APPLIED AND INDUSTRIAL MICROBIOLOGY

Untapped potential: exploiting fungi in bioremediation of hazardous chemicals

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Abstract | Fungi possess the biochemical and ecological capacity to degrade environmental organic chemicals and to decrease the risk associated with metals, metalloids and radionuclides, either by chemical modification or by influencing chemical bioavailability. Furthermore, the ability of these fungi to form extended mycelial networks, the low specificity of their catabolic enzymes and their independence from using pollutants as a growth substrate make these fungi well suited for bioremediation processes. However, despite dominating the living biomass in soil and being abundant in aqueous systems, fungi have not been exploited for the bioremediation of such environments. In this Review, we describe the metabolic and ecological features that make fungi suited for use in bioremediation and waste treatment processes, and discuss their potential for applications on the basis of these strengths.

Industrial processes, agricultural practices and the use of chemicals in many areas of our daily lives result in the deliberate or accidental release of potentially toxic chemicals into the environment. After their release, these chemicals can be transported through the atmosphere and water and, in many cases, find their way into sediments and soils. Environmental chemicals of particular concern include petroleum hydrocarbons, halogenated solvents from industrial sources, endocrine-disrupting agents and drugs, explosives, agricultural chemicals, heavy metals, metalloids and radionuclides. To become biologically degraded or detoxified, environmental chemicals need to be exposed to appropriate microbial catalysts. In heterogeneous environments, this is impeded by the tendency of many organic and inorganic chemicals to escape the aqueous microhabitats of degrader organisms. Chemicals precipitate, adsorb to surfaces or accumulate in organic matter and in tiny pores of solid matrices, leading to a decline in their bioavailability[1]. Such accumulation often occurs in inhospitable, toxic environments that lack the nutrients, water or appropriate electron acceptors that would be needed to support the growth of microorganisms capable of remediation. The ideal decontamination machinery would be able to cope with these conditions, and could explore the contaminated environment thoroughly and track chemicals even into pores and organic matter. The catabolic capacity of this machinery would not rely on the availability of the pollutants as substrates, but would be maintained by other compounds or by trophic interactions with plants instead. It would also have to be active in a wide range of conditions, including extreme (dry, toxic or acidic) environments. Finally, this ideal machinery would transport water, electron acceptors, essential nutrients and catabolically active organisms to contaminated spots.

Fungi, alone or in collaboration with bacteria and plants, display many of these features and could be important components of biotechnologies designed to remediate polluted soil, water and air. To date, bioremediation by fungi has not been an unfettered success story, as it tends to disregard the ecological demands of fungi and often uses ecologically displaced organisms in competition with bacteria more suited to the polluted environment. As such, the potential use for fungi in bioremediation and waste treatment has not received the attention merited by the extensive metabolic capabilities of these organisms.

The aquatic and terrestrial habitats of fungi (FIG. 1) are heavily exposed to anthropogenic chemicals, of which the organics are inherently suitable as heterotrophic substrates, and the metals may share features of natural micronutrients. These environmental chemicals are therefore subject to fungal activities[2,3]. In this Review,

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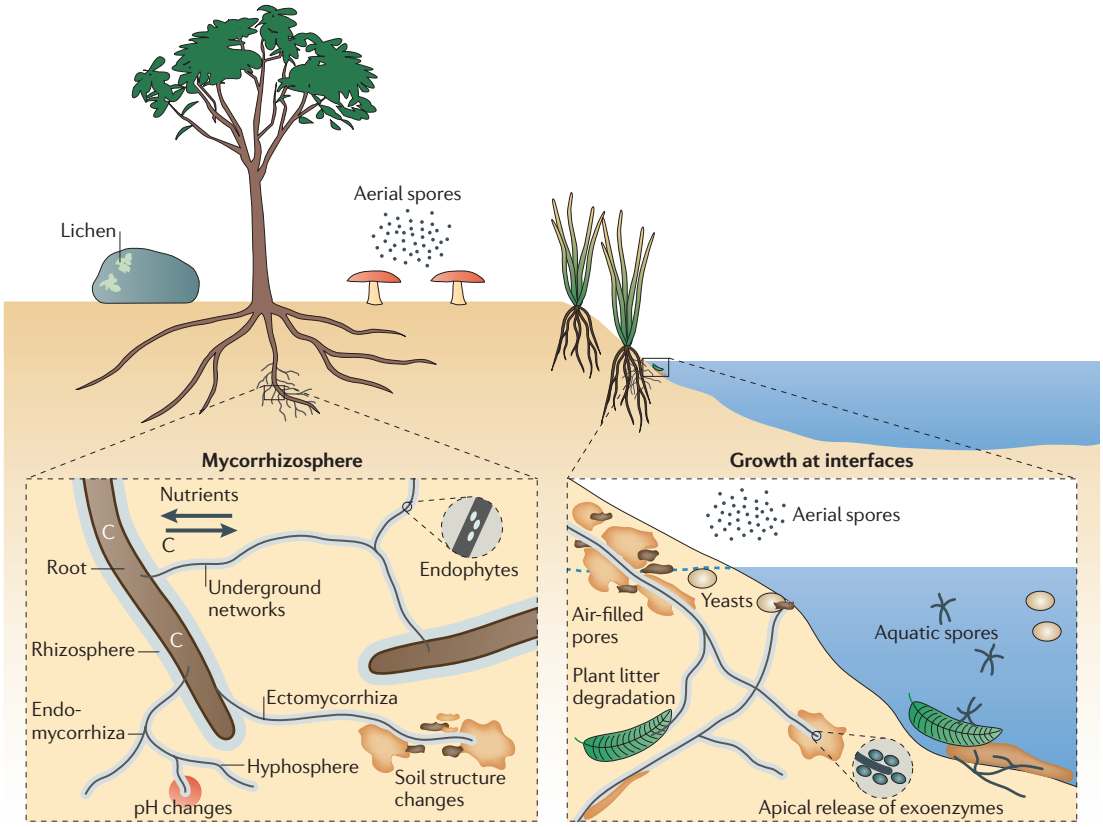

Figure 1 | **Typical habitats of terrestrial and aquatic fungi, and some of their ecological features.** Fungi in the environment can exist as terrestrial and aquatic saprobes, yeasts, symbiotic partners of lichens, and mycorrhizal symbionts associated with plant roots. They can form ectomycorrhizal and endomycorrhizal associations and can influence soil structure (by enmeshment of particles), soil chemistry (by excretion of acids, for example) and plant growth (through the mobilization and provision of nutrients in exchange for photosynthetic assimilates). Saprotrophic fungi adapted to water-unsaturated soil or aqueous environments can propagate through spores adapted to dispersal through the atmosphere or water, respectively. Growing hyphae explore food sources by deploying extracellular enzymes (exoenzymes).

we introduce the fungal way of life and its function in ecosystems. We then discuss the important groups of fungi that act on environmental chemicals, and describe the enzymatic and genetic mechanisms behind these activities. Finally, we identify the unique characteristics of fungi that qualify them for pollutant degradation and bioremediation, and propose several fields of environmental biotechnology in which the use of fungi promises to be particularly effective.

Occurrence and ecological features of fungi

The kingdom Fungi includes species that live as moulds, mushrooms, lichens, rusts, smuts and yeasts — eukaryotes with remarkably diverse life cycles[4]. Fungi exist as saprobes, parasites or mutualists of, for example, plants (mycorrhizae) or algae (lichens). Less than 100,000 of the 1.5 million estimated fungal species have been described[4]. Fungi have been defined as eukaryotic, heterotrophic, absorptive organisms that typically develop a branched, tubular body called a mycelium and reproduce by means of sporulation[2].

Filamentous fungi have evolved a spatially extensive growth form of thread-like hyphae, which maintain a highly polarized internal cellular organization, supporting apical growth[5]. Despite the microscopic diameters of these hyphae (2–10 μm), some of the largest living organisms on earth are fungi with networks extending over hundreds of hectares[6]. Accordingly, fungi can be regarded as 'macroorganisms packaged in microscopic units' (REF. 7); that is, they exhibit a unique lifestyle that is adapted to heterogeneous environments.

Soil facilitates the development of filamentous fungi because it experiences only little mechanical disturbance by shear forces that would potentially disrupt fungal mycelia. Fungi account for up to 75% of the soil microbial biomass (total soil microbial biomass is 50–1,000 μg per g dry weight or 2–45 t per Ha), and the length of hyphae can be up to 10^2 m per g, 10^3 m per g and 10^4 m per g for arable, pasture and forest topsoils, respectively[8]. Some fungi tolerate extreme environmental conditions (temperatures of –5 to +60 °C; pH of 1 to 9) and grow at a water activity of only 0.65, or with 0.2% oxygen[2]. Unlike bacteria, which are immobilized at matric potentials of <–50 kPa[9], fungi do not require continuous water phases for active dispersal. Their hyphae grow across air–water interfaces, bridge air-filled soil

Saprobe
A heterotrophic organism that feeds on dead or decaying organic material.

Water activity
A measure of the water (in a substrate) that is available for microbial growth, expressed as the decimal fraction of the amount of water present when the substrate is in equilibrium with a saturated atmosphere.

Matric potential
The force (measured in units of negative pressure) that the soil exerts on water owing to capillary and adsorptive forces.

pores, grow into soil pores with a diameter as little as 2 μm[7] and even penetrate rock matrices[10]. The morphology of mycelia reflects an effective foraging strategy, combining scattered explorative expansion under poor nutrient conditions with massive exploitative growth in optimal environments[8]. Mycelia influence soil structure through electrostatic, adhesive and enmeshment mechanisms[11] and organic matter decomposition[12]. They also affect water infiltration properties of soils by producing large quantities of hydrophobic compounds such as hydrophobins[13]. An important feature of fungi is the secretion of oxidative exoenzymes at their constantly lengthening hyphal tips. Saprobic fungi exploiting cellulose and lignin have key roles in global nutrient cycling. Their ubiquity and dominance in many environments indicates that they process enormous quantities of plant debris[12], although the fungal contribution to the global cycling of carbon and nitrogen is not exactly known. Transformations by fungal exoenzymes also provide substrates to catabolically interacting 'fungiphilic' bacteria in the hyphosphere[14,15].

To maintain their integrity despite damage and predation, mycelial networks transport nutrients between spatially separated source and sink regions[16]. The organization of fungal networks has parallels with that of other foraging biological systems (for example, plant roots) and man-made infrastructures such as public suburban commuter railway systems[17]. Both diffusive and active translocation of compounds occur in fungal hyphae[18,19]. Mycorrhizal symbioses rely on the effective fungal uptake of mineral nutrients from the soil and their transfer to the plant symbiont[18] in exchange for photosynthates accounting for 10–30% of the host plant's net carbon fixation[20]. Isotopic labelling has shown that fungi that form arbuscular mycorrhizae (AM) actively translocate plant-derived lipids (triacylglycerol) to the extra-radical mycelium at speeds of up to 11 mm per s, or at a rate of up to 1.3 mg lipids per h per runner hypha[21]. Mycorrhizae contribute up to 25% of the nitrogen absorbed by plants, as well as 80% of the phosphorus, 10% of the potassium, 25% of the zinc and 60% of the copper[20]. Little is known about translocation of substances in non-mycorrhizal fungi, but it is suspected that all fungi use similar mechanisms. Bidirectional intravacuolar transport occurs over cm distances[19] and permits solute transport even against the mass flow that effects the turgor-driven tip extension of hyphae[22]. Uptake of hydrophobic organic contaminants[23] and an estimated contaminant transport rate as high as 5 ng per h by a single runner hypha have also been described[24]. Further, fungal mycelia have been found to host specific endomycotic bacteria[14] and to facilitate the movement of extra-hyphal bacteria in air-filled pores[25,26].

Fungal diversity and pollutant catabolism

Fungi transform a large range of organic pollutants[27–31]. In the kingdom Fungi (estimated to contain 80,000–100,000 described species)[4,32], most pollutant degraders belong to the phyla Ascomycota and Basidiomycota, followed by the subphylum Mucoromycotina (FIG. 2). There are very few documented examples of degradation capabilities for other fungi (FIG. 2).

Meiosporic ascomycetes and mitosporic ascomycetes, the largest fungal group in terrestrial and aquatic environments[33], account for roughly 64% of all described fungi[4]. Numerous members of the subphylum Pezizomycotina, the most diverse group of the filamentous ascomycetes[4], attack environmental organic chemicals (FIG. 2). For instance, species of the genera *Cladophialophora* and *Exophiala* (of the order Chaetothyriales) assimilate toluene[29]. *Aspergillus* and *Penicillium* spp. (of the order Eurotiales) degrade aliphatic hydrocarbons, chlorophenols, polycyclic aromatic hydrocarbons (PAHs), pesticides, synthetic dyes and 2,4,6-trinitrotoluene (TNT)[3,31,33–35]. Metabolization of polychlorinated dibenzo-*p*-dioxins (PCDDs) is reported for the genera *Cordyceps* and *Fusarium* (of the order Hypocreales), as well as for *Pseudallescheria* spp. (of the order Microascales)[30,31]. The mitosporic *Acremonium* spp. degrade PAHs and Royal Demolition Explosive (RDX)[30,31], and *Graphium* spp. degrade methyl-*tert*-butylether (MTBE)[36]. Outside of the Pezizomycotina, *Phoma* spp. degrade PAHs, pesticides and synthetic dyes[3,31,37]. The subphylum Saccharomycotina (FIG. 2) mostly consists of yeasts and includes degraders of *n*-alkanes, *n*-alkylbenzenes, crude oil, the endocrine disrupting chemical (EDC) nonylphenol, PAHs and TNT (in the genera *Candida*, *Kluyveromyces*, *Neurospora*, *Pichia*, *Saccharomyces* and *Yarrowia*)[3,34,35,38–40].

The phylum Basidiomycota accounts for about 34% of all described fungal species[4] (FIG. 2). Basidiomycetes mostly inhabit terrestrial environments and are rare in aquatic habitats[41]. Filamentous wood- and soil litter-decaying members of the subphylum Agaricomycotina (FIG. 2) exploit nonspecific oxidative exoenzymes used in lignin and lignocellulose decomposition to mineralize numerous organic chemicals co-metabolically — that is, in the presence of an additional substrate serving as a carbon and energy source. For instance, wood-inhabiting white-rot fungi such as *Phanerochaete chrysosporium* (in the order Corticiales), *Nematoloma* spp. and *Pleurotus* spp. (both in the order Agaricales), and *Trametes* spp. (in the order Polyporales) mineralize various chloroaromatics, PAHs and TNT[3,28,34,42,43]. Soil-dwelling, litter-decomposing agaric basidiomycetes such as *Agrocybe* spp. and *Stropharia* spp. are also known to mineralize organic pollutants[34,44,45]. Though mineralization of environmental chemicals is less common in brown-rot basidiomycetes, *Gloeophyllum* spp. (in the order Gloeophyllales) mineralize chlorophenols and fluoroquinolone antibiotics[43,46]. The basidiomycete subphylum Pucciniomycotina (FIG. 2) contains mitosporic yeasts of the genus *Rhodotorula*, which metabolize cresols, crude oil constituents, PAHs and RDX, whereas other genera degrade crude oil and dibenzothiophene[35,47–50]. The subphylum Mucoromycotina (FIG. 2), part of the basal fungal lineage, accounts for less than 1% of all described fungi[4]. In this subphylum, the genera *Cunninghamella*, *Mucor* and *Rhizopus* (members of the order Mucorales) include degraders of PAHs, pesticides, textile dyes and TNT[3,31,34,35].

The potential roles of mycorrhizal associations for bioremediation of organic chemicals have primarily been addressed with ectomycorrhizae (ECM). The fungal symbionts of ECM belong predominantly to the

Hydrophobin
One of a class of small, cysteine-rich proteins that are secreted by filamentous fungi and that self-assemble at hydrophilic–hydrophobic interfaces into an amphipathic membrane.

Exoenzyme
An enzyme that is secreted by a cell and that is usually used for breaking up large molecules that would otherwise be unable to enter the cell.

Photosynthate
A chemical (and its biogenic derivatives) that is produced by photosynthesis.

Meiosporic ascomycete
A member of the phylum Ascomycota that undergoes sexual reproduction, in which haploid meiospores produced by meiosis serve as propagules.

Mitosporic ascomycete
A member of the phylum Ascomycota that can exist in an asexual reproductive state (anamorph), using diploid mitospores produced by mitosis as propagules, or a sexual reproductive state (teleomorph).

Agaric basidiomycete
A basidiomycete of the order Agaricales, having a stem with an umbrella-like cap containing lamellae (gills) on the underside; commonly called a mushroom.

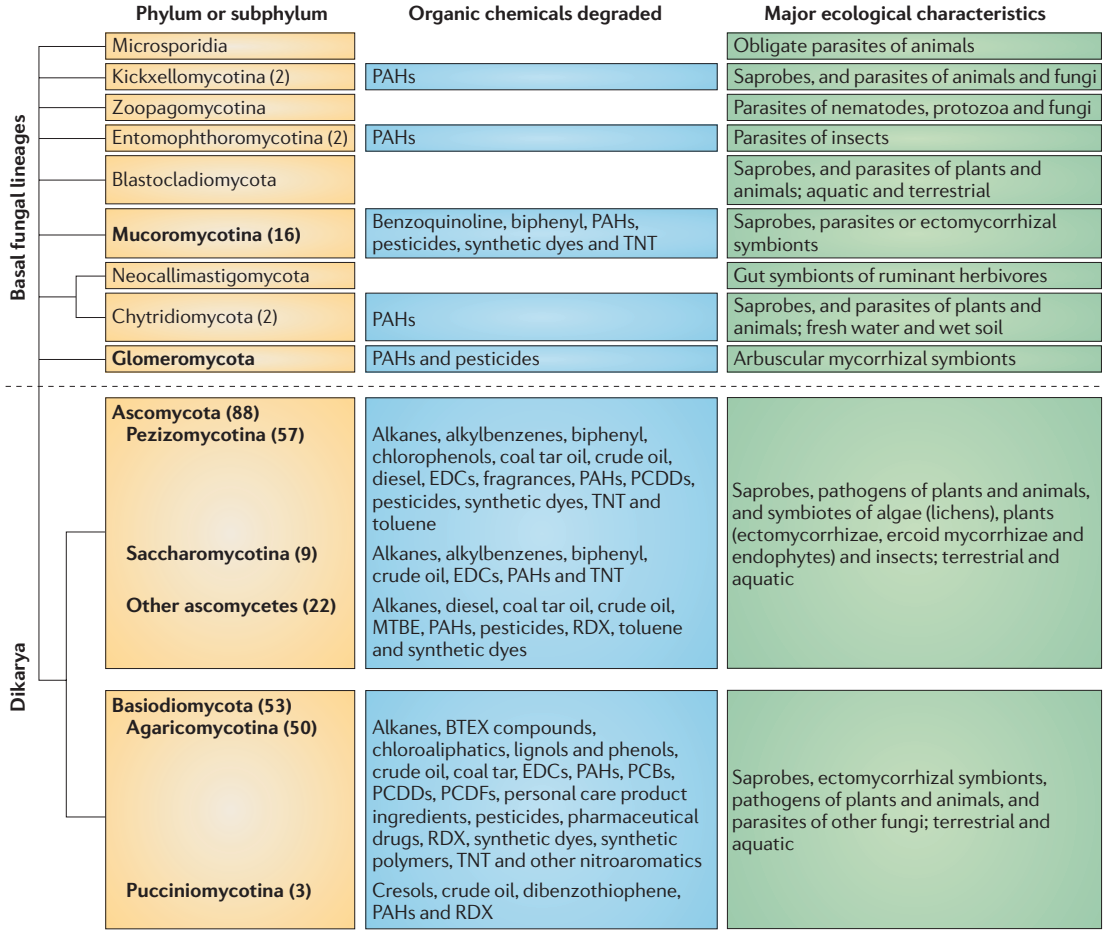

Figure 2 | **Major organic chemicals degraded by various fungal phyla and subphyla.** The classification of Fungi[136], the major organic chemicals that are degraded by various fungal phyla and subphyla[3,27–31,33–40,42–59,74,75,82–86,88,89,91,93,94,101,102,105–107], and the important ecological characteristics[4] of these fungi. Taxa that are relevant for the bioremediation of organic chemicals are bold. The less relevant ascomycete and basidiomycete subphyla *Taphrinomycotina* and *Ustilaginomycotina*, respectively, are omitted. The number of genera in each taxonomic group with alkane, biphenyl, coal tar, coal tar oil, crude oil, dibenzothiophene, diesel, polycyclic aromatic hydrocarbon (PAH) and toluene degraders are given in parentheses[35]. The placement of genera and species into the respective phyla and subphyla was aided by the National Center for Biotechnology Information (NCBI) Taxonomy browser (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?). Basal fungal lineages is the collective term for all fungi excluding the Dikarya. BTEX, benzene, toluene, ethylbenzene and xylenes; EDCs, endocrine disrupting chemicals; MTBE, methyl-*tert*-butylether; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; RDX, Royal Demolition Explosive; TNT, 2,4,6-trinitrotoluene.

Basidiomycota and less frequently to the Ascomycota (FIG. 2). ECM involve about 10,000 fungal and 8,000 plant species[51–53]. Axenic cultures of ECM fungi degrade various chloroaromatics, PAHs and TNT, and enhanced degradation was occasionally observed when these fungi were grown in symbiosis with plants[52,53]. AM involve as many as 250,000 plant species. About 150 to 200 fungal species belonging to the phylum Glomeromycota (FIG. 2) have been identified as AM symbionts, but the diversity of AM symbionts may be much higher[53]. The scant data suggest that there is enhanced dissipation of organic pollutants such as PAHs and atrazine in the presence of AM[53,54]. Ericoid mycorrhizae are formed by around 3,400 plant species of the order Ericales with various ascomycetes (FIG. 2). Axenic cultures of ericoid mycorrhizal fungi have been shown to degrade chloroaromatic herbicides[53].

Axenic culture
A culture in which an organism grows alone, with no other organisms (hosts, symbionts or parasites) present.

Catabolic enzymes and degradation

The low specificity of many fungal enzymes enables the organisms producing them to co-metabolize structurally diverse compounds belonging to different pollutant classes, the most prominent example for this being *Phanerochaete chrysosporium* degrading benzene, toluene, ethylbenzene and xylenes (BTEX) compounds, nitroaromatic and *N*-heterocyclic explosives (TNT and RDX, respectively), organochlorines (chloroaliphatics, chlorolignols, chlorophenols, polychlorinated biphenyls and PCDDs), PAHs, pesticides, synthetic dyes and synthetic polymers[28,55,56]. Moreover, structurally diverging representatives of a particular pollutant class (for example, various low- and high-molecular-mass PAHs and different PCDD congeners) can be degraded by the same fungal organism[3,57,58], even in mixture[59].

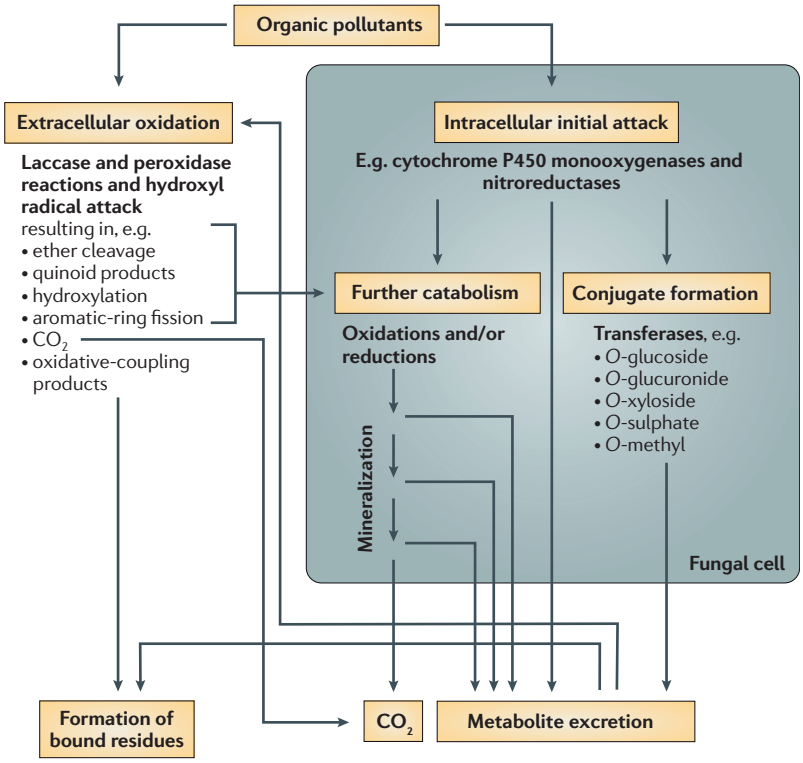

Figure 3 | **Principal methods used by fungi to degrade organic chemicals.** Although fungi primarily co-metabolize organic pollutants, they do grow on some aliphatic or aromatic compounds, including volatile organics[3,29,38,39,137]. Initial pollutant attack may occur extracellularly or intracellularly. Metabolites generated during extracellular pollutant oxidation may be subject to intracellular catabolism or may form bound residues of soil constituents. Metabolites arising from intracellular initial attack may be excreted and can then either undergo further extracellular enzymatic reactions or form bound residues through abiotic oxidative coupling. They may also be secreted in the form of conjugates (which usually persist) or may undergo further intracellular catabolism. This may result in mineralization or, again, in metabolite excretion at various oxidation stages if subsequent oxidation is impeded.

Extracellular oxidoreductases. A unique characteristic of fungi is their ability to attack organic compounds using a range of extracellular oxidoreductases with relatively nonspecific activities (FIG. 3; TABLE 1). These enzymes have probably evolved to support fungal growth on recalcitrant substrates of random structure such as lignocellulose — substrates that are not accessible for most bacteria — through the depolymerization and removal of lignin and its humic substance derivatives[28,60,61]. Hence, these enzymes give fungi an ecological advantage over bacteria in particular decomposition niches[61].

Laccases are copper-containing oxidase enzymes that are widespread among basidiomycetes and ascomycetes. They use molecular oxygen to oxidize pollutants and frequently occur as multiple isoenzymes[62] (TABLE 1). Potential applications of laccases related to bioremediation and waste treatment include: the decolorization and detoxification of dye-containing effluents from the textile and dyestuff industries, of bleach plant effluents from the pulp and paper industries, of olive mill wastes and of coffee pulp; the degradation and detoxification of recalcitrant wastewater pollutants (chlorophenols, EDCs, PAHs, pesticides and others) and hazardous compounds arising from coal processing (sulphur-containing compounds and phenols); and the immobilization of soil pollutants by coupling them to soil humic substances[62–66]. Small, diffusible redox mediators considerably expand the substrate range of laccases[62,63] (TABLE 1). Genetic engineering is another strategy that can be used to improve the catalytic features of laccases and to enable the cost-efficient production of laccases in homologous or heterologous hosts; ascomycetes are more amenable to genetic manipulation than basidiomycetes[65]. Other copper-containing oxidases such as tyrosinases oxidize phenols (TABLE 1), including those that are highly chlorinated, and hence have a potential application in the removal of such compounds from contaminated media; ongoing attempts in this area are aiming at large-scale enzyme production through the development of homologous or heterologous expression systems[67].

The lignin-modifying class II haem peroxidases manganese peroxidase (MnP), lignin peroxidase and versatile peroxidase, as well as the recently revisited dye-decolorizing haem peroxidases (DyP) and the newly discovered peroxygenases of the haem–thiolate peroxygenase–peroxidase superfamily, are produced by basidiomycetes and oxidize pollutants with high redox potentials[68–71] (TABLE 1). Typically, multiple genes encode the basidiomycete peroxidase isoenzymes produced during idiophasic growth[68–72]. Other peroxidases such as *Caldariomyces fumago* haem–thiolate chloroperoxidase (CPO) and *Coprinopsis cinerea* peroxidase (CiP) act on phenols and other pollutants with lower redox potentials[70] (TABLE 1). Similar to laccases and tyrosinases, extracellular peroxidases are being studied as biocatalysts for degrading chlorophenols and other phenols, EDCs, PAHs, pesticides, and synthetic dyes in waste streams, for example, and for the treatment of effluents from pulp and cotton mills, food-processing and fruit-processing plants, and breweries[64,66,68,70]. Genetic engineering of peroxidases to improve their catalytic properties allows enhancement of the H_2O_2 resistance of the enzymes, or the structural modification of already commercially available enzymes to enable the oxidation of other compounds with high redox potentials[68]. Although efficient production of recombinant CiP, CPO and DyP, and of wild-type CPO has been established, ongoing efforts concentrate on improving the recombinant expression of other peroxidases[68–70].

Laccases and typical peroxidases produce organic radicals through one-electron abstractions from organic substrates. The radicals released can undergo various spontaneous follow-up reactions, including ether cleavage in dioxins[55], quinone formation from PAHs and chlorophenols[3,73,74], and the abiotic oxidative coupling of EDCs (nonylphenol and bisphenol A) or PAHs[73,75,76] (FIG. 3). Radical reactions may also covalently bind metabolites (for example, of TNT) to soil organic matter[27,77] (FIG. 3). MnP can cleave aromatic rings of monoaminodinitrotoluenes and chlorophenols even in the absence of cells, and can produce substantial amounts of CO_2 from these compounds (FIG. 3), whereas efficient oxidation of TNT and PAHs requires the additional presence

One-electron abstraction
Oxidation of a compound (the electron-donating substrate) through the removal of one electron, which is then transferred to an electron acceptor.

Abiotic oxidative coupling
Spontaneous chemical oxidation of an organic compound (for example, by air oxygen or by oxidized forms of transition metals such as manganese and iron), leading to the formation of oligomeric or polymeric coupling products.

Table 1 | **Major classes of enzymes involved in the fungal catabolism of organic pollutants***

Enzymes (ExPASy ENZYME accession‡)	Fungal taxa	Localization and occurrence§	Reaction mechanism	Comments	Refs
Laccases (EC 1.10.3.2)‖¶	Ascomycota and Basidiomycota	Extracellular	• O_2-dependent one-electron oxidation of organic compounds	• Redox potential of around 0.4–0.8 V • Direct oxidation of various phenols, aromatic amines and anthraquinone dyes • A wide range of pollutants oxidized in the presence of natural and synthetic redox mediators • Activity mostly in the acidic and rarely in the neutral or alkaline pH range	62,63
Tyrosinases (EC 1.14.18.1)	Ascomycota, Basidiomycota and Mucoromycotina	Sometimes extracellular but mainly intracellular	• O_2-dependent hydroxylation of monophenols to *o*-diphenols (cresolase activity) • Oxidation of *o*-diphenols to catechols (catecholase activity)	• Oxidation of various phenols, including those that are highly chlorinated • Activity from the acidic to the alkaline pH range	67,87
Lignin peroxidases (EC 1.11.1.14)‖¶	Basidiomycota	Extracellular	• H_2O_2-dependent one-electron oxidation of aromatic compounds	• Redox potential of 1.4–1.5 V • Direct oxidation of various aromatics with high redox potentials, but rapid inactivation during oxidation of phenols • Direct oxidation of PAHs with an ionization potential of ≤7.55 eV • Extended substrate range (including dyes with high redox potentials and phenols) in the presence of the redox mediator veratryl alcohol • Activity in the acidic pH range	68,70, 150
Manganese peroxidases (EC 1.11.1.13)‖¶	Basidiomycota	Extracellular	• H_2O_2-dependent one-electron oxidation of Mn^{2+} to Mn^{3+}, which subsequently oxidizes organic compounds	• Redox potential of 1.0–1.2 V • Mn^{3+}-mediated oxidation of various phenols and aromatic amines • Extended substrate range in the presence of co-oxidants (organic SH-containing compounds, unsaturated fatty acids and their derivatives) • Activity in the acidic pH range	60,68, 70
Versatile peroxidases (EC 1.11.1.16)‖	Basidiomycota	Extracellular	• H_2O_2-dependent direct one-electron oxidation of aromatic compounds • H_2O_2-dependent one-electron oxidation of Mn^{2+} to Mn^{3+}, which subsequently oxidizes organic compounds	• Redox potential of around 1.4–1.5 V • Direct oxidation of phenols and aromatics with high redox potentials, including dyes • Mn^{3+}-dependent reactions as for manganese peroxidase • Activity in the acidic pH range	68–70
Coprinopsis cinerea peroxidase (EC 1.11.1.7)‖¶	Basidiomycota	Extracellular	• H_2O_2-dependent one-electron oxidation of aromatic compounds	• Redox potential of around 0.9–1.1 V • Direct oxidation of phenols and dyes with low redox potentials • Activity from the acidic to the alkaline pH range	70,72
Dye-decolorizing peroxidases (EC 1.11.1.x)‖	Basidiomycota	Extracellular	• H_2O_2-dependent one-electron oxidation of organic compounds • Additional hydrolysing activity	• Redox potential of around 1.2–1.5 V • Oxidation of anthraquinone dyes with high redox potentials (only rarely oxidized by other peroxidases) • Highly stable at high pressure, high temperature and very low pH • Activity in the acidic pH range	70
Caldariomyces fumago haem–thiolate chloroperoxidase (EC 1.11.1.10)‖¶	Ascomycota	Extracellular	• H_2O_2-dependent halogenation of organic compounds in the presence of halides (one-electron transfer) • H_2O_2-dependent one-electron oxidations of phenols and anilines in the absence of halides • H_2O_2-dependent peroxygenation (two-electron oxidation), leading to epoxidation of (cyclo)alkenes, hydroxylation of benzylic carbon and sulphoxidation of S-containing organic compounds	• Redox potential not known • No activity on non-substituted aromatic rings and *n*-alkanes • Activity in the acidic pH range	70

Table 1 (cont.) | **Major classes of enzymes involved in the fungal catabolism of organic pollutants***

Enzymes (ExPASy ENZYME accession‡)	Fungal taxa	Localization and occurrence§	Reaction mechanism	Comments	Refs
Haem–thiolate peroxygenases‖	Basidiomycota	Extracellular	• H_2O_2-dependent peroxygenation of aromatic, aliphatic and heterocyclic compounds, leading to aromatic and alkylic carbon hydroxylation, double-bond epoxidation, ether cleavage, sulphoxidation or *N*-oxidation reactions (depending on the substrate); • H_2O_2-dependent one-electron abstractions from phenols; • H_2O_2-dependent bromination of organic substrates	• Redox potential not known • Peroxygenation of various monoaromatic to polyaromatic pollutants, including PAHs, dibenzofuran, and monohydroxylated and polyhydroxylated products • Ether bond cleavage between aromatic and aliphatic parts of molecules and in alicyclic and aliphatic ethers (for example, MTBE) • Activity from the acidic to the alkaline pH range	70
Cytochrome P450 monooxygenases‖#	Ascomycota, Basidiomycota, Mucoromycotina and Chytridiomycota	Cell bound	• Incorporation of a single atom from O_2 into a substrate molecule, with concomitant reduction of the other atom to H_2O	• Epoxidation and hydroxylation of aromatic or aliphatic structures of many pollutants, including PAHs, PCDDs, alkanes and alkyl-substituted aromatics	3, 80–82, 87
Phenol 2-monooxygenases (EC 1.14.13.7)	Ascomycota and Basidiomycota	Cell bound	• Incorporation of a single atom from O_2 into a substrate molecule, with concomitant reduction of the other atom to H_2O	• *Ortho*-hydroxlyation of various (halo) phenols to the corresponding catechols	33,87
Nitroreductases‖	Ascomycota and Basidiomycota and Mucoromycotina	Cell bound	• NAD(P)H-dependent reduction of nitroaromatics to hydroxylamino and amino(nitro) compounds, and of nitro functional groups of N-containing heterocycles	• Reduction of TNT to hydroxylamino-dinitrotoluene and amino-dinitrotoluenes • Formation of mononitroso derivatives and ring cleavage products from cyclic nitramine explosives • Widespread among fungi	34, 90–94
Quinone reductases‖	Basidiomycota	Cell bound	• NAD(P)H-dependent reduction of quinones	• Functions in quinone detoxification, in the conversion of quinones arising from extracellular pollutant oxidation into substrates for extracellular and intracellular oxidoreductases, and in pollutant attack by hydroxyl radicals arising from quinone redox cycling • Occurrence in white-rot and brown-rot basidiomycetes	95–98, 102
Reductive dehalogenases‖	Basidiomycota and perhaps Ascomycota	Cell bound	• Two-component system comprising a membrane-bound glutathione *S*-transferase that produces glutathionyl conjugates with concomitant chlorine removal, and a soluble glutathione conjugate reductase that releases reductively dechlorinated compounds	• Reductive dechlorination of chlorohydroquinones arising from chlorophenol degradation and of diphenyl ether herbicides (basiodiomycetes) • Perhaps responsible for reductive dechlorination of chlorocatechols arising from PCDD degradation (ascomycetes)	58,86, 102, 104
Miscellaneous transferases	Ascomycota, Basidiomycota and Mucoromycotina	Cell bound	• Formation of glucoside, glucuronide, xyloside, sulphate or methyl conjugates from hydroxylated compounds	• Phase II enzymes are prominent in fungal PAH metabolism but also act on other pollutants • Widespread among fungi	3,89

MTBE, methyl-*tert*-butylether; PAH, polycyclic aromatic hydrocarbon; PCDD, polychlorinated dibenzo-*p*-dioxin; TNT, 2,4,6-trinitrotoluene. *See Supplementary information S1 (table) for an extended version of this table, additionally exemplifying known enzyme genes and providing information about the regulation of catabolic-enzyme production in fungi. ‡See the ExPASy ENZYME database. §Further sequence information related to extracellular enzymes can be found in the Fungal Oxidative Lignin Enzymes (FOLy) database (laccases, and lignin, manganese, versatile and generic peroxidases) and the PeroxiBase database (peroxidases). ‖Particularly promising for the bioremediation of organic chemicals. ¶Commercialized for textile dye-bleaching in finishing dyed cotton fabric (DeniLite, a laccase, and Baylase, which uses *C. cinerea* peroxidase), for preparing cork stoppers for wine bottles (Suberase, a laccase) and for diagnostic and research applications (lignin and manganese peroxidases, and *C. fumago* haem–thiolate chloroperoxidase)[66,70]. #Further sequence information about fungal cytochrome P450s is available at the Fungal P450 page maintained by D. R. Nelson.

of appropriate co-oxidants[45,60,78] (TABLE 1). Laccases are further involved in quinone redox cycling, leading to the production of the hydroxyl radical, which is then available for subsequent pollutant attack[79] (FIG. 3). Unlike typical peroxidases, haem–thiolate peroxygenases produced by certain basidiomycetes of the order Agaricales catalyse H_2O_2-dependent hydroxylations of various pollutants, including PAHs and dibenzofuran, and cleave ether bonds between aromatic and aliphatic parts of molecules as well as in alicyclic and aliphatic ethers[70]

(TABLE 1). Hence, they produce activated metabolites that are more susceptible to further degradation than their parent compounds.

Cell-bound enzymes. Many intracellular fungal enzymes involved in chemical catabolism also lack substrate specificity. Mixed-function cytochrome P450 oxidases of various fungi catalyse epoxidations and hydroxylations of numerous pollutants, with prominent examples being PAHs and dioxins[3,80] (TABLE 1). The presence of multiple cytochrome P450-encoding genes contributes to the enormous catabolic versatility of ligninolytic fungi[81]. For instance, cytochrome P450s can initiate the metabolism of dioxins, nonylphenol and PAHs in these organisms[3,80,82]. However, the fact that extracellular oxidoreductases can also oxidize such pollutants[3,55,76] raises the question of whether an intracellular or extracellular primary attack on pollutants predominates under a particular environmental situation, as this would be expected to influence the expression of degrading enzymes. Cytochrome P450s were further implicated in fungal metabolism of anti-inflammatory drugs, lipid regulators, anti-epileptic and anti-analgesic pharmaceuticals[83–85], and diphenyl ether herbicides[86]. Cytochrome P450 monooxygenases also contribute to the catabolic versatility of fungi lacking extracellular oxidoreductases. For example, they enable *Cunninghamella elegans* (in the subphylum Mucoromycotina) to oxidize PAHs with 2–5 aromatic rings[3]. Non-haem mixed-function oxidases such as phenol 2-monooxygenases produce catechols from phenols[33,87] (TABLE 1). Metabolites arising from initial intracellular hydroxylation of, for example, chlorophenols are released if further metabolization is impeded, and may undergo abiotic oxidative coupling[33] (FIG. 3). Otherwise, primary metabolites may undergo further metabolism until mineralization is completed[3]. This further metabolization may also lead to the secretion of metabolites at various stages of oxidation if subsequent degradation is slow or impeded[33,40,88] (FIG. 3).

Various transferases act on hydroxyl groups of pollutants and their metabolites to catalyse the formation of conjugates (TABLE 1), which are typically not subject to further fungal degradation. Detoxification through the excretion of water-soluble conjugates (FIG. 3) is well documented for the fungal metabolism of PAHs[3] and other organic pollutants[89].

Aromatic nitroreductases (TABLE 1) are widespread among fungi and reduce TNT to hydroxylamino- and amino-dinitrotoluenes, which are excreted and may undergo various further enzymatic and non-enzymatic reactions[27,34,90,91] (FIG. 3). Amino-dinitrotoluenes can be converted efficiently by laccase and MnP[77,78]. Other nitroreductases convert explosives containing *N*-heterocyclic structures, such as RDX and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), into their respective mononitroso derivatives[92–94].

Quinone reductases of white-rot and brown-rot basidiomycetes[95,96] (TABLE 1) are involved in quinone redox cycling, which initiates extracellular Fenton reactions that lead to the production of hydroxyl radicals[97,98]. Extracellular pollutant attack by hydroxyl radicals results in spontaneous hydroxylations and dehalogenations of aromatic and aliphatic pollutants[46,99,100] (FIG. 3). Quinone reductases also reduce quinones that result from the transformation of pollutants by extracellular oxidoreductases. This detoxifies quinones and converts them back into substrates of extracellular oxidoreductases, as demonstrated for chlorophenol metabolism in ligninolytic basidiomycetes[74,101].

Whereas extracellular laccases and peroxidases catalyse oxidative dechlorinations[55,74,101–103], reductive dehalogenases of ligninolytic basidiomycetes reductively dechlorinate chlorohydroquinones arising from chlorophenol degradation[74,102,104] (TABLE 1). Similar enzymatic activities seem to reductively dechlorinate diphenyl ether herbicides[86] and chlorocatechols[59].

Suitability of fungi for bioremediation

Fungi and bacteria can both degrade and transform organic environmental chemicals. One might therefore ask which characteristics or environmental circumstances make fungi particularly suitable for application in environmental biotechnology. Obviously, fungal degradation should be considered for pollutant classes that are inefficiently degraded by bacteria. For example, bacteria might be disadvantaged if substrates contain rare structural elements, have a low bioavailability, contain little energy or occur permanently at minute concentrations (see BOX 1). The problem of concentration applies to a range of structurally quite different chemicals that combine low environmental concentrations with worryingly high biological activities, traits that are attributable to their design as human and veterinary drugs (including synthetic hormones or antibiotics). Such chemicals, together with ingredients of many other consumer products, are now found in environmental matrices (water, aquatic sediments and soil), as they are not retained in wastewater treatment plants. Ligninolytic basidiomycetes and mitosporic ascomycetes, including aquatic fungi, are known to degrade EDCs (nonylphenol, bisphenol A and 17α-ethinylestradiol); analgesic, anti-epileptic and non-steroidal anti-inflammatory drugs; X-ray contrast agents; polycyclic musk fragrances; and ingredients of personal care products[75,76,82–85,89,105–107]. However, there are other characteristics that could make the use of fungi more attractive irrespective of whether the bacterial option is available.

Long-range transport. The use of filamentous fungi may be advantageous in cases for which the translocation of essential factors (nutrients, water, the pollutant itself, and so on) is required for the transformation or detoxification of environmental chemicals. In their natural environments, fungi cope with resource heterogeneity by translocating resources between different parts of their mycelium[20]. Time-lapse recordings visualize the immense traffic of resources in fungal hyphae, particularly in the direction of growth (see the Fungal Cell Biology Group website for images and movies). Hyphal transport of autofluorescent PAHs was also visualized[24]. Resource translocation includes the recycling of hyphal biomass located in substrate-depleted regions

Box 1 | **Bacterial approaches to pollutant degradation**

Bacterial degradation of pollutants differs in two aspects from most cases of fungal degradation:

- Bacteria typically use the pollutants as growth substrates. The efficiency of bacterial degradation thus relies on a positive feedback loop between pollutant degradation and the formation of more bacteria. However, unlike chemical catalysts, organisms require minimum substrate fluxes to persist. Below a crucial 'per cell' flux of maintenance energy, the biocatalyst concentration and its catabolic capacity decrease. This occurs when pollutant concentrations are very low (some trace chemicals in wastewater treatment) or when pollutants are poorly bioavailable (high-molecular-mass hydrocarbons) or contain very little energy (highly oxidized chemicals, such as chlorinated or nitrated compounds). Under such conditions, an organism persists only if it succeeds in reducing its maintenance requirements — usually coupled with a loss in activity (for example, entering dormancy or undergoing sporulation) — or in using other substrates along with the pollutant.
- Bacteria use specific biochemical pathways. Degradation pathways for new pollutants are mostly modifications or extensions of existing pathways. Alternatively, they may be newly assembled but based on existing enzymes gained, for example, by genetic transfer between species. However, degradative pathways will only evolve and radiate when there is a selective benefit for their encoding bacterium. Specific pathways are thus unlikely to exist for environmental chemicals that always occur at concentrations below those required for multiplication or for chemicals containing rare or novel structural elements. The difficulty in finding bacteria that productively degrade drugs, agricultural chemicals or ingredients of consumer products (cosmetics, dyes, detergents, and so on) can be explained in this way. However, bacteria are particularly successful degraders of structurally simple mass chemicals (for example, those that are aliphatic or aromatic with low numbers of functional groups).

for the benefit of exploration for food in other regions. Fungi have also been found to stimulate pollutant degradation by bacteria in soil environments in which the active movement of bacteria to pollutant reservoirs is limited by physical barriers such as air-filled pores or dense aggregates. Owing to their wedge-shaped, hydrophobic tips, growing fungal hyphae penetrate air–water interfaces and soil aggregates. The surfaces of the resulting mycelia have been found to be surrounded by water films, representing continuous pathways for motile bacteria[25]. Experiments and model simulations showed that random and chemotactic swimming of bacteria along these mycelia facilitate bacterial degradation of heterogeneously distributed chemicals[108,109].

Catabolism of recalcitrant organic pollutants. PCDDs and polychlorinated dibenzofurans (PCDFs) are highly oxidized owing to the electronegativity of the chlorine atoms withdrawing electrons from the aromatic rings. These compounds are therefore poor electron donors. Moreover, they always occur at very low environmental concentrations. Accordingly, there is no data to suggest that congeners of these compounds containing more than one chlorine atom are used as carbon and energy sources by bacteria[30]. Aerobic and anaerobic bacterial biotransformation of the particularly toxic PCDD 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is slow (in the range of months) and stops at the stage of less chlorinated aromatic products[30]. By contrast, the white-rot fungus *Phanerochaete sordida* transforms 2,3,7,8-TCDD into chlorocatechols in 10 days[57], whereas *P. chrysosporium* even mineralizes the compound[56]. PCDDs with 6–8 chlorines and PCDFs with 4–8 chlorines were degraded by *P. sordida* at similar rates[57], indicating a lack of specificity in this organism. The ascomycete *Cordyceps sinensis* removed highly chlorinated dioxins as fast as white-rot fungi[58,59]. TNT is also highly oxidized owing to the electronegativity of its nitro groups. Most aerobic bacteria transform TNT into monoamino-dinitrotoluenes, diamino-nitrotoluenes and hydroxylamino-dinitrotoluenes, which may accumulate and form highly mutagenic azoxy-tetranitrotoluenes, whereas TNT conversion by anaerobic bacteria mostly stops at the stage of triaminotoluene. By contrast, white-rot and litter-decaying basidiomycetes mineralize TNT rapidly[27,34,91]. Research into the biotechnological use of nonspecific enzymes must address the difficulty in controlling the reactions. In cases for which the fungal transformation products from PAHs include small amounts of mutagenic and carcinogenic metabolites, most of these transformation products were less toxic than their parent compounds[3]. By contrast, the formation of PCDDs and PCDFs on enzymatic oxidative coupling of chlorophenols[110,111] indicates that a careful assessment of the structure, biological activity, stability and environmental behaviour of products is required. The laccase-catalysed transformation of EDCs to oligomeric coupling products reduced the endocrine-disrupting activity of these compounds[76]. Similarly, detoxification was achieved when laccases and other phenoloxidases were used to covalently link various chlorophenols, other phenols and TNT metabolites to constituents of soil humic substances[77,103,112].

PAHs are good substrates from an energetic perspective, and low-molecular-mass PAHs are readily used by bacteria[113]. As bioavailability strongly decreases with increasing molecular mass, only a few bacteria grow on high-molecular-mass PAHs with five or more aromatic rings[113,114]. Fungi of the Ascomycota, Basidiomycota and Mucoromycotina hydroxylate PAHs intracellularly and further convert them to water-soluble products that can be excreted, thus using a nonspecific mechanism for their detoxification[3,113,114]. Moreover, various fungi use extracellular oxidoreductases in PAH degradation and mineralize even high-molecular-mass PAHs, such as the highly carcinogenic benzo[*a*]pyrene (FIG. 4) and others[3,114].

Remediation of metals, metalloids and radionuclides. Morphological, ecological and biochemical features qualify fungi for remediation of metals, metalloids and radionuclides (referred to collectively as metals here). Toxic metals can enter the environment during all stages of the life cycle of metallic goods, from the mining of metal ores to the final disposal of metal-containing wastes. Metal mobilization from geogenic sources, agricultural practices and accidents such as fires further contribute to the constant input of metals into waters and the atmosphere, from where they are transported into soils and aquatic sediments. Environmental contamination with metals can be so severe — for example, in the neighbourhood of ore mines and smelters or at disposal sites — that active remediation is inevitable. The risk from chemical forms of metals is intimately linked with the inherently non-degradable elements that they

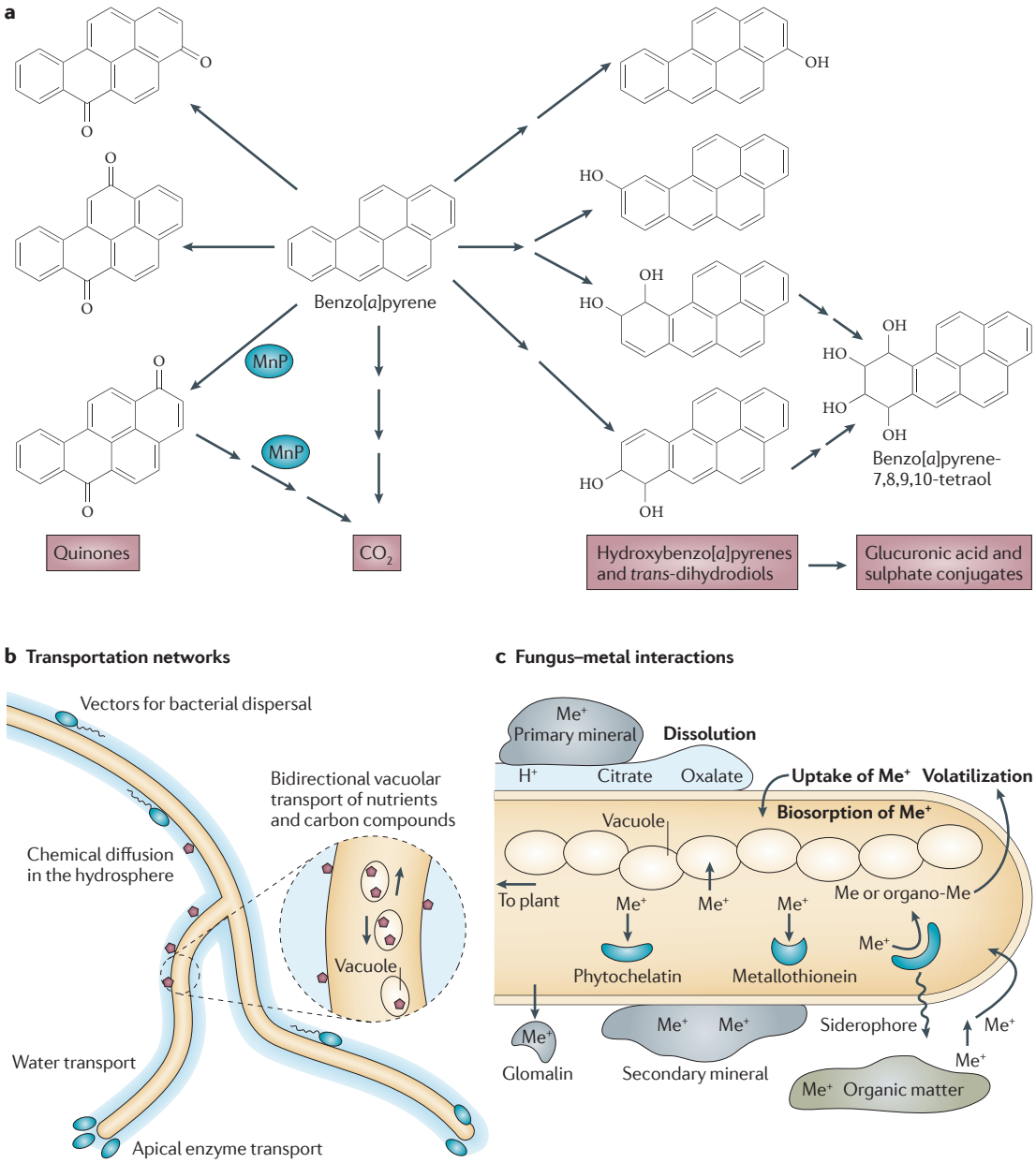

Figure 4 | **Fungal interactions with recalcitrant organic contaminants and metals. a** | An example of fungal polycyclic aromatic hydrocarbon biodegradation: the biochemical pathway and products reported for the co-metabolic fungal degradation of benzo[*a*]pyrene[3,45]. Note that the epoxide intermediates that arise from initial intracellular attack by cytochrome P450s and precede the formation of hydroxylated products[3] are not shown. **b** | The influences of fungal hyphae on the subsurface mobility of bacteria, carbon compounds, nutrients and water in both the saturated and the vadose soil zones. **c** | Typical interactions of fungi with metals (Me) are shown. Metal mobilization results from the production and excretion of organic acids (for example, citrate and oxalate), which increase metal solubility through acidification of the mycosphere and provision of metal-complexing structures[138]. This frequently occurs as a side effect of the dissolution of primary minerals containing phosphate, carried out by mycorrhizal fungi. Siderophores are chelators excreted for the acquisition of iron, and they may cross-react with other metals[139]. Extra-hyphal immobilization occurs through the formation of secondary minerals[140], biosorption to cell wall constituents such as chitin and chitosan[129,130], complexation by glomalin (that is, metal-sorbing glycoproteins excreted by arbuscular mycorrhizal fungi)[141] and effects of fungal mycelia and glomalin on soil aggregate stability against wind and water erosion[120]. Metal uptake occurs, for example, through specific transporters for the acquisition of essential metals[142,143], and these transporters may cross-react with other metals. Intracellular metal immobilization involves storage in vacuoles and complexation by cytoplasmic metallothioneins[144] and phytochelatins[145] (that is, proteins and peptides, respectively, that are rich in SH groups). Metal transformations such as reactions involving organometals (for example, methylations)[146] and redox reactions frequently result in metal volatilization. Streams of cytoplasmic vesicles and vacuoles along fungal hyphae[147] may translocate metals to other parts of the mycelium[148] and to the plant symbionts of the fungi[149]. MnP, manganese peroxidase.

contain. Hence, biological processes cannot eliminate metal toxicity in an irreversible way. The risk arising from these elements can nonetheless be reduced by biological transformation into more harmless species or by separation from susceptible biota. This separation can be in the form of transport away from receptor organisms or a reduction of chemical bioavailability. From a technological perspective, there is also much interest in the removal of metals from wastewater streams and in the reclamation of precious metals. Fungi interact with metals in various ways, as schematically shown in FIG. 4. These include processes of mobilization and immobilization in the mycosphere, sorption to cell walls and uptake into fungal cells. After being incorporated into the fungus, metals can be chemically transformed, stored in different parts of the cell or translocated along fungal hyphae and into plant symbionts. In many cases, environmental pollution is neither purely organic nor purely inorganic.

Potential environmental applications

Despite their biochemical and ecological qualities, fungi are rarely the biological agents of choice for environmental biotechnology. There are hardly any examples that one could class as 'using fungal environmental technology'. Indeed, under many environmental conditions and in many applications, bacteria are chosen or self-establish because they exhibit superior performance. Bacteria tolerate a broader range of habitats, use biochemical reactions of higher specificity, more often degrade contaminants productively (leading to independence from auxiliary organic substrates), grow faster and are more mobile in aqueous environments, to name just a few advantages. One of the main reasons for the underexploitation of fungi has been their requirement for oxygen and the cost of providing it to polluted environments; another reason seems to be the reported failure of filamentous fungi in remediation schemes (namely, land farming and soil reactors) that have been developed for bacteria. Whereas bacteria benefit from the improvement in bioavailability caused by mechanical homogenization of soil, this stirring and ploughing prevents fungi from developing mycelia[115]. Although wood-inhabiting ligninolytic basidiomycetes possess interesting biochemical features, their application to contaminated soil environments, which differ greatly from their natural habitats, turned out to be difficult and resulted in poor remediation success[61]. The much better performance of these fungi in sterile soil indicates their difficulty in competing with the autochthonous microbial community[61]. However, the application of filamentous fungi could be a promising alternative or a valuable complement in situations of bacterial malfunction. Examples include circumstances in which contaminants are physically inaccessible to unicellular organisms; are present in habitats that are too polluted, too acidic or too dry for degrader bacteria; are biochemically inaccessible because their structures are too complex or xenobiotic for specific degradation; do not represent good substrates from the nutritional and evolutionary perspective, as they occur at minute concentrations or contain little energy; or, finally, are inherently impossible to detoxify, thus requiring long-distance transport out of the contaminated environment. Other important prerequisites for the application of filamentous fungi in mycoremediation and environmental biotechnology include the supply of organic substrates, the presence of oxygen and the limitation of mechanical disturbance. In spite of this extensive catalogue of boundary conditions, there are many real cases of environmental contamination for which filamentous fungi are presently used, either deliberately or unwittingly (the reader is referred to a recent overview[116]), or would merit more consideration, such as the co-metabolic removal of air-borne organic pollutants that partition to vegetation, carried out by fungi that decay plant litter.

Organic or metal contamination in surface soils. Free-living and mycorrhizal fungi fulfil various functions in passive or semi-passive *in situ* soil remediation schemes. As well as mobilizing nutrients, fungi mobilize, incorporate and/or transform metals to either translocate them towards host plants that support phytoextraction[117], or immobilize them in the mycorrhizosphere, leading to plant protection[118]. This facilitates plant settlement and promotes plant growth. These fungi transport hydrophobic organic chemicals inside their hyphae with positive effects on contaminant bioavailability[24], and they also transport water[119]. They transform organic contaminants metabolically or co-metabolically[31] and transfer plant-derived organic substrates and mycorrhizal biomass to non-symbiotic soil bacteria and fungi[2], as well as facilitating bacterial movement to pollutants[25,108]. Finally, the penetration of soil by fungal mycelia[10] and the enmeshment of soil aggregates improve the soil structure[120]. All of these processes result in a general stimulation of biological activity. However, the direct cooperation of fungi and bacteria in the catabolism of pollutants requires more research, as there are indications that fungal metabolites resist bacterial degradation. It was found that the fungus *C. elegans* converts PAHs into sulphate-conjugated metabolites, which resist further degradation by the soil microbial community but, owing to their solubility, potentially threaten groundwater resources[121]. Plant-based, and particularly tree-based, remediation schemes promote fungal activities in the rhizosphere (rhizoremediation). The total contribution of fungi to remediation success is difficult to quantify, as true controls deviating only in the absence of fungi are highly artificial and, in many cases, impossible to establish. Nonetheless, owing to the potential positive influences of root-associated fungi for the *in situ* treatment of contaminated soil, planting of contaminated sites can generally be recommended. Other ways of promoting soil fungi, such as ploughing in appropriate plant biomass, have clear drawbacks[122]. Although on-site experiments have demonstrated durable pollutant removal by artificially established fungi, they required tedious empirical studies to select the appropriate fungus that was capable of transforming the pollutants and was viable and active in the soil in question. Moreover, the need for large amounts of co-substrates and their

Box 2 | **Active remediation versus environmental restoration by natural attenuation**

Bioremediation is now regarded as the default method for the rehabilitation of polluted environments because of its cost efficiency and environmental friendliness. However, the extent to which these advantages apply depends on the degree of technical intervention. The intensity at which bioremediation can be conducted ranges from the most intensive *ex situ* treatments involving specialized organisms, mechanical forces, heat, and solvents or detergents, through the stimulation of indigenous microbial communities with nutrients or oxygen, to the entirely passive monitoring of the progress of *in situ* natural attenuation. Concentration-based clean-up goals often justified intensive remediation measures in the early years of this technology. Since the 1990s, there has been a clear trend towards passive methods, driven by the recognition of unfavourable cost–benefit ratios of intensive treatment, the realization that polluted 'mega-sites' cannot be excavated and the insight that soil, in particular, attains a better ecological status when it is gently cleaned rather than intensely processed. This shift of attitude was accompanied by the implementation of risk-based remediation targets, which also paved the way for biological treatments that lead to physical or chemical stabilization of pollutants rather than their elimination. Extraction and degradation typically require the mobilization of pollutants, with potential drawbacks for organisms other than the pollutant degraders. Moreover, degradation of metals, metalloids and radionuclides is inherently impossible, thus leaving risk reduction by stabilization as the only option.

incorporation into the soil material limits the economic feasibility and increases the energy demand of such approaches[61]. Surprisingly, litter-decaying basidiomycetes, which may be particularly well adapted to soil conditions, still remain to be tested[61].

Potential roles of mycorrhizal associations for bioremediation purposes have primarily been addressed with ECM[52–53]. ECM fungi are of interest because the carbon supply from their host plants may support fungal growth into contaminated matrices and stimulate co-metabolic reactions[53]. ECM mycelia may also support microbial biofilms harbouring degrader bacteria[53]. The few data available suggest that the enhanced dissipation of organic pollutants may increase their bioavailability to bacteria in the presence of AM[53]. Inoculation of maize, alfalfa and leek with various species of AM fungi substantially improved the transformation of atrazine, phenanthrene and BTEX, respectively[54,123,124]. However, how strongly pollutants affect AM and what the contributions of these pollutants are to mycorrhizosphere processes such as phytostimulation remain largely unresolved.

Concentrated organic contaminants in water streams. Fungal extracellular oxidoreductases hold promise for the detoxification and degradation of concentrated pollutants in waste effluents for which conventional wastewater processes are not efficient. Examples include acidic olive oil mill wastewaters containing toxic phenols and lipids; alkaline effluents of high salinity from the textile and dyestuff industries, containing structurally diverse dyes and pigments; highly toxic molasses wastewaters containing melanoidin-type compounds; and toxic pulp and paper bleach plant effluents containing phenolic, chlorinated and coloured compounds[64,125,126]. Ascomycetes and basidiomycetes from freshwater and marine environments combine adaptation to aqueous environments with an enzymatic inventory necessary for nonspecific pollutant degradation, suggesting that their potential for use in aquatic remediation processes has to date been underestimated[37,126,127].

Trace organic contaminants in water streams. Environmental trace contaminants that are strongly xenobiotic (for example, EDCs, drugs, fragrances and other ingredients of consumer products, and agrochemicals) are abundant nowadays and cause much concern. Municipal wastewater contains small concentrations of the ingredients of many consumer products and drugs[128]. Many of these contaminants do not lend themselves to bacterial degradation because of distinctly xenobiotic structures. Using the nonspecific nature of fungal metabolism seems like a more realistic option for their transformation than waiting for specific catabolic pathways to evolve in bacteria. Process waters of different industries (fine chemical, agrochemical, pharmaceutical and textile industries) contain chemical mixtures that are minimally held back by conventional wastewater treatment. Water-adapted aquatic fungi should be considered for the co-metabolic transformation of chemicals such textile dyes, drugs, nonylphenol and polycyclic musk fragrances in specifically designed treatment modules.

Metal removal from water streams. Fungal biomass functions as biosorbent and nucleus for metal precipitation[129]. Two envisaged process schemes are immobilized fungal-waste biomass (typically from synthetic biotechnological processes) percolated with metal-loaded process water[130], and a fungal contribution to the biology of constructed wetlands designed for metal removal[131]. However, the enormous body of scientific information and the many forecasts of a bright future for biosorption technology stand in clear contrast to the almost complete lack of full-scale technical application of biosorption processes[129]. Despite this, it seems possible that the increasing demand for a growing number of industrial metals, in particular rare earth elements, together with the ongoing exhaustion of accessible ore deposits, will lead to more interest in biosorption as a component of a recycling- and biomass-based economy. In this case, fungal biomass may attain an important role.

Isolated extracellular enzymes. As extracellular fungal oxidoreductases can be easily produced and applied in an immobilized state[63], they can also be used in biotechnological applications — that is, the complications relating to the control of processes involving living organisms and to the requirement of intracellular enzymes for cofactor or redox equivalents are avoided.

Laccases are particularly interesting because they do not require exogenous H_2O_2 but use molecular oxygen as the oxidant[63]. Known disadvantages of commonly applied laccase redox mediators are the high costs of these non-recyclable compounds and the occasional formation of toxic by-products[63]. Extracellular fungal peroxidases produced by basidiomycetes have the advantage of a higher redox potential and a broader substrate range than laccases[70]. Drawbacks of these enzymes are their requirement for H_2O_2 and their inhibition by excess H_2O_2 (REFS 64,132). The biotechnological application of MnPs is restricted because they require a suitable manganese chelator (an organic acid, such as oxalate, malonate or lactate) and, for particularly recalcitrant compounds, suitable co-oxidants such as organic sulphur compounds or unsaturated fatty acids[60].

Removal of contaminants from air streams. Environmental conditions favouring the establishment and activity of fungi prevail in gas-phase biofilters designed to eliminate volatile organic chemicals. The prevalence of fungi in such filters can be explained by the high degree of air saturation (low water potential) in biofilters, through which humidified waste air flows, and by the choice of lignocellulose-rich natural materials (compost, bark, peat and heather) as the solid support and substrate source for the establishment of degrader organisms[29,133]. Fungi are even more favoured in slightly acidic biofilters. A comprehensive overview of many technical and biological aspects of modern biofilter technology and of various applications for air treatment is given in REF. 134. Unfortunately, some biofilters have also been found to host pathogenic fungi[29].

Conclusions and future directions

We have shown that the particular lifestyle of fungi and their biochemical potential can be of importance for a wide range of biotechnological applications to remove or stabilize organic and inorganic contaminants. Given the actual demand for sustainable, cheap and tailor-made technologies (see BOX 2), there is considerable impetus to translate powerful ecosystem services, such as those provided by fungi, into ecology-based technologies. Regarding the reclamation of contaminated land, there is an ongoing trend towards passive remediation schemes, referred to as monitored natural attenuation. Such measures take more time than active *ex situ* treatment, as they rely on the autochthonous microbial communities. However, they are energy efficient and result in ecologically intact environments. The low degree of mechanical intervention in natural attenuation of soil very probably favours the establishment of filamentous fungi. Most fungi are also known to produce large quantities of exudates that can serve as auxiliary carbon sources for pollutant-degrading bacteria. The inclusion of plants for the alimentation of mycorrhizal or other autochthonous fungi seems to be a promising approach for enhanced rhizosphere bioremediation of organic contaminants, all the more effective because hyphal penetration of soil aggregates containing entrapped pollutants or bacterial microcolonies may increase pollutant bioavailability. Unfortunately, relative contributions of fungi to degradation and detoxification processes have seldom been quantified, and the same is true for the positive effects of fungi on bacterial remediation processes. Limited knowledge of the methodologies and ecological approaches required to sustain sufficient fungal biomass, activity and enzyme production in contaminated environments is still a great hindrance to mycoremediation. In addition, studies on the spatiotemporal stability of bacteria–fungi associations are needed to improve our understanding of these interactions and to develop novel, ecologically sound bioremediation approaches. With the advent of powerful isotope techniques, such analyses come into reach and might lead to a better appreciation of fungal contributions to the purification of nature, such as the fungal removal of air-borne persistent organic pollutants that partition into plant litter or humus, a process that is considered to provide a relevant reservoir and secondary source in the global cycling of these compounds[135]. An important driver of passive remediation technologies involving fungi, besides their low cost, will be the common acceptance of risk-based clean-up standards, such as those currently included in the legislation of countries such as the United States and the United Kingdom[1]. Therefore, there are important financial, ecological and legal reasons for gaining a better understanding of fungal activities and their implementation in environmental technology.

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Competing interests statement
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DATABASES

ExPASy ENZYME: http://expasy.org/enzyme/
FOLy: http://foly.esil.univ-mrs.fr/index.html
Fungal P450 page (maintained by D. R. Nelson): http://drnelson.uthsc.edu/fungal.genomes.html
NCBI Taxonomy: http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi
PeroxiBase: http://peroxibase.toulouse.inra.fr
US Department of Energy Joint Genome Institute (for fungal genome projects): http://www.jgi.doe.gov/genome-projects/

FURTHER INFORMATION

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Assembling the Fungal Tree of Life project: https://aftol.umn.edu/
Fungi Images on the Net: http://www.in2.dk/fungi
The Fungal Cell Biology Group: http://fungalweb.icmb.ed.ac.uk/index.html
The fungal kingdom: diverse and essential roles in Earth's ecosystem: http://academy.asm.org/images/stories/documents/fungalkingdom.pdf
The Humongous Fungus: ten years later: http://botit.botany.wisc.edu/toms_fungi/apr2002.html

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