BIOAVAILABILITY AS A MICROBIAL SYSTEM PROPERTY: LESSONS LEARNT FROM BIODEGRADATION IN THE MYCOSPHERE

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ABSTRACT

Bioavailability for contaminant degradation requires a deep understanding of the ecology of degrader microbial systems and, hence, should be perceived as microbial system property. In this chapter we summarize recent research on microbial ecology of contaminant biodegradation in the mycosphere that is defined as the microhabitat surrounding and affected by mycelia. By forming unique transport networks, mycelial fungi are highly adapted to cope with complex heterogeneous habitats and to grow under conditions of uneven availability of their vital resources, such as nutrients and water. Combining concepts from bioavailability, ecophysiology, and microbial ecology, our chapter discusses the impact of fungal networks on chemical and bacterial transport and its effect on contaminant bioavailability and degradation at different organizational levels (i.e. from the molecular up to the ecosystem level). It thereby provides generic information on key factors, processes, and ecological principles that drive contaminant biotransformation in the mycosphere.

KEYWORDS

Biodegradation, ecology, fungal-bacterial interactions, microbial systems, mycosphere.

1. BIOAVAILABILITY AND CONTAMINANT DEGRADING MICROBIAL SYSTEMS

1.1. Introduction

Anthropogenic chemicals (contaminants) accumulate in the environment if their emission and degradation are unbalanced. Being main drivers of biogeochemical cycles, microbial systems [1] are also key players for the degradation of chemicals in the environment. Rate and extent of degradation, however, depends on the molecular property and the availability of the chemical to degrading organisms [2], [3] [4] as well as environmental conditions that sustain the activity and abundance of degrader biomass. After release, anthropogenic chemicals typically end up in terrestrial systems; i.e. ecosystems that form important habitats of biogeochemical nutrient cycling by fungi and bacteria [5]. The fungal kingdom comprises a vast diversity of taxa expressing various morphologies that range from single-celled yeasts to large multicellular organisms with a complex interconnected network (mycelium) of minute, protoplasm-filled tubes called hyphae [6]. Mycelia efficiently spread in heterogeneous habitats such as soil, where they effectively influence microbial contaminant degradation by both their own catabolic potential and multifarious interactions with degrader bacteria [7], [8]. Whereas other reviews focus on fungal ecology [5] [9], the biochemical versatility of fungi [10], [11], [12], [13] or

the untapped fungal potential for remediation [7], this chapter addresses the question, how interactions between fungi, bacteria and their habitats influence the bioavailability and biodegradation of organic chemicals. It focuses on mycelial fungi and biodegradation in their mycosphere being defined as the microhabitats surrounding and affected by hyphae and mycelia [14]. Please note that the terms `biodegradation` and `biotransformation` are not consistently used in the literature: while some associate 'biodegradation' to the ultimate breakdown into CO_2, NH_4^+, or H_2O (also referred to as 'mineralization') others use 'biodegradation' to refer to the disappearance of the contaminants and do not distinguish between incomplete transformation to metabolites ('biotransformation') and 'mineralization'. Here, we mostly use 'biodegradation' in a sense encompassing both 'biotransformation' and 'mineralization'.

1.2. Bioavailability driving biodegradation

The term 'bioavailability' is used to denote the '*degree of interaction of chemicals with living organisms*' [15] and several biological and chemical methods for assessing bioavailability have been described [4]. For contaminant biodegradation we here adopt the approach by Bosma et al [16] defining bioavailability as the *rate of a chemical's mass transfer to microbial cells relative to their intrinsic catabolic degradation potential*. This perspective points at the relevance of mass fluxes for 'degradation processes' and discriminates bioavailability for degradation from bioavailability for 'non-degradation' processes that lead to poisoning or inhibition of the receptor organism [17].

Low bioavailability for biodegradation may arise if the environmental concentration of a chemical is small (e.g. as for organic micropollutants) or if the flux of a chemical to the degrading cells is minimal (e.g. as for poorly water-soluble or sorbed chemicals [18]). The flux may also become nearly zero as for compounds such as plastic polymers [19]. The mode and rate of emission of chemicals also has significant effects on the spatial distribution of a chemical, which is also crucial for its bioavailability. While accidental spills of industrial chemicals lead to massive localized contaminations and potentially high chemical fluxes to cells, waterborne transport of e.g. micropollutants may result in diffuse low-level contamination that may not sustain sufficient degrader biomass. Rein et al. for example found that concentrations of < 5-10 nM of polycyclic aromatic hydrocarbons (PAH) did not meet the maintenance requirements of the degrader population [20]. According to Bosma et al. several processes (Fig. 1) determine the bioavailability and biodegradation of chemicals by a degrader cell: the release and transport of the chemical from the source to the cell ('availability to degrader') and the cell's uptake and rate of biodegradation and the respective changes of cell activity ('activity of degrader'). Productive biodegradation (i.e. biotransformation that promotes the build-up and maintenance of biomass) only takes place if chemicals can be transported across the microbial cell membranes into the cytosol where they are metabolized and used for cell maintenance and growth; a process that may in particular influence the bioavailability of chemicals present at low concentrations [21, 22]. Large molecules need to be depolymerized by extracellular enzymes prior to uptake (e.g. performed by fungi), whereas uptake of ions, polar molecules or molecules with very low saturation concentrations (e.g. high molecular weight PAHs) may rely on energy-dependent cellular uptake systems. Such uptake, however, can only take place if the microbes draw advantage from degradation of such chemicals. Co-metabolic contaminant biodegradation, by contrast, is not a growth-linked process and often depends on the use of non-specific enzymes to degrade environmental compounds. It is an often

underappreciated facet of microbial contaminant biodegradation, which may increase the bioavailability of contaminants and produce more available metabolites (cf. the metabolites in Fig. 1) in spite of little benefit for the degrader. Co-metabolism can occur under various aerobic and anaerobic environmental conditions and for a wide variety of contaminants and co-substrates [23]. Since the microorganisms do not rely on the contaminants for growth, the co-metabolic degradation has the potential to degrade chemicals at minute concentrations ('micropollutants') and, hence, to achieve cleanup endpoints in the parts per trillion range [23]. As for metabolic degradation the environmental conditions have to allow high abundance and activity of the degraders.

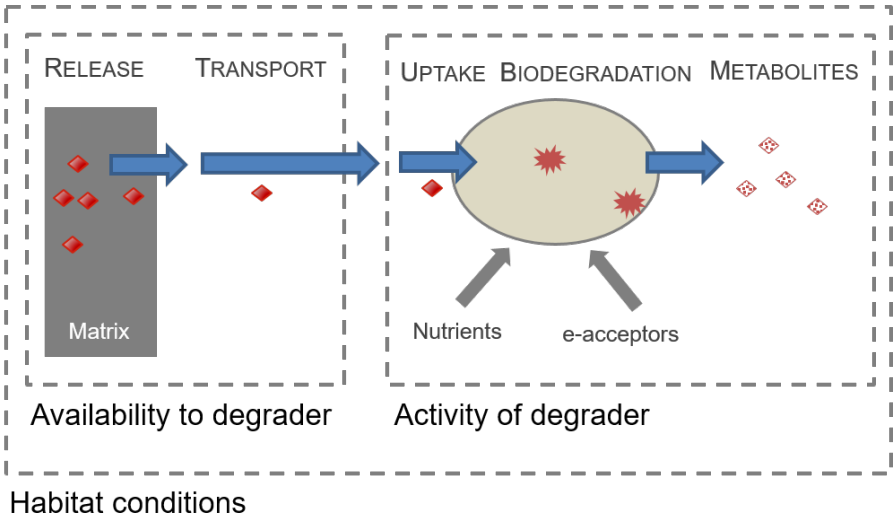

Figure 1. Conceptualization of the main processes driving the bioavailability and biodegradation of an inherently biodegradable chemical. Bioavailability is a dynamic feature that is determined by the release (of bound chemicals), transport, uptake and transformation of the chemicals at the cellular site of response. It depends on the presence of the chemical, the abundance and catabolic activity of degrader cells and the prevalent habitat conditions.

1.3. Microbial system properties driving bioavailability

The activity and abundance of microorganisms at the micro- and macro-scale [24] of terrestrial systems is controlled by the spatial heterogeneity including highly heterogeneous distribution of nutrients, pH, temperature, water or terminal electron acceptors [25] [26]. Spatial variations in soil are simultaneously considered to be key drivers for the high diversity and abundance of soil microorganisms. One gram of surface soil can contain up to 10^9–10^{10} prokaryotic cells, hundreds of meters of fungal hyphae and 10^8–10^9 viruses [26]. Such values convert to > 5 tons of prokaryotic and 1-15 tons of fungal biomass per hectare [26]. Despite of such high biomass only a small fraction (0.17%-0.02%) of the specific surface area of soil is considered to be covered with microorganisms [27, 28]. Any contaminant biodegradation in such microbial systems hence relies on appropriate fluxes of matter and energy to and between degrader organisms to ensure sufficient microbial activity [29]. Thereby, a chemical's transport to and uptake into a cell also depends on morphological, physiological and behavioral characteristics (functional traits) of the microbes [18]. These traits may include possibilities to adjust the uptake of chemicals, for instance, by excretion of surface active molecules (biosurfactants) or expression of high affinity uptake systems. Mechanisms of active self-locomotion, allowing dispersal and chemotaxis of microorganisms, are traits that let them actively control their exposure to chemicals

[30]. For bacteria, the effectiveness of these traits depends on the presence of water in their direct surrounding, which is why water is the major factor controlling bacterial movement in soil. Finally, the activity of a particular organism is always affected by interactions with other organisms in the same habitat. Major microbial interactions during chemical biodegradation are the competition for substrates and nutrients, but also predation or cooperation, for instance by syntrophy or by protection against pathogens or predators. The above-mentioned spatiotemporal variations of habitat conditions in terrestrial systems may cause stress and disturbances [31] [32] [33] of the degraders. Such harsh conditions may request high cellular adaptive capacity and high degree of intercellular interactions [34] [35]. Moreover, the presence of contaminating chemicals itself also likely exerts a selective pressure on microbial communities [36] and may trigger the evolution of microbial communities towards the degradation of even new chemical structures. If degradable, anthropogenic chemicals can serve as sources of carbon and energy, thus favoring the degrader organisms in microbial communities. Differential metabolic potential and sensitivity of organisms to toxic effects of chemicals will hence contribute to the microbial biodiversity of a contaminated microbial system, by favoring organisms that take profit from contaminant input and simultaneously distressing organisms that may suffer e.g. from potential limitation of nutrients and electron acceptors utilized by contaminant-degraders.

2. MYCELIAL FUNGI HAVE TRAITS AND FOSTER PROCESSES RELEVANT FOR CONTAMINANT BIODEGRADATION

Fungi colonize nearly all habitats of our planet and shape many terrestrial ecosystem functions. Comprising estimated 12 Gt of carbon, fungi are ubiquitous and form the third most abundant biomass on our planet after plants (450 Gt C) and bacteria (70 Gt C) [37]. In the following we outline three major characteristics of mycelial fungi that make the mycosphere a hotspot for high contaminant bioavailability and biodegradation:

2.1. Fungi are ubiquitous and also present in contaminated habitats

With more than 144,000 known species [6] and up to 3.8 million undiscovered species, fungi significantly contribute to the taxonomic diversity of microbial systems. In moist, aerobic terrestrial habitats containing complex natural organic matter [38] up to 300 taxa in 0.25 g of soil have been described [38] thereby accounting for up to 50-75% of the microbial biomass. Such abundance is triggered by the capacity of saprotrophic fungi to depolymerize constituents of animals, wood and other plant material [39], [6]. Some fungi are highly adapted to extreme environmental conditions. They may grow at low oxygen partial pressures, temperatures ranging from -5 to +60 °C, pH values of 1 to 9 or at a water activity of as low as 0.6533. Or they may be tolerant to drought, enabled by a transport of water in fungal hyphae [40], and thereby preserving relevant ecosystem functions [41]. Melanized fungi have even been found to use radioactivity for growth [42] or even to survive simulated Martian environmental conditions [43]. Fungi are also often found in contaminated environments [7] although still poor knowledge exists on fungal community responses to contaminant mixtures and remediation approaches [8], [44].

2.2. Fungi have a broad catabolic potential and decouple contaminant transformation from biomass formation

The species richness and abundance of fungi often goes along with a substantial diversity in biochemical functionality. Saprophytic fungi play a significant role in the decomposition, sequestration, and production of

polymeric organic matter (e.g. lignin, lipids, carbohydrates and proteins [39]). They normally attack high molecular weight compounds with extracellular oxidoreductases. The low specificity of these enzymes also enables the co-metabolic transformation of structurally diverse pollutant classes [7]. Metabolites produced may then either be subject to intracellular catabolism (and used for biomass production), secreted in the form of conjugates or form bound residues of soil constituents [7]. Despite of commonly being considered as aerobic organisms reports on the presence of fungi in anoxic habitats [45], [46] and anaerobic transformations of contaminants by fungi exist (e.g. [47] [48], [49]). Although saprophytic bacteria and fungi often share similar biogeochemical functions, they express different suitability to degrade contaminants. The overall catabolic potential of fungi for degrading organic contaminants seems broader than the often highly specific bacterial and archaeal biodegradation pathways. Bacteria and archaea use contaminants as sole sources of carbon and energy by a series of highly specific biochemical pathways requiring corresponding terminal electron acceptors [7]. The availability of a contaminant to specialized degrader organisms thus becomes central for biomass production, i.e. a main driver of the feedback loop of contaminant uptake and biomass formation [18]. For poorly available chemicals (and unlike for fungal enzymes that remain expressed even at low contaminants concentrations [13]) specific degradation pathways may not be expressed in bacteria. At such conditions bacteria and archaea may enter dormancy, undergo sporulation or start using more available substrates (while at best co-metabolizing the contaminants). Even though often termed as 'phytoremediation' the degradation of soil contaminants in presence of plants has to be regarded as a result of the complete root zone including bacteria, fungi, and plants [50]. Given the often oligotrophic nature of soil, plant root derived exudates are a major driver of co-metabolic fungal degradation. The plant-associated microbial communities can be seen as 'a sunlight driven hotspot for the turnover of organic chemicals' [51]. Mycorrhizal symbioses rely on the effective mycelial transfer of mineral nutrients and water to the plant symbiont in exchange for photosynthates that account for up to 30% of the host plant's net carbon fixation. The 'mycorrhizosphere' (i.e. the mycosphere around a mycorrhizal fungus in the root zone of plants) is a prominent hotspot of microbial activity and the biodegradation of contaminants such as chloroaromatics, polycyclic aromatic hydrocarbons (PAH), and explosives.

2.3. Fungi adapt well to habitat heterogeneity and create suitable niches for contaminant biodegradation

The highly fractal structure of interconnected mycelial networks enables fungi to exploit the three-dimensional space of their habitats. They are also less sensitive to environmental heterogeneities (e.g. in water and nutrient availability) than bacteria, because they are able to decouple activity from local habitat conditions [52]. Driven by a turgor pressure of up to 600 kPa (i.e. ca. the pressure inside a bike tire) [53] hyphae may grow at speeds of >20 µm min^{-1} [53] and extend even into submicron soil pores of matrices [54]. Fungi thereby may exhibit mycelial lengths of ≈10^2 m g^{-1} in arable and up to 10^4 m g^{-1} in forest topsoil [7]. Expressing hydrophobic cell-wall proteins (hydrophobins), many hyphae are also able to overcome air-water interfaces and bridge air-filled soil pores and access heterogeneously distributed soil nutrient and carbon sources. The concurrence of an adaptive mycelial morphology in response to environmental conditions and a bi-directional cytoplasmic streaming promotes an effective mycelial foraging strategy that combines growth of feeder hyphae in favorable (nutrient-rich) environments with expansion of exploratory hyphae into new areas. Although the diameter of their hyphae measures 2-10 µm, mycelial networks can extend over an area of hectares [55]. Fungi also act as

engineers for microbial, in particular bacterial, habitats [56]. Thus, their mycelia create suitable habitats for efficient bacterial degradation of contaminants in several ways: (i) Intrahyphal translocation and release provides C-metabolites and N and P nutrients that can be used for bacterial activity and co-metabolic degradation [57] (Fig. 2b). (ii) Production of large quantities of hydrophobins that shape soil water infiltration properties and water availability to bacteria. (iii) Extraction and transport of soil nutrients and water from areas of high to areas of low water potential [40] enables microbial activity [58] [41, 59] (Fig. 2b). (iv) Hyphae of filamentous fungi also mobilize a wide range of hydrophobic contaminants by vesicle-bound cytoplasmic transport ('fungal pipelines', [60] or 'nutrient mobile links' [9]) and transported compounds become available to distant bacterial degraders [61] [51]. As a consequence of these multiple interactions with bacteria, fungal networks should be considered as key players in microbe-driven chemical ecology [62].

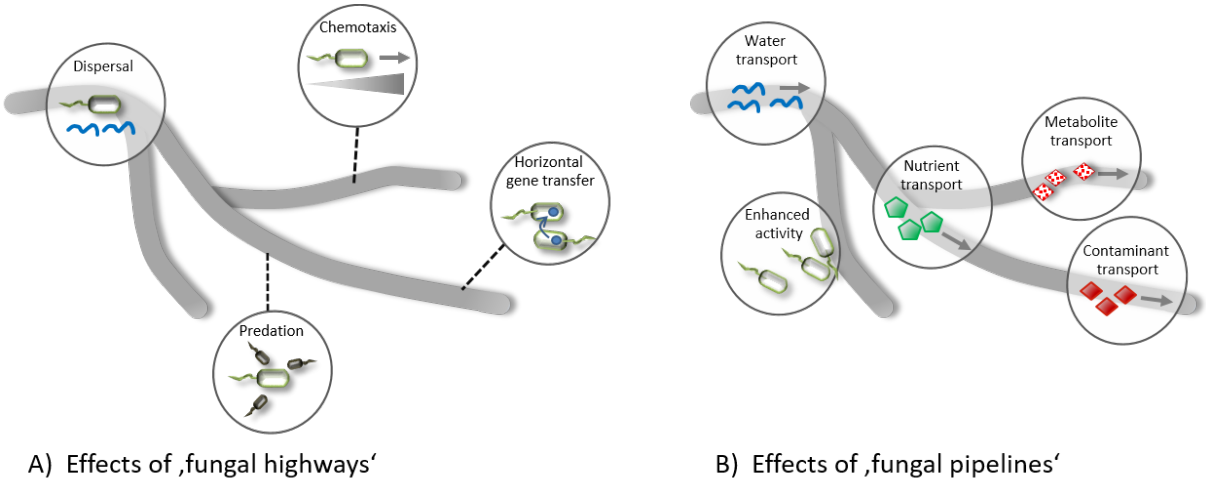

Fig. 2. (A) Liquid films along hyphae ('fungal highways') facilitate various ecological processes relevant for contaminant biodegradation including bacterial dispersal, potentially chemotaxis-driven towards contaminant hotspots, horizontal gene transfer and predation by bacterivores. (B) Matter transport by hyphae ('fungal pipelines') comprises the translocation and release of water, nutrients, metabolites but also contaminants. Both transport processes increase the bioavailability of resources and contaminants for bacteria, their activity and hence contaminant biodegradation in the mycosphere.

Beside active degradation of PAHs, fungi hence also exert a selective force on the bacteria in the mycosphere ('fungiphiles' [63]) due to their release of nutrients and signaling molecules [64]. The bacterial diversity in the mycosphere can range from a few to several hundreds of species and depends on the fungus [65] [66]. Bacterial activity may also be beneficial for fungi, e.g. when fungiphilic bacteria provide specific nutrients or degrade antifungal toxins in exchange for fungal products. Taking into account such multifaceted interactions, the concept of bipartite bacterial–fungal interactions is shifting towards complex interaction networks (sometimes also conceptually named as 'metaorganisms' [67]). The scaffold of the mycelia also serves as efficient dispersal vectors ('fungal highways', [68] or 'genetic mobile links' [9] [69]) for bacteria thereby promoting their (random or taxis-driven) access to soil habitats [70]. Contaminated soil habitats often remain poorly accessible to bacteria as their active dispersal is restricted by the poor connectivity of surfaces and discontinuity of water phases in the absence of episodes of water flow or bioturbation. Such restrictions can be overcome by 'fungal highways'. Liquid films forming along hyphae further enable transport and close cell-to-cell contact of initially spatially separate bacterial conjugation partners along the network structures. Hyphae thereby form a hotspot for horizontal gene

transfer between bacteria (HGT) and evolution endowing bacteria with new genetic traits for contaminant degradation [71].

3. Linking mycosphere traits and processes to bottlenecks of contaminant bioavailability

Three conceptual bottlenecks limit the bioavailability and biodegradation of contaminants in soil: (i) Insufficient concentration of contaminants for cellular uptake (bottleneck 1: 'Availability to degrader'), (ii) insufficient activity and abundance of microbial communities carrying the necessary catabolic potential (bottleneck 2: 'Activity and abundance of degrader'), and (iii) poor temporal stability of the degraders' performance (bottleneck 3: 'Functional stability of degrader`). In the following, we link the above outlined fungal traits and mycosphere processes to these mutually interwoven bottlenecks (cf. also Table 1 and Fig. 3):

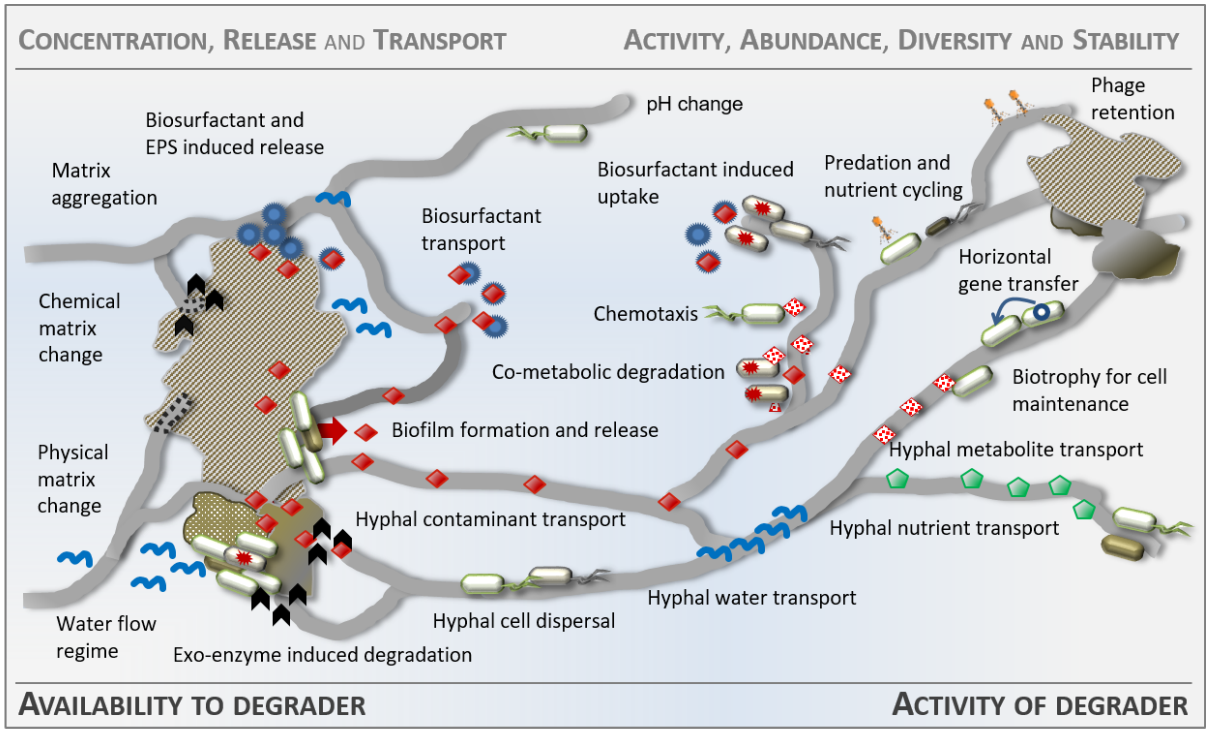

Figure 3. Conceptualization of mycosphere processes promoting the bioavailability and biodegradation of contaminants. These processes (i) stimulate the release and transport of matrix-bound chemicals, (ii) increase the contact probability between contaminants and degrader cells, (iii) fuel synergetic bacterial fungal exchange of nutrients, (iv) promote co-metabolic degradation of contaminants, (v) endorse evolution in bacterial communities and (vi) modulate physical and chemical habitat conditions. For detailed descriptions please refer also to Table 1.

3.1. Bottleneck 1 – Availability to degrader

Insufficient uptake arises if contaminant fluxes towards degrader cells are too low to address their full catabolic potential [18] (Fig. 1). In microbial systems such restriction typically occurs if contaminants are present at minute concentrations of if they are matrix-bound (sorbed), poorly water soluble, or present as solid chemicals. The high surface area of mycelia and good sorption in chitosan or chitin of hyphal cell walls is an important process to increase the availability of poorly concentrated chemicals with octanol-water partition coefficients of log D_{ow} of ≤ 3.0 [72]. Assuming a hyphal diameter of 10^{-5} m and mycelial length of 100 to 10000 m g^{-1} the surfaces of fungi

may amount up to ≈0.03 - 0.3 m^2 g^{-1}_{soil} and comprise up to 0.01 - 1% of the specific surface area of soil [73]. By physical [74] and chemical [7] weathering of surfaces mycelia may further promote the release of matrix-associated [53] [75], or polymer-bound chemicals [76]. Fungi thereby produce significant amounts of amphiphilic surface active compounds that mediate the release, of contaminants [77] and metals [78] and promote chemical transport and uptake to organisms [79]. Typical structures of fungal biosurfactants comprise sophorolipids, protein-lipid/polysaccharide complexes, glycolipids, or glycolipoproteins. Fungi often also decreases the pH in the mycosphere [80] [81] and can modulates speciation, release, and availability of pH-sensitive and/or matrix-bound chemicals. Acidic habitat conditions also enhance the release of insoluble mineral elements and – in conjunction to fungal metabolites serving as bacterial carbon and energy sources - lead to improved nutrient availability and activity of mineral/matrix surface weathering bacteria [72][77][78, 81]. The ubiquity and the widespread networks of mycelia in soils are further prone to transport sorbed chemicals over distances up to the cm-range [60] and, hence, to increase the contaminant availability to degrader cells distant from the sources. This is a particularly important process in heterogeneous vadose environments [61], where air-filled pores restrict the transfer of water-bound chemicals and bacteria.

3.2. Bottleneck 2 – Activity and abundance of degrader

Efficient contaminant transfer to cells however occurs only if cells are able to efficiently take and degrade the chemicals. This is the case if the draw advantage from degradation or if co-substrates promote their uptake and degradation. Next to high contaminant fluxes microbial habitats hence also must provide sufficient nutrient and energy fluxes to cells to sustain their activity and abundance. Low matter transfer to cells may not only limit the functional performance of cells but also evokes inadequate intracellular regulation and expression of metabolic contaminant degradation pathways and, thus, impair the survival, abundance and evolution of bacterial degrader communities. The impact of fungi on the physiology, regulation, and expression of metabolic pathways in bacteria is still poorly studied. Mycelia however, have been described to serve as networks for random and chemotactic dispersal of bacteria (`fungal highways`, Fig. 2A) thereby endorsing the contact probability between degrader cells and contaminant sources and, hence, the activity and abundance of degrader organisms [82] [83]. Mycelia further enable transport of contaminants and fungal metabolites (e.g. low molecular weight peptides, organic acids, sugars or sugar alcohols, metal-mobilizing or antimicrobial compounds [84]) to distant bacteria and sustain bacterial fungal cross-feeding [85] and bacterial activity and abundance, resp. [82] [86, 87]. Such cross-feeding is particularly important [88] under oligotrophic conditions [85] where fungi increase the habitat carrying capacity of bacteria [89] [20]. It also allows for carbon flows within the mycorrhizosphere where bacteria have been split into “plant-feeders” and “fungus-feeders” [88]. Independent of the habitat bacterial fungal interactions play an important role in biogeochemical nutrient and carbon cycling [86] [87] and thus, also for the turnover of contaminants. Another trait of (mainly saprotrophic) fungi comprises the use of unspecific extracellular enzymes and/or radical mediated transformations that also allow for the co-metabolic breakdown of complex polymeric compounds, recalcitrant chemicals or organic micropollutants. Subsequent transformation products act as carbon and energy sources for energy-driven bacterial contaminant uptake and/or co-metabolic degradation as detailed above. Mycelial cytoplasmic streaming also goes along with ‘active’ transport of water by the `fungal pipelines` enabling bacterial activity in otherwise dry areas [58]. A recent study

for instance has shown that hyphal release of water and nutrients induced the germination of spores of *Bacillus subtilis* cells [58]. By enmeshing soil aggregates [80] some fungi also modulate hydraulic conductivity [90] and water flow regimes and thereby promote waterborne transport and mutual contact of chemicals, nutrients and bacteria. An often overseen aspect is the effect of mycelia on predation and subsequent cycling of nutrients within contaminant degrading communities. Recent studies have analyzed the joint effect of predation and dispersal networks on contaminant degradation by linking spatial abundances of degrader and predator bacteria to the degradation of the major soil contaminant phenanthrene [91]. The data found suggested that predation facilitated by (mycelial) dispersal networks support the build-up of an effective bacterial biomass and, henceforth, contaminant biodegradation in heterogeneous systems such as soil [92] [91].

3.3. Bottleneck 3 – Functional stability and diversity of degrader

Adopting this chapter's concept of bioavailability it becomes clear that contaminant bioavailability comprises both a spatial [93] and a temporal dimension. Even though sufficient microbial capacity for contaminant degradation may exist at a given point of time, environmental conditions (e.g. temperature, water or nutrient availability) fluctuate and cause disturbances to microhabitat of degrading communities and, hence, impede their contaminant degrading functional stability. By exerting selective pressure [36] environmental fluctuations alter the composition of microbial communities and their functional diversity [94]. This also includes losses of the genotypic and phenotypic diversity of degrader organisms and/or their expression of contaminant-specific functional traits (Fig. 4). The presence of contaminants and varying fluxes thereof to degrader cells, resp. are form also disturbance *per se* and impact the fitness and performance of competing degrader organisms. For instance, bacteria and fungi may strive for the same pools of nutrients and electron acceptors pools. Depending on the situation bacterial fungal interactions may thereby be competitive (e.g. induced by the production by antimicrobial compounds [95]) or range from apparently random physical interactions to specific commensal or symbiotic associations [96] [95]. Bacterial biotrophy of extracellular fungal products (e.g. organic acids, sugars, or polyols [84]) may be a commensal interaction that has been discussed as effective strategy to fuel bacterial dispersal along hyphae. Such dispersal promotes bacterial access to new contaminant sources [83] or may help bacteria to efficiently drop out of unsuitable habitats. Although many studies exist on the use of bacteria and fungi in bioremediation approaches (cf. [97]) still poor information exists on their [62] metabolic interactions allowing for contaminant degradation in complex environments. Experiments preformed in synthetic and *in silico* microbial habitats however revealed that hyphae promote the stability of contaminant biodegradation by (i) translocating bacteria and nutrients for enhanced recolonization and recovery of degrader communities after disturbances [98], (ii) distributing water from wet to dry habitats and thereby shaping suitable local matric potentials for improved bacterial degradation [58] and fungal compound mineralization [41], and (iii) sustaining bacterial dispersal and compound degradation capacity at low osmotic and matric potentials [99]. Mycelia also have been shown to serve as novel ecological routes for enrichment and dissemination of antibiotic resistance genes [100] and as a focal point [71][100] for horizontal gene transfer between genetically distinct bacteria; i.e. as a main mechanism of evolution endowing bacteria with new genetic traits in favor of contaminant degradation [71]. Recent work has also discussed the mycosphere as arena for bacteriophage retention [101] and phage-induced exchange of genetic elements among microbial communities [102], [103]. Phage predation [104] in the

mycosphere may form an important evolutionary force for microbial degrader communities and their adaptation to changing environmental and contaminant conditions, respectively.

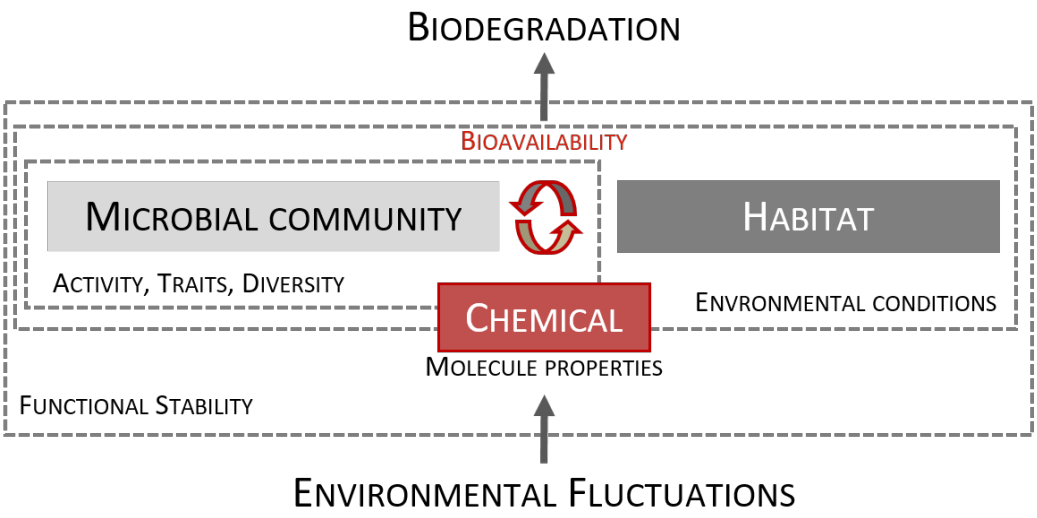

Figure 4. Bioavailability for contaminant degradation requires a deep understanding of the ecology of contaminant degrading microbial systems in the mycosphere. Key drivers of bioavailability of inherently degradable chemicals thereby stretch over different organizational levels and scales including the molecular, cellular, community and microbial system level. The biodegradation of a chemical depends on its physico-chemical properties and intrinsic structural stability toward (bio)-chemical reactions (molecule properties), its presence and distribution in a given habitat and also the functional potential and effectiveness of microbial communities (traits). All these aspects are subject to and result from ever-changing environmental fluctuations.

4. LESSONS LEARNT: CONTAMINANT BIOAVAILABILITY STRETCHES OVER VARIOUS ORGANIZATIONAL LEVELS AND REQUIRES DEEP UNDERSTANDING OF THE ECOLOGY OF DEGRADER MICROBIAL SYSTEMS

Bioavailability for contaminant degradation in soil requires a deep understanding of the ecology of degrader microbial systems. The drivers of bioavailability in the mycosphere thereby stretch over different organizational levels and scales including the molecular, cellular, community and system level (Fig. 4). At the molecular level the structure and the physicochemical properties of the chemical will determine the abiotic interactions and potential intrinsic recalcitrance towards existing biochemical degradation pathways (and the evolution of new pathways, respectively). Such chemicals are unlikely to be quickly degraded without the production of harmful or persistent degradation products. At the cellular level mass transfer into the cell (i.e. by active or diffusion-driven cellular uptake) and biochemical transformation capacity by the cell will determine the biodegradation (cf. Fig. 1). These processes have been shown to be modulated by the mycosphere in several ways. At the community level the presence of contaminants themselves also exerts selective pressure on the abundance and diversity of microbial communities [36]. Increased exchange of genetic elements in the mycosphere also triggers the evolution of their genetic potential to degrade new chemical structures or biodiversity changes. Finally, at the system level the prevailing habitat conditions must provide sufficient proliferation to allow for ongoing contaminant bioavailability and degradation ('Functional stability'). This may also require microbial interactions with plants (e.g. in the rhizosphere [51]) or other higher organisms such soil-dwelling animals. Last but not least even though we here focus on biodegradation, microbial system considerations on compound bioavailability also need to account for competing abiotic degradation processes (e.g. photo-degradation, heterogeneous catalysis

at matrix surfaces or hydrolysis) that may affect available concentration of chemicals and their epi-metabolome [105] in any microbial system.

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Table 1: Overview of mycelial traits and processes on habitat and bacteriome conditions being presumed to be relevant for microbial (bacterial!) activity and the bioavailability and biodegradation of organic chemicals.

KEY TRAITS OF MYCELIAL FUNGI	MAIN EFFECTS ON BIOAVAILABILITY	AVAILABILITY TO DEGRADER (BOTTLENECK 1)		ACTIVITY AND ABUNDANCE OF DEGRADER (BOTTLENECK 2)			DIVERSITY AND STABILITY OF DEGRADER (BOTTLENECK 3)	
		RELEASE	TRANSPORT	UPTAKE	ACTIVITY	ABUNDANCE	DIVERSITY	STABILITY
RELEASE OF PRIMARY AND SECONDARY METABOLITES	Mycelial release leads to enhanced availability of nutrients and carbon compounds and cross-feeding [106].	Metabolites are resource for matrix weathering organisms that promote release of chemicals. Organic acids change pH and affect release of pH sensitive chemicals.	Organic acids change pH and solubility and transport of pH sensitive chemicals.	Metabolites are resource for energy-driven or co-metabolic chemical uptake.	Metabolites are nutrient source for (co-metabolic) degradation [41], Khan et al (2020) unpublished. Bacteria and fungi mutually exchange nutrients [107]and metabolites (cross-feeding) [108] [57, 69, 107]. Release of secondary metabolites alters the availability of nutrients in soil [109].	Metabolites enable biofilm formation on hyphae [110] and fuel bacterial dispersal along hyphae. Metabolites are source for tactic colonization [111, 112]. Synergetic cross kingdom interactions under oligotrophic conditions [85] and increase of habitat carrying capacity of bacteria [89] [20]	Metabolites select for fungiphilic bacteria [63] [56] [113] [64] Secondary metabolites modulate community composition (by acting as antibiotics [114] or signals to other organisms) [115] [62].	Metabolites form a carbon and energy source to fulfill maintenance requirements for degrader organisms [20], [16]. Fungal bacterial synergetic cross kingdom interactions stabilize ecosystem functions [116].
PRODUCTION OF BIOSURFACTANTS AND EXOPOLYMERIC SUBSTANCES	Biosurfactant and EPS release promotes solubilisation of hydrophobic chemicals [117] [118] and hyphal growth.	Surfactants and EPS mediate chemical release from matrix [119].	Micelles promote transport of dissolved chemicals and mobilize liquid chemicals by emulsification [120] [79].	Micelles promote cellular chemical uptake [79].	EPS and micelles promote degradation by bacteria and fungi [79] [121].			Hydrophobins increase bacterial access to zones in air-filled matrices [7].
SAPROTROPHIC GROWTH USING UNSPECIFIC EXTRACELLULAR ENZYMES OR RADICAL-MEDIATED TRANSFORMATIONS	Saprophytic growth decouples fungal biomass abundance from contaminant availability. Unspecific attack of complex and	Unspecific attack promotes biochemical weathering of matrix and release of entrapped chemicals and nutrients [122].	Enzymes modulate compound mobilization [123].	Degradation of complex polymers to units available for bacterial uptake [124].	Transformation of complex polymers [125], [126]. Hydrophobic, recalcitrant chemicals and micropollutants [124] [127] produce		Metabolites shape mycosphere bacterial community composition [63] [56].	Metabolites form carbon and energy sources to fulfill maintenance requirements for degrader organisms [20], [16].

	polymeric molecules increases release of chemicals and metabolites [7].				metabolites modulating bacterial activity.			
NETWORK-BASED GROWTH: ***EFFECT ON MATTER TRANSPORT***	Mycelia decouple resource location from location of growth and help to overcome heterogeneous distribution of carbon sources and/or nutrients [128] [129]	Mycelial enmeshment of soil matrix [130] [131] promotes close contact and direct chemical uptake by hyphae and bacteria.	Mycelia form scaffold for matter transport in air-filled and heterogeneous matrices. Hyphae promote matter and contaminant transport [128]. Mycelia enrich hydrophobic compounds by biosorption and increase accessible concentration to mycosphere bacteria [132].	Hyphae transport chemicals to immediate vicinity of bacterial surfaces [61].	Mycelia increase bacterial degradation activity [61] [133]. Mycelia bridge NAPL-water interfaces [133] and increase degradation.	Hyphae take up and transport chemicals/nutrients to communities distant from source [60] [134]. This increases habitat carrying capacity of degrader bacteria [89]. Mycelial matter transport provides carbon and energy source for spore germination and bacterial growth in oligotrophic habitats [58].	Hyphae form networks for signaling molecules and modulate cell to cell interactions [62]. Mycelia retain bacteriophages [101] infecting bacteria.	Networks allow for spatial decoupling of resource and bacterial/fungal growth location [135]. Hyphae fuel bacterial dispersal to zones of heterogeneously distributed chemicals (increased accessibility) [83] or out of zones of disturbance.
NETWORK-BASED GROWTH: ***EFFECT ON BACTERIA TRANSPORT***	Mycelia are a scaffold for bacterial dispersal and promote transport through air-filled pores [14].	Mycelia enable transport of bacteria to sorbing matrices promoting mixed bacterial fungal biofilm formation, increased biodegradation [77] and bacteria-induced weathering of matrix.	Bacteria are transported to contaminant source [136].		Mycelia enable chemotactic movement along [70] and to mycelia. Dispersal networks promote bacterial transport and degradation at low matric and osmotic potentials [99]. Dispersal of predators along hyphae affects degradation [91, 136, 137].	Mycelia form scaffolds for tactic movement to chemoattractor sources [70]. Dispersal of predators decreases bacterial biomass [91, 136, 137]. Dispersal networks promote bacterial transport and abundance at low matric and osmotic potential [99].	Mycelia form scaffold for transport of predators [91, 137] Mycelia promote horizontal gene transfer among bacteria and evolution [71] [100]. Mycelia transport chemical signals and shape cell to cell interactions [62]. Mycelia increase co-migration induced	Mycelia allow for recolonization of disturbed areas [98] Mycelia enable dispersal and habitat colonization at low matric potentials [68].

							invasion effects [138].	
MODULATION OF WATER REGIME, PH AND SALINITY	Mycelia induce change of hydraulic flow regime and water availability. Hyphae modulate pH and salinity at the microscale [139].	Mycelia modulate surface wettability [140] and chemical release due to changed surface wetting.	Mycelia increase water-borne solute transport. Mycelia decrease water intrusion by hydrophobins [141]. Mycelia change pH and affect transport of pH sensitive compounds.	Mycelia potentially change uptake of pH sensitive chemicals [139].	Mycelia increase microbial activity due to water transport [58].	Mycelia increase bacterial abundance in areas of low water availability due to water transport [58].	Mycelial influence presence and diversity of fungiphilic degrader bacteria [63] [142] [56].	Mycelia alleviate of drought stress and salt stress. [99].
MODULATION OF MATRIX INTEGRITY	Turgor pressure apical growth promotes physical effects on matrix integrity and subsequent release of entrapped chemicals [53] [75].	Mycelia promote bio-weathering of matrices and surfaces [143] [75] [74] and availability of entrapped chemicals and nutrients.	Mycelia influence soil aggregate structure [80] and change hydraulic conductivity and solute transport. [144].					Mycelial bioturbation allows for colonization of new matrices, nutrients sources and habitats.

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5. REFERENCES

1. Louca, S., et al., *Function and functional redundancy in microbial systems.* Nature Ecology & Evolution, 2018. **2**(6): p. 936-943.
2. Semple, K.T., et al., *Microbial interactions with organic contaminants in soil: Definitions, processes and measurement.* Environmental Pollution, 2007. **150**(1): p. 166-176.
3. Wick, L.Y., T. Colangelo, and H. Harms, *Kinetics of mass transfer-limited bacterial growth on solid PAHs.* Environmental Science & Technology, 2001. **35**(2): p. 354-361.
4. Ortega-Calvo, J.-J., et al., *From Bioavailability Science to Regulation of Organic Chemicals.* Environmental Science & Technology, 2015. **49**(17): p. 10255-10264.
5. van der Heijden, M.G.A., et al., *Mycorrhizal ecology and evolution: the past, the present, and the future.* New Phytologist, 2015. **205**(4): p. 1406-1423.
6. Willis, K.J., *State of the World's Fungi.* Ima Fungus, 2018. **9**(1): p. 1.
7. Harms, H., D. Schlosser, and L.Y. Wick, *Untapped potential: exploiting fungi in bioremediation of hazardous chemicals.* Nature Reviews Microbiology, 2011. **9**(3): p. 177-192.
8. Harms, H., L.Y. Wick, and D. Schlosser, *The Fungal Community in Organically Polluted Systems*, in *The Fungal Community: Its Organization and Role in the Ecosystem*, J.F.W. John Dighton, Editor. 2016, RC Press, Boca Raton/FL.
9. Bielcik, M., et al., *The role of active movement in fungal ecology and community assembly.* Movement Ecology, 2019. **7**(1): p. 12.
10. Ullrich, R. and M. Hofrichter, *Enzymatic hydroxylation of aromatic compounds.* Cellular and Molecular Life Sciences, 2007. **64**(3): p. 271-293.
11. Hofrichter, M., et al., *New and classic families of secreted fungal heme peroxidases.* Applied Microbiology and Biotechnology, 2010. **87**(3): p. 871-897.
12. Baldrian, P., *Wood-inhabiting ligninolytic basidiomycetes in soils: Ecology and constraints for applicability in bioremediation.* Fungal Ecology, 2008. **1**: p. 4-12.
13. Schlosser, D., *Fungal Attack on Environmental Pollutants Representing Poor Microbial Growth Substrates.*, in *Grand Challenges in Fungal Biotechnology. Grand Challenges in Biology and Biotechnology.* , N.H. (eds), Editor. 2020, Springer: Cham.
14. Worrich, A., L.Y. Wick, and T. Banitz, *Ecology of Contaminant Biotransformation in the Mycosphere: Role of Transport Processes*, in *Advances in Applied Microbiology, Vol 104*, G.M. Gadd and S. Sariaslani, Editors. 2018, Elsevier Academic Press Inc: San Diego. p. 93-133.
15. Council, N.R., *Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications*. 2003, Washington, DC: The National Academies Press. 432.
16. Bosma, T.N.P., et al., *Mass transfer limitation of biotransformation: Quantifying bioavailability.* Environmental Science & Technology, 1997. **31**(1): p. 248-252.
17. Hanzel, J., et al., *Walking the tightrope of bioavailability: growth dynamics of PAH degraders on vapour-phase PAH.* Microbial Biotechnology, 2012. **5**(1): p. 79-86.
18. Johnsen, A.R., L.Y. Wick, and H. Harms, *Principles of microbial PAH-degradation in soil.* Environmental Pollution, 2005. **133**(1): p. 71-84.
19. Krueger, M.C., H. Harms, and D. Schlosser, *Prospects for microbiological solutions to environmental pollution with plastics.* Applied Microbiology and Biotechnology, 2015. **99**(21): p. 8857-8874.
20. Rein, A., et al., *Impact of bacterial activity on turnover of insoluble hydrophobic substrates (phenanthrene and pyrene)-Model simulations for prediction of bioremediation success.* Journal of Hazardous Materials, 2016. **306**: p. 105-114.
21. Kundu, K., et al., *Defining lower limits of biodegradation: atrazine degradation regulated by mass transfer and maintenance demand in Arthrobacter aurescens TC1.* Isme Journal, 2019. **13**(9): p. 2236-2251.
22. Elsner, M. and G. Imfeld, *Compound-specific isotope analysis (CSIA) of micropollutants in the environment - current developments and future challenges.* Current Opinion in Biotechnology, 2016. **41**: p. 60-72.
23. Hazen, T.C., *Cometabolic Bioremediation*, in *Handbook of Hydrocarbon and Lipid Microbiology*, K.N. Timmis, Editor. 2010, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 2505-2514.
24. Crawford, J.W., et al., *Towards an evolutionary ecology of life in soil.* Trends in Ecology & Evolution, 2005. **20**(2): p. 81-87.
25. Or, D., et al., *Physical constraints affecting bacterial habitats and activity in unsaturated porous media - a review.* Advances in Water Resources, 2007. **30**(6-7): p. 1505-1527.

26. Tecon, R. and D. Or, *Biophysical processes supporting the diversity of microbial life in soil.* Fems Microbiology Reviews, 2017. **41**(5): p. 599-623.
27. Crawford, J.W., et al., *Microbial diversity affects self-organization of the soil-microbe system with consequences for function.* Journal of the Royal Society Interface, 2012. **9**(71): p. 1302-1310.
28. Young, I.M. and J.W. Crawford, *Interactions and self-organization in the soil-microbe complex.* Science, 2004. **304**(5677): p. 1634-1637.
29. Harms, H. and L.Y. Wick, *Dispersing pollutant-degrading bacteria in contaminated soil without touching it.* Engineering in Life Sciences, 2006. **6**(3): p. 252-260.
30. Krell, T., et al., *Bioavailability of pollutants and chemotaxis.* Current Opinion in Biotechnology, 2013. **24**(3): p. 451-456.
31. Botton, S., et al., *Resilience of microbial systems towards disturbances.* Crit Rev Microbiol, 2006. **32**(2): p. 101-12.
32. Shade, A., et al., *Fundamentals of microbial community resistance and resilience.* Front Microbiol, 2012. **3**: p. 417.
33. Griffiths, B.S. and L. Philippot, *Insights into the resistance and resilience of the soil microbial community.* FEMS Microbiol Rev, 2013. **37**(2): p. 112-29.
34. Nikel, P.I., et al., *The private life of environmental bacteria: pollutant biodegradation at the single cell level.* Environmental Microbiology, 2014. **16**(3): p. 628-642.
35. Ren, X.Y., et al., *Sorption, transport and biodegradation - An insight into bioavailability of persistent organic pollutants in soil.* Science of the Total Environment, 2018. **610**: p. 1154-1163.
36. van der Meer, J.R., *Environmental pollution promotes selection of microbial degradation pathways.* Frontiers in Ecology and the Environment, 2006. **4**(1): p. 35-42.
37. Bar-On, Y.M., R. Phillips, and R. Milo, *The biomass distribution on Earth.* Proceedings of the National Academy of Sciences of the United States of America, 2018. **115**(25): p. 6506-6511.
38. Lee Taylor, D. and R.L. Sinsabaugh, *Chapter 4 - The soil fungi: occurrence, phylogeny, and ecology.* . In E. A. Paul (Ed.), Soil microbiology, ecology and biochemistry (fourth edition) (pp. 77-109). Boston: Academic Press., 2015.
39. Treseder, K.K. and J.T. Lennonb, *Fungal Traits That Drive Ecosystem Dynamics on Land.* Microbiology and Molecular Biology Reviews, 2015. **79**(2): p. 243-262.
40. Allen, M.F., *Mycorrhizal fungi: Highways for water and nutrients in arid soils.* Vadose Zone Journal, 2007. **6**(2): p. 291-297.
41. Guhr, A., et al., *Redistribution of soil water by a saprotrophic fungus enhances carbon mineralization.* Proceedings of the National Academy of Sciences of the United States of America, 2015. **112**(47): p. 14647-14651.
42. Dadachova, E., et al., *Ionizing Radiation Changes the Electronic Properties of Melanin and Enhances the Growth of Melanized Fungi.* Plos One, 2007. **2**(5): p. 13.
43. Zakharova, K., et al., *Protein patterns of black fungi under simulated Mars-like conditions.* Scientific Reports, 2014. **4**.
44. Ventorino, V., et al., *Comparative assessment of autochthonous bacterial and fungal communities and microbial biomarkers of polluted agricultural soils of the Terra dei Fuochi.* Scientific Reports, 2018. **8**.
45. Orsi, W.D., *Ecology and evolution of seafloor and subseafloor microbial communities.* Nature Reviews Microbiology, 2018. **16**(11): p. 671-683.
46. Ivarsson, M., et al., *Fungi in Deep Subsurface Environments*, in *Advances in Applied Microbiology, Vol 102*, S. Sariaslani and G.M. Gadd, Editors. 2018, Elsevier Academic Press Inc: San Diego. p. 83-116.
47. Wang, G.Y., et al., *Biodegradation of phenol and m-cresol by Candida albicans PDY-07 under anaerobic condition.* Journal of Industrial Microbiology & Biotechnology, 2009. **36**(6): p. 809-814.
48. Russell, J.R., et al., *Biodegradation of Polyester Polyurethane by Endophytic Fungi.* Applied and Environmental Microbiology, 2011. **77**(17): p. 6076-6084.
49. Aydin, S., et al., *Aerobic and anaerobic fungal metabolism and Omics insights for increasing polycyclic aromatic hydrocarbons biodegradation.* Fungal Biology Reviews, 2017. **31**(2): p. 61-72.
50. El Amrani, A., et al., *"Omics" Insights into PAH Degradation toward Improved Green Remediation Biotechnologies.* Environmental Science & Technology, 2015. **49**(19): p. 11281-11291.
51. Fester, T., et al., *Plant-microbe interactions as drivers of ecosystem functions relevant for the biodegradation of organic contaminants.* Current Opinion in Biotechnology, 2014. **27**: p. 168-175.
52. de Vries, F.T., et al., *Land use alters the resistance and resilience of soil food webs to drought.* Nature Climate Change, 2012. **2**(4): p. 276-280.
53. Lew, R.R., *How does a hypha grow? The biophysics of pressurized growth in fungi.* Nature Reviews Microbiology, 2011. **9**(7): p. 509-518.

54. Bornyasz, M.A., R.C. Graham, and M.F. Allen, *Ectomycorrhizae in a soil-weathered granitic bedrock regolith: Linking matrix resources to plants.* Geoderma, 2005. **126**(1-2): p. 141-160.
55. Ferguson, B.A., et al., *Coarse-scale population structure of pathogenic Armillaria species in a mixed-conifer forest in the Blue Mountains of northeast Oregon.* Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 2003. **33**(4): p. 612-623.
56. Boer, W., et al., *Living in a fungal world: impact of fungi on soil bacterial niche development.* FEMS Microbiol Rev, 2005. **29**(4): p. 795-811.
57. Zhang, L., et al., *Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium.* New Phytologist, 2016. **210**(3): p. 1022-1032.
58. Worrich, A., et al., *Mycelium-mediated transfer of water and nutrients stimulates bacterial activity in dry and oligotrophic environments.* Nature Communications, 2017. **8**: p. 9.
59. de Vries, F.T., et al., *Soil bacterial networks are less stable under drought than fungal networks.* Nature Communications, 2018. **9**: p. 12.
60. Furuno, S., et al., *Mycelia Promote Active Transport and Spatial Dispersion of Polycyclic Aromatic Hydrocarbons.* Environmental Science & Technology, 2012. **46**(10): p. 5463-5470.
61. Schamfuss, S., et al., *Impact of Mycelia on the Accessibility of Fluorene to PAH-Degrading Bacteria.* Environmental Science & Technology, 2013. **47**(13): p. 6908-6915.
62. Schmidt, R., et al., *Microbe-driven chemical ecology: past, present and future.* Isme Journal, 2019. **13**(11): p. 2656-2663.
63. Warmink, J.A., R. Nazir, and J.D. van Elsas, *Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi.* Environmental Microbiology, 2009. **11**(2): p. 300-312.
64. Nazir, R., et al., *Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats.* Fems Microbiology Ecology, 2010. **71**(2): p. 169-185.
65. Schulz-Bohm, K., et al., *Fungus-associated bacteriome in charge of their host behavior.* Fungal Genetics and Biology, 2017. **102**: p. 38-48.
66. Ghodsalavi, B., et al., *A novel baiting microcosm approach used to identify the bacterial community associated with Penicillium bilaii hyphae in soil.* Plos One, 2017. **12**(10): p. 14.
67. Olsson S, B.P., Pawlowska TE. , *Ecology and evolution of Fungal-Bacterial interactions*, in *The Fungal Community: Its Organization and Role in the Ecosystem,*, W.J.e. Dighton J, Editor. 2017, CRC Press, : Boca Raton.
68. Kohlmeier, S., et al., *Taking the fungal highway: Mobilization of pollutant-degrading bacteria by fungi.* Environmental Science & Technology, 2005. **39**(12): p. 4640-4646.
69. Zhang, W., et al., *Mycelial network-mediated rhizobial dispersal enhances legume nodulation.* The ISME Journal, 2020.
70. Furuno, S., et al., *Fungal mycelia allow chemotactic dispersal of polycyclic aromatic hydrocarbon-degrading bacteria in water-unsaturated systems.* Environmental Microbiology, 2010. **12**(6): p. 1391-1398.
71. Berthold, T., et al., *Mycelia as a focal point for horizontal gene transfer among soil bacteria.* Scientific Reports, 2016. **6**: p. 8.
72. Hofmann, U. and D. Schlosser, *Biochemical and physicochemical processes contributing to the removal of endocrine-disrupting chemicals and pharmaceuticals by the aquatic ascomycete Phoma sp. UHH 5-1-03.* Appl Microbiol Biotechnol, 2016. **100**(5): p. 2381-99.
73. Pennell, K.D., *Specific surface area*, in *Reference Module in Earth Systems and Environmental Sciences*, S.A. Elias, Editor. 2016, Elsevier: Oxford. p. 1–8.
74. Lybrand, R.A., et al., *A coupled microscopy approach to assess the nano-landscape of weathering.* Sci Rep, 2019. **9**(1): p. 5377.
75. Gadd, G.M., *Geomicrobiology of the built environment.* Nature Microbiology, 2017. **2**(4): p. 9.
76. Zumstein, M.T., et al., *Biodegradation of synthetic polymers in soils: Tracking carbon into CO2 and microbial biomass.* Science Advances, 2018. **4**(7): p. 8.
77. Perera, M., et al., *Biofilm mediated synergistic degradation of hexadecane by a naturally formed community comprising Aspergillus flavus complex and Bacillus cereus group.* BMC Microbiology, 2019. **19**(1): p. 84.
78. Tourney, J. and B.T. Ngwenya, *The role of bacterial extracellular polymeric substances in geomicrobiology.* Chemical Geology, 2014. **386**: p. 115-132.
79. Garcia, J.M., L.Y. Wick, and H. Harms, *Influence of the nonionic surfactant Brij 35 on the bioavailability of solid and sorbed dibenzofuran.* Environmental Science & Technology, 2001. **35**(10): p. 2033-2039.

80. Lehmann, A., et al., *Fungal Traits Important for Soil Aggregation.* Frontiers in Microbiology, 2020. **10**: p. 13.
81. Liu, Y., et al., *Bacterial diversity among the fruit bodies of ectomycorrhizal and saprophytic fungi and their corresponding hyphosphere soils.* Scientific Reports, 2018. **8**(1): p. 11672.
82. Wick, L.Y., et al., *Effect of fungal hyphae on the access of bacteria to phenanthrene in soil.* Environmental Science & Technology, 2007. **41**(2): p. 500-505.
83. Banitz, T., et al., *Highways versus pipelines: contributions of two fungal transport mechanisms to efficient bioremediation.* Environmental Microbiology Reports, 2013. **5**(2): p. 211-218.
84. Leveau, J.H.J. and G.M. Preston, *Bacterial mycophagy: definition and diagnosis of a unique bacterial-fungal interaction.* New Phytologist, 2008. **177**(4): p. 859-876.
85. Velez, P., et al., *Nutrient Dependent Cross-Kingdom Interactions: Fungi and Bacteria From an Oligotrophic Desert Oasis.* Frontiers in Microbiology, 2018. **9**: p. 15.
86. Llado, S., R. Lopez-Mondejar, and P. Baldrian, *Forest Soil Bacteria: Diversity, Involvement in Ecosystem Processes, and Response to Global Change.* Microbiol Mol Biol Rev, 2017. **81**(2).
87. Brabcova, V., et al., *Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community.* New Phytol, 2016. **210**(4): p. 1369-81.
88. Ballhausen, M.-B. and W. de Boer, *The sapro-rhizosphere: Carbon flow from saprotrophic fungi into fungus-feeding bacteria.* Soil Biology and Biochemistry, 2016. **102**: p. 14-17.
89. de Boer, W., et al., *Living in a fungal world: impact of fungi on soil bacterial niche development.* Fems Microbiology Reviews, 2005. **29**(4): p. 795-811.
90. Bitterlich, M., P. Franken, and J. Graefe, *Arbuscular Mycorrhiza Improves Substrate Hydraulic Conductivity in the Plant Available Moisture Range Under Root Growth Exclusion.* Frontiers in plant science, 2018. **9**: p. 301-301.
91. Otto, S., et al., *Catch me if you can: dispersal and foraging of Bdellovibrio bacteriovorus 109J along mycelia.* ISME J, 2016.
92. Otto, S., H. Harms, and L.Y. Wick, *Effects of predation and dispersal on bacterial abundance and contaminant biodegradation.* Fems Microbiology Ecology, 2017. **93**(2): p. 7.
93. Ladau, J. and E.A. Eloe-Fadrosh, *Spatial, Temporal, and Phylogenetic Scales of Microbial Ecology.* Trends in Microbiology, 2019. **27**(8): p. 662-669.
94. Escalas, A., et al., *Microbial functional diversity: From concepts to applications.* Ecology and Evolution, 2019. **9**(20): p. 12000-12016.
95. Deveau, A., et al., *Bacterial-fungal interactions: ecology, mechanisms and challenges.* Fems Microbiology Reviews, 2018. **42**(3): p. 335-352.
96. Ul Haq, I., et al., *The Interactions of Bacteria with Fungi in Soil: Emerging Concepts*, in *Advances in Applied Microbiology, Vol 89*, S. Sariaslani and G.M. Gadd, Editors. 2014, Elsevier Academic Press Inc: San Diego. p. 185-215.
97. Jambon, I., et al., *Harnessing plant-bacteria-fungi interactions to improve plant growth and degradation of organic pollutants.* Journal of Plant Interactions, 2018. **13**(1): p. 119-130.
98. Konig, S., et al., *Functional Resistance to Recurrent Spatially Heterogeneous Disturbances Is Facilitated by Increased Activity of Surviving Bacteria in a Virtual Ecosystem.* Frontiers in Microbiology, 2018. **9**: p. 14.
99. Worrich, A., et al., *Mycelium-Like Networks Increase Bacterial Dispersal, Growth, and Biodegradation in a Model Ecosystem at Various Water Potentials.* Applied and Environmental Microbiology, 2016. **82**(10): p. 2902-2908.
100. Nazir, R., et al., *Fungal networks serve as novel ecological routes for enrichment and dissemination of antibiotic resistance genes as exhibited by microcosm experiments.* Scientific Reports, 2017. **7**: p. 13.
101. Ghanem, N., et al., *Mycelial Effects on Phage Retention during Transport in a Microfluidic Platform.* Environmental Science & Technology, 2019. **53**(20): p. 11755-11763.
102. Pratama, A.A. and J.D. van Elsas, *Gene mobility in microbiomes of the mycosphere and mycorrhizosphere -role of plasmids and bacteriophages.* Fems Microbiology Ecology, 2019. **95**(5): p. 16.
103. Zhang, M.Z., et al., *The mycosphere constitutes an arena for horizontal gene transfer with strong evolutionary implications for bacterial-fungal interactions.* Fems Microbiology Ecology, 2014. **89**(3): p. 516-526.
104. Fernández, L., A. Rodríguez, and P. García, *Phage or foe: an insight into the impact of viral predation on microbial communities.* The ISME journal, 2018. **12**(5): p. 1171-1179.
105. de Lorenzo, V., *Systems biology approaches to bioremediation.* Current Opinion in Biotechnology, 2008. **19**(6): p. 579-589.

106. D'Souza, G., et al., *Ecology and evolution of metabolic cross-feeding interactions in bacteria.* Natural Product Reports, 2018. **35**(5): p. 455-488.
107. Rashid, M.I., et al., *Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils.* Microbiological Research, 2016. **183**: p. 26-41.
108. Uehling, J.K., et al., *Microfluidics and Metabolomics Reveal Symbiotic Bacterial–Fungal Interactions Between Mortierella elongata and Burkholderia Include Metabolite Exchange.* Frontiers in Microbiology, 2019. **10**(2163).
109. Nagy, L.G., et al., *Six Key Traits of Fungi: Their Evolutionary Origins and Genetic Bases.* Microbiology Spectrum, 2017. **5**(4).
110. Guennoc, C.M., et al., *Bacterial biofilm formation on the hyphae of ectomycorrhizal fungi: a widespread ability under controls?* Fems Microbiology Ecology, 2018. **94**(7).
111. Ul Haq, I., et al., *Chemotaxis and adherence to fungal surfaces are key components of the behavioral response of Burkholderia terrae BS001 to two selected soil fungi.* Fems Microbiology Ecology, 2016. **92**(11).
112. Rudnick, M.B., J.A. van Veen, and W. de Boer, *Oxalic acid: a signal molecule for fungus-feeding bacteria of the genus Collimonas?* Environmental Microbiology Reports, 2015. **7**(5): p. 709-714.
113. Johnston, S.R., L. Boddy, and A.J. Weightman, *Bacteria in decomposing wood and their interactions with wood-decay fungi.* Fems Microbiology Ecology, 2016. **92**(11): p. 12.
114. Keller, N.P., *Fungal secondary metabolism: regulation, function and drug discovery.* Nature Reviews Microbiology, 2019. **17**(3): p. 167-180.
115. Effmert, U., et al., *Volatile Mediated Interactions Between Bacteria and Fungi in the Soil.* Journal of Chemical Ecology, 2012. **38**(6): p. 665-703.
116. de Menezes, A.B., A.E. Richardson, and P.H. Thrall, *Linking fungal-bacterial co-occurrences to soil ecosystem function.* Current Opinion in Microbiology, 2017. **37**: p. 135-141.
117. Shekhar, S., A. Sundaramanickam, and T. Balasubramanian, *Biosurfactant Producing Microbes and their Potential Applications: A Review.* Critical Reviews in Environmental Science and Technology, 2015. **45**(14): p. 1522-1554.
118. Santos, D.K.F., et al., *Biosurfactants: Multifunctional Biomolecules of the 21st Century.* International Journal of Molecular Sciences, 2016. **17**(3): p. 31.
119. Kiran, G.S., et al., *Optimization and production of a biosurfactant from the sponge-associated marine fungus Aspergillus ustus MSF3.* Colloids and Surfaces B-Biointerfaces, 2009. **73**(2): p. 250-256.
120. Mnif, I. and D. Ghribi, *High molecular weight bioemulsifiers, main properties and potential environmental and biomedical applications.* World Journal of Microbiology & Biotechnology, 2015. **31**(5): p. 691-706.
121. Jia, C.Y., et al., *Composition and morphology characterization of exopolymeric substances produced by the PAH-degrading fungus of Mucor mucedo.* Environmental Science and Pollution Research, 2016. **23**(9): p. 8421-8430.
122. Wicke, D. and T. Reemtsma, *Mobilization of hydrophobic contaminants from soils by enzymatic depolymerization of soil organic matter.* Chemosphere, 2010. **78**(8): p. 996-1003.
123. Vetter, Y.A., et al., *A predictive model of bacterial foraging by means of freely released extracellular enzymes.* Microbial Ecology, 1998. **36**(1): p. 75-92.
124. Schlosser, D., *Fungal Attack on Environmental Pollutants Representing Poor Microbial Growth Substrates*, in *Grand Challenges in Fungal Biotechnology*, H. Nevalainen, Editor. 2020, Springer International Publishing: Cham. p. 33-57.
125. Amaral-Zettler, L.A., E.R. Zettler, and T.J. Mincer, *Ecology of the plastisphere.* Nature Reviews Microbiology: p. 13.
126. Zumstein, M.T., et al., *High-Throughput Analysis of Enzymatic Hydrolysis of Biodegradable Polyesters by Monitoring Cohydrolysis of a Polyester-Embedded Fluorogenic Probe.* Environmental Science & Technology, 2017. **51**(8): p. 4358-4367.
127. Jambon, I., et al., *Fenton-Mediated Biodegradation of Chlorendic Acid - A Highly Chlorinated Organic Pollutant - By Fungi Isolated From a Polluted Site.* Frontiers in Microbiology, 2019. **10**: p. 13.
128. Fricker, M.D., et al., *The Mycelium as a Network.* Microbiology Spectrum, 2017. **5**(3): p. 32.
129. Fukasawa, Y., M. Savoury, and L. Boddy, *Ecological memory and relocation decisions in fungal mycelial networks: responses to quantity and location of new resources.* Isme Journal, 2020. **14**(2): p. 380-388.
130. Rillig, M.C. and D.L. Mummey, *Mycorrhizas and soil structure.* New Phytologist, 2006. **171**(1): p. 41-53.
131. Rillig, M.C., et al., *Plant root and mycorrhizal fungal traits for understanding soil aggregation.* New Phytologist, 2015. **205**(4): p. 1385-1388.

132. Olicón-Hernández, D.R., J. González-López, and E. Aranda, *Overview on the Biochemical Potential of Filamentous Fungi to Degrade Pharmaceutical Compounds.* Frontiers in Microbiology, 2017. **8**(1792).
133. Sungthong, R., et al., *Mycelium-Enhanced Bacterial Degradation of Organic Pollutants under Bioavailability Restrictions.* Environmental Science & Technology, 2017. **51**(20): p. 11935-11942.
134. Oliver, J.P. and J.S. Schilling, *Harnessing fungi to mitigate CH4 in natural and engineered systems.* Applied Microbiology and Biotechnology, 2018. **102**(17): p. 7365-7375.
135. Whiteside, M.D., et al., *Mycorrhizal Fungi Respond to Resource Inequality by Moving Phosphorus from Rich to Poor Patches across Networks.* Current Biology, 2019. **29**(12): p. 2043-+.
136. Yang, P. and J.D. van Elsas, *Mechanisms and ecological implications of the movement of bacteria in soil.* Applied Soil Ecology, 2018. **129**: p. 112-120.
137. Otto, S., et al., *Effects of Facilitated Bacterial Dispersal on the Degradation and Emission of a Desorbing Contaminant.* Environmental Science & Technology, 2016. **50**(12): p. 6320-6326.
138. Nazir, R., et al., *The capacity to comigrate with Lyophyllum sp strain Karsten through different soils is spread among several phylogenetic groups within the genus Burkholderia.* Soil Biology & Biochemistry, 2012. **50**: p. 221-233.
139. Hyde, S.M. and P.M. Wood, *A mechanism for production of hydroxyl radicals by the brown-rot fungus Coniophora puteana: Fe(III) reduction by cellobiose dehydrogenase and Fe(II) oxidation at a distance from the hyphae.* Microbiology-Uk, 1997. **143**: p. 259-266.
140. Cicatiello, P., et al., *Marine fungi as source of new hydrophobins.* International Journal of Biological Macromolecules, 2016. **92**: p. 1229-1233.
141. Winandy, L., O. Schlebusch, and R. Fischer, *Fungal hydrophobins render stones impermeable for water but keep them permeable for vapor.* Scientific Reports, 2019. **9**: p. 8.
142. Warmink, J.A. and J.D. van Elsas, *Selection of bacterial populations in the mycosphere of Laccaria proxima: is type III secretion involved?* Isme Journal, 2008. **2**(8): p. 887-900.
143. Li, Z.B., et al., *Cellular dissolution at hypha- and spore-mineral interfaces revealing unrecognized mechanisms and scales of fungal weathering.* Geology, 2016. **44**(4): p. 319-322.
144. Lehto, T. and J.J. Zwiazek, *Ectomycorrhizas and water relations of trees: a review.* Mycorrhiza, 2011. **21**(2): p. 71-90.