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Review

Resource seeking strategies of zoosporic true fungi in heterogeneous soil habitats at the microscale level

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ABSTRACT

Zoosporic true fungi have frequently been identified in samples from soil and freshwater ecosystems using baiting and molecular techniques. In fact some species can be components of the dominant groups of microorganisms in particular soil habitats. Yet these microorganisms have not yet been directly observed growing in soil ecosystems. Significant physical characteristics and features of the three-dimensional structures of soils which impact microorganisms at the microscale level are discussed. A thorough knowledge of soil structures is important for studying the distribution of assemblages of these fungi and understanding their ecological roles along spatial and temporal gradients. A number of specific adaptations and resource seeking strategies possibly give these fungi advantages over other groups of microorganisms in soil ecosystems. These include chemotactic zoospores, mechanisms for adhesion to substrates, rhizoids which can penetrate substrates in small spaces, structures which are resistant to environmental extremes, rapid growth rates and simple nutritional requirements. These adaptations are discussed in the context of the characteristics of soils ecosystems. Recent advances in instrumentation have led to the development of new and more precise methods for studying microorganisms in three-dimensional space. New molecular techniques have made identification of microbes possible in environmental samples.

1. Introduction

Soil ecosystems provide excellent habitats for a large number of species of organisms from all domains of life including archaea, bacteria, fungi, protists, animals and plants. In this paper we focus on one group of eukaryotic microorganisms, the zoosporic true fungi. In general these fungi have been poorly sampled in biodiversity studies in soil. However, assemblages of some species of zoosporic true fungi appear to be a predominant group of eukaryotic microorganisms present in some soil ecosystems, such as in high altitude soils (Freeman et al., 2009) and are well represented in many other ecosystems (Sparrow, 1960; Powell, 1993; Gleason et al., 2010a). Zoosporic true fungi possibly play keystone roles in the functioning of both soil and freshwater ecosystems (Sparrow, 1960; Powell, 1993; Gleason et al., 2008), but their apparent patchy and spatially limited distribution makes their population densities difficult to assess using current quantitative methods. Also their population sizes may change rapidly over time and across space (Sparrow, 1960). Sometimes they can be very abundant for a limited time, but rare for the rest of the time. Indeed, their persistence in soil depends on their capacity for growth, survival and subsequent radiation from isolated microenvironments. In this review we focus on the adaptations of zoosporic true fungi to small scale habitats within soils at the microscale level, both spacial (μm) and temporal (s).

Zoosporic true fungi (often called chytrids) include a very large and diverse group of microorganisms which have been commonly observed growing on many substrates in samples collected from both soil and freshwater habitats (Sparrow, 1960; Powell, 1993; Barr, 2001; Shearer et al., 2007). These fungi characteristically produce zoospores with a single posteriorly directed whiplash flagellum (Sparrow, 1960; Barr, 2001). Actively motile zoospores are the primary mechanism for dispersal in liquid phase over short distances (Sparrow, 1960; Gleason and Lilje, 2009). The asexual life cycle is relatively simple.

Most of the species of zoosporic true fungi that are found in soil are currently placed into the Phyla Chytridiomycota, Blastocladiomycota and Monoblepharidomycota (recently proposed by

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M. Powell, unpublished). The obligate anaerobic species in the Phylum Neocallimastigomycota are usually restricted to the digestive system of herbivorous vertebrates (Trinci et al., 1994; Liggenstoffer et al., 2010), although there is some evidence that they can survive in the soil as resistant structures (Davies et al., 1993). Species in the genus *Olpidium* still await taxonomic placement (James et al., 2006). These species are common parasites of flowering plants in agricultural soils (Powell, 1993). In addition species in the genus *Rozella* are common in both soil and aquatic ecosystems (Sparrow, 1960; Lara et al., 2010; Jones et al., 2011). These parasites of zoosporic true fungi and heterotrophic stramenopiles have recently been place in the Phylum Cryptomycota (Jones et al., 2011) or Rozellida (Lara et al., 2010) and are considered to be basal to the Fungi in the Supergroup Opisthokonta.

Baiting methods have historically been used to identify saprotrophic species growing in soil. A small sample of soil is usually mixed with sterile water and baited with plant or animal materials (Couch, 1939; Sparrow, 1960; Dogma, 1973; Barr, 1987, 2001). The species which colonize the baits are then identified by morphological characteristics. For example, Letcher and Powell (2001, 2002) used this method to study the diversity of zoosporic true fungi in different soils and habitats in the mountains of Virginia. In contrast, the study by Lozupone and Klein (2002) used molecular methods to identify zoosporic fungi in soils from Colorado. Although both baiting and molecular methods have provided much useful information about the distribution of species in ecosystems at the macroscale level, little information about the distribution of species in small scale habitats within soils at the microscale level is available, primarily because the sample sizes are too large.

Since both abiotic and biotic parameters in soil can greatly influence the distribution, abundance and diversity of zoosporic true fungi, these parameters would be expected to alter the distribution patterns of microorganisms in the sampling data. For example, some species of saprobic zoosporic true fungi prefer to colonize specific substrates (Sparrow, 1960; Powell, 1993; Gleason et al., 2011). Parasitic species often infect only one invertebrate host and must therefore be studied together with their host, for example, some Blastocladiomycota (Gleason et al., 2010b). Species which are parasites of the roots of flowering plants, such as *Olpidium*, are usually observed growing on host tissue in the rhizosphere (Hartwright et al., 2010). Some of these factors are considered in the context of current models in this review.

Information on the morphology, development and physiology of genetically diverse zoosporic true fungi has been provided from investigations with a relatively small number of species grown in pure culture in liquid or on solid media in the laboratory (Cantino and Mills, 1976; Powell, 1993, 1994; Barr, 2001; Lilje and Lilje, 2008; Digby et al., 2010; Gleason et al., 2010a, 2011). Nonetheless, some of the information obtained from these studies can be used to explain the ecology of zoosporic true fungi in soil ecosystems. In the laboratory the environment in liquid media is somewhat similar to aquatic ecosystems, and the environment on the surface of solid media is somewhat similar to terrestrial ecosystems.

Under certain conditions soil ecosystems share some characteristics with aquatic ecosystems, particularly during major flooding, intensive irrigation of agricultural lands and seasonally high water tables. Alpine sites receive large inputs of water during the spring (Freeman et al., 2009). These sites can be expected to provide an excellent habitat for zoosporic true fungi. In addition, soil ecosystems have some characteristics similar to terrestrial ecosystems, particularly when the water films in pores are thin, such as during long dry seasons. In this review we define terrestrial ecosystems as those which occur on the top of or above the soil and remain dry for long periods of time. In addition, soils also have many unique characteristics.

Newly developed methods allow examination of the structures of soils in thin sections (2D) and in X-ray microtomography (3D) (Young et al., 2008). Three-dimensional models can, in principle, be constructed from serial biological thin sections, but identification of species *in vivo* is currently very difficult at present and impossible using X-ray microtomography. However, identification of species may be possible in thin sections using the newly developed fluorescence *in situ* hybridization (FISH) technique (Jobard et al., 2010). Although recent research has provided a preliminary understanding of the three-dimensional structure of different types of soil (Ritz and Young, 2004; Young et al., 2008), the current models for soil structure and function have not adequately considered the diversity, distribution and functions of the microorganisms present within the soil at the microscale level.

In this review we examine the physical structures of soil and some of the environmental gradients present, and relate these to the morphological and physiological characteristics of zoosporic true fungi observed in culture. We also discuss the interactions between zoosporic true fungi and other groups of soil organisms and the relevance of some general principles of microbial ecology which apply to zoosporic true fungi in soil ecosystems. From this information we will predict how zoosporic true fungi have adapted to soil habitats and the advantages and disadvantages that these fungi have compared to other groups of microorganisms. Finally, some novel and innovative techniques for examining the structures of soils and the microorganisms living in them will be discussed.

We expect that many species of zoosporic true fungi are well adapted to growth and survival and that a large variety of niches are present in the soil. Morphological and physiological characteristics of some zoosporic true fungi make them particularly well suited for seeking resources and for rapid growth, survival and dispersal in many soil habitats. Yet all soil microorganisms face challenges such as spatial constraints in pores and channels, lack of moisture during droughts, anaerobic conditions in flooded soils, lack of utilizable nutrients and other extreme environmental conditions. These hypotheses remain to be carefully tested in future research.

2. Zoosporic true fungi and their special features

2.1. The life cycle

The asexual life cycle of zoosporic true fungi in liquid culture, on solid growth media and on solid substrates submerged in water will be described here. The thalli in monocentric species form only one sporangium and growth is determinant, while in polycentric species the nuclei migrate through hyphae and form multiple sporangia and growth is indeterminant. Because these fungi propagate by releasing actively mobile zoospores, at least a thin layer of water is necessary for the completion of the life cycle.

Fungal zoospores vary considerably in size and shape. Koch (1968) and Bernstein (1968), in their pioneering studies of motility, measured the diameter and shape of the zoospore bodies and length of the flagella in zoospores from a small number of isolates swimming in a thin film of water under controlled conditions in the laboratory. These isolates included species in several genera commonly found in soil: *Spizellomyces*, *Chytriomyces*, *Rhizophydium* and *Rhizophlyctis*. Shape of zoospores varied from spherical to ovoid to amoeboid, the diameters of the bodies of zoospores varied from 2 to 8 μm and flagellar lengths varied from 15 to 33 μm. These variables were influenced by growth conditions, including substrates and environmental factors. The data from Sparrow (1960), Koch (1968) and Bernstein (1968) give us an approximate size range for fungal zoospores. Most fungal zoospores are in the size range defined for motile cells of

heterotrophic flagellates (approximately ≤ 5 μm) (Sime-Ngando et al., 2011).

When a 0.2 ml drop of sterile distilled water with zoospores is placed on the surface of solid growth media in Petri dishes, the zoospores swim about and randomly populate the volume of the thin film of water (Gleason et al., 2011). The zoospores soon settle down, attach to the surface of the agar, encyst and then germinate. Zoospores often attach to the sides of plastic tubes and to sand grains (Henderson, unpublished data) when grown in liquid media. However, in liquid media in the laboratory many zoospores never attach to any solid surfaces prior to encystment. The length of time during which zoospores remain motile varies. Barr (1969a) recorded maximum times of 5–72 h in water, but the average time was less than 2 h. However, Cantino and Mills (1976) estimated a maximum of 10 h.

The rhizoidal systems produced after germination of the zoospores vary considerably in morphology, size and complexity (Sparrow, 1960). The rhizoids of some species have extremely elaborate branching patterns (Barr, 1969a, 1984). The diameters of some of the smaller branches approach the limits of resolution in the light microscope (0.2 μm) and therefore are difficult to estimate. In polycentric species the diameter of the rhizomycelium can also be small.

The zoosporangia also vary greatly in size. For example, Bostick (1968) observed that zoosporangia in *Chytriomyces hyalinus* varied in diameter from 5 to 75 μm depending on temperature and type of substrate. Finally, mature zoosporangia can release zoospores under appropriate conditions. A maturation process is required before the zoospores are ready for release (Cantino and Mills, 1976). The environmental cues for zoospore release are not understood, but flooding thalli grown on solid growth media with water often stimulates zoospore release (Koch, 1968; Barr, 1987, 2001; Gleason and Lilje, 2009). Zoosporic true fungi have adapted to the drying-wetting cycle in soil ecosystems to varying degrees by the formation of resistant sporangia (Gleason et al., 2010a).

The rhizoids of zoosporic true fungi can easily penetrate solid substrates. For example, saprobic species can penetrate the thick walls of pollen grains (Barr, 1970; Phuphumirat et al., 2011) and fibre in the rumen (Joblin, 1989; Trinci et al., 1994). Zoospores of the parasites of flowering plants and invertebrate animals attach to surfaces, and the rhizoids penetrate and grow into the host tissue (Gleason et al., 2010b). The rhizoids of *Zygorhizidium affluens* and *Zygorhizidium planktonicum*, parasites of the diatom, *Asterionella formosa*, penetrate the host cell by squeezing between the upper and lower girdle lamellae, a space of only approximately 0.1–0.2 μm in diameter (Beakes et al., 1992). Zoosporic true fungi are common parasites of many species of phytoplankton and cyanobacteria with tough cell walls (Kagami et al., 2007; Gutman et al., 2009; Rasconi et al., 2011).

2.2. Nutrition

There is no evidence that zoosporic true fungi have phagotrophic nutrition during any stage of their life cycle (Gleason and Lilje, 2009). Since the rhizoids release enzymes for extracellular digestion, their nutrition is osmotrophic. Sparrow (1960) lists a large number of plant and animal materials which are good substrates for zoosporic true fungi. Many saprophytic zoosporic true fungi can be grown on media in which a single carbon source is the only significant organic substance present (Barr, 1969b; Digby et al., 2010). Commonly available carbon sources include cellulose, chitin, starch, protein and lipids (macromolecules). Nitrate, ammonium, sulphate and phosphate ions provide good sources of nitrogen, sulphur and phosphorous for some of these fungi (Barr, 1969b; Digby et al., 2010). Some species are even able to dissolve insoluble phosphate sources (Midgley et al., 2006). In addition, zoosporic true fungi are able to survive in nutrient depleted environments for long periods of time, possible aided by re-absorption of organic compounds from dead thalli (Lilje and Lilje, 2008).

2.3. Mechanisms for dispersal

Zoosporic true fungi have a number of mechanisms for dispersal (Koch, 1968; Gleason et al., 2008, 2010a; Gleason and Lilje, 2009). **Active, directed and short distance**: (1) The whiplash flagellum can propel zoospores in liquid water for short distances. (2) Amoeboid zoospores can creep along solid surfaces or through gels using their pseudopods. **Passive, by abiotic factors, not directed and short or long distance**: (3) Entire thalli attached to substrates can be carried passively in water currents. (4) Resistant structures, such as zoospore cysts and resistant sporangia, can be carried passively by air or water currents or by animals in the soil. (5) Zoosporic true fungi can be dispersed by movement of the soil itself. **Passive, by biological vectors and short to medium distance**: (6) Earthworms transport a variety of zoosporic true fungi in the digestive system or on the surface as they burrow through soil (Thornton, 1970). Nematodes may carry parasitic species (Gleason et al., 2010b). (A number of other soil invertebrates, such as mites, are known to feed on fungi and may transmit fungi through the soil.) Powell and Blackwell (1991) described a proposed passive dispersal mechanism for *Septosperma rhizophydii*. This species produces rocket-shaped resting spores which could be carried by water currents through small channels and pores in soil.

2.4. Ecological strategies

Three ecological strategies of true fungi are recognized by Dix and Webster (1995): (i) competitive (C-selected); (ii) stress-tolerant (S-selected), and (iii) ruderal (R-selected). Under suitable environmental conditions such as the presence of new substrates or nutrients or when the temperature warms some zoosporic true fungi can grow rapidly (Sparrow, 1960; Marano et al., 2011). For example, Sparrow (1960) observed "chytrid epidemics" in ponds in the spring in cold climates. These fungi are considered to be ruderals (R-selected). Many zoosporic true fungi can form resistant structures so they are stress-tolerant (S-selected) as well. Experimental evidence suggests that zoosporic true fungi can survive a wide range of environmental conditions ranging from very cold to very hot environments (reviewed by Gleason et al., 2010a). Different strategies may be adopted under different environmental conditions, so that zoosporic true fungi can occupy many ecological niches and contribute significantly to the biomass under suitable conditions.

3. Zoosporic true fungi and the abiotic environment in soils

3.1. Physical characteristics of the soil

3.1.1. Soil layers

Many substrates for the growth of microorganisms can be found on the surface of soils. For example, pollen grains, like all other airborne particles, frequently settle on the surface of soil and water. This can be seen clearly after rains during the flowering seasons of the dominant plant species. For instance, in coniferous forests pollen grains form yellow spots on the soil surface. Pollen grains are excellent substrates for the growth of zoosporic true fungi in natural ecosystems (Goldstein, 1960; Sparrow, 1960; Lee, 2000; Phuphumirat et al., 2011). Zoospores attach to the surface of pollen grains either floating or submerged in water or on the surface of

moist soil, encyst and germinate. The rhizoids must penetrate the exine walls of the pollen grains which contain sporopollenin and other recalcitrant molecules in order to reach the starch reserves which are good carbon sources for some species (Gleason et al., 2011). Molecules and ions adsorbed by rhizoids have been shown to be transported to the sporangium in thalli of *Blastocladiella emersonii*, an obligate aerobe (Kropp and Harold, 1982). However, the surface of the soil is subject to rapid drying.

Highly vegetated soils often have thick layers of leaf litter at the surface (O horizon). These layers can provide a wide diversity of utilizable plant and animal substrates, adequate moisture and high oxygen tensions and are thought to be excellent habitats for zoosporic true fungi (Sparrow, 1960). Since these layers are loosely packed, there are relatively large volumes filled with either water and/or air and the composition within these volumes can change very rapidly. It is very likely that saprobic zoosporic fungi are abundant and diverse within these litter layers at least during times when the litter is wet or water saturated. This is supported by recent studies on the role of zoosporic fungi in the decomposition of leaves in aquatic environments: it was shown that zoosporic true fungi are more diverse within the fungal community that promotes the decomposition of leaves than are mitosporic hyphomycetes (Nikolcheva and Bärlocher, 2004; Seena et al., 2008). Therefore soils covered by a distinct leaf litter layer can provide excellent habitats for zoosporic true fungi.

The top soil (A horizon) lies beneath the leaf litter and is the upper-most mineral layer of the soil. In many agricultural and natural soils this layer can also contain a high concentration of organic matter (such as in humus), but it is more densely packed than leaf litter. Zoosporic true fungi have the ability to grow here as well (Sparrow, 1960). Because zoosporic true fungi present in soil ecosystems are mainly aerobic and strongly depend on the availability of water and oxygen for survival and propagation, it is also very likely that within this soil layer a large number of species and a large portion of the biomass of zoosporic true fungi can be found. Within soils steep vertical gradients of resources such as air, water or nutrients can be found (Hinsinger et al., 2009). Plant parasitic species associated with roots will be mainly restricted to the A horizon assuming that the highest root densities can be found within this layer.

Beneath the top soils lie various other layers of mineral soils (E, B and C horizons) above unweathered parent material (R horizon). Although the concentration of organic matter is generally low, microorganisms are known to interact with organic and mineral particles of varying sizes in subsoil horizons. The change in availability of soil carbon and other resources with depth was found to significantly explain changes in the distribution of soil microbial communities (Steenwerth et al., 2006).

Finally the rhizosphere includes plant roots, a large diversity of bacteria, fungi, protists and metazoa as well as organic and inorganic matter. There is both dissolved and insoluble organic and inorganic matter in soil water. The rhizosphere and its relevance for zoosporic fungi will be discussed later in this review.

3.1.2. Soil structure

In general, data from soil profiles, types of vegetation present, geological formations from which the soil is derived and the chemical characteristics of soil samples provide little useful information for characterization of microscale habitats, because soil is a heterogeneous medium. Analysis of the physical environment across spatial and temporal microscales is necessary.

The complex structure of soil determines the rates of many of its key physical and biological processes including diffusion and convection, and the distribution of oxygen and water (Young et al., 2008). The structure also provides the habitat for the resident microbial community, and its fractal-like structure reflects the broad range in scale over which heterogeneity predominates from nanometres to the landscape (Perrier et al., 1999; Turcotte, 2007). At the pore scale, this heterogeneity is manifest in the broad range of effective pore sizes that store water across a correspondingly wide range of environmental conditions (Sukop et al., 2001). Indeed this heterogeneity is responsible for the fact that soil structure affects the balance of air and water which is a major determinant of the rate and nature of microbial processes (Ritz and Young, 2004; Young et al., 2008).

The structure of soil is not static and is subject to continuous change, largely through the action of surface tension and physical reworking by biological processes. During wet–dry cycles, water-film dynamics creates internal forces that pull particles apart to form cracks. The creation of such cracks is a strong function of the percentage and chemical composition of the clay fraction. During a wetting event, soil structure can be severely disrupted through slaking as surface tension forces water into the structure and increases the pressure of trapped air up to the failure point. The re-aggregation of soil structure is promoted by water films, but the process is known to be modified at the pore scale by microbial activity. Tisdall and Oades (1982) originally proposed the mechanism where the by-products of microbial metabolism bind soil particles. This has subsequently been demonstrated, for example, with arbuscular mycorrhizal fungi that produce glomalin-related soil protein, an insoluble exudate which is strongly correlated with degree of soil microaggregation (Rillig, 2004). At larger scales, the growth of plant roots and fungal hyphae and the movement of animals, such as earthworms (Young et al., 2008), nematodes and insects [such as termites and ants] (Evans et al., 2011), create structure. However, it is at the pore scale that structural genesis is so important for impacting on microbial processes.

All these processes as well as structure will strongly influence the survival and active dispersal of zoosporic true fungi. Microscopic cracks formed during drying cycles can provide routes for the dispersal of the zoospores during phases of water saturation. On the one hand, the water films which are formed provide a “highway” for actively swimming zoospores, and also for passive dispersal of zoosporic fungi with the soil water currents. On the other hand, soil microbes involved in microaggregation influence the dispersal and survival of zoosporic fungi, because they restrict the pore size in soil and consequently the pores will not permit the passage of the zoospores. Soil microbes can also compete for nutrients and space within the microaggregates and consequently inhibit the further colonisation of microhabitats. However, these aggregations of bacteria and fungi attract a wide range of predators such as protists or small metazoans. These predators can be infected by parasitic zoosporic fungi and after their death can be subsequently colonized by saprobic zoosporic fungi.

As the water evaporates from the surface of solid media in the laboratory the growing thalli come in direct contact with the air and dry out quickly. When the thin layer of water recedes on the surface of solid media, zoospores must stop swimming and encyst, and when a zoospore cyst or the entire thallus dries out, it becomes dormant. Later when moisture returns, the zoospore cyst can germinate and develop into a mature thallus, and the dormant thalli can resume growth. Zoosporic true fungi can persist as dormant stages under unfavourable conditions for a long period of time (Gleason et al., 2010a). We expect that this ability of zoosporic true fungi to recover growth makes them highly compatible with soil environments.

Microorganisms depend upon the rapid movement of solutes in the liquid phase, especially oxygen, carbon dioxide, organic and inorganic nutrients. The liquid phase in pores and channels often consists of thin films of water. Many microorganisms including

zoosporic true fungi live within these thin films. The thin films adhere to solute active pore surfaces and are controlled by matric potential, pore scale structure and the influence of exudates including those from plant roots (Read et al., 2003). Importantly, the distribution of these thin films affects the flow of solutes and gases (e.g. oxygen and carbon dioxide). Soil water under a high matric potential will predominantly comprise thin films and a relatively high volume of air-filled pore space. Under these conditions oxygen will move freely, but solute flow will be limited. By contrast, soil water under low matric potentials will have relatively few thin films, and oxygen flow will be restricted in comparison to solute movement. This trade-off between solute and gas flow leads to the creation of complex gradients of resources in soil and to an enormous diversity of microenvironments. The 'hostility' of soil to colonisation is reflected in the fact that only around 0.01% of the potential surface area is occupied by microorganisms (Young and Crawford, 2004). It is only because of the high surface area per unit volume resulting from soil's fractal structure that the numbers of microorganisms per unit volume are high.

Zoosporic true fungi grow either submerged in shallow liquids or at the gas/liquid interfaces. Since most species are obligate aerobes (Gleason et al., 2007) they need to remain relatively close to the surface so that oxygen tension is not limiting in order to attain optimum growth rates. It is often necessary to shake liquid cultures to increase oxygen tension and to obtain good growth rates (Gleason et al., 2010a, 2011). This suggests that the rate of diffusion of oxygen can be a limiting factor and that zoosporic true fungi are better adapted to colonizing the surface of exposed microaggregates where oxygen is plentiful.

Bacterial diversity in soil has been found to increase with decreasing water potential (Carson et al., 2010). As soil begins to dry from the saturated state there is a reduction in the connectivity of soil water within the pore spaces, providing protective microhabitats. For zoosporic fungi, it is also likely that the thickness of the water films will affect motility, and hence the effective connectivity of the pore space because of surface effects. Wallace (1958) notes that water film thickness has an optimum in regard to nematode movement. When soil is too dry, the nematodes become trapped by surface tension, and when soil is too wet, the thickness of the water films prevents efficient swimming. The result is a relatively narrow range of matric potentials between which nematodes move freely in soil. Whether this is also true of zoosporic fungi is not known, but as the underlying physics will be the same, it is likely to be relevant, though the relevant film thickness will be different because zoospores are smaller than nematodes. Indeed there may be considerable value in applying some of the theoretical and experimental concepts in regard to the impact of soil structure on nematode movement to research with zoospores (Feltham et al., 2002; Rodger et al., 2004; Hapca et al., 2007). The corresponding change in the scale (from nematodes to zoospores) is likely to create additional factors that influence movement as a result of the low Reynolds number experienced by these small organisms in water (Purcell, 1977).

Fungal hyphae are known to tunnel into mineral particles (Smits, 2006). For example, Hoffland et al. (2003) and van Schöll et al. (2008) suggested that ectotrophic mycorrhizae are responsible for the formation of tunnels, 3–10 μm in diameter, in feldspar grains. Organic ions produced by fungi may facilitate the process of tunnelling and the release of soluble inorganic cations into soil water from the mineral particles. Mineral tunnelling occurs predominantly in feldspar grains in the upper few centimetres of the eluvial (E) horizon of podzol soils (Smits, 2006). Feldspar is an aluminosilicate mineral which contains Si, Al and Na with either K or Ca. Although there is no evidence currently available for tunnel formation by zoosporic fungi, zoosporangia are known to attach, via rhizoids, to mineral surfaces (Henderson, unpublished data). It is also known that some zoosporic fungi species dissolve insoluble minerals (Midgley et al. 2006).

3.1.3. Other interactions between fungal structures and the soil

Zoospores of some species have been shown to be positively chemotactic, that is, they respond to chemical gradients (Machlis, 1969; Held, 1974; Orpin and Bountiff, 1978; Mitchell and Deacon, 1986; Moss et al., 2008). The chemicals to which they respond vary with species, but they include utilizable organic substrates and mineral ions. Once the zoospores reach the surface of the substrate, they attach. As previously stated zoospores of some species can swim for several hours. Very little is known about the process of adhesion to substrates, but this process has been studied briefly in a few species (Travland, 1979; Tunlid et al., 1991; Deacon and Saxena, 1997; Richardson et al., 1998). Most of this research has focused on parasitic rather than on saprobic species. The environmental cues which trigger the encystment of zoospores are not thoroughly understood. Some information on the encystment process in *B. emersonii*, *Allomyces macrogynus* and *Catenaria anguillulae* has been published by Cantino and Mills (1976), Barstow and Pommerville (1980) and Deacon and Saxena (1997). However, in many species, changes in temperature, changes in moisture, depletion of endogenous reserves, arrival at utilizable substrates and other environmental factors have been observed to trigger encystment. It is very likely, although not yet proven, that these triggers play a decisive role in resource seeking in the environment and in their reaction to changes in the abiotic environment.

Rhizoids formed by zoosporic true fungi have at least four important functions: attachment to the substrate, mechanical disruption of tissues, accessing utilizable substrates and release of enzymes for digestion of substrates. All four processes have been intensively studied in the rumen and hindgut of mammals where the rumen fungi (Phylum Neocallimastigomycota) play key roles in digestion of fibre (Joblin, 1989; Trinci et al., 1994). Rumen fungi are not usually found growing in soil because they are obligately anaerobic (Trinci et al., 1994). However, we expect the rhizoids of other groups of zoosporic true fungi to have similar functions in the soil.

Rhizoids may also contribute to colony formation. Zoosporic true fungi have been observed to form colonies in both solid and liquid culture media (Lilje and Lilje, 2008). In liquid culture the colonial structure of an isolate of *Rhizophydium* was facilitated by the entanglement of zoospores in branching rhizoids of mature thalli (Lilje and Lilje, 2008). As a result these colonies were composed of thalli at different stages of the life cycle. The potential of zoosporic true fungi to form colonies suggests a possible mechanism by which they may survive harsh conditions in the environment, and therefore this process will very likely occur in soil ecosystems.

In general the filamentous growth of true fungi and heterotrophic stramenopiles gives them a distinct ecological advantage over bacteria in heterogeneous soil environments. Apical growth allows translocation of nutrients through the mycelia network. However the larger size of eukaryotic cells (>3 μm) relative to the size of prokaryotic cells (0.5–1 μm) excludes many eukaryotic microorganisms from the small micropores. For this reason we would expect larger eukaryotic microorganisms to be more susceptible to predation and more vulnerable to the wet–dry cycle than the smaller bacteria. The larger filamentous true fungi and heterotrophic stramenopiles and many groups of heterotrophic protists would prefer exploitation of soils through the larger air and water-filled pores and channels and soil water in larger volumes. In

contrast, the smaller size of polycentric zoosporic true fungi would allow them to grow in small micropores and possibly to translocate nutrients through rhizoids and the rhizomycelium. Kropp and Harold (1982) clearly documented the translocation of nutrients through the rhizoids of *B. emersonii*, a monocentric species.

3.2. Environmental gradients

Much research on the distribution of zoosporic true fungi has been conducted over many years in lentic ecosystems, for example, in a number of lakes in Northern Michigan (Paterson, 1967) and in the English Lakes District (Willoughby, 1961) and more recently in several lakes in Poland (Czeczuga and Muszyńska, 2004) and France (Lefèvre et al., 2007) and in a stream in Argentina (Marano et al., 2011). Extensive data on physical (abiotic) factors is available for many of these sites. In lakes and large streams physical factors usually change slowly over broad temporal and spatial scales during the seasons. As a consequence transects are often used to sample across different habitats in freshwater ecosystems.

In contrast in soils the physical factors can change rapidly over time, primarily because of the drying-wetting and freezing–thawing cycles. Also, because of microscale patchiness and heterogeneity, gradients can operate over a very small spatial scale such as μm or smaller (Young et al., 2001). Therefore in soils the temporal and spatial scales are very different from those in most freshwater environments such as lakes and large streams. These small scale volumes are not considered in most procedures for soil sampling, but the temporal and spatial gradients are very important for the microorganisms present (Baker et al., 2009). Methods for sampling at the microscale level are necessary to monitor and understand the processes that contribute to soil formation.

Important gradients include moisture content, temperature, dissolved oxygen tension, dissolved carbon dioxide concentration, pH, osmotic and matric potential and concentration of nutrients. Zoosporic true fungi have adapted to grow and survive within a range of values for many abiotic factors (Gleason et al., 2010a), but in soils these values constantly change. Nonetheless, a number of abiotic factors in soil can stabilise conditions - such as small scale storage of water and the immobilisation of mineral ions by soil pores. It is also possible that frequent change in physical factors may reduce competition by eliminating genotypes which have not adapted. Therefore, perhaps adaptation to frequent environmental change is important for many types of soil microorganisms.

We expect that the wide range of microhabitats in soil due to heterogeneity results in increased niche diversity. For example, a study of intra-aggregate microbial soil communities found both obligate aerobic and anaerobic bacteria present (Hansel et al., 2008). This suggests that oxygen gradients create microhabitats within soil aggregates.

4. Zoosporic true fungi and the biotic environment

4.1. Zoosporic true fungi and the microbial loop in soil

The concept of the microbial loop was originally proposed to describe the roles of bacteria in aquatic food webs. Recently Bonkowski (2004) applied this concept to eukaryotic microorganisms in the rhizosphere. The rhizosphere is the portion of soil that surrounds the plant roots and is heavily influenced by root exudates and the microorganisms associated with the plant. The rhizosphere provides an interface where soil organisms strongly interact, and the biogeochemical cycles are central components of these interactions (Dessaux et al., 2009). Within soils, plant roots are also very important drivers of the formation of biotic microhabitats. Many plants are known to secrete a large diversity of organic compounds, some of which contain nitrogen, into the rhizosphere as exudates. Some estimates suggest that up to half of the net carbon fixed by photosynthesis in vascular plants can be released into the rhizosphere. Mycorrhizal fungi and nitrogen fixing bacteria could absorb 20–40% of this carbon, but the remainder is available to support the growth of other groups of prokaryotic and eukaryotic microorganisms in the rhizosphere (Jones et al., 2009).

Grazing bacterivorous protozoa, especially naked amoebae, and bacterivorous nematodes control the population sizes of bacteria by top-down regulation. Naked amoebae graze biofilms and colonies attached to the surface of roots and soil particles (Bonkowski, 2004). The diameter of many “protozoa” is between 10 and 100 μm so that many of them are unable to enter small pores in the soil. However, pseudopods can reach into soil pores and thin water films in search for bacterial colonies. Bacterivorous nematodes can migrate quickly into large volumes with high bacteria and protozoan densities. The presence of plant roots, protists, nematodes and other suitable hosts can boost the numbers of zoosporic fungi as well. However, zoosporic true fungi can enter the soil microbial loop simply as food sources for grazing protists and soil animals. The role of zoosporic true fungi within terrestrial food webs might be similar to that in aquatic ecosystems. Algal and plant parasites and litter decomposers are primary consumers. Parasites of animals which eat algae are secondary consumers. These zoosporic true fungi can transfer energy to higher trophic levels in soil ecosystems. Zoosporic true fungi need to be included in the microbial loop concept for soil as a link between trophic levels, because like bacteria they decompose detritus and dissolved organic matter, and they are subsequently grazed by protists and metazoans (Gleason et al., 2008). Along with other saprobic true fungi and heterotrophic stramenopiles, zoosporic true fungi contribute to the turnover of nutrients in the rhizosphere. However, quantitative data is not yet available.

4.2. Food webs

Fungal zoospores in freshwater ecosystems are known to provide valuable food resources to grazing and filter feeding metazoans (Kagami et al., 2007; Gleason et al., 2008). Various groups of ciliates, amoeba and metazoans are known to feed on spores and hyphae of higher fungi (Old and Darbyshire, 1978; Bärlocher and Brendelberger, 2004). Cladocerans, ciliates and amoeba also feed on zoospores and thalli of zoosporic true fungi in both soil and freshwater ecosystems (Sparrow, 1960; Kagami et al., 2007; Gleason et al., 2008; Gleason, unpublished observations).

Many species of zoosporic true fungi found in soil are saprobes and release zoospores into soil water (Sparrow, 1960). But other species can be parasites. Species of *Olpidium*, *Physoderma* and *Synchytrium* are common parasites of plant roots, tubers and other parts of plants in contact with soil (Powell, 1993). The zoospores of parasites are likely to remain in the soil water in the rhizosphere near their hosts, but they can be washed away by water currents. Also hyperparasitic zoosporic true fungi are frequent parasites of other species of zoosporic true fungi (Karling, 1942). *Rozella*, a genus in the Cryptomycota, includes species which are parasites of several common species of zoosporic true fungi found in soil (Held, 1981). Jones et al. (2011) found zoospores putatively belonging to *Rozella* in many environmental samples using molecular techniques.

Matter and energy in zoospores are transferred from these primary consumers to secondary consumers in food webs. We expect that zoosporic true fungi have mechanisms to avoid predation by growing in small volumes or by becoming inaccessible

by the production of biofilms. However, the dynamics of these populations have not been studied in soil ecosystems.

In both soil and freshwater habitats zoosporic true fungi play important roles in the biogeochemical cycles (Gleason et al., 2008). Over time the growing fungi will reduce the concentration of inorganic nutrients and add more organic compounds to the soil. Many species can degrade recalcitrant dissolved and insoluble organic compounds. Since many species can use nitrate as a nitrogen source and sulphate as a sulphur source (Digby et al., 2010), the enzymatic systems for their reduction are present. These enzymatic systems may also be able to reduce ferric, manganous and other oxidized metallic ions in soils as well (Ottow and von Klopotek, 1969; Timonin et al., 1972). However zoosporic true fungi must compete with plant roots and other fungi for nitrate and other inorganic ions. Later nitrogen and other elements are mineralized by the microbial loop.

5. Application of specific resource seeking strategies in soil ecosystems

One important resource seeking strategy is the ability of zoospores to locate utilizable carbon sources quickly. Zoospores which have been studied in the laboratory can detect chemical gradients and orient their swimming behaviour appropriately (chemotaxis) (Gleason and Lilje, 2009). Because of their small size (approximately 2–8 μm in diameter), they can swim through some of the smaller water-filled channels, thin films and pores in the soil. Furthermore, they can swim quickly through the larger channels freshly formed by the movement of small invertebrates in search of new substrates but the actual speed of swimming is unknown.

In the soil rhizoids of monocentric zoosporic true fungi have an advantage over the hyphae of many other groups of fungi and heterotrophic stramenopiles because of their small diameter which allows penetration into volumes which are too small for most hyphae. Furthermore rhizoids appear to be chemotropic because they tend to grow up the chemical gradient towards utilizable substrates. Rhizoids can release enzymes to soften the tissues as they grow (Trinci et al., 1994). Growth in diameter of rhizoids can exert hydrolic pressure strong enough to break apart fibrous substrates (Joblin, 1989). Growth of fungal hyphae can displace soil particles (Young et al., 2008) and therefore alter the structure of the soil. Consequently growth of fungal rhizoids and hyphae also influences the availability of substrates on a microscale.

Both in growth media and on natural substrates, zoospores and rhizoids adhere tightly to surfaces such as glass, plastic, sand, baits, plant fibre, agar and other rhizoids (Sparrow, 1960; Barr, 1987, 2001; Gleason et al., 2011; Henderson and Lilje, unpublished observations). In the soil we expect adhesion to prevent dislodgement of the developing thallus from the substrate by water currents.

Zoospore cysts, resistant sporangia and small dormant thalli can be carried passively by water currents or by animals in the soil such as earthworms (Thornton, 1970), nematodes or dipteran larvae (Gleason et al., 2010b) or by movement of the soil itself and are important in dispersal. Some species have structures which are resistant to drying, freezing, high temperatures, high salinity and extremes of pH which possibly insure survival under extreme conditions (Gleason et al., 2010a).

Many species of zoosporic true fungi have rapid growth strategies (R-strategies) when the temperature increases or when new substrates become available, such as pollen grains and algal hosts in the spring in cold climates. Rapid growth of both saprobes and parasites contribute to the epidemics in freshwater habitats described by Sparrow (1960). Rapid growth of some species, such as *Rhizophlyctis rosea*, has also been observed in soil (Marano et al., 2011). High population densities can result.

Monocentric zoosporic true fungi appear to grow well at the solid/liquid/gas interfaces such as on the surface of nutrient agar (Barr, 1987; Gleason et al., 2011). Here they have access to dissolved nutrients, adequate moisture and sufficient dissolved oxygen. Over time if the colonies dry out, growth stops, but growth can resume once conditions favourable for growth return.

Most species of zoosporic true fungi are obligate aerobes (Gleason et al., 2007). Yet they are able to survive for short periods of time under anaerobic conditions. This is an important adaptation when oxygen is temporarily unavailable in the soil, such as when soil is fully saturated with water during flooding.

Many species have relatively simple nutrient requirements which make growth more possible in mineral soils. In some species the only organic requirement is the carbon source (Barr, 1969b; Digby et al., 2010). Some species of zoosporic fungi release organic acids from the fermentation of carbohydrates (Gleason et al., 2007) which may accelerate the decomposition of rocks and provide mineral ions essential for growth of microorganisms and plants.

Finally zoospores can swim into small volumes of water (micropores) where large predators are less likely to reach the developing thalli. Small volumes possibly exclude large protists and metazoans such as amoebae, ciliates, and cladocerans and other common predators. However, some species of vampyrellid amoebae can produce pseudopods which can extend into small volumes and penetrate their prey (Old and Darbyshire, 1978). In addition small volumes of water may provide some protection from desiccation since they are remote from the drier external atmosphere.

In summary, zoosporic true fungi are well adapted to a number of the diverse conditions in soils: they (i) tolerate wide ranges of environmental factors, (ii) can use many abundant materials as energy sources, (iii) can attach to soil particles, (iv) can be dispersed with soil water over long distances, and (v) can persist under adverse conditions for long times, and (vi) can multiply quickly when a suitable nutrition source is available and environmental conditions are appropriate.

6. Future perspectives

Recent advances in conventional and synchrotron-based X-ray instrumentation and molecular techniques such as FISH have provided new opportunities for soil microbiologists to examine the fungal–mineral interface. Atomic force microscopy has been used to look at mineral weathering by fungal exudates (Sutheimer et al., 1999). The combining of previously mutually exclusive microscopic techniques into a single platform has resulted in expanding our understanding of fungal–mineral interactions by providing an alternative insight into this complex system. For instance, scanning transmission electron microscopy (STEM) coupled with energy dispersive spectroscopy has provided high resolution elemental distribution maps of soil in addition to mapping the oxidative state of elements (reviewed in Smits, 2009).

The three-dimensional visualisation of the soil–microbe complex is becoming more sophisticated with the advancements in instrumentation. X-ray microtomography provides a flexible alternative for the three-dimensional visualisation and modelling of the fungal–mineral interface (Grose et al., 1996; Feeney et al., 2006; Mooney et al., 2006; Nunan et al., 2006; Deacon et al., 2008; Young et al., 2008; Hallett et al., 2009). The non-destructive scanning of samples has increased our understanding of the distribution patterns and relationships between soil particulates, pores, organic matter and microorganisms. Attempts to directly visualise fungi in soil using X-ray microtomography have

had limited success as a result of the very poor X-ray signal generated by fungi. An alternative approach may be to simulate properties of soil in an artificial culture matrix that enhances the three-dimensional soil constructs but allow electron signals from the stained fungi to be visualised and quantified using X-ray tomography (Lilje et al., unpublished data). The simplified culture modelling approach may provide yet another insight into the highly complex interactions in soil.

FISH and other molecular techniques are increasingly being used to identify unknown microorganisms in environmental samples (Jobard et al., 2010) and these procedures can be adapted for use at the microscale level (Jones et al., 2011). The construction of suitable probes is time consuming but critical (Sanz and Köchling, 2007). This will be particularly true for the identification of zoosporic fungi. However, with further refinement of these techniques and their adaptation to soil ecosystems, it is expected that an increasing number of zoosporic true fungi will be detected.

7. Conclusions

A thorough knowledge of the structure of soil at the microscale level is necessary for studying the distribution of assemblages of zoosporic true fungi along temporal and spatial gradients. Soil is a heterogeneous medium. In this paper we have described the physical characteristics of soil ecosystems in general, the life history of zoosporic true fungi, some aspects of interaction with other groups of microorganisms, and some specific resource seeking strategies which zoosporic true fungi can use in soil ecosystems.

Using baiting techniques zoosporic true fungi are frequently found growing in samples from soil ecosystems. It is clear that these fungi have adapted well to soil ecosystems. Some physiological capacities necessary for zoosporic true fungi to adapt to particular features of the soil have been discussed. In fact, all of the microorganisms present in soil must be able to adapt to rapid changes in the physical environment, particularly moisture, temperature, oxygen concentration and currents. However, the mechanisms for adaption are largely unknown.

The distributions of these fungi need to be studied along transects with spatial and temporal microscales in order to better understand the specific ecological roles of these fungi. Not much is known about the interactions between zoosporic true fungi and other microorganisms in the soil. Recent advances in instrumentation have lead to the development of new and more precise methods. Three-dimensional imaging may provide information on the spatial relationship between species of zoosporic true fungi, their substrates and other competing species of microorganisms in the soil. FISH techniques have provided new methods to identify microorganisms in environmental samples. Particularly the roles of zoosporic true fungi in biogeochemical cycles and in food webs at the microscale level remain to be documented. We hope that the information provided in this review will be useful in future research.

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