Microbial Involvement in the Bioremediation of Total Petroleum Hydrocarbon Polluted Soils: Challenges and Perspectives

Ilaria Chicca 1, Simone Becarelli 1,2* and Simona Di Gregorio 1,*

1 Department of Biology, University of Pisa, 56126 Pisa, Italy; ilaria.chicca@biologia.unipi.it (I.C.); simone.becarelli@biologia.unipi.it (S.B.)
2 BD Biodigressioni srl, 56126 Pisa, Italy
* Correspondence: simona.digregorio@unipi.it

Abstract: Nowadays, soil contamination by total petroleum hydrocarbons is still one of the most widespread forms of contamination. Intervention technologies are consolidated; however, full-scale interventions turn out to be not sustainable. Sustainability is essential not only in terms of costs, but also in terms of restoration of the soil resilience. Bioremediation has the possibility to fill the gap of sustainability with proper knowledge. Bioremediation should be optimized by the exploitation of the recent “omic” approaches to the study of hydrocarbonolytic microbiomes. To reach the goal, an extensive and deep knowledge in the study of bacterial and fungal degradative pathways, their interactions within microbiomes and of microbiomes with the soil matrix has to be gained. “Omic” approaches permits to study both the culturable and the unculturable soil microbial communities active in degradation processes, offering the instruments to identify the key organisms responsible for soil contaminant depletion and restoration of soil resilience. Tools for the investigation of both microbial communities, their degradation pathways and their interaction, will be discussed, describing the dedicated genomic and metagenomic approaches, as well as the interpretative tools of the deriving data, that are exploitable for both optimizing bio-based approaches for the treatment of total petroleum hydrocarbon contaminated soils and for the correct scaling up of the technologies at the industrial scale.

Keywords: bioremediation; culturable and unculturable microbes; machine learning; metagenomic and genomic sequencing; predictive functional metagenomics

1. Introduction

In the era of renewable energies, crude oil is still one of the most important commodities in the world. Its uses span from energy generation and fuel for transportation to petrochemical productions such as plastics and solvents. Oil industry is one of the most powerful branches in the world economy, but it is based on finite reserves and, since an increasing number of conventional underground reserves are almost depleted, unconventional processes of oil extraction are nowadays feasible and consolidated. Examples are shale oil and tar sands, noxious for the environment, as well as the conventional oil drilling. United States and Canada are the major providers of shale oil and tar sands respectively, accounting for nearly one fourth of the world’s crude oil production. Most of the rest of crude oil reserves are in the Middle East, housing the greatest segment of oil reservoirs [1]. The crude oil to consumers supply-chain consists in three segments. Potentially, the three segments damage the environment. The upstream one, comprises exploration and drilling activities. The midstream activities, comprise transportation and refinery. The downstream activities are represented by the supply chains of heating oil and fuels. Oil spillage, pipeline accident and vandalization are the main sources of contamination for the environment and they have already destroyed coastal marine and terrestrial areas, polluted ground and surface aquifers and led to geopolitical crisis. Moreover, crude oil processing is a source...
of atmosphere pollution, interferences with ecological resources and release of hazardous materials with health and safety implications for the biosphere. A sustainable approach to the managing of noxious situations is mandatory and bio-based technologies may be an opportunity.

Crude oil is composed by different fractions, \( n \)-alkanes, aromatics, nitrogen-sulphur-oxygen compounds (NSO), resins and asphaltenes. The \( n \)-alkane or saturated fraction, ranging from \( \text{C}_1 \) (methane) to more than \( \text{C}_{40} \), is composed by branched and not-branched chains of saturated hydrocarbons [2]. The aromatic fraction ranges from benzene, toluene, ethylbenzene and xylene (BTEX) or single-ring aromatic fractions, to multi-ring polycyclic aromatic hydrocarbons (PAH) or aromatic rings substituted with different alkyl groups [3]. Resins and asphaltenes have very complex and mostly unknown carbon structures with the addition of many nitrogen, sulfur and oxygen atoms [4,5].

Total petroleum hydrocarbon (TPH) is the term used to describe a large family of several hundreds of chemical compounds that originally come from crude oil. Since the chemical composition of crude oil is very complex, it is not practical to measure each component separately, but it is feasible, standardized and useful to measure and refer to the total amount of TPH when evaluating the level of contamination at a site. Most of the scientific literature on the biodegradation of crude oil refers to the TPH fraction, which includes both volatile and extractable petroleum hydrocarbons, encompassing the gasoline range organics (\( \text{C}_6 - \text{C}_{10} \)), diesel range organics (\( \text{C}_{11} - \text{C}_{28} \)) and oil range organics (\( \text{C}_{29} - \text{C}_{35} \)). All these compounds are described as toxic to the environment.

The technologies dedicated to the protection of the environment or to the restoration of environmental quality, that have to face this complex and noxious scenario, must integrate the consolidated chemical-physical and less consolidated bio-based technologies to reach significant goals in terms of success. Bio-based technologies can be more sustainable in terms of costs because they are less energy-intensive. They are also eco-friendly since they are based on exploiting the potential of ecosystems to recover resilience in the various ecological niches. Here we review what is known, from a biological point of view, on the metabolic potential of microorganisms towards TPH, and which instruments we have to increase our capacity to exploit their potentiality, focusing on the adoption of “omics” in the environmental protection sector, with a focus on soil, one of the main ecosphere compartments affected by crude oil contamination [6].

2. The Soil Scenario

Soil crude oil and TPH contaminations are mainly consisting of weathered ones, except for fresh accidental spills. Indeed, processes of adsorption, photo transformation, biological transformation and volatilization of the diverse crude oil and TPH components are at the base of the transformation of fresh contamination in weathered ones in soil [7]. The process of weathering chemical structures in the environment is strictly dependent on the complexity of their chemical structures, which determine potential volatilization, water solubility, and loss of bioavailability [8]. In soil, the weathering of TPH is principally consistent with their sorption to the soil organic matter (SOM). The SOM in terms of amount [9] and nature [10] is mainly responsible for the decrease of bioavailability of TPH in soil. In fact, partitioning processes of TPH in the SOM entrap the contamination in soil micropores [11,12]. Once the contamination is entrapped in the SOM, the establishment of diverse processes of interaction among chemical structures with similar moieties (e.g., humic fraction of the SOM and organic contaminants with aromatic moieties) occurs. These interactions consist in dipole–dipole, dipole-induced dipole and hydrogen bonding [13]. Moreover, interactions between TPH and the soil mineral constituents also occur [14–16], strengthening the decrease in TPH bioavailability in soil, especially with the aging of the contamination. The higher is the molecular weight of the organic contaminants, the higher the number of aromatic moieties and functional groups capable to interact with the SOM. Consequently, a weathered oil-contaminated soil is usually dominated by high molecular weight hydrocarbons, significantly recalcitrant to biodegradation [17,18]. In this context,
the high molecular weight hydrocarbons are actually less toxic than the low molecular one, since these latter ones are less bioavailable for plants and microorganisms. The lag phase of bacterial growth was described as longer in the presence of higher concentrations of bioavailable hydrocarbons, and plant growth is inhibited by their presence since their hydrophobicity decreases the soil capacity to absorb water and nutrients [7].

On the other hand, it is generally accepted that microbes can eventually transform all the surrounding organic material over time. In particular, contaminants in environmental matrices exert a selective pressure on microbial communities. Speciation processes eventually occur and with time microbes, develop strategies to transform contaminants. Historically contaminated matrices are a useful reservoir of these organisms. Microbial strategies for contaminant transformation include not only degradative pathways but also the increment in their bioavailability and even their immobilization to contrast their toxicity (e.g., polycondensation or humification of the organic matter). Consequently, it is not surprising that the persistence of the TPH fractions in the soil is associated with the speciation of microbial strains that develop the capacity to transform the fraction with time [19].

Microbial biodegradation and biotransformation of organics vary in accordance with their chemical structures and the functional capabilities of the microbial community, specialized for the transformation of the parent molecule and of its intermediates of degradation. These latter ones are actually significantly important for the fate of pollutants in the environment, since in some cases, degradation products of the primary pollutants are more prone to subsequent transformations than the parent contaminant and, therefore, may be mineralized [20]. Other degradation products, such as polyaromatic chemical structures, are more susceptible to interaction with the SOM [21]. At the same time, the microbial catabolism is driven by pollutant concentration, since the activation of the microbial-degrading metabolisms needs a minimum threshold of pollutant concentration [22]. To reach these threshold levels, the bioavailability or the water solubility of the contaminant is mandatory. Consequently, in soil, TPH biodegradation rates are based also on the sorbed phase of the contamination [23–27]. Indeed, microorganisms reach the sorbed phase of the contamination because of their capacity to produce surfactants, responsible for both the increase in contaminant bioavailability and for facilitating the fixation of microorganisms onto the surface of the sorbed phase contaminants [28,29]. Thus, it is evident that there are a plethora of chemical-physical and biological elements that determine the destiny of organic pollutants in soil, and the condition is complicated by the complexity of the composition of the contamination.

3. Hydrocarbueroclastic Bacterial Pathways

The aerobic bacterial metabolic pathways involved in TPH transformation are mainly intracellular. Regarding the aromatic fraction of TPH, the mechanisms developed by bacterial cells to assimilate aromatic compounds have been fixed and optimized by natural selection, giving rise to functionally catabolic pathways organized in a funnel-like topology. In fact, a wide diversity of aromatics is directed via different peripheral pathways to a few key central intermediates. The latter are subjected to de-aromatization and further conversion to intermediary metabolites, such as acetyl-CoA, succinyl-CoA or pyruvate, via central pathways [30]. Hydroxylating oxygenases and ring-cleavage dioxygenases are responsible, respectively, for the hydroxylation and oxygenolytic cleavage of the aromatic rings. The products of these oxidative passages converge both on catecholic structures that are subjected to ortho or meta cleavage by intradiol or extradiol (type I and II) dioxygenases, respectively, and on non-catecholic structures such as gentisate, homogentisate, monohydroxylated aromatic acids and heteroaromatic flavonols, that are subject of ring cleavage by dedicated type III extradiol dioxygenases [31]. Moreover, dioxygenases such as the CO-forming 1-H-3-hydroxy-4-oxoquinaline 2,4-dioxygenase (HOD) and 1-H-3-hydroxy-4-oxoquinoline 2,4-dioxygenase (QDO) are involved in the transformation of N-heteroaromatic compounds [32]. In Figure 1, the scheme of the main biochemical strate-
gies to degrade aromatic structure (benzoate) in aerobic bacteria is reported. The aerobic hybrid pathway (Scheme B, Figure 1) shares the same activation reaction involved in anaerobic degradation.

Figure 1. Benzoate can be aerobically catabolized following two major strategies: (A) classical aerobic biodegradation pathway and (B) aerobic hybrid pathway. In both strategies, an activation step (blue), dearomatization/ring-cleavage step (pink) and further degradation to central metabolites, that is, lower pathway step (green), can be identified. The ortho cleavage of catechol (b-ketoadipate central pathway) and the benzoyl-CoA hybrid pathway converge into the common b-ketoadipyl-CoA intermediate. The anaerobic degradation of benzoate shares a similar initial reaction with the aerobic hybrid pathway catalyzed by a benzoate-CoA ligase.

Regarding the TPH saturated fraction, this is composed by n-alkane whose oxidation is intracellular and initiated by oxygenases that introduce oxygen atoms into n-alkanes by four different pathways (Figure 2). The terminal oxidation pathway [33] is involved in the oxidation of the n-alkanes terminal methyl group. The product of the reaction is a primary alcohol, further oxidized by alcohol dehydrogenases and aldehyde dehydrogenases to fatty acid that enters in β-oxidation [34]. The termini of the n-alkane can be oxidated to the corresponding fatty acid without breaking the carbon chain by the biterminal oxidation pathway. The product of the reaction is a ω-hydroxy fatty acid, further converted to a dicarboxylic acid, entering in β-oxidation [34–36]. Subterminal oxidation has been also observed to form primary alcohols and secondary alcohols or methyl acetone with the same chain length as the substrate [37]. In Acinetobacter sp. strain HO1-N [38], the n-alkanes are oxidized to form n-alkyl hydroperoxides and then peroxy acids, alkyl aldehydes and finally, fatty acids. A dioxygenase is responsible for the first step of oxidation [39].
The water solubility and consequent bioavailability of both the aromatic and saturated fraction of TPH are low. The latter is eventually increased by the capability of most of the hydrocarburoclastic bacteria to produce biosurfactants. Biosurfactants are of diverse chemical compositions such as glycolipids, fatty acids, lipopeptides and lipopolysaccharide and neutral lipids [40]. Biosurfactants are amphiphilic molecules with alkyl chains linked to sugar molecules, resulting in hydrophobic and hydrophilic regions, respectively [41], reducing the surface tension at the water/oil interface, leading to emulsification of hydrophobic moieties [42]. Indeed, biosurfactants enhance the bioavailability of contaminants for microbial degradation, by improving the solubilization of hydrocarbons in water layers, where bacterial metabolism and spreading occur [43]. Hydrocarburoclastic bacteria have been described as capable to produce biosurfactants in situ, which promote their survival in hydrophobic compound-dominated environments [44]. Different bacterial genera are described as capable to produce biosurfactants, among others, *Pseudomonas, Bacillus, Acinetobacter, Alcaligenes, Rhodococcus* and *Corynebacterium* spp. [40,45,46]. The exploitation of hydrocarburoclastic bacteria producing biosurfactants find application in the acceleration of the bioremediation of polluted soil and sediments [46–48], even though fragmented information are available on chemical and physical properties of biosurfactants produced by hydrocarburoclastic bacteria during the hydrocarbon degradation process [49].

4. Fungal Pathways

Fungi are very competitive in disturbed ecological niches since they are able to spread in the environment via hyphae elongation and adopt growth strategies to resist physical stresses, such as lack of nutrients and water by osmo- and xero-tolerance [50–52]. Fungi have been described also as more efficient than bacteria in the degradation of high molecular weight hydrocarbons in soils [53,54], due to their capacity to secrete extracellular a-specific polyphenols oxidases and laccases that are capable to transform macromolecules recalci-

Figure 2. Possible branches of the aerobic degradation of *n*-alkanes. A bifurcation is possible at the end of terminal oxidation pathway: the obtained carboxylic acid can ether go through β-oxidation or be further oxidized by the ω-fatty acid mono-oxygenases to form dicarboxylic acid (biterminal oxidation). The products of Subterminal oxidation pathway are secondary alcohols or methyl ketones, which can be further oxidized by Baeyer–Villiger mono-oxygenases and esterases to generate fatty acids and primary alcohols. The first step of Finnerty pathway is the formation of *n*-alkyl hydroperoxides by alkane dioxygenases that are oxidated to fatty acids.
trant to biodegradation, such as lignin and soil organic matter [55,56]; these latter ones show structural similarities to the TPH aromatic fraction [57,58]. The reaction catalyzed by these enzymes is the a-specific extraction of electrons from polyphenolic macromolecules to yield radical cation intermediates. These intermediates are channeled to the aromatic ring opening, with the breakdown of phenolic and aromatic compounds, or to polycondensation reactions [57]. In soil, the polycondensation reaction of organic compounds is responsible for the synthesis of the SOM [59]; indeed, microorganisms catalyze the stabilization of the organic matter by polycondensation (humification) reactions [60]. In contaminated soils, the organic portion consists in part or mainly in the contamination; consequently, it is reasonable to assess that microbial specimens catalyze the stabilization of the bioavailable portion of the contamination by its humification. Basidiomycetes have been extensively described as ligninolytic fungi and are consequently capable to produce the extracellular polyphenols oxidases and laccases previously described. However, Ascomycetes have been found to be dominant in TPH-contaminated soils [61]. Numerous studies have demonstrated the ability of Ascomycetes to transform recalcitrant compounds [46,62–64], as well as their involvement in the synthesis of soil organic matter and their ability to catalyze extracellular polymerization of polyphenols [65]. On the other hand, Ascomycetes have been described for the involvement of also cytochrome P450 monoxygenases (CYPs) in the oxidation of aromatic structures [56,61,66]. The fungal CYP biodegradation pathway of the polyaromatic structures consists of initial oxidation of the aromatic ring and conversion in hydroxy, dihydroxy, dihydrodiol and quinone derivatives [33]. Oxidized metabolites are conjugated and stored in cellular organelles and lipid-vesicles [67,68], or secreted in a more soluble and biodegradable form [69,70] (Figure 3). Fungal Cytochrome P450, by catalyzing oxidation of hydrocarbon C–H bonds to the corresponding hydroxy (C–OH) products, are responsible also for the initial hydroxylation of n-alkane [71–75]. Similarly, to bacterial metabolism, the alcohol is oxidized to the corresponding aldehyde and then to the corresponding fatty acid [54]. Fungal di-terminal and subterminal oxidation have been also observed [2,54,76,77]. Indeed, fungi, in relation to the aromatic and saturated fraction of TPH adopt both intracellular and extracellular strategies of degradation.

Figure 3. General pathways and enzymes involved in ring cleavage and/or oxidation of poly-cyclic aromatic hydrocarbons.

5. Microbial Interactions

Synergisms between microorganisms in the environment might be explained by the generalization of the ecological concept of K-r strategy, which basically explains two different approaches of nutrition of “organisms” sharing the same niche. The r-strategy consists
of high growth rates with high availability of carbon sources, while K-strategy consists of slow rates of growth and low availability of carbon sources. K-strategists are favored under nitrogen limitation since K-strategists are able to decompose the SOM, recalcitrant to biodegradation, for mineral nitrogen acquisition [78,79]. In parallel, r-strategists commonly utilize the SOM-derived compounds solubilized by the K-strategists. The r-strategists are favored in presence of available carbon and higher nitrogen availability [80]. A significant shift in microbial community structure from K-strategists to r-strategists is observed with the increased availability of nitrogen and carbon [81]. The shift might be observed also in terms of fungal abundances (K-strategists) in favor of bacterial ones (r-strategists), in consequence of the capacity of fungi to transform the SOM. It might be possible that the real situation is more complicated but it is evident that fungi and bacteria share microhabitats, assembled into dynamic co-evolving communities, described in almost every ecosystem [82]. These communities include microbial species from a wide diversity of fungal and bacterial families [83]. Microbial interactions contribute to soil functions as well as, and eventually even more than, species diversity [84]. Generally, the co-occurrence and synergism between fungi and bacteria in the soil might be based on the bacterial capacity to utilize fungal-secreted metabolites and overcome fungal defense mechanisms [85]. On the other hand, an example of the synergisms between the two kingdoms is the one of fungal lignocellulose decomposers, *Clitocybe* and *Mycena* spp., that interact with potential N₂-fixing bacteria, responsible for nitrogen deposition in soil during the decay of leaves [86,87]. Bacteria may contribute to nitrogen nutrition of fungi while fungi produce carbon sources for bacteria. Moreover, in oligotrophic habitat, fungi promote bacterial growth by nutrient and water transfer from fungal hyphae to the bacterial cells [50]. Model simulations show that fungal hyphae are spreading bacteria towards a source of contamination in environments where the active movement of bacteria towards pollutant reservoirs is limited by physical barriers [88–90]. In the context of crude oil bioremediation, it is worth mentioning that fungal hyphae have been described to mobilize PAHs by entrapping and transporting the latter in cytoplasmic vesicles [91], providing entrapped PAHs to hydrocarburoclastic bacteria [92]. In laboratory models of water-unsaturated environments, fungi transport the contamination from the water-depleted niches to the less-dried ones, where bacteria are blooming [93]. Moreover, fungi excrete organic molecules such as organic acids or polyols that activate bacterial chemotaxis towards the hyphae [94,95]. Indeed, fungi promote ecosystem functioning in heterogeneous habitats by transporting resources from high nutrient and water levels to nutrient-poor and dry areas.

Other than chemotaxis between fungi and bacteria, another useful chemical signal tool in the ecology is quorum sensing (QS). Quorum sensing enables bacteria to communicate by exchanging signaling molecules also referred to as “autoinducers”. Two types of autoinducers are described: intraspecific signals, which are used by the same organism within a population and interspecific signals, which are used for communication among the entire microbial community [96]. A strong correlation between the expression of genes involved in hydrocarbon depletion and quorum sensing has been observed; as an example, a positive correlation between quorum sensing and the expression of the 2,3-catechol dioxygenase of the meta-cleavage (lower) pathway for hydrocarbon degradation has been reported [97]. On the other hand, the same correlation has been observed for quorum sensing modulation of the level of transcription of dioxygenase genes in the upper BTEX oxidation pathway [98]. QS is involved also in fungal morphogenesis, fungal biofilm development, apoptosis and eventually, pathogenicity [99]. Moreover, QS has been described as involved in interkingdom signaling in mixed fungal-bacterial biofilms [100–103].

6. Plasticity of Microbial Metabolic Profiles

Among bio-based approaches, mycoremediation has achieved a significant number of reasonable successes, especially in the case of bioaugmentation of indigenous fungi to soils and sediments contaminated by TPH [46,104]. In cases where bacterial ecology
has been studied during the contaminant degradation process, a significant effect of the bioaugmentation of fungal strain on the latter has been observed [46,104]. Bioaugmentation of autochthonous fungi to aged contamination has been observed as capable to promote the establishment of active hydrocarbon-degrading bacterial populations, competent for the degradation of both the aliphatic and the aromatic hydrocarbons [104]. On the other hand, the bioaugmentation of an autochthonous Ascomycetes to a TPH-contaminated soil, a Ciboria sp. strain, accelerated the onset of specialist bacterial species, competent for the transformation of the aromatic fraction of the contamination [46]. In this study, a functional predictive metabarcoding analysis of the bacterial ecology was adopted, and bacterial genera such as Arthrobacter, Dietzia, Brachybacterium, Brevibacterium, Gordonia, Leucobacter, Lysobacter and Agrobacterium spp. were identified as generalist saprophytes, essential for the onset of hydrocarbonoclastic specialist bacterial species, identified as Streptomyces, Nocardoides, Pseudonocardia, Solirubrobacter, Parvibaculum, Rhodanobacter, Luteimonas, Planomicrobium and Bacillus spp. The functional traits that resulted to be indicative of the functional diverse roles of the two groups of bacterial species was the Dye decolorizing peroxidases (DyP). DyP has been retrieved both in eukaryotic and prokaryotic organisms, showing a higher diversity in prokaryotes [105]. Bacterial DyP may have high redox potentials, capable of oxidizing phenolic [106] and non-phenolic lignin model compounds [107]. The DyP, in the case study, was associated with the saprophytic metabolisms of the harbor ing bacterial genera, more than their capacity to deplete the contamination. As a matter of the fact, the saprophytic metabolism of the bioaugmented Ciboria sp. was positively synergizing with the bacterial one, accelerating the onset of bacterial specialists for the degradation of the aromatic fraction of the contamination, eventually accelerating its depletion. In this context, it should be mentioned that the saprophytic metabolisms of fungi and bacteria might be responsible for the transformation process of the organic matter in the soil, determining both the mobilization of carbon source via a partial transformation of the SOM and the stabilization of the latter, by eliciting polycondensation reactions. On the other hand, it is reasonable to assess that the aging of the contamination might render the polluted soil an oligotrophic environment, due to the lack of bioavailability of the dominant carbon source due to weathering processes. Microbial saprophytic metabolisms might be pivotal to the increase in bioavailability of the contaminants, deriving from the saprophytic partial oxidation of the main carbon sources in contaminated soils, that is consisting with the SOM-sorbed contamination. Thus, synergizing saprophytic metabolisms of fungi and bacteria might be pivotal to prime the actual depletion of the SOM-sorbed contaminants by both direct mechanisms (stabilization) and an indirect one, activating the metabolisms of specialist species, recalling the ecological concept of K-r strategy.

As previously assessed, a saprophytic metabolism mediated by microbial peroxidases is responsible for the humification (stabilization) of the organic matter in the soil. The humification of organic matter consists of a process defined as composting. Composting is an aerobic oxidative process that relies on the actions of microorganisms to degrade organic materials, resulting in the thermogenesis and production of organic and inorganic compounds, and on humification of the organic matter with a consequent stabilization. Composting strategies in biodegradation/bioremediation of organic pollutants have been seriously adopted for the last 20 years. In relation to the application of the protocol to soil remediation, there are a variety of composting systems, mainly consisting of more or less controlled and engineered windrows and open piles. Initially, many of these systems were developed for the stabilization of sewage sludges, catalyzing processes of stabilization, or humification of the corresponding organic fraction. With reference to the scenario of interest in the context of TPH contamination of soil, it is reasonable to assess that, where pollutants are completely bioavailable and biodegradable, composting processes should be favorable. On the other hand, the limited bioavailability, eventually due to aging of the contamination, might be accompanied by processes of stabilization of the contamination, determining a depletion of the contamination in terms of measurable parental components [108]. This assessment is corroborated by the evidence that during
composting of high molecular weight hydrocarbons, their mineralization, quantified by the
development of CO$_2$ during the soil composting process, is limited and inversely correlated
to depletion of the contamination [109], suggesting the occurring of stabilization processes
more than mineralization of highly recalcitrant to biodegradation pollutants. This aspect,
if inducing perplexity from the toxicological point of view [48], is advantageous for the
optimization of bio-based processes. In fact, if saprophytic microorganisms are involved in
increasing contaminant bioavailability and eventually in determining the conditions for the
onset of microorganisms capable of transformation of the contamination (specialist species),
but also in processes of stabilization of the contamination, the bioaugmentation approach
to bioremediation might be re-designed by the exploitation not only of the specialist
species for contaminant transformation but also of the saprophytic specimen showing
resistance to the contamination. In a study concerning the exploitation of co-composting
of lignocellulosic material and contaminated soils, it was observed that a wide variety of
microorganisms (bacteria and fungi) are involved in the biodegradation of TPH. Fungi
were described as producing enzymes for a partial oxidation of TPH with bacteria utilizing
these partially oxidized products as carbon and energy sources. The bacterial ecology was
described as changing during the successive phases of the composting process. The fungal
ecology was described as mainly associated or deriving from the bulking lignocellulosic
material, resulting as microbial effectors priming both the whole composting process and
the interaction with the bacterial ecology, at the base of the efficiency of the biodegradative
microbiome [110].

In another study, it was observed that the addition of bulking agents, such as rice straw
or sawdust, improved the contaminant degradation rate. With a metagenomic analysis on
fungal and bacterial communities, the authors concluded that the removal efficiency was
related to a selective effect of the bulking agents on specific microbial communities. The
community composition analysis shows that the abundance of petroleum-degrading taxa
such as the bacterial *Sphingomonas* and *Phenylobacterium* spp. and the fungal *Humicola*
and *Graphium* spp. increased synergistically after addition of rice straw and sawdust, with a
significant acceleration of TPH depletion over time [111].

As a matter of the fact, the interaction of bacteria and fungi seems to be pivotal for the
recovery of polluted soils and widely diversified microbial metabolic pathways, charac-
terizing diverse prokaryotic and eukaryotic microbial species which actively sustain the
decontamination process. In fact, it is already accepted that the degradation of recalcitrant
and polluting organic chemical structures, in a complex matrix like the soil, cannot be
achieved individually, but by complex community interactions using different metabolic
strategies of microorganisms adapted to the latter [19]. However, many of the bio-based pro-
cesses dedicated to the decontamination of polluted environments are basically limited to
the isolation, characterization and bioaugmentation of specialist species. As previously as-
sessed, it is not the single specialist, but microbial assemblages with syntrophic associations
that are principally responsible for the transformation of contaminants in the environment.
Moreover, in bioremediation, the depletion of the contamination must be accompanied
by the “re-shaping” of the soil as a substrate offering ecosystem services [112,113]. In this
context, the involvement of plant growth-promoting (PGP) bacteria that contribute to the
restoration of soil quality, by their interaction with the primary producers colonizing the
soil, might be of interest since it has also been described as involved in the degradation of
soil contaminants, both directly and indirectly, improving plant performances in phytore-
mediation [114]. Microbial isolates combining both xenobiotic degradation capacity and
PGP potentials might be an efficient and sustainable tool to be exploited in the planning of
bio-based remediation interventions for the restoration of contaminated soils [115]. The
design of synthetic microbial microbiota for biaugmentation, combining degradative and
re-shaping functions, is desirable.
7. Taxonomical and Functional Metagenomics for Bioremediation

The updated scenario of the microbial processes of TPH transformation in contaminated soils reveals levels of complexity that risk to associate the exploitation of bio-based processes to uncertainty. In order to transform the bio-based approaches to soil decontamination in robust technologies, a comprehensive understanding of the physiology, ecology and phylogeny of the colonizing microbial consortia is mandatory. The capacity to isolate microbial specialists for the biodegradation of TPH and crude oils is crucial for evaluating the biodegradation process they catalyze, as well as the regulatory mechanisms that influence their activities in contaminated soils. Moreover, the sequencing of their genomes is mandatory to deposit sequences of interest for in silico analysis of all the soil matrices contaminated by the same class of contaminants. Nowadays, the application of “omics” techniques in the investigation of pure cultures gives the opportunity to assess the genetic diversity of specialist species and of all environmental microbes, to investigate genes responsible for contaminant degradation and their regulation. In this context, it should be mentioned that “omics” investigation can be exploited for both isolates and environmental samples [116–118] and the adoption of metagenomics, transcriptomics and proteomics offers the opportunity to design new approaches to the management of processes dedicated to the restoration of the environment [119,120]. These techniques can be exploited to study the organization, interdependence, physiology, ecology and phylogeny of environmental microbiomes, comprising the hydrocarburolastic ones. The study of microbiomes cannot be limited to culturomic approaches. Indeed, the large majority of microorganisms colonizing natural habitats are either uncultivable or extremely difficult to cultivate. Metagenomics is designed to study these microbes [121], with the recovering of DNA sequences from environmental DNA, offering the opportunity to discover genes and biodegradative pathways [122,123]. The correspondence between the kinetics of decontamination and the variations of the microbial ecology is mandatory to assess the microbial populations that are involved in the decontamination of TPH-contaminated soils [48]. The predictive functional metagenomics have been exploited to dissect bacterial functions that are dominant in processes of TPH depletion in soils, synergically catalyzed by fungi and saprophytic bacteria, introducing possible mechanisms of interaction between the fungal and the bacterial hydrocarburolastic community in an aged TPH soil contamination [48].

The extremely useful functional metagenomic analysis can be successfully based also on the cloning of environmental DNA fragments in selected microbial hosts, successively screened for environmental functions of interest. The sequencing of the fragments cloned in the selected hosts leads to the sequencing of gene coding for these functions [124], enriching environmental databases on pollutant-degrading genomic sequences of known and unknown, isolated and not yet isolated, microbial candidates, facilitating in silico analysis of all the environmental matrices, affected by the same contamination. The described functional analysis combined with taxonomic metabarcoding and the derived predictive functional analysis should be considered as the bases for developing a robust predictive instrument to infer the functions that the microbial communities and colonizing contaminated matrices can express and, consequently, the functions to be exploited to complete and even accelerate a decontamination process. Due to the availability of a plethora of metagenomic, in silico functional and wet-lab functional data, bioinformatic processing applications for environmental DNA sequence-based screening of metagenomes are extremely important and they tend to be adopted among other methodological approaches to design bio-based technologies for the recovery of contaminated matrices. Among others, a list of the most important, frequently utilized and open-source pipelines of analysis follows.

Meta Genome Analyzer (MEGAN) analyzes large amounts of metagenomic sequence data [125] comparing metagenomic and metatranscriptomic data both functionally and taxonomically, mapping reads to the NCBI, SEED, COG and KEGG classificators. The tool is robust and user-friendly.
Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA) is a database and computational tools providing the possibility for depositing, locating, analyzing and sharing data about microbial biology [126,127]. CAMERA offers the opportunity to deposit and work with metadata relevant to environmental metagenome datasets with annotations in a semantically aware environment. The user can use semantic queries to search in the database. CAMERA might be defined as a complete genome-analysis tool allowing users to analyze both metagenome and genome data.

Subsystem Technology for metagenomes (MG-RAST) is an automated platform providing quantitative insight into microbiomes based on their sequence data [128]. The pipeline performs similarity-based annotation on nucleic acid datasets, clustering and protein prediction, phylogenetic and metabolic reconstructions of genomes and metagenomes.

Phylogenetic metabarcoding is fundamental to study the microbial ecologies of any environmental niches. However, the molecular tool does not provide direct evidence of the community functional capabilities. PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states), now PICRUSt2 [129], offers a computational approach to predict the functional composition of a metagenome using marker gene data and a database of reference genomes. PICRUSt uses an extended ancestral-state reconstruction algorithm to predict which gene families are present and then combines gene families to estimate the composite metagenome. Using phylogenetic information, PICRUSt2 infers key findings from the Human Microbiome Project and accurately predicts the abundance of gene families in host-associated and environmental communities, with quantifiable uncertainty.

Indeed, metagenomics is fundamental to understanding the composition and the ecology of hydrocarburoclastic microbiota that can be defined as bucket-brigades for the degradation of TPH and crude oils in soils. Their functional analysis is fundamental to recover hydrocarburoclastic microbiomes, combining taxonomy and functional information, providing the instruments to deepen the information provided by a conceptual model of a contaminated site; in fact, the latter can comprise not only data about the stratigraphy, geological characteristics and chemical profiles of the contamination in the contaminated site. Indeed, the site will be characterized also in terms of the colonizing microbial communities, whose functional traits will be automatically inferred by the exploitation of data provided by the constantly increasing number of metagenomic studies. In this direction, it is worth mentioning that the predictive metagenomic profiling successfully described phylogenetic and functional compositions of diverse oil-polluted sites around the world, allowing the inferring of metagenomic features including taxonomical markers and functional modules, to be used as biomarkers for the effective distinction between diverse oil-polluted sites [130].

8. Conclusions

Biodegradation is defined as a sustainable approach to the decontamination of soils, however, for the transferability of the technology onto the industrial scale, repeatability, robustness and predictability of the rate of success are mandatory. The upscaling step requires an extensive knowledge of the biological processes underlying biodegradation, of the actors involved in and their interplay and of the chemical and physical factors contributing to reach the goal [131]. “Omics” approaches, by taxonomical and functional analyses of the data, offer a whole picture of the process with the identification of the key organisms within the microbiomes involved in the TPH degradation. At the same time, they offer an instrument to optimize the culturomic approach for the isolation of key actors of the whole process of decontamination, providing the instruments for the integration of culturomic and non-culturomic approaches, mandatory to boost the upscaling of the bioremediation technology and the number of sites that might be treated in the future. At the same time, the derived knowledge progressively increases the amount of the taxonomical and functional pool of genomic data for in silico interpretation of the microbiological characteristics of contaminated sites, eventually contaminated by vast arrays of contaminants. The further goal is the integration of analytical approaches based
on machine learning, such as the one described here for the analysis of omic-derived data, and the stochastic ones typically adopted in the modelization of physical events, occurring in real situations, by a pipeline of analysis equipped with data that have been validated and calibrated with laboratory experiments and field cases. These latter are implemented in the design of processes on the real scale, since normally, providing instruments for the geotechnical and geological characterization and rheological engineering (e.g., MADflow, Available on line: http://madflow.ca, accessed on 1 March 2022). With reference to the extension and frequency of TPH contamination of soils all around the world and to the reach of already acquired data, the integration of all the analytical approaches will provide a robust instrument of prediction and control of the rate of success of a dedicated bio-based process.

**Author Contributions:** Conceptualization and investigation, I.C., S.B. and S.D.G.; writing—original draft preparation, I.C. and S.D.G.; writing—review and editing, I.C., S.B. and S.D.G.; supervision, S.D.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** No new data were created or analyzed in this study. Data sharing is not applicable to this article.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Available online: www.ibisworld.com/global/market-size/global-oil-gas-exploration-production/ (accessed on 1 March 2022).
2. Abbasian, F.; Lockington, R.; Mallavarapu, M.; Naidu, R. A Comprehensive Review of Aliphatic Hydrocarbon Biodegradation by Bacteria. *Appl. Biochem. Biotechnol.* 2015, 176, 670–699. [CrossRef] [PubMed]
3. Meckenstock, R.U.; Boll, M.; Mouttaki, H.; Koelschbach, J.S.; Cunha Tarouco, P.; Weyrauch, P.; Dong, X.; Himmelberg, A.M. Anaerobic Degradation of Benzene and Polycyclic Aromatic Hydrocarbons. *Microb. Physiol.* 2016, 26, 92–118. [CrossRef] [PubMed]
4. Harayama, S.; Kasai, Y.; Hara, A. Microbial communities in oil-contaminated seawater. *Curr. Opin. Biotechnol.* 2004, 15, 205–214. [CrossRef] [PubMed]
5. Chandra, S.; Sharma, R.; Singh, K.; Sharma, A. Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. *Ann. Microbiol.* 2013, 63, 417–431. [CrossRef]
6. Du, W.; Wan, Y.; Zhong, N.; Fei, J.; Zhang, Z.; Chen, L.; Hao, J. Status quo of soil petroleum contamination and evolution of bioremediation. *Pet. Sci.* 2011, 8, 502–514. [CrossRef]
7. Khan, M.A.I.; Biswas, B.; Smith, E.; Naidu, R.; Megharaj, M. Toxicity assessment of fresh and weathered petroleum hydrocarbons in contaminated soil- a review. *Chemosphere* 2018, 212, 755–767. [CrossRef]
8. Ollivier, B.; Magot, M. *Petroleum Microbiology*; Wiley Online Library: Hoboken, NJ, USA, 2005. [CrossRef]
9. Hatzinger, P.B.; Alexander, M. Effect of Aging of Chemicals in Soil on Their Biodegradability and Extractability. *Environ. Sci. Technol.* 2002, 29, 537–545. [CrossRef] [PubMed]
10. Platt, J.J.; Brusseau, M.L. Rate-limited sorption of hydrophobic organic compounds by soils with well-characterized organic matter. *Environ. Sci. Technol.* 1998, 32, 1604. [CrossRef]
11. Reid, B.J.; Jones, K.C.; Semple, K.T. Bioavailability of persistent organic pollutants in soils and sediments—Perspective on mechanisms 1608, consequences and assessment. *Environ. Pollut.* 2000, 108, 103–112. [CrossRef]
12. Nam, K.; Alexander, M. Role of nonaporosity and hydrophobicity in sequestration and bioavailability: Tests with model solids. *Environ. Sci. Technol.* 1998, 32, 71–74. [CrossRef]
13. Pignatello, J.J.; Xing, B. Mechanisms of Slow Sorption of Organic Chemicals to Natural Particles. *Environ. Sci. Technol.* 1995, 29, 1–11. [CrossRef]
14. Ball, W.P.; Roberts, P.V. Long-Term Sorption of Halogenated Organic Chemicals by Aquifer Material. 1. Equilibrium. *Environ. Sci. Technol.* 1991, 25, 1223. [CrossRef]
15. Ball, W.P.; Roberts, P.V. Long-Term Sorption of Halogenated Organic Chemicals by Aquifer Material. 2. Intraparticle Diffusion. *Environ. Sci. Technol.* 1991, 25, 1237. [CrossRef]
16. Mader, B.T.; Uwe-Goss, K.; Eisenreich, S.J. Sorption of nonionic, hydrophobic organic chemicals to mineral surfaces. *Environ. Sci. Technol.* 1997, 31, 1079. [CrossRef]
17. Trindade, P.V.O.; Sobral, L.G.; Rizzo, A.C.L.; Leite, S.G.F.; Soriano, A.U. Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: A comparison study. *Chemosphere* 2005, 58, 515–522. [CrossRef]
18. Maletić, S.; Dalmacija, B.; Rončević, S.; Agaba, J.; Petrović, O. Degradation kinetics of an aged hydrocarbon-contaminated soil. *Water Air Soil Pollut.* 2009, 202, 149–159. [CrossRef]
19. Zhang, T.; Zhang, H. Microbial Consortia Are Needed to Degrade Soil Pollutants. Microorganisms 2022, 24, 261. [CrossRef]
20. Minna Laine, M.; Jørgensen, K.S. Straw compost and bioremediated soil as inocula for the bioremediation of chlorophenol-contaminated soil. Appl. Environ. Microbiol. 1996, 62, 1507. [CrossRef]
21. Kästner, M.; Streibich, S.; Beyrer, M.; Richnow, H.H.; Fritsche, W. Formation of bound residues during microbial degradation of [14C]anthracene in soil. Appl. Environ. Microbiol. 1999, 65, 1834. [CrossRef]
22. Boethling, R.S.; Alexander, M. Effect of Concentration of Organic Chemicals on Their Biodegradation by Natural Microbial Communities. Appl. Environ. Microbiol. 1979, 37, 1211–1216. [CrossRef]
23. Fu, C.; Planstiel, S.; Gao, C.; Yan, X.; Govind, R.; Tabak, H.H. Studies on Contaminant Biodegradation in Slurry 1211, Wafer, and Compacted Soil Tube Reactors. Environ. Sci. Technol. 1996, 30, 743–750. [CrossRef]
24. Park, J.H.; Zhao, X.; Voice, T.C. Biodegradation of Non-desorbable Naphthalene in Soils. Environ. Sci. Technol. 2001, 35, 2734. [CrossRef] [PubMed]
25. Woo, S.H.; Lee, M.W.; Park, J.M. Biodegradation of phenanthrene in soil-slurry systems with different mass transfer regimes and soil contents. J. Biotechnol. 2004, 110, 235–250. [CrossRef] [PubMed]
26. Zhao, X.; Voice, T.C. Assessment of bioavailability using a multicolumn system. Environ. Sci. Technol. 2000, 34, 1506. [CrossRef]
27. Zhao, X.; Szafrański, M.J.; Maraga, M.A.; Voice, T.C. Sorption and bioavailability of carbon tetrachloride in a low organic content sandy soil. Environ. Toxicol. Chem. 1999, 18, 1755. [CrossRef]
28. Harms, H.; Zehnder, A.J.B. Bioavailability of sorbed 3-chlorodibenzofuran. Appl. Environ. Microbiol. 1976, 62, 27–33. [CrossRef]
29. Pigunatello, J.J.; Martinson, M.M.; Steiert, J.G.; Carlson, R.E.; Crawford, R.L. Biodegradation and Photoysis of Pentachlorophenol in Artificial Freshwater Streams. Appl. Environ. Microbiol. 1983, 46, 1024–1031. [CrossRef] [PubMed]
30. Fuchs, G.; Boll, M.; Heider, J. Microbial degradation of aromatic compounds—From one strategy to four. Nat. Rev. Microbiol. 2011, 9, 803–816. [CrossRef]
31. Fetzner, S. Ring-cleaving dioxygenases with a cupin fold. Appl. Environ. Microbiol. 2012, 78, 2505. [CrossRef]
32. Steiner, R.A.; Janssen, H.J.; Roversi, P.; Oakley, A.J.; Fetzner, S. Structural basis for cofactor-independent dioxygenation of N-heteroaromatic compounds at the α/β-hydrolase fold. Proc. Natl. Acad. Sci. USA 2010, 107, 657. [CrossRef]
33. Li, L.; Liu, X.; Yang, W.; Xu, F.; Wang, W.; Feng, L.; Bartlam, M.; Wang, L.; Rao, Z. Crystal Structure of Long-Chain Alkane Monoxygenase (LadA) in Complex with Coenzyme FMN: Unveiling the Long-Chain Alkane Hydroxylase. J. Mol. Biol. 2008, 376, 453–465. [CrossRef] [PubMed]
34. Watkinson, R.J.; Morgan, P. Physiology of aliphatic hydrocarbon-degrading microorganisms. Physiol. Biochem. Microorg. 1991, 1, 79–92. [CrossRef] [PubMed]
35. Kester, A.S.; Foster, J.W. Ditermal oxidation of long-chain alkanes by bacteria. J. Bacteriol. 1965, 85, 859–869. [CrossRef] [PubMed]
36. Coon, M.J. Omega Oxygenases: Nonheme-iron enzymes and P450 cytochromes. Biochem. Biophys. Res. Commun. 2005, 338, 378–385. [CrossRef]
37. Forney, F.W.; Markovetz, A.J. Subterminal Oxidation of Aliphatic Hydrocarbons. J. Bacteriol. 1970, 102, 281. [CrossRef]
38. Fennerty, W. Lipids of Acinetobacter. In Proceedings of the World Conference on Biotechnology for the Fats and Oil Industry; Applewhite, T.H., Ed.; Amer Oil Chemists Society: Urbana, IL, USA, 1988; pp. 184–188.
39. Maeng, J.H.O.; Sakai, Y.; Tani, Y.; Kato, N. Isolation and characterization of a novel oxygenase that catalyzes the first step of n-alkane oxidation in Acinetobacter. Biochem. Biophys. Res. Commun. 2008, 376, 378–385. [CrossRef] [PubMed]
40. Cameostra, S.S.; Makkar, R.S. Biosurfactant-enhanced bioremediation of hydrophobic pollutants. Pure Appl. Chem. 2010, 82, 97–116. [CrossRef]
41. Costa, S.G.V.A.O.; Nitschke, M.; Lépine, F.; Déziel, E.; Contiero, J. Structure, properties and applications of rhamnolipids produced by Pseudomonas aeruginosa L2-1 from cassava wastewater. Process Biochem. 2010, 45, 1511. [CrossRef]
42. Harkins, W.D.; Jordan, H.F. A method for the determination of surface and interfacial tension from the maximum pull on a ring. J. Am. Chem. Soc. 2002, 52, 1751. [CrossRef]
43. Banat, I.M.; Satpute, S.K.; Cameotra, S.S.; Patil, R.; Nyayanit, N.V. Cost effective technologies and renewable substrates for biosurfactants’ production. Front. Microbiol. 2017, 5, 697. [CrossRef]
44. Ganes, A.; Lin, J. Diesel degradation and biosurfactant production by Gram-positive isolates. Afr. J. Biotechnol. 2011, 8, 5847. [CrossRef]
45. Abbassian, F.; Lockington, R.; Megharaj, M.; Naidu, R. A Review on the Genetics of Aliphatic and Aromatic Hydrocarbon Degradation. Appl. Biochem. Biotechnol. 2016, 178, 224–250. [CrossRef] [PubMed]
46. Becarelli, S.; Chicca, I.; la China, S.; Siracusa, G.; Bardi, A.; Gullo, M.; Petroni, G.; Levin, D.B.; Di Gregorio, S. A New Ciboria sp. for Soil Mycoremediation and the Bacterial Contribution to the Depletion of Total Petroleum Hydrocarbons. Front. Microbiol. 2021, 12, 647373. [CrossRef] [PubMed]
47. Kumar, M.; Leon, V.; de Sisto Materano, A.; Ilzins, O.A. Enhancement of oil degradation by co-culture of hydrocarbon degrading and biosurfactant producing bacteria. Pol. J. Microbiol. 2006, 55, 139–146.
48. Di Gregorio, S.; Becarelli, S.; Siracusa, G.; Ruffini Castiglione, M.; Petroni, G.; Masini, G.; Gentini, A.; de Lima e Silva, M.R.; Lorenzi, R. Pleurotus ostreatus spent mushroom substrate for the degradation of polycyclic aromatic hydrocarbons: The case study of a pilot dynamic biopile for the decontamination of a historically contaminated soil. J. Chem. Technol. Biotechnol. 2016, 91, 1654. [CrossRef]
49. Chandankere, R.; Yao, J.; Cai, M.; Masakorala, K.; Jain, A.K.; Choi, M.M.F. Properties and characterization of biosurfactant in crude oil biodegradation by bacterium Bacillus methylotrophicus USTBa. *Fuel* 2014, 122, 140–148. [CrossRef]

50. Worrich, A.; Struyhanuk, H.; Musat, N.; König, S.; Banitz, T.; Centler, E.; Frank, K.; Thullner, M.; Harms, H.; Richnow, H.H.; et al. Mycelium-mediated transfer of water and nutrients stimulates bacterial activity in dry and oligotrophic environments. *Nat. Commun.* 2017, 8, 1. [CrossRef]

51. González-Abredalo, D.; Pérez-Llano, Y.; Peidro-Guzmán, H.; del Sánchez-Carbente, M.R.; Folch-Mallol, J.L.; Aranda, E.; Vaidyanathan, V.K.; Cabana, H.; Gunde-Cimerman, N.; Batista-Garcia, R.A. First demonstration that ascomycetous halophilic fungi (*Aspergillus sydowi* and *Aspergillus destruens*) are useful in xenobiotic mycoremediation under high salinity conditions. *Bioreour. Technol.* 2019, 279, 287–296. [CrossRef]

52. Peidro-Guzmán, H.; Pérez-Llano, Y.; González-Abredalo, D.; Fernández-López, M.G.; Dávila-Ramos, S.; Aranda, E.; Hernández, D.R.O.; García, A.O.; Lira-Ruan, V.; Pliego, O.R.; et al. Transcriptomic analysis of polyaromatic hydrocarbon degradation by the halophilic fungus *Aspergillus sydowi* at hypersaline conditions. *Environ. Microbiol.* 2021, 23, 3435. [CrossRef]

53. Aranda, E.; Godoy, P.; Reina, R.; Badía-Fabregat, M.; Rosell, M.; Marco-Urrea, E.; García-Romera, I. Isolation of Ascomycota fungi with capability to transform PAHs: Insights into the biodegradation mechanisms of Penicillium oxalicum. *Int. Biodeterior. Biodegr.* 2017, 122, 141–150. [CrossRef]

54. Prenafeta-Boldú, F.X.; de Hoog, G.S.; Summerbell, R.C. Fungal Communities in Hydrocarbon Degradation. In *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, Handbook of Hydrocarbon and Lipid Microbiology; McGenity, T., Ed.; Springer: Cham, Switzerland, 2019; pp. 1–36. [CrossRef]

55. Harms, H.; Schlosser, D.; Wick, L.Y. Untapped potential: Exploring fungi in bioremediation of hazardous chemicals. *Nat. Rev. Microbiol.* 2011, 9, 177–192. [CrossRef] [PubMed]

56. Marco-Urrea, E.; García-Romera, I.; Aranda, E. Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. *New Biotechnol.* 2015, 32, 620–628. [CrossRef] [PubMed]

57. Baldrian, P. Increase of laccase activity during interspecific interactions of white-rot fungi. *FEMS Microbiol. Ecol.* 2004, 50, 245–253. [CrossRef] [PubMed]

58. Schmidt, K.R.; Chand, S.; Gostomski, P.A.; Boyd-Wilson, K.S.H.; Ford, C.; Walter, M. Fungal Inoculum Properties and Its Effect on Growth and Enzyme Activity of Trametes versicolor in Soil. *Biotechnol. Prog.* 2005, 21, 377–385. [CrossRef]

59. Wong, D.W.S. Structure and action mechanism of ligninolytic enzymes. *Appl. Biochem. Biotechnol.* 2009, 157, 174–209. [CrossRef]

60. Ghosal, D.; Ghosh, S.; Dutta, T.K.; Ahn, Y. Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *Front. Microbiol.* 2016, 7, 1369. [CrossRef]

61. Aranda, E. Promising approaches towards biotransformation of polycyclic aromatic hydrocarbons with Ascomycota fungi. *Curr. Opin. Biotechnol.* 2016, 38, 1–8. [CrossRef]

62. Covino, S.; D’Annibale, A.; Stazi, S.R.; Cattabeni, T.; Čvaničarová, M.; Stella, T.; Petruccioli, M. Assessment of degradation potential of aliphatic hydrocarbons by autochthonous filamentous fungi from a historically polluted clay soil. *Sci. Total Environ.* 2015, 505, 207–216. [CrossRef]

63. Becarelli, S.; Chicca, I.; Siracusa, G.; la China, S.; Gentini, A.; Lorenzi, R.; Munz, G.; Petroni, G.; Levin, D.B.; Di Gregorio, S. Hydrocarbonoclastic Ascomycetes to enhance co-composting of total petroleum hydrocarbon (TPH) contaminated dredged sediments and lignocellulosic matrices. *New Biotechnol.* 2019, 50, 27–36. [CrossRef]

64. Siracusa, G.; Yuan, Q.; Chicca, I.; Bardi, A.; Spennati, F.; Becarelli, S.; Levin, D.B.; Munz, G.; Petroni, G.; Di Gregorio, S. Mycoremediation of Old and Intermediate Landfill Leachates with an Ascomycete Fungal Isolate, *Lambertella sp.* *Water 2020*, 12, 800. [CrossRef]

65. Zavarzina, A.G.; Lisov, A.A.; Zavarzin, A.A.; Leonovskiy, A.A. Fungal Oxidooreductases and Humification in Forest Soils. In *Bioresour. Technol.* 2014, 133, 283–291. [CrossRef] [PubMed]

66. Loss, E.M.O.; Lee, M.K.; Wu, M.Y.; Martien, J.; Chen, W.; Amador-Noguez, D.; Jefcoate, C.; Remucal, C.; Jung, S.; Kim, S.C.; et al. Cytochrome P450 monoxygenase-mediated metabolic utilization of benzo[a]pyrene by aspergillus species. *mBio* 2019, 10, e00558-19. [CrossRef]

67. Verdin, A.; Lounès-Hadj Sahraoui, A.; Newsam, R.; Robinson, G.; Durand, R. Polycyclic aromatic hydrocarbons storage by halophilic fungus Aspergillus sydowii at hypersaline conditions. *Environ. Sci. Pollut. Res. Int.* 2014, 21, 3515. [CrossRef]

68. Meulenbergh, R.; Rijnaarts, H.H.M.; Doddem, H.J.; Field, J.A. Partially oxidized polycyclic aromatic hydrocarbons show an increased bioavailability and biodegradability. *FEMS Microbiol. Lett.* 1997, 152, 45–49. [CrossRef] [PubMed]

69. Capotorti, G.; Cesti, P.; Lombardi, A.; Guglielmetti, G. Formation of sulfate conjugates metabolites in the degradation of phenanthrene, anthracene, pyrene and benzo[a]pyrene by the ascomycete *Aspergillus terreus*. *Polycycl. Aromat. Compd.* 2005, 25, 197–213. [CrossRef]

70. Chen, W.; Lee, M.K.; Jefcoate, C.; Kim, S.C.; Chen, F.; Yu, J.H. Fungal cytochrome p450 monoxygenases: Their distribution, structure, function, family expansion, and evolutionary origin. *Genome Biol. Evol.* 2014, 6, 1620. [CrossRef]
72. Sepic, E.; Leskovsek, H.; Trior, C. Aerobic bacterial degradation of selected polyaromatic compounds and n-alkanes found in petroleum. J. Chromatogr. 1995, 697, 515–523. [CrossRef]

73. Koma, D.; Hasumi, F.; Yamamoto, E.; Ohla, T.; Chung, S.Y.; Kubo, M. Biodegradation of long-chain n-paraffins from waste oil of car engine by Acinetobacter sp. J. Biosci. Bioeng. 2001, 91, 94–96. [CrossRef]

74. van Hamme, J.D.; Singh, A.; Ward, O.P. Recent Advances in Petroleum Microbiology. Microbiol. Mol. Biol. Rev. 2003, 67, 503–549. [CrossRef]

75. Meng, L.; Li, H.; Bao, M.; Sun, P. Metabolic pathway for a new strain Pseudomonas synxantha LSH-7' : From chemotaxis to uptake of n-hexadecane. Sci. Rep. 2017, 7, 1–13. [CrossRef]

76. Li, X.W.; Liu, Z.P. Microbial biodegradation of petroleum hydrocarbon. Acta Microbiol. Sinica. 2002, 42, 764–767.

77. Das, N.; Chandran, P. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotechnol. Res. Int. 2011, 2011, 1–13. [CrossRef] [PubMed]

78. Warmink, J.A.; Nazir, R.; van Elsas, J.D. Universal and species-specific bacterial “fungiphiles” in the mycospheres of different basidiomycetous fungi. Environ. Microbiol. 2009, 11, 300–312. [CrossRef] [PubMed]

79. Fontaine, S.; Henault, C.; Aamor, A.; Bdouli, N.; Blooër, J.M.G.; Maire, V.; Mary, B.; Revaillet, S.; Maron, P.A. Fungi mediate long-term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol. Biochem. 2011, 43, 86–96. [CrossRef]

80. Blagodatskaya, E.V.; Blagodatsky, S.A.; Anderson, T.H.; Kuzyakov, Y. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. Appl. Soil Ecol. 2007, 30, 1–95. [CrossRef]

81. Milcu, A.; Heim, A.; Ellis, R.J.; Scheu, S.; Manning, P. Identification of General Patterns of Nutrient and Labile Carbon Control on Soil Carbon Dynamics Across a Successional Gradient. Ecosystems 2011, 14, 710–719. [CrossRef]

82. Purahong, W.; Wubet, T.; Lentendu, G.; Schloter, M.; Pecyna, M.J.; Kapturska, D.; Hofrichter, M.; Krüger, D.; Buscot, F. Life in leaf litter: Novel insights into community dynamics of bacteria and fungi during litter decomposition. Mol. Ecol. 2016, 25, 4059. [CrossRef] [PubMed]

83. Scherlach, K.; Hertweck, C. Mediators of mutualistic microbe–microbe interactions. Nat. Prod. Rep. 2018, 35, 303–308. [CrossRef]

84. Ma, B.; Wang, H.; Dsouza, M.; Lou, J.; He, Y.; Dai, Z.; Brookes, P.C.; Xu, J.; Gilbert, J.A. Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. ISME J. 2016, 10, 1891. [CrossRef]

85. Stopnisek, N.; Zuhlke, D.; Carlier, A.; Barberan, A.; Fierer, N.; Becher, D.; Riedel, K.; Eberl, L.; Weisskopf, L. Molecular mechanisms underlying the close association between soil microorganisms and fungi. ISME J. 2015, 10, 253–264. [CrossRef]

86. Chen, R.; Senbayram, M.; Blagodatsky, S.; Myachina, O.; Dittert, K.; Lin, X.; Blagodatskaya, E.; Kuzyakov, Y. Soil C and N availability determine the priming effect: Microbial N mining and stoichiometric decomposition theories. Glob. Chang. Biol. 2014, 20, 2356. [CrossRef] [PubMed]

87. Echter, V.; Brabcová, V.; Etrovský Tý Vomora, M.; López-Mondejar, R.; Oliveira Monteiro, L.M.; Saraiva, J.P.; Human, Z.R.; Cjatham, T.; Nunes Da Rocha, U.; Baldrian, P. Complementary Roles of Wood-Inhabiting Fungi and Bacteria Facilitate Deadwood Decomposition. MSystems 2021, 6, e01078-20. [CrossRef] [PubMed]

88. Banitz, T.; Fetzer, I.; Johst, K.; Wick, L.Y.; Harms, H.; Frank, K. Assessing biodegradation benefits from dispersal networks. Ecol. Model. 2011, 222, 2522. [CrossRef]

89. Tecon, R.; Tecon, R.; Oh, D. Bacterial flagellar motility on hydrated rough surfaces controlled by aqueous film thickness and connectedness. Sci. Rep. 2016, 6, 1–11. [CrossRef]

90. Worrich, A.; König, S.; Miltner, A.; Banitz, T.; Centler, F.; Frank, K.; Thuillier, M.; Harms, H.; Kästner, M.; Wick, L.Y. Mycelium-like networks increase bacterial dispersal, growth, and biodegradation in a model ecosystem at various water potentials. Appl. Environ. Microbiol. 2016, 82, 2902. [CrossRef]

91. Furuno, S.; Soss, S.; Wild, E.; Jones, K.C.; Semple, K.T.; Harms, H.; Wick, L.Y. Mycelia promote active transport and spatial dispersion of polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 2012, 46, 5463. [CrossRef]

92. Schamfuß, S.; Neu, T.R.; van der Meer, J.R.; Tecon, R.; Harms, H.; Wick, L.Y. Impact of mycelia on the accessibility of fluorene to PAH-degrading bacteria. Environ. Sci. Technol. 2013, 47, 6908. [CrossRef]

93. Furuno, S.; Päzolt, K.; Rabe, K.; Neu, T.R.; Harms, H.; Wick, L.Y. Fungal mycelia allow chemotactic dispersal of polycyclic aromatic hydrocarbon-degrading bacteria in water-unsaturated systems. Environ. Microbiol. 2010, 12, 1391. [CrossRef]

94. Rudnick, M.B.; van Veen, J.A.; de Boer, W. Oxalic acid: A signal molecule for fungus-feeding bacteria of the genus Collimonas? Environ. Microbiol. Rep. 2015, 7, 709–714. [CrossRef]

95. Ul Haq, I.; Oliveira da Rocha Calixto, R.; Yang, P.; Maria Pires dos Santos, G.; Barreto-Bergter, E.; Dirk van Elsas, J. Chemotaxis and adherence to fungal surfaces are key components of the behavioral response of Burkholderia terrae BS001 to two selected soil fungi. FEMS Microbiol. Ecol. 2016, 92, fiw164. [CrossRef]

96. Mangwani, N.; Dash, H.R.; Chauhan, A.; Das, S. Bacterial quorum sensing: Functional features and potential applications in biotechnology. J. Mol. Microbiol. Biotechnol. 2012, 22, 215–227. [CrossRef] [PubMed]

97. Yong, Y.C.; Zhong, J.J. Regulation of aromatics biodegradation by rhl quorum sensing system through induction of catechol meta-cleavage pathway. Bioresour. Technol. 2013, 136, 761–765. [CrossRef] [PubMed]

98. Chicca, L; Becarelli, S.; Dariel, C.; Ia China, S.; de Kievet, T.; Petroni, G.; Di Gregorio, S.; Levin, D.B. Degradation of BTEX mixture by a new Pseudomonas putida strain: Role of the quorum sensing in the modulation of the upper BTEX oxidative pathway. Environ. Sci. Pollut. Res. Int. 2020, 27, 36203–36214. [CrossRef] [PubMed]
99. Wongsuk, T.; Pumeesat, P.; Luptertlop, N. Fungal quorum sensing molecules: Role in fungal morphogenesis and pathogenicity. *J. Basic Microbiol.* 2016, 56, 440–447. [CrossRef]

100. Stanley, C.E.; Stöckli, M.; van Swaay, D.; Sabotić, J.; Kallio, P.T.; Künzler, M.; Demello, A.J.; Aebi, M. Probing bacterial–fungal interactions at the single cell level. *Integr. Biol.* 2014, 6, 935–945. [CrossRef]

101. Sztajer, H.; Szafranski, S.P.; Tomasz, J.; Beck, M.; Nimitz, M.; Rohde, M.; Wagner-Döbler, I. Cross-feeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*. *ISME J.* 2014, 8, 2256. [CrossRef]

102. Dixon, E.F.; Hall, R.A. Noisy neighbours: Quorum sensing in fungal–microbial infections. *Cell. Microbiol.* 2015, 17, 1431. [CrossRef]

103. Lindsay, A.K.; Hogan, D.A. *Candida albicans*: Molecular interactions with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Fungal Biol. Rev.* 2014, 28, 85–96. [CrossRef]

104. Medaura, M.C.; Guivernau, M.; Moreno-Ventas, X.; Prenafeta-Boldú, F.X.; Víñas, M. Bioaugmentation of Native Fungi, an Efficient Strategy for the Bioremediation of an Aged Industrially Polluted Soil With Heavy Hydrocarbons. *Front. Microbiol.* 2021, 12, 713. [CrossRef]

105. Chen, C.; Li, T. Bacterial dye-decolorizing peroxidases: Biochemical properties and biotechnological opportunities. *Phys. Sci. Rev.* 2016, 1. [CrossRef]

106. Chen, C.; Shrestha, R.; Jia, K.; Gao, P.F.; Geisbrecht, B.V.; Bossmann, S.H.; Shi, J.; Li, P. Characterization of Dye-decolorizing Peroxidase (DyP) from *Thermomonospora curvata* reveals unique catalytic properties of A-type DyPs. *J. Biol. Chem.* 2015, 290, 23447–23463. [CrossRef] [PubMed]

107. Min, K.; Gong, G.; Woo, H.M.; Kim, Y.; Um, Y. A dye-decolorizing peroxidase from *Bacillus subtilis* exhibiting substrate-dependent optimum temperature for dyes and β-ether lignin dimer. *Sci. Rep.* 2015, 5, 1–8. [CrossRef] [PubMed]

108. Bosma, T.N.P.; Middeldorp, P.J.M.; Schraa, G.; Zehnder, A.J.B. Mass Transfer Limitation of Biotransformation: Quantifying Bioavailability. *Environ. Sci. Technol.* 1996, 31, 248–252. [CrossRef]

109. Semple, K.T.; Reid, B.J.; Fermor, T.R. Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environ. Pollut.* 2001, 112, 269–283. [CrossRef]

110. Tran, H.T.; Lin, C.; Bui, X.T.; Ngo, H.H.; Cheruuyot, N.K.; Hoang, H.G.; Vu, C.T. Aerobic composting remediation of petroleum hydrocarbon-contaminated soil. Current and future perspectives. *Sci. Total Environ.* 2021, 753, 14. [CrossRef]

111. Huang, Y.; Pan, H.; Wang, Q.; Ge, Y.; Liu, W.; Christie, P. Enrichment of the soil microbial community in the bioremediation of a petroleum-contaminated soil amended with rice straw or sawdust. *Chemosphere* 2019, 224, 265–271. [CrossRef]

112. Hawkes, C.V.; Kivlin, S.N.; Rocca, J.D.; Huguet, V.; Thomsen, M.A.; Suttle, K.B. Fungal community responses to precipitation. *Glob. Change Biol.* 2011, 17, 1637. [CrossRef]

113. Jiao, S.; Chen, W.; Wei, G. Resilience and Assemblage of Soil Microbiome in Response to Chemical Contamination Combined with Plant Growth. *Appl. Environ. Microbiol.* 2019, 85, e02523-18. [CrossRef]

114. Lázaroae, M.M. Multiple Responses of Gram-Positive and Gram-Negative Bacteria to Mixture of Hydrocarbons. *Braz. J. Microbiol.* 2010, 41, 649. [CrossRef]

115. Chicca, I.; Becarelli, S.; Bernabei, G.; Siracusa, G.; Di Gregorio, S. Innovative Culturomic Approaches and Predictive Functional Metagenomic Analysis: The Isolation of Hydrocarbonoclastic Bacteria with Plant Growth Promoting Capacity. *Water* 2022, 14, 142. [CrossRef]

116. Ofaim, S.; Ofek-Lalzar, M.; Sela, N.; Jinag, J.; Kashi, Y.; Minz, D.; Freilich, S. Analysis of microbial functions in the rhizosphere using a metabolic-network based framework for metagenomics interpretation. *Front. Microbiol.* 2017, 8, 1606. [CrossRef] [PubMed]

117. Chemerys, A.; Pelletier, E.; Cruaud, C.; Martin, F.; Violet, F.; Jouanneau, Y. Characterization of Novel Polycyclic Aromatic Hydrocarbon Dioxygenases from the Bacterial Metagenomic DNA of a Contaminated Soil. *Appl. Environ. Microbiol.* 2014, 80, 6591–6600. [CrossRef] [PubMed]

118. Röling, W.F.M. Maths on microbes: Adding microbial ecology to metagenomics. *Microb. Biotechnol.* 2015, 8, 21–22. [CrossRef] [PubMed]

119. Rodríguez, A.; Castrejon-Godinez, M.L.; Salazar-Bustamante, E.; Gama-Martínez, Y.; Sánchez-Salinas, E.; Mussali-Galante, P.; Tovar-Sánchez, E.; Ortiz-Hernández, M.L. Omics Approaches to Pesticide Biodegradation. *Curr. Microbiol.* 2020, 77, 545–563. [CrossRef]

120. Plewniak, F.; Crognale, S.; Rosssetti, S.; Bertin, P.N. A genomic outlook on bioremediation: The case of arsenic removal. *Front. Microbiol.* 2018, 9, 820. [CrossRef]

121. Bilal, T.; Malik, B.; Hakeem, K.R. Metagenomic analysis of uncultured microorganisms and their enzymatic attributes. *J. Microbiol. Methods* 2018, 155, 65–69. [CrossRef]

122. Pacwa-Plonciuczak, M.; Baniecka, P.; Bondarczuk, K.; Piotrowska-Geget, Z. Metagenomic Functional Profiling Reveals Differences in Bacterial Composition and Function During Bioaugmentation of Aged Petroleum-Contaminated Soil. *Front. Microbiol.* 2020, 11, 2106. [CrossRef]

123. Yergeau, E.; Sanschagrin, S.; Beaumier, D.; Greer, C.W. Metagenomic analysis of the bioremediation of diesel-contaminated Canadian high arctic soils. *PLoS ONE* 2012, 7, e30058. [CrossRef]
124. Jacquiod, S.; Demanèche, S.; Franqueville, L.; Ausec, L.; Xu, Z.; Delmont, T.O.; Dunon, V.; Cagnon, C.; Mandic-Mulec, I.; Vogel, T.M.; et al. Characterization of new bacterial catabolic genes and mobile genetic elements by high throughput genetic screening of a soil metagenomic library. *J. Biotechnol.* 2014, 190, 18–29. [CrossRef]

125. El Hadidi, M.; Ruscheweyh, H.J.; Huson, D. Improved metagenome analysis using MEGAN5. In Proceedings of the Joint 21st Annual International Conference on Intelligent Systems for Molecular Biology (ISMB) and 12th European Conference on Computational Biology (ECCB), Berlin, Germany, 21–23 July 2013.

126. Seshadri, R.; Kravitz, S.A.; Smarr, L.; Gilna, P.; Frazier, M. CAMERA: A Community Resource for Metagenomics. *PLoS Biol.* 2007, 5, e75. [CrossRef]

127. Sun, S.; Chen, J.; Li, W.; Altintas, I.; Lin, A.; Peltier, S.; Stocks, K.; Allen, E.E.; Ellisman, M.; Grethe, J.; et al. Community cyberinfrastructure for Advanced Microbial Ecology Research and Analysis: The CAMERA resource. *Nucleic Acids Res.* 2011, 39, D546–D551. [CrossRef] [PubMed]

128. Meyer, F.; Paarmann, D.; D’Souza, M.; Olson, R.; Glass, E.M.; Kubal, M.; Paczian, T.; Rodriguez, A.; Stevens, R.; Wilke, A.; et al. The metagenomics RAST server—A public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinform.* 2008, 9, 1–8. [CrossRef] [PubMed]

129. Douglas, G.M.; Maffei, V.J.; Zaneveld, J.R.; Yurgel, S.N.; Brown, J.R.; Taylor, C.M.; Huttenhower, C.; Langille, M.G.I. PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* 2020, 38, 685–688. [CrossRef] [PubMed]

130. Mukherjee, A.; Chettri, B.; Langpoklakpam, J.S.; Basak, P.; Prasad, A.; Mukherjee, A.K.; Bhattacharyya, M.; Singh, A.K.; Chattopadhyay, D. Bioinformatic Approaches Including Predictive Metagenomic Profiling Reveal Characteristics of Bacterial Response to Petroleum Hydrocarbon Contamination in Diverse Environments. *Sci. Rep.* 2017, 7, 1–22. [CrossRef] [PubMed]

131. Cai, P.; Ning, Z.; Ning, Z.; Liu, Y.; He, Z.; He, Z.; Shi, J.; Niu, M. Diagnosing bioremediation of crude oil-contaminated soil and related geochemical processes at the field scale through microbial community and functional genes. *Ann. Microbiol.* 2020, 70, 1–15. [CrossRef]