ACTA
PROTOZOOLOGICA

Special issue:
Protists in Soil Processes

Introduction to special issue

The Past, Present and Future of Soil Protist Ecology

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Abstract. This essay introduces the special issue of this journal on the ecology of soil protists. This issue marks approximately the first 100 years of soil protistology as a discipline as some of the first studies to show that protozoa were an important part of soil ecology took place at Rothamsted, in southern England, towards the end of the first decade of the twentieth century. Much of the work on soil protists – and indeed the papers in this special issue – concentrate on traditional protozoa. In addition it is now realised that slime molds (eumycetozoans) can potentially make an important contribution to the numbers and diversity of soil amoebae. We also argue that diatoms and other algae are likely important in soils and in need of more detailed study. By its nature microbiology (including soil protist ecology) is a science limited by technology – for example the subject could not really exist before the invention of the microscope. We suggest ways in which newer technologies (molecular methods, stable isotopes etc.) may contribute to future studies on the ecology of soil protists.

Key words: Testate amoebae, naked amoebae, flagellates, ciliates, diatoms, slime molds, algae, history, protozoology.

'A key aspect of soil's fertility derives from life itself... Soil everywhere receives a flux of fairly similar dead life, which is then mixed, digested, and aggregated with particles of minerals into a matrix.' (Volk 1998, p. 108).

INTRODUCTION – THE OFTEN OVERLOOKED IMPORTANCE OF PROTISTS

The protists are a hugely diverse group of organisms, from traditional protozoa – such as amoebae and ciliates – to diatoms, slime molds and water molds (Mar-

gulis and Chapman 2009). However, protists have been strangely invisible in much of microbial ecology, a subject where prokaryotes have tended to dominate (e.g. McArthur 2006, Madigan *et al.* 2012). Indeed microbes themselves have been ignored in much of classical ecology (Wilkinson 1998, Nee 2004). There is an old English proverb 'Out of sight, out of mind' and clearly without microscopy most microorganisms are – by definition – out of sight, so it is perhaps not surprising that ecologists have tended to overlook them. However, microbes are not only crucial to many ecosystem processes but are also exceedingly diverse, a point not lost on some of the more thoughtful non-microbiologists. For example, the late Stephen Jay Gould (1996) eloquently argued that microbes have always dominated Earth history both in the past and the present, but he focused only on bacteria to make his case. As various

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authors have pointed out (e.g. Caron *et al.* 2009, Nee 2004) although such bacteria centred arguments are relatively common, protists have been rather overlooked with most microbiological work focusing on the importance of prokaryotes – and to a lesser extent viruses – rather than the diversity of eukaryotic microorganisms. However, as the papers in this special issue of *Acta Protozoologica* show, a diverse range of protists may be of great ecological significance in soils, making them a key aspect of life in the soil and so contributors to soil fertility as suggested by this papers epigraph.

## THE FIRST 100 YEARS; THE EARLY HISTORY OF SOIL PROTIST STUDIES, FROM DELFT TO ROTHAMSTED

The study of microbiology effectively began in the 17th century with the work of the early microscopists such as Robert Hooke and, especially, Antoni van Leeuwenhoek from Delft in Holland. As Ernst Mayr (1982, p. 100) pointed out 'microscopy opened up a new world for the biologist... [which revealed] the existence of an entirely unexpected living microcosmos particularly of aquatic organisms invisible to the naked eye.' Indeed many of these 'animals' described by van Leeuwenhoek were aquatic protists – although oddly he did not record any amoebae (Corliss 2002). It is perhaps not surprising that many of these first studies looked at fresh water and sea water rather than soil. Anyone who has spent time searching for protists on a slide covered with soil particles will not be surprised these organisms were first described from cleaner aquatic samples. Indeed this bias continued for several hundred years. For example at the start of the 20th century the Rhizopoda (amoebae) were described as inhabiting 'ponds and lakes, marshes and swamps; wherever, in fact, enough moisture exists to support a tuft of moss' (Cash and Hopkinson 1905, p. 30). They are certainly common in many of these habitats but also occur widely in soils too.

This watery emphasis is nicely illustrated by the one of the few species of free living amoebae that a general biologist is likely to have heard of because introductory biology textbooks are a common 'habitat' for it – namely *Amoeba proteus* (Fig. 1). This species was named by Joseph Leidy in the 19th century using specimens from freshwater samples (Leidy 1879, Warren 1998) – although the organism had been known under a range of pseudonyms since the 18th century (Cash and Hopkin-

son 1905). Throughout the 19th century this genus was considered aquatic (Griffith and Henfrey 1875, Leidy 1879, Cash and Hopkinson 1905) and even modern textbooks describe *Amoeba* solely as 'a common freshwater genus' (Madigan *et al.* 2012, p. 627). However, they are also widespread in soils, with the well-known *A. proteus* found in sites ranging from garden soils in Tristan de Cuna (Sandon and Cutler 1924) to the rhizospheres of conifer seedlings in North American greenhouse experiments (Ingham and Massicotte 1994). The point about the aquatic emphasis prior to the 20th century is even more strongly made by the ciliates in the genus *Colpoda*. This genus has been described as 'certainly the most common soil ciliate world-wide' (Smith and Crook 1995, p. 184) and the diversity of colpodids is actually greater in soil than in freshwater habitats (Foissner 1998). However, Kent (1881) only described *Colpoda* spp. from aquatic habitats in his three volume *Manual of the infusoria*!

The serious study of soil protozoa started in the first few decades of the 20th century, barely one hundred years ago. The first attempt to review the topic in a book length treatment, at least in the Anglophone world, seems to have been Harold Sandons 1927 book *The composition and distribution of the protozoan fauna of the soil*. In the book he pointed out that although the presence of protozoa in soils had been known since the early 19th century (he particularly cites the work of Ehrenberg) it had mainly been assumed that soil protozoa 'were present in the soil merely as stragglers brought in from other more natural habitats' (Sandon 1927, p. 1). He suggested that it was the end of the first decade of the 20th century before people started to study soil protozoa properly following the demonstration by Russell and Hutchinson (1909) that removing soil protozoa appeared to greatly increase the fertility of some soils – because of their assumed role as major predators of bacteria and so interactions with nutrient cycling. As Russell recollected years later, the idea that protozoa were an impotent part of the soil biota was initially controversial (Russell 1967). Russell, Hutchinson and Sandon all worked at Rothamsted Experimental Station in southern England (Fig. 2), arguably the birth place of serious studies on the ecology of soil protists.

Rothamsted was one of the first scientific agricultural research stations, with the early experiments started during the 1830's. It was founded by John Bennet Laws on his family estate, only half an hour or so by train from central London (Silvertown 2005). In 1843
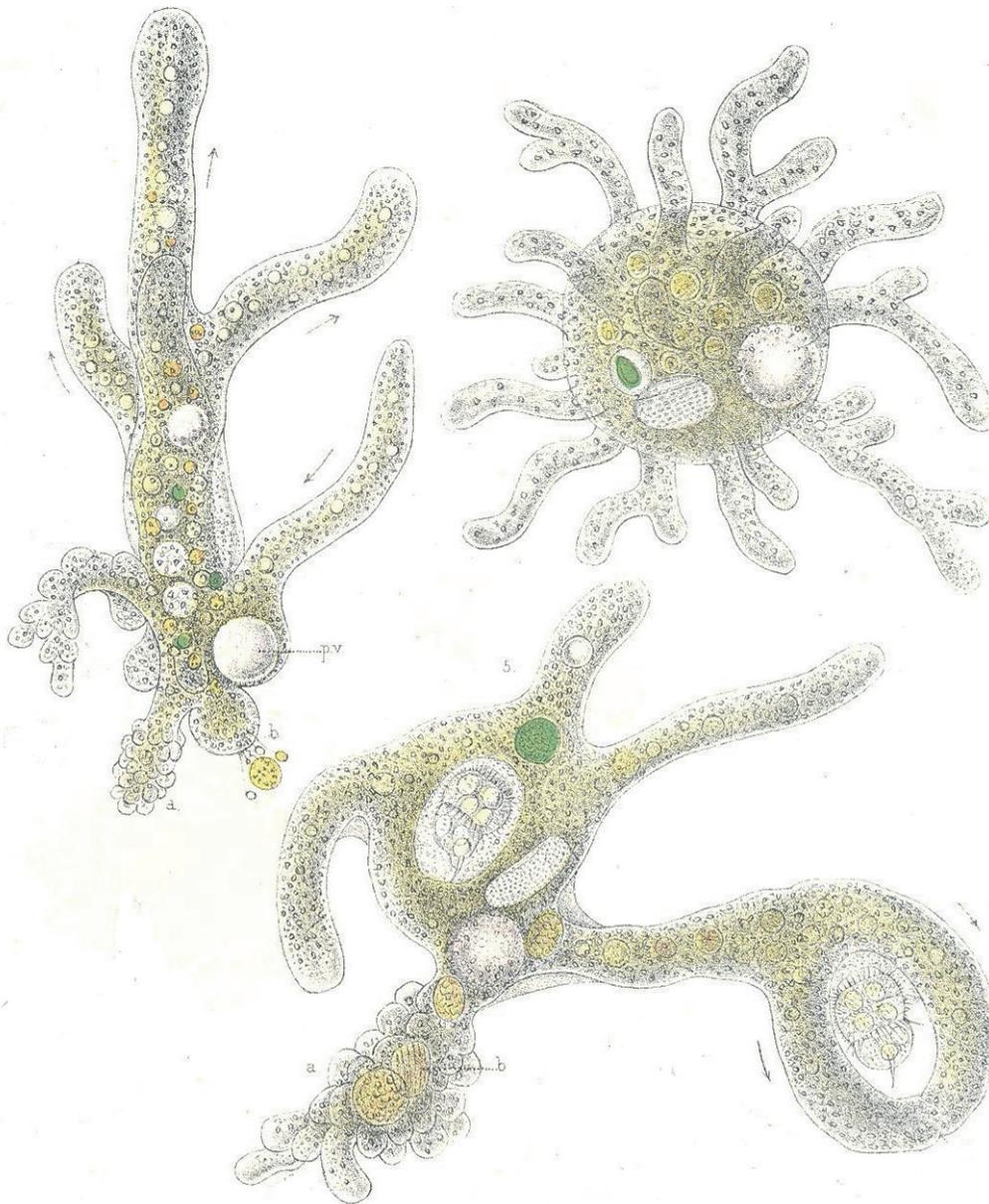
Fig. 1. The textbook classic protozoan – *Amoeba proteus* from Leidy (1879).

Laws hired Joseph Henry Gilbert as his scientific collaborator and later came to view this date as the start of Rothamsted as a proper research institute (Stevenson 1989). Understanding how soil fertility interacted with plant growth was a key question in early Rothamsted research, unsurprisingly as Laws wealth came in part from the manufacture of fertilizers. From around 1910 – following the demonstration that partial soil sterilization increased plant growth apparently through altering the balance between bacteria and protozoa – research on free-living soil microbes became an important part of Rothamsted research (Stevenson 1989).

With the founding of serious studies of soil protozoa around 1910 this special issue on the ecology of soil protists marks roughly 100 years of serious work on the topic. It focuses on the ecology of protists mainly in relatively natural habitats, rather than in the agricultural soils where their significance was first recognised.

Fig. 2. The Broadbalk experiment at Rothamstaed. Wheat has been grown continually here since 1843 with different fertilizer regimes used in different parts of the field. From 1910 onwards a greater attention was given to the microbiology of the soil following the demonstration that protozoa were important in the soil ecology and hence the plant production on the field (Stevenson 1989). For example, it was shown that amoebae were more common on plots receiving farmyard manure (Russell 1967).

Clearly there is still an need to explain the importance of soil protists to the wider scientific community – for example in a recent otherwise excellent book on the biology of soils (Bardgett 2005) the index only has four entries under 'protozoa.' However, from an applied microbiology perspective slowly things are starting to change (Gardi *et al.* 2009). This was demonstrated by a recent conference held in London in June 2012 by the Institute of Ecology and Environmental Management which argued for a more holistic approach to soil management – bringing together experts from a wide range of relevant disciplines. Indeed the publicity for this conference acknowledged that soil microorganisms are often neglected by the ecological community at large, yet their importance in all soil ecosystems makes them worthy of further study.

THE DIVERSITY OF SOIL PROTISTS

Soils contain a wide diversity of protists (Fig. 3). For example 365 species of protozoa (ciliates, flagellates, testate and naked amoebae) were found in an intensive study of 1 ha of upland grassland in Southern Scotland (Esteban *et al.* 2006). In general higher numbers of both individuals and taxa can be expected in wetter soils with higher proportions of organic matter – a generalisation that certainly seems to be true for most testate amoebae assemblages (Wilkinson and Mitchell 2010). However, reasonable numbers of protists can be found even in the microbiotic crusts of warm deserts – where water is only sporadically available (Bamforth 2004). The key aspect of the environment for protists is presumably the pres-

Fig. 3. Examples of protist diversity in a range of soil habitats. Top Left: The summit of Green Mountain (845 m a. s. l.), Ascension Island in the tropical South Atlantic. A single soil sample from under the bamboo produced three species of flagellates, two non-testate amoebae (gymnamoeba), one ciliate and three testate amoebae species (Wilkinson and Smith 2006). In addition Landolt *et al.* (2005) isolated eight species of protostelid slime moulds from leaf litter samples collected near the pond shown in the photograph. All the vegetation seen in the photograph is comprised of introduced plant species (Wilkinson 2004).Top Right: Summit of Mot Sper Chamana Sevenna (2424 m a. s. l.) in the European Alps, on the Swiss/Italian boarder. Two moss samples from the summit gave a total of 17 testate amoebae species (Wilkinson and Mitchell, unpublished). Bottom Left: Island in a small lake at Mere Sands Wood nature reserve in North West England. The island is heavily used by roosting and breeding water birds, so the soils probably have a high nutrient status. Seventeen species of testate amoebae and 29 species of diatoms were found in the litter and upper soil horizons taken from within the quadrat shown in the photograph (Creevy *et al.*, unpublished). Bottom Right: Holcroft Moss – a peat bog in Cheshire, England. Twenty four species of testate amoebae were found in the surface vegetation and underlying soil of a single sample (Valentine *et al.*, unpublished).

ence of a water film on the soil particles. As Rønn *et al.* (2012) point out most soil protists are 'fundamentally aquatic creatures visiting a terrestrial World,' hence the requirement for a water film, however temporary it may be. Despite the diversity of protists the papers in this special issue suggest that much of the work on them in soils is focused on the 'traditional' protozoa – amoebae (testate and non-testate), ciliates and flagellates. Only one paper considers non-traditional protozoa, namely that by Stephenson and Feest (2012) on 'slime molds' which are now clearly recognised as protists, and can contribute considerably to the total number of amoebae in a soil. Traditionally these amoebae were classified amongst the fungi rather than as protists (Stephenson

2010), indeed this group is informally referred to as either 'molds' or 'moulds' (authors differ on this) because of their previously presumed fungal nature.

To many biologists the most well known slime molds are the cellular slime molds – especially *Dictyostelium discoideum* which has been widely used as a model organism in developmental and evolutionary biology (Bonner 2009). In the wild this iconic species has a strange disjunct distribution being found in Eastern North America, Eastern Asia and Japan (Swanson *et al.* 1999). Surprisingly we understand rather little about the ecology of this species in nature, considering it is so widely studied in the laboratory and its full genome has been published (Eichinger *et al.* 2005). Indeed the

ecology of dictyostelids in general is poorly known. However, it is known that they are found in soils world-wide – especially forest soils – where they tend to be associated with the litter layer (Stephenson and Feest 2012). In South-Western Europe their diversity peaks in colder, wetter soils (Romeralo *et al.* 2011). The largest group of slime molds are not the dictyostelids but the myxomycetes – or plasmodial slime molds (Stephenson 2010). This group has been known before the invention of the microscope, as during their life cycle they can form macroscopic multi-nucleated plasmodia, which are often brightly coloured, easily seen and also easily mistaken for fungi (Feest 1996, Stephenson 2010). These are potentially hugely important in soil ecology and are in need of much greater ecological study.

One potentially important group of soil protists not considered by the other papers in this special issue are the algae *sensu lato* – especially the diatoms. Described as 'beautiful *aquatic* protists' (Margulis and Chapman 2009. Our italics), they can be widespread in soils (see bottom left of Fig. 3); indeed in our experience they are very commonly seen when examining a wide range of soils under the microscope. Their diversity is illustrated by the study of van Kerckvoorde *et al.* (2000) which found 81 taxa of diatoms from 30 soil samples collected in Northeast Greenland – a part of the world that is not usually associated with high biodiversity. Similarly, this high diversity of diatom taxa has been found from the opposite end of the Earth with a total of 163 taxa identified in 108 soil samples (Moravcova *et al.* 2010) collected on the largest island of the Crozet Archipelago, northwards of the Antarctic Convergence.

There are perhaps good reasons why, compared with the aquatic forms, soil diatoms have been relatively overlooked. According to Schuttler (1986) species lists and relative species composition of soil diatom assemblages are difficult to obtain because soil diatoms are small, often sparse and detection amongst soil particles is difficult. As with other protists, traditional taxonomy of diatoms is based on morphology, yet the striae in soil diatoms are very faint compared with the aquatic forms which can make identification more difficult (Lund 1946). As a result, it's argued that the density of striae and other features of the diatom frustule cannot be relied on when comparing soil diatoms with the aquatic forms (Hayek and Hulbury 1956). It seems quite plausible that taxonomic uncertainty still arises with some soil species undoubtedly described under more than one name. Interestingly, it's long been argued that the smallness of diatoms in soil could be an adaptation to

the soil environment – making it easier for them to use the moisture films surrounding the soil particles of the soil (Lund 1946).

Recent preliminary studies of soil diatoms under different agricultural regimes in Switzerland suggest that they may be potentially useful indicators of soil 'health' with more diatoms (and more testate amoebae) being found in soils subjected to less intensive agriculture (Heger *et al.* 2012). One difficulty highlighted by this study was the very time-consuming nature of identifying and counting diatoms and testate amoeba by direct counts of soil slides. Because of this Heger *et al.* (2012) suggested that molecular methods may be the way forward in this area. Diatoms are not the only eukaryotic algae found in soils, indeed often green algae are the most common soil algal group – even more common than cyanobacteria (Zancan *et al.* 2006). As with diatoms these other soil algae are potentially useful indicators of soil 'heath.' For example Zancan *et al.* (2006) found that less disturbed soils contained a higher diversity of algae in a study of agricultural fields in Italy.

Diatoms preserved in salt marsh sediments have often been used in attempts to reconstruct past sea level, however other protists, most commonly foraminifera, can be of use. In this issue Ooms *et al.* (2012) extends this approach to using testate amoebae in a brackish tidal marsh habitat. Testate amoebae from sediment cores (peats and lake sediments) have also been widely used for reconstructing past environmental changes – such as changes in bog surface wetness over thousands of years (e.g. Warner and Charman 1994). For example the bog shown in Fig. 3 (lower right hand panel) has 4.22 meters of peat formed over the last four thousand years containing testate 'shells' which can be compared to the current testate communities on the bog surface (Valentine *et al.*, unpublished data). So not only can we study modern testate communities but we can also obtain an historical perspective on how they change over time.

THE ECOLOGICAL ROLES OF PROTISTS IN SOILS

Protists – such as testate amoebae – are potentially important organisms in the cycling of nutrients and energy in soils (Wilkinson and Mitchell 2010). The early Rothamsted studies focused on the role of protozoa as predators of bacteria. As several of the papers in this special issue show, the feeding relationships, energy

and nutrient flows between bacteria, protozoa and small metazoans such as nematodes, are an active and still controversial area of research (Anderson 2012, Bonkowski and Clarholm 2012, Rønn 2012, Stephenson and Feest 2012). This is not an easy topic to investigate and the paper by Bonkowski and Clarholm (2012) in this issue discusses some of the challenges of experimental design in this area. Many recent studies have emphasised the positive effect of protozoan predation of bacteria on plant growth through mechanisms such as increased nitrogen mineralization (e.g. Bonkowski and Clarholm 2012 and references therein). However, this raised obvious questions about the classic studies of John Russell and colleagues at Rothamsted which ushered in the science of soil protist ecology by apparently showing that *removing* protozoa improved plant growth! Indeed for almost 100 years there has been a generally accepted idea that predation on bacteria by protozoa results in an inverse relationship between bacterial and protozoan densities in soils (Pussard *et al.* 1994). Part of the answer to this apparent contradiction is given by the comments of Rønn *et al.* (2012) in this issue that under some conditions 'selective protozoan grazing can favour plant inhibiting bacteria.' So removing protozoa can have both positive and negative effects on mineralisation and plant growth depending on the ecology of the particular soil.

The obvious assumption is that bacteria are likely to possess adaptations that reduce the probability of them falling prey to protozoa. However, it should be noted that it is not always in the interests of bacteria to avoid being eaten. Some bacteria can survive and grow in protozoan food vacuoles and this may be a mutualistic relationship, with bacteria acquiring a protected micro-habitat and the protists benefitting from bacterial metabolites (Pussard *et al.* 1994). One mechanism by which bacteria may protect themselves from both competitors and predators is chemical – that is by antibiotic production. Although it seems obvious that this is the likely reason for the evolution of antibiotic production, historically this has been difficult to prove and indeed many microbiologists were sceptical that this was the correct explanation for the evolution of antibiotic production (Postgate 1994). However, the 'chemical warfare' explanation for antibiotics now seems well established and the nature of the soil habitat – with more spatial structure than aquatic systems – seems well suited to this particular adaptation (Wiener 2000). Much of the work on the ecology of antibiotic production has focused on competition between various bacteria

and/or micro fungi. However, Winding and Oberender (2012) show, with a clever use of genetically modified bacteria, that antibiotic production can impact on soil protozoan populations too. Such studies are of applied importance since there is an interest in using bacteria, which can produce anti-fungal compounds, as an alternative to more conventional chemical fungicides – raising questions about potential unintended effects on other soil biota such as protozoa.

As the early Rothamsted studies made clear protozoa can potentially alter soil microbial communities in ways that have important implications for plants. At a global scale a major change in terrestrial plant ecology is the expansion of novel ecosystems dominated by a mix of non-native plant species (Hobbs *et al.* 2006, Mascaro *et al.* 2012). A particularly dramatic example of this is the bamboo forest on the summit of Green Mountain Ascension Island shown in Fig. 3. One of the more problematic invasive plant species in Britain is *Rhododendron ponticum* and Vohník *et al.* (2012) show that the testate amoebae community in soils under *Rhododendron* in Britain has changed to resemble that found under this shrub in its native range in Spain. This work also raises the possibility of using testate amoebae to monitor changes in soil ecology following clearance of invasive *Rhododendron* in conservation management schemes. As well as radically altering terrestrial vegetation human actions have the potential to cause significant climate change (see Gardi *et al.* 2009). In this context the amount of carbon stored in soil becomes of particular interest and therefore so do microbial processes in the soil that can affect carbon storage (Lenton and Huntingford 2003). This makes the role of protists in the processes affecting soil respiration of great applied importance (Anderson 2011, 2012).

THE WAY FORWARD – SOME SUGGESTIONS FOR FUTURE RESEARCH

By its very nature microbiology is a branch of science very much driven by technology. Certainly before the invention of the microscope its very subject matter was in most cases invisible (slime mold plasmodia being one of the few exceptions to this generalization). Today technological change is still important – for example in the last few decades molecular methods have greatly changed our understanding of microbial phylogeny (Sapp 2009) – and technology is likely to be

crucial to advances in our understanding of the role of protists in soil ecology. Certainly progress using just light and electron microscopy has been relatively slow. For example Wilkinson and Mitchell (2010) drew attention to the comments by Chardez (1960) that for testate amoebae their role in chemical transformations in soil was not well known, but that since they could be very common in some soils they were likely to be playing a role in humification. Fifty years later Wilkinson and Mitchell (2010) suggested that 'these comments still stand!' More recently – 15 years ago – Coûteaux and Derbyshire (1998) pointed out that protozoa appeared to be important in both carbon and nitrogen cycles in soils but that 'it has not been established, however, what level of protozoan diversity is essential for these transformations.' Once again this is still the case! This lack of progress is, in part, due to the limitations of microscopy in studying such problems and also partly due to the relatively small number of people who have worked in this field – as well as the intrinsic difficulty in studying processes that are out of sight in the soil. These difficulties are nicely illustrated by the lack of good data on what phagotrophic protists, such as testate amoebae, eat (Gilbert *et al.* 2000). For example, in a direct observation study investigating the food preferences of the testate amoeba species complex *Nebela collaris sensu lato*, Gilbert *et al.* (2003) found that (on average) 71 ± 27% of the prey could not be identified by direct observation due to their poor preservation state, making interpretation of the results difficult.

Some soil protists, such as diatoms and testate amoebae, can be directly counted under the microscope. This can be very time consuming but has the advantage of potentially producing population data very similar to that collected in studies of the ecology of macroscopic organisms. Other groups (e.g. ciliates, flagellates or cellular slime molds) have traditionally more often been studied by culture based methods. The disadvantage here is that some taxa can be difficult to culture and the data is less useful for quantifying the actual population sizes in the soil – rather than in the culture – so is less ecologically informative. Molecular methods address some of the problems associated with both direct counts and culture based approaches, while raising other new problems (Finlay 2004)!

There has been a widespread suspicion that morphospecies in protists may be hiding cryptic diversity – with multiple different species hiding within a single morphospecies. During the 1990's various studies started to use molecular methods to try and address this problem in a range of protist groups (Mann 1999, Wanner *et al.* 1997). So far the results are mixed with some studies failing to find evidence of hidden diversity when applying molecular methods (e.g. Foissner *et al.* 2001, Lara *et al.* 2011) while others have done so (e.g. Kosakyan *et al.* 2012). However, trying to define species (or any other taxa) by molecular methods also raises problems – what level of genetic similarity should be used as the cut off point to identify a new taxon, and indeed, does it make biological sense to define species in such a way (Fenchel 2005, Finlay 2004). Because species concepts in protists are unclear there is a real possibility that in many cases what we call a 'species' in protists may be more equivalent to higher taxa – such as genera – in larger well studied groups such as animals (Bass and Boenigk 2011). In many cases we currently cannot rule out the possibility that a species list of protists from a soil is more comparable with a list of genera of plants or birds from a woodland – if so it follows that soils are much more diverse than suggested by many of the papers in this special issue. There may be some hope for clarifying these issues in the future. For example the fact that molecular methods are suggesting that sexual reproduction is more widespread in amoebae than used to be thought (Lahr *et al.* 2011) could make it easier to define protist species in a way that is comparable with many macroscopic organisms by utilizing the biological species concept (Mayr 1942). However, we are still some way from understanding how to use molecular methods to census soil microbes with the confidence that an animal ecologist might have in a census of woodland birds.

In field based experiments, Stable Isotope Ecology (SIE) is particularly useful in providing a schematic representation of what's occurring in the soil food web (Crotty *et al.* 2012). As different microbes take up various isotopes at different rates (Kump *et al.* 2010), stable isotopes are useful in tracking feeding relationships as they do not decay and spontaneously change into a different isotope. Using a multi-proxy analyses of observations of digestive vacuole content and ^{13}C and ^{15}N, recent research (Jassey *et al.* 2012) has shown that two species of testate amoebae (*Nebela tincta* and *Hyalosphenia papillo*) both commonly dominant in *Sphagnum* peatlands did not have the same trophic position in the microbial food web along the "fen-bog" gradient, highlighting the complexity of studying within group feeding interactions. It's suggested the ^{15}N enrichment of *N. tincta* may result from mycophagous behaviour whilst *H. papillo* also contained endosymbiotic algae,

which Wilkinson and Mitchell (2010) hypothesized represents a potential alternative source of energy. Considering the potential importance of microalgae, this energy source in *H. papillo* demonstrates the need to further quantify the potential energetic benefits of endosymbiotic algae to soil protists. Stable isotopes are potentially an important tool in working out what soil microbes feed on, however, as with any new approach care is needed to avoid overconfidence in interpreting the results. For example fractionation effects can make these data difficult to interpret (Schoeller 1999) and it would not be surprising if there were considerable differences between different protists in the way they accumulate different isotopes.

It is also possible to combine several of these new technologies so, for example, molecular biology and stable isotope chemistry can come together with Stable Isotope Probing (SIP) which can be used in conjunction with RNA or DNA analysis, to identify the organisms involved within the food web. This recent coupling of molecular biological methods with stable isotope abundance in biomarkers is said to have provided a cultivation independent means of linking the identity of bacteria with their function in the environment (Radajewski *et al.* 2000), which the authors believe has the potential for wide application in microbial ecology. SIP seems a promising tool where organisms are extractable in numbers too small to test through bulk stable isotope analysis (Crotty *et al.* 2012), which is likely to be the case with many of the rarer soil protists. Indeed, present and future advances in technology will drive our understanding of the soil protist food web, gaining new insight into the complex interactions.

As well as these longstanding problems of quantifying natural soil biodiversity and elucidating soil food webs the ecology of soil protists is an area ripe with many important and understudied questions. To conclude this introduction we illustrate this with just two examples. In the classical plant based on primary successions common in ecology textbooks the identity of the species changes, with early successional species being replaced with other species over time (Keddy 2007). There is some – so far limited – evidence that this well-known pattern may not be found in soil protists. For testate amoebae Wanner and Xylander (2005) found that although new species arrived over time there was not the classic textbook species turnover – as the early successional species continued to survive into later succession stages. It would be interesting to replicate this work for other soils and other groups of soil pro-

tists, to establish if there are significant differences in successional processes between microbes and the better studied macrobes such as plants. If this is the case it raises interesting questions about what this means for understanding soil microbial ecology. This could also be another area where cryptic diversity in morphospecies matters, and so molecular methods may be of importance. Potentially this lack of taxonomic turnover could be an artefact of cryptic diversity in small early successional testate species – so the early species are being replaced by species that are morphologically very similar and so not recognised. Clearly smaller taxa tend to have fewer obvious characters to help with morphospecies identification.

An additional area of research with very few studies is the potential role of humans in the dispersal of soil protists. This has been well studied in aquatic systems; however there are almost no studies of human dispersal for soil living microorganisms (Wilkinson 2010). One recent exception to this is the work of Perrigo *et al.* (2012) on the occurrence of dictyostelids in mud on boots. Although it was a small scale study (18 pairs of boots) they found viable slime molds from 4 boots. Again this is an area where molecular techniques may be particularly useful – indeed such methods were used in the slime mold study. If we are transporting large numbers of soil protists around the world what are the implications of this for soil ecology and biogeography? For example not only plants but also soil was introduced to Ascension Island (Fig. 3 top left) during the 19th century (Wilkinson 2010). Clearly there is no shortage of interesting and important questions for the second 100 years of soil protist studies.

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