"Omics" Insights into PAH Degradation toward Improved Green Remediation Biotechnologies

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"Omics" Insights into PAH Degradation toward Improved Green Remediation Biotechnologies

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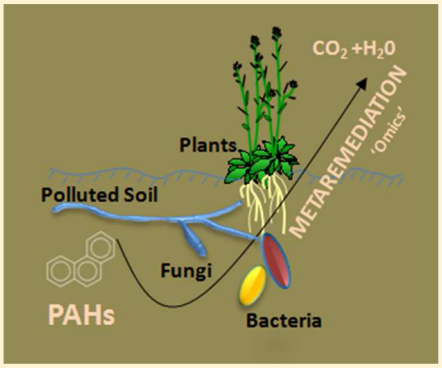
Supporting Information

ABSTRACT: This review summarizes recent knowledge of polycyclic aromatic hydrocarbons (PAHs) biotransformation by microorganisms and plants. Whereas most research has focused on PAH degradation either by plants or microorganisms separately, this review specifically addresses the interactions of plants with their rhizosphere microbial communities. Indeed, plant roots release exudates that contain various nutritional and signaling molecules that influence bacterial and fungal populations. The complex interactions of these populations play a pivotal role in the biodegradation of high-molecular-weight PAHs and other complex molecules. Emerging integrative approaches, such as (meta-) genomics, (meta-) transcriptomics, (meta-) metabolomics, and (meta-) proteomics studies are discussed, emphasizing how "omics" approaches bring new insight into decipher molecular mechanisms of PAH degradation both at the single species and community levels. Such knowledge address new pictures on how organic molecules are cometabolically degraded in a complex ecosystem and should help in setting up novel decontamination strategies based on the rhizosphere interactions between plants and their microbial associates.



INTRODUCTION

Aromatic compounds are largely produced by plants in the form of soluble secondary products such as phenols or flavonoids, or as cell wall components such as the polymer lignin. These molecules constitute 20% of the earth's biomass and their turnover, which is mostly performed by living organisms, is tightly related to biogeochemical cycles.[1] Simultaneously, the catabolism of these molecules and the knowledge of the mechanisms involved at the organism, community, and consortium levels remain fragmentary.

Throughout the last century, industrial revolution has produced a myriad of aromatic end-products, since the increasing human activity leads to a massive use of fossil fuels, and the generation of manifold aromatic such as polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous products of the combustion of carbon-based substances, they are naturally occurring products due to forest fires and volcanic activities. In addition a large variety of PAHs may be found as substituted molecules with polar functional moieties such as carboxyl groups of primary amino groups. These aromatic compounds may have some similarities to structures found in wood and humic substances.[2] In this work we focus our analysis on PAHs because they constitute ubiquitous worldwide pollutants and pose serious environmental problems on a global scale, they are very harmful to human health as they have been classified as contaminants of emerging concern[3] and may affect dramatically natural ecosystems biodiversity.[4] In addition they combine high recalcitrance with high (geno)-toxicity.[5] A driving factor of their recalcitrance in contaminated ecosystems is their low bioavailability, which limits biotransformation despite often widespread PAH-degrading catabolic potential.[6] Such a paradox may limit bioremediation efforts and makes ecotoxicological risk assessment challenging. It is conceivable that many evolutionary and coevolutionary processes have occurred allowing microorganisms and plants to coadapt and cosurvive in ecosystems containing complex aromatic molecules. For instance, many PAH-degrading bacteria and fungi express genes that increase their adaptation to low bioavailability, including features like active migration and chemotaxis of bacteria toward PAHs, biofilm formation near PAHs sources, high-affinity PAH-uptake systems, low

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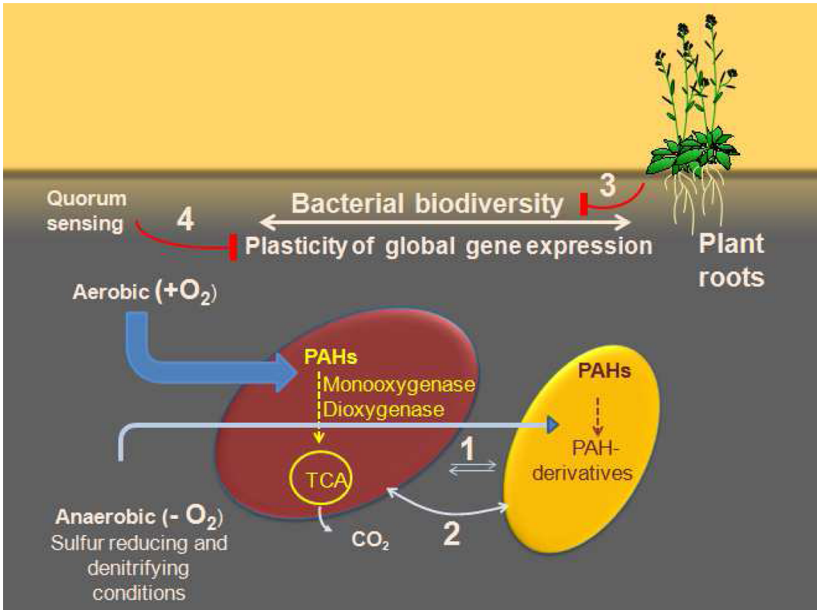

Figure 1. Bacterial ecology and plasticity of PAH catabolism in a polluted environment. Effective degradation of complex aromatic compounds in ecosystems usually involves the exchange of metabolic byproducts by microbial consortia. (1) Complete degradation may be the result of the catabolic activity of several microorganism species through the exchange of metabolic intermediates. (2) The rapid spread of genes involved in derivative pathways may occur by horizontal gene transfer. (3) Plant root exudates and mechanical soil aeration strongly influence the distribution of microorganisms. (4) Quorum sensing coordinates global gene expression in a population density-dependent manner. Soil bacterial consortia therefore are highly plastic, as they adapt their PAH catabolism to changing environmental conditions. Aerobic catabolism in soil bacteria seems to be the major environmental bacterial PAH-degradation process (bold blue arrow). The bacteria may use PAHs as the sole carbon source and energy. This degradation first involves the regulation of genes encoding monooxygenases and dioxygenases followed by cleavage of the aromatic ring. Active anaerobic PAH catabolism (the fine blue arrow), a rather rare PAH-degradation process observed in polluted environments, depends on optimal denitrifying and sulfur-reducing conditions. Brown and yellow bacteria represent different strains harboring distinct PAH-degrading metabolic pathways. TCA: Tricarboxylic acid cycle.

energy requirements for cell maintenance, or the ability to simultaneously metabolize PAHs and other nutrient sources.[7] On the other hand, it has been shown that plants trigger an increase of both diversity and total number of microorganisms in PAH-contaminated soil, while unplanted soils showed less variety of PAH degraders.[8] Concomitant effective rhizodegradation may occur naturally because roots release a myriad of compounds, such as flavonoids[9,10] and fatty acids,[11] which increase microbial growth and PAH-degradation activity. For example, in the rhizosphere of *Spartina* plants, PAH-degrading bacteria were found to be highly increased as compared to unplanted sediments.[12] It has been found that microorganisms use root exudates as a carbon source for the initial ring hydroxylation in the catabolism of high-molecular-weight PAHs (referred as compounds containing four or more fused benzene rings) during rhizodegradation.[13] In addition to chemical compounds released from roots; plants growth and death both provide nutrients and mechanically enhance PAH oxidation by allowing soil aeration.[14]

The complex interactions of plants and their associated microorganisms play a pivotal role in the biodegradation of high-molecular-weight PAHs and other complex molecules. It is therefore crucial to understand the complex interplay between bacterial, fungal and plant ecological, physiological, and molecular mechanisms involved in the biodegradation of contaminants.[15] Ecological engineering approaches combining myco-, phyto-, and rhizoremediation are needed to establish sustainable alternatives for effective clean up and restoration of polluted soil ecosystems. Such approach needs to perceive the biodegradation of a chemical as an ecosystem property, and consequently link knowledge about the chemicaĺs molecular determinants governing (bio-) transformation with the biogeochemical and ecological drivers of ecosystem functioning.[15,16] Integrating plants with microbial bioremediation (termed as rhizodegradation) leads to a more efficient sustainable remediation.[8,13,17] Bioremediation technologies are still erratic and have to be optimized, since these strategies addressed separately phytoremediation, mycoremediation and bioremediation to clean up polluted environment.

Interestingly, recent emerging integrative "omics"-based approaches can help investigate the genome, transcriptome, proteome and metabolome of single organisms and even mixed communities, opening new opportunities to decipher molecular mechanisms of PAH degradation in polluted environment.[18] Metagenomics, metatranscriptomics, metaproteomics, and meta−metabolomics address the whole complement of DNA, mRNA, proteins and metabolites, respectively, in an environmental sample. Metagenomics can identify the functional potential and the taxonomic identity of all organisms in an environmental sample, but yields no information as for the actual active members of the community. There are two different flavours of metagenomics, (i) shotgun metagenomics where all DNA is sheared and sequenced and functions and taxonomy are derived from homology search in databases, and (ii) functional metagenomics where large DNA pieces are inserted in vectors and expressed in hosts which are then screened for activity and only clones showing the desired activity are sequenced. Combining these approaches can help determining which organisms are actually carrying out specific functions in situ and how much this function is expressed in an

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environmental sample. Additionally, Amplicon sequencing consist in the next-generation sequencing of marker genes (e.g., the 16S rRNA gene, the ITS region, *cpn60*, *rpoB*) is also widely used to identify the microbial community composition and diversity in environmental samples. When combined with other methods, like stable-isotope probing (SIP), amplicon sequencing can also yield information on community members that metabolized a particular substrate. The principle of DNA- and RNA-SIP is to feed a microbial community with a labeled substrate (e.g., C_{13}-naphthalene) and retrieve labeled (heavy) DNA or RNA through centrifugation of nucleic acids in a density gradient. The labeled DNA or RNA is then sequenced directly or amplified to sequence marker genes. For instance, using SIP combined with 16S rRNA gene amplicon sequencing, it was possible to link taxonomic group identity to PAH degradation.[19,20] This approach helped highlighting the idea that complex organic pollutants are metabolized or mineralized by microbial consortia in the field,[18] which largely differ from results obtained under laboratory conditions.

In the present paper and in the light of emerging "omics" biology, we summarize recent advances in PAH bioremediation, emphasizing how plant influence microbial communities in the rhizosphere and how they interplay as a metaorganism or holobiont to degrade such complex pollutants.

DIVERSITY AND PLASTICITY OF BACTERIAL PAH CATABOLISM IN COMPLEX ECOSYSTEMS

Even if bacterial consortia have the capacity to actively express genes, high rates of PAH-degradation are often largely dependent on complex interactions between soil microbiota, plants, and the soil environment (Figure 1).[15] Plant root exudates and mechanical soil aeration can strongly influence the distribution and activity of microorganisms. Additionally, indications of bacterial movement along roots have been published,[21] such effects being generally plant species-specific.[22−25] The plant species colonizing polluted environments was shown to closely affect bacterial biodiversity.[26] A better understanding of the interactions between the plant and its microbiome could help optimize phytoremediation through microbiome engineering.[27] The use of omics approaches has already helped better explain the interactions between plants and the soil microbial communities during PAH degradation in the rhizosphere.[23,24,28] Interestingly, many of the plant secondary metabolites that are exuded in the rhizosphere are analogous to PAH contaminants and might stimulate PAH degradation pathways.[28] Hence, the rhizosphere is often enriched in PAH degraders even when the plant is growing in noncontaminated soils.[24]

Many PAH-degrading bacteria have been isolated and characterized from PAH-contaminated environments.[29] Based on cultivation-dependent methods, efficient degrader strains have been shown to belong mainly to a limited number of genera groups, such as *Sphingomonas*, *Burkholderia*, *Pseudomonas*, and *Mycobacterium*.[7] Most of these bacteria have the ability to use PAHs as a sole source of carbon and energy. Using these isolated strains, the biochemical catabolic pathways of aromatic compounds (in particular PAHs) have been elucidated and described.[30] Conventionally, the PAH degradation pathways have been elucidated using straightforward "step-by-step" techniques that include analytical chemistry, biochemical characterization, and identification of metabolic intermediates and key enzymes. Such approaches allowed deciphering several metabolic processes and mechanisms involved in PAH degradation in single strains. Likewise to many metabolic pathways, several genes involved in PAH catabolism are clustered in operons and are localized on bacterial plasmids. In addition, several genes are a part of, or flanked by, transposon or transposon-like sequences;[7,31] suggesting that PAH degradation genes are able to spread rapidly within bacterial communities by horizontal gene transfer. Indeed, PAH catabolism in bacteria is tightly related to environmental and ecological conditions (Figure 1). Hence, in aerobic conditions, molecular oxygen is used to initiate enzymatic attack,[30] involving the action of key mono- or dioxygenase enzymes that introduce oxygen atoms forming *cis*-dihydrodiols that are further channelled through the ortho- or meta-cleavage pathways.[7,29] Inversely, degradation of PAHs under oxygen-limited conditions has been reviewed,[29] highlighting that many questions remain unanswered. Under anaerobic conditions, nitrate and sulfate are thought to be used as terminal electron acceptors, but this has only been unambiguously observed in enriched cultures.[32,33] In order to assess PAH degradation under denitrifying conditions, pyrene, phenanthrene, and fluorene were added to soil enriched with a mixed population of microorganisms, but poor degradation rates have been observed when PAHs were the only source of carbon.[32] Interestingly, the presence of other carbon sources in the medium improved PAH biotransformation, suggesting cometabolic degradation of PAHs under anaerobic conditions.[32] Mixed bacterial communities were reported to degrade two- and three-ring compounds,[32] but anaerobic degradation of larger PAHs has not been observed yet.

"Omics"-based approaches recently revitalised the study of PAH catabolism by allowing for an integrative view of the biochemical processes responsible for PAH degradation in polluted sites. Some of these approaches, like metatranscriptomics, can associate taxonomic identity and catabolic gene clusters, to a given catabolic activity.[34,24,28] For instance, de Menezes et al.[34] revealed that the exposure of soil microbial communities to phenanthrene led to an increase in the abundance of transcripts of genes related to dioxygenase, stress response and detoxification. Similarly, the rhizosphere of willows growing in contaminated soils was significantly enriched in transcripts related to PAH degradation,[24] which were subsequently shown to be mainly related to members of the orders *Actinomycetales*, *Rhodospirillales*, *Burkholderiales*, *Alteromonadales*, *Solirubrobacterales*, *Caulobacterales*, and *Rhizobiales*.[28] Proteomic approaches have also been successfully used to describe bacterial degradation of high molecular weight PAHs.[35−37] For instance, among 1122 proteins, these studies identified 27 and 54 proteins essential in the complete degradation of pyrene and fluoranthene, respectively.[35,36] Another recent study compared metaproteomic and metagenomic profiles, in term of diversity and interactions between community members which resulted in the reconstruction of the naphthalene degradation pathway for specific groups of bacteria from complex microbial communities.[37] This type of specific pathway reconstruction based on metaproteomic and taxonomic data is facilitated by the development of new bioinformatic tools.[38]

In order to identify microbial communities involved in PAH metabolism in contaminated soil, stable isotope probing (SIP) can be coupled with metaproteomics, amplicon sequencing or metagenomics to identify organisms that have incorporated isotopically labeled atoms of a substrate (generally ^{13}C- or ^{15}N atoms) in their proteins or DNA.[39,40] Based on SIP followed by

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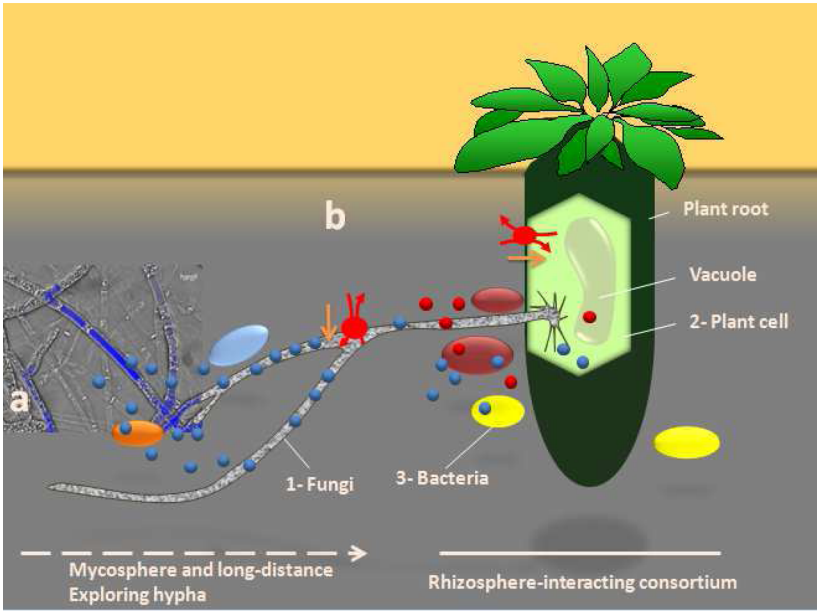

Figure 2. Dynamics of PAHs and meta-cleavage degradation pathways by soil microbial communities and plants in the rhizosphere. a: Mycelia increase the mobility of a wide range of hydrophobic contaminants and improve the accessibility of PAHs to bacteria and plants in soil. The figure is an overlay of light transmission and blue fluorescence micrograph corresponding to hyphae and vesicle-bound fluorene, respectively. It illustrates the mycelia-mediated transport of fluorene within hyphae of *Pythium ultimum*. The micrograph was kindly provided by Dr. S. Schamfuß, Helmholtz Centre for Environmental Research, UFZ, Department of Environmental Microbiology, Permoserstraße 15, D-04318 Leipzig, Germany. b: (1) Fungi constitute one of the most important soil organisms involved in dynamics and degradation of PAHs (blue dots). The mycosphere, defined as the microhabitats surrounding the dense fungal hyphae, host selected bacteria, and is clearly a main factor deriving the enormous biodiversity of soil bacteria and influence profoundly bacterial community. Additionaly, Mycelial networks (i) act as an effective dispersal network for both undirected and chemotactic mobilization of contaminant-degrading bacteria (referred to as "fungal highways") and (ii) increase the mobility of a wide range of PAHs due to their translocation during cytoplasmic streaming (referred to as "fungal pipelines"). Thus, mycelia improve the accessibility of bacteria and plants to soil contaminants. (2) Plant cells, (3) bacteria, and fungi interactions lead to meta-cleavage of organic pollutants such as PAHs and involve the exchange of byproducts (red dots) between species. Absorption of these compounds is controlled by simple diffusion through cell membranes (simple arrows) or by transporters (yellow circle), which, in turn, lead to complete catabolism of PAHs.

16S rRNA gene amplicon sequencing, three groups of uncultivated *Proteobacteria* and *Sphingomonas* were identified as the primary degraders of pyrene, with a majority of 16S rRNA gene sequences being unrelated to any previously described bacteria.[41] Similarly, ^{15}N-SIP followed by alkane monooxygenase (*alkB*) and 16S rRNA gene amplicon sequencing allowed to identify the taxonomic groups efficiently incorporating nitrogen from N fertilizers during diesel fuel degradation in arctic soils.[18] In the context of plant-microbe interaction, labeling plants with $^{13}CO_2$ would enable tracking the transfer of C from the plant to the PAH degrading microorganisms.

Functional metagenomics using large-insert libraries constructed from environmental DNA were used to screen for genes encoding specific enzymatic activities, such as oxygenases, by using the ability of these enzymes to produce a PAH substrate-dependent coloration when expressed in *Escherichia coli*.[42,43] These studies revealed a high diversity and abundance of meta-cleavage pathways with an exceptional high density of particular genes involved in PAH metabolism. The shotgun metagenomic analysis of the diversity of ring-opening oxygenase genes that had been selected under stringent polluted environmental conditions, underlined a strong selection for genes involved in meta-cleavage pathways.[44,45] Although microorganisms play a major role in soil decontamination, PAH degradation efficiency is limited by the spatiotemporal distribution of PAHs in the heterogeneous soil environment as well as the unequal distribution of microbial communities providing the suitable catabolic activities. In contrast to classical approaches, metagenomics studies allow the identification and monitoring of PAH-degrading microbial populations even in complex ecosystems.[46] For instance, the adaptation of *Pseudomonas fluorescens* HK44 was monitored along with its ability to degrade PAHs in the soil following inoculation in conjunction with its impact on the indigenous bacterial population.[47] As reviewed by Desai et al.,[48] one of the main advantages of "omics" methods is the identification of PAH-degrading bacteria that cannot grow on artificial media. Using these methods, bacterial populations growing on PAH-contaminated soils were successfully monitored,[49] and it was determined that factors such as oxygenation, nutrients, soil depth, and duration of treatment impacted the survival of inoculated genetically engineered bacterial strain. Such promising technologies will bring a more global and integrative view on how microbial consortia colonise and adapt to PAH-contaminated ecosystems and interact with plants.

Microbe−microbe interactions and syntrophy play a pivotal role in the catabolic process of complex molecules in natural ecosystems.[50,51] Syntrophic interactions between bacteria result in the exchange of byproducts and shared metabolic pathways and contribute to the regulation of expression of genes involved in catabolism (Figure 1). The degradation of aromatic compounds was also shown to trigger the production and the exchange of metabolic factors involved in microbe−microbe communication.[43] The quorum sensing factors are secreted signal molecules that bacteria use to communicate; they were

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suggested to enable the coordination of global gene expression in a given population.[50] Indeed, functional analysis of phenanthrene-degrading bacteria revealed the presence of a genomic island containing genes involved in the entire phenanthrene-catabolic pathway that are associated with genes encoding quorum sensing signal molecules.[52]

ROLE OF FUNGI AND THEIR INTERACTIONS WITH BACTERIA AND PLANTS IN RECYCLING OF PAHS

Lignolytic fungi specialized in the degradation of lignin, a phenolic polymer, may achieve metabolism of structurally similar molecules such as PAHs. They produce extracellular enzymes of very low substrate specificity such as lignin peroxidase, Mn-peroxidase, or versatile peroxidases and laccases, which enable them to also degrade other aromatic compounds in the environment. These enzymes can partially or completely mineralize many phenolic compounds which can lead to the production of more water-soluble molecules, such as quinones, that can be further metabolized by soil bacterial community. The activity of these enzymes against environmental chemicals has been recently reviewed.[53,54] For example, the lignin peroxidase and Mn-peroxidase enzymes catalyze one-electron oxidation of PAHs with high potential ionization that is followed by aromatic ring cleavage. Interestingly, PAHs containing more than six rings was shown to be metabolizable via Mn-peroxidase-dependent lipid peroxidation, both in vivo and in vitro.[55] In addition to the extracellular enzymatic PAH-degrading system mentioned above, fungal cytochrome P450 (CYP) monooxygenases have been shown to be involved in the catabolism of PAHs. The first step in phenanthrene intracellular mineralization involves the O_2 incorporation by a CYP monooxygenase, which has strong ability to catabolise PAHs with a high number of fused rings.[56] Since the whole genome of the model fungi *Phanerochaete chrysosporium* have been sequenced and is now available in the public database;[57] it revealed the presence of about 150 putative CYP-encoding genes, most of them are considered orphan genes with unknown function. Hence, a genome-wide CYPs microarray screen and genome-to-function characterization allowed the first identification and characterization of several CYP monooxygenase-encoding genes involved in the oxidation of PAHs.[56] Their cloning and expression in a heterologous system demonstrated their involvement in the oxidation of many recalcitrant PAH containing more than four rings.

Beside active degradation of PAHs, fungi exert a selective force on the soil bacteria. But how these fungi influence bacterial soil community and the potential stimulation of PAH degradation is still an unresolved question. It is highly relevant to mention that the mycosphere, defined as the microhabitats surrounding the dense fungal hyphae, host selected bacteria, and is clearly an important driver of the enormous biodiversity of soil bacteria (e.g., by increased horizontal gene transfer).[58] Even if the exact mechanism involved in how the bacterial community is selected in the mycosphere still is ignored, the concept of species-specific fungiphiles bacteria have been introduced, as it is suggested that many bacteria are adapted to mycosphere environment, due to the release of nutrients and signaling molecules by fungi.[59] In addition, mycelia of fungi form a dense and extended physical network of thread-like hyphae in soil (Figure 2). These mycelia have been found to (i) interact with bacteria,[59] (ii) act as effective dispersal networks for both undirected and chemotactic mobilization of contaminant-degrading bacteria (referred to as "fungal highways"),[60−62] (iii) increase the mobility of a wide range of PAHs due to their translocation during cytoplasmic streaming (referred to as "fungal pipelines");[63] and (iv) therefore improve the accessibility of bacteria and plants to soil contaminants and, consequently, their biodegradation.[62,64] Given their ubiquity and length of mycelial networks, fungi appear to play a pivotal role and exhibit untapped biochemical and biophysical potential in the ecology of PAH biodegradation in contaminated ecosystems.[53]

On the other hand, mycorrhizal fungi establish symbiotic associations with most of plants,[65] they are considered as major determinants of plants interactions in these challenging ecosystems, as they alleviate stress caused by pollutants.[66] Unfortunately, mycorrhizal fungi show complex genetic organization as they exist in heterokaryotic form where the nuclei are genetically different or in homokaryotic form containing identical nuclei. In addition, very high diversity can be found even in single isolate and one single root system may be colonised by multiple different mycorrhizal fungi. Hence, the use of "omics" strategies to understand plant-mycorrhiza interactions is still in its infancy but will hopefully bring new insights to decipher these complex interactions in PAH polluted environments. Nevertheless, during the preparation of this review, a relevant investigation have been published highlighting the identification of the most transcriptionally dominant fungal taxa associated with pine roots using an optimized method, combining DNA/RNA extraction which allow the identification of specific taxonomic groups. Thus, the availability in public databases of genome of one taxon allowed the identification of patterns of gene content and transcripts abundance.[67] Until very recently, difficulties were encountered to study taxonomic groups and species of fungi in a complex ecosystem containing DNA originating from multiple organisms. However, the evaluation of many DNA regions as barcodes specific for fungi by Fungal Barcoding Consortium,[68] should contribute to improve the understanding of how the association of plants−fungi of soils communities is involved in the dissipation of hazardous molecules such PAHs. In addition, an overview of "omics" tools and systems biology used to highlight the role of mycorrhizal fungi and their interaction with plants have been published recently.[69] The authors point out that the emergence of public databases that integrate "omics" data is fundamental to fill the existing gap between field and laboratory observations, and will help to understand the contribution of mycorrhizal fungi in ecosystem services.

PAHS UPTAKE AND METABOLISM BY PLANTS

Although PAH absorption and transport by plant tissues remains poorly documented, the model plant *Arabidopsis thaliana* was shown to be able to absorb and internalize phenanthrene.[70,71] Additional data based on fluorescence microscopy observations indicated that phenanthrene accumulates in trichomes, suggesting a particular role of trichome cells in accumulation and/or dissipation of phenanthrene molecules.[70] Interestingly, a comparative analysis of *Arabidopsis* and the halophytic plant model *Thellungiella salsuginea* revealed a potential role of stomata in phenanthrene volatilization.[72] Furthermore, phenanthrene uptake by wheat roots involves two biological mechanisms: (i) a fast passive diffusion, occurring just after the transfer of wheat to phenanthrene-supplemented medium, and (ii) a slow, active absorption, putatively mediated by transporters that remain uncharacterized.[71] In the presence of PAHs, disturbance of ROS scavenging was observed in

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Arabidopsis, associated with phenotypical changes such growth inhibition, leaf deformation, and necrosis. Application of Omic approaches such microarrays analyses revealed a profound transcriptional changes. Additional data based on mutant experiments showed that phenanthrene inhibit growth through an ethylene-independent pathway.[73]

In contrast to bacteria and fungi, the mechanisms of PAH metabolism and accumulation in plants remain unknown. A model for the detoxification of organic pollutants by plant cells has been proposed and dubbed the "green liver" because of its analogy to the mammalian liver (Figure 3).[74] The processes involved in this model have been divided into three steps: (i) signaling, (ii) transport and biotransformation to less toxic molecules, and (iii) compartmentalization. At the end of these processes, the conjugated xenobiotic is transferred to the vacuole by ATP-binding cassette (ABC) transporters.[74−76]

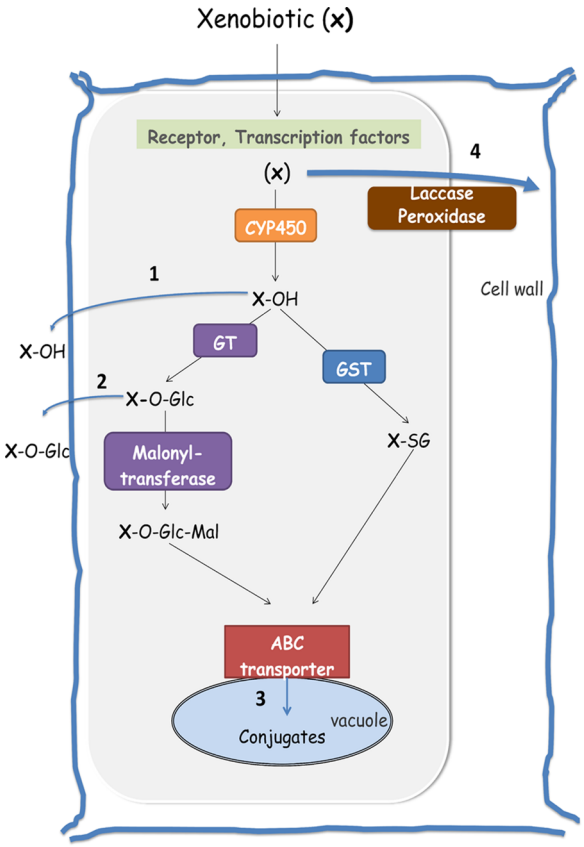

Figure 3. Simplified representation of the plant detoxification system. Detoxification occurs in three phases. (i) Transformation: xenobiotics are chemically modified using oxidation, reduction, or hydrolysis. This stage involves cytochrome P450s (CYPs). (ii) Conjugation: the xenobiotics are conjugated to endogenous molecules. Glycosyltransferases (UGTs) transfer nucleotide-diphosphate-activated sugars such as UDP-glucose to low-molecular-weight substrates. The glycosylated xenobiotics are also conjugated with malonate by malonyltransferases (MTs). The conjugation step also can be performed by the gluthatione transferases (GSTs) by attaching the tripeptide gluthatione to xenobiotics. (iii) Compartmentalisation: The conjugated xenobiotic is transferred to the vacuole by ATP-binding cassette (ABC) transporters or to the cell wall. Blue arrows indicate detoxification strategies, either by transferring conjugated xenobiotics to extracellular space (1−2), to vacuole (3) or incorporating them into the cell wall (4). The competition between these pathways determines plant tolerance to organic pollutants.

However, some differences in these processes do occur. For instance, the malonylation reaction depends on environmental conditions,[77] but also on plant species.[78] According to these factors, Plants may transform PAHs to less toxic molecules, and store them as conjugated organic contaminants in their vacuoles or excrete them to the extracellular environment.[78] Additionally, some organic pollutants may undergo alternative metabolic routes as they are integrated to plant cell macromolecules, predominantly lignin.[78] Indeed, laccase- and peroxidase-encoding genes are involved in this process. For example, data obtained from molecular cloning and partial characterization of a peroxidase expressed in plant roots suggested its involvement in the removal of organic pollutants by cross-linking them to cell wall polysaccharides or proteins.[79]

Based on genomic analysis, it was proposed that "the whole expressed genome responsible for the signaling, transport, and detoxification of xenobiotics in the cell" constitute the xenome.[80] Thereby genome-wide analysis have been performed by Ramel et al.,[81] the authors showed that organic pollutants induced in *Arabidopsis* expression of gene families including ascorbate peroxidases, glutathione-S-transferases, CYPs, and the early induction of an original set of transcription factors potentially involved in organic pollutant signaling. Although for the PAH-model molecule phenanthrene detection and signaling remains unknown in plants, the first transformation rendering PAHs more water-soluble, have been proposed to involve CYPs. Indeed, such enzymes contribute to PAH degradation in fungi.[56,57] In addition to CYPs, glycosyltransferases (UGT), gluthatione transferases (GST), and malonyltransferases have been described to participate in the conjugation step and the ATP-binding cassette (ABC) transporters in the compartmentalization step. Except for malonyltransferase, which has been reported to be encoded by two genes in *Arabidopsis*,[77] all the other components of the "green-liver" model are encoded by multigenic families. The *Arabidopsis* genome annotation revealed 252 putative CYPs, 53 putative GSTs, 107 putative glycosyltransferases, two malonyltransferase and 136 ABC putative transporters.[80,82,83]

A transcriptomic analysis of 21-day-old *Arabidopsis* plantlets cultivated in vitro showed that PAH treatment induced expression of genes encoding proteins involved in oxidative stress regulation.[73] Following its uptake, it has been hypothesized that phenanthrene could be oxidized by mono- or dioxygenases, resulting in increased reactive oxygen species levels responsible for oxidative stresses, and accordingly several physiological changes occurred after exposure to phenanthrene.[70,73] To elucidate the early molecular processes involved in plant response to PAHs and to identify genes strongly differentially regulated after short-term exposure to PAHs, which could play a pivotal role in PAH signaling and metabolism, we performed a genome-wide analysis of 15-days-old *Arabidopsis* plantlets subjected to short-term phenanthrene exposure (unpublished data) using the CATMA version 5 array containing 31 776 gene-specific tags corresponding to 22 089 genes from *Arabidopsis*.[84,85] The results enabled to dived the early plant response to phenanthrene in three phases: (1) a quick response, occurring within the first 30 min, in which the plants seem to rapidly sense phenanthrene, as differentially expressed genes are mainly involved in perception and signaling; (2) a reaction phase, observed after 2−8 h of incubation, characterized by regulation of numerous genes involved in detoxification; (3) and a third phase, starting after 8 h, in which many differentially expressed genes were involved in

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metabolic pathways, mostly in degradation of macromolecules. During this last phase, plant responsiveness to phenanthrene was characterized by the induction of numerous genes often involved in regulation of plant responses to stresses. Therefore, it can be hypothesized that common genes shared with other xenobiotic- and pollutant-degrading pathways exist. To test that assumption, we collected pertinent information on publicly available genome-wide analyses of plant-derived xenobiotic degradation. Data included experiments performed with a series of organic and inorganic contaminants including aluminum,[86] atrazine,[81] benzoxazolin-2(3H)-one,[87] cadmium,[88] polychlorinated biphenyl,[89] phenol,[90] selenium,[91] and trinitrotoluene.[92] A list of all the genes encoding proteins involved in the xenome (CYP, UGT, MT, GST, and ABC transporters) that appeared differentially expressed in at least one transcriptomic analysis after *Arabidopsis* exposure to the cited xenobiotics was compiled and subsequently compared to previous data obtained after long-term phenanthrene exposure.[73] This line-up revealed that most potential xenome genes involved in phenanthrene detoxification were also regulated in other xenobiotic-induced stress experiments, except for one CYP (*AT5G47990*) and two ATP-binding cassette transporters (*AT1G51500* and *AT5G44110*). This suggests that plant xenobiotic detoxification shares common molecular features. However, short-term phenathrene exposure also involves specific sets of genes. The functional characterization of these specific genes might bring new insight into better understand specific detoxification pathway of PAHs, as they could be used as target genes for plant transformation to increase plant specific PAH degradation.

Even if the molecular mechanisms associated with the metabolism of PAHs by plants remain under investigation, a number of species, such as *Populus nigra*, *Salix exigua*, *Festuca arundinacea*, and *Miscanthus giganteus*, among others, (see Supporting Information Table 1 online), grow on PAH-contaminated soil and reduce PAH levels in the environment, since they accumulate PAHs and derivatives molecules in their tissues. These species could be considered for potential degradation of PAHs molecules and might be useful for remediation strategies. Screening of plant species involved in PAH remediation is often performed in vitro, or using soil or growth media supplemented either with a single or a mixture of PAHs. Most studies evaluate the quantity of PAHs removal from the soil by plants compared to bare soil or the amount of PAHs absorbed by the plants to identify those species adapted for phytoremediation. However, it remains difficult to identify the best plant species for PAH phytoremediation because environmental conditions and plant-associated microorganisms can largely modify PAH remediation capacity and have to be taken into account.[77,78] In addition, significant degradation rate of high-molecular-weight PAHs was only observed when several plant species were combined. These observations were explained by the involvement of different metabolisms and degradation abilities of each plant species.[93,94] Alternatively, plant diversity appears to drive changes in microbial communities[95,96] which could enhance high-molecular-weight PAH removal.

Several genes coding for proteins that degrade PAHs from bacteria[35,97] and fungi[56] have been used to generate transgenic plants to be used for PAH remediation. For example, a gene encoding a fungal glutathione transferase was introduced into tobacco plants[98] and the resulting transgenic plants showed a higher uptake and catabolism of anthrancene that result in an increased tolerance to this PAH. Likewise, several prokaryotic genes linked to the decontamination of explosives have been successfully incorporated into plants.[99] Another approach to enhance the phytoremediation potential of plants would involve transgenic plants secreting PAH-degrading enzymes in order to increase PAH bioavailability. Even if this strategy has been achieved for organic pollutants such as bisphenol A, pentachlorophenol, 2, 3-dihydroxybiphenyl, and 1-chlorobutane,[100] such approaches have not yet been described for PAH phytoremediation.

Additionally, recent advances in plants high-throughput technologies, such as genome-wide association mapping, transcriptomics, genomics, proteomics, and metabolomics analyses provide new opportunities to decipher mechanisms involved in plant responses to PAH pollution. The identification of events or genes involved in the perception, absorption, transformation, conjugation, and compartmentalization/transport of PAHs may help to generate plants with higher and more efficient PAH-remediation potential, either by improvement of natural breeding methods or by the production of transgenic plants harboring an enhanced degradation capacity, a higher tolerance to xenobiotics and a better capacity to recruit bacteria and fungi with degradation capacity.

FUTURE PROSPECTS

Recent reviews have addressed aspects of plant-microbe interaction in various other contexts. In this work we particularly discussed metagenomic and "omics"-associated approaches, along with in situ stable isotope probing which give new picture of PAH metabolism, since these integrative investigations allowed the identification of the whole set of species that are collaborating as a consortium in field conditions. Some evidence suggests that the joint action of plant-fungal-bacterial consortia can result in the rapid degradation of complex molecules.[101] Hence, phytoremediation, mycoremediation, bioremediation, and rhizoremediation, wherein only one or few species are taken into account, have to be revised. Consequently, in polluted field sites, remediation of organic xenobiotics must to be reasoned at the metaorganism level.[102] Since not only metabolic, genomic, and biochemical determinants have to be considered but also ecological aspect are of high importance. Applying metagenomics, metatranscriptomics, metabolomics, and other complementary omics technologies to predict the catabolic potential at the metaorganism/holobiont scale in polluted soils open up new opportunities to increase the efficiency of biological-based remediation. For example, omics approaches have the capacity to identify target active indigenous hydrocarbon-degrading microorganisms in the rhizosphere which have been selected by plants.[103] The isolation of these microorganisms and their utilization as inoculums will be highly valuable for bioaugmentation and stimulation biotechnologies. The "omics"-based knowledge on catabolic potential and metabolite flow should be included in ecological modeling as input for prediction of the fate of PAHs in the plant-microbe complex, it can also be used for predictive risk assessment. Likewise better knowledge on the spatiotemporal distribution of the PAH biotransformation capacity should be available in order to better understand and predict the ecosystem function of PAH biodegradation.

Plants, archaea, fungi, bacteria, and microfaunal organisms constantly interact and influence each other, resulting in complex multispecies interactions across many of the domains

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of life (Figures 1 and 3). The advent of single- and multispecies omics tools now allows for the integrative and comprehensive assessment of this metaorganism/holobiont involved in green remediation technologies such as phytoremediation, mycoremediation and bioremediation. The future steps would be to harness the interaction occurring within the metaorganism to optimize the remediation efficiency and thereby generalize the usage of green remediation technologies.[27] This approach, that one could dub "meta-remediation", could consist in, for example, planting a species with enhanced recruitment capacity for PAH degraders at the same time as a bacterial and fungal consortium with high PAH degradation rates.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01740.

Table 1: List of plants used for PAH phytoremediation technology (PDF)

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Notes

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