Mycotransformation of organic and inorganic substrates

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This article outlines the important biogeochemical roles that fungi play in the degradation, utilization and transformation of organic and inorganic substrates. Fungal populations are intimately involved in element cycling at local and global scales and such processes have major implications for living organisms, notably plant productivity and human health. While most attention has focussed on carbon cycling, the involvement of fungi in most other elemental cycles is emphasised. Some fungal transformations have applications in environmental biotechnology, e.g. in metal leaching, recovery and detoxification, xenobiotic and organic pollutant degradation, as well as result in adverse effects when these mechanisms are involved in degradation of foodstuffs, natural products and building materials. This account highlights the interdisciplinary approach that is necessary to further understanding of the important roles that fungi play in the biogeochemical cycling of elements, the chemical and biological mechanisms that are involved, and their biotechnological significance.

Keywords: fungi, xenobiotics, metals, metalloids, degradation, transformation, biogeochemistry, minerals

Introduction

The most important perceived environmental roles of fungi are as decomposer organisms, plant pathogens and symbionts (mycorrhizas), and in the maintenance of soil structure due to their filamentous branching growth habit and exopolymer production (see Ritz & Young, this volume). However, a broad appreciation of fungi as agents of biogeochemical change is lacking, and apart from obvious connections with the carbon cycle, they are frequently neglected within broader microbiological and geochemical spheres in contrast to bacteria. While the profound geochemical activities of bacteria receive considerable attention, especially in relation to carbon-limited and/or anaerobic environments, in aerobic environments fungi are of great importance, especially when considering the plant root-soil interface. For example, symbiotic mycorrhizal fungi are associated with ~80% of plant species and are responsible for major mineral transformations and redistributions of inorganic nutrients, e.g. essential metals and phosphate, as well as carbon flow, while free-living fungi have major roles in decomposition of plant and other organic materials, including xenobiotics, as well as mineral solubilization. Fungi are often dominant members of the soil microflora, especially in acidic environments, and may operate over a wider pH range than many heterotrophic bacteria. Fungi are also major biodeterioration agents of stone, wood, plaster, cement and other building materials, and it is now realised that they are important components of rock-inhabiting microbial communities with significant roles in mineral dissolution and secondary mineral formation (Burford *et al.*, 2003). The purpose of this article is to outline some of the main mechanisms by which fungi can transform organic and inorganic substrates (Fig 1) and the significance of these processes for environmental cycling of elements on global and local scales, and some of their potential applications in environmental biotechnology.

Organic substrates

In relation to organic matter decomposition, most attention has probably been given to carbon and nitrogen cycles, and the ability of fungi to utilise a wide spectrum of organic compounds is well known. These range from simple compounds such as sugars, organic acids, and amino acids which can easily be transported into the cell to more complex molecules which are first broken down to smaller molecules by extracellular enzymes before cellular entry. Such compounds include natural substances such as cellulose, pectin, lignin, lignocellulose, chitin and starch to
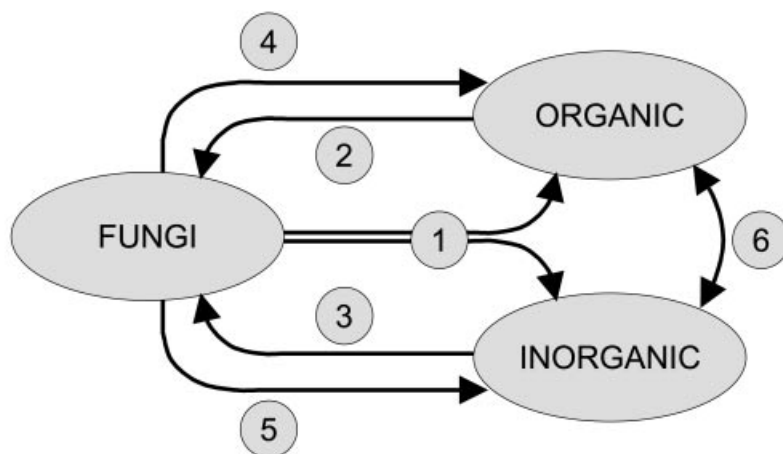
Fig 1 Simplified representation of fungal action on organic and inorganic substrates which may be naturally-occurring and/or anthropogenically derived. 1. organic and inorganic transformations mediated by enzymes and metabolites, e.g. H^+, CO_2, and organic acids, and physico-chemical changes occurring as a result of metabolism; 2. uptake, metabolism or degradation of organic substrates; 3. uptake, accumulation, sorption, metabolism of inorganic substrates; 4. production of organic metabolites, exopolymers, and biomass; 5. production of inorganic metabolites, secondary minerals and transformed metal(loid)s; 6. chemical interactions between organic and inorganic substances, e.g. complexation and chelation, which can modify bioavailability, toxicity and mobility. Translocation phenomena may also be associated with the biotic components of this model.

anthropogenic products like hydrocarbons, pesticides, and other xenobiotics (Evans & Hedger, 2001; Harvey & Thurston, 2001).

Some fungi have remarkable degradative properties and lignin-degrading white rot fungi, such as *Phanerochaete chrysosporium*, can degrade several xenobiotics including aromatic hydrocarbons, chlorinated organics, polychlorinated biphenyls, nitrogen-containing aromatics and many other pesticides, dyes and xenobiotics (Table 1). Such activities are of potential in bioremediation where appropriate ligninolytic fungi have been used to treat

Table 1. Examples of organic pollutants degraded by white rot fungi (see Reddy & Mathew, 2001; Singleton, 2001; Cerniglia & Sutherland, 2001; Knapp et al., 2001)

Polycyclic aromatic hydrocarbons	Anthracene and derivatives, benzo[a]pyrene, fluorine, naphthalene, acenaphthene, acenaphylene, phenanthrene, pyrene, biphenylene
Chlorinated aromatic compounds	Chlorophenols (e.g. pentachlorophenol (PCP), trichlorophenol (TCP), dichlorophenol (DCP)); chlorolignols, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), polychlorinated biphenyls (PCBs), dioxins, chlorobenzenes
Dyes	Azure B, Congo Red, Disperse Yellow 3 (DY3), Orange II, Poly R, Reactive Black 5, Reactive Orange 96, Reactive Violet 5, Remazol Brilliant Blue R (RbbR), Solvent Yellow 14, Tropaeolin, Triphenylmethane dyes (e.g. Crystal Violet)
Nitroaromatics	2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene, 2-amino-4,6-dinitrotoluene, 1-chloro-2,4-dinitrobenzene, 2,4-dichloro-1-nitrobenzene, 1,3-dinitrobenzene and derivatives
Pesticides	Alachlor, Aldrin, Chlordane, 1,1,1-trichloro-2,2-bis(40-chlorophenyl)ethane (DDT), Heptachlor, Lindane, Mirex, Atrazine, Benomyl
Other pollutants	Benzene, toluene, ethylbenzene, o-, m-, p-xylenes (BTEX compounds), linear alkylbenzene sulfonate (LAS), trichloroethylene
Organometallic compounds	Alkyltins, e.g. tributyltin chloride, oxide and naphthenate; alkylleads, e.g. trimethyllead compounds; organomercurials, e.g. phenylmercury compounds

soil contaminated with substances like pentachlorophenol (PCP) and polycyclic aromatic hydrocarbons (PAHs), the latter being constituents of creosote (Fig 2). In general, treatment processes involve inoculation of the contaminated soil with the chosen fungal strain followed by nutrient addition, irrigation and aeration and maintenance by general land farming procedures. Correct preparation of the fungal inoculum can be crucial: fungi may be grown on lignocellulosic substrates prior to introduction into the soil (Singleton, 2001). Treatment can take weeks to months or longer depending on the level of contamination and environmental factors. In many cases, xenobiotic-transforming fungi need additional utilisable carbon sources because although capable of degradation, they cannot utilize these substrates as an energy source for growth. Therefore inexpensive utilisable lignocellulosic wastes such as corn cobs, straw and sawdust can be used as nutrients to obtain enhanced pollutant degradation (Reddy & Mathew, 2001). Similar phenomena are of course important in all manner of composting approaches. Wood-rotting

and other fungi are also receiving attention for the decolourization of dyes and industrial effluents, various agricultural wastes such as forestry, pulp and paper by-products, sugar cane bagasse, coffee pulp, sugar beet pulp, apple and tomato pulp, and cyanide (Knapp *et al.*, 2001; Barclay & Knowles, 2001; Cohen & Hadar, 2001).

Fungi are also important in the degradation of naturally-occurring complex molecules in the soil, an environment where the hyphal mode of growth provides several advantages, and also aquatic habitats. Since 95% of plant tissue is composed of carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur, the decomposition activities of fungi clearly are important in relation to redistribution of these elements between organisms and environmental compartments (Table 2). As well as C, H, O, N, P, and S, another 15 elements are typically found in living plant tissues - K, Ca, Mg, B, Cl, Fe, Mn, Zn, Cu, Mo, Ni, Co, Se, Na, Si - most of these having some essential role. However, all 90 or so naturally-occurring elements may be found in plants, most at low concentrations
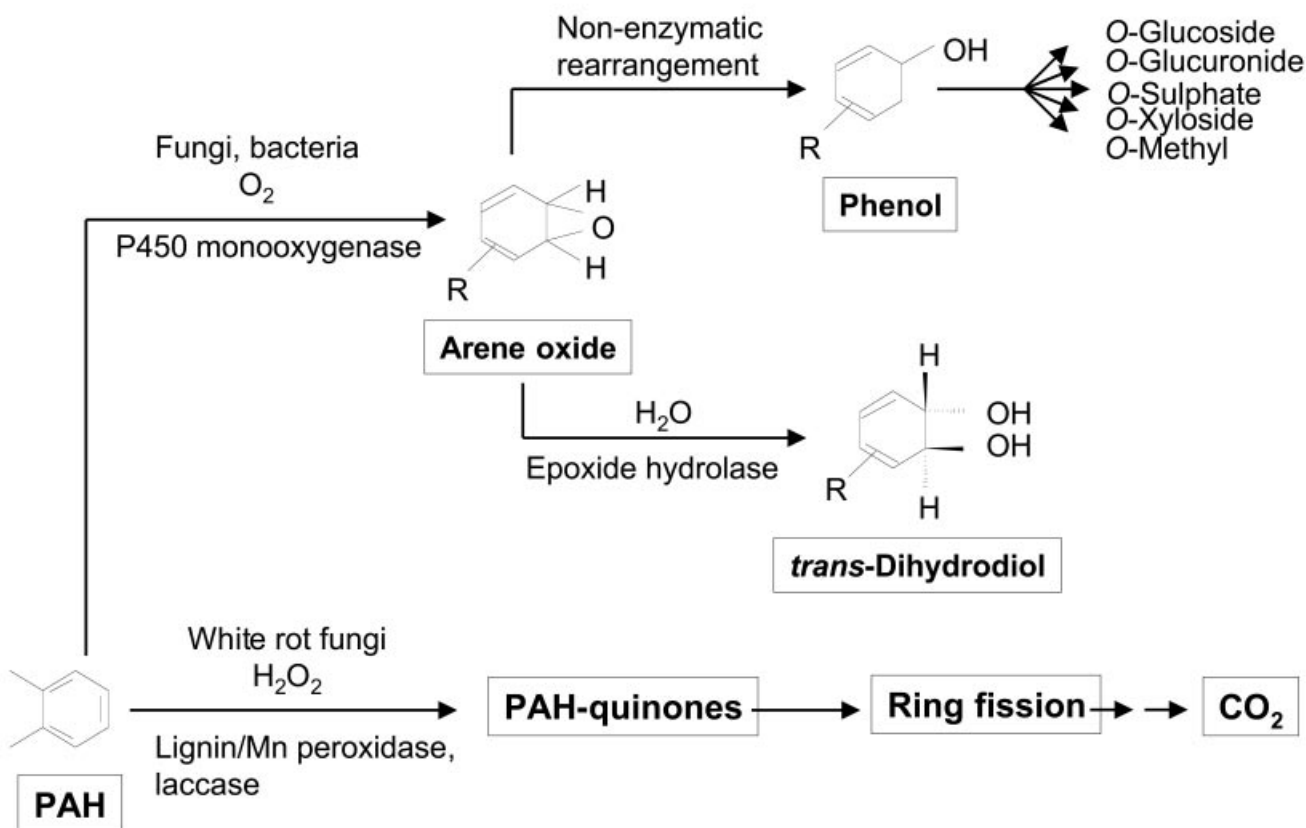

Fig 2 Initial reactions of the two main routes of polycyclic aromatic hydrocarbon (PAH) degradation by fungi. Fungi, and bacteria, metabolize a wide variety of PAHs converting them to CO_2 or various dead-end products that do not result in CO_2 production. Non-ligninolytic fungi metabolize PAHs by cytochrome P450 monooxygenase and epoxide-catalysed reactions to form trans-dihydrodiols, and other metabolites including phenols and quinones. Ligninolytic fungi degrade PAHs by non-specific radical oxidation catalysed by extracellular ligninolytic enzymes that lead to PAH quinones, which may be further metabolized to CO_2 (see Sutherland & Cerniglia, 2001).

although this may be highly dependent on environmental conditions. These include Au, As, Hg, Pb and U, and there are even plants that accumulate relatively high concentrations of metals like Ni and Cd. In fact, plant metal concentrations may reflect environmental conditions and provide an indication of toxic metal pollution or metalliferous ores. Such plants are also receiving attention in bioremediation contexts (= phytoremediation when plants are involved). Animals likewise contain a plethora of elements in varying amounts. For example, the human body is mostly water and so 99% of the mass comprises oxygen, carbon, hydrogen, nitrogen, calcium and phosphorus (Table 2). However, many other elements are present in lower amounts including substances taken up as contaminants in food and water. A similar situation occurs throughout the plant, animal and microbial world and therefore, any decomposition, degradative and pathogenic activities of fungi must be linked to the redistribution and cycling of all these constituent elements, both on local and global scales (Fig 3).

Organometals (compounds with at least one metal-carbon bond) can also be attacked by fungi with the organic moieties being degraded and the metal compound undergoing changes in speciation. Fungal organometal transformations may be envisaged for the removal of alkylleads or organotins from water (Macaskie & Dean, 1987; 1990; Gadd, 1993a, 2000b). Degradation of organometallic compounds can be carried out by fungi, either by direct biotic action (enzymes) or by facilitating abiotic degradation, for instance by alteration of pH and excretion of metabolites. Organotin compounds, such as tributyltin oxide and tributyltin naphthenate, may be degraded to mono- and dibutyltins by fungal action, inorganic Sn(II) being the ultimate degradation product. Organomercury compounds may be detoxified by conversion to Hg(II) by fungal organomercury lyase, the Hg(II) being subsequently reduced to Hg(0) by mercuric reductase, a system broadly analogous to that found in mercury-resistant bacteria (Gadd, 1993a).

Inorganic substrates

Rocks, minerals, and building materials

Minerals are naturally-occurring inorganic solids of definite chemical composition and possessing an ordered internal structure; rocks can be considered to be any solid mass of mineral or mineral-like material and may therefore often contain several kinds of minerals. The most common minerals are the silicates, with non-silicates constituting less than 10% of the Earth's crust, the most common being carbonates, oxides, sulphides and phosphates. Rocks and minerals represent a vast reservoir of elements, many of which are essential to life, and which must be released from their inanimate prison into forms that may be assimilated by the biota. These include essential metals as well as anionic nutrients like phosphate (Table 3).

Due to their filamentous growth habit and ability to produce and exude organic acids, protons and other metabolites, fungi are ideal biological weathering agents of rocks, minerals and building materials (Fig 4). Sub-aerial rock surfaces may be thought an inhospitable habitat for fungal growth due to moisture deficit and nutrient limitation although many species are able to deal with varying extremes in such factors as light, salinity, pH, and water potential, over considerable periods of time. It is likely that fungi are

Table 2. Major elemental composition of living organisms (data derived from a number of sources). Since most of living tissue is water, oxygen comprises most of the elemental mass. Carbon, the basic elemental unit for all organic molecules is second. C,H,N, and O comprise >95% by weight of all living organisms with around 99% of an organism's mass comprising the elements below (CHNOPS) with Ca being up to 1.5% of this in the human body (major skeletal component). Other important macronutrient elements are K and Mg with micronutrient elements including Fe, Cl, Cu, Mn, Zn, Mo, B, Ni, Co, Na, and Se. Si is a major component of diatoms and other siliceous algae. Other elements accumulated by living organisms which may be involved in specialized biological roles, e.g. V, I, Ba, or are inessential include F, Br, Li, Sr, Al, Pb, As, other metals, actinides and lanthanides. It is likely that the majority of stable elements can potentially be associated with living organisms, albeit at vastly different concentration ranges, and dependent on environmental factors and nutrition.

Element	Human	Plant	Bacterium
Carbon	19.4%	11.3%	12.1%
Hydrogen	9.3	8.7	9.9
Nitrogen	5.1	0.8	3.0
Oxygen	62.8	77.9	73.7
Phosphorus	0.6	0.7	0.6
Sulphur	0.6	0.1	0.3
CHNOPS total	97.8	99.5	99.6

Table 3. Abundance of elements in the earth's crust. Eight elements account for nearly 99% of all crustal rocks. The two most common elements in the Earth's crust, oxygen and silicon, combine to form the silicates, the most abundant minerals. With Al, alumina-silicates result: these three elements account for more than 80% of the Earth's crust (see Lutgens & Tarbuck, 2000).

Element	% by weight
Oxygen	46.71
Silicon	27.69
Aluminium	8.07
Iron	5.05
Calcium	3.65
Sodium	2.75
Potassium	2.58
Magnesium	2.08
Sub-total	98.58
Titanium	0.62
Hydrogen	0.14
Phosphorus	0.13
Carbon	0.094
Manganese	0.09
Sulphur	0.052
Barium	0.05
Chlorine	0.045
Chromium	0.035
Fluorine	0.029
Zirconium	0.025
Nickel	0.019
All others	0.091
Total	100

ubiquitous components of the microflora of all rocks and building stone and they have been reported from a wide range of rock types including limestone, marble, granite, sandstone, basalt, gneiss, dolerite and quartz, even from the most harsh environments, e.g. hot and cold deserts (Staley *et al.*, 1982; Gorbushina *et al.*,
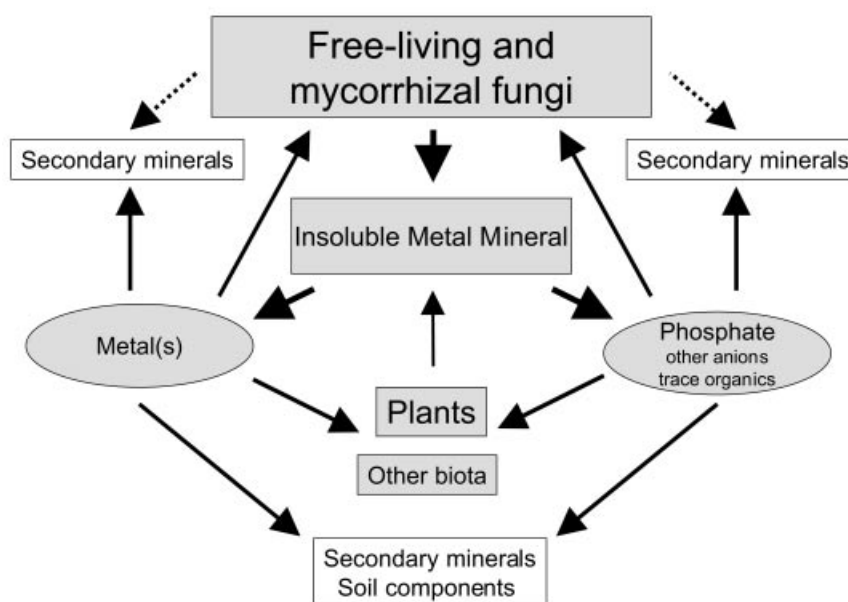

Fig 4 Action of free-living and mycorrhizal fungi on insoluble metal minerals in the terrestrial environment resulting in release of mineral components - metal(s), anionic substances, trace organics and other impurities – which can be taken up by the biota as well as forming secondary minerals with soil components or fungal metabolites/biomass, and also be sorbed or otherwise removed by organic and inorganic soil components. The dashed arrows imply secondary mineral formation as a result of excreted metabolites as well as fungal action on non-biogenic minerals. Possible losses to groundwater are not shown.
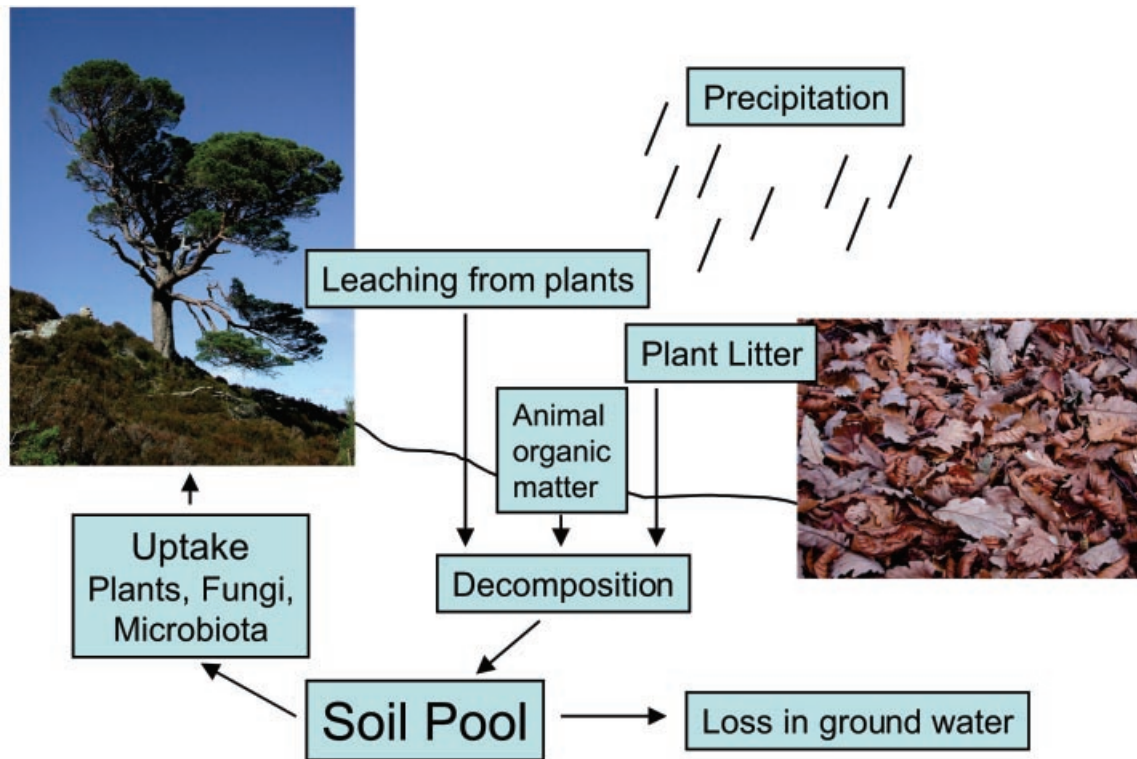
Fig 3 A simplified elemental biogeochemical cycle in a forest ecosystem where decomposition processes, and therefore a prime fungal role, leads to cycling of many other elements besides C. The cycle depicted could be of Ca or K for example (see Schlesinger, 1997).

1993; Sterflinger, 2000; Verrecchia, 2000). The presence of organic and inorganic residues on mineral surfaces or within cracks and fissures within the mineral substrate can encourage proliferation of fungi and other microbes as well as the waste products of algae and bacteria, dead cells, decaying plant material, dust particles, aerosols and animal faeces (Sterflinger, 2000).

Soil elements reflect the composition of the Earth's crust, though some modification occurs by weathering, biogenic and anthropogenic activities which on a local scale may be pronounced: chemical changes include dissolution of rock minerals while biological activity can result in enrichment of C, N and S. Elements and minerals that remain can reorganize into secondary minerals. In the soil, fungus-mineral interactions are an integral component of environmental cycling processes (Gadd, 1993b; 1999; 2001a). Mycorrhizal fungi in particular are one of the most important ecological groups of soil fungi in terms of mineral weathering and dissolution of insoluble metal compounds (Paris *et al.*, 1995; Jongmans *et al.*, 1997; Martino *et al.*, 2003a) (Fig 4). Fungi are also important components of lithobiotic communities (associations of microorganisms forming a biofilm at the mineral-microbe interface), where they interact with the substrate both geophysically and geochemically (Burford & Gadd, 2003) and this can result in the

formation of patinas, films, varnishes, crusts and stromatolites (Gorbushina & Krumbein, 2000).

Biomechanical deterioration of rocks can occur through hyphal penetration and burrowing into decaying material and along crystal planes in, for example, calcitic and dolomitic rocks (Kumar & Kumar, 1999; Sterflinger, 2000). Cleavage penetration can also occur with lichens (Banfield *et al.*, 1999). Spatial exploration of the environment to locate and exploit new substrates is facilitated by a range of sensory responses that determine the direction of hyphal growth. Thigmotropism (or contact guidance) is a well-known property of fungi that grow on and within solid substrates with the direction of fungal growth being influenced by grooves, ridges and pores. However, biochemical actions are believed to be more important processes than mechanical degradation (Sterflinger, 2000). Microbes and plants can induce chemical weathering of rocks and minerals through the excretion of H^+, organic acids and other metabolites (Ehrlich, 1998; Sterflinger, 2000; Gadd & Sayer, 2000) (Fig 5). Such biochemical weathering of rocks can result in changes in the mineral micro-topography through pitting and etching of surfaces, and even complete dissolution of mineral grains (Ehrlich, 1998; Kumar & Kumar, 1999). Fungi generally acidify their micro-environment via a number of mechanisms which include the excretion of protons via the plasma
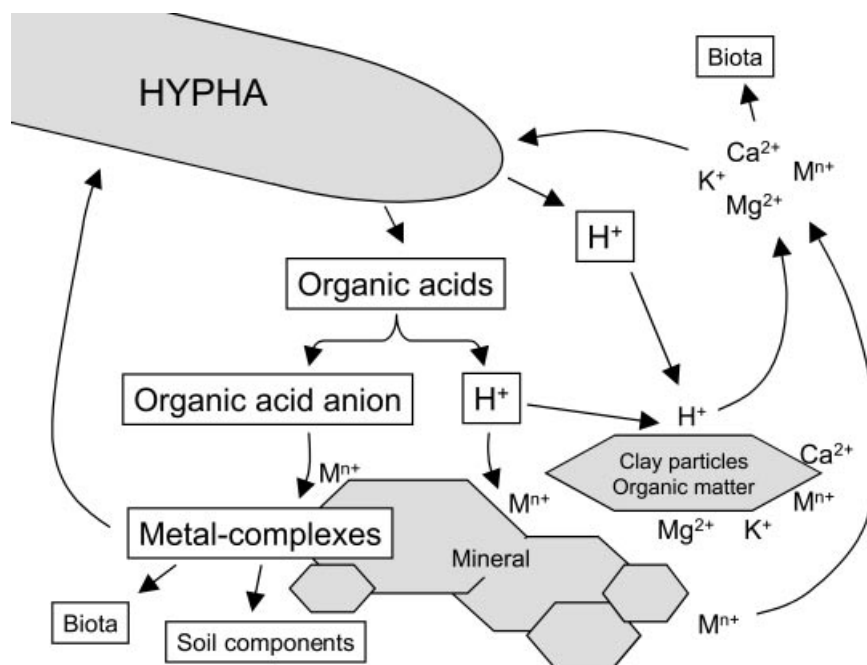
Fig 5 Proton- and organic acid ligand-mediated dissolution of metals from soil components and minerals. Proton release results in cation exchange with sorbed metal ions on clay particles, colloids etc. and metal displacement from mineral surfaces. Released metals can interact with biomass as described and also be taken up by other biota, and react with other environmental components. Organic acid anions, e.g. citrate, may cause mineral dissolution or removal by complex formation. Metal complexes can interact with biota as well as environmental constituents: in some circumstances complex formation may be followed by crystallization, e.g. metal oxalate formation (see Gadd, 1999).

membrane proton translocating ATPase or in exchange for nutrients. They can also excrete organic acids, while respiratory activity may result in carbonic acid formation (Burgstaller & Schinner, 1993). In addition, fungi excrete a variety of other primary and secondary metabolites with metal-chelating properties (e.g., siderophores, carboxylic acids, amino acids and phenolic compounds (Gadd & Sayer, 2000). The weathering of sandstone monuments by fungi has been attributed to the production of acetic, oxalic, citric, formic, fumaric, glyoxylic, gluconic, succinic and tartaric acids (Gomez-Alarcon *et al.*, 1994; Hirsch *et al.* 1995). Fungi can also attack rock surfaces through redox attack of mineral constituents (Grote & Krumbein, 1992; De la Torre & Gomez-Alarcon, 1994).

On the beneficial side, fungi have recently been proposed as agents for the decontamination of asbestos-polluted soils where it is suggested that adhesion of the asbestos fibrils to the mycelium could limit their dispersal, but also that the action of fungal iron(III)-binding siderophores (crocidolite, the most studied carcinogenic asbestos contains around 30% iron) could modify the surfaces and remove the free radical generating sites that are involved in triggering carcinogenic mechanisms (Martino *et al.*, 2003b).

Metal and metalloid transformations

Fungi can transform metals, metalloids (elements with properties intermediate between those of metals and non-metals – the group includes arsenic, selenium and tellurium) and organometallic compounds by reduction, methylation and dealkylation, again processes of environmental and biotechnological importance since transformation of a metal or metalloid may modify its mobility and toxicity. For example, methylated selenium derivatives are volatile and less toxic than inorganic forms while reduction of metalloid oxyanions, such as selenite or tellurite to amorphous elemental selenium or tellurium respectively, results in immobilization and detoxification (Thompson-Eagle & Frankenberger, 1992; Morley *et al.*, 1996). The mechanisms by which fungi (and other microorganisms) effect changes in metal speciation and mobility are not only important survival determinants but also components of biogeochemical cycles for metals, and many other elements including carbon, nitrogen, sulphur and phosphorus (Gadd, 1999).

Metals and their compounds interact with fungi in various ways depending on the metal species, organism and environment, while fungal metabolism also influences metal speciation and mobility. Many metals are essential for life, e.g. Na, K, Cu, Zn, Co, Ca, Mg, Mn, and Fe, but all can exert toxicity when present above certain threshold concentrations (Gadd, 1993*b*). Other metals, e.g. Cs, Al, Cd, Hg and Pb, have no known biological function but all can be accumulated by fungi

(Gadd, 1993*b*; 2001a,*b*). Metal toxicity is greatly affected by environmental conditions and the chemical behaviour of the particular metal species in question. Despite apparent toxicity, many fungi survive, grow and flourish in apparently metal-polluted locations and a variety of mechanisms, both active and incidental, contribute to tolerance. Fungi have many properties which influence metal toxicity including the production of metal-binding proteins, organic and inorganic precipitation, active transport and intracellular compartmentalization, while major constituents of fungal cell walls, e.g. chitin and melanin, have significant metal binding abilities (Gadd & Griffiths, 1978; Gadd, 1993*b*; Fomina & Gadd, 2002).

Metal mobilization

Metal mobilization from rocks, minerals, soil and other substrates can be achieved by chelation by excreted metabolites and siderophores, and methylation which can result in volatilization. As mentioned above, biochemical actions of fungi on minerals are more important than biomechanical deterioration especially in the context of elemental cycles (Kumar & Kumar, 1999). Fungi can solubilize minerals by means of proton efflux, the production of Fe(III)-binding siderophores, and as a result of respiratory carbon dioxide accumulation. In addition, other excreted metabolites with metal-complexing properties, e.g. amino acids, phenolic compounds, and organic acids may also be involved. Fungal-derived carboxylic acids can play an integral role in chemical attack of mineral surfaces and these provide a source of protons for solubilization and metal-chelating anions to complex the metal cation (Gadd, 1999, 2001a; Burgstaller & Schinner, 1993; Gadd & Sayer, 2000) (Fig 5). Many metal citrates are highly mobile and not readily degraded. Oxalic acid can act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe (Strasser *et al.*, 1994). Solubilization phenomena can also have consequences for mobilization of metals from toxic metal containing minerals, e.g. pyromorphite ($Pb_5(PO_4)_3Cl$), contaminated soil and other solid wastes (Sayer *et al.*, 1999). Fungi can also mobilize metals and attack mineral surfaces by redox processes (Grote & Krumbein, 1992; Gadd, 1993a; De la Torre & Gomez-Alarcon, 1994): Fe(III) and Mn(IV) solubility is increased by reduction to Fe(II) and Mn(II) respectively. Reduction of Hg(II) to volatile elemental Hg(0) can also be mediated by fungi (Gadd, 1993*a,b*).

The removal of metals from industrial wastes and by-products, low grade ores and metal-bearing minerals by fungal "heterotrophic leaching" is relevant to metal recovery and recycling and/or bioremediation of contaminated solid wastes (Burgstaller & Schinner, 1993). Most processes for the winning of metals from ores by solubilization processes involve chemoautotrophic bacteria, particularly the genus *Thiobacillus*. However, the pH of growth media may be increased by many industrial metal-containing wastes, e.g. filter dust from metal processing, and most *Thiobacillus* spp. cannot solubilize metals effectively above pH 5.5. Although fungi need a source of carbon and aeration, they can solubilize metals at higher pH values and so could perhaps become more important where leaching with bacteria is not possible and in bioreactors. Leaching of metals with fungi can be very effective although a high level of organic acid production may need to be maintained. Other possible applications of fungal metal solubilization are the removal of unwanted phosphates, and metal recovery from scrap electronic and computer materials (Brandl, 2001).

The ability of fungi, along with bacteria, to transform metalloids has been utilized successfully in the bioremediation of contaminated land and water. Selenium methylation results in volatilization, a process which has been used to remove selenium from the San Joaquin Valley and Kesterson Reservoir, California, using evaporation pond management and primary pond operation (Thompson-Eagle *et al.*, 1991; Thompson-Eagle & Frankenberger, 1992).

Metal immobilization

Fungal biomass provides a metal sink, either by metal biosorption to biomass (cell walls, pigments and extracellular polysaccharides), intracellular accumulation and sequestration, or precipitation of metal compounds onto and/or around hyphae (Fig 6). Fungi are highly effective biosorbents for a variety of metals including Ni, Zn, Ag, Cu, Cd and Pb (Gadd, 1990; 1993*b*) and this can be an important passive process in both living and dead biomass (Gadd, 1990; 1993*b*; Sterflinger, 2000). The presence of chitin, and pigments like melanin, strongly influences the ability of fungi to act as biosorbents (Gadd & Mowll, 1985; Manoli *et al.*, 1997; Fomina & Gadd, 2002). In a biotechnological context, fungi and their by-products have received considerable attention as biosorbent materials for metals and radionuclides (Gadd & White, 1992; Gadd, 2002). Their case as suitable material is supported by the ease with which they are grown and their availability as waste products from industry, for example A. *niger* (citric acid production), and *S. cerevisiae* (brewing). Many studies have shown their
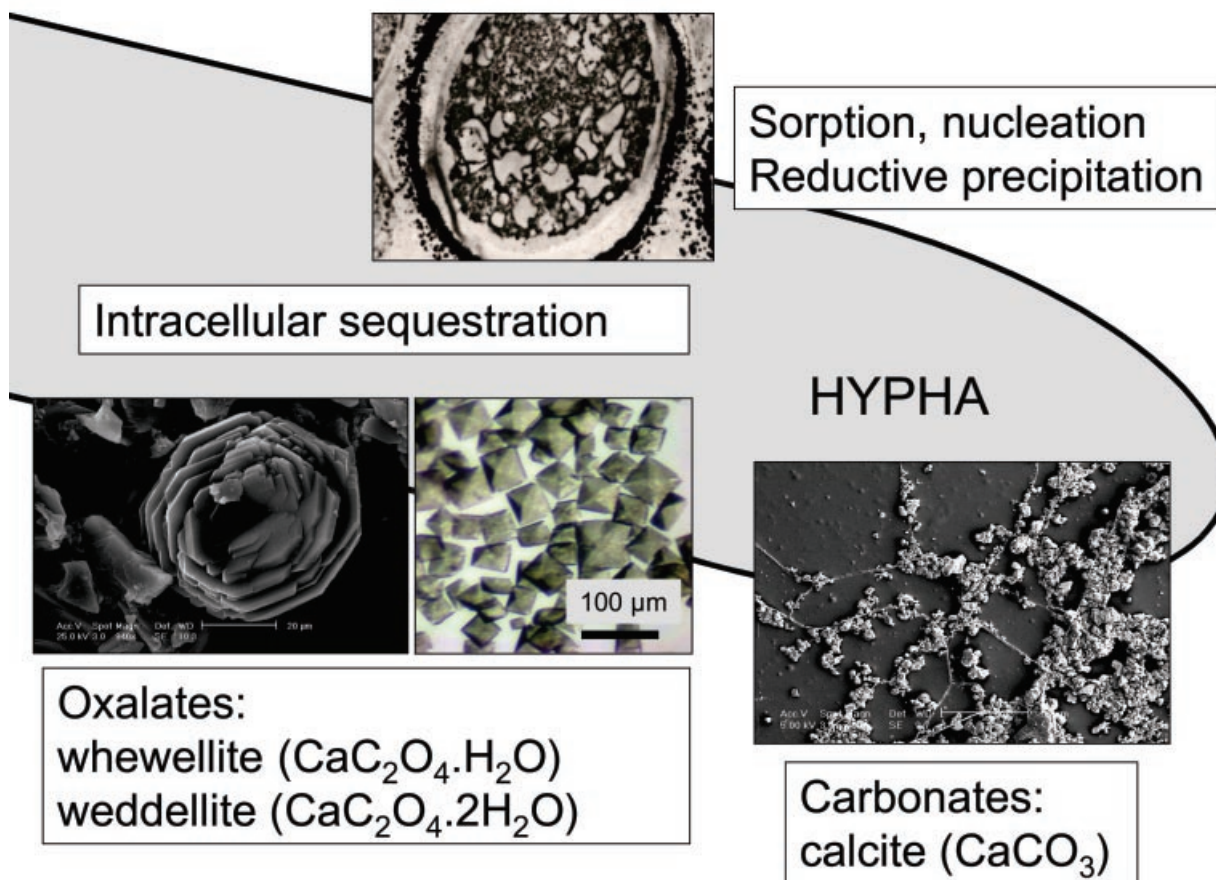
Fig 6 Simplified diagram of metal immobilization mechanisms exhibited by fungi. Sorption, nucleation and reductive precipitation can result in extensive secondary mineral or related deposits associated with biomass (the picture shows elemental silver around *Aureobasidium pullulans*); insoluble metal oxalate minerals may result from oxalic acid excretion (picture shows calcium oxalate produced by *Trametes (Coriolus) versicolor* (left) and by *Beauveria caledonica* right)); fungal biomass may also act as a nucleation site for calcium carbonate formation (picture shows calcite deposition on a *Chaetomium* sp. isolated from limestone). A variety of mechanisms are involved in intracellular sequestration including transport systems on plasma and vacuolar membranes, intracellular metal-binding peptides such as metallothioneins and _-glutamyl peptides, and redox reactions (See Gadd, 1993*b*; oxalate/carbonate images courtesy of Marina Fomina and Euan P. Burford – unpublished data).

efficacy in sorbing metal contaminants either as living or dead biomass, in pelleted whole-cell or dissembled forms, and as mobile or immobilized sorbents in batch and continuous processes. However, attempts to commercialise biosorption have been limited, primarily due to competition with commercially-produced ion exchange media of high specificity.

Fungi can precipitate a number of inorganic and organic compounds, e.g. oxalates, oxides and carbonates (Arnott, 1995; Verrecchia, 2000; Gadd, 1999; Grote & Krumbein, 1992; Gharieb & Gadd, 1999) and this can lead to formation of biogenic minerals (mycogenic precipitates). Precipitation, including crystallization, will immobilize metals but also leads to release of nutrients like sulphate and phosphate (Gadd, 1999). Calcium oxalate crystals are commonly associated with free-living, pathogenic and plant symbiotic fungi and this has a profound effect on

biogeochemical processes in soils, acting as a reservoir for calcium, but also influencing phosphate and sulphate availability (Gadd, 1999; 2001a; Gharieb *et al.*, 1998) (Fig 6). Fungi can also produce other metal oxalates with a variety of different metals and metal-bearing minerals e.g. Cd, Co, Cu, Mn, Sr, Zn and Ni (Gadd, 1999) which may provide a mechanism whereby fungi can tolerate toxic metal-containing environments. A similar mechanism occurs in lichens growing on copper-sulphide bearing rocks, where substantial precipitation of copper oxalate occurs within the thallus (Purvis & Halls, 1996). The precipitation of carbonates on fungal hyphae, particularly calcite ($CaCO_3$), occurs in calcareous soils and near surface limestones (calcretes) (Verrecchia *et al.*, 1990; Monger & Adams, 1996; Bruand & Duval, 1999) (Fig 6). Many fungi precipitate reduced forms of metals and metalloids in and around fungal hyphae, for

example; Ag(I) can be reduced to elemental silver Ag(0) (Fig 6); selenate [Se(VI)] and selenite [Se(IV)] to elemental selenium; tellurite [Te(IV)] to elemental tellurium [Te(0)]. These reductive abilities of fungi are clearly demonstrated since they result in black (Ag, Te) and red (Se) colonies in fungi due to precipitation of elemental forms of these metal(loid)s (Gharieb *et al.*, 1995, 1999).

Conclusions

Fungi play important biogeochemical roles in the degradation, utilization and transformation of organic and inorganic substrates. Such activities underpin element cycling at local and global scales and have major implications for living organisms, notably plant productivity and human health. Many processes have application in environmental biotechnology, e.g. metal leaching, recovery and detoxification, xenobiotic and organic pollutant degradation, as well as result in adverse effects when these mechanisms are involved in degradation of foodstuffs and building materials. It is timely to expand our appreciation and understanding of fungi as agents of global environmental change, and to emphasise the interdisciplinary approach that is necessary to further understanding of the important roles that fungi play in the biogeochemical cycling of elements, the chemical and biological mechanisms that are involved, and their biotechnological significance.

Acknowledgements

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