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Review paper

Ecology of Soil Eumycetozoans

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Abstract. Eumycetozoans, commonly referred to as slime moulds, are common to abundant organisms in soils. Three groups of slime moulds (myxogastrids, dictyostelids and protostelids) are recognized, and the first two of these are among the most important bacterivores in the soil microhabitat. The purpose of this paper is first to provide a brief description of all three groups and then to review what is known about their distribution and ecology in soils.

Key words: Amoebae, bacterivores, dictyostelids, myxogastrids, protostelids.

INTRODUCTION

One of the idiosyncratic branches of the eukaryotic tree of life consists of an assemblage of amoeboid protists referred to as the supergroup Amoebozoa (Fiore-Donno *et al*. 2010). The most diverse members of the Amoebozoa are the eumycetozoans, commonly referred to as slime moulds. Since their discovery, slime moulds have been variously classified as plants, animals or fungi. Because they produce aerial spore-bearing structures that resemble those of certain fungi and also typically occur in some of the same types of ecological situations as fungi, slime moulds have been traditionally studied by mycologists (Martin and Alexopoulos 1969). However, molecular data have confirmed that they are amoebozoans and not fungi (Bapteste *et al*. 2002, Yoon *et al*. 2008, Baudalf 2008).

Three groups of slime moulds (myxogastrids, dictyostelids and protostelids) are recognized (Olive 1970, 1975). Members of the three groups exhibit considerable diversity in the type of aerial spore-bearing structures produced, which can range from exceedingly small examples (most protostelids) with only a single spore to the very largest examples (certain myxogastrids) that contain many millions of spores. However, all slime moulds are fundamentally alike in that they are characterized by a life cycle in which there is a trophic stage represented by a single amoeboid cell (albeit multinucleate and often macroscopic in a second trophic stage characteristic of myxogastrids) that alternates with a spore-bearing stage. The microscopic amoeboid cell and a resistant (resting) cyst that the latter can produce under adverse conditions are the forms in which the vast majority of slime moulds typically occur in the soil microhabitat.

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Protostelids

The simplest slime moulds are the protostelids, which have been known for only about half a century (Olive 1975). These organisms, perhaps more appropriately referred to as protosteloid amoebae (Brown *et al*. 2011), are morphologically similar to some of the giant soil-inhabiting amoebae. Indeed, some of the apparently arachnoid-type of soil amoebae might be protostelids, since the trophic forms appear to be very similar. Unless an observer using a microscope is prepared to change focus away from the surface of the particular object being observed, the possible presence of a spore (or spores) upon a unicellular stalk – the distinguishing feature of a protostelid – will not come into focus. The indication is that an amoeba previously observed has apparently disappeared when in fact it has transformed into the fruiting body of a protostelid (Fig. 1). These giant amoebae can be very extensive, and they have been observed to cover the full surface of a 9 cm Petri dish (Feest, personal observation). Very little is known about the activity of protostelids in soil, since these organisms are mostly studied by direct observation of fruiting bodies obtained in laboratory culture from living or recently dead plant material (Moore and Stephenson 2003, Spiegel *et al*. 2004, Kosheleva *et al*. 2009). Nevertheless, their presence in soil in low numbers was confirmed by Feest (1987), who also reported (Feest and Campbell 1986) a negative correlation between the numbers of protostelids/arachnoid amoebae in soil and the incidence of "take all" disease in wheat (take-all is caused by the fungus *Gaeumannomyces graminis* var. *triticis* J. Walker). Chakraborty and Old (1982) showed that consumption of the spores of *G. graminis* by arachnoid amoebae occurs, so there may be a serious role for these organisms in the suppression of a very important disease of wheat. Macroscopic members of the genus *Ceratiomyxa* form relatively large fruiting bodies in which the exposed surface is covered with spores borne individually on tiny stalks. Each one of these is morphologically similar to the fruiting body of a protostelid with a single-spored fruiting body. *Ceratiomyxa* has been considered as a giant protostelid, but recent molecular evidence suggests that it is actually a sister group to the myxogastrids (Fiore-Donno *et al*. 2010), with which it shares a number of features in common (e.g. relatively large plasmodium, macroscopic fruiting body, and ecological association with decaying wood and other types of plant debris).

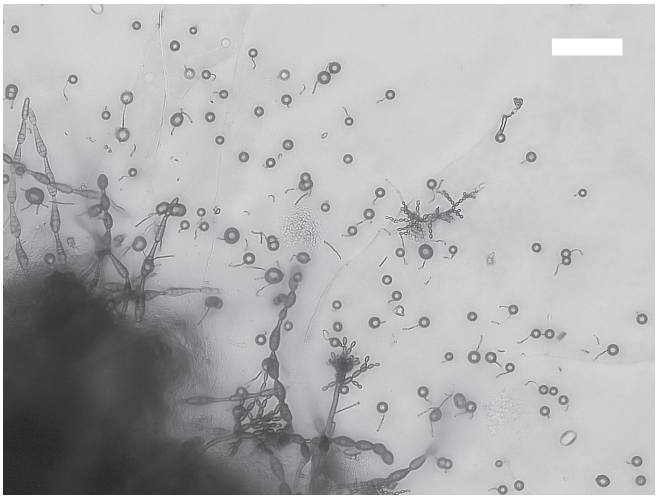

Fig. 1. Fruiting bodies of *Protostelium mycophaga* L. S. Olive and Stoian (photo by John Shadwick). Scale bar: 50 μm.

Dictyostelids

Dictyostelids, the second group of slime moulds, derive their common name from the net-like arrangement of the cells that make up the stalk of the fruiting body (the name of the type genus *Dictyostelium* literally translates to "net-stalks"). The amoeboid trophic cells that represent the vegetative stage in the life cycle of a dictyostelid do not form giant amoebae. Instead, under the influence of chemo-attractants, the production of which is stimulated by population density, these amoeboid trophic cells aggregate to form macroscopic (albeit still very small) fruiting bodies in which each cell retains its individual integrity. A single fruiting body of a dictyostelid consists of a stalk, unbranched in some species (e.g. many species of *Dictyostelium*) but displaying branching in others (all species of *Polyspondylium*), and one or more sori of spores (Fig. 2). The cells that make up the stalk can be said to represent an excellent example of altruism, since they sacrifice themselves to provide a structure (the stalk) from which their fellow amoebae can be distributed!

The spores produced in the fruiting body of a dictyostelid are embedded in a mucilaginous matrix that slowly dries and hardens. As such, these spores have a rather limited potential for being dispersed by wind (Olive 1975). However, it has been demonstrated that many different animals, ranging from microscopic invertebrates to birds and small mammals (Stephenson and Landolt 1992), can serve as vectors for dictyostelid

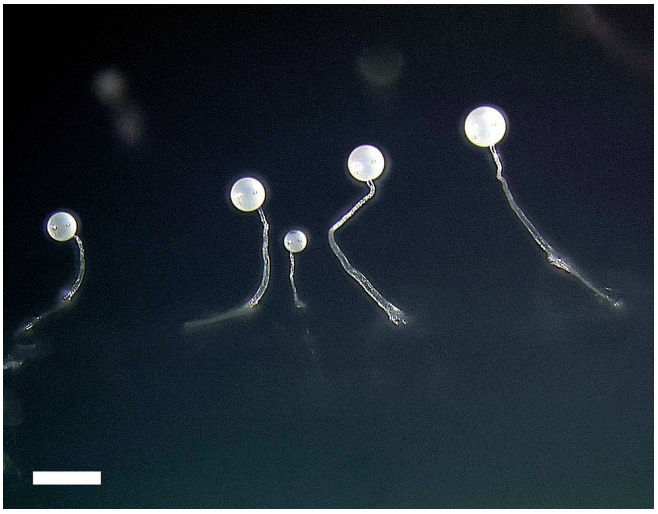

Fig. 2. Fruiting bodies of *Dictyostelium sphaerocephalum* (Oudem.) Sacc. and Marchal (photo by Andy Swanson). Scale bar: 0.3 mm.

spores in nature. The presence of suitable vectors is likely to be an important factor for dictyostelids associated with the soil microhabitat.

Aggregation and the subsequent formation of fruiting bodies represent an asexual dispersal process, but sex is known for many species of dictyostelids (Raper 1984, Cavender 1990, Kessin 2001). The latter involves the formation of what are referred to as macrocysts. In brief, the process begins with amoeboid cells of two mating types fusing to form a giant cell, which is essentially a diploid zygote (Chang and Raper 1981). The giant cell then ingests some of the other amoeboid cells present in the same microsite prior to encysting. Ultimately, meiosis takes place in the resulting macrocyst, and numerous "new" haploid amoeboid cells emerge though a rupture in the macrocyst wall. Macrocysts were not recognized as the sexual stage of dictyostelids until the 1960s, and these structures have not yet been observed for many species. Although most species of dictyostelids investigated to date appear to be heterothallic, with mating types required, homothallic strains have been reported for some species. Macrocysts also serve as a resistant stage in the life cycle, allowing the organism to survive under suboptimal conditions. Individual amoeboid cells also can encyst, thus forming microcysts. Microcysts represent yet another way that these organisms can deal with unfavorable environmental conditions (Kessin 2001). Indeed, the microcysts of dictyostelids are likely to be more common than active amoeboid cells in some soils.

Dictyostelids appear to be more common in forest soils than in agricultural, grassland or desert soils (Cavender and Raper 1965c, Raper 1984, Feest 1987, Cavender 1990), and certain species are abundant in cultivated garden soil that is amended organically (Kauffman 1986). More species are found at lower latitudes than at higher latitudes (Cavender 1973), and at a given latitude more species are found at lower elevations than at higher elevations (e.g. Hagiwara 1976, Traub *et al*. 1981, Stephenson *et al*. 1999). Higher densities of dictyostelids are present in moist soils than in dry soils, although they are rare in soils saturated with water. Singh (1947) described the relationship that exists for fruiting ability and the level of soil moisture, while Cavender and Raper (1965c) showed that different species vary in abundance along a forest moisture gradient and also that species abundances can be related to differences in forest composition. Romeralo *et al*. (2011) also reported that differences in forest composition represent an important factor in determining the distribution of these organisms. Horn (1971) found that there was competitive exclusion between species that depended on the same kind of bacteria, and Ketcham *et al*. (1988) demonstrated that biological interactions influence population sizes of different species of dictyostelids.

Some species of dictyostelids appear to be strictly tropical, others are strictly temperate, and others, although cosmopolitan, are more common in either tropical or temperate regions of the world (Cavender 1973, Raper 1984, Swanson *et al*. 1999). Many of the tropical species are unable to survive a temperate winter. Suthers (1985) recovered tropical species of dictyostelids from the fecal material of migratory birds freshly arrived in North America and from soils where the birds had landed, but these species were not present later in the year (i.e. during winter). The highest biodiversity of dictyostelids has been reported from Neotropical rainforest soils (Vadell and Cavender 1995), but a few species can be surprisingly abundant even in tundra soils (Cavender 1978, Stephenson *et al*. 1991). It appears that some dictyostelids display an affinity for marginal or disturbed habitats not often sampled for these organisms in the past, whereas others may be confined to a single limited geographical region of the world. They can be very abundant in microsites of soil enrichment (e.g. animal droppings) and were once thought to be primarily coprophilous (Raper 1984).

Dictyostelids are especially abundant in those forest soils with a well-developed humus layer (Cavender and Raper 1965b; Cavender 1973, 1990; Raper 1984; Feest 1987; Hagiwara 1989; Landolt and Stephenson 1989). They are most abundant in the layer of leaf litter found at the surface and decrease in number and diversity with increasing depth (Cavender and Raper 1965b, Stephenson and Landolt 1996). Raper (1937) and Singh (1947) showed that dictyostelids can consume a variety of soil bacteria but prefer coliform bacteria if these are available. As such, they may play a role in keeping the soil environment free of the pathogenic forms found in this group of bacteria.

Dictyostelids are usually isolated from soil by using some variation of the "Cavender method" (Cavender and Raper 1965a, Raper 1984, Stephenson and Cavender 1996). In brief, this method involves collecting samples from a number of sites in a given habitat, returning these to the laboratory, and then diluting and suspending a portion (often 5.0 grams) of each sample in a known volume (e.g. 45 ml) of distilled water. A small (but measured) amount of this suspension is spread evenly on a plate of a weak nutrient agar such as hay infusion agar (Raper 1984) or weak malt extract-yeast extract agar (Spiegel *et al*. 2004) and then overlain with a turbid suspension of *E. coli* in water. Plates are incubated at ambient temperatures for 3 or 4 days and then examined for colonies of dictyostelid fruiting bodies. Identification to species is made from direct observation of features of the fruiting bodies.

Due to the ease with which dictyostelids are recovered from soils and the early establishment of the "Cavender method" for estimating their density in soil, the activity of dictyostelids as soil microbivores is well established (Stephenson and Cavender 1996). However, whether or not they represent a significant limiting factor for bacterial populations in soil is not yet known, although it has been observed that dictyostelids do respond to increases in soil bacterial populations (Horn 1971). Actual densities of dictyostelids in soil vary widely, and in particular microsites numbers may exceed 1,000 colonies per gram of soil. However, in most temperate soils, typical numbers of colonies per gram generally range from ca 20 to several hundred (Raper 1984). When these figures are projected to an area of several cm^2 or even a single m^2, the incredible abundance of dictyostelids present in the soil microhabitat becomes more clearly apparent.

Myxogastrids

The plasmodial slime moulds (myxogastrids or myxomycetes), like other eumycetozoans, have a life cycle that is fundamentally based around a single uninucleate amoeboid cell. This cell can be either a rather undistinguished amoeba (sometimes referred to as a myxamoeba) or a distinctive flagellated form (called a swarm cell or amoeboflagellate). The latter of these two forms is often induced by the presence of free water in the immediate environment in which the cell occurs, and the conversion between phases takes place very rapidly (< 1 second) (Feest, personal observation).

However, at certain times in the life cycle, the amoeboid cell ceases to multiply by binary fission and simply increases in size and synchronous doubling of the nuclei leading to a cell with enormous numbers of nuclei, becoming what is known as a plasmodium (plural: plasmodia) (Fig. 3). Some plasmodia may well be the largest known cells, and in one species (*Brefeldia maxima* [Fr.] Rostaf.) can be meters across and weigh several kilograms. Under favorable conditions, the plasmodium gives rise to one or more fruiting bodies (also referred to as sporocarps or sporophores) containing spores (Fig. 4). Under adverse conditions, such as drying out of the immediate environment or low temperatures, a plasmodium may convert into a hardened, resistant structure called a sclerotium, which is capable of reforming the plasmodium upon the return of favorable conditions.

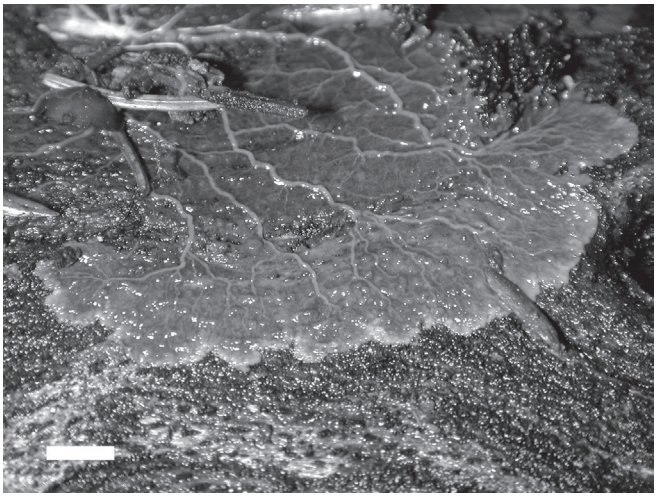

Fig. 3. Plasmodium of a myxomycete (photo by Randy Darrah). Scale bar: 25 mm.

Fig. 4. Fruiting bodies of *Hemitrichia calyculata* (Speg.) M. L. Farr (photo by Kim Fleming). Scale bar: 1.0 mm.

Moreover, amoeboid cells can undergo a reversible transformation to dormant microcysts. Both sclerotia and microcysts can remain viable for long periods of time (several years) and are probably very important in the continued survival of myxogastrids in some ecological situations and/or habitats, including soils and all of the other substrates potentially available for these organisms in deserts (Estrada-Torres *et al*. 2009, Lado *et al*. 2011, Wrigley de Basanta *et al*. 2011).

The fruiting bodies produced by myxogastrids are somewhat suggestive of those produced by certain macrofungi, although they are considerably smaller (often no more than 1–2 mm tall). The spores of the vast majority of myxogastrids range in size from 5 to 15 μm in diameter, with most species producing spores 10 ± 2 μm in diameter. The spores are largely wind-dispersed, but animal vectors also play some role (e.g. Murray *et al*. 1985). Upon reaching a favorable microsite, the spores complete the life cycle by germinating to produce the uninucleate amoeboid cells.

In the "textbook" life cycle outlined above, two haploid amoeboid cells fuse to form a diploid zygote, and the latter then develops into a multinucleate plasmodium in which all of the nuclei present are diploid. Under appropriate conditions, a plasmodium gives rise to a fruiting body, within which meiosis occurs when the spores are produced. An amoeboid cell emerges from the spore to begin the life cycle anew. However, some myxogastrids are known to be apomictic and thus do not follow this general pattern. Clark and Haskins (2010) listed 51 different species in which the reproductive system has been examined for one or more isolates. Fourteen of these were found to have both heterothallic and non-heterothallic (presumably apomictic) systems, eight had only heterothallic systems, and 29 were reported to be non-heterothallic. Relatively little is known about the relative proportions of heterothallic versus non-heterothalic reproduction in nature, but the latter may be more common.

For practical reasons, identification of myxogastrids is based almost exclusively upon features of the fruiting body (Martin and Alexopoulos 1969). Fruiting bodies that have developed under natural conditions in the field can be collected and preserved for study by collecting a portion of the substrate upon which the fruiting bodies occur, allowing the fruiting bodies to dry and then placing this material in a small pasteboard box for permanent storage. The entire procedure is described in some detail by Nannenga-Bremekamp (1991) and Stephenson and Stempen (1994). Some species of myxogastrids that apparently occur in soil produce plasmodia that migrate to substrates (including living plants) above the soil surface, where the fruiting bodies develop. However, the majority of species present in the soil do not appear to form fruiting bodies on a regular basis and thus must be cultured directly from the soil. Direct environmental sampling through the use of molecular methods to document the presence of myxogastrids in soil has been demonstrated in a few rather limited studies (Stephenson *et al*. 2011). Interestingly, the still rather limited data available on the taxonomic diversity of the myxogastrids associated with soil suggest that members of a single genus (*Didymium*) are often dominant.

Feest (1987) described a sampling technique that allowed the precise estimation of populations of soil myxogastrids. Following the observation that most myxogastrids were to be found in the top 4 cm of soil, 1 cm^2 soil cores taken to a depth of 4 cm were collected from 50 randomly selected points in a grid of 10 by 10 cm subplots established within a larger 1 m^2 plot. The cores were broken up and a weighed 50 cm^2 subsample added to 950 cm^2 of diluent (containing a wetting agent to release cysts adhering to soil particles). After being mixed thoroughly, the resulting soil suspension was diluted through a tenfold series. This allowed the results to be expressed as numbers per cm^2, per gram (1 to 40,000) and per m^2 and avoided errors arising from the differences in soil

density that can exist from one microsite to another. The latter can result in errors with a tenfold difference in magnitude. A small amount (10 cm^2) of each of the tenfold soil suspensions was added to each of five half strength corn meal agar plates to which 1 cm^2 of a 1% packed cell volume of yeast cells (*Saccharomyces cerevisiae* Meyen) had been added. The yeast cells were added to provide large "prey" for the myxogastrid cells to consume and thus avoid competition with other predators. These conditions allowed all three forms of myxogastrids to be cultured and observed from a single soil sample. Cultures were examined after 7 days for the presence of the flagellated cells ("swarmers") of myxogastrids (the swarmers are very distinct and unlikely to be mistaken for anything else after having been observed once). Dictyostelids and protostelid amoebae also show up in these cultures over the period of several weeks. Relating the number of records for each organism per five plates per dilution to a most probable number system allows the estimation of the soil population of the organism in question. Using this method, estimates of numbers per cm^2 of soil and per m^2 are possible. The maximum value of > 18,000 per cm^2 allowed the estimation of a population > 720×10^6 per m^2, which was not uncommon. Following experiments with dry soils where very few myxogastrids were recovered, Feest (1987) also found that freezing samples of soil caused cysts to excyst, so each soil sample was examined as both fresh and frozen. On some occasions, the results obtained indicated that more than 95% of the soil population of myxogastrids was in the cyst form. As such, sampling soil as a fresh sample alone would produce only a gross underestimation of the actual population of myxogastrids (Feest 1987).

In work that extended over a period of time, Feest (1987) showed that as a soil dried out, the progressive loss of soil moisture first limited activity of ciliates in the soil and then affected the activity of myxogastrids. Following the decline in trophic amoebae (and a rise in encysted forms), the bacterial population increased 15 fold, suggesting that feeding activities by myxogastrids and other amoebae maintained the bacterial population at a level only about a fifteenth of what it potentially would be. The reawakening of the myxogastrids would initiate the release of substantial amounts of nutrients as a result of their feeding upon the large bacterial population.

Clearly, the myxogastrids are organisms of considerable intrinsic interest, and the plasmodia have been the subject of extensive study. They have been shown to respond to their immediate environment through chemotaxis and phototaxis. Other properties of plasmodia, such as the presence of actin and myosin fibrils that act as the motive force for their movement and as models for muscle action or the synchronous division of the millions of nuclei that have allowed cell biologists to elucidate the nuclear cell cycle, have been the focus of numerous studies (Burland *et al*. 1993, Nair 1994, Bailey 1995, Haindl and Holler 2005). Their ecological role is much less well known, but they are both bacteriovorous and fungivorous.

ECOLOGICAL IMPORTANCE

A major portion of the net annual primary production in forests and other terrestrial ecosystems becomes directly or indirectly available to the decomposers in the detritus food chain, many of which are associated with the soil microhabitat. These decomposers (bacteria and fungi) are, in turn, an important food resource for various phagotrophic invertebrates and protozoans. Naked amoebae, which can make up 95% of the protozoan population in some soils (Feest 1987), are the single most important group in terms of bacterial consumption. In addition to their direct influence on the structure of soil microbial communities, these amoebae play a key role in nutrient cycling. Mineralization is stimulated and decomposition enhanced by the amoebae releasing nutrients tied up in the microbial biomass. For example, amoebae are known to release ammonia to plant roots when feeding on bacteria and can produce increases in dry weight and nitrogen content (Clarholm 1981, Rosswall and Paustian 1984). It is not known what percentage of the total population of soil amoebae is made up of the amoeboid stages of dictyostelids and myxogastrids, but judging from the data available from a number of recent studies, it is significant. For example, Feest and Campbell (1986) reported that myxogastrid amoebae alone represented > 50% of the total amoebae for some agricultural soils. Their study was based upon the use of a culture-based method (Feest and Madelin 1985), but Urich *et al*. (2008) used a RNA-centered mega-transcriptomic approach to generate the largest dataset for the entire soil protozoan community available to date. Eumycetozoans were found to represent the single largest component of total soil protozoan biodiversity, which appears to underscore the major ecological role these organisms play in the soil microhabitat.

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