge et al. (2018). Following the temporal dynamics of MAI values, it is possible to highlight the resistance of soil under beech and the resilience of the investigated soils in a simple and accurate way, further pointing out that a single value allows obtaining robust information on soil stability. The temporal dynamics obtained with the MAI are comparable with those obtained with the Lemanowicz index (Lemanowicz, 2019), proving the robustness and reliability of our index. The Lemanowicz index application, as well as in the case of several other indices (Wyszkowska et al., 2013), is limited by the different units of measurement of the employed parameters or by the fact that the metabolic activity of the soil can also depend on organic resources (Grosso et al., 2018; Tian et al., 2015). With our index we are not only able to obtain information about resistance and resilience, but we also overcome the problem of considering different parameters generally measured in such studies (and otherwise unsuitable in a synthetic index due to their different units of measurements), normalizing the results to a common source of variation.

Another possible approach for exploring the community responses to perturbations is the analysis of food webs (Bardgett and Caruso, 2020). The idea is based on food webs structured into the ‘fast’ energy channel, formed by bacteria and their consumers, and the ‘slow’ energy channel formed by fungi and their consumers. The first energy channel quickly circulates nutrients and quickly recovers from perturbations, increasing resilience; the second energy channel slowly circulates nutrients and slowly recovers from perturbations, increasing resistance (de Ruiter et al., 1995; Moore and Hunt, 1988). These theoretical ideas are still to be fully tested but in general, soil food webs with a greater biomass in the slow fungal energy channel are more resistant than those dominated by the fast bacterial energy channel (Bardgett and Caruso, 2020). In our case, we found greater resistance in soils under beech and holm oak, which showed the lowest content of fungal biomass, compared to soils under black pine. On the contrary, we found greater resilience in the soils under pine and beech, which contained the highest bacterial biomass values. The complementary and asymmetric functions of the energy channels, which process organic matter at different rates, provide a mechanism for responding to perturbations, with a stable recovery of the food web, in accordance with Rooney et al. (2006). We can consider a shift of the distribution of biomass and energy flow to the bacterial channel due to the additional source of energy (supplied by PAHs in our study), as also shown in managed agroecosystems (De Vries et al., 2013).

The analysis of food webs as a possible approach for exploring the community responses to perturbations appears a sophisticated method, easily affected by too many parameters. Conversely, the proposed MAI can be applied in different fields of study, obtaining important information, about a particular ecosystem, in a single and robust value. In addition, the normalization on a reference factor, in our case soil organic carbon, is important, since soil metabolic activity may depend on soil organic resources rather than on possible stresses. Thus, normalizing on a factor that can influence the measured responses, is of paramount importance to highlight the differences regardless this factor.

5. Conclusions

In this mesocosm study, in all the investigated soils, phenanthrene (3 aromatic rings) and pyrene (4 aromatic rings) during 360 days of incubation degraded faster than benzo[a]pyrene (5 aromatic rings), according to their molecular weights. In soil under pine, the PAH degradation proceeds with a minor extent in respect to soils under holm oak and beech, mainly in relation to the sequestration of PAHs in soil richer in organic matter. In accordance with our first expectation, we found higher microbial resistance to perturbation in “broad scale” soil hydrolytic enzyme activity for holm oak soil (and to a lesser extent for pine soil) in respect to the two “narrow-niche” soil enzymes (laccase and peroxidase). Our results demonstrated a higher functional stability in soils under pine and beech compared with soil under holm oak. This was indicated by the responses of the laccase and peroxidase activities, which were less affected (higher resistance) or recovered more quickly (higher resilience) in these soils after the PAH contamination perturbation, likely thanks to the high amount of organic matter. In

accordance with the second expectation, the three soils showed different responses, likely in relation to the different soil properties, dominant canopies and microbial community diversity. The proposed synthetic index, i.e. MAI (Metabolic Activity Index), not only estimates soil quality and functioning, but also highlights soil stability, overcoming the problem of the different parameters measured in different studies and their different units of measurements.

CRediT authorship contribution statement

Enrica Picariello: Investigation, Data curation, Writing - original draft, Writing - review & editing. **Daniela Baldantoni:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing. **Soledad Muniategui-Lorenzo:** Resources, Methodology, Writing - review & editing. **Estefania Concha-Graña:** Investigation, Writing - review & editing. **Flavia De Nicola:** Conceptualization, Methodology, Resources, Funding acquisition, Supervision, Writing-original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107844.

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