REVIEW ARTICLE

Fungi in freshwaters: ecology, physiology and biochemical potential

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Abstract

Research on freshwater fungi has concentrated on their role in plant litter decomposition in streams. Higher fungi dominate over bacteria in terms of biomass, production and enzymatic substrate degradation. Microscopy-based studies suggest the prevalence of aquatic hyphomycetes, characterized by tetraradiate or sigmoid spores. Molecular studies have consistently demonstrated the presence of other fungal groups, whose contributions to decomposition are largely unknown. Molecular methods will allow quantification of these and other microorganisms. The ability of aquatic hyphomycetes to withstand or mitigate anthropogenic stresses is becoming increasingly important. Metal avoidance and tolerance in freshwater fungi implicate a sophisticated network of mechanisms involving external and intracellular detoxification. Examining adaptive responses under metal stress will unravel the dynamics of biochemical processes and their ecological consequences. Freshwater fungi can metabolize organic xenobiotics. For many such compounds, terrestrial fungal activity is characterized by cometabolic biotransformations involving initial attack by intracellular and extracellular oxidative enzymes, further metabolization of the primary oxidation products via conjugate formation and a considerable versatility as to the range of metabolized pollutants. The same capabilities occur in freshwater fungi. This suggests a largely ignored role of these organisms in attenuating pollutant loads in freshwaters and their potential use in environmental biotechnology.

Aquatic hyphomycetes are essential providers of ecosystem functions

What are aquatic hyphomycetes?

Aquatic hyphomycetes (also known as freshwater hyphomycetes, amphibious fungi or Ingoldian fungi) are a polyphyletic group of true fungi (Bärlocher, 1992a; Belliveau & Bärlocher, 2005). Their taxonomy and identification have traditionally been based on the morphology and development of asexually produced spores (mitospores or conidia). There is no unambiguous definition of what makes a fungus an aquatic hyphomycete, but two crucial characteristics are the ability (1) to sporulate under water and (2) to thrive on deciduous leaves decaying in streams and rivers. In addition, a majority of aquatic hyphomycetes produce relatively large, multiradiate (often tetraradiate) or sigmoid spores (Ingold, 1975; Webster & Descals, 1981), whose tips may be covered with sticky mucilage (Read *et al.*, 1992). These properties facilitate the attachment of the spore to leaves and other smooth surfaces (Webster, 1959; Dang *et al.*, 2007; Kearns & Bärlocher, 2008). The major milestones in the discovery and taxonomic analysis of aquatic hyphomycetes and some useful keys are listed in Fig. 1 (for more details, see Bärlocher, 1992a) (Box 1).

Spore production

Ingold's discovery of the main habitat of aquatic hyphomycetes has been called the starting point of a 'minor mycological industry' (Ainsworth, 1976). Kegel (1906) was the first to demonstrate that sporulation can be induced when an agar block overgrown with a pure culture is submerged in nutrient-poor water. Sporulation is considerably enhanced when submerged agar cultures are agitated with a stream of

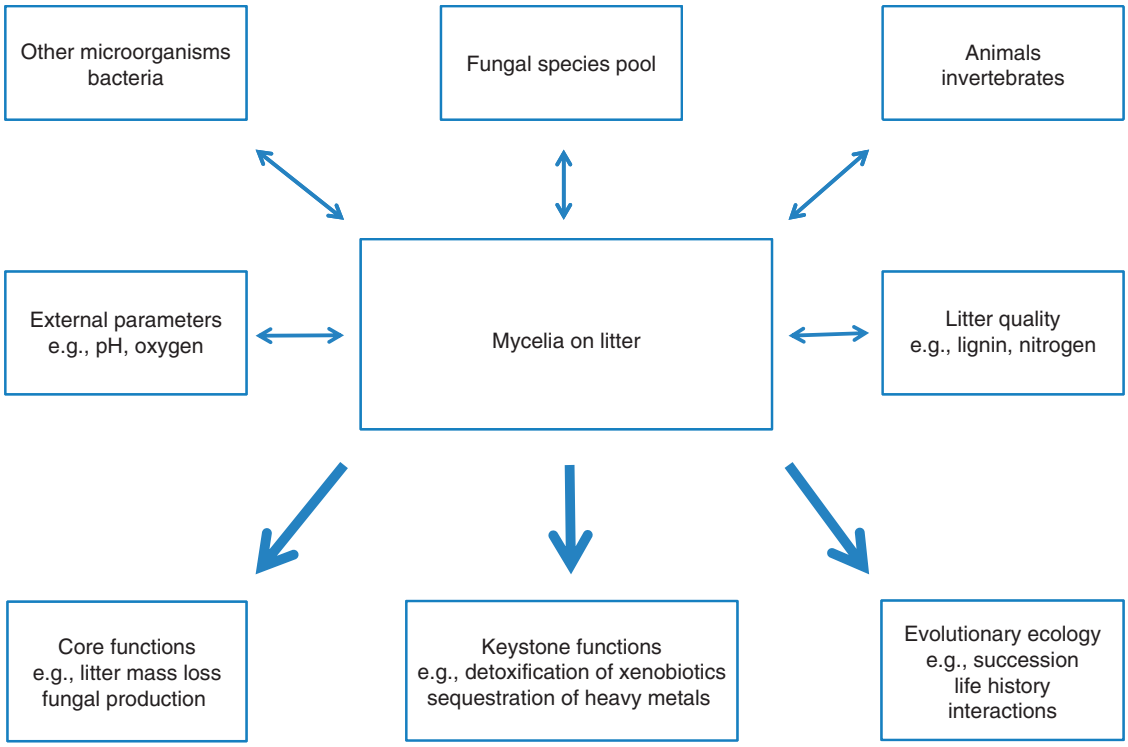

Fig. 1. The involvement of fungi in litter breakdown (modified from Gessner *et al.*, 1997; Zak *et al.*, 2006). The pool of fungal species in the environment, other groups of organisms and internal and external variables determine the quantities and diversity of fungal mycelia on the substrate. The mycelia are agents regulating ecosystem processes, which we subdivide into core (major effects involving large quantities) and keystone (effect disproportionate to quantities involved). The evolutionary point of view puts individual mycelia at its center, and investigates how these units are able to maintain themselves by extracting nutrients in the litter, effectively allocating the acquired energy to growth and reproduction and by interacting with other organisms.

compressed air (Webster & Towfik, 1972). Reliance on spore counts and identifications have dominated research on aquatic hyphomycete community ecology: Iqbal & Webster (1973) introduced a filtering technique to follow seasonal changes in spore numbers and types suspended in the water column. The first major surveys of aquatic hyphomycete successions in streams were based on spores formed on leaves that were incubated in Petri plates (Bärlocher & Kendrick, 1974; Suberkropp & Klug, 1976). Subsequently, Bärlocher (1981) aerated leaves for 1–2 days before capturing newly formed spores on a membrane filter. This approach has been widely adopted to characterize the dynamics of fungal populations on leaves. Several studies have reported numbers of up to eight spores released per day per microgram of detrital dry mass (Gessner, 1997), which accounts for close to or greater than 50% of estimated fungal production on leaves (Suberkropp, 1984, 1991; Findlay & Arsuffi, 1989; Gessner & Chauvet, 1993; Sridhar & Bärlocher, 2000). Detailed information on useful methods to isolate and maintain pure cultures of aquatic hyphomycetes is provided by Descals (1997, 2005, 2008) and Marvanová (2005).

Involvement in decomposition and food web

While mycologists were busy investigating the taxonomy, biology and ecology of aquatic hyphomycetes and documenting their global occurrence, few limnologists seemed to be aware of these (or any other) fungi. This changed when the major contribution of allochthonous plant detritus to food webs in streams was rediscovered in the 1960s (for reviews, see Webster & Benfield, 1986; Allan & Castillo, 2007). In temperate streams, the most important source of this imported material is usually deciduous leaves. Kaushik & Hynes (1968) demonstrated that protein levels of some leaf species gradually increased when they were incubated in stream water. Because they did not observe a concurrent increase in bacterial numbers, they attributed protein accumulation to fungal growth. They confirmed this by showing that protein levels increased in the presence of antibacterial, but not antifungal antibiotics. Similar increases were also found on leaves decaying in streams (Kaushik & Hynes, 1971). In food choice experiments, several stream invertebrates clearly preferred 'conditioned' leaves, i.e. leaves that had been incubated for days to weeks under conditions

Box 1. Major milestones in our knowledge of aquatic hyphomycetes

Taxonomy and Phylogeny
- Hartig (1880) and Saccardo (1880) describe the first two fungal species that qualify as aquatic hyphomycetes from terrestrial habitats.
- De Wildeman (1893, 1894, 1895) discovers four additional aquatic hyphomycete species in ponds.
- Ingold (1942) identifies leaves in a stream as the main habitat of aquatic hyphomycetes.
- Ranzoni (1956) identifies the first sexual state of an aquatic hyphomycete as an ascomycete.
- Ingold (1959) documents the first aquatic hyphomycete with a basidiomycete teleomorph.
- Relying on the direct observation of developing fruitbodies, Webster (1992) documents that most aquatic hyphomycete are related to ascomycetes, and a minority to basidiomycetes.
- Molecular analyses confirm that aquatic hyphomycetes are polyphyletic and mostly related to ascomycetes (Belliveau & Bärlocher 2005; Baschien *et al.*, 2006; Campbell *et al.*, 2009).

Selected taxonomic surveys of aquatic hyphomycetees with keys
- Petersen (1962, 1963a, b)
- Nilsson (1964)
- Ingold (1975)
- Brathen (1982, 1984)
- Nawawi (1985)
- Goh (1997)
- Marvanová (1997)
- Santos-Flores & Betancourt-López (1997)
- Gulis *et al.* (2005)

allowing fungal colonization, which resulted in increased protein levels. Triska (1970) observed the same feeding preferences in two detritivorous caddisfly larvae. Mackay & Kalff (1973) showed that fungal-conditioned leaves were much more palatable than bacterial-conditioned or sterile leaves. These early studies suggested that microbial community during leaf decomposition is dominated by fungi in terms of biomass and degradative activity, and that they play a greater role than bacteria in preparing the leaf litter for ingestion by detritus-feeders. It should be noted, however, that the studies generally did not distinguish between aquatic hyphomycetes and phylloplane fungi, which are common on terrestrial leaves and persist for weeks in the stream (Bärlocher & Kendrick, 1974; Gessner *et al.*, 1997). Gessner *et al.* (2007) and Gulis *et al.* (2006) have recently reviewed the role of fungi and other microorganisms in leaf litter processing.

Nutrition of stream invertebrates

Kaushik & Hynes (1971) proposed that the leaf itself, consisting primarily of structural polysaccharides, is essentially an inert substrate for the accumulation of fungal cells, which provide the actual nutrition to the invertebrates. Numerous studies have confirmed that fungal growth (primarily, but not exclusively by aquatic hyphomycetes) on freshly shed leaves generally improves survival and growth of invertebrates, but is not an absolute requirement (for reviews, see Bärlocher, 1985; Suberkropp, 1992; Canhoto & Graça, 2008). Fungal mycelia generally provide a more concentrated and more efficiently digested food source than leaves; survival on fungal diets varied from excellent to poor. Survivorship on bacterial-conditioned food was lower than on fungal-conditioned food (Kostalos & Seymour, 1976). The contribution of fungal mycelia to the growth of various shredders typically varies between 1% and 59% (Suberkropp, 1992); in *Pycnopsyche gentilis*, it was sufficient to account for 100% of the observed growth of third instars, but only for 50% of fifth instars (Chung & Suberkropp, 2009). A second conditioning mechanism is the partial breakdown of plant polysaccharides by fungal exoenzymes into smaller units that are more easily digested by invertebrates (Bärlocher, 1982). It would be wrong, however, to conclude that fungal colonization inevitably improves the nutritional quality of the substrate: by inoculating a suitable fungus, the preference of an amphipod for certain leaf species could be reversed (Bärlocher & Kendrick, 1973). Caddis fly larvae differentiated between leaf patches colonized by different fungi (Arsuffi & Suberkropp, 1985).

Biomass and growth

Because microbial cells clearly contribute to invertebrate nutrition, accurate estimates of bacterial and fungal biomass and their production on decaying leaves are essential. In a first attempt, fungal biomass was estimated by measuring the length of stained fungal hyphae in decolorized leaves (Bärlocher & Kendrick, 1974). This is believed to result in considerable underestimates (Gessner & Newell, 2002). Nevertheless, direct counts suggested that fungal biomass typically contributed 90–95% to the total microbial biomass during early stages of leaf decay (Findlay & Arsuffi, 1989), confirming earlier conclusions on the relative importance of fungi vs. bacteria (Kaushik & Hynes, 1968, 1971).

More recently, indicator molecules such as ATP, chitin and ergosterol have been used to quantify fungal biomass (Suberkropp, 2008). ATP degrades rapidly after cell death, and therefore reflects living biomass, but it occurs in all organisms and does not allow differentiation between bacterial and fungal cells. Chitin occurs in many invertebrates, is refractory and persists long after fungal hyphae have died. Ergosterol has become the index molecule of choice for fungal biomass. It is the major membrane sterol in higher fungi and is rarely produced by other organisms (Gessner & Newell, 2002; Suberkropp, 2008). It is also believed to rapidly degrade upon cell death, although this has been disputed (Mille-Lindblom *et al.*, 2004). Ideally, ergosterol levels should strictly correlate with fungal biomass, but, depending on species and environmental conditions, the ratio of ergosterol to biomass in pure cultures can

vary by a factor of up to 14 (Gessner & Chauvet, 1993; Charcosset & Chauvet, 2001; Raviraja *et al*., 2004). Estimates of fungal biomass based on ergosterol were up to three times higher than those calculated from ATP concentrations (Suberkropp *et al*., 1993); with other conversion factors, the discrepancy was less severe (Abelho, 2009). Clearly, ergosterol measurements from environmental samples have to be interpreted with caution. Nevertheless, they are useful for following the dynamics of fungal biomass during leaf decomposition and for comparing bacterial and fungal biomasses. Typically, ergosterol levels increase when deciduous leaves enter streams, reach a plateau and eventually decline. At its peak, fungal biomass (estimated by ergosterol level) may account for as much as 18–23% of detrital mass (Gessner, 1997; Methvin & Suberkropp, 2003). It is generally lower in floodplain ponds than in rapidly flowing river sections (Baldy *et al*., 2002). On leaves, fungal biomass consistently outweighs bacterial biomass (estimated by epifluorescence microscopy) by a factor of ≥ 10 (Baldy *et al*., 2002; Hieber & Gessner, 2002; Pascoal *et al*., 2005). At later stages of decay and on smaller organic particles, fungal biomass declines while bacterial biomass remains relatively constant (Findlay *et al*., 2002).

Microbial production can be determined by following biomass over time if losses due to death, conversion to spores, etc., are negligible. If they are not, direct estimates of growth rates are essential. For fungi, Newell & Fallon (1991) introduced a method based on ^{14}C-acetate incorporation into ergosterol. Bacterial growth is generally estimated by measuring incorporation of leucine or thymidine into proteins and DNA, respectively. Application of these techniques to leaves in streams again demonstrated that production rates of fungi in most instances considerably exceeded those of bacteria (Findlay & Arsuffi, 1989; Weyers & Suberkropp, 1996; Baldy & Gessner, 1997; Baldy *et al*., 2002; Pascoal *et al*., 2005). Repeated measurements of fungal production rates and standing crops of leaf litter have been combined to estimate annual fungal production, which has ranged from 16 to 193 g m^{-2} (Suberkropp, 1997; Methvin & Suberkropp, 2003; Carter & Suberkropp, 2004; Gulis *et al*., 2006).

While neither ergosterol nor acetate incorporation provide any information on the fungal taxa growing on leaves, $\geq 50\%$ of this production is channeled into identifiable conidia of aquatic hyphomycetes (Suberkropp, 1984, 1991; Findlay & Arsuffi, 1989; Gessner & Chauvet, 1993; Sridhar & Bärlocher, 2000), which can be captured by filter-feeding invertebrates (Bärlocher & Brendelberger, 2004).

Enzymes

Vascular plant detritus consists primarily of cellulose, hemicelluloses, pectin and lignin (Webster & Benfield, 1986). Because leaf mass loss proceeded most quickly when fungal growth was possible (Kaushik & Hynes, 1968, 1971), fungi were assumed to actively degrade and use some of these polymers. Suberkropp *et al*. (1976) were the first to show significant declines in hemicellulose and cellulose during leaf processing in a stream. By contrast, there was an absolute increase in lignin, which the authors attributed to the complexing of nitrogenous compounds by lignin and polyphenols. Similar results were reported for conifer needles by Rosset *et al*. (1980). Single and mixed pure cultures of both aquatic hyphomycetes and common phyllosphere taxa such as *Alternaria*, *Cladosporium* and *Aureobasidium* caused weight loss of sterilized leaf disks, although at low incubation temperatures, aquatic hyphomycete species were generally more effective (Bärlocher & Kendrick, 1974). Mass loss of submerged leaves due to terrestrial fungi has also been reported by Godfrey (1983) and Graça & Ferreira (1995). Suberkropp & Klug (1980) reported specific enzymatic capabilities of selected aquatic hyphomycetes; due to strong pectinolysis, several species macerated intact leaves into fine particles, thus adding to the food supply of stream filter-feeders. Many other reports have since demonstrated that most aquatic hyphomycetes can degrade cellulose, various hemicelluloses and pectin (Chamier, 1985; Zemek *et al*., 1985; Abdullah & Taj-Aldeen, 1989; Chandrashekar & Kaveriappa, 1991; Abdel-Raheem & Ali, 2004). The breakdown of lignin is more complex and involves a variety of enzymes, among them monooxygenases, dioxygenases and peroxidases (Hendel *et al*., 2005). Several authors have reported limited activities of some of these enzymes in several aquatic hyphomycetes (Fisher *et al*., 1983; Zemek *et al*., 1985), and peroxidase activities on leaf litter in streams is generally low (Tank *et al*., 1998). Lignin decomposition in leaves is therefore likely to proceed slowly if at all, which partly explains the rise in lignin content reported by Suberkropp *et al*. (1976) and Rosset *et al*. (1980). As mentioned earlier, some of the increase may also be due to artifact lignins formed by microbial enzyme/tannin precipitates.

The significance of laccases or related enzymes extends beyond the breakdown of lignin: their activity in *Clavariopsis aquatica* correlated with the removal of technical nonylphenol (t-NP), which can disrupt endocrine function in vertebrates (Solé *et al*., 2008c).

Involvement of other fungi

Exponential decay rates of seven leaf species in a third-order stream in the French Pyrenees correlated strongly with maximum ergosterol concentration, net mycelial production and spore production by aquatic hyphomycetes (Gessner & Chauvet, 1994), suggesting that these fungi largely control this process. Similar correlations were found by Maharning & Bärlocher (1996), although the slopes of the

regression curves were significantly lower. While spore production is clearly connected to aquatic hyphomycetes, ergosterol occurs in other higher fungi, although it is absent in chytrids (Gessner & Newell, 2002). Because a substantial proportion of estimated fungal biomass on leaves is channeled into the production of aquatic hyphomycete conidia, it seems reasonable to assume that these fungi generally dominate leaf decay in streams, which is further supported by their enzymatic diversity. Nevertheless, several lines of evidence suggest that other groups cannot be ignored. Terrestrial fungi have consistently been observed or isolated from leaves decaying in streams (Bärlocher & Kendrick, 1974; Rossi *et al.*, 1983). More recently, molecular analyses have revealed the ubiquitous presence of DNA from fungal taxa that have few or no aquatic hyphomycete representatives (Nikolcheva & Bärlocher, 2004; Bärlocher *et al.*, 2008; Seena *et al.*, 2008). The importance of these taxa may be greater when dissolved oxygen levels are low, and water temperatures and organic pollution high (Chergui & Pattée, 1988; Raviraja *et al.*, 1996).

Perspectives in research on aquatic hyphomycetes

Aquatic hyphomycetes participate in the decomposition of plant remains, which has been defined as 'the sum of biotic and abiotic transformations resulting in the formation of biomass (fungal, bacterial or animal), carbon dioxide and other mineral substances, dissolved organic matter (DOM) and fine particulate organic matter (FPOM)' (Gessner *et al.*, 1997). These transformations can be interpreted from a process or from a taxon oriented view (Fig. 1). The starting points for both are fungal mycelia on plant remains. Quantities and identities of these mycelia are involved in a feedback system with the local fungal species pool, external environmental variables (e.g. oxygen, pH, inorganic nutrients), leaf litter quality and quantity (chemistry, timing of production) and both microbial (primarily bacterial) and animal (primarily invertebrate) groups. Fungal mycelia perform core ecosystem functions such as regulating the rate of litter mass loss, providing food for leaf-shredders (accumulation of biomass) and for filter-feeders (production of conidia and fine leaf particles), and releasing inorganic nutrients. In addition, they are instrumental in keystone processes (processes with a disproportionate effect on the ecosystem relative to the quantities involved): they sequester metal ions and may break down estrogen mimics such as nonylphenol and other xenobiotics (see: Distinctive features of fungal organopollutant metabolism and metabolization of organic xenobiotics by freshwater fungi). However, even thorough knowledge of these processes and their rates provide only limited insights into mechanisms that govern the composition and persistence of observed fungal communities (Bärlocher, 2010). Topics in evolutionary ecology such as biodiversity, life history or adaptation to global climate change require more detailed knowledge of taxon-specific properties. Both structure (taxa) and function (processes) are considered essential in modern biodiversity research (Pascoal & Cássio, 2008).

Freshwater fungal biodiversity is controlled by the environmental context and governs ecological functions

Based on extensive collections on several continents, Ingold (1975) concluded that the diversity of aquatic hyphomycetes is highest in nonpolluted, relatively cool, well-aerated streams running through deciduous forests. It seems likely that their contribution to stream ecosystem functions also peaks under these conditions. While most mycologists and limnologists would probably agree with these statements, it has been surprisingly difficult to find unequivocal and consistent connections between natural or human-altered deviations from the 'ideal' stream and diversity and functions of aquatic hyphomycete communities. There is no doubt, moreover, that some aquatic hyphomycete species are remarkably resilient and occur, for example, in arctic or subarctic streams where terrestrial input is dominated by grasses (Engblom *et al.*, 1986), in sulfur springs with temperatures reaching 36.5 °C (Chandrashekar *et al.*, 1991) and in surface water (Krauss *et al.*, 2001), sediment (Sridhar *et al.*, 2008) and groundwater (Krauss *et al.*, 2003b, 2005) with extremely high levels of heavy metals.

Riparian vegetation

It has repeatedly been established that leaves of some tree species (e.g. *Alnus glutinosa*) are colonized more rapidly and by more species of aquatic hyphomycetes than others (e.g. *Fagus sylvatica*) (Bärlocher, 1992a, b). This is not due to strict substrate specificity by fungal species, but rather to shifts in their relative proportions (differential colonization is more pronounced when leaves are compared with grass or wood; Gulis, 2001). Assuming this is based on some degree of resource specialization, one would therefore expect a positive correlation between the diversity of riparian vegetation (which provides fungal substrates) and fungal diversity. Surprisingly, this has not always been observed. For example, Bärlocher & Rosset (1981) reported over twice as many fungal species from a stream running through a practically pure stand of *Picea abies* compared with two other streams bordered by mixed deciduous forest. In a more extensive study with 16 streams, no correlation was found between riparian tree diversity and fungal species richness (Wood-Eggenschwiler & Bärlocher, 1983). By contrast, Fabre (1996) found a significant correlation between fungal species

richness and richness and diversity of the riparian tree community. However, partial correlations showed that fungal and tree communities were structured simultaneously, but independently by environmental conditions summarized by elevation. Laitung & Chauvet (2005) and Lecerf *et al.* (2005) also found a significant correlation between leaf and fungal species richness in 10 streams.

The studies discussed above dealt with essentially naturally grown forests. Changes can also occur due to deliberate introductions (e.g. eucalypt in Portugal and Spain) or due to invasive species (e.g. Japanese knotweed in Europe, willow in Australia, saltcedar in the United States; for a review, see Lecerf & Chauvet, 2008). A meta-analysis of 16 studies revealed no consistent impact of afforestation on fungal diversity, although streams running through some Portuguese eucalypt plantations had a clearly reduced community (Bärlocher & Graça, 2002; Ferreira *et al.*, 2006); in comparable Spanish streams, increased levels of inorganic nutrients may have negated this effect and similar numbers of fungal species colonized exposed leaves (Chauvet *et al.*, 1997; Ferreira *et al.*, 2006). Meta-analysis revealed no overall significant effect of altered forests on microbial breakdown rates or maximal mycelial biomass, but possibly a small stimulatory effect on spore production (Lecerf & Chauvet, 2008).

pH and alkalinity

Based on a number of studies, Bärlocher (1987) concluded that the number of aquatic hyphomycete species is generally high within a relatively broad pH region between 5 and 7 and declines below and above these values. Low pH values as such do not appear to be harmful to aquatic hyphomycetes: on solid media, 10 species grew best at values between 4 and 5 (in liquid media, the optimum was generally 1 U higher; Rosset & Bärlocher, 1985). Weight loss of maple leaves due to microbial activity peaked between 5.5 and 6.0 (Thompson & Bärlocher, 1989). However, anthropogenic acidification can raise aluminium concentration in the stream water, which severely depressed fungal richness and activity (Chamier, 1985; Chamier & Tipping, 1997; Baudoin *et al.*, 2008). No difference in fungal richness was found between two humic streams in Sweden, both exposed to acid precipitation and one treated with lime (Bärlocher & Peterson, 1988).

Low alkalinity or levels of Ca^{2+}, which coincide with low pH values, make aquatic hyphomycetes more vulnerable to heavy metal exposure (Abel & Bärlocher, 1984). In a recent study (Cornut, 2010), anthropogenic acidification strongly depressed leaf mass loss on the stream bed surface and, to a lesser extent, in the hyporheic zone. This was attributed to the drastic impact of acidity on leaf-shredders and the reduced fine particle production by the impoverished fungal community. The latter may be connected to a reduced pectin lyase activity at lower pH values (Chamier, 1985). By contrast, fungal biomass showed no obvious trend and was in fact highest in the most acidic stream.

Pollution by metals

Mining pollution very consistently has a negative effect on fungal species richness and associated performance metrics such as maximum spore production and biomass and fungal leaf-processing rates (meta-analysis, Lecerf & Chauvet, 2008). This has been shown for effluents from mining for coal (e.g. Maltby & Booth, 1991; Bermingham *et al.*, 1996; Schlief, 2004), gold (Lecerf & Chauvet, 2008), copper (Cu) (Sridhar *et al.*, 2000, 2001; Krauss *et al.*, 2001), zinc (Zn) (Niyogi *et al.*, 2002, 2009) and uranium (Ferreira *et al.*, 2010b). At moderate pollution, the effects are generally more severe on fungal diversity and less so on fungal biomass/growth and degradational activity (Duarte *et al.*, 2008; Solé *et al.*, 2008b), which may suggest some compensation by resistant strains or species. Exposure to higher levels drastically impoverishes fungal communities and reduces leaf decomposition (Krauss *et al.*, 2001; Duarte *et al.*, 2008; Solé *et al.*, 2008b;Fernandes *et al.*, 2009a).

Sridhar *et al.* (2005) demonstrated partial adaptation of some fungi to heavy metal pollution by transplantation experiments. They isolated two strains of *Heliscus lugdunensis* and one strain of *Tetracladium marchalianum* from two habitats differing in severity of heavy metal pollution. After precolonization in the laboratory, leaf disks were transferred to the two sites. Two out of three strains performed better at the site of their origin. The authors also observed a relatively swift recovery of the fungal community and metabolism upon removal from acute heavy metal exposure. Similar observations were recently made by Duarte *et al.* (2009). Leaves incubated in a reference stream were exposed in microcosms supplemented with Cu or Zn, which reduced leaf mass loss, fungal reproduction and altered the fungal community. After release from metal stress, the structure and activity of the fungal community quickly recovered. Higher fungal diversity may mitigate the impact of heavy metal pollution on leaf decomposition in streams (Duarte *et al.*, 2008).

Eutrophication

Lecerf & Chauvet (2008) reviewed the effects of eutrophication on fungal diversity and their growth and involvement in leaf breakdown. The only significant effect in their meta-analysis was declining fungal diversity, even though individual studies often found clear negative or positive effects. This inconsistency is not surprising, because eutrophication is a rather broad concept and can include both inorganic and organic sources. Especially in field studies, it is difficult to separate effects of the two and to consider potential

interactions with other factors. Nitrates and phosphates in particular are well known to stimulate fungal growth and leaf breakdown. In fact, extremely low levels of these nutrients may have been responsible for impoverished fungal communities in some streams studied by Suberkropp (1995). At higher concentrations, the positive correlation between nutrients and fungal diversity may reverse itself: Sridhar *et al*. (2009) found fast decay rates, but low fungal diversity in a more nutrient-enriched stream. Similarly, Raviraja *et al*. (1998) observed very low aquatic hyphomycete diversity in a eutrophied stream without significant impact on leaf breakdown rates. It seems unlikely that nutrients at the levels observed directly inhibited some species and led to their extinction (but see Solé *et al*., 2008b). Possibly, oxygen depletion on leaf surfaces due to sedimentation of fine particles or increased microbial respiration negatively affected aquatic hyphomycete metabolism and ultimately growth (Pascoal & Cássio, 2004; Medeiros *et al*., 2009). Or, increased levels of toxic organic and inorganic micropollutants typically associated with eutrophication may be responsible for reduced fungal diversity (Lecerf & Chauvet, 2008). Neither of these scenarios, however, explains the continued strong degradational activity, unless some species are resistant to low oxygen levels or to the postulated toxins, or other microbial groups take over their functions. Alternatively, increased nutrient levels may selectively stimulate some aquatic hyphomycete species, allowing them to displace weaker competitors (Bärlocher, 2005). This assumes a hump-shaped relationship between diversity and community productivity or growth, which is well documented for some plant-dominated systems (Gough *et al*., 2000).

Temperature and climate change

Temperature is generally considered the most important environmental factor affecting metabolic functions and ultimately growth and survival of microorganisms (Madigan *et al*., 2009). It is characterized by three cardinal points: T_{min}, below which no growth is possible, T_{opt}, at which growth is most rapid and T_{max}, above which growth is not possible. The three temperatures usually differ for growth and reproduction.

Aquatic hyphomycete species in temperate regions are less inhibited by water temperatures close to 0 °C than phylloplane fungi (Bärlocher & Kendrick, 1974). Their growth generally peaks between 15 and 25 °C, while sporulation is highest at somewhat lower temperatures (Koske & Duncan, 1974; Webster *et al*., 1976; Suberkropp, 1984; Chauvet & Suberkropp, 1988; Sridhar & Bärlocher, 1993; Dang *et al*., 2009). Beyond minima and maxima, temperatures that kill the individual colony or propagule are important. For example, temperate species were shown to be more likely to survive freezing than tropical species (Sridhar & Bärlocher, 1993); the reverse may be true for survival at high temperatures. The occurrence and frequency of such extreme events may have a profound effect on fungal community composition and activities and override the impact of the overall trend.

Another point to consider is the fact that temperature rarely remains stationary. Growth averaged over a 24-h period is only identical with the growth at the average temperature during the same period if the relationship between growth and temperature is linear. If the curve is concave down (e.g. n-shaped), it is lower; if the curve is concave up (e.g. u-shaped), it is higher. Thus, temperature oscillations around 8 °C accelerated decomposition by 18% (5 ° oscillation) and 31% (9 ° oscillation), respectively (Dang *et al*., 2009).

These responses to temperature can be expected to influence both seasonal and regional abundance of individual species and therefore fungal community composition, although the evidence is rarely straightforward and suggest that the ecological context (nutrients, oxygen, biological interactions) can strongly modulate temperature preferences (Bärlocher, 1992b). Thus, *Flagellospora penicillioides* and *Lunulospora curvula* do not grow below 5 °C; not surprisingly, they disappear from streams during colder seasons (Webster *et al*., 1976; Suberkropp, 1984). In a Michigan stream, distinct winter and summer assemblages could be identified; however, temperature growth responses in cultures only partially explained their seasonal cycles (Suberkropp, 1984). On a larger scale, temperature preferences of individual species are broadly linked to geographical distribution (Bärlocher, 1992b), and species such as *F. penicillioides*, *L. curvula*, *Lunuluspora cymbiformis* and *Triscelophorus acuminatus* are more common in subtropical or tropical streams (Wood-Eggenschwiler & Bärlocher, 1985; Goh, 1997; Santos-Flores & Betancourt-López, 1997).

The continued use of fossil fuels is projected to raise the atmospheric CO_2 level from approximately 380 μL L^{-1} to between 490 and 1060 μL L^{-1} by the end of this century, which will contribute to an average global temperature increase of between 1 and 6 °C (IPCC, 2007). Additionally, the frequency of extreme weather events (e.g. record high or low temperatures, floods, droughts) and overall variability are expected to increase. These changes will influence the activities of aquatic fungi, and by extension their distribution and occurrence, and the composition of fungal communities. Because of numerous and intimate fungal feed-back loops (Fig. 1) with resources (plant detritus), potential competitors (bacteria, invertebrates) and predators (invertebrates), predicting fungal responses to environmental change and the resulting effects on ecosystem processes are daunting challenges.

Because the water temperature in temperate streams is generally below the optimal temperature for most fungi (see

references above), an immediate primary consequence of increasing it by a few degrees would be an accelerated rate of decomposition. This has been confirmed for the hyporheic habitat in a stream, whose temperature was artificially raised by 4.3 °C (Bärlocher *et al.*, 2008). Compared with an unheated control section, it lowered the number of leaf fragments, which were the primary substrates of aquatic hyphomycetes (Bärlocher *et al.*, 2006). In addition, the number of fungal species on fragments from the heated habitat declined. Bärlocher *et al.* (2008) concluded that the accelerated rate of decomposition shrinks the supply of suitable substrates; in addition, their ability to sustain fungal colonies rapidly deteriorates. Leaf-eating invertebrates were not studied explicitly, but they had free access to the leaf fragments. It seems likely, however, that their impact is much lower in the hyporheic zone than on the streambed surface (Cornut, 2010; Cornut *et al.*, 2010).

Accelerated decomposition was also observed when leaves preincubated in a stream (ambient temperature 18 °C) were exposed in microcosms to a 'warming' treatment (25 °C, vs. control at 18 °C) (Fernandes *et al.*, 2009b). A freeze–thaw treatment between stream and microcosm exposure, as an example of an extreme event that may become more frequent due to climate change, retarded decomposition and caused *L. curvula*, a typical summer species, to become extinct.

In a microcosm experiment with a mixture of six pure cultures, the rate of decomposition increased between 5 and 15 °C (Ferreira & Chauvet, 2010). The rate of increase was significantly enhanced at the highest temperature, when nutrients (nitrate and phosphate) were added. Increased eutrophication is common in many scenarios of global change (IPCC, 2007).

Many studies have investigated the effects of the root cause of climate change, elevated CO_2 levels, on the quality of leaf litter. Generally, this leads to greater leaf biomass, C/N ratio, concentrations of tannins and other phenolics and lower nitrogen levels (for reviews, see Norby *et al.*, 2001; Stiling & Cornelissen, 2007; Ferreira *et al.*, 2010a), with negative consequences for herbivores. However, in an extensive review of primarily terrestrial studies, Norby *et al.* (2001) failed to detect a consistently negative impact of these changes on short-term decay rates or respiration from decomposing litter.

Decomposition experiments in streams have also given inconsistent results. Leaves grown at elevated CO_2 levels may decay more slowly (e.g. Rier *et al.*, 2002), but fungal biomass can be as high as or higher than on control leaves (Rier *et al.*, 2002; Ferreira *et al.*, 2010a). Mosquito larvae had higher mortality rates and slower development rates when fed on leaves from elevated CO_2 levels (Tuchman *et al.*, 2003). Negative effects were also shown for *Tipula abdominalis* (Tuchman *et al.*, 2002). The effects of elevated CO_2-induced leaf quality on *Sericostoma vittatum* (*Trichoptera*: *Sericostomatidae*) were complex (Ferreira *et al.*, 2010a). When offered at 10 °C, leaves with the elevated treatment were preferred (no difference at 15 °C). Growth and survival rates were generally higher at the lower temperature.

The ultimate goal of climate biology is to predict changes in stream ecosystems due to elevated CO_2 and temperature. Several shortcomings and pitfalls of currently used approaches have been discussed in Bärlocher *et al.* (2008). To begin with, we cannot assume that increasing temperature and CO_2 levels have the same effect and act independently (additive). For example, elevated CO_2 increased allocation to phenolics in three deciduous trees, but elevated temperature had the opposite effect. In combination, the overall effect was nil (Veteli *et al.*, 2007). The same principle applies to decomposition experiments: both temperature and CO_2 levels should be elevated to simulate the effects of global change. Leaf chemistry influenced by CO_2 levels and temperature during a feeding trial strongly interacted when its suitability as food to a detritus-feeder was studied (Ferreira *et al.*, 2010a). Another potentially decisive point concerns the transition from current to projected conditions: a mycorrhizal community responded strongly to an abrupt doubling of CO_2 concentration, but not to a gradual change of the same magnitude spread over 6 years (Klironomos *et al.*, 2005). The anticipated climate changes will likely be spread out over several decades. In any given catchment area, members of the existing riparian plant community will become extinct unless they can adapt to the changing conditions. They may also be joined by invasive species (which, being microorganisms, may be 'invisible'; Litchman, 2010). Local aquatic hyphomycetes in turn have to adapt to the changes in climate and resource chemistry and to invasive microbial competitors or become extinct. The next trophic levels, leaf-eating invertebrates, will have to deal with the changed leaf–fungus complex, climatic changes and invaders. The magnitude and directions of these interlocking feedback loops will depend on the initial context and evolutionary potential of local and neighboring species pools; the resulting cascades can become extremely complex and the outcome(s) difficult to predict. A large-scale simulation of the joint effects of temperature and flow regimes in Mediterranen streams suggested increases in instream metabolism and export of organic carbon (Acuña & Tockner, 2010).

In view of the ubiquitous occurrence of aquatic hyphomycetes on all continents, their ability to colonize essentially all types of plant litter and short life cycles facilitating rapid evolutionary change (Bärlocher, 1992b), extensive extinctions due to climate change seem unlikely. In the short term, however, an impoverished riparian vegetation may lower local fungal diversity, which in turn may lower the nutritional value of conditioned leaves (e.g. Lecerf *et al.*, 2005).

Climate change over the last 55 years generally had a positive impact on forest productivity when water was not

limiting (Boisvenue & Running, 2006), and the fruiting period of forest fungi has been expanding since 1985 (Gange *et al.*, 2007), suggesting increased mycelial growth and organic matter turnover in response to global warming.

Connections between biodiversity and ecological functions

Much of the concern about declining biodiversity is based on the fear that it will threaten ecosystem functions and services (Kinzig *et al.*, 2001). Many obervations and experiments in plant communities have indeed demonstrated an asymptotic relationship between species richness and production or accumulation of biomass (Loreau *et al.*, 2001). Studies involving aquatic hyphomycetes have started only recently (Bärlocher & Corkum, 2003), are few in numbers (Pascoal & Cássio, 2008) and have involved relatively few species (up to eight, Dang *et al.*, 2005). A problem unique to microbial systems is the difficulty of keeping track of individual species. While the composition of the initial inoculum can be controlled, it cannot be assumed that it will be maintained throughout the experiment. It is therefore uncertain whether the measured function is indeed the result of the initially established diversity. Estimates of the realized diversity throughout the experiment have been based on spore productions by the introduced species (Dang *et al.*, 2005) or on intensities of denaturing gradient gel electrophoresis (DGGE) bands of their amplified DNA (Pascoal *et al.*, 2010).

Investigated functions have most commonly included mass loss of leaves, followed by build-up of fungal biomass and release of conidia. Thus far, the evidence suggests that traits of certain species have a greater impact on these ecological functions than diversity *per se* (i.e. numbers of species present in a stream or microcosm; Pascoal & Cássio, 2008), and nutrient enrichment can overwhelm the often weak diversity effects (Bärlocher & Corkum, 2003). There appears to be sufficient redundancy in the fungal community to buffer its core functions against loss of biodiversity. However, the variability of decay rates increased at lower diversity (Dang *et al.*, 2005), particularly under Zn stress (Pascoal *et al.*, 2010), suggesting that the effects of fungal species loss on ecosystem stability may be exacerbated with increasing pollution. In addition, species-specific fungal traits may have a greater impact on higher trophic levels: food selection by leaf-eating invertebrates is often strongly influenced by the identity of fungi that have colonized the leaves (Bärlocher & Kendrick, 1973; Arsuffi & Suberkropp, 1985; Lecerf *et al.*, 2005). Again, this raises the topic of keystone properties: even though all aquatic hyphomycete species tested appear to perform the same basic functions at similar rates (breakdown of structural polysaccharides, addition of mycelia, release of conidia), their effect on the ecosystem may be selectively amplified or attenuated at higher trophic levels.

Sophisticated tools unravel structure and functions of fungal communities

Fungal community structure

Fungal communities on leaves decaying in streams have been detected and characterized by aerating substrates in nutrient-poor solutions (Bärlocher, 2005; see Aquatic hyphomycetes are essential providers of ecosystem functions). Clearly, this favors the discovery of aquatic hyphomycetes. The majority of the fungal biomass on decaying leaves, however, consists of nonreproductive mycelia (e.g. Bärlocher & Kendrick, 1974), which may or may not be able to form identifiable propagules under aeration. The traditional reliance on identifying newly formed spores to characterize dominant species therefore includes an element of circular reasoning (Bärlocher, 2005). In contrast, molecular methods, which isolate nucleic acids directly from environmental samples, facilitate the detection of fungal taxa regardless of life stage (i.e. reproducing or nonreproducing hyphae, mitospores and meiospores, dormant structures). Moreover, molecular methods are a powerful alternative when fungal biomass and sporulation are drastically reduced due to unfavorable environmental conditions (Solé *et al.*, 2008a; Fig. 2). Isolated DNA is amplified with fungal group-specific primers (Nikolcheva *et al.*, 2003, 2005; Nikolcheva & Bärlocher, 2004; Bärlocher, 2007; Das *et al.*, 2007; Duarte *et al.*, 2008; Seena *et al.*, 2008; Harrop *et al.*, 2009) and usually analyzed by fingerprint methods. Community fingerprinting techniques (e.g. terminal restriction fragment length polymorphism, DGGE, thermal gradient gel electrophoresis) have been successfully applied to study aquatic fungi in natural environments or in microcosms (Nikolcheva *et al.*, 2003; Nikolcheva & Bärlocher, 2004; Raviraja *et al.*, 2005; Das *et al.*, 2007; Duarte *et al.*, 2008; Solé *et al.*, 2008a; Moreirinha *et al.*, 2010).

Most of these studies resulted in estimates of fungal diversity in terms of operational taxonomic units or phylotypes, some of which did not correspond to fungal taxa dominating the spore production (Nikolcheva *et al.*, 2005; Solé *et al.*, 2008a). However, community fingerprints rarely allow unequivocal identification of all fungi present. Only the excision, cloning and sequencing of the 'fingerprint bands' can confirm or refute their phylogenetic affiliation to aquatic hyphomycetes, and, ideally, allow assigning them to described taxa. Alternatively, the initial mixture of amplicons with fungal-specific primers can directly be cloned and sequenced. Two such studies have revealed the dominance of ascomycete-affiliated taxa (most aquatic hyphomycetes are ascomycetes, but not all ascomycetes are

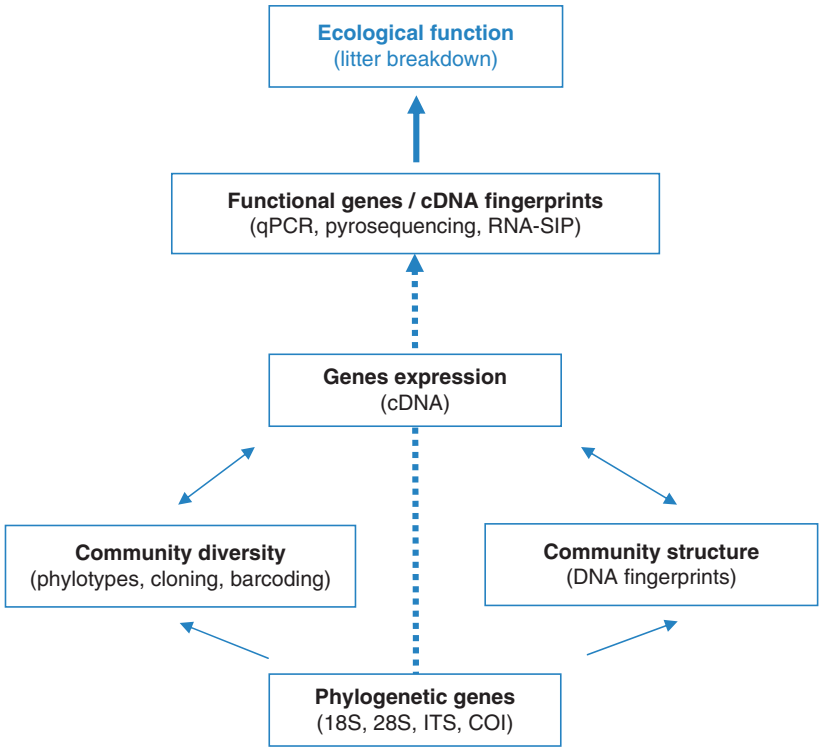

Fig. 2. Schematic diagram of molecular approaches linking diversity and structure of fungal communities involved in litter breakdown. Laccases are an example of functional genes putatively involved in ecological process. When based on phylogenetic genes, the scheme illustrates the absence of a direct connection of community diversity and structure to litter breakdown. In this case, only cDNA fingerprints might allow a more direct assessment of the ecological function (dashed arrow).

aquatic hyphomycetes). In both cases, however, substantial numbers of clones were assigned to fungal groups that rarely or never contain aquatic hyphomycetes (Bärlocher *et al.*, 2008; Seena *et al.*, 2008). A practical, standardized method for the identification of fungal structures at any life stage therefore remains a top priority. Currently, the most promising approach is the use of molecular barcodes. These are short DNA sequences from a specified region of the genome that are unique for each species (Hebert *et al.*, 2003). Suitable barcodes should be sequences that can be reliably extracted and amplified, and are of low intraspecific and sufficient interspecific variability. For animals, a 648-bp fragment at the 5′ end of the mitochondrial cytochrome *c* oxidase 1 gene (COX1 or CO1) has been recommended due to its high variability, low intron interference and robust amplification ability (Hebert *et al.*, 2003). Identification is straightforward when a sequence is constant within one species and does not occur anywhere else. Failing this, intraspecific differences have to be much lower than those among species. Based on many studies in various animal groups, ranging from invertebrates to fish to birds, Hebert *et al.* (2004) suggest a threshold of a factor of 10 between sequence variation within and among species. For fungi, the entire internal transcribed spacer (ITS) sequence was recommended as a first step for species identification, with a second sequence added for more precise identification and phylogenetic placement (Fungi Barcode Initiative; Rossman, 2007). The suitability of this region to resolve the taxonomy of the aquatic hyphomycete genus *Tetracladium* was recently demonstrated by Letourneau *et al.* (2010). Seena *et al.* (2010) sequenced and compared the ITS site in 94 fungal isolates belonging to 19 species collected in Portuguese streams. The Portuguese isolates and sequences from GenBank exhibited taxonomic cohesiveness. All isolates grouped with their respective species; however, all *Tricladium* species did not group within the same genus.

Due to species-specific food preferences of leaf-eating invertebrates (Bärlocher & Kendrick, 1973; Arsuffi & Suberkropp, 1985; Lecerf *et al.*, 2005), quantitative measurements of biomass and enzyme activities of individual aquatic hyphomycete species in streams are of prime importance for documenting and understanding their ecological significance in food webs. As a first step, several studies attempted to assess the relative contribution of all fungi to aquatic microbial communities. Lefèvre *et al.* (2010) developed a quantitative real-time PCR assay, based on 18S rRNA gene, to assess zoosporic fungi. Application to natural samples provided only semi-quantitative estimates, because the number of rRNA operons per cell is not well known. Quantitative real-time PCR was also used by Manerkar *et al.* (2008) to estimate fungal biomass in a stream during leaf decomposition. The data supported greater contribution of Fungi than Bacteria or Archaea to total microbial biomass as suggested by other methods (see Biomass and growth), but again, the data were only semi-quantitative.

Thus far, quantitative real-time PCR used in environmental studies have focussed on large taxonomic units

(zoosporic fungi, higher Fungi). It seems feasible to design primers for selective amplification of basidiomycetes or ascomycetes; selective primers for aquatic hyphomycetes are unlikely due to the polyphyletic origin of this group (Belliveau & Bärlocher, 2005). Because quantitative real-time PCR works best when a limited number of species is targeted, this method nevertheless promises to be an excellent tool for microcosm experiments. It could be used to monitor the relative abundance of individual species within a fungal community (i.e. to determine 'key-players' for a defined ecosystem function), or to elucidate the role of selected taxa within simplified microbial food webs. More complete information would be provided by a cloning/sequencing approach targeting barcodes to establish relative frequencies of occurrence of the various species, or using new high-throughput pyrosequencing technology with barcodes. Such methods are becoming increasingly common in medical microbiology, and can undoubtedly be adapted to aquatic fungal ecology (Armougom & Raoult, 2008; Petrosino *et al.*, 2009). This would overcome the current difficulty of assessing relative abundances of fungal species in natural communities, which remains one of the major limitations in functional ecology, because specific traits of some species often have a greater influence on ecosystem functioning than species number *per se* (Duarte *et al.*, 2006).

Ecological functions

It is assumed that DNA in dead cells is rapidly broken down, so that the vast majority of amplified sequences are derived from living fungi or bacteria. This may generally be the case, but the presence of clay or colloids can prolong the integrity of environmental DNA (Pietramellara *et al.*, 2009). Pure cultures of *Escherichia coli* cells killed by heat, acid, drying or starvation still provided positive PCR signals (Sheridan *et al.*, 1998). On the other hand, RNAs were less stable. This is particularly true for mRNA, which rapidly turns over in living cells, and may be the best indicator of viability (Sheridan *et al.*, 1998). These compounds can directly be isolated from environmental samples, reverse-transcripted into cDNA and amplified by PCR (RT-PCR). Pyrosequencing both genomic DNA and cDNA (from mRNA) allows simultaneous assessment of structure and (potential) functions of microbial communities (Urich *et al.*, 2008; DeLong, 2009; Fig. 2). At least in principle, taxonomic composition and metabolic pathways can be connected, such that we can determine which taxa are present and whether or not they are actively transcribing specified genes. However, the correlation between mRNA and protein levels is often poor (Gygi *et al.*, 1999; MacDonald *et al.*, 2003). A realistic description of the actual processes taking place would therefore additionally require measuring synthesized proteins (enzymes) and their activities (e.g. using protocols by Sinsabaugh *et al.*, 1994; Jackson *et al.*, 1995, 2006).

Another approach to link function to specific taxa is stable isotope probing (DNA-SIP or RNA-SIP; Boschker *et al.*, 1998; Boschker & Middelburg, 2002; Manefield *et al.*, 2002;Fig. 2). This involves the incorporation of stable-isotope-labelled substrates into cellular biomarkers (e.g. specific DNA or RNA sequences) that can be used to identify organisms assimilating the substrate. A precondition, of course, is that no cross-feeding occurs, which may not necessarily apply when polysaccharides are digested in mixed cultures (e.g. Tiunov & Scheu, 2005; Schneider *et al.*, 2010).

The interpretation of these complex data sets with DNA, RNA and proteins presents significant computational and statistical challenges (DeLong, 2009; Huttenhower & Hofmann, 2010). Potential pitfalls include biases in extraction of nucleic acids and proteins, and in amplification or activity measurements. In addition, these methods usually involve a homogenization step, which destroys the spatial arrangement of cells and enzymes. To reveal the distribution of mycelia belonging to different species, monoclonal antibodies (Bermingham *et al.*, 1995, 1996, 2001; this method could also be applied to localize key enzymes) or FISH (McArthur *et al.*, 2001; Baschien *et al.*, 2008) may be useful. However, neither method has been sufficiently standardized for routine application.

We are not aware of any studies addressing fungal functional gene diversity in aquatic environments. However, primers have been designed to study the diversity of laccase genes in ascomycetes colonizing detritus in saltmarshes (Lyons *et al.*, 2003). Ten species contained 39 distinct sequences, with several species yielding multiple distinct laccase types. Two laccase types from a natural-decay clone library were novel and did not match any of the sequences obtained from the pure cultures. Luis *et al.* (2005) compared RNA and DNA laccase of terrestrial basidiomycete sequences and showed that $< 30\%$ of the laccase genes detected in a soil core were expressed. Solé *et al.* (2008c) identified two putative laccase gene fragments in the aquatic hyphomycete *C. aquatica*, and suggested the existence of additional laccase genes.

Fungal polyphenol oxidases such as laccases catalyze the oxidation of various substrates coupled to the reduction of oxygen to water. They are instrumental in the biodegradation of biopolymers such as lignin (Baldrian, 2006) and therefore play a major role in litter breakdown. Thus, the laccase gene family is a good candidate for attempts to link the structure and function of fungal communities. The results reinforce the point that in addition to studying functional genes, knowing their expression pattern is essential for characterizing fungal activities.

Schneider *et al.* (2010) investigated extracellular degradative enzymes in single and mixed cultures of the bacterium

Pectobacterium carotovorum and a terrestrial fungus, *Aspergillus nidulans*, growing on beech leaves. Semi-quantitative proteome analyses were combined with analyses of extracellular degradative enzyme activities (proteases, pectinases, cellulases and various hemicellulases). Most of the enzyme activity could be attributed to the fungus. Interestingly, the levels of cellulase and pectinase activities were very similar in pure fungal and bacterial/fungal co-cultures, suggesting comparable decompositional activities (actual mass loss of the leaves was not reported). However, ergosterol levels (indicator of fungal biomass) were less than half in the mixed culture, indicating that the bacterium benefited from the degradation products of *A. nidulans* at the expense of fungal growth. Antagonistic effects of bacteria on fungal growth in aquatic model systems have been reported before (e.g. Mille-Lindblom & Tranvik, 2003; Mille-Lindblom *et al.*, 2006), while in mixtures of fungi both facilitative and antagonistic interactions have been documented (Bärlocher & Corkum, 2003; Tiunov & Scheu, 2005). These studies point to the potential disconnect between diversity and various ecological functions: a more diverse microbial community may well stimulate one aspect (e.g. mass loss rate) and inhibit another (e.g. fungal growth). It may therefore be futile to look for consistent rules connecting microbial diversity and ecological function. It must be remembered that communities are best understood as being structured by natural selection on individuals (or genes) pursuing their own interests (Bärlocher, 2010).

Future perspectives – transcriptome, proteome and metabolome analysis

Although fungal interactions with the biotic and abiotic environment usually involve multiple genes, gene expression studies frequently target just one or only a very few genes. The development of new tools such as cDNA microarrays has opened new perspectives for assessing microbial activities. cDNA microarrays offer the possibility of recording the whole transcriptome on a single chip and have been applied successfully to fungi, thus facilitating investigations of the exceedingly complex interactions among thousands of genes expressed simultaneously (Jacob *et al.*, 2004; Doddapaneni & Yadav, 2005; Wright *et al.*, 2005; Jakupović *et al.*, 2006; Le Quéré *et al.*, 2006). Adapting microarrays to environmental studies presents significant challenges (Zhou, 2003). However, microarrays appear promising for monitoring selected genes in the environment or for identifying genes involved in fungal interactions with the environment (Wu *et al.*, 2001; Rhee *et al.*, 2004). Rhee *et al.* (2004) demonstrated that in bacteria, microarray-based quantification was highly consistent with real-time PCR quantification. These results illustrate the potential of the method for qualitative (i.e. specific) and quantitative monitoring of microbial communities other than bacteria.

Like transcriptome analysis with cDNA microarrays, the analysis of the entire proteome (Blee *et al.*, 2001; Vido *et al.*, 2001) is a multivariate approach allowing investigations of complex interactions of fungi with their environments at the protein level, and has successfully been applied to fungal species (Kniemeyer *et al.*, 2006; de Oliveira & de Graaff, 2011). Further identification of proteins may be achieved by sequencing with tandem MS, which nowadays is commonly performed using hyphenated techniques, for example through amino acid-coded mass tagging and liquid chromatography tandem MS as shown for *Schizosaccharomyces pombe* (Bae & Chen, 2004). While most proteomic studies conducted in the past have focused on whole cell extracts, cytosolic proteins and fungal secretomes, fungal organelle proteomics are becoming increasingly important (de Oliveira & de Graaff, 2010). Careful sequential extraction or single-cell sampling methods may be used to obtain more information on membrane proteins, DNA-binding proteins and cell-to-cell signalling proteins.

GC and to an increasing extent liquid chromatography coupled with MS combine high sample throughput with acceptable measurement times and accurate metabolite identification (Moco *et al.*, 2009). Parallel controlling of numerous metabolites in multiple samples may provide more detailed insights into dynamic metabolomic networks and their perturbations by specified environmental stresses as already shown for plants (Saitō *et al.*, 2006). Fungi show less cellular differentiation than plants, but their complex metabolism produces a broad range of often bioactive metabolites (Turner & Aldrigde, 1983; Cole *et al.*, 2003; Frisvad *et al.*, 2004). Metabolite profiling, combined with genetic, proteomic and biochemical approaches (Reaves & Rabinowitz, 2010), can be expected to foster a comprehensive understanding of fungal metabolism especially under stress conditions. Analytical methods to study metabolic networks with respect to data acquisition, network statistics and biochemical interpretation have been extensively discussed and reviewed in the literature (e.g. Fiehn & Weckwerth, 2003; Goodacre *et al.*, 2004; Reaves & Rabinowitz, 2010).

Physiological and biochemical versatility allows aquatic fungi to cope with environmental challenges

Response to metals

High concentrations of essential metals and presence of toxic metals cause major environmental and human health problems due to toxic effects in different biota, trophic transfer and biomagnification in food chains. Ecological and ecobiochemical research on heavy metals is an interdisciplinary effort considering both biotic and abiotic factors.

There is growing interest in understanding the ecological, physiological and biochemical properties that allow some fungi to colonize and live in heavy metal-polluted habitats. Together with bacteria, aquatic fungi are essential constituents of aquatic habitats (see Aquatic hyphomycetes are essential providers of ecosystem functions). The resources locked within substrates are efficiently released by mycelia, i.e. filamentous networks of hyphae. Filamentous fungi have a high surface area-to-volume ratio and therefore provide a large contact area with metals in the environment. They grow by extending the tips of hyphae to form an expanding mycelial network (Steinberg, 2007). Hyphae navigate around biological or abiotic surfaces. Their orientation is a vital aspect of orientated growth, morphogenesis and spatial ecology (Brand & Gow, 2009)

The speciation of heavy metals in the environment and their bioavailability have exerted selective pressures on prokaryotic and eukaryotic microorganisms, many of which have evolved mechanisms to withstand high concentrations of such pollutants (Nies & Silver, 2007; Krauss *et al.*, 2008; Gadd, 2010). Terrestrial and aquatic fungi have developed stress responses to environmental changes: resistance [immediate response, e.g. response to reactive oxygen species (ROS), intracellular synthesis of chelating peptides and proteins] and tolerance (based on intrinsic biochemical and structural properties of fungal cells, e.g. changes in cell wall permeability, extracellular slimes and enzymes, metabolite excretion) (Krauss *et al.*, 2008; Schlosser *et al.*, 2008). Fungal tolerance mechanisms depend (1) on the metabolic and nutritional status of the fungus, which may induce an adaptive stress response, and (2) on the concentration of anions and metals in water, pH value and stability constants of specific extracellular complexes. Because they accumulate more biomass, filamentous fungi can sequester greater amounts of metals than bacteria (Massaccesi *et al.*, 2002). Examining adaptive responses under metal stress will help us to explore the functioning and regulation of cellular metabolism in fungi and unravel the dynamics of metal-influenced biochemical processes and their ecological consequences. Metal avoidance and tolerance in freshwater fungi involve a sophisticated network of mechanisms involving both external and internal detoxification of metal ions.

Extracellular metal precipitation

Precipitation of metal ions onto or around hyphal cells represents a sink for metals. Metal-binding capacity is influenced by environmental pH, hyphal density and excretion of organic compounds (Gadd, 2007). Freshwater fungi were able to grow on and decompose alder leaves exposed in a small spring-fed stream in Central Germany (site H4) under heavy metal-precipitating conditions (Ehrman *et al.*, 2008). The water of this site, a small spring-fed stream originating from a smelting waste dump, contained close to $3\,g\,L^{-1}$ of dissolved Zn, several $mg\,L^{-1}$ of other heavy metals and metalloids, and high levels of nitrate and sulfate (Krauss *et al.*, 2001; Ehrman *et al.*, 2008). The surface of exposed leaves quickly became covered with zincwoodwardite containing significant amounts of aluminum, sulfur, Cu and Zn (Ehrman *et al.*, 2008). The growth of hyphae completely encased in the precipitate continued without marked loss of biomass. It appears that some properties of natural leaves are necessary for colonization because artificial substrates were not colonized to the same extent (Ehrman *et al.*, 2008). It is possible, although unproven, that new filaments catalyze additional precipitation, and that fungal hyphae actively accelerate heavy metal precipitation. Regardless, the addition of inexpensive and environment-friendly leaves to contaminated water might be a valuable addition to chemical remediation methods.

More detailed studies of how aquatic fungi regulate adsorption of heavy metals may provide evidence as to the passive or active nature of this process. The interaction of fungi with toxic metals and xenobiotics in the habitat could be exploited to shield ecosystem functions and services, and to facilitate natural attenuation of pollutants (bioremediation).

Biosorption to cell walls

In terrestrial fungi, increased biosorption of heavy metals from soil has been attributed to the specific structure of the cell wall with chitin and chitosan as main constituents (Gadd, 2007). Ahrazem *et al.* (2001) identified glucose, galactose, mannose and glucuronic acid as the main components of the cell wall of *Sesquicillium* sp. (*Hypocreales*) and *Nectria cinnabarina*. These results were confirmed for two strains of *H. lugdunensis* (Teleomorph: *Nectria*) (G.-J. Krauss *et al.*, unpublished data), an aquatic hyphomycete closely related to *N. cinnabarina*. The cell wall has the capacity to bind metal ions at negatively charged sites such as glucuronic acid. External biosorption of cadmium (Cd) in *H. lugdunensis* was up to 10 times greater than intracellular content (Jaeckel *et al.*, 2005b; Braha *et al.*, 2007). Similar characteristics were shown for Cu. In contrast, intracellular Zn levels were higher than sorptively bound Zn (Jaeckel *et al.*, 2005b).

When exposed to metal ions, swollen hyphal cells were observed in aquatic hyphomycetes (Azevedo *et al.*, 2007; Braha *et al.*, 2007), similar to reports from the zygomycete *Mucor rouxii* for Cu (Gardea-Torresdey *et al.*, 1996) and from *Fusarium* sp. and *Alternaria tenuis* for Cd (Gharieb, 2001). The function of these induced morphological changes is unknown. The response, however, does not appear to be specific. Other abiotic stresses (e.g. low pH, potassium or oxygen deficiency) can result in similar morphological changes (Gharieb, 2001).

Plasma membrane identity

When metals are taken up, the plasma membranes of hyphal cells are primary targets for metal action. Uptake and subsequent sequestration of metals to various subcellular localizations require transition metal transporters. Similar to plants, fungi take up heavy metals from the environment by transport systems of varying specificity, located in the plasma membrane (Clemens, 2006; Krämer *et al*., 2007). *Saccharomyces cerevisiae* has been used as model organism to study the processes of metal homoestasis and resistance in fungi, which include transport processes (Van Ho *et al*., 2002). These transport systems through the plasma membrane could be regulated in aquatic fungi such that only small amounts of toxic metals are taken up. Two strains of *H. lugdunensis* showed different metal uptake capacity for Cd and Cu (Braha *et al*., 2007). Distinct regulated transport systems might be involved in Cd and Cu uptake of the two strains isolated from differently contaminated sites. In the freshwater fungi *Varicosporium elodeae* and *Heliscus submersus* (Azevedo *et al*., 2007), Cu (up to 150 μM) induced a more severe disruption of plasma membrane integrity than Zn (up to 200 μM). It can be hypothesized that the redox-active nature of Cu and its ability to generate free radicals promote lipid peroxidation in plasma membranes and interruption of transport processes.

Heavy metal ATPases (HMAs) specifically transporting heavy metals (e.g. Cu, Cd, Zn, Pb, Mn) have been found in organisms ranging from bacteria to humans. Performing a phylogenetic analysis of fungal HMA, a remarkable diversity was found (Saitoh *et al*., 2009). HMAs can be divided into groups A, B and C. Group A is predicted to deliver Cu ions to Cu-containing proteins, while groups B and C are thought to function as cell membrane Cu-efflux pumps. While groups B and C contain fungal-specific HMAs, group A consists of fungal orthologues that have been well conserved in eukaryotes (Saitoh *et al*., 2009). A genomic assay is needed to determine the occurrence and nature of HMAs in freshwater fungi.

Intracellular changes of macromolecular complexes

Heavy metals elicit a set of fundamental metabolic responses. Stress can lead to the interruption of central metabolic processes, in particular those performed by macromolecular complexes such as spliceosomes. One study reports the effects of stress on the mechanisms of pre-mRNA splicing in ascomycetes (Bond, 2006). Using the knowledge of gene organization and transcriptome analysis in the aquatic chytridiomycete *Blastocladiella emersonii*, the inhibitory effect of Cd on intron splicing was documented (Georg & Gomes, 2007; Georg *et al*., 2009). Because this fungus has proteins with Zn-related domains involved in pre-mRNA processing, Cd might inhibit splicing by displacing Zn ions in metalloproteins, including those present in the spliceosome system. Global gene expression analysis of *B. emersonii* cells exposed to Cd revealed 189 upregulated and 110 downregulated genes (Georg & Gomes, 2007). Four genes encoding for heat shock proteins (HSP) 10, 20, 90 and 100 were upregulated threefold upon exposure to Cd. HSPs are ubiquitous tools for organisms to cope with stress-induced denaturation of cellular protein. Upon Cd exposure, HSP 70 synthesis was induced in a time- and dose-dependent manner in the aquatic hyphomycete *H. lugdunensis* (Miersch & Grancharov, 2008).

Modulation of Reactive Oxygen Species (ROS)

Several studies have indicated that exposure of fungi to a diverse array of metals elicits oxidative stress (Penninckx, 2002; Pocsi *et al*., 2004; Krauss *et al*., 2008). ROS play important roles in the pathways of cellular redox homeostasis based on gene expression and gene product regulation. Low molecular antioxidants [e.g. ascorbate, glutathione (GSH)] participate in redox signalling in a complex manner. Metal-induced formation of ROS together with changes in the cellular redox state elicit signals leading to alterations in the cellular pattern of redox-mediating enzymes and their substrates and cofactors.

Cu is of utmost significance for fungi. The occurrence of two oxidation states Cu(I) and Cu(II) allows the metal to function as a reducing or oxidizing agent in numerous biochemical reactions. This property also makes Cu potentially toxic. Exposure to Cu(II) can catalyze the formation of ROS in some aquatic hyphomycetes (Azevedo *et al*., 2007). In *H. submersus* both superoxide dismutase and catalase activity were stimulated under Cu stress, and catalyse activity increased with increasing Cu(II) concentration. The magnitude of the increase in catalase activity was more pronounced in *H. submersus* than in *V. elodeae*, probably contributing to the higher tolerance of *H. submersus* to Cu and to its ability to survive in metal-polluted streams, such as the site where the fungus was isolated (Azevedo *et al*., 2007). In *H. lugdunensis*, Cu diminished the activity of glutathione reductase (Braha *et al*., 2007). One reason for decreased glutathione reductase could be a lack of redox equivalents such as NADPH. This effect might explain the inhibition of glucose-6-phosphate dehydrogenase after Cu exposure, limiting the essential production of NADPH as shown for *V. elodeae, H. submersus* (Azevedo *et al*., 2007) and *H. lugdunensis* (Braha *et al*., 2007).

In contrast, metals without redox-active properties (e.g. Cd and Zn) induce oxidative stress via indirect mechanisms such as interacting with the antioxidant defense system, for example by influencing the GSH pool. However, Cd, which is similar to Zn in many aspects, generally causes toxic effects in fungi (Pocsi *et al*., 2004; Baudouin-Cornu & Labarre, 2006; Krauss *et al*., 2008). The level of GSH in *H.*

lugdunensis was strain specific (Miersch *et al.*, 1997, 2001; Jaeckel *et al.*, 2005b; Braha *et al.*, 2007). Under Cd exposure, GSH level increased drastically in two *H. lugdunensis* strains (Braha *et al.*, 2007). The increased GSH to GSSG ratio suggests an increase in the GSH levels without drastic changes in GSSG concentrations. *Varicosporium elodeae* and *T. marchalianum* responded in a similar manner, while Cu exposure had no effect (Miersch *et al.*, 1997, 2005). An increased production of thiol peptides in different freshwater fungi was found under exposure to Cd (Miersch *et al.*, 1997, 2005; Braha *et al.*, 2007; Guimarães-Soares *et al.*, 2007) and Zn/Cu (Guimarães-Soares *et al.*, 2007). In the zygomycete *Mucor racemosus*, Cd caused a decrease in GSH, but induced GSH-derived phytochelatins (Miersch *et al.*, 2001).With Cd exposure, peroxidase activity increased in two *H. lugdunensis* strains, while dehydroascorbate reductase and glucose-6-phosphate dehydrogenase declined progressively (Braha *et al.*, 2007). Exposure to Zn did not increase the GSH level (Jaeckel *et al.*, 2005b). As shown for Zn, catalase activity was stimulated in both *H. submersus* and *V. elodeae* (Azevedo *et al.*, 2007). The responses of superoxide dismutase in *H. submersus* and of catalase in *V. elodeae* were stronger when exposed to Cu/Zn mixtures than to Cu alone, suggesting a synergistic effect of metal mixtures (Azevedo *et al.*, 2007).

Azevedo *et al.* (2009) described a link between metal-induced oxidative stress and the appearance of programmed cell death in freshwater fungi. Programmed cell death destroys cells that represent a threat to the organism as a whole. It is mediated by a complex intracellular program in freshwater fungi (Azevedo *et al.*, 2009); both Cu and Zn exposure induce apoptotic events. The fungi most tolerant to Zn (*V. elodeae*) or Cu (*H. submersus*) exhibited higher levels of programmed cell death markers, suggesting that programmed cell death processes might be linked to fungal resistance/tolerance to metal stress. Exposure of *V. elodeae* to Cu promoted nuclear morphological alterations, caspase-like activity and DNA strand breaks, but did not lead to detectable intracellular ROS accumulation. Cu exposure of *Flagellospora curta* did not result in DNA strand breaks. No enhanced caspase-like activity was measured after Zn exposure of *F. curta* mycelia. The results reflect a high plasticity of the programmed cell death pathways allowing the sacrifice of certain hyphal cells for the benefit of the mycelium as a whole (Richie *et al.*, 2007). The occurrence of programmed cell death in heavy metal-exposed freshwater fungi might be important for their acclimation in metal-polluted waters.

Thiol peptide stress response

Sulfate assimilation and Cd response in fungi are metabolically connected (Vido *et al.*, 2001; Bae & Chen, 2004; Mendoza-Cozatl *et al.*, 2005; Wysocki & Tamás, 2010). Like plants, fungi synthesize GSH from inorganic sulfate via reductive sulfate assimilation (Mendoza-Cozatl *et al.*, 2005; Fig. 3). In *H. lugdunensis*, Cd causes an upregulation of mRNA for sulfite reductase (P. Nathan *et al.*, unpublished data). Georg & Gomes (2007) described the upregulation of genes responsible for cysteine as well as GSH synthesis in response to Cd stress in the aquatic chytridiomycete *B. emersonii*. Cysteine, the final product of sulfate assimilation is metabolized to GSH in two consecutive ATP-dependent reactions via γ-glutamylcysteine (Mendoza-Cozatl *et al.*, 2005). The levels of both GSH precursors increased under Cd exposure in *H. lugdunensis* (G.-J. Krauss *et al.*, unpublished data).

Regarding enhanced synthesis of reduced GSH in Cd-stressed *H. lugdunensis*, Braha *et al.* (2007) hypothesized that a substantial proportion of this GSH pool was removed to detoxify the metal by intracellular chelation to bis(glutathionato)Cd complexes ($CdGS_2$). In *S. cerevisiae* and *S. pombe*, Cd detoxification depends on binding by glutathione as $CdGS_2$ and the sequestration of these complexes into the vacuole mediated by the tonoplast-located ABC transporter ScYCF1 (Li *et al.*, 1997), SpHTM1 (Preveral *et al.*, 2009) and Abc2 (Mendoza-Cozatl *et al.*, 2010, Fig. 3). Such translocation may contribute to the depletion of cytosolic GSH pools causing changes in cellular redox homeostasis. No $CdGS_2$ complexes were found in vacuoles of aquatic hyphomycetes. However, in *T. marchalianum,* Cd was present in vacuolar polyphosphate precipitates (Miersch *et al.*, 2005; Fig. 3).

Phytochelatins differing in length were first isolated from fungi and described as cadystins (Kondo *et al.*, 1984; Murasugi, 2008). They are GSH-derived peptides of the general structure $(\gamma\text{-Glu-Cys})_{2-6}$Gly (Wesenberg *et al.*, 2010) and are synthesized by phytochelatin synthase (PCS; Clemens & Persoh, 2009). However, the synthesis of metal-binding PC is not tied to PCS activity. The fungi *S. cerevisiae* and *Neurospora crassa* synthesize only phytochelatin2 (PC2) upon exposure to metal ions (Kneer *et al.*, 1992). *Saccharomyces cerevisiae* lacks a PCS homologue. In this yeast, vacuolar serine carboxypeptidases are responsible for the synthesis of PC2 (Wünschmann *et al.*, 2007). In *H. lugdunensis*, 100 μM Cd increased the GSH content and induced the synthesis of one phytochelatin (PC2, Jaeckel *et al.*, 2005a). Because *H. lugdunensis* shows (1) a Cd-induced PC2 synthesis (Jaeckel *et al.*, 2005a) and (2) does not have a PCS gene (D. Meissner *et al.* unpublished data), carboxypeptidases might catalyze the reaction in this fungus (Fig. 3). Phytochelatins in other Cd-stressed freshwater hyphomycetes have been postulated, but not identified (Guimarães-Soares *et al.*, 2006). In the zygomycete *M. racemosus*, Cd caused an increase of the thiol pool with a simultaneous decrease of glutathione and induction of two phytochelatins (PC2, PC3), whereas Cu had no effect (Miersch *et al.*, 2001). In contrast to *S. pombe* and *S. cerevisiae* (Kneer *et al.*, 1992;

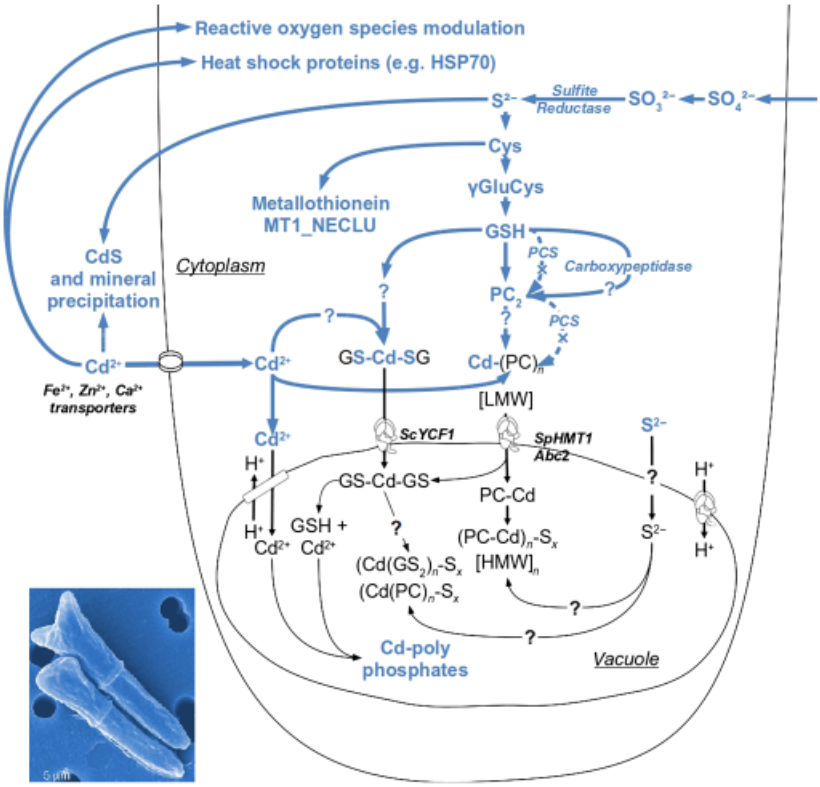

Fig. 3. Cd(II) stress response in a hyphal tip of aquatic hyphomycetes. Blue (bold): results based on *Heliscus lugdunensis* (Jaeckel *et al.*, 2005a; Braha *et al.*, 2007; Miersch & Grancharov, 2008) and *Tetracladium marchalianum* (Miersch *et al.*, 2005). Black: supplemental results from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* (Ernst *et al.*, 2008; Saitoh *et al.*, 2009; Mendoza-Cozatl *et al.*, 2010). Insert: spores of *H. lugdunensis*. PCn, phytochelatin $n > 2$; ScYCF1, *S. cerevisiae* yeast Cd factor 1; SpHMT1, *S. pombe* yeast heavy metal transporter; Abc2, *S. pombe* ABC-type phytochelatin cadmium transporter.

Ow *et al.*, 1994; Murasugi, 2008), neither intracellular PC–metal complexes nor Cd-GS_2- or phytochelatin-encapped CdS crystallites have so far been detected in freshwater fungi.

Cd exposure at a concentration of 50 μM raised cellular soluble sulfide levels in *T. marchalianum* strains between three- and sevenfold, while Cu and Zn had no effect (Miersch *et al.*, 2005). Sulfide was not detectable in the culture (Miersch *et al.*, 2005). Probably due to the upregulation of sulfite reductase in *H. lugdunensis*, high concentrations of soluble sulfide were found in Cd-exposed cells (Nathan *et al.*, unpublished data). *Heliscus lugdunensis* seems to be able to detoxify Cd extracellularly by the formation of CdS crystallites, probably by initiation of a matrix-mediated mineralization process (J. Miersch *et al.*, unpublished data, Fig. 3).

Heliscus lugdunensis seems to be the first organism described that under Cd stress synthesized a novel small metallothionein (MT) MT1-NECLU together with a PC (Jaeckel *et al.*, 2005a, Fig. 3). This was accompanied by increased GSH levels (Jaeckel *et al.*, 2005a). Metallothioneins are gene-encoded, Cys-rich metal-binding proteins of low molecular mass. They have been found in fungi and representatives of other kingdoms (Blindauer & Leszczyszyn, 2010). Metallothioneins chelate heavy metals through clusters of thiolate bonds. Fungal metallothioneins are also involved in other intracellular reactions, for example redox regulation, and developmental processes (Singh & Ashby, 1999; Clemens & Simm, 2003; Tucker *et al.*, 2004). Generally, fungal metallothioneins are induced by Cu. Thus, the ascomycete *N. crassa* synthesizes a small metallothionein (Lerch, 1991; Cobine *et al.*, 2004; Kumar *et al.*, 2005), which belongs to family 8 of the metallothionein classes (http://www.expasy.ch/cgi-bin/lists?metallo.txt). Small Cu-binding cysteine-rich (30%) metallothioneins were isolated from the ectomycorrhizal fungi *Laccaria laccata* and *Paxillus involutus*, and characterized by their molecular masses and spectroscopic features (Howe *et al.*, 1997). In addition to Cu, Cd induced the expression of a metallothionein gene in the fungus *Colletotrichum gloeosporioides* (Hwang & Kolattukudy, 1995). *Candida glabrata* produces a metallothionein in response to high concentrations of Cu, but under Cd exposition it mainly synthesizes phytochelatins (Mehra *et al.*, 1988, 1989).

The synthesis of MT1_NECLU (molecular mass 2330 Da) from *H. lugdunensis* can be induced by Cd (Jaeckel *et al.*, 2005a), but not by other heavy metals (G.-J. Krauss *et al.*, unpublished data). Due to similarities in amino acid sequence of other small metallothioneins from the ascomycete *N. crassa* (Münger *et al.*, 1985) and the basidiomycete *Agaricus bisporus* (Münger & Lerch, 1985), the novel metallothionein can be categorized as a member of family 8 of the metallothionein classes (Jaeckel *et al.*, 2005a). The Cd metallated metallothionein has a loop-like structure

(Meissner *et al.*, unpublished data). In another *H. lugdunensis* strain isolated from a highly polluted habitat, Cd induced another small metallothionein, which slightly differs from MT1_NECLU (Meissner *et al.*, unpublished data). This could indicate that the two *H. lugdunensis* strains represent distinct ecotypes, which is supported by other metabolic differences (Braha *et al.*, 2007). Due to the lack of structure identification, the proposed occurrence of high molecular metallothioneins in metal-exposed freshwater fungi remains speculative (Guimarães-Soares *et al.*, 2006; Miersch & Grancharov, 2008).

Despite growing knowledge regarding responses to high amounts of essential metals or to toxic metals and their detoxification, information on metal sensing, intracellular signal transduction and metabolic changes in fungal cells is limited. More details are needed for understanding the impact of these compounds on the metabolic network (Wesenberg *et al.*, 2010). Biochemical single-cell analysis performed by laser microdissection after harvesting the cells might allow the description of the initial metabolic impact of metals on hyphal tips, but needs a chamber allowing the growth of single filaments and cutting hyphal cells in a standardized manner (B. Leyh *et al.*, unpublished data).

Effects of xenoorganics on freshwater fungi

Several studies have addressed the effects of organic pollutants on aquatic hyphomycetes in the field and the laboratory, with pesticides being most frequently investigated among pollutants with defined chemical structures. Here, we briefly present only effects of major relevance because they have been reviewed and summarized previously (Hodkinson, 1976; Bärlocher, 1992a; Sridhar & Raviraja, 2001; Krauss *et al.*, 2003a).

In field studies, organic contaminants have often, but not always, been implicated in a decline in species richness of aquatic fungi, accompanied by decreased leaf decay rates (reviewed by Bärlocher, 1992a; Sridhar & Raviraja, 2001; Krauss *et al.*, 2003a). However, the loss of fungal diversity is not necessarily related to a loss of ecological functions. Leaf decay was not significantly affected in an Indian stream polluted with organics, whereas the number of aquatic hyphomycetes species was reduced by 80% (Raviraja *et al.*, 1998). In laboratory studies, low concentrations of the insecticide 1,1,1-trichloro-2,2-bis(4′-chlorophenyl)ethane (DDT) enhanced the growth of the aquatic hyphomycetes *H. submersus*, *Tetracladium setigerum*, *V. elodeae* and *C. aquatica*, as well as that of the oomycetes *Isoachlya* sp., *Isoachlya monilifera*, *Saprolegnia* sp. and *Pythium* sp. Similarly, the growth of the terrestrial fungi *Aureobasidium pullulans*, *Cephalosporium acremonium*, *Cladosporium cladosporioides* and *Cylindrocarpon orthosporium*, which were isolated from aquatic habitats, was affected (summarized by Hodkinson, 1976). Furthermore, DDT was found to increase the respiratory quotients of various sugars in *H. submersus*, indicating that the insecticide inhibits aerobic oxidation of sugars and shifts the catabolism to anaerobic fermentation (Hodkinson, 1976). Like terrestrial species, aquatic hyphomycetes accumulate DDT (Hodkinson, 1976). Pentachlorophenol was also reported to accumulate in mycelia of aquatic hyphomycetes. Fungal reproduction and conidium production of aquatic hyphomycetes declined upon pentachlorophenol exposure (reviewed by Sridhar & Raviraja, 2001).

Distinctive features of fungal organopollutant metabolism and metabolization of organic xenobiotics by freshwater fungi

Physiological and biochemical interactions of fungi from freshwater environments with organic environmental pollutants have received considerably less attention than those of terrestrial fungi. There are few studies on the ability of aquatic hyphomycetes and other freshwater fungi to metabolize organic xenobiotics, and very little is known about the enzymes involved in such processes. However, there is no reason to assume that aquatic fungi differ fundamentally from terrestrial species in terms of their general responses to environmental pollutants. Fungal metabolization of hydrocarbon pollutants is predominantly aerobic. A limited number of simple aromatic and aliphatic hydrocarbons such as *n*-alkanes, *n*-alkylbenzenes, aliphatic ketones, ethylbenzene, styrene and toluene can be used as sole sources of carbon and energy for growth of filamentous fungi (Gerasimova *et al.*, 1975; Murphy & Perry, 1984; Fedorak & Westlake, 1986; Weber *et al.*, 1995; Qi & Moe, 2002) and yeasts (Jigami *et al.*, 1974; Cox *et al.*, 1993, 1996; Craft *et al.*, 2003). Mitosporic fungi (Hofrichter & Scheibner, 1993; Jones *et al.*, 1993, 1994; García-Peña *et al.*, 2005) and yeasts (Middelhoven & Spaaij, 1997; Middelhoven *et al.*, 2004) are able to use phenol, *o*-cresol, *m*-cresol, *p*-cresol and 4-ethylphenol as growth substrates.

However, apart from the aforementioned examples and in contrast to bacteria, fungi cometabolize the majority of aromatic environmental pollutants, resulting in the formation of organic biotransformation products or in mineralization to CO_2 (Prenafeta-Boldú *et al.*, 2006) (Fig. 4). The initial intracellular oxidative attack on organic pollutants commonly involves hydroxlyation, resulting from the activation of dioxygen and the insertion of one oxygen atom into the substrate. Cytochrome P450 monooxygenase systems are frequently responsible for such reactions in both ligninolytic and nonligninolytic terrestrial fungi (Cerniglia & Sutherland, 2001; Matsuzaki & Wariishi, 2004; Hiratsuka *et al.*, 2005; Prenafeta-Boldú *et al.*, 2006; Yadav *et al.*, 2006) (Fig. 4). Evidence for the involvement of cytochrome P450

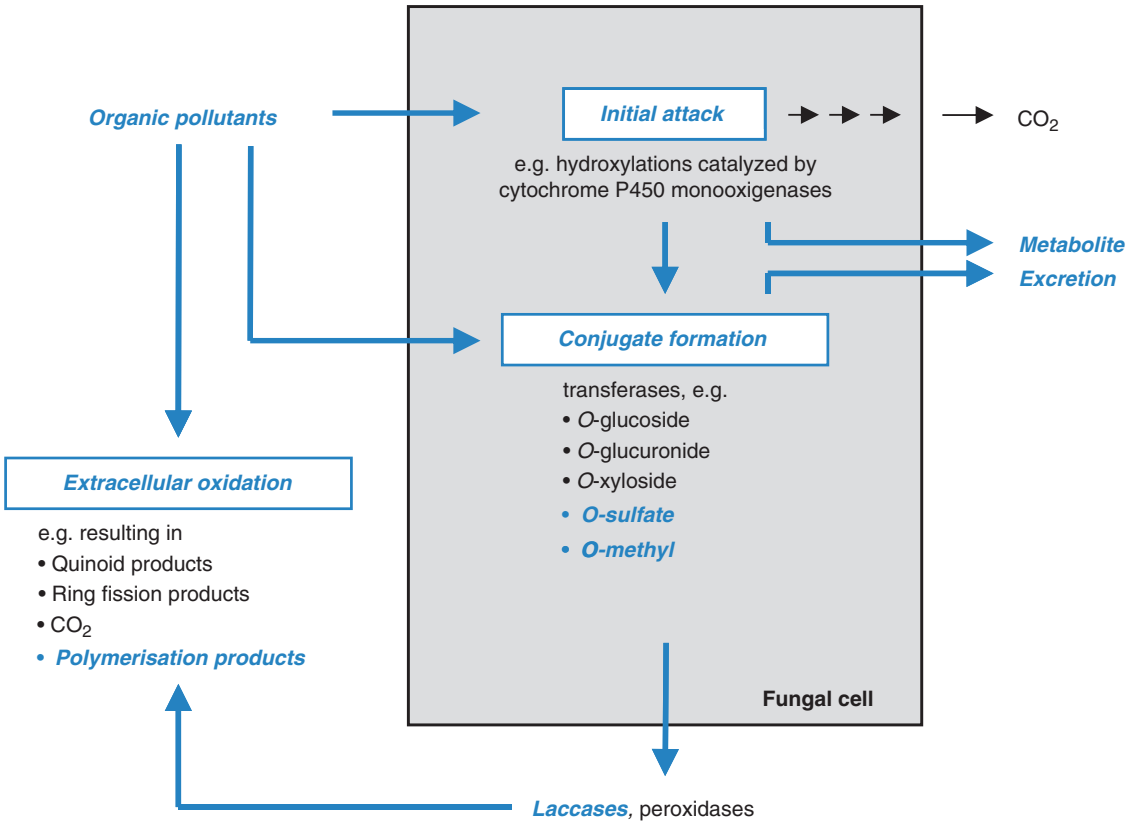

Fig. 4. Major reactions and enzymes involved in fungal metabolism of xenobiotics. Reactions and enzymes described for freshwater fungi according to Augustin *et al*. (2006), Junghanns *et al*. (2005) and Martin *et al*. (2007, 2009) are highlighted in blue and italics. Figure modified from Schlosser *et al*. (2008).

monooxygenases in the metabolism of xenobiotics by freshwater fungi has not yet been provided. Nevertheless, cytochrome P450 reactions can be inferred from hydroxylated metabolites produced during intracellular biotransformation of environmentally relevant micropollutants such as the endocrine-disrupting chemical t-NP (Corvini *et al*., 2006) and the polycyclic musk compounds HHCB and AHTN (Martin *et al*., 2007) by aquatic hyphomycetes and other mitosporic freshwater isolates (Fig. 4). Primary fungal pollutant oxidation is often followed by additional oxidation steps, leading to the excretion of biotransformation products at various stages of oxidation. Intracellular t-NP metabolization by the aquatic hyphomycete *C. aquatica*, a species frequently observed in rivers and streams (Baldy *et al*., 2002; Nikolcheva *et al*., 2003), and another mitosporic freshwater isolate involved oxidation of the differently branched side chains of the nonylphenol isomers contained in t-NP, whereas their phenolic moieties remained unchanged (Junghanns *et al*., 2005; Martin *et al*., 2009). Metabolites of t-NP detected in *C. aquatica* indicated nonyl chain hydroxylation, further oxidation into keto or aldehyde compounds and the subsequent formation of carboxylic acid derivatives (Martin *et al*., 2009).

Pollutant attack by aquatic hyphomycetes via reductive reactions is suggested by the reported formation of the metabolite 1,1-dichloro-2,2-bis(4′-chlorophenyl)ethane (DDD) from DDT. Within 330 h, *H. submersus* converted DDT into DDD at 27% and into 1,1-dichloro-2,2-bis(4′-chlorophenyl)ethylene (DDE) at 8%, whereas *C. aquatica* transformed 6% of DDT into these metabolites (Hodkinson, 1976). Reductive dechlorination is well known from anaerobic bacteria, but has also been reported for terrestrial fungi (Reddy & Gold, 1999).

Phase II reactions such as methylation or conjugate formation with glucose, xylose, glucuronic acid or sulfate involve polar functional groups of pollutant metabolites or of the pollutants themselves, leading to the excretion of products that are more water-soluble than their parent compounds and usually not amenable to further metabolization (Cerniglia *et al*., 1992; Cerniglia & Sutherland, 2001; Gadd, 2004; Zhang *et al*., 2004; Prenafeta-Boldú *et al*., 2006) (Fig. 4). Conjugate formation is well known from the metabolization of pollutants by terrestrial fungi (Reddy *et al*., 1997; Hundt *et al*., 2000; Cerniglia & Sutherland, 2001; Hammer *et al*., 2001; Gesell *et al*., 2004), but has also been reported for aquatic hyphomycetes (Augustin *et al*.,

2006; Martin *et al.*, 2007, 2009), suggesting a general role in fungal detoxification through the excretion of water-soluble metabolites (Fig. 4). The aquatic hyphomycete *H. lugdunensis* transformed the pesticide and polycylic aromatic hydrocarbon (PAH) metabolite 1-naphthol to 1,4-naphthoquinone, 1-methoxynaphthalene and 1-naphthylsulfate, indicating the involvement of intracellular phase II enzymes such as methyl- and sulfotransferases (Augustin *et al.*, 2006). *Heliscus lugdunensis* was consistently found among the top-ranked fungal species identified at different polluted sites related to Cu shale mining (Krauss *et al.*, 2001; Sridhar *et al.*, 2005), which suggests a role of aquatic hyphomycetes in metabolizing polyaromatic hydrocarbon byproducts of Cu shale smelting processes. The formation of methyl conjugates from t-NP and HHCB biotransformation metabolites was implicated in *C. aquatica* (Martin *et al.*, 2007, 2009).

Terrestrial wood- and soil litter-decomposing white-rot basidiomycetes possess an outstanding potential for mineralizing many xenobiotic compounds to CO_2. This is attributed to a complex and nonspecific cometabolic degradation mechanism also active during lignin degradation (Bumpus *et al.*, 1985; Field *et al.*, 1993; Hammel, 1995; Reddy, 1995; Sack *et al.*, 1997; Scheibner *et al.*, 1997; Mester & Tien, 2000; Cerniglia & Sutherland, 2001; Steffen *et al.*, 2002). Extracellular radical-generating enzymes such as laccases (EC 1.10.3.2) and certain lignin-modifying peroxidases are involved in lignin degradation (Hofrichter, 2002; Martínez, 2002; Baldrian, 2006; Hammel & Cullen, 2008; Ruiz-Duenas *et al.*, 2009; Giardina *et al.*, 2010), but also oxidatively attack many xenobiotics, for example PAHs (Johannes & Majcherczyk, 2000; Hofrichter, 2002), chlorophenols (Hofrichter *et al.*, 1998; Hofrichter, 2002), nitroaromatic compounds (Hofrichter *et al.*, 1998; Hofrichter, 2002), dyes (Heinfling *et al.*, 1998; Mayer & Staples, 2002; Wesenberg *et al.*, 2003; Camarero *et al.*, 2005), herbicides, pesticides (Mayer & Staples, 2002; Davila-Vazquez *et al.*, 2005) and endocrine disruptors (Tsutsumi *et al.*, 2001; Dubroca *et al.*, 2005; Cabana *et al.*, 2007). Manganese peroxidase (EC 1.11.1.13) can even cleave aromatic rings and catalyze the mineralization of xenobiotics to CO_2 in the absence of cells (Hofrichter *et al.*, 1998; Hofrichter, 2002). Concerning the occurrence of lignin-modifying enzymes in fungi from aquatic environments, laccases have repeatedly been demonstrated in aquatic hyphomycetes and other mitosporic freshwater isolates (Abdel-Raheem & Ali, 2004; Junghanns *et al.*, 2005, 2009; Martin *et al.*, 2007, 2009). Sound evidence for lignin-modifying peroxidases in aquatic fungi at this point is limited to marine fungi (Raghukumar *et al.*, 1999, 2008). Besides intracellular metabolization of t-NP, the biotransformation of this compound by *C. aquatica* and another freshwater ascomycete anamorph involved concomitant extracellular t-NP oxidation by laccase, leading to the formation of oxidative coupling products in the range of di- to pentamers (Junghanns *et al.*, 2005; Martin *et al.*, 2009) (Fig. 4). In *C. aquatica*, evidence for a nonspecific oxidation of the various nonylphenol isomers contained in t-NP by laccase was provided. Such reactions contrast with the selective degradation of t-NP isomers by bacteria and could prevent the enrichment of highly estrogenic isomers in remaining t-NP. In contrast to laccase reactions, intracellular fungal biotransformation was much more isomer-specific and caused a significant shift in the isomeric composition of remaining t-NP. As a result, certain t-NP constituents related to more estrogenic isomers were less efficiently degraded than others (Martin *et al.*, 2009). Likewise, the polycyclic musk fragrances HHCB and AHTN were oxidized by extracellular laccases from aquatic hyphomycetes and mitosporic freshwater isolates, leading to the identification of the environmental metabolite HHCB-lactone as a product of HHCB laccase oxidation (Martin *et al.*, 2007). As for t-NP, these results suggest the involvement of both extra- (laccase) and intracellular reactions in the biotransformation of HHCB and AHTN by freshwater fungi (Martin *et al.*, 2007) (Fig. 4). More recently, aquatic hyphomycetes and other mitosporic freshwater isolates were shown to metabolize several anthraquinone and azo dyes typical of textile industry effluents (Junghanns *et al.*, 2008). The demonstrated ability of laccases from freshwater ascomycete anamorphs to oxidize synthetic dyes (Junghanns *et al.*, 2009) may indicate their involvement in dye biotransformation.

Evidence for the mineralization of xenobiotic organic pollutants in freshwater fungi has not yet been provided (Fig. 4). The further metabolic fates of metabolites formed from various xenobiotics by aquatic hyphomycetes and other mitosporic freshwater isolates (Hodkinson, 1976; Augustin *et al.*, 2006; Martin *et al.*, 2007, 2009; Junghanns *et al.*, 2008) remain open.

The demonstrated capability of terrestrial species (Bumpus *et al.*, 1985; Reddy, 1995; Pointing, 2001) and of freshwater isolates (Junghanns *et al.*, 2005, 2008; Martin *et al.*, 2007) to metabolize representatives of more than one particular chemical class of xenobiotics considerably differs from bacterial degradation, which generally seems to be much more species- and compound-specific. In addition to aquatic hyphomycetes occurring predominantly in streams, ubiquitous mitosporic fungi and zygomycetes such as *Penicillium*, *Aspergillus*, *Fusarium*, *Paecilomyces*, *Mucor* and *Rhizopus* spp., as well as yeasts, can be found in freshwater environments (Niewolak, 1975; Tóthová, 1999; Amaral Zettler *et al.*, 2002). Members of these genera degrade several organic environmental pollutants such as PAHs (Cerniglia & Sutherland, 2001; Verdin *et al.*, 2004), fungicides (Gesell *et al.*, 2001) and chlorophenols (Hofrichter *et al.*, 1994), suggesting that they may also contribute to the degradation of other organic contaminants in aquatic environments.

Outlook

The broad concepts of the taxonomy and ecology of aquatic hyphomycetes were worked out several decades ago, and have largely been confirmed, even though many details remain to be filled in. Major new insights concerning their phylogeny and biochemistry, however, can be expected from the increased use of molecular methods (Bärlocher, 2010). These new approaches have already revealed the consistent presence of other fungal groups and are shedding light on how aquatic hypomycetes and other microorganisms deal with heavy metals and xenoorganics. The responses of this vital group of stream organisms to these and other anthropogenic stresses is one of the most pressing concerns today. A first step may be identifying species or communities, whose presence/absence indicates a specified stress. Solé *et al*. (2008b) studied aquatic hyphomycete communities in rivers and aquifers in the Mansfeld region (Central Germany), which has long been the site of intensive mining and numerous chemical industries. Eleven sites were investigated, with between 1 and 13 species of aquatic hyphomycetes (which is considerably lower than what would be expected in pristine streams). Within this set, stress factors such as heavy metals, sulfate, nitrate and low oxygen concentrations were connected to reduced fungal diversity and biomass. Redundancy analysis indicated that 86.2% of the changes in fungal community structure was due to variations in water chemistry. The observed species could be divided into two groups based on their correlation with DOC/TOC (dissolved organic carbon/total organic carbon), Cl^- and E_h. The first group (*T. marchalianum*, *T. setigerum*, *Cylindrocarpon* sp., *Tricladium angulatum*, *Lemonniera centrosphaera*, *Alatospora acuminata* and *Alatospora flagellata*) were not correlated, while the second group (*Anguillospora longissima*, *Clavatospora longibrachiata*, *C. aquatica*, *Flagellospora curvula*, *H. lugdunensis*, *Tumularia aquatica* and *Lemonniera aquatica*) were strongly negatively correlated to these parameters. Clearly, more analyses incorporating other regions are required to confirm the consistency of these associations. The study nevertheless points to the potential use of aquatic hyphomycete communities as bioindicators of anthropogenic stresses. This approach will be useful, but it does not provide much insight into mechanisms that allow certain strains or species to persist, maybe even flourish, in the face of severe anthropogenic stress. It is unlikely that they are based on 'core' enzymatic properties, such as the ability to use plant polysaccharides, which seems to be nearly universal among aquatic hyphomycetes (Chamier, 1985). More likely they are based on 'keystone' genes (Fig. 1) that allow, for example, sequestration and detoxification of heavy metals or other toxins. If this is the case, screening aquatic fungal communities for such genes (PCR with specific primers) or their products (RT-PCR to produce mRNA, or proteome analyses) may facilitate predictions of their resilience when challenged by selected anthropogenic stressors. Of these, factors associated with climate change have attracted a huge amount of interest. A commonly expected response of affected species is a migration toward greater altitude and latitude. Ingold once claimed that if he were 'transported to a part of the world without knowledge of its locality', he could 'make a very rough guess as to its latitude by examining the spora of a suitable stream' (Money, 2010). If the aquatic hyphomycete species in a given region respond in a similar manner, spore samples would provide an easy and cheap approach to evaluate the progress of climate change and its impact on stream communities.

References

Abdel-Raheem A & Ali E (2004) Lignocellulolytic enzyme production by aquatic hyphomycetes species isolated from the Nile's delta region. *Mycopathologia* **157**: 277–286.

Abdullah SK & Taj-Aldeen SJ (1989) Extracellular enzymatic activity of aquatic and aero-aquatic conidial fungi. *Hydrobiologia* **174**: 217–223.

Abel TH & Bärlocher F (1984) Effects of cadmium on aquatic hyphomycetes. *Appl Environ Microb* **48**: 245–251.

Abelho M (2009) ATP and ergosterol as indicators of fungal biomass during leaf decomposition in streams: a comparative study. *Int Rev Hydrobiol* **94**: 3–15.

Acuña V & Tockner K (2010) The effects of alterations in temperature and flow regime on organic carbon dynamics in Mediterranean river networks. *Glob Change Biol* **16**: 2638–2650.

Ahrazem O, Prieto A, Gómez-Miranda B, Bernabé M & Leal JA (2001) Comparison of cell-wall polysaccharides from *Nectria cinnabarina* with those from the group of *Nectria* with *Sesquicillium anamorphs*. *Microbiology* **147**: 1839–1849.

Ainsworth GC (1976) *Introduction to the History of Mycology*. 1st edn. Cambridge University Press, Cambridge, UK.

Allan JD & Castillo MM (2007) *Stream Ecology: Structure and Function of Running Waters*. Springer, Berlin.

Amaral Zettler LA, Gomez F, Zettler E, Keenan BG, Amils R & Sogin ML (2002) Eukaryotic diversity in Spain's River of Fire. *Nature* **417**: 137.

Armougom F & Raoult D (2008) Use of pyrosequencing and DNA barcodes to monitor variations in Firmicutes and Bacteroidetes communities in the gut microbiota of obese humans. *BMC Genomics* **9**: 576.

Arsuffi TL & Suberkropp K (1985) Selective feeding by stream caddisfly (Trichoptera) detritivores on leaves with fungal-colonized patches. *Oikos* **45**: 50–58.

Augustin T, Schlosser D, Baumbach R, Schmidt J, Grancharov K, Krauss G & Krauss G-J (2006) Biotransformation of 1-naphthol by a strictly aquatic fungus. *Curr Microbiol* **52**: 216–220.

Azevedo M, Carvalho A, Pascoal C, Rodrigues F & Cássio F (2007) Responses of antioxidant defenses to Cu and Zn stress in two aquatic fungi. *Sci Total Environ* **377**: 233–243.

Azevedo M, Almeida B, Ludovico P & Cássio F (2009) Metal stress induces programmed cell death in aquatic fungi. *Aquat Toxicol* **92**: 264–270.

Bae W & Chen X (2004) Proteomic study for the cellular responses to Cd^{2+} in *Schizosaccharomyces pombe* through amino acid-coded mass tagging and liquid chromatography tandem mass spectrometry. *Mol Cell Proteomics* **3**: 596–607.

Baldrian P (2006) Fungal laccases – occurrence and properties. *FEMS Microbiol Rev* **30**: 215–242.

Baldy V & Gessner MO (1997) Towards a budget of leaf litter decomposition in a first-order woodland stream. *CR Acad Sci III Vie* **320**: 747–758.

Baldy V, Chauvet E, Charcosset J & Gessner MO (2002) Microbial dynamics associated with leaves decomposing in the mainstem and floodplain pond of a large river. *Aquat Microb Ecol* **28**: 25–36.

Bärlocher F (1981) Fungi on the food and in the faeces of *Gammarus pulex*. *T Brit Mycol Soc* **76**: 160–165.

Bärlocher F (1982) The contribution of fungal enzymes to the digestion of leaves by *Gammarus fossarum* Koch (Amphipoda). *Oecologia* **52**: 1–4.

Bärlocher F (1985) The role of fungi in the nutrition of stream invertebrates. *Bot J Linn Soc* **91**: 83–94.

Bärlocher F (1987) Aquatic hyphomycete spora in 10 streams of New Brunswick and Nova Scotia. *Can J Bot* **65**: 76–79.

Bärlocher F (1992a) Research on aquatic hyphomycetes: historical background and overview. *The Ecology of Aquatic Hyphomycetes* (Bärlocher F, ed), pp. 1–15. Springer-Verlag GmbH, New York, NY.

Bärlocher F (1992b) Community organization. *The Ecology of Aquatic Hyphomycetes* (Bärlocher F, ed), pp 38–76. Springer-Verlag, Berlin.

Bärlocher F (2005) Freshwater fungal communities. *The Fungal Community: Its Organization and Role in the Ecosystem*. 3rd edn (Dighton J, White JF Jr & Oudemans P, eds), pp. 39–59. CRC Press, Boca Raton, FL.

Bärlocher F (2007) Fungal community structure and decomposition in aquatic environments. *Manual of Environmental Microbiology* (Hurst CJ, Crawford RL, Garland JL, Lipson DA & Mills AL, eds), pp. 469–478. ASM Press, Washington, DC.

Bärlocher F (2010) Molecular approaches promise a deeper and broader understanding of the evolutionary ecology of aquatic hyphomycetes. *J N Am Benthol Soc* **29**: 1027–1041.

Bärlocher F & Brendelberger H (2004) Clearance of aquatic hyphomycete spores by a benthic suspension feeder. *Limnol Oceanogr* **49**: 2292–2296.

Bärlocher F & Corkum M (2003) Nutrient enrichment overwhelms diversity effects in leaf decomposition by stream fungi. *Oikos* **101**: 247–252.

Bärlocher F & Graça MAS (2002) Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. *Freshwater Biol* **47**: 1123–1135.

Bärlocher F & Kendrick B (1973) Fungi and food preferences of *Gammarus pseudolimnaeus*. *Arch Hydrobiol* **72**: 501–516.

Bärlocher F & Kendrick B (1974) Dynamics of the fungal population on leaves in a stream. *J Ecol* **62**: 761–791.

Bärlocher F & Peterson RCJ (1988) Aquatic hyphomycetes in an acidified and limed umic stream in Sweden. *Verh Internat Verein Limnol* **23**: 1279–1286.

Bärlocher F & Rosset J (1981) Aquatic hyphomycete spora of two Black Forest and two Swiss Jura streams. *T Brit Mycol Soc* **76**: 479–483.

Bärlocher F, Nikolcheva LG, Wilson KP & Williams DD (2006) Fungi in the hyporheos of a springbrook. *Microb Ecol* **52**: 708–715.

Bärlocher F, Seena S, Wilson K & Williams DD (2008) Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. *Freshwater Biol* **53**: 368–379.

Baschien C, Marvanová L & Szewzyk U (2006) Phylogeny of selected aquatic hyphomycetes based on morphological and molecular data. *Nova Hedwigia* **83**: 311–352.

Baschien C, Manz W, Neu TR, Marvanova L & Szewzyk U (2008) *In situ* detection of freshwater fungi in an alpine stream by new taxon-specific fluorescence *in situ* hybridization probes. *Appl Environ Microb* **74**: 6427–6436.

Baudoin J, Guérold F, Felten V, Chauvet E, Wagner P & Rousselle P (2008) Elevated aluminium concentration in acidified headwater streams lowers aquatic hyphomycete diversity and impairs leaf-litter breakdown. *Microb Ecol* **56**: 260–269.

Baudouin-Cornu P & Labarre J (2006) Regulation of the cadmium stress response through SCF-like ubiquitin ligases: comparison between *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and mammalian cells. *Biochimie* **88**: 1673–1685.

Belliveau MJR & Bärlocher F (2005) Molecular evidence confirms multiple origins of aquatic hyphomycetes. *Mycol Res* **109**: 1407–1417.

Bermingham S, Dewey FM & Maltby L (1995) Development of a monoclonal antibody-based immunoassay for the detection and quantification of *Anguillospora longissima* colonizing leaf material. *Appl Environ Microb* **61**: 2606–2613.

Bermingham S, Maltby L & Cooke RC (1996) Effects of a coal mine effluent on aquatic hyphomycetes. II. Laboratory toxicity experiments. *J Appl Ecol* **33**: 1322–1328.

Bermingham S, Dewey F, Fisher P & Maltby L (2001) Use of a monoclonal antibody-based immunoassay for the detection and quantification of *Heliscus lugdunensis* colonizing alder leaves and roots. *Microb Ecol* **42**: 506–512.

Blee KA, Wheatley ER, Bonham VA, Mitchell GP, Robertson D, Slabas AR, Burrell MM, Wojtaszek P & Bolwell GP (2001) Proteomic analysis reveals a novel set of cell wall proteins in a transformed tobacco cell culture that synthesises secondary walls as determined by biochemical and morphological parameters. *Planta* **212**: 404–415.

Blindauer CA & Leszczyszyn OI (2010) Metallothioneins: unparalleled diversity in structures and functions for metal ion homeostasis and more. *Nat Prod Rep* **27**: 720–741.

Boisvenue C & Running SW (2006) Impacts of climate change on natural forest productivity – evidence since the middle of the 20th century. *Glob Change Biol* **12**: 1–21.

Bond U (2006) Stressed out! Effects of environmental stress on mRNA metabolism. *FEMS Yeast Res* **6**: 160–170.

Boschker HTS & Middelburg J (2002) Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiol Ecol* **40**: 85–95.

Boschker HTS, Nold SC, Wellsbury P, Bos D, de Graaf W, Pel R, Parkes RJ & Cappenberg TE (1998) Direct linking of microbial populations to specific biogeochemical processes by 13C-labelling of biomarkers. *Nature* **392**: 801–805.

Braha B, Tintemann H, Krauss G, Ehrman JM, Bärlocher F & Krauss G-J (2007) Stress response in two strains of the aquatic hyphomycete *Heliscus lugdunensis* after exposure to cadmium and copper ions. *BioMetals* **20**: 93–105.

Brand A & Gow NA (2009) Mechanisms of hypha orientation of fungi. *Curr Opin Microbiol* **12**: 350–357.

Brathen I (1982) The aquatic, non-stauroconidial hyphomycetes of Norway with notes on the Nordic species. *Nord J Bot* **2**: 575–582.

Brathen I (1984) The aquatic, stauroconidial hyphomycetes of Norway with notes on the Nordic species. *Nord J Bot* **4**: 375–392.

Bumpus J, Tien M, Wright D & Aust S (1985) Oxidation of persistent environmental pollutants by a white rot fungus. *Science* **228**: 1434–1436.

Cabana H, Jones JP & Agathos SN (2007) Preparation and characterization of cross-linked laccase aggregates and their application to the elimination of endocrine disrupting chemicals. *J Biotechnol* **132**: 23–31.

Camarero S, Ibarra D, Martinez MJ & Martinez AT (2005) Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. *Appl Environ Microb* **71**: 1775–1784.

Campbell J, Marvanová L & Gulis Vladislav (2009) Evolutionary relationships between aquatic anamorphs and teleomorphs: *Tricladium* and *Varicosporium*. *Mycol Res* **113**: 1322–1334.

Canhoto C & Graça MAS (2008) Interactions between fungi and stream invertebrates: back to the future. *Novel Techniques and Ideas in Mycology, Fungal Diversity Research Series* (Sridhar KR, Bärlocher F & Hyde KD, eds), pp. 305–325. Fungal Diversity Press, Yunnan, P.R. China.

Carter MD & Suberkropp K (2004) Respiration and annual fungal production associated with decomposing leaf litter in two streams. *Freshwater Biol* **49**: 1112–1122.

Cerniglia CE & Sutherland JB (2001) Bioremediation of polycyclic aromatic hydrocarbons by ligninolytic and non-ligninolytic fungi. *Fungi in Bioremediation* (Gadd GM, ed), pp. 136–187. Cambridge University Press, Cambridge, UK.

Cerniglia CE, Sutherland JB & Crow SA (1992) Fungal metabolism of aromatic hydrocarbons. *Microbial Degradation of Natural Products* (Winkelman G, ed), pp. 193–217. John Wiley & Sons Ltd, Weinheim, Germany.

Chamier A (1985) Cell-wall-degrading enzymes of aquatic hyphomycetes: a review. *Bot J Linn Soc* **91**: 67–81.

Chamier A & Tipping E (1997) Effects of aluminium in acid streams on growth and sporulation of aquatic hyphomycetes. *Environ Pollut* **96**: 289–298.

Chandrashekar KR & Kaveriappa KM (1991) Production of extracellular cellulase by *Lunulospora curvula* and *Flagellospora penicillioides*. *Folia Microbiol (Praha)* **36**: 249–255.

Chandrashekar KR, Sridhar KR & Kaveriappa KM (1991) Aquatic hyphomycetes of a sulphur spring. *Hydrobiologia* **218**: 151–156.

Charcosset J & Chauvet E (2001) Effect of culture conditions on ergosterol as an indicator of biomass in the aquatic hyphomycetes. *Appl Environ Microb* **67**: 2051–2055.

Chauvet E & Suberkropp K (1998) Temperature and sporulation of aquatic hyphomycetes. *Appl Environ Microb* **64**: 1522–1525.

Chauvet E, Fabre E, Elósegui A & Pozo J (1997) The impact of eucalypt on the leaf-associated aquatic hyphomycetes in Spanish streams. *Can J Bot* **75**: 880–887.

Chergui H & Pattée E (1988) The dynamics of hyphomycetes on decaying leaves in the network of the river Rhone (France). *Arch Hydrobiol* **114**: 3–10.

Chung N & Suberkropp K (2009) Contribution of fungal biomass to the growth of the shredder, *Pycnopsyche gentilis* (Trichoptera: Limnephilidae). *Freshwater Biol* **54**: 2212–2224.

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* **88**: 1707–1719.

Clemens S & Persoh D (2009) Multi-tasking phytochelatin synthases. *Plant Sci* **177**: 266–271.

Clemens S & Simm C (2003) Heavy metal-binding proteins and peptides. *Polyamides and Complex Proteinaceous Materials II, Biopolymers* (Fahnestock SR & Steinbüchel A, eds), pp. 255–288. Wiley-VCH, Weinheim.

Cobine PA, McKay RT, Zangger K, Dameron CT & Armitage IM (2004) Solution structure of Cu6 metallothionein from the fungus *Neurospora crassa*. *Eur J Biochem* **271**: 4213–4221.

Cole RJ, Schweikert MA & Jarvis BB (2003) *Handbook of Secondary Fungal Metabolites (3-Volume Set)*. 1st edn. Academic Press, San Diego, CA.

Cornut J (2010) Décomposition de détritus végétaux dans la zone hyporhéique de cours d'eau forestier: implication du compartiment fongique. PhD Thesis, Université Toulouse 3, Paul Sabatier, France.

Cornut J, Elger A, Lambrigot D, Marmonier P & Chauvet E (2010) Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. *Freshwater Biol* **55**: 2541–2556.

Corvini P, Schäffer A & Schlosser D (2006) Microbial degradation of nonylphenol and other alkylphenols – our evolving view. *Appl Microbiol Biot* **72**: 223–243.

Cox HHJ, Houtman JHM, Doddema HJ & Harder W (1993) Growth of the black yeast *Exophiala jeanselmei* on styrene and styrene-related compounds. *Appl Environ Microb* **39**: 372–376.

Cox HHJ, Faber BW, Van Heiningen WN, Radhoe H, Doddema HJ & Harder W (1996) Styrene metabolism in *Exophiala jeanselmei* and involvement of a cytochrome P-450-dependent styrene monooxygenase. *Appl Environ Microb* **62**: 1471–1474.

Craft DL, Madduri KM, Eshoo M & Wilson CR (2003) Identification and characterization of the CYP52 family of *Candida tropicalis* ATCC 20336, important for the conversion of fatty acids and alkanes to alpha,omega-dicarboxylic acids. *Appl Environ Microb* **69**: 5983–5991.

Dang CK, Chauvet E & Gessner MO (2005) Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. *Ecol Lett* **8**: 1129–1137.

Dang CK, Gessner MO & Chauvet E (2007) Influence of conidial traits and leaf structure on attachment success of aquatic hyphomycetes on leaf litter. *Mycologia* **99**: 24–32.

Dang CK, Schindler M, Chauvet E & Gessner MO (2009) Temperature oscillations coupled with fungal communities can modulate warming effects on litter decomposition. *Ecology* **90**: 122–131.

Das M, Royer TV & Leff LG (2007) Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. *Appl Environ Microb* **73**: 756–767.

Davila-Vazquez G, Tinoco R, Pickard MA & Vazquez-Duhalt R (2005) Transformation of halogenated pesticides by versatile peroxidase from *Bjerkandera adusta*. *Enzyme Microb Tech* **36**: 223–231.

DeLong EF (2009) The microbial ocean from genomes to biomes. *Nature* **459**: 200–206.

De Oliveira JM & de Graaff LH (2011) Proteomics of industrial fungi: trends and insights for biotechnology. *Appl Microbiol Biot* **89**: 225–237.

Descals E (1997) Ingoldian Fungi: some field and laboratory techniques. *Boll Soc Hist Nat Balears* **40**: 169–221.

Descals E (2005) Techniques for handling Ingoldian fungi. *Methods to Study Litter Decomposition* (Graça M, Bärlocher F & Gessner MO, eds), pp. 129–141. Springer, Dordrecht, the Netherlands.

Descals E (2008) Procedures for the identification of Ingoldian fungi. *Novel Techniques and Ideas in Mycology, Fungal Diversity Research Series* (Sridhar KR, Bärlocher F & Hyde KD, eds), pp. 215–259. Fungal Diversity Press, Yunnan, P.R. China.

De Wildeman E (1893) Notes mycologiques. *Fasc II Ann Soc Belge Microscopie* **17**: 35–68.

De Wildeman E (1894) Notes mycologiques. *Fasc III Ann Soc Belge Microscopie* **18**: 135–161.

De Wildeman E (1895) Notes mycologiques. *Fasc II Ann Soc Belge Microscopie* **19**: 193–206.

Doddapaneni H & Yadav JS (2005) Microarray-based global differential expression profiling of P450 monooxygenases and regulatory proteins for signal transduction pathways in the white rot fungus *Phanerochaete chrysosporium*. *Mol Genet Genomics* **274**: 454–466.

Duarte S, Pascoal C, Cássio F & Bärlocher F (2006) Aquatic hyphomycete diversity and identity affect leaf litter decomposition in microcosms. *Oecologia* **147**: 658–666.

Duarte S, Pascoal C & Cássio F (2008) High diversity of fungi may mitigate the impact of pollution on plant litter decomposition in streams. *Microb Ecol* **56**: 688–695.

Duarte S, Pascoal C & Cássio F (2009) Functional stability of stream-dwelling microbial decomposers exposed to copper and zinc stress. *Freshwater Biol* **54**: 1683–1691.

Dubroca J, Brault A, Kollmann A, Touton I, Jolivalt G, Kerhoas L & Mougin C (2005) Biotransformation of nonylphenol surfactants in soils amended with contaminated sewage sludges. *Environmental Chemistry: Green Chemistry and Pollutants in Ecosystems* (Lichtfouse E, Schwarzbauer J & Robert D, eds), pp. 305–315. Springer, Berlin/Heidelberg, Germany.

Ehrman JM, Bärlocher F, Wennrich R, Krauss G-J & Krauss G (2008) Fungi in a heavy metal precipitating stream in the Mansfeld mining district, Germany. *Sci Total Environ* **389**: 486–496.

Engblom E, Lingdell P, Marvanova L & Muller-Haeckel A (1986) Foam spora in running waters of southern Greenland. *Polar Res* **4**: 47–51.

Ernst WHO, Krauss G-J, Verkleij JAC & Wesenberg D (2008) Interaction of heavy metals with the sulphur metabolism in angiosperms from an ecological point of view. *Plant Cell Environ* **31**: 123–143.

Fabre E (1996) Relationships between aquatic hyphomycetes communities and riparian vegetation in 3 Pyrenean streams. *CR Acad Sci III Vie* **319**: 107–111.

Fedorak PM & Westlake DWS (1986) Fungal metabolism of n-alkylbenzenes. *Appl Environ Microb* **51**: 435–437.

Fernandes I, Duarte S, Cássio F & Pascoal C (2009a) Mixtures of zinc and phosphate affect leaf litter decomposition by aquatic fungi in streams. *Sci Total Environ* **407**: 4283–4288.

Fernandes I, Uzun B, Pascoal C & Cássio F (2009b) Responses of aquatic fungal communities on leaf litter to temperature-change events. *Int Rev Hydrobiol* **94**: 410–418.

Ferreira V & Chauvet E (2010) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Glob Change Biol* in press. DOI: 10.1111/j.1365-2486.2010.02185.x.

Ferreira V, Elosegi A, Gulis V, Pozo J & Graça MAS (2006) Eucalyptus plantations affect fungal communities associated with leaf-litter decomposition in Iberian streams. *Arch Hydrobiol* **166**: 467–490.

Ferreira V, Gonçalves AL, Godbold DL & Canhoto C (2010a) Effect of increased atmospheric CO_2 on the performance of an aquatic detritivore through changes in water temperature and litter quality. *Glob Change Biol* **16**: 3284–3296.

Ferreira V, Goncalves AL, Pratas J & Canhoto C (2010b) Contamination by uranium mine drainages affects fungal growth and interactions between fungal species and strains. *Mycologia* **102**: 1004–1011.

Fiehn O & Weckwerth W (2003) Deciphering metabolic networks. *Eur J Biochem* **270**: 579–588.

Field JA, de Jong E, Feijoo-Costa G & de Bont JAM (1993) Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics. *Trends Biotechnol* **11**: 44–49.

Findlay S & Arsuffi TL (1989) Microbial growth and detritus transformations during decomposition of leaf litter in a stream. *Freshwater Biol* **21**: 261–269.

Findlay S, Tank J, Dye S *et al.* (2002) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microb Ecol* **43**: 55–66.

Fisher P, Davey R & Webster J (1983) Degradation of lignin by aquatic and aero-aquatic hyphomycetes. *T Brit Mycol Soc* **80**: 166–168.

Frisvad JC, Smedsgaard J, Larsen TO & Samson RA (2004) Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. *Studies Mycol* **49**: 201–241.

Gadd GM (2004) Mycotransformation of organic and inorganic substrates. *Mycologist* **18**: 60–70.

Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycol Res* **111**: 3–49.

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology* **156**: 609–643.

Gange AC, Gange EG, Sparks TH & Boddy L (2007) Rapid and recent changes in fungal fruiting patterns. *Nature* **316**: 71.

García-Peña I, Hernández S, Auria R & Revah S (2005) Correlation of biological activity and reactor performance in biofiltration of toluene with the fungus *Paecilomyces variotii* CBS115145. *Appl Environ Microb* **71**: 4280–4285.

Gardea-Torresdey JL, Cano-Aguilera I, Webb R, Tiemann KJ & Gutiérrez-Corona F (1996) Copper adsorption by inactivated cells of *Mucor rouxii*: effect of esterification of carboxyl groups. *J Hazard Mater* **48**: 171–180.

Georg RC & Gomes SL (2007) Transcriptome analysis in response to heat shock and cadmium in the aquatic fungus *Blastocladiella emersonii*. *Eukaryot Cell* **6**: 1053–1062.

Georg RC, Stefani R & Gomes S (2009) Environmental stresses inhibit splicing in the aquatic fungus *Blastocladiella emersonii*. *BMC Microbiol* **9**: 231.

Gerasimova NM, Lin LZ & Bekhtereva MN (1975) Lipid composition of *Cunninghamella elegans* cultivated on n-alkanes. *Mikrobiologia* **44**: 460–464.

Gesell M, Hammer E, Specht M, Francke W & Schauer F (2001) Biotransformation of biphenyl by *Paecilomyces lilacinus* and characterization of ring cleavage products. *Appl Environ Microb* **67**: 1551–1557.

Gesell M, Hammer E, Mikolasch A & Schauer F (2004) Oxidation and ring cleavage of dibenzofuran by the filamentous fungus *Paecilomyces lilacinus*. *Arch Microbiol* **182**: 51–59.

Gessner MO (1997) Fungal biomass, production and sporulation associated with particulate organic matter in streams. *Limnetica* **13**: 33–44.

Gessner MO & Chauvet E (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Appl Environ Microb* **59**: 502–507.

Gessner MO & Chauvet E (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology* **75**: 1807–1817.

Gessner MO & Newell SY (2002) Biomass, growth rate, and production of filamentous fungi in plant litter. *Manual of Environmental Microbiology* (Hurst CJ, Crawford RL, Knudsen GR, McInerney MJ & Stetzenbach LD, eds), pp. 390–408. ASM Press, Washington, DC.

Gessner MO, Suberkropp K & Chauvet E (1997) Decomposition of plant litter by fungi in marine and freshwater ecosystems. *The Mycota*, *Vol. IV* (Wicklow DT & Söderström BE, eds), pp. 303–322. Springer, Berlin/Heidelberg, Germany.

Gessner MO, Gulis V, Kuehn KA, Chauvet E & Suberkropp K (2007) Fungal decomposers of plant litter in aquatic ecosystems. *The Mycota: Microbial and Environmental Relationships*, *Vol. 4* (Kubicek CP & Druzhinina IS, eds), pp. 301–324. Springer, Berlin/Heidelberg, Germany.

Gharieb M (2001) Pattern of cadmium accumulation and essential cations during growth of cadmium-tolerant fungi. *BioMetals* **14**: 143–151.

Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S & Sannia G (2010) Laccases: a never-ending story. *Cell Mol Life Sci* **67**: 369–385.

Godfrey B (1983) Growth of two terrestrial microfungi on submerged alder leaves. *T Brit Mycol Soc* **81**: 418–421.

Goh TK (1997) Tropical freshwater hyphomycetes. *Biodiversity of Tropical Microfungi* (Hyde KD, ed), pp. 189–227. Hong Kong University Press, Hong Kong.

Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG & Kell DB (2004) Metabolomics by numbers: acquiring and understanding global metabolite data. *Trends Biotechnol* **22**: 245–252.

Gough L, Osenberg CW, Gross KL & Collins SL (2000) Fertilization effects on species density and primary productiity in herbaceous plant communities. *Oikos* **89**: 428–439.

Graça MAS & Ferreira RCF (1995) The ability of selected aquatic hyphomycetes and terrestrial fungi to decompose leaves in freshwater. *Sydowia* **47**: 167–179.

Guimarães-Soares L, Felícia H, Bebianno MJ & Cássio F (2006) Metal-binding proteins and peptides in the aquatic fungi *Fontanospora fusiramosa* and *Flagellospora curta* exposed to severe metal stress. *Sci Total Environ* **372**: 148–156.

Guimarães-Soares L, Pascoal C & Cássio F (2007) Effects of heavy metals on the production of thiol compounds by the aquatic fungi *Fontanospora fusiramosa* and *Flagellospora curta*. *Ecotox Environ Safe* **66**: 36–43.

Gulis V (2001) Are there any substrate preferences in aquatic hyphomycetes? *Mycol Res* **105**: 1088–1093.

Gulis V, Kuehn KA & Suberkropp K (2006) The role of fungi in carbon and nitrogen cycles in freshwater ecosystems. *Fungi in Biogeochemical Cycles* (Gadd GM, ed), pp. 404–435. Cambridge University Press, Cambridge, UK.

Gulis V, Marvanová L & Descals E (2005) An illustrated key to the common temperate species of aquatic hyphomycetes. Methods to Study Litter Decomposition (Graça MAS, Bärlocher F & Gessner MO, eds), pp. 153–167. Springer, Dordrecht, the Netherlands.

Gygi SP, Rochon Y, Franza BR & Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol* **19**: 1720–1730.

Hammel KE (1995) Organopollutant degradation by ligninolytic fungi. *Microbial Transformation and Degradation of Toxic Organic Chemicals* (Young LY & Cerniglia CE, eds), pp. 331–346. Wiley-Liss, New York.

Hammel KE & Cullen D (2008) Role of fungal peroxidases in biological ligninolysis. *Curr Opin Plant Biol* **11**: 349–355.

Hammer E, Schoefer L, Schäfer A, Hundt K & Schauer F (2001) Formation of glucoside conjugates during biotransformation of dibenzofuran by *Penicillium canescens* SBUG-M 1139. *Appl Microbiol Biot* **57**: 390–394.

Harrop BL, Marks JC & Watwood ME (2009) Early bacterial and fungal colonization of leaf litter in Fossil Creek, Arizona. *J N Am Benthol Soc* **28**: 383–396.

Hartig R (1880) Der Ahornkeimlingpilz, *Cercospora acerina*. *Untersuch Forstbot Inst München* **1**: 58–61.

Hebert PDN, Cywinska A, Ball SL & deWaard JR (2003) Biological identifications through DNA barcodes. *Proc Biol Sci* **270**: 313–321.

Hebert PDN, Stoeckle MY, Zemlak TS & Francis CM (2004) Identification of birds through DNA barcodes. *PLoS Biol* **2**: e312.

Heinfling A, Martinez MJ, Martinez AT, Bergbauer M & Szewzyk U (1998) Transformation of industrial dyes by manganese peroxidases from *Bjerkandera adusta* and *Pleurotus eryngii* in a manganese-independent reaction. *Appl Environ Microb* **64**: 2788–2793.

Hendel B, Sinsabaugh RL & Marxsen J (2005) Lignin-degrading enzymes: phenoloxidase and peroxidase. *Methods to Study Litter Decomposition* (Graça MAS, Bärlocher F & Gessner MO, eds), pp. 273–278. Springer, Dordrecht, the Netherlands.

Hieber M & Gessner MO (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology* **83**: 1026–1038.

Hiratsuka N, Oyadomari M, Shinohara H, Tanaka H & Wariishi H (2005) Metabolic mechanisms involved in hydroxylation reactions of diphenyl compounds by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Biochem Eng J* **23**: 241–246.

Hodkinson M (1976) Interactions between aquatic fungi and DDT. *Recent Advances in Aquatic Mycology* (Jones EBG, ed), pp. 447–467. Wiley, New York.

Hofrichter M (2002) Review: lignin conversion by manganese peroxidase (MnP). *Enzyme Microb Tech* **30**: 454–466.

Hofrichter M & Scheibner K (1993) Utilization of aromatic compounds by the *Penicillium* strain Bi 7/2. *J Basic Microbiol* **33**: 227–232.

Hofrichter M, Bublitz F & Fritsche W (1994) Unspecific degradation of halogenated phenols by the soil fungus *Penicillium frequentans* Bi 7/2. *J Basic Microbiol* **34**: 163–172.

Hofrichter M, Scheibner K, Schneegaß I & Fritsche W (1998) Enzymatic combustion of aromatic and aliphatic compounds by manganese peroxidase from *Nematoloma frowardii*. *Appl Environ Microb* **64**: 399–404.

Howe R, Evans RL & Ketteridge SW (1997) Copper-binding proteins in ectomycorrhizal fungi. *New Phytol* **135**: 123–131.

Hundt K, Martin D, Hammer E, Jonas U, Kindermann MK & Schauer F (2000) Transformation of triclosan by *Trametes versicolor* and *Pycnoporus cinnabarinus*. *Appl Environ Microb* **66**: 4157–4160.

Huttenhower C & Hofmann O (2010) A quick guide to large-scale genomic data mining. *PLoS Comput Biol* **6**: e1000779.

Hwang C & Kolattukudy PE (1995) Isolation and characterization of genes expressed uniquely during appressorium formation by *Colletotrichum gloeosporioides* conidia induced by the host surface wax. *Mol General Genet* **247**: 282–294.

Ingold CT (1942) Aquatic hyphomycetes of decaying alder leaves. *T Brit Mycol Soc* **25**: 339–417.

Ingold CT (1959) Aquatic spora of Omo Forest, Nigeria. *T Brit Mycol Soc* **42**: 479–485.

Ingold CT (1975) *An illustrated guide to aquatic and water-borne hyphomycetes (fungi imperfecti): With notes on their biology. Freshwater Biol Assoc Sci Publ* **No 3**: 1–96.

International Panel on Climate Change (IPCC). 2007 Climate change 2007: the physical science basis. *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M & Miller HL, eds), Cambridge University Press, Cambridge, UK.

Iqbal S & Webster J (1973) Aquatic hyphomycete spora of the River Exe and its tributaries. *T Brit Mycol Soc* **61**: 331–346.

Jackson CR, Foreman CM & Sinsabaugh RL (1995) Microbial enzyme activities as indicators of organic matter processing rates in a Lake Erie coastal wetland. *Freshwater Biol* **34**: 329–342.

Jackson EF, Echlin HL & Jackson CR (2006) Changes in the phyllosphere community of the resurrection fern, *Polypodium polypodioides*, associated with rainfall and wetting. *FEMS Microbiol Ecol* **58**: 236–246.

Jacob C, Courbot M, Martin F, Brun A & Chalot M (2004) Transcriptomic responses to cadmium in the ectomycorrhizal fungus *Paxillus involutus*. *FEBS Lett* **576**: 423–427.

Jaeckel P, Krauss G, Menge S, Schierhorn A, Rücknagel P & Krauss G-J (2005a) Cadmium induces a novel metallothionein and phytochelatin 2 in an aquatic fungus. *Biochem Bioph Res Co* **333**: 150–155.

Jaeckel P, Krauss G-J & Krauss G (2005b) Cadmium and zinc response of the fungi *Heliscus lugdunensis* and *Verticillium* cf. *alboatrum* isolated from highly polluted water. *Sci Total Environ* **346**: 274–279.

Jakupović M, Heintz M, Reichmann P, Mendgen K & Hahn M (2006) Microarray analysis of expressed sequence tags from haustoria of the rust fungus *Uromyces fabae*. *Fungal Genet Biol* **43**: 8–19.

Jigami Y, Omori T, Minoda Y & Yamada K (1974) Screening of n-alkylbenzenes assimilating yeasts and identification of oxidation products from n-alkylbenzenes. *Agr Biol Chem* **38**: 401–408.

Johannes C & Majcherczyk A (2000) Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. *Appl Environ Microb* **66**: 524–528.

Jones KH, Trudgill PW & Hopper DJ (1993) Metabolism of p-cresol by the fungus *Aspergillus fumigatus*. *Appl Environ Microb* **59**: 1125–1130.

Jones KH, Trudgill PW & Hopper DJ (1994) 4-Ethylphenol metabolism by *Aspergillus fumigatus*. *Appl Environ Microb* **60**: 1978–1983.

Junghanns C, Moeder M, Krauss G, Martin C & Schlosser D (2005) Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. *Microbiology* **151**: 45–57.

Junghanns C, Krauss G & Schlosser D (2008) Potential of aquatic fungi derived from diverse freshwater environments to decolourise synthetic azo and anthraquinone dyes. *Bioresource Technol* **99**: 1225–1235.

Junghanns C, Pecyna MJ, Böhm D, Jehmlich N, Martin C, von Bergen M, Schauer F, Hofrichter M & Schlosser D (2009) Biochemical and molecular genetic characterisation of a novel laccase produced by the aquatic ascomycete *Phoma* sp. UHH 5-1-03. *Appl Microbiol Biot* **84**: 1095–1105.

Kaushik NK & Hynes HBN (1968) Experimental study on the role of autumn-shed leaves in aquatic environments. *J Ecol* **56**: 229–243.

Kaushik NK & Hynes HBN (1971) The fate of the dead leaves that fall into streams. *Arch Hydrobiol* **68**: 465–515.

Kearns SG & Bärlocher F (2008) Leaf surface roughness influences colonization success of aquatic hyphomycete conidia. *Fungal Ecol* **1**: 13–18.

Kegel (1906) *Varicosporium elodeae*, ein Wasserpilz mit auffallender Konidienbildung. *Ber Deut Bot Ges* **24**: 213–216.

Kinzig AP, Tilman D & Pacala S (2001) *The Functional Consequences of Biodiversity: Empirical Progress and Theoretical Extensions*. Princeton University Press, Princeton, NJ.

Klironomos JN, Allen MF, Rillig MC, Piotrowski J, Makvandi-Nejad S, Wolfe BE & Powell JR (2005) Abrupt rise in atmospheric CO_2 overestimates community response in a model plant–soil system. *Nature* **433**: 621–622.

Kneer R, Kutchan TM, Hochberger A & Zenk MH (1992) *Saccharomyces cerevisiae* and *Neurospora crassa* contain heavy metal sequestering phytochelatin. *Arch Microbiol* **157**: 305–310.

Kniemeyer O, Lessing F, Scheibner O, Hertweck C & Brakhage AA (2006) Optimisation of a 2-D gel electrophoresis protocol for the human-pathogenic fungus *Aspergillus fumigatus*. *Curr Genet* **49**: 178–189.

Kondo N, Imai K, Isobe M, Goto T, Murasugi A, Wada-Nakagawa C & Hayashi Y (1984) Cadystin a and b, major unit peptides comprising cadmium binding peptides induced in a fission yeast – separation, revision of structures and synthesis. *Tetrahedron Lett* **25**: 3869–3872.

Koske RE & Duncan JW (1974) Temperature effects on growth, sporulation, and germination of some 'aquatic' hyphomycetes. *Can J Bot* **52**: 1387–1391.

Kostalos M & Seymour RL (1976) Role of microbial enriched detritus in the nutrition of *Gammarus minus* (Amphipoda). *Oikos* **27**: 512–516.

Krämer U, Talke IN & Hanikenne M (2007) Transition metal transport. *FEBS Lett* **581**: 2263–2272.

Krauss G, Bärlocher F, Schreck P, Wennrich R, Glasser W & Krauss G-J (2001) Aquatic hyphomycetes occur in hyperpolluted waters in Central Germany. *Nova Hedwigia* **72**: 419–428.

Krauss G, Bärlocher F & Krauss G-J (2003a) Effects of pollution on aquatic hyphomycetes. *Freshwater Mycology, Fungal Diversity Research Series 10*, *Vol. 10* (Tsui CKM & Hyde KD, eds), pp. 211–230. Fungal Diversity Press, Hong Kong.

Krauss G, Sridhar KR, Jung K, Wennrich R, Ehrman JM & Bärlocher F (2003b) Aquatic hyphomycetes in polluted groundwater habitats of Central Germany. *Microbial Ecol* **45**: 329–339.

Krauss G, Sridhar KR & Bärlocher F (2005) Aquatic hyphomycetes and leaf decomposition in contaminated groundwater wells in Central Germany. *Arch Hydrobiol* **162**: 416–428.

Krauss G-J, Wesenberg D, Ehrman JM, Solé M, Miersch J & Krauss G (2008) Responses to heavy metals. *Novel Techniques and Ideas in Mycology, Fungal Diversity Research Series* (Sridhar KR, Bärlocher F & Hyde KD, eds), pp. 149–182. Fungal Diversity Press, Yunnan, P.R. China.

Kumar KS, Dayananda S & Subramanyam C (2005) Copper alone, but not oxidative stress, induces copper-metallothionein gene in *Neurospora crassa*. *FEMS Microbiol Lett* **242**: 45–50.

Laitung B & Chauvet E (2005) Vegetation diversity increases species richness of leaf-decaying fungal communities in woodland streams. *Arch Hydrobiol* **164**: 217–235.

Lecerf A & Chauvet E (2008) Diversity and functions of leaf-decaying fungi in human-altered streams. *Freshwater Biol* **53**: 1658–1672.

Lecerf A, Dobson M, Dang CK & Chauvet E (2005) Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia* **146**: 432–442.

Lefèvre E, Jobard M, Venisse J, Bec A, Kagami M, Amblard C & Sime-Ngando T (2010) Development of a real-time PCR assay for quantitative assessment of uncultured freshwater zoosporic fungi. *J Microbiol Meth* **81**: 69–76.

Le Quéré A, Eriksen KA, Rajashekar B, Schützendübel A, Canbäck B, Johansson T & Tunlid A (2006) Screening for rapidly evolving genes in the ectomycorrhizal fungus *Paxillus involutus* using cDNA microarrays. *Mol Ecol* **15**: 535–550.

Lerch K (1991) Purification of *Neurospora crassa* copper-metallothionein. *Method Enzymol* **205**: 278–283.

Letourneau A, Seena S, Marvanová L & Bärlocher F (2010) Potential use of barcoding to identify aquatic hyphomycetes. *Fungal Diversity* **40**: 51–64.

Li Z, Lu Y, Zhen R, Szczypka M, Thiele D & Rea P (1997) A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of bis(glutathionato)cadmium. *P Natl Acad Sci USA* **94**: 42–47.

Litchman E (2010) Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. *Ecol Lett* **13**: 1560–1572.

Loreau M, Naeem S, Inchausti P *et al.* (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* **294**: 804–808.

Luis P, Kellner H, Zimdars B, Langer U, Martin F & Buscot F (2005) Patchiness and spatial distribution of laccase genes of ectomycorrhizal, saprotrophic, and unknown basidiomycetes in the upper horizons of a mixed forest cambisol. *Microb Ecol* **50**: 570–579.

Lyons J, Newell S, Buchan A & Moran M (2003) Diversity of ascomycete laccase gene sequences in a southeastern US salt marsh. *Microb Ecol* **45**: 270–281.

MacDonald TM, Dubois L, Smith LC & Campbell DA (2003) Sensitivity of cyanobacterial antenna, reaction center and CO2 assimilation transcripts and proteins to moderate UVB: light acclimation potentiates resistance to UVB. *Photochem Photobiol* **77**: 405–412.

Mackay RJ & Kalff J (1973) Ecology of two related species of caddis fly larvae in the organic substrates of a woodland stream. *Ecology* **54**: 499–511.

Madigan MT, Martinko JM, Dunlap PV & Clark DP (2009) *Biology of Microorganisms*. Pearson/Benjamin Cummings, San Francisco.

Maharning AR & Bärlocher F (1996) Growth and reproduction in aquatic hyphomycetes. *Mycologia* **88**: 80–88.

Maltby L & Booth R (1991) The effect of coal-mine effluent on fungal assemblages and leaf breakdown. *Water Res* **25**: 247–250.

Manefield M, Whiteley AS, Griffiths RI & Bailey MJ (2002) RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Appl Environ Microb* **68**: 5367–5373.

Manerkar M, Seena S & Bärlocher F (2008) Q-RT-PCR for assessing archaea, bacteria, and fungi during leaf decomposition in a stream. *Microb Ecol* **56**: 467–473.

Martin C, Moeder M, Daniel X, Krauss G & Schlosser D (2007) Biotransformation of the polycyclic musks HHCB and AHTN and metabolite formation by fungi occurring in freshwater environments. *Environ Sci Technol* **41**: 5395–5402.

Martin C, Corvini PFX, Vinken R, Junghanns C, Krauss G & Schlosser D (2009) Quantification of the influence of extracellular laccase and intracellular reactions on biotransformation of the xenoestrogen technical nonylphenol by the aquatic hyphomycete *Clavariopsis aquatica* from an isomer-specific viewpoint. *Appl Environ Microb* **75**: 4398–4409.

Martínez AT (2002) Molecular biology and structure–function of lignin-degrading heme peroxidases. *Enzyme Microb Tech* **30**: 425–444.

Marvanová L (1997) Freshwater hyphomycetes: a survey with remarks on tropical taxa. *Tropical Mycology* (Janardhanan KK, Rajendran C, Natarajan K & Hawksworth DL, eds), pp. 168–226. Science Publishers, Enfield, NH.

Marvanová L (2005) Maintenance of aquatic hyphomycete culture. *Methods to Study Litter Decomposition* (Graça M, Bärlocher F & Gessner MO, eds), pp. 129–152. Springer, Dordrecht, the Netherlands.

Massaccesi G, Romero MC, Cazau MC & Bucsinszky AM (2002) Cadmium removal capacities of filamentous soil fungi isolated from industrially polluted sediments, in La Plata (Argentina). *World J Microb Biot* **18**: 817–820.

Matsuzaki F & Wariishi H (2004) Functional diversity of cytochrome P450 s of the white-rot fungus *Phanerochaete chrysosporium*. *Biochem Bioph Res Co* **324**: 387–393.

Mayer AM & Staples RC (2002) Laccase: new functions for an old enzyme. *Phytochemistry* **60**: 551–565.

McArthur FA, Baerlocher MO, MacLean NA, Hiltz MD & Bärlocher F (2001) Asking probing questions: can fluorescent *in situ* hybridization identify and localise aquatic hyphomycetes on leaf litter? *Int Rev Hydrobiol* **86**: 429–438.

Medeiros AO, Pascoal C & Graça MAS (2009) Diversity and activity of aquatic fungi under low oxygen conditions. *Freshwater Biol* **54**: 142–149.

Mehra RK, Tarbet EB, Gray W & Winge DR (1988) Metal-specific synthesis of two metallothioneins and gamma-glutamyl peptides in *Candida glabrata*. *P Natl Acad Sci USA* **85**: 8815–8819.

Mehra RK, Garey J, Butt T, Gray W & Winge D (1989) *Candida glabrata* metallothioneins. Cloning and sequence of the genes and characterization of proteins. *J Biol Chem* **264**: 19747–19753.

Mendoza-Cozatl D, Loza-Tavera H, Hernandez-Navarro A & Moreno-Sanchez R (2005) Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. *FEMS Microbiol Rev* **29**: 653–671.

Mendoza-Cozatl DG, Zhai Z, Jobe TO *et al.* (2010) *Schizosaccharomyces pombe* Abc2 transporter mediates phytochelatin accumulation in vacuoles and confers cadmium tolerance. *J Biol Chem* **285**: 20416–20426.

Mester T & Tien M (2000) Oxidation mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants. *Int Biodeter Biodegr* **46**: 51–59.

Methvin BR & Suberkropp K (2003) Annual production of leaf-decaying fungi in 2 streams. *J N Am Benthol Soc* **22**: 554–564.

Middelhoven WJ & Spaaij F (1997) *Rhodotorula cresolica* sp. nov., a cresol-assimilating yeast species isolated from soil. *Int J Syst Bacteriol* **47**: 324–327.

Middelhoven WJ, Scorzetti G & Fell JW (2004) Systematics of the anamorphic basidiomycetous yeast genus *Trichosporon*

behrend with the description of five novel species: *Trichosporon vadense*, *T. smithiae*, *T. dehoogii*, *T. scarabaeorum* and *T. gamsii*. *Int J Syst Evol Micr* **54**: 975–986.

Miersch J & Grancharov K (2008) Cadmium and heat response of the fungus *Heliscus lugdunensis* isolated from highly polluted and unpolluted areas. *Amino Acids* **34**: 271–277.

Miersch J, Bärlocher F, Bruns I & Krauss G-J (1997) Effects of cadmium, copper, and zinc on growth and thiol content of aquatic hyphomycetes. *Hydrobiologia* **346**: 77–84.

Miersch J, Tschimedbalshir M, Bärlocher F, Grams Y, Pierau B, Schierhorn A & Krauss G-J (2001) Heavy metals and thiol compounds in *Mucor racemosus* and *Articulospora tetracladia*. *Mycol Res* **105**: 883–889.

Miersch J, Neumann D, Menge S, Bärlocher F, Baumbach R & Lichtenberger O (2005) Heavy metals and thiol pool in three strains of *Tetracladium marchalianum*. *Mycol Prog* **4**: 185–194.

Mille-Lindblom C & Tranvik LJ (2003) Antagonism between bacteria and fungi on decomposing aquatic plant litter. *Microb Ecol* **45**: 173–182.

Mille-Lindblom C, von Wachenfeldt E & Tranvik LJ (2004) Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death. *J Microbiol Meth* **59**: 253–262.

Mille-Lindblom C, Fischer H & Tranvik LJ (2006) Antagonism between bacteria and fungi: substrate competition and a possible tradeoff between fungal growth and tolerance towards bacteria. *Oikos* **113**: 233–242.

Moco S, Schneider B & Vervoort J (2009) Plant micrometabolomics: the analysis of endogenous metabolites present in a plant cell or tissue. *J Proteome Res* **8**: 1694–1703.

Money NP (2010) Cecil Terence Ingold (1905–2010). *Nature* **465**: 1025.

Moreirinha C, Duarte S, Pascoal C & Cássio F (2010) Effects of cadmium and phenanthrene mixtures on aquatic fungi and microbially mediated leaf litter decomposition. *Arch Environ Con Tox* in press. DOI: 10.1007/s00244-010-9610-6.

Münger K & Lerch K (1985) Copper metallothionein from the fungus *Agaricus bisporus*: chemical and spectroscopic properties. *Biochemistry* **24**: 6751–6756.

Münger K, Germann UA & Lerch K (1985) Isolation and structural organization of the *Neurospora crassa* copper metallothionein gene. *EMBO J* **4**: 2665–2668.

Murasugi A (2008) Cadystin (phytochelatin) synthesis induced by cadmium and resulted formation of cadmium sulphide nanoparticles in *Schizosaccharomyces pombe*. *Curr Top Biotechnol* **4**: 65–73.

Murphy GL & Perry JJ (1984) Assimilation of chlorinated alkanes by hydrocarbon-utilizing fungi. *J Bacteriol* **160**: 1171–1174.

Nawawi A (1985) Basidiomycetes with branched, water-borne conidia. *Bot J Linn Soc* **91**: 51–60.

Newell SY & Fallon RD (1991) Toward a method for measuring instantaneous fungal growth rates in field samples. *Ecology* **72**: 1547–1559.

Nies DH & Silver S (2007) *Molecular Microbiology of Heavy Metals*. Springer, Berlin/Heidelberg, Germany.

Niewolak (1975) The occurrence if microorganisms in the water of some lakes in the district of Wêgorzewo. *Acta Hydrobiol* **17**: 371–390.

Nikolcheva LG & Bärlocher F (2004) Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. *Mycol Prog* **3**: 41–49.

Nikolcheva LG, Cockshutt AM & Bärlocher F (2003) Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. *Appl Environ Microb* **69**: 2548–2554.

Nikolcheva LG, Bourque T & Bärlocher F (2005) Fungal diversity during initial stages of leaf decomposition in a stream. *Mycol Res* **109**: 246–253.

Nilsson S (1964) Freshwater hyphomycetes: taxonomy, morphology and ecology. *Symb Bot Upsal* **18**: 1–130.

Niyogi DK, McKnight DM & Lewis WM (2002) Fungal communities and biomass in mountain streams affected by mine drainage. *Arch Hydrobiol* **155**: 255–271.

Niyogi DK, Cheatham CA, Thomson WH & Christiansen JM (2009) Litter breakdown and fungal diversity in a stream affected by mine drainage. *Arch Hydrobiol* **175**: 39–48.

Norby RJ, Cotrufo MF, Ineseon P, O'Neill EG & Canadell JP (2001) Elevated CO_2, litter chemistry, and decomposition: a synthesis. *Oecologia* **127**: 153–165.

Ow DW, Ortiz DF, Speiser DM & McCue KF (1994) Moloecular genetic analysis of cadmium tolerance in *Schizosaccharomyces pombe*. *Metal Ions in Fungi* (Winkelmann G & Winge DR, eds), pp. 339–359. Marcel Dekker, New York.

Pascoal C & Cássio F (2004) Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. *Appl Environ Microb* **70**: 5266–5273.

Pascoal C & Cássio F (2008) Linking fungal diversity to the functioning of freshwater ecosystems. *Novel Techniques and Ideas in Mycology, Fungal Diversity Research Series* (Sridhar KR, Bärlocher F & Hyde KD, eds), pp. 1–19. Fungal Diversity Press, Yunnan, P.R. China.

Pascoal C, Cássio F, Marcotegui A, Sanz B & Gomes P (2005) Role of fungi, bacteria, and invertebrates in leaf litter breakdown in a polluted river. *J N Am Benthol Soc* **24**: 784–797.

Pascoal C, Cássio F, Nikolcheva L & Bärlocher F (2010) Realized fungal diversity increases functional stability of leaf litter decomposition under zinc stress. *Microb Ecol* **59**: 84–93.

Penninckx MJ (2002) An overview on glutathione in *Saccharomyces* versus non-conventional yeasts. *FEMS Yeast Res* **2**: 295–305.

Petersen RH (1962) Aquatic hyphomycetes from North America. I Aleuriosporae (Part 1), and key to the genera. *Mycologia* **54**: 117–151.

Petersen RH (1963a) Aquatic hyphomycetes from North America. II Aleuriosporae (Part 2), and Blastosporae. *Mycologia* **55**: 18–29.

Petersen RH (1963b) Aquatic hyphomycetes from North America. III Phialosporae and miscellaneous species. *Mycologia* **55**: 570–581.

Petrosino JF, Highlander S, Luna RA, Gibbs RA & Versalovic J (2009) Metagenomic pyrosequencing and microbial identification. *Clin Chem* **55**: 856–866.

Pietramellara G, Ascher J, Borgogni F, Ceccherini M, Guerri G & Nannipieri P (2009) Extracellular DNA in soil and sediment: fate and ecological relevance. *Biol Fert Soils* **45**: 219–235.

Pocsi I, Prade RA & Penninckx MJ (2004) Glutathione, altruistic metabolite in fungi. *Adv Microb Physiol* **49**: 1–76.

Pointing S (2001) Feasibility of bioremediation by white-rot fungi. *Appl Microbiol Biot* **57**: 20–33.

Prenafeta-Boldú FX, Summerbell R & Sybren de Hoog G (2006) Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard? *FEMS Microbiol Rev* **30**: 109–130.

Preveral S, Gayet L, Moldes C *et al.* (2009) A common highly conserved cadmium detoxification mechanism from bacteria to humans: heavy metal tolerance conferred by the atp-binding cassette (ABC) transporter SpHMT1 requires glutathione but not metal-chelating phytochelatin peptides. *J Biol Chem* **284**: 4936–4943.

Qi B & Moe W (2002) Biodegradation of volatile organic compounds by five fungal species. *Appl Microbiol Biot* **58**: 684–689.

Raghukumar C, D'Souza TM, Thorn RG & Reddy CA (1999) Lignin-modifying enzymes of *Flavodon flavus*, a basidiomycete isolated from a coastal marine environment. *Appl Environ Microb* **65**: 2103–2111.

Raghukumar C, D'Souza-Ticlo D & Verma AK (2008) Treatment of colored effluents with lignin-degrading enzymes: an emerging role of marine-derived fungi. *Crit Rev Microbiol* **34**: 189–206.

Ranzoni FV (1956) The perfect state of *Flagellospora penicillioides*. *Am J Bot* **43**: 13–17.

Raviraja NS, Sridhar KR & Bärlocher F (1996) Breakdown of introduced and native leaves of two Indian streams. *Int Rev Hydrobiol* **81**: 529–539.

Raviraja NS, Sridhar R & Bärlocher F (1998) Breakdown of *Ficus* and *Eucalyptus* leaves in an organically polluted river in India: fungal diversity and ecological functions. *Freshwater Biol* **39**: 537–545.

Raviraja NS, Nikolcheva LG & Bärlocher F (2004) Pellet size affects mycelial ergosterol content in aquatic hyphomycetes. *Mycologia* **96**: 388–392.

Raviraja N, Nikolcheva L & Bärlocher F (2005) Diversity of conidia of aquatic hyphomycetes assessed by microscopy and by DGGE. *Microb Ecol* **49**: 301–307.

Read SJ, Moss ST & Jones EBG (1992) Attachment and germination of conidia. *The Ecology of Aquatic Hyphomycetes* (Bärlocher F, ed), pp. 135–151. Springer, Berlin/Heidelberg, Germany.

Reaves ML & Rabinowitz JD (2010) Metabolomics in systems microbiology. *Curr Opin Biotechnol* in press. DOI: 10.1016/j.copbio.2010.10.001.

Reddy CA (1995) The potential for white-rot fungi in the treatment of pollutants. *Curr Opin Biotech* **6**: 320–328.

Reddy GVB & Gold MH (1999) A two-component tetrachlorohydroquinone reductive dehalogenase system from the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Biochem Bioph Res Co* **257**: 901–905.

Reddy GVB, Joshi DK & Gold MH (1997) Degradation of chlorophenoxyacetic acids by the lignin-degrading fungus *Dichomitus squalens*. *Microbiology* **143**: 2353–2360.

Rhee S, Liu X, Wu L, Chong SC, Wan X & Zhou J (2004) Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. *Appl Environ Microb* **70**: 4303–4317.

Richie DL, Miley MD, Bhabhra R, Robson GD, Rhodes JC & Askew DS (2007) The *Aspergillus fumigatus* metacaspases CasA and CasB facilitate growth under conditions of endoplasmic reticulum stress. *Mol Microbiol* **63**: 591–604.

Rier ST, Tuchman NC, Wetzel RG & Teeri JA (2002) Elevated-CO_2-induced changes in the chemistry of quaking aspen (*Populus tremuloides* Michaux) leaf litter: subsequent mass loss and microbial response in a stream ecosystem. *J N Am Benthol Soc* **21**: 16–27.

Rosset J & Bärlocher F (1985) Aquatic hyphomycetes: influence of pH, Ca^{2+} and HCO_3^- on growth *in vitro*. *T Brit Mycol Soc* **84**: 137–145.

Rosset J, Bärlocher F & Oertli JJ (1980) Decomposition of conifer needles and deciduous leaves in two Black Forest and two Swiss Jura streams. *Int Rev Hydrobiol* **67**: 1–5.

Rossi L, Fano EA, Basset A, Fanelli C & Fabbri AA (1983) An experimental study of a microfungal community on plant detritus in a mediterranean woodland stream. *Mycologia* **75**: 887–896.

Rossman A (2007) Report of the planning workshop for all fungi DNA barcoding. *Inoculum* **58**: 1–5.

Ruiz-Duenas FJ, Morales M, Garcia E, Miki Y, Martinez MJ & Martinez AT (2009) Substrate oxidation sites in versatile peroxidase and other basidiomycete peroxidases. *J Exp Bot* **60**: 441–452.

Saccardo PA (1880) Conspectus generum fungorum Italiae inferiorum. *Michelia* **2**: 1–38.

Sack U, Heinze T, Deck J, Cerniglia CE, Martens R, Zadrazil F & Fritsche W (1997) Comparison of phenanthrene and pyrene degradation by different wood-decaying fungi. *Appl Environ Microb* **63**: 3919–3925.

Saitō K, Dixon RA & Willmitzer L, eds. (2006) *Plant Metabolomics*. Birkhäuser (Springer), Berlin, Germany.

Saitoh Y, Izumitsu K & Tanaka C (2009) Phylogenetic analysis of heavy-metal ATPases in fungi and characterization of the copper-transporting ATPase of *Cochliobolus heterostrophus*. *Mycol Res* **113**: 737–745.

Santos-Flores CJ & Betancourt-López (1997) Aquatic and water-borne hyphomycetes (Deuteromycotina) in streams of Puerto Rico (including records from other Neotropical locations. *Carib J Sci Spec Pub* **2**: 1–116.

Scheibner K, Hofrichter M, Herre A, Michels J & Fritsche W (1997) Screening for fungi intensively mineralizing 2,4,6-trinitrotoluene. *Appl Microbiol Biot* **47**: 452–457.

Schlief J (2004) Leaf associated microbial activities in a stream affected by acid mine drainage. *Int Rev Hydrobiol* **89**: 467–475.

Schlosser D, Solé M, Wesenberg D, Geyer R & Krauss G (2008) Responses to organic pollutants. *Novel Techniques and Ideas in Mycology, Fungal Diversity Research Series 20* (Sridhar KR, Bärlocher F & Hyde KD, eds), pp. 119–148. Fungal Diversity Press, Yunnan, P.R. China.

Schneider T, Gerrits B, Gassmann R, Schmid E, Gessner MO, Richter A, Battin T, Eberl L & Riedel K (2010) Proteome analysis of fungal and bacterial involvement in leaf litter decomposition. *Proteomics* **10**: 1819–1830.

Seena S, Wynberg N & Bärlocher F (2008) Fungal diversity during leaf decomposition in a stream assessed through clone libraries. *Fungal Divers* **30**: 1–14.

Seena S, Pascoal C, Marvanová F & Cássio F (2010) DNA barcoding of fungi: a case study using ITS sequences for identifying aquatic hyphomycete species. *Fungal Diver* **44**: 77–87.

Sheridan GE, Masters CI, Shallcross JA & Mackey BM (1998) Detection of mRNA by reverse transcription – PCR as an indicator of viability in *Escherichia coli* cells. *Appl Environ Microb* **64**: 1313–1318.

Singh G & Ashby AM (1999) Cloning of the mating type loci from *Pyrenopeziza brassicae* reveals the presence of a novel mating type gene within a discomycete MAT 1-2 locus encoding a putative metallothionein-like protein. *Mol Microbiol* **32**: 1115–1115.

Sinsabaugh RL, Osgood MP & Findlay S (1994) Enzymatic models for estimating decomposition rates of particulate detritus. *J N Am Benthol Soc* **13**: 160–169.

Solé M, Chatzinotas A, Sridhar K, Harms H & Krauss G (2008a) Improved coverage of fungal diversity in polluted groundwaters by semi-nested PCR. *Sci Total Environ* **406**: 324–330.

Solé M, Fetzer I, Wennrich R, Sridhar KR, Harms H & Krauss G (2008b) Aquatic hyphomycete communities as potential bioindicators for assessing anthropogenic stress. *Sci Total Environ* **389**: 557–565.

Solé M, Kellner H, Brock S, Buscot F & Schlosser D (2008c) Extracellular laccase activity and transcript levels of putative laccase genes during removal of the xenoestrogen technical nonylphenol by the aquatic hyphomycete *Clavariopsis aquatica*. *FEMS Microbiol Lett* **288**: 47–54.

Sridhar KR & Bärlocher F (1993) Effect of temperature on growth and survival of five aquatic hyphomycetes. *Sydowia* **45**: 337–387.

Sridhar KR & Bärlocher F (2000) Initial colonization, nutrient supply, and fungal activity on leaves decaying in streams. *Appl Environ Microb* **66**: 1114–1119.

Sridhar KR & Raviraja NS (2001) Aquatic hyphomycetes and leaf litter processing in polluted and unpolluted habitats. *Trichomycetes and Other Fungal Groups* (Misra JK & Horn BW, eds), pp. 293–314. Science Publishers, Enfield.

Sridhar KR, Krauss G, Bärlocher F, Wennrich R & Krauss G-J (2000) Fungal diversity in heavy metal polluted waters in Central Germany. *Fungal Divers* **5**: 119–129.

Sridhar KR, Krauss G, Bärlocher F, Raviraja NS, Wennrich R, Baumbach R & Krauss G-J (2001) Decomposition of alder leaves in two heavy metal-polluted streams in Central Germany. *Aquat Microb Ecol* **26**: 73–80.

Sridhar KR, Bärlocher F, Krauss G-J & Krauss G (2005) Response of aquatic hyphomycete communities to changes in heavy metal exposure. *Int Rev Hydrobiol* **90**: 21–32.

Sridhar KR, Bärlocher F, Wennrich R, Krauss G-J & Krauss G (2008) Fungal biomass and diversity in sediments and on leaf litter in heavy metal contaminated waters of Central Germany. *Arch Hydrobiol* **171**: 63–74.

Sridhar KR, Duarte S, Cássio F & Pascoal C (2009) The role of early fungal colonizers in leaf-litter decomposition in Portuguese streams impacted by agricultural runoff. *Int Rev Hydrobiol* **94**: 399–409.

Steffen KT, Hatakka A & Hofrichter M (2002) Removal and mineralization of polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. *Appl Microbiol Biot* **60**: 212–217.

Steinberg G (2007) Hyphal growth: a tale of motors, lipids, and the spitzenkorper. *Eukaryotic Cell* **6**: 351–360.

Stiling P & Cornelissen T (2007) How does elevated carbon dioxide (CO_2) affect plant–herbivore interactions? A field experiment and meta-analysis of CO2-mediated changes on plant chemistry and herbivore performance. *Glob Change Biol* **13**: 1823–1842.

Suberkropp K (1984) Effect of temperature on seasonal occurrence of aquatic hyphomycetes. *T Brit Mycol Soc* **82**: 53–62.

Suberkropp K (1991) Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. *Mycol Res* **95**: 843–850.

Suberkropp K (1992) Interactions with invertebrates. *The Ecology of Aquatic Hyphomycetes* (Bärlocher F, ed), pp. 118–134. Springer, Berlin/Heidelberg, Germany.

Suberkropp K (1995) The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. *Can J Bot* **73**: 1361–1369.

Suberkropp K (1997) Annual production of leaf-decaying fungi in a woodland stream. *Freshwater Biol* **38**: 169–178.

Suberkropp K (2008) Fungal biomass and growth. *Novel Techniques and Ideas in Mycology, Fungal Diversity Research Series 20* (Sridhar KR, Bärlocher F & Hyde KD, eds), pp. 203–213.

Suberkropp K & Klug MJ (1976) Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology* **57**: 707–719.

Suberkropp K & Klug MJ (1980) The maceration of deciduous leaf litter by aquatic hyphomycetes. *Can J Bot* **58**: 1025–1031.

Suberkropp K, Godshalk GL & Klug MJ (1976) Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology* **57**: 720–727.

Suberkropp K, Gessner MO & Chauvet E (1993) Comparison of ATP and ergosterol as indicators of fungal biomass associated with decomposing leaves in streams. *Appl Environ Microb* **59**: 3367–3372.

Tank JL, Webster J, Benfield EF & Sinsabaugh RL (1998) Effect of leaf litter exclusion on microbial enzyme activity associated with wood biofilms in streams. *J N Am Benthol Soc* **17**: 95–103.

Thompson PL & Bärlocher F (1989) Effect of pH on leaf breakdown in streams and in the laboratory. *J N Am Benthol Soc* **8**: 203–210.

Tiunov AV & Scheu S (2005) Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. *Ecol Lett* **8**: 618–625.

Tóthová L (1999) Occurrence of mitosporic fungi in the Slovak section of the Danube river. **54**: 379–385.

Triska FJ (1970) Seasonal distribution of aquatic hyphomycetes in relation to the disappearance of leaf litter from a woodland stream. PhD Thesis, University of Pittsburgh, Pittsburgh, PA.

Tsutsumi Y, Haneda T & Nishida T (2001) Removal of estrogenic activities of bisphenol A and nonylphenol by oxidative enzymes from lignin-degrading basidiomycetes. *Chemosphere* **42**: 271–276.

Tuchman NC, Wetzel RG, Riert ST, Wahtera KA & Teeri JA (2002) Elevated atmospheric CO2 lowers leaf nutrutional quality for strea ecosystem food webs. *Glob Change Biol* **8**: 163–170.

Tuchman NC, Wahtera KA, Wetzel RG, Russo NM, Kilbane GM, Sasso LM & Teeri JA (2003) Nutritional quality of leaf detritus altered by elevated atmospheric CO_2: effects on development of mosquito larvae. *Freshwater Biol* **48**: 1432–1439.

Tucker SL, Thornton CR, Tasker K, Jacob C, Giles G, Egan M & Talbot NJ (2004) A fungal metallothionein is required for pathogenicity of *Magnaporthe grisea*. *Plant Cell* **16**: 1575–1588.

Turner WB & Aldrigde DC (1983) *Fungal Metabolites*. Academic Press, London, UK.

Urich T, Lanzén A, Qi J, Huson DH, Schleper C & Schuster SC (2008) Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. *PLoS ONE* **3**: e2527.

Van Ho A, Ward DM & Kaplan J (2002) Transition metal transport in yeast. *Annu Rev Microbiol* **56**: 237–261.

Verdin A, Sahraoui AL & Durand R (2004) Degradation of benzo[a]pyrene by mitosporic fungi and extracellular oxidative enzymes. *Int Biodeter Biodegr* **53**: 65–70.

Veteli TO, Mattson WJ, Niemelä P, Julkunen-Tiitto R, Kellomäki S, Kuokkanen K & Lavola A (2007) Do elevated temperature and CO_2 generally have counteracting effects on phenolic phytochemistry of boreal trees? *J Chem Ecol* **33**: 287–296.

Vido K, Spector D, Lagniel G, Lopez S, Toledano MB & Labarre J (2001) A proteome analysis of the cadmium response in *Saccharomyces cerevisiae*. *J Biol Chem* **276**: 8469–8474.

Weber F, Hage K & de Bont J (1995) Growth of the fungus *Cladosporium sphaerospermum* with toluene as the sole carbon and energy source. *Appl Environ Microb* **61**: 3562–3566.

Webster J (1959) Experiments with spores of aquatic hyphomycetes: I. Sedimentation, and impaction on smooth surfaces. *Ann Bot* **23**: 595–611.

Webster J (1992) Anamorph–teleomorph relationships. *The Ecology of Aquatic Hyphomycetes* (Bärlocher F, ed), pp. 99–117. Springer, Berlin, Germany.

Webster J & Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. *Annu Rev Ecol Syst* **17**: 567–594.

Webster J & Descals E (1981) Morphology, distribution, and ecology of conidial fungi in freshwater habitats. *Biology of Conidial Fungi*, *Vol. 2* (Kendrick B, ed), pp. 419–451. National Museum of Natural Sciences, Ottawa, Canada.

Webster J & Towfik FH (1972) Sporulation of aquatic hyphomycetes in relation to aeration. *T Brit Mycol Soc* **59**: 353–364.

Webster J, Moran ST & Davey RA (1976) Growth and sporulation of *Tricladium chaetocladium* and *Lunulospora curvula* in relation to temperature. *T Brit Mycol Soc* **67**: 491–495.

Wesenberg D, Kyriakides I & Agathos SN (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnol Adv* **22**: 161–187.

Wesenberg D, Krauß G-J & Schaumlöffel D (2010) Metallo-thiolomics: investigation of thiol peptide regulated metal homeostasis in plants and fungi by liquid chromatography-mass spectrometry. *Int J Mass Spectrom* in press. DOI: 10.1016/j.ijms.2010.10.026.

Weyers HS & Suberkropp K (1996) Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. *J N Am Benthol Soc* **15**: 408–420.

Wood-Eggenschwiler S & Bärlocher F (1983) Aquatic hyphomycetes in sixteen streams in France, Germany and Switzerland. *T Brit Mycol Soc* **81**: 371–379.

Wood-Eggenschwiler S & Bärlocher F (1985) Geographical distribution of Ingoldian fungi. *Verh int Ver Limn* **22**: 2780–2785.

Wright DP, Johansson T, Quéré AL, Söderström B & Tunlid A (2005) Spatial patterns of gene expression in the extramatrical mycelium and mycorrhizal root tips formed by the ectomycorrhizal fungus *Paxillus involutus* in association with birch (*Betula pendula*) seedlings in soil microcosms. *New Phytol* **167**: 579–596.

Wu L, Thompson DK, Li G, Hurt RA, Tiedje JM & Zhou J (2001) Development and evaluation of functional gene arrays for detection of selected genes in the environment. *Appl Environ Microb* **67**: 5780–5790.

Wünschmann J, Beck A, Meyer L, Letzel T, Grill E & Lendzian KJ (2007) Phytochelatins are synthesized by two vacuolar serine carboxypeptidases in *Saccharomyces cerevisiae*. *FEBS Lett* **581**: 1681–1687.

Wysocki R & Tamás MJ (2010) How *Saccharomyces cerevisiae* copes with toxic metals and metalloids. *FEMS Microbiol Rev* **34**: 925–951.

Yadav JS, Doddapaneni H & Subramanian V (2006) P450ome of the white rot fungus *Phanerochaete chrysosporium*: structure, evolution and regulation of expression of genomic P450 clusters. *Biochem Soc T* **34**: 1165–1169.

Zak DR, Blackwood CB & Waldrop MP (2006) A molecular dawn for biogeochemistry. *Trends Ecol Evol* **21**: 288–295.

Zemek J, Marvanová L, Kuniak Ľ & Kadlečíková B (1985) Hydrolytic enzymes in aquatic Hyphomycetes. *Folia Microbiol* **30**: 363–372.

Zhang M, Bourbouloux A, Cagnac O, Srikanth CV, Rentsch D, Bachhawat AK & Delrot S (2004) A novel family of transporters mediating the transport of glutathione derivatives in plants. *Plant Physiol* **134**: 482–491.

Zhou J (2003) Microarrays for bacterial detection and microbial community analysis. *Curr Opin Microbiol* **6**: 288–294.