MICROBIAL DIVERSITY OF A NATURE RESERVE: EUKARYOTIC MICROORGANISMS OF MERE SANDS WOOD IN N.W. ENGLAND

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ABSTRACT

The idea of incorporating eukaryotic microorganisms into biological surveys is a new idea in nature conservation. Using Testate amoebae and Diatoms as model organisms, this study aimed at expanding the Wildlife Trust's knowledge of biodiversity at its Mere Sands Wood Nature Reserve by describing and quantifying microbial diversity and distribution using traditional protist taxonomy based on morphology. The methods employed were similar to that adopted in the studies of macroscopic organisms, allowing for a comparison between the diversity of microscopic and previously studied macroscopic organisms.

The results of this study highlighted the dramatic increase in species richness estimates by incorporating just two groups of eukaryotic microorganisms. Species richness was a useful measure of diversity and these results were largely consistent with diversity indices. Terrestrial soil and lake sediment microhabitats supported distinct assemblages of testate amoebae and diatom communities which enabled inferences to be made about the potential factors influencing microbial diversity and distribution in terrestrial and aquatic habitats. Lower microbial diversity was found in lake sediment associated with invasive aquatic plants and native monocultures. Taxon richness and diversity varied between different microhabitats and also at the replicate scale within a $1m^2$ quadrat, further highlighting the small-scale variability in microbial communities. The lack of correlation between different microbial groups demonstrated the complexities in understanding the structure of microbial communities. The relationship between species and genus richness highlighted the potential for using surrogate measures for future rapid biodiversity surveys.

The use of evolutionary life strategies in protists is an area for future study which may provide useful insights into nature conservation management for microscopic organisms in habitats prone to disturbance and stress. This study illustrates the potential for incorporating eukaryotic microorganisms into biodiversity surveys. It would be useful to extend this research to other nature reserves and representative habitats using a nested design, facilitating replication and randomisation.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 The diversity of distribution of eukaryotic microorganisms

The diversity and distribution of free-living microbial eukaryotes is a vastly understudied question in classical ecology (Wilkinson 2007). Ecologists have focused much attention on trying to explain broad patterns of biodiversity, yet published literature on, for instance, the loss of biological diversity or 'biodiversity' (both terms are often used interchangeably) invariably focuses on the conspicuous higher taxa and culturally important groups of plants and animals, often with a limited distribution, compared with protists (protozoa, diatoms and other algal groups, slime molds and water molds), which have often been considered 'cosmopolitan' or 'ubiquitous' in their distribution (Finlay 2002). In an ecological sense, this represents a fundamental bias in ecological research which should be addressed given the fact that microbial communities are essential components of all ecosystems (Margulis *et al* 1986, Wilkinson 2008, Wilkinson and Mitchell 2010, Heger *et al* 2011). There are several possible explanations for this bias. Firstly, it's probably about how we perceive microbes, for instance, it's not clear how many naturalists today view microbes as 'germs' or disease as opposed to normal components of the ecosystem (Margulis *et al* 1986). Secondly, owing to their small size, protists are hidden without the use of a microscope, highlighting the obvious logistical difficulty for naturalists to determine their presence and generate species abundance data comparable to macroscopic organisms (Wilkinson *et al* 2012). Further, the notion of 'species' which has been borrowed from the animal kingdom is possibly invalid for some microbial groups (Margulis *et al* 1986) and it's argued the concept of species diversity can break down where microorganisms are concerned (Magurran 2004). For instance, the drastic underestimation of protist diversity and importance in more general biodiversity papers (see Dolman *et al* 2012, Corbet 2011), could be partly due to the fact that there is no generally accepted basis for delimiting species in protists (Boenigk *et al* 2012), with aggregates of microspecies treated as a single species. This is perhaps reflected in recent, wide ranging estimates of eukaryotic diversity reported to be

between two and one hundred million! (Costello *et al* 2013). Indeed, ambiguous species boundaries are not confined to microscopic organisms, problems occur when using the species concept for larger macroscopic organisms, with implications for biodiversity and nature conservation (Agapow et al 2004).

Fontaneto (2011) highlights the problems associated with identifying microbial 'species' as units of diversity. One practical solution is the concept of 'morphotypes' or 'morphospecies' which is used to distinguish taxa based on their morphology, treating morphospecies as equivalent to species in richness estimates (Magurran 2004). For emotive reasons we attach great significance to the morphological dimension of diversity and Nee (2004) argues that biologists are largely attracted to the subject in the first place by this emotion. Thus, it's perhaps not surprising that the 'visible' macroscopic organisms, especially those with K-selected traits, have been given prominence in biological surveys, biodiversity estimates and nature conservation strategies, compared with the vastly smaller, out of sight microscopic organisms. This is reflected in local, national and international nature conservation strategies, for example, the IUCN Red list of threatened species (IUCN 2012), which is underpinned by describing species, estimating species richness and extinction rates (Costello *et al* 2013). Few microscopic taxa are known to the 'Red list'. Some are included because the habitats which support them are especially prone to disturbance, for instance, European diatom taxa in oligotrophic and dystrophic aquatic habitats (Lange-Bertalot 1997). In terrestrial habitats relatively few soil organisms have been given Red Data Book status (Anderson 2009). Biodiversity conservation priorities arise through quantitative knowledge of the species present and their population sizes and decision-making hinges on understanding how biota will respond to stresses and disturbances of ecosystems (Cotterill *et al* 2008). Yet, despite these efforts it is realised that many species will go extinct before they have been discovered (Costello *et al* 2013).

In its simplest sense species richness (or the number of taxa in the unit of study) is arguably the most traditional and widely used measure of biodiversity. This concept underlies many ecological models and conservation strategies, for instance, for macroscopic organisms current and background rates of species extinction are calibrated against patterns of species richness (Gotelli and Colwell 2001, Costello *et al* 2013). Indeed, concern about the unprecedented rate of species extinction globally has focused on members of the plant and

animal kingdoms, often on species with limited distributions, whereas microbial species with a perceived cosmopolitan distribution are assumed to be protected by their ubiquitous occurrence (Cairns 1993). Cotterill *et al* (2008) argue it is this reason why the conservation of micro-organisms has been largely ignored in Red Data books. Recent research however could suggest an error in this assumption documenting extinction of eukaryotic microbes in Britain (see Hambler *et al* 2011). In an attempt to quantify regional biodiversity and conservation priorities for two extensive regions of Eastern England, Dolman *et al* (2012) found a dramatic bias in taxonomic coverage towards the more easily recognised macroscopic organisms. For example, flowering plants comprised the largest proportion of records (50%) in Breckland, compared with 0.03% of records for diatom and algal species. Despite being a diverse group in the animal kingdom, strikingly, only three nematode records in total were obtained! On a smaller scale, a biodiversity perspective on a small nature reserve managed by the Scottish Wildlife Trust found only nine species of microorganisms confidently identified (Corbet 2011), compared with 1775 species recorded overall! Reports on findings of species and species lists also form the basis for biogeographic analyses and such analyses have mainly focused on macroscopic organisms with a restricted distribution. The extent and factors controlling the distribution of microorganisms is far less well understood, although there is some evidence (Fontaneto 2011) that restricted distribution in microorganisms is more common than previously believed (see Wilkinson 2001, Heger *et al* 2011, Yang *et al* 2010), which could suggest the ecological differences between micro and macro-organisms may not be as prevalent as previously thought (Finlay 2004). Nevertheless, we are still a long way from understanding the fundamental ecological differences between free-living protists and macroscopic organisms. To investigate such differences Foissner (2006) argues vigorous sampling and reliable investigations on the number of morphospecies in different habitats and representative ecosystems could be the way forward. More recently, using molecular data for Euglyphid testate amoebae, Lara *et al* (2013 *in review*) suggest that the patterns of diversity and community structure of microorganisms may not be fundamentally different from those of multicellular organisms.

Testate amoebae and diatoms are diverse and abundant groups of taxonomically distinctive 'shelled' protists, preserved in a range of terrestrial and aquatic environments. Morphospecies in both testate amoebae and diatoms are principally defined by variations in

the composition, morphology and size of the shell. Using the morphospecies concept both microbial groups can be counted directly under the microscope potentially producing population size data very similar to those collected in studies of the ecology of macroscopic organisms (Wilkinson *et al* 2013). Owing to their preservation potential and response to environmental conditions, both groups are being increasingly used in a wide range of applications. Diatoms are routinely used to monitor water quality. The Trophic Diatom Index (TDI) was developed to monitor the trophic status of rivers based on diatom composition (Kelly and Whitton 1995) and existing diatom-based metrics are used in the Water Framework Directive to assess the ecological status of aquatic ecosystems (Kelly *et al* 2009). Analyses of fossil diatom species assemblages continue to be used by paleolimnologists to reconstruct a wide-range of environmental variables (Smol and Stoermer 2010) and they are also being used to establish reference conditions and restoration targets for lakes (Bennion *et al* 2011). Testate amoebae have been used as palaeoenvironmental indicators in peat (e.g. Charman 2001, Lamentowicz and Mitchell 2005, Mitchell *et al* 2008, Swindles *et al* 2009) and lake sediments (e.g. Bennion *et al* 2011, Roe *et al* 2012), additionally, they have been used as indicators of ecosystem health in agricultural ecosystems (Heger *et al* 2011) and as restoration indicators in ombrotrophic peatlands (Davis and Wilkinson 2004) and floodplains (Fournier *et al* 2012).

Despite their applied importance, understanding of the factors that govern patterns in diversity and distribution in modern samples is less understood. Few studies have used both testate amoebae and diatoms as model organisms. Heger *et al* (2011) used both groups of eukaryotic microorganisms as bioindicators in soils under different farming practices. Other polar studies into the effects of animal perturbations on community composition have tended to publish testates (Vincke *et al* 2006) and diatoms (Moravcova *et al* 2010) separately. Using the traditional taxonomy based on morphology, the main aim of the present study was to determine eukaryotic diversity and distribution on a nature reserve in North West England using testate amoebae and diatoms as model groups. The following hypotheses were investigated: (i) species richness and diversity of diatoms and testate amoebae would be correlated across the different habitats (ii) there would be no difference in species richness and diversity of diatoms and testate amoebae between habitats (iii) there would be a relationship between environmental factors (organic matter content and pH) and species

richness or diversity (iv) that measures of species richness and diversity indices would be correlated and give a consistent indication of which habitat was the most diverse.

1.2 Potential factors influencing microbial diversity and distribution

One of the central goals in ecological studies is to determine why organisms inhabit the place they do. A variety of biotic and abiotic factors have been put forward as potential explanations for microbial diversity and distribution, such as: colonisation, dispersal, competition, predation, productivity, resources, and environmental factors. In freshwater environments, Barker *et al* (2010) argue it's a question of scale, as at appropriate scales there are likely to be highly heterogeneous distributions of every organism to every environment shaped by multiple forces. In terrestrial soils, the factors influencing the diversity and distribution of microorganisms are probably less understood compared with aquatic systems. Vohnik *et al* (2012) suggest comparatively less is known about the role of biotic interactions between microbial groups in soils compared with abiotic factors. Furthermore, the question of how microbial diversity is regulated and maintained between soil trophic levels is also suggested to be another major gap in our understanding (Ledeganck *et al* 2003). Arguably, compared with biotic interactions, environmental variables are easier to measure. This point is nicely demonstrated by Mitchell *et al* (2008) using testate amoebae. This group of protists need to find the required material to build their shell which may be one of the constraints that determines the micro-distribution of species, thus it's far easier to measure, for instance pH, compared with availability of particles for shell construction.

Disturbance and loss of habitats is another a key factor governing the diversity and distribution of all organisms. On a global scale human activities are causing rapid, novel and substantial changes to earth's ecosystems (Vitousek *et al* 1997). For instance, human disturbance of natural communities can increase the colonisation success of invasive species, often displacing the native biota and leading to a loss of biodiversity. Importantly, as human populations expand their land use, activities such as agriculture, mining and forestry will continue to be a direct force dominating terrestrial and freshwater ecosystems. Despite this, humans are generally not considered as a driving force in, for instance, microbial biogeography (Wilkinson 2010, Foissner 2011). However, a recent study by Perrigo *et al* (2012) has found support for the idea that anthropogenic dispersal could be leading microbiologists to find 'unnatural' cosmopolitan distributions (c.f. Wilkinson 2010). Clearly, considering few 'natural' ecosystems exist, we cannot rule out the role of humans influencing the diversity and distribution of all organisms.

1.2.1 Terrestrial environment

Soils are a major reservoir for global diversity in terrestrial ecosystems (Bardgett 2005) but the majority of soil organisms are microbial and have yet to be described (Gardi *et al* 2009). Indeed, it's been known for some time that organisms themselves are one of the five major soil forming factors (Jenny 1946), along with minerals, organic matter, water and air. Studies (Foissner 1987, Esteban *et al* 2006) have shown that protists are highly diverse and abundant groups of eukaryotes in soil with several different functional groups (Adl and Gupta 2006), yet this unseen majority has been largely ignored in relation to the soil food web (Crotty *et al* 2012), biogeochemical cycling (Wilkinson 2008) and microbial ecology (Wilkinson and Mitchell 2010). By nature, soils are complex, spatially heterogeneous systems and it's argued (Crotty *et al* 2012) that they should be viewed as a three-dimensional space including not only the vertical and horizontal soil profile but also the water and air filled pore spaces which add to this heterogeneity of the soil environment. The key aspect of the terrestrial environment for soil protists is thought to be the presence of a water film on the soil particles (Wilkinson *et al* 2012) and experimental studies using Testacea (Lousier 1974) have found a positive correlation between the number of active and living Testacea with soil moisture content.

It has been hypothesized that humus forms (mull, moder and mor) can be attributed to the development of terrestrial life, to which Ponge (2003) argues could be the main factor explaining variation in terrestrial ecosystems. Many organisms play a direct role in the humification process, for instance, Szanser *et al* (2011) found algal production and biomass (measured by the amount of chlorophyll a within the soil and its fluctuation over time) an important component of the humification process during decomposition. Testate amoebae are heterotrophic protists and are fundamental players in nutrient cycling in soils. They have a particularly important role in soils with low pH and high organic matter, such as those with moder or mor humus (Wilkinson and Mitchell 2010). In some terrestrial ecosystems they are often the dominant microorganisms and may represent a substantial proportion of the microbial biomass (Vohnik *et al* 2012). Despite, this, we are still some way from understanding the complex interactions and trophic feeding channels within soil food webs (Crotty *et al* 2012). Recent research in nutrient poor soils (Vohnik *et al* 2009, Vohnik *et al* 2012) has suggested a role for testates in nutrient cycling via interactions with mycorrhizal fungi.

Mycorrhizal networks play a key role in plant communities, for instance, by supplying and recycling nutrients (Marcel *et al* 2009), highlighting the potential interactions and feedbacks in soils involving members from the plant, fungi and protist kingdoms. Recent research (Jassey *et al* 2012) combining direct observations with stable isotope analysis has shown spores and mycelia of fungi were the main food sources for some testate amoebae. Interestingly, this study found different testate amoebae species have different trophic positions in the microbial food web probably as a result of different feeding strategies.

Testate amoebae have received comparatively more attention in soils (see Wilkinson and Mitchell 2010, Sutton and Wilkinson 2007, Vincke *et al* 2006, Ledeganck *et al* 2003) and mosses (Jassey *et al* 2012, Gilbert *et al* 2003, Heal 1964) compared with other protists, such as diatoms. Whilst most diatom species occur in aquatic habitats (Heger *et al* 2011), limited published literature suggests specific diatom communities do occur in soils (Lund 1946, Schuttler 1986, Moravcova *et al* 2010, Heger *et al* 2011), where they grow with other microalgae and cyanobacteria (Lund 1946, Zancan *et al* 2006). Both groups have different functions in terrestrial environments. Diatoms and other algae are primary producers (autotrophs) and their major benefits in terrestrial habitats are the product of their photoautrophic nutrition and their contribution to soil formation and nitrogen fixation (Zancan *et al* 2006). In the soil food web they serve as a food source for soil protozoa and micro and meio-fauna such as nematodes and collembolans (Heger *et al* 2011). As in the aquatic forms associated with sediments and surfaces, soil diatoms have been found to possess raphes (Lund 1946) and so the power to move within the soil microenvironment. The main environmental factors influencing soil diatom communities are moisture and nutrients (Moravcova *et al* 2010) and pH (Lund 1946).

In times of disturbance and stress, for instance in agricultural soils, some microorganisms such as testate amoebae form cysts which emerge when conditions become favourable (Foissner 2011), raising interesting questions about the role of humans in the introduction of testate amoebae cysts by soil dispersal in terrestrial ecosystems (Wilkinson 2010). Foissner (2011) argues resting cysts are the most likely mode of anthropogenic dispersal. Birds can also potentially exert a large impact on the microbial species composition in terrestrial ecosystems, especially in their breeding environment, in the form of trampling, manuring and physical disturbance of the vegetation (Vincke *et al* 2007, Moravcova *et al* 2010)

and also as a dispersal mechanism (Suthers 1985). Evidence from a study investigating testate species composition in soils influenced by the Wandering albatross *Diomedea exulans* suggested a limited albatross influence may actually increase the living fraction within the testacean soil community (Vincke *et al* 2007), *D. exulans* also seemed to influence diatom species composition in perturbed soils (Moravcova *et al* 2010).

1.2.2 Freshwater environment

Lakes are complex, highly heterogeneous biological systems with different organisms associated with different zones and substrates such as rocks (epilithic), sediments (epipelic) and plants (epiphytic). The littoral and benthic zones are suggested to be more complex than the open water, however, activities in the benthic zone may be affected by, and in turn, influence the ecology of the open water (Moss, 1998, Moss 2010). Further, the ecology of freshwater changes over a diurnal cycle often linked to physicochemical variables, nutrient availability (Barker *et al* 2010) and predator avoidance (Han *et al* 2011). Submerged plants support a distinctive periphyton community which includes: bacteria, fungi, algae, protozoa and other small animals, and different plants have different associated species and population sizes and populations may vary in different parts of the plant (Moss 1998). There are many abiotic and biotic factors potentially influencing microbial diversity and distribution in lakes, for instance, primary productivity, trophic interactions i.e. predation, lake size and volume, differences in the physical and chemical environment. In order to understand biodiversity in lakes, applied research (Dodson *et al* 2000) has focused on understanding the relationship between species richness and productivity. Some (Vyverman *et al* 2007) suggest historical processes, for example, colonisation and extinction, dispersion and migration constrain patterns in regional and local diatom diversity.

Substrate type at the microhabitat scale has also been found to influence microbial diversity and distribution. For example, in a study investigating the effectiveness of using diatoms as an assessment tool for the Water Framework Directive (WFD), Fisher and Dunbar (2007) found high variation in diatom frustule frequency and biovolume at the individual replicate scale, highlighting that the periphytic diatom species composition varies in response to the substrate sampled. In a paleolimnological study Burbidge and Schroder-Adams (1998)

hypothesised that the main factor controlling the distribution of testate amoebae populations in Lake Winnipeg, Canada was the type of organic material in the lake sediment, yet, in paleolimnological and indeed modern ecological studies, the precise sources of organic matter on which they feed is not known (Moss 1998) and less still is known about biotic interactions. For instance, Barker *et al* (2010) illustrate marked variability in available nutrient distributions in both open water and in macrophyte stands of shallow lakes, highlighting that simple sampling regimes may not account for biotic interactions and abiotic factors occurring at different spatial and temporal scales. Testate amoebae are one of many diverse and important components of the microbial trophic level within the benthic community of lakes and wetlands, playing a crucial role in food webs as the intermediate between bacterial and benthic invertebrate communities. Recent research (Han *et al* 2011) suggests trophic conditions (abundance of food) are a major factor controlling the population dynamics of testate amoebae and zooplankton in an oligo-mesotrophic reservoir.

Human disturbance of freshwater and terrestrial ecosystems is another potential factor influencing biodiversity in lakes. Lake sediments are historical archives of past activity and paleolimnological research shows anthropogenic stresses have degraded many European lakes (Bennion et al 2011), which have been affected by: eutrophication, acidification, toxic pollution, water level change and the introduction of non-native, invasive species (Moss 1998, Moss 2010). Current major human impacts coupled with the additional threat of climate change are likely to increase some of these symptoms, for instance, eutrophication and deoxygenation (Moss *et al* 2009). Some (Bennion *et al* (2011) suggest a multi proxy palaeoecological approach is needed to use the past as a key to establish a baseline 'natural' state to inform restoration. Whilst eutrophication can occur naturally, paleolimnological studies have found evidence of human disturbance to lake ecosystems since the introduction of agriculture as early as 6000 years ago in Denmark (Bradshaw *et al* 2006) and later examples include the eutrophication of Llyn Mire in Wales during Tudor times as a by-product of cannabis retting (French and Moore 1986). Importantly, other paleolimnological studies have demonstrated nutrient enrichment linked to human activity in lake sediments from the mid nineteenth century onwards (Bennion *et al* 2011). This coincides with the intensification of human activity associated with rapid industrialisation and population growth which Bennion *et al* (2011) argue has degraded the ecological quality of UK lakes, for example, through

agricultural intensification and phosphorus transfer in runoff from agricultural land. The main focus for reducing eutrophication remains with phosphorus (P) and the EU Water Framework Directive (2000/60/EC) provides a legislative framework to implement catchment controls over P inputs to EU waters from all sources, including those from agriculture (Withers and Haygarth 2007). Until recently, research has focused on using diatoms to infer changes in nutrient status and trophic status change (Smol and Stoermer 2010, Battarbee *et al* 2002). It's now also recognised testate amoebae may have potential as indicators of eutrophication in lakes and may be able to provide an estimate of baseline conditions (Roe *et al* 2010, Roe *et al* 2012), thus allowing for cross validation of proxy-based inference models using existing diatom data. The problem of eutrophication highlights that terrestrial elements in the landscape can indirectly alter the structure and function of freshwater environments, for which human disturbance plays a key part.

1.3 Model organisms

1.3.1 Biology of Testate amoebae

Testate amoebae (traditionally phylum Sarcodaria, superclass Rhizopoda) are a functional, polyphyletic group of unicellular protists (Smith *et al* 2008) and are the subject of recent revised classification (Adl *et al* 2012). A single eukaryotic cell is enclosed within a test (shell) ranging in size from 5 - 300µm with an oral aperture through which filose or lobose pseudopodia protrude to perform the dual functions of motility and feeding (Charman 1999). Pseudopod morphology divides testate amoebae into two groups: lobe-shaped or filiform. Testate lobose amoebae include the larger species (> 100 µm) and include those in the families *Difflugiidae*, *Centropyxidae*, *Arcellidae* and *Hyalospheniidae*. The tests either comprise of mineral particles, diatom frustules, recycled siliceous scales (xenosomes) or plates or are composed of siliceous, calcite, or chitinoid self-secreted plates (idiosomes) held together by organic cement (Adl *et al* 2012). In contrast, testate filose amoebae include the families *Euglyphidae* and *Trimenatidae* which contain smaller species (< 100 µm) with self-secreted siliceous tests composed of idiosomes. Most reproduction is asexual by simple division during which the parent constructs and entirely new test and then divides leaving one daughter nucleus and

cytoplasm inside this structure (Charman 1999), but molecular phlyogenetic studies suggest they also reproduce sexually (Lahr *et al* 2011).

1.3.2 Biology of Diatoms

Diatoms (division Bacillariophyta or class Bacillariophyceae) are a monophyletic group of unicellular or colonial autotrophic eukaryotes (Chepurnov *et al* 2004) ranging in size from 5 – 500 µm. Diatoms are either solitary, free-living cells whilst others are attached to other cells forming colonies or to different organic or mineral substrates. The chloroplast is enclosed within a siliceous cell wall called the frustule (or valve) and striae which are the lines occurring on the valve are highly resistant to decay. Two major groups have traditionally been recognised: centric diatoms and pennate diatoms. The valve outline in centric diatoms is often circular with a radial pattern of markings on the valve whilst the valves of most pennate diatoms are bipolar. Pennate diatoms are subdivided into two groups: raphid pennates which possess one or two slits (the raphe system) and araphid pennates which lack a raphe. Motility is restricted to the uniflagellate sperm of centric diatoms and to pennate diatoms that possess the raphe system. Movement of the cell relative to the substratum is believed to be mediated by the secretion of material from the raphe (Round *et al* 1990). The diatom life cycle has been suggested to comprise two principal phases: vegetative phase during which the cells divide mitotically and a comparatively shorter phase that includes sexual reproduction (Chepurnov *et al* 2004).

1.4 Aims of this study

This study addressed the idea of describing and quantifying 'cryptic' microbial diversity for nature conservation, which was recently suggested in Nature (Esteban and Finlay 2010). Microbial communities have escaped description at Mere Sands Wood Nature Reserve probably because they are invisible to the scrutiny of most naturalists (Margulis *et al* 1986). The broad aims of this study were to compare eukaryotic microbial diversity and distribution on a nature reserve with previously studied macroscopic organisms and analyse differences in microbial diversity between different terrestrial and aquatic microhabitats. Linking research with conservation, the present study aimed to critically appraise the ways in which free-living eukaryote species could potentially be included in biodiversity conservation surveys. Diatoms and Testate amoebae are ecologically widespread, species-rich groups of 'shelled' Protists found in a range of habitats. Using the traditional protist taxonomy based on shell morphology both groups were counted directly under the microscope producing species richness and population size data for microorganisms which were compared with existing data collected in studies of the ecology of macroscopic organisms. Microscopical analysis and the subsequent creation of species lists provided not only quantitative data on which taxa occurred in terrestrial and aquatic microhabitats, but also an initial understanding of how these taxa may be organised into communities. Species assemblages in different terrestrial and aquatic microhabitats provided insights into the potential biotic and abiotic factors influencing microbial ecology.

The ecology of protists in soils raises many important and understudied questions. For instance, some studies (Foissner 1987) have illustrated graphically that some protists such as testate amoebae are most abundant in the upper soil layers. Yet, despite being perhaps the most biologically active and functionally important zone of the soil profile, the litter horizon is often overlooked (Bardgett 2005). This study aimed to quantify and contrast the diversity and abundance of testate amoebae and diatoms vertically through the soil profile in fresh organic matter (FOM) and soil organic matter (SOM). It is reported (Foissner 1987, Wilkinson and Mitchell 2010) that for testate amoebae a positive relationship exists between the organic matter content of soils and the abundance and diversity however, it is not yet known whether the same relationship exists for diatoms. Organic matter content in terrestrial soils and

aquatic lake sediment was determined to test this hypothesis for both groups. Additionally, there is limited evidence (Wanner *et al* 2008, Smith *et al* 2008) that microbes do not conform to the classical successional processes observed for vascular plants, for instance, the process of gradual replacement of a pioneering plant community by more stable plant communities. In testing this hypothesis this study aimed to analyse the ratio of testate amoebae considered as r-K strategists in different successional stages of vegetation on an island within a shallow lake, thus providing further insights into potential differences in the ecology of macroscopic and microscopic organisms.

In lakes, studies have shown that further research into the structure of microbial communities is needed. For instance, in a study investigating the concordance of taxonomic richness patterns in lakes, Allen *et al* (1999) found a lack of concordance between different taxonomic groups, highlighting community structural aspects should also be investigated. Using a correlative approach examining different measures of diversity, this study aimed to analyse whether there was any correlation between the diversity of testate amoebae and diatoms in terrestrial and freshwater habitats. Further, an important aspect of ecological studies is the detection of structured spatial variation and identification of spatial scales (Krebs 1999). Fisher and Dunbar (2007) and Barker *et al* (2010) have found that microorganisms exhibit small-scale patchiness in lakes and this has implications for assessments of waterbodies required for legislation such as the European Water Framework Directive. This study aimed to follow this research by intensive field sampling of terrestrial and aquatic microhabitats using a nested (hierarchal) design.

The main research questions asked were: (1) how does microbial diversity compare with that of previously studied macroscopic organisms on a single nature reserve? (2) Is there any difference in testate amoebae and diatom diversity between different terrestrial and aquatic microhabitats? (3) Is there a correlation between taxon richness and the diversity of different microbial groups? (4) Are there any relationships between taxon richness, diversity and environmental variables? (5) Can we add any further insights into the ecology of eukaryotic microorganisms? (6) What surrogate measures could be used in rapid microbial biodiversity surveys?

CHAPTER TWO

STUDY SITE

2.1 Site Description

Mere Sands Wood Nature Reserve located in Rufford, Lancashire (British National Grid Reference SD 447 157: latitude and longitude 53.6355^0N, 2.8371^0W) is owned and managed by the Wildlife Trust for Lancashire, Manchester and North Liverpool to encourage wildlife and provide facilities for visitors. The 42 hectare reserve resembles an island surrounded by an intensive agro ecosystem on the Lancashire Plains. The site is characterised by a series of artificially created lakes surrounded by mature deciduous and coniferous woodland with patches of grassland, heath and scrub. Within close proximity to Martin Mere Wildfowl and Wetland Trust (WWT), the reserve is nationally important for migratory wildfowl and resident birds and other notable species of interest to nature conservation, yet, the main feature of its Site of Special Scientific Interest (SSSI) status is its geological interest. The reserve stands on stratified aeolian sands and peat overlying boulder clay of glacial origin and it is this stratigraphy that has been internationally recognised for its potential contribution for interpreting geomorphological processes and environmental changes during the late glacial and early post glacial periods. The soils of MSW provide the best sections available for understanding the deposition of the Shirdley Hill Sands of Lancashire (Chiverell *et al* 2004).

The site was suitable for this study not only for its habitat heterogeneity but also for existing biological data for macroscopic organisms, available for higher plants, birds, fungi and some invertebrates. The existence of such biological data heavily relies upon voluntary participation as a means of scientific data collection as does the practical management of the reserve for its nature conservation interest.

2.2 Site History

The reserve has an interesting history of human disturbance documented since the late 17th century onwards. The name 'Mere Sands' dates back to medieval times when the site was situated on the shore of 'Martin Mere', once the largest freshwater lake in England which began as a depression in the glacial drift which filled with water as the ice from the last ice age retreated. From about 1697 onwards, Martin Mere and its surrounding peatlands were gradually drained for agriculture (Hale and Coney 2005). Before the mid-nineteenth century the Hesketh estate planted woodland, mainly of oak *Quercus,* to provide game cover. A *Rhododendron ponticum* understory was added later. The site and its planted woodland remained relatively undisturbed until about 1920 when the land was sold and much of the mature timber was felled. The remaining woodland around the periphery subsequently suffered serious fire damage. The result was regenerated woodland of Birch *Betula* and *Rhododendron* in which stands of Scots Pine *Pinus sylvestris* were planted in the 1930s. Between 1974 and 1982 sand extractors quarried the site recognizing its economic value for use in glass-making. Following this, extracted areas were landscaped into shallow edged lakes. During this period Trust members and the local community worked with (what was then) Lancashire County Council to require the extraction company under a planning agreement to landscape the site into a nature reserve once extraction was completed. On completion of the sand winning in 1982 the then Lancashire Naturalists Trust acquired the site. Restoration also involved tree planting on a relatively large scale in subsequent years.

2.3 Site character and ecology

Mere Sands Wood Nature Reserve is a spatially heterogeneous, largely man-made ecosystem but with attributes of many natural ecosystems. A large proportion of the reserve is composed of woodland and open water with ecotones between terrestrial and aquatic vegetation communities. For instance, dense patches of *Phragmites australis* and *Menyanthes trifoliata* which were planted and subsequently naturally colonised are a common form of lakeside ecotone at MSW. These beds accumulate organic matter which is then colonised by trees such as *Betula spp, Alnus glutinosa and Salix spp.* Human disturbance is necessary to

maintain the mosaic of habitats which support different assemblages of plants and animals. Practical conservation management involves halting and reversing natural succession to maintain areas of open water and terrestrial habitats and controlling the spread of invasive species.

2.3.1 Terrestrial habitats

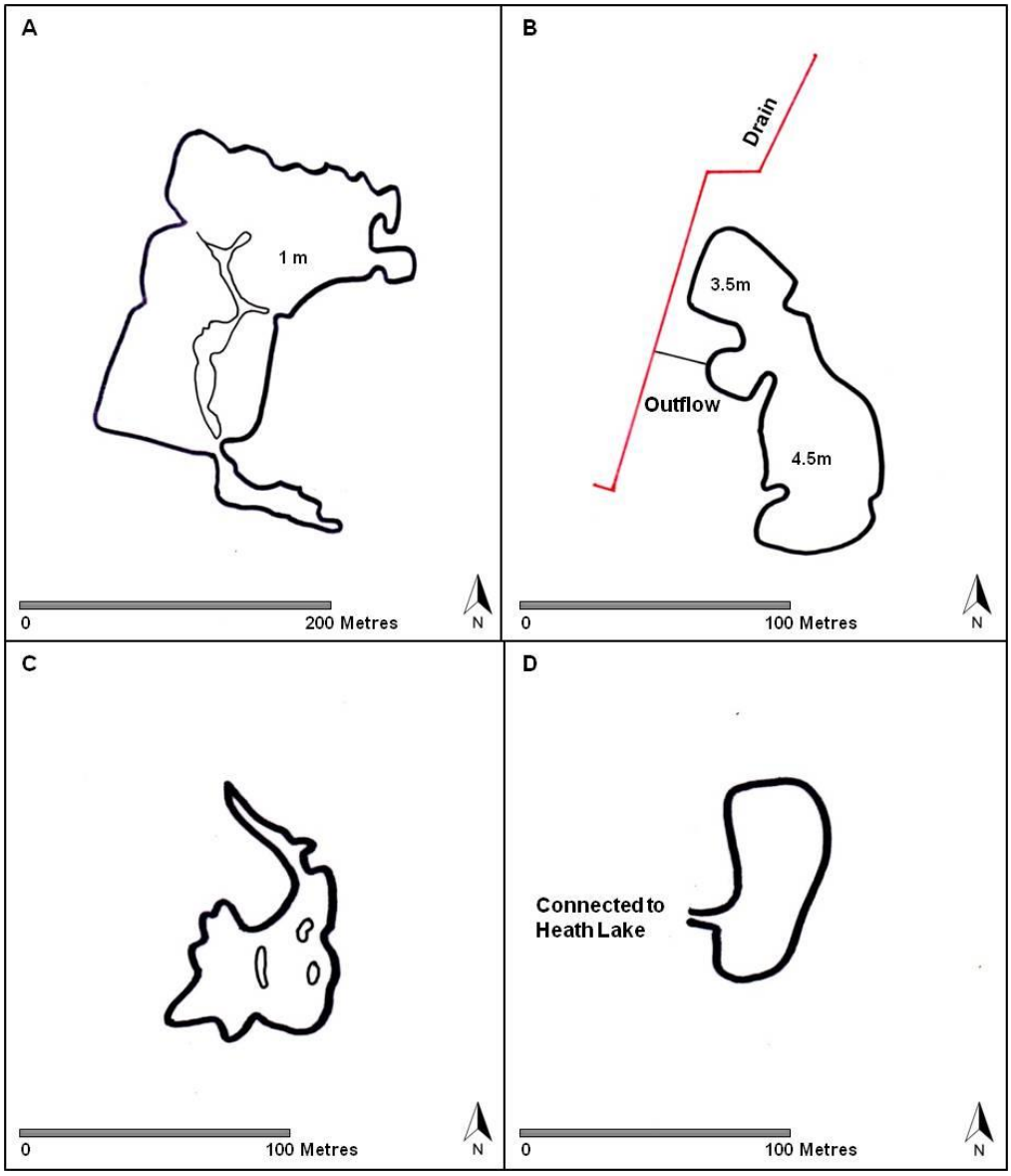
Woodland covers a large proportion of the reserve, particularly around the periphery of the site where it acts as a screen from the surrounding arable landscape and surrounding the individual waterbodies. The woodland is composed of mainly Scots pine (*Pinus sylvestris)* and birch (*Betula spp*.) with oak (*Quercus robur),* beech (*Fagus sylvatica)* and sycamore (*Acer pseudoplatantus)* and different standing biomass has played a role in the development of different terrestrial humus forms on the reserve (Ponge 2003). *S*tands are managed to create different successional stages for the benefit of associated plants and animals and felled trees are often left in situ and are decomposed by a vast array of fungi for which the site is noted. The woodland understory mainly consists of native species including bramble (*Rubus fruticosus agg)*, ferns (*Drypoteris spp)* and bracken (*Pteridium aquilinum)* with a ground cover of grasses (*Poaceae)*, mosses (*Bryophyta)* and wildflowers beneath open canopies in spring. The woodland also consists of patches of non-native *Rhododendron ponticum* creating a dark understory with little or no ground cover. It is considered an invasive species at the reserve and conservation management involves cutting and burning on site. Conservation grazing is also part of the management on the reserve and Hebridean sheep are used to control the dominance of rank grasses in some habitats such as the meadow.

2.3.2 Freshwater habitats

A major feature of MSW nature reserve is the series of artificial lakes, which are entirely man-made and are a by-product of sand extraction and subsequent restoration. These lakes which are all very different in terms of size, shape and depth (Figures 1 and 2 a – d) are important for over-wintering wetland birds. All lakes are shallow (<3m) with the exception of Mere End (Figure 2d), which is 4.5m in deeper areas. The largest of the lakes in

terms of surface area 'Twin Lakes' (Figure 2a) is considered the most important for wetland birds (L. Beaton *pers comms*) and within this shallow lake an island was created by the sand extractors formed from mixed layers of sand and peat from within this area. Today, human disturbance on the island is minimal and it is heavily used by birds for nesting, loafing, feeding and roosting. Compared with the other lakes, Twin Lake is more open with relatively smaller patches of marginal aquatic vegetation, and overhanging trees. In general, lake littoral zones are predominantly composed of dense patches of native and exotic wetland plants. Some native species, such as *Phragmites australis* and *Menyanthes trifoliata* were initially introduced to the reserve and have since naturally established and form dense patches in the littoral zones of many of the lakes and ponds, with *P. australis* also naturally occurring in many of the ditches. *Crassula helmsii* has been accidentally introduced and has since spread dramatically across many of the wetland areas of the reserve which may have resulted in the displacement of submerged macrophytes. Other macrophytes present include: *Myriophyllum spicatum*, *Ceratophyllum demersum Iris pseudacorus*, *Typha latifolia, Nuphar lutea* and a floating exotic plant *Nymphaea* cf *marliacea*.

Figures 1 a - d. Photographs of sampled lakes at Mere Sands Wood Nature Reserve:
a – Twin lake, **b** – Mere End, **c** – Scrape, **d** – End Lake.



Figures 2 a - d. Sketch maps of sampled lakes at Mere Sands Wood Nature Reserve: **a** – Twin lake, **b** – Mere End, **c** – Scrape, **d** – End Lake

CHAPTER THREE

METHODS

3.1 Field sampling design

Field sampling was undertaken over two consecutive days in September 2011 and followed a nested design (Figure 3) which has been recommended as a way to overcome the problems of patterns and scales in ecological studies (Levin 1992, Krebs 1999). Sampling was directed towards capturing the range of environmental variability within terrestrial and aquatic habitats (Figure 4). Three randomly placed quadrats ($1m^2$) were placed within each habitat and random numbers (terrestrial samples) were used to obtain three replicate subsamples within each quadrat, therefore allowing for assessment of multiple scales of variability. Each sample comprised about 20g of soil/lake sediment placed in a labelled sealed bag. Fresh and soil organic matter was sampled from terrestrial habitats using a spatula. Aquatic sediment samples were collected from four lakes from a boat using a sediment sampler and a grapnel was used to determine benthic vegetation composition. A one metre squared area was identified and three sediment samples were taken at random from within it. All samples were frozen prior to laboratory preparation.

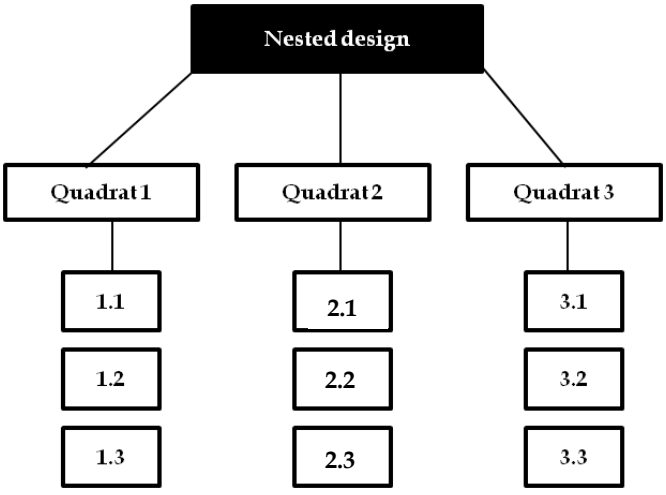


Figure 3. Example of the nested design – each of the levels of the main effect can be subdivided into randomly selected subgroups, the classification of the groups is said to be nested (Krebs 1999)

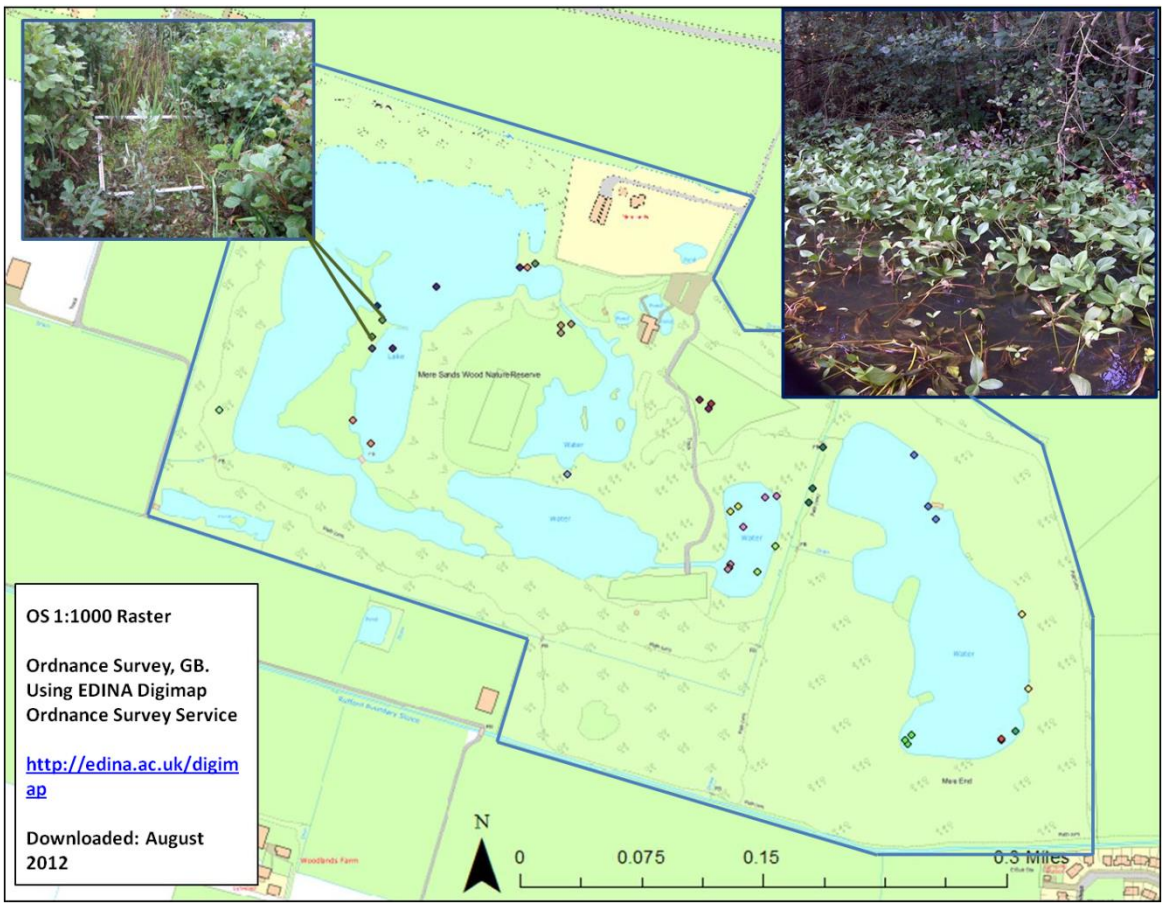


Figure 4. Map of the study site boundary, sampling locations and illustrations of some of the areas sampled. Top left: quadrat ($1m^2$) on an island within a shallow lake. Top right: Littoral zone of Mere End Lake dominated by *Menyanthes trifoliata*.

3.2 Testate amoebae data collection

3.2.1 Laboratory preparation

Testate amoebae do not require chemical reagents for microscopical preparation and were isolated from litter, soils and sediment using a sieving procedure (Charman *et al* 2000, Booth *et al* 2010). A known weight of oven dried (50^{O}C) sample was soaked and disaggregated in 30 ml de-ionised water using a clean stirring rod. The boiling stage recommended as a way to disaggregate samples by Charman *et al* (2000) was omitted in order to allow dead and alive tests to be distinguished. One tablet of *Lycopodium* spores were added as an exotic marker to allow the calculation of test concentrations. The material was washed though a sieve (250µm) to remove plant and course particulate matter, the fine-sieving process of Charman *et al* (2000) was omitted as it can result in the loss of small taxa (Payne 2009). The material was washed into a 30ml centrifuge tube and centrifuged at 3000 rpm for three minutes and the supernatant was discarded. In modern samples, empty and living tests can be distinguished by staining with Rose Bengal (Scott *et al* 2001, Vincke *et al* 2006) which can potentially indicate tests that must have been alive at the time of collection by preferentially staining the cytoplasm. Evidence suggests that staining requires a cautious approach as in live Foraminifera tests, Boltovskoy (1963) reported failure of the stain to penetrate the test wall of some living specimens; often different strengths of stain are required for different species (Scott *et al* 2001). In the present study samples were stained with 1-4 drops (depending on the volume of sediment) of a 0.25% Rose Bengal solution. Samples were stored and frozen in water which is recommended for viewing stained specimens (Scott *et al* 2001) and for potential scanning electron microscopy work (Booth *et al* 2010). Slides were mounted for light microscopy using glycerol as opposed to water to aid with the identification of morphotypes. Scanning electronic microscopy (SEM) was required for the identification of some morphotypes. For this, 0.3ml of the same solution used for optical microscopy was placed onto an aluminium stub. The samples were dried for 3 days in a desiccator prior to SEM analysis.

3.2.2 Quantification and identification

Tests were counted at 400x magnification using a Meiji Techno microscope in phase contrast. In each sample 150 tests were counted where possible, a number which is considered to be the standard search effort for testate amoebae (Davis and Wilkinson 2004, Vincke *et al* 2006, Payne 2009, Booth *et al* 2010). The total testate amoebae sum also includes the shells of a similarly sized unknown organism present in large numbers, particularly in aquatic samples. Experimenting using taxon sampling curves found this number of individuals and level of search effort appropriate to quantify species richness estimates. For example, in the island soil samples the curve increased relatively rapid at first and then more slowly in later samples as increasingly rare taxa were added (Figure 5). Relative abundances are taxa were expressed as percentages of the total counted (Booth *et al* 2010), as used in comparable studies (Trappeniers *et al* 1999). Microscopical analysis of testate amoebae also permitted the quantification of nematode abundance.

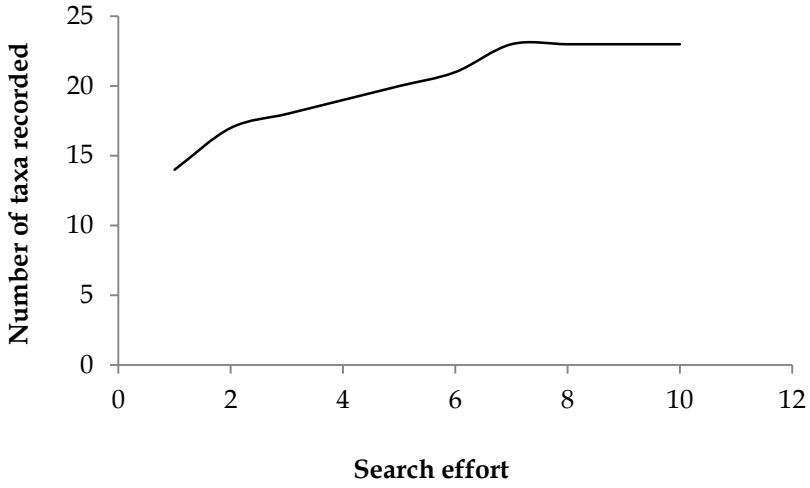


Figure 5. Taxon sampling curve for island soil samples, search effort is defined as the number of transects.

Morphological identifications of testate amoebae were mainly based on the work of Charman et al (2000), Clarke (2003), Scott *et al* (2001), Ogden and Hedley (1980) and Mazei and Tsyganov (2006). Most taxa were identified to morphospecies level or genera if in doubt, in particular, testate amoebae of the genus *Difflugia* which are currently undergoing taxonomic revision (Mazei and Warren 2012) and are a difficult group to confidently identify to species level.

3.3 Diatom data collection

3.3.1 Laboratory preparation

Terrestrial and freshwater samples were treated the same in laboratory preparation. A known weight of sample was oven dried at 105^0C for 48 hours. Diatom samples require digestion to remove organic matter. There are different methods described and evaluated for this process (see Ming *et al* 2007). As in other comparable studies (Heger *et al* 2011) diatom frustules were treated with 50ml of 30% hydrogen peroxide (H_2O_2) to remove organic matter. Hydrochloric acid (10%) was added to remove calcium carbonate from the samples. The supernatant was removed after >12 hours of sedimentation. Each sample was then re-suspended and cleaned in 30ml de-ionised water and a 0.3ml aliquot of the diatom suspension was dried on a glass cover slip and the cleaned diatom valves were mounted in Naphrax.

3.3.2 Quantification and identification

Diatom frustules were counted under oil immersion at x1000 magnification using a Meiji Techno microscope in phase contrast. Where possible a minimum of 400 frustules was counted, and identified when more than half of the valve remained intact (Smol and Stoermer 2010). It was realised that this number of counts may only characterise the dominant taxa. However, it was a number in which coarse ecological inferences could be made in line with the aims and objectives of the study. Where possible, diatom frustules were identified to morphospecies level using the taxonomy of Krammer and Lange-Bertalot (1999), which was particularly useful for aquatic samples. According to Schuttler (1986) species lists and relative species composition of soil diatom assemblages are often more difficult to obtain because soil diatoms are often sparse and detection amongst soil particles is difficult. Furthermore, (Lund 1946) argues the striae in soil diatoms are very faint compared with the aquatic forms and the density of these and the carinal dots cannot be trusted when comparing soil diatoms with the aquatic forms. As a result of such differences, the taxonomy of soil diatoms mainly followed that of Lund (1946).

3.4 Environmental variables

3.4.1 Determination of bulk organic matter content

Organic matter influences biological properties and activities in soil. In this study bulk organic matter content was determined using loss on ignition (Avery and Bascomb 1982) to support diatom and testate analyses in order to determine whether a positive relationship exists between the organic matter content of terrestrial litter and soil and lake sediment and the diversity of microscopic organisms. Loss on ignition was calculated: where W_C is the crucible weight, W_S is the crucible and sample weight after 24 hours in the muffle furnace at 105^{O}C and W_A is the crucible and sample weight after combustion of organic material in the muffle furnace at 450°C for a further 8 hours. The organic content was measured and expressed as percentage of the dry matter.

$$\frac{(W_S) - (W_A)}{(W_S) - (W_C)} * 100\%$$

3.4.2 Indicative pH measurements

Soil pH influences biological diversity, carbon and nutrient cycling and the physical properties. In September 2012 additional replicate soil samples were collected from terrestrial microhabitats (with the exception of the island samples). Soil pH values were determined in the laboratory using standard methods; 1:2.5 soil: water suspensions being prepared for pH measurement based on 10 g <2 mm soil particles to 25ml deionised water (Hesse 1971). A pH meter and probe was used in the field to measure pH of the lakes after calibration at pH 4, 7 and 10. Sample water was collected in a glass container and the pH reading was taken from the meter. As pH is measured on a logarithmic scale median pH values are presented in this study.

3.5 Existing biological records

The LWT holds an extensive database of biological records for MSW nature reserve, predominantly for terrestrial habitats. Of particular interest to this study were biological records for plants and fungi. The fungi records were utilized as they were considered to be more informative in terms of defining the substrate and habitat. Testate amoebae are said to encounter fungi in all terrestrial ecosystems (Vohnik et al 2012). To further investigate potential relationships between previously studied macro fungi and eukaryotic microorganisms in the present study spatial diversity and distribution was compared and contrasted. One of the problems with this method was timing of data collection. A further limitation was the organisation of data which were not spatially comparable. For instance, although the macro fungi dataset provided an indication of the locality of some of the records, it was not possible to fully ascertain whether the macro fungi record was taken from the same locality as the microbial sampling site. The macro fungi database was filtered by habitat type; available data existed for *Betula*, *Pinus, Poaceae* and *Phragmites*. Species lists and species richness estimates were generated for macro fungi and compared with the species lists of testate amoebae and diatoms. Due to the problems and limitations of using this method caution should be applied to the qualitative results.

The species list and presence/absence data includes some supplementary data for Testate amoebae in soils associated with *Rhododendron ponticum* at MSW.

3.6 Data analysis

The generation of species lists and population density for different microhabitats was the basis of this spatial analysis. In addition, diversity statistics were used to measure species diversity and evenness (Magurran 2004). Comparable studies (Neville *et al* 2010) have used species diversity indices not only as a measure of diversity, but also to indicate the relative health of the community from which the sample was taken. PAST software (Hammer *et al* 2001) originally aimed at paleontology data was used to calculate diversity indices and SPSS (Version 18) was used for statistical analysis. Diversity indices were calculated for all replicate subsamples and the mean and standard deviation calculated for analyses.

3.6.1 The Margalef diversity index

The Margalef diversity index (Clifford and Stephenson 1975) is one of the most common species richness indices and attempts to compensate for sampling effects by dividing richness, *S,* the number of species recorded, by *N*, the total number of individuals in the sample. When applying the Margalef index Gamito (2010) suggests the data must be organized as absolute numbers and not as a density data matrix. In other words, by the total number of individuals of each species in each sampling point as opposed to the number of individuals of each species per square metre in each sampling point.

$$D_{Mg} = \frac{(S - 1)}{\ln N}$$

3.6.2 Simpson Diversity Index

The Simpson diversity index (Simpson 1949), sometimes described as a nonparametric diversity index, is one of the most robust and meaningful dominance or evenness measures available and captures the variance of the species abundance distribution (Magurran 2004). Being less sensitive to sample size, the Simpson index is a useful for smaller populations and is expressed: where n_i = the number of individuals in the *i*th species; and N = the total number of individuals. As D increases diversity decreases. In other words, the index ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely) (Hammer *et al* 2001).

$$D = \sum \left(\frac{n_i (n_i - 1)}{N (N-1)} \right)$$

3.6.3 Shannon Diversity Index

The Shannon Index (SDI) is the most widely used measure and takes into account the degree of evenness in species abundances (Magurran 2004). The SDI varies from 0 for communities with a single taxon to high values for communities with many taxa each with few individuals (Hammer *et al* 2001). Neville *et al* (2010) used these values to indicate the relative condition of the community from which the sample was taken: harsh unfavourable

environmental conditions were characterised with an SDI between 0.5-1.5, intermediate conditions range from 1.5-2.5 and favourable/stable conditions have an SDI >2.5. The SDI is expressed: where n_i is the number of individuals.

$$H = -\sum_{i} \frac{n_i}{n} \ln \frac{n_i}{n}$$

3.6.4 LF Index

As in other comparable studies (Mattheeussen *et al* 2005), the LF-index (Bonnet 1976) was used to estimate the developmental stage of testate amoebae communities in different microhabitats. This index is based on the assumption that filose testate amoebae display r-strategic traits (for example, smaller body size, high fecundity, short generation time, ability to disperse offspring widely), while lobose testates follow a more K-based strategy (larger body size, production of fewer offspring, longer life expectancy) (Smith *et al* 2008). Data are presented as the proportion of testates with lobose vs filose pseudopodia based on the assumption that higher values indicate more developed communities. The index is calculated as the ratio between the number of testate amoebae with lobose and filose pseudopodia (LF = (lobose – filose) / (lobose + filose), and varies theoretically from -1 (undeveloped communities) to +1 (developed stabilised communities). In the absence of an alternative index used to investigate potential life strategies in protists, the LF index should be treated with caution.

CHAPTER FOUR

RESULTS

4.1 Overview of biodiversity at Mere Sands Wood Nature Reserve

4.1.1 How does microbial species richness compare with previously studied macroscopic organisms?

In a total of 181 soil and sediment samples from different microhabitats at MSW, 209 eukaryotic microbial species and subspecies have been identified. A list of all taxa recorded (Appendix 1) and photomicrographs of some taxa (Appendix 2) are shown. Before the present study 970 eukaryote species were recorded at MSW belonging to the Plant, Fungi and Animal kingdoms. With the incorporation of members of just two groups of the kingdom Protista the number of biological records has increased by 17.73%. The relative proportions of existing biological records held at the reserve for three of the five kingdoms: animals, plants and fungi were quantified and compared with data for protists generated in the present study (Figure 6).

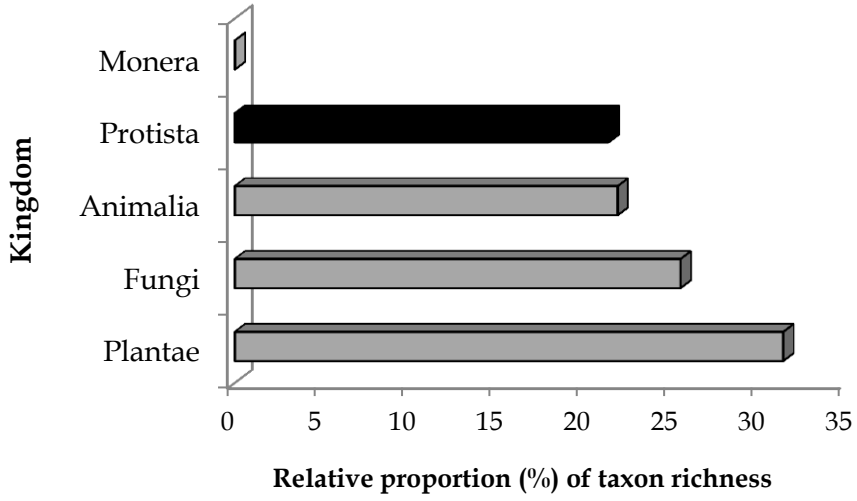


Figure 6. Taxonomic composition (%) of recorded biodiversity at Mere Sands Wood Nature Reserve using Whittaker's Five Kingdom System (Margulis and Schwartz 1998, Hagen 2012)

There was a general trend of greater taxon richness for macroscopic organisms compared with microscopic organisms. The highest proportion of biological records available was for members of the plant kingdom (31.34%) followed by macro fungi (25.46%). The kingdom Protista consists of the two model groups: diatom taxon richness (58.45%) was slightly higher than testate amoebae taxon richness (41.55%). Birds comprised the largest proportion of sightings of species in the animal kingdom (80.19%) compared with invertebrates (18.87%). No records exist for prokaryotic microorganisms in the Kingdom Monera.

4.1.2 Qualitative observations on the spatial distribution of testate amoebae and diatoms with previously studied macro-fungi

Qualitative observations suggested that habitat type appeared to influence the presence and number of testate, diatom and macro fungi taxa. Despite this, there was no clear trend in the spatial distribution of testate amoebae and diatoms with previously studied macro fungi (Figure 7). For instance, with the exception of grassland, macro fungi were generally more species rich in terrestrial habitats compared with aquatic. In terms of broad habitat types it's perhaps not surprising that woodland substrates contained the highest number of macro fungi taxa compared with grassland and marginal aquatic substrates such as *Phragmites australis*. These findings suggested that testate amoebae do potentially encounter fungi in all terrestrial habitats; of particular interest was the observed similarity between the numbers of testate and fungi taxa associated with *Pinus sylvestris* woodland substrates and the absence of diatoms. In contrast, higher numbers of macro fungi taxa were observed for *Betula pendula* woodland compared with testates, however, the present study sampled from litter and soil organic matter beneath early successional *B. pendula* woodland, whereas the macro-fungi records associated with *Betula* came from different substrates including soil, twigs, branches, stumps and leaves.

The existing macro fungi records at MSW contained data appropriate and relevant to four broad habitats sampled in the present study. It should be noted that caution should be applied to these qualitative findings as due to temporal and spatial limitations of the data,

statistical analyses to determine potential relationships between groups were deemed inappropriate.

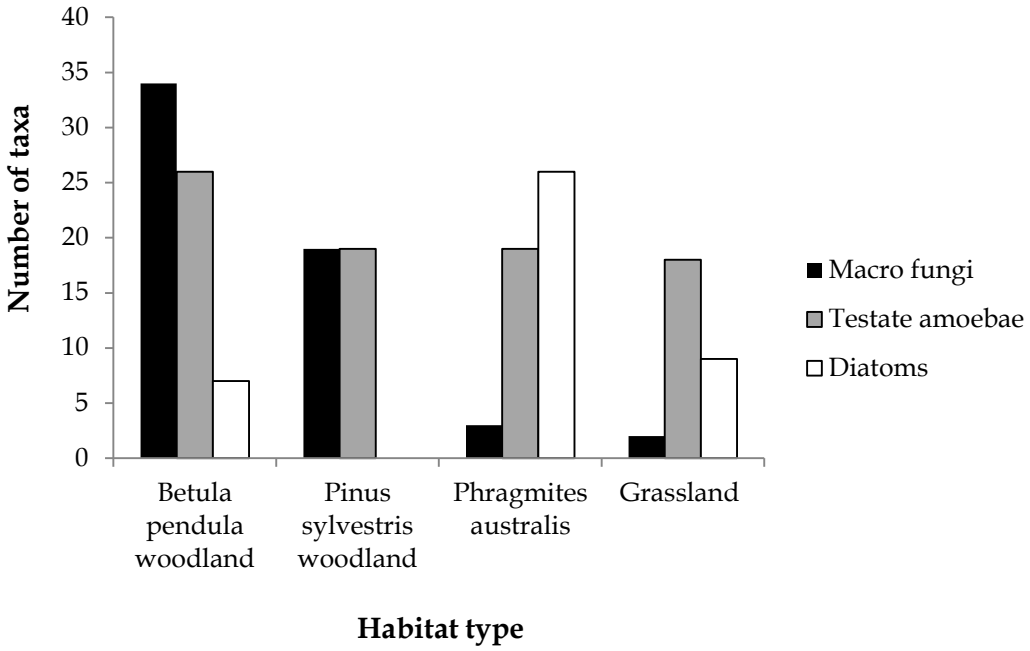


Figure 7. Qualitative observations on the spatial distribution of testate amoebae and diatoms with previously studied macro fungi at Mere Sands Wood Nature Reserve

4.1.3 Microbial taxon and genus richness

Overall, this study recorded 83 testate amoebae taxa from 26 genera (Figure 8) and 126 diatoms from 24 genera (Figure 9). The mean (± SD) number of testate taxa per sample (n=94) was 11.0 ± 4.6. Most taxa belonged to the lobose genera *Difflugia* (11 taxa), *Centropyxis* (9 taxa) and filose genus *Euglypha* (8 taxa). In contrast, the highest proportion of all counted tests was constituted by filose testates in the genus *Trinema* (Table 1). The mean number of diatom taxa per sample was greater 19.0 ± 9.3. Most taxa belonged to sediment associated diatom genera *Navicula* (17 taxa), *Pinnularia* (12 taxa), *Fragilaria* (10 taxa) and plant associated *Achnanthes* (10 taxa). The highest proportion of all counted frustules belonged to the genus *Fragilaria* (Table 2).

A summary of overall microhabitat taxon and genus richness is presented for terrestrial microhabitats (Table 3) and aquatic microhabitats (Table 4) and relative abundance tables for genera are shown (Appendix 5 and 6). The highest number of taxa recorded for diatoms in terrestrial microhabitats was in soil organic matter sampled beneath bryophyte vegetation on an island within a shallow lake. Testate amoebae taxon richness was highest on the same island in fresh organic matter associated with marginal aquatic vegetation composed of *Iris pseudacorus* and *Typha latifolia*. For aquatic microhabitats testate amoebae taxon richness was highest in the benthic sediment of Mere End Lake. Overall, Mere End Lake (Figures 2 and 3 b) showed a higher taxon and genus richness for both diatoms and testate amoebae compared with the other lakes studied, although, this is probably a result of a larger sample size and increased search effort. In all lakes taxon and genus richness was higher in benthic lake sediment (open water microhabitats), compared with sediment associated with homogenous patches of native and exotic littoral vegetation.

The Pearson correlation coefficient test was used to test the null hypothesis that there was no relationship between taxon and genus richness between terrestrial microhabitats (testate amoebae n = 14 and diatoms n = 10). Both groups showed a positive statistical significant correlation between taxon and genus richness in terrestrial microhabitats (Table 5). A similar trend was observed for testate amoebae (n = 14) and diatoms (n = 15) in aquatic microhabitats.

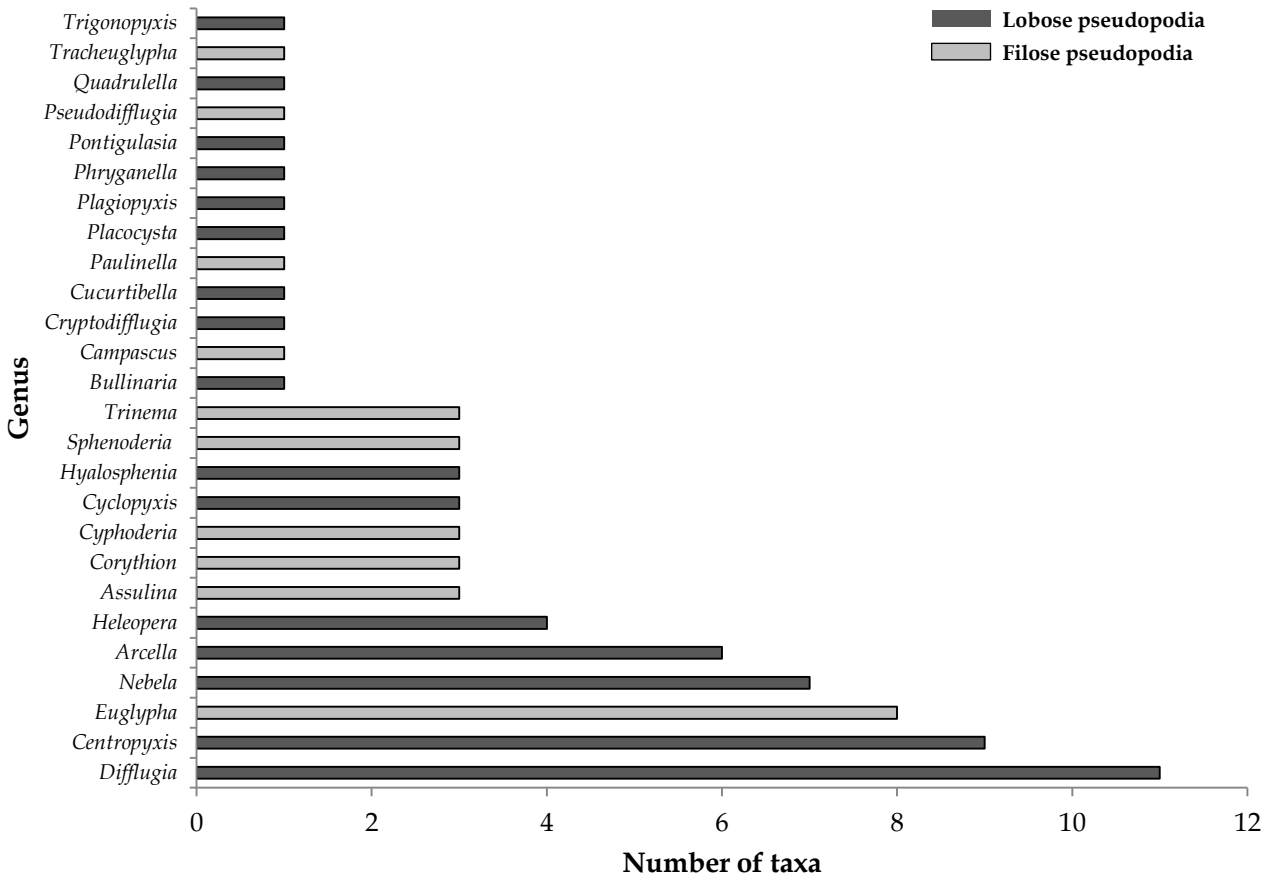


Figure 8. Distribution of MSW testate amoebae species per genus in terrestrial (n=42) and aquatic (n=52) microhabitats with qualitative information relating to life strategy

Table 1. The main observed testate amoebae genera with their relative abundance (%) and the number of present taxa

	Relative abundance (%)	**Number of taxa**
Trinema	33.32	3
Euglypha	20.32	9
Difflugia	9.53	12
Corythion	7.00	3
Centropyxis	5.39	9
Other	24.44	47

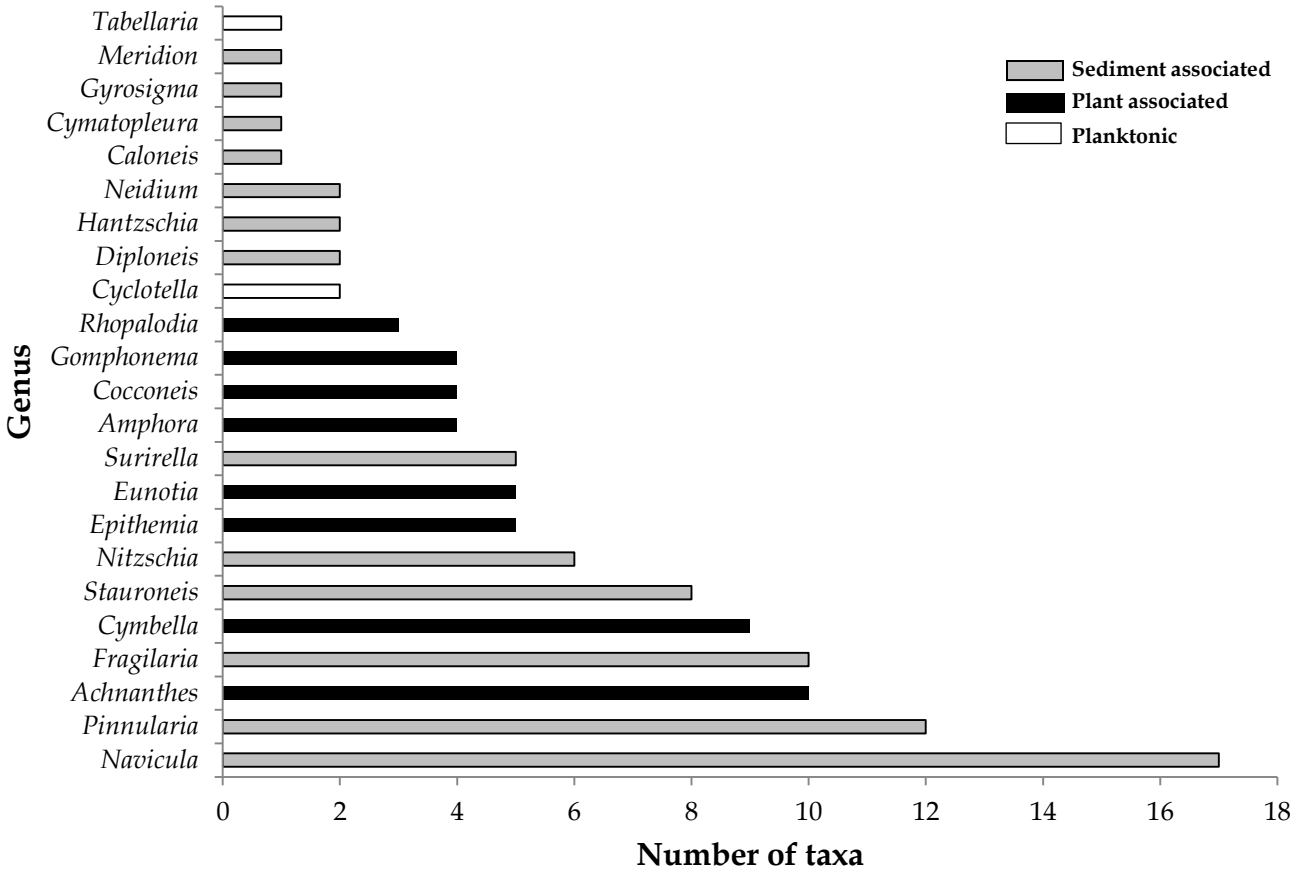


Figure 9. Distribution of MSW diatom species per genus in terrestrial (n=36) and aquatic (n=51) microhabitats with qualitative information on substrate type

Table 2. The main observed diatom genera with their relative abundance (%) and the number of present taxa

	Relative abundance (%)	**Number of taxa**
Fragilaria	28.86	10
Achnanthes	21.74	10
Cocconeis	13.33	4
Navicula	11.21	17
Gomphonema	3.83	5
Other	21.03	78

Table 3. A summary of overall of taxon and genus richness in terrestrial microhabitats

	Number of testate taxa	Number of genera	Number of diatom taxa	Number of genera
Fresh organic matter				
Betula pendula early succession	22	9	1	1
Mature *Pinus sylvestris* woodland	17	12	0	0
Grazed *Poaceae* meadow	14	8	7	4
*Open vegetation *Bryophytes*	19	11	24	11
*Mixed semi-open vegetation	28	13	-	-
*Thick shrub A*lnus glutinosa*	27	13	-	-
Soil organic matter				
Betula pendula early succession	17	9	6	6
Mature *Pinus sylvestris* woodland	18	11	0	0
Grazed *Poaceae* meadow	11	8	9	5
*Open vegetation *Bryophytes*	19	10	45	13
*Mixed semi-open vegetation	22	13	-	-
*Thick shrub A*lnus glutinosa*	26	13	-	-
Rhododendron ponticum dominated	27	12	0	0
Cleared *Rhododendron ponticum*	19	10	5	5

* island within a shallow lake

Table 4. A summary of overall taxon and genus richness in aquatic microhabitats

	Number of testate taxa	Number of genera	Number of diatom taxa	Number of genera
Mere End Lake				
Open water	34	14	50	18
Phragmites australis	13	7	17	13
Menyanthes trifoliata	10	8	32	16
Crassula helmsii	10	7	34	16
End Lake				
Open water	10	8	42	14
Mixed vegetation community	12	7	35	12
Menyanthes trifoliata	8	7	30	12
Crassula helmsii	10	8	25	13
Twin Lake				
Open water	22	12	44	17
Menyanthes trifoliata	16	10	31	13
Crassula helmsii	-	-	23	11
Scrape				
Open water	26	10	43	17
Phragmites australis	15	8	36	16
Menyanthes trifoliata	9	6	34	17
Drainage ditch	16	8	34	14

Table 5. Pearson's correlation between taxon and genus richness

	Microhabitat	Correlation coefficient	P-value
Testate amoebae	Terrestrial	0.795	0.001
	Aquatic	0.903	<0.001
Diatoms	Terrestrial	0.922	<0.001
	Aquatic	0.707	0.003

4.2 Microbial diversity and distribution in terrestrial environments

4.2.1 Differences in microbial diversity between terrestrial microhabitats

Overall, there was a general trend of higher testate diversity compared with diatoms in all terrestrial microhabitats (Figures 10 a - d). The mean number of testate taxa per terrestrial sample (n=42) was 14.05 ± 0.665 compared to the mean number of diatom taxa per sample (n=36) of 6.50 ± 1.726. Soil organic matter (SOM) and fresh organic matter (FOM) within each microhabitat/vegetation zone were different from each other in terms of bulk organic matter content and pH (Table 6).

Table 6. Summary of environmental variables for different microhabitats/vegetation zones

	Bulk FOM (%)	Bulk SOM (%)	pH *
Betula pendula early succession	48.4 ± 9.4	4.4 ± 2.0	6.29*
Mature *Pinus sylvestris* woodland	95.3 ± 1.3	95.6 ± 0.3	4.07*
Grazed *Poaceae* meadow	51.1 ± 7.8	17.5 ± 1.9	6.45*
*Open vegetation *Bryophytes*	81.7 ± 8.4	71.9 ± 4.5	-
*Mixed semi-open vegetation	84.7 ± 6.8	87.0 ± 3.5	-
*Thick shrub A*lnus glutinosa*	80.9 ± 12.3	45.7 ± 12.8	-
Rhododendron ponticum dominated	94.6 ± 0.7	86.1 ± 2.9	4.89*
Cleared *Rhododendron ponticum*	90.8 ± 5.0	37.3 ± 20.3	4.34*

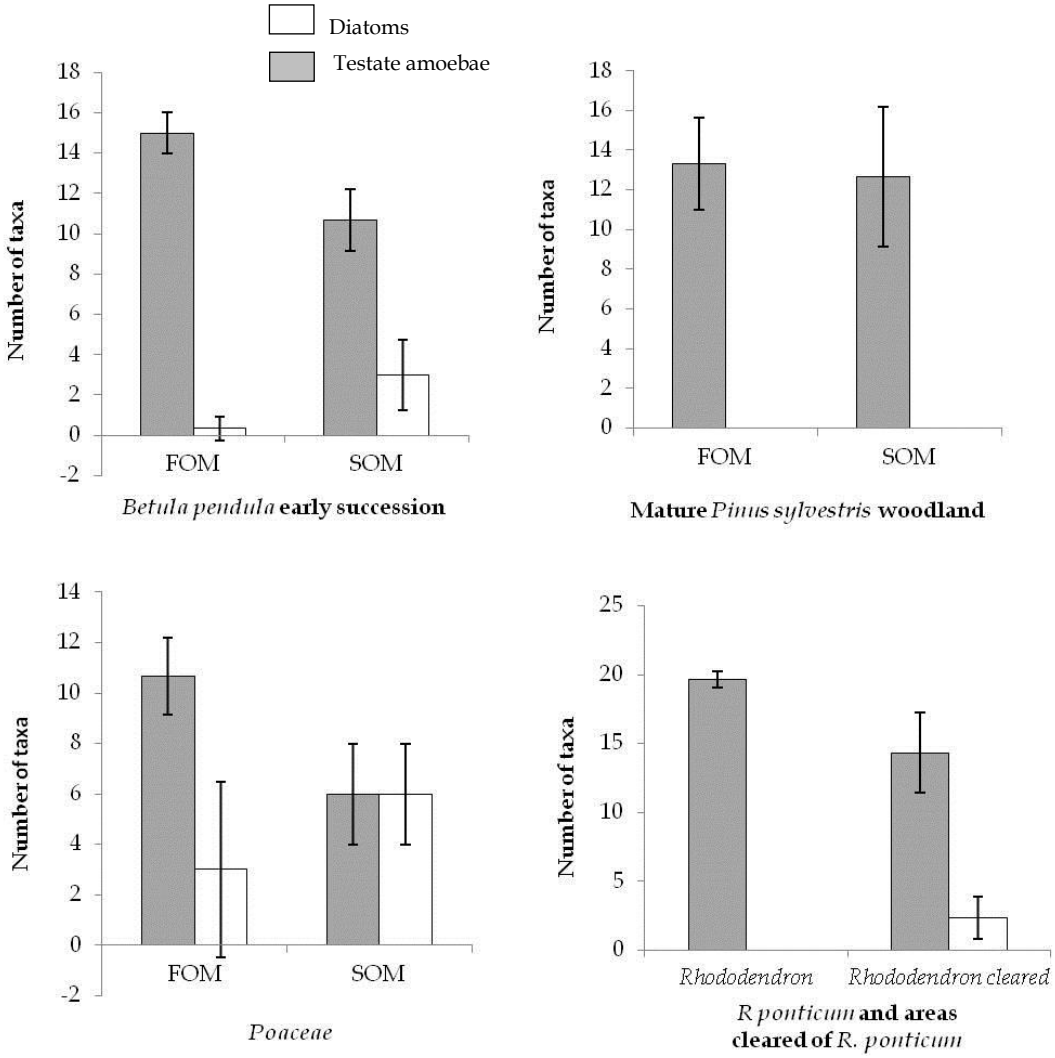
* indicative pH measurement
* Samples taken from a man-made island within a shallow lake

The mean number of testate taxa was highest in soil associated with *Rhododendron ponticum* 19.67 ± 0.577 and the lowest number of taxa was found in soils associated with *Poaceae* 6.0 ± 2.0. Analysis of Variance (ANOVA) was used to test the null hypothesis that there was no difference in mean testate diversity among terrestrial microhabitats (n=14). There was a statistically significant difference in the Margalef index of testate diversity among terrestrial microhabitats ($F_{13,28}$ = 2.961 p=0.008). The Kruskal Wallis test suggested a significant difference in the number of testate taxa ($K_{13,28}$ = 27.315, p=0.011). Conversely, there was no significant difference between the Simpsons ($K_{13,28}$ = 21.313, p=0.067) and Shannon ($K_{13,28}$ = 21.895, p=0.057) indexes of testate diversity among terrestrial habitats.

There was a greater number of diatom taxa found in fresh and soil organic matter from samples taken on an artificial island within a shallow lake compared with other terrestrial microhabitats. These island soils also contained the highest abundance of nematodes (89%), (appendix 5, table 1) among all terrestrial habitats. The mean number of diatom taxa found in island soils directly below a vegetation community composed of mainly *Bryophytes* was 27.00 ± 3.606 compared with 0.33 ± 0.577 in fresh organic matter associated with early successional *Betula pendula* woodland. In contrast with testate amoebae, the number of diatom taxa in soil associated with *Poaceae* was relatively high 6.0 ± 2.0 compared with other terrestrial habitats.

Diatom taxon richness followed a similar trend as testate amoebae as there was a significant difference between the mean number of diatom taxa between terrestrial microhabitats ($F_{7, 16}$= 50.427, p=<0.001) and the Margalef index of diversity ($K_{7, 16}$= 17.980, P=0.012). In contrast with testate amoebae, there was also a significant difference between the Simpson ($K_{7, 16}$ = 18.227, p=0.011) and Shannon ($K_{7, 16}$ =18.435, p=0.010) diversity indices.

Testate amoebae were present in all terrestrial samples; in contrast, diatoms were not detected in soil and litter samples associated with mature *Pinus sylvestris* woodland and soils associated with *Rhododendron ponticum*. Soil samples taken from an area cleared of *R.ponticum* did contain diatoms although the mean number of taxa was relatively low at 2.33 ± 1.528 compared with other terrestrial microhabitats. Testate amoebae taxon richness was generally higher in FOM compared with SOM (Figures 10 a – d) with the exception of soils associated with thick shrub on an island within a shallow lake (Figure 11).



Figures 10 a - d. The mean (±SD) number of testate amoebae and diatom taxa in soil and fresh organic matter in terrestrial microhabitats

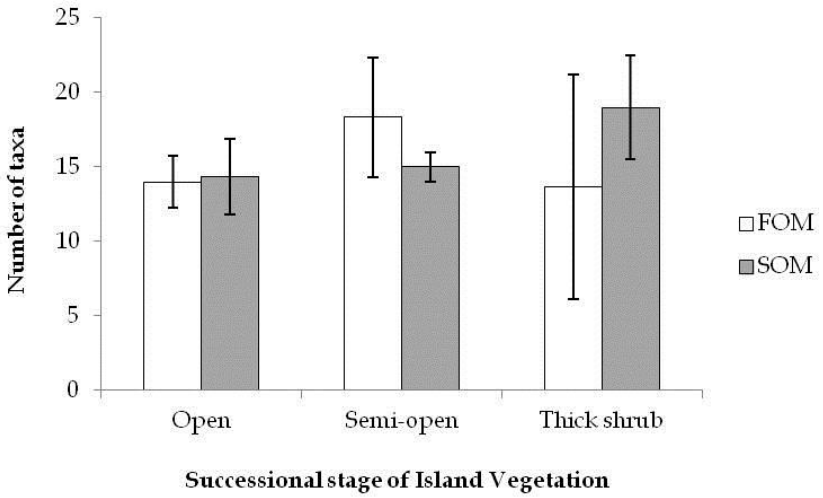


Figure 11. The mean (±SD) number of testate amoebae taxa in fresh (n=3) and soil organic matter (n=3) under different stages of vegetation succession on an island in a shallow lake

4.2.2 Correlations between species richness and diversity of testate amoebae and diatoms

After testing the normality of the diversity data for both groups the Pearson's correlation coefficient was used to test the alternative hypothesis that there was a significant relationship between species richness and the diversity of testate amoebae and diatoms in the terrestrial microhabitats (n= 9). Diversity summary statistics for number of taxa (n=3), Simpson (n=3), Shannon (n=3) and Margalef (n=3) indexes of diversity are shown in Appendix 3. The hypothesis was rejected as there was no correlation among the number of taxa ($r = 0.172$, $p = 0.658$), Simpson ($r = 0.047$, $p = 0.904$), Shannon ($r = 0.054$, $p = 0.890$) and Margalef ($r = -0.037$, $p = 0.924$) indexes of diversity between diatoms and testate amoebae.

4.2.3 Correlations between species richness, diversity and environmental variables

There were no statistically significant relationships ($p>0.05$) found between the number of taxa and diversity of testate amoebae and diatoms and environmental variables: among all terrestrial microhabitats. The terrestrial microhabitats were classified into humus forms with mor humus soil classified with a pH <5 which included *Pinus sylvestris* and *Rhododendron ponticum* microhabitats and mull humus soils with pH >6 which included *Betula pendula* and *Poaceae*. There was a significant negative correlation between the number of

testate taxa and the organic matter content of mor humus soil ($r = -0.763$, $p = 0.017$) (Figure 12) and between the number of testate taxa and organic matter content of mull humus soil ($r = -0.828$, $p = 0.042$) (Figure 13).

4.2.4 Correlations between species richness and diversity indices

For diatoms, the Pearson's Correlation co-efficient showed consistency between species richness and all diversity indices in terrestrial microhabitats ($p = <0.05$). This may be expected though as the two measures are auto-correlated. In contrast, there was no significant relationship between testate taxon richness and the Simpson index ($r = 0.514$, $p = 0.157$) and Simpson and Margalef index ($r = 0.369$, $p = 0.329$).

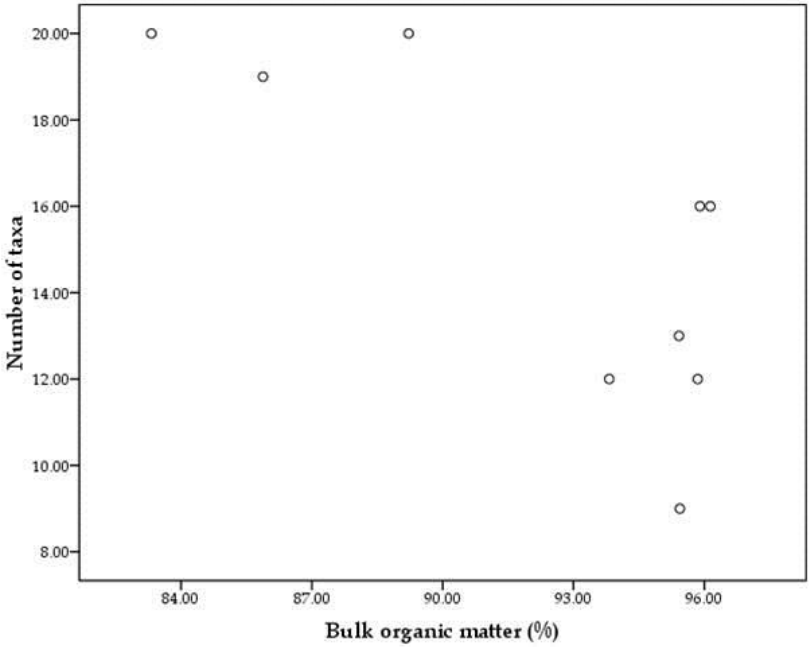


Figure 12. There was a significant negative correlation between the mean number of testate taxa and bulk organic matter content in mor humus type soil (n=9)

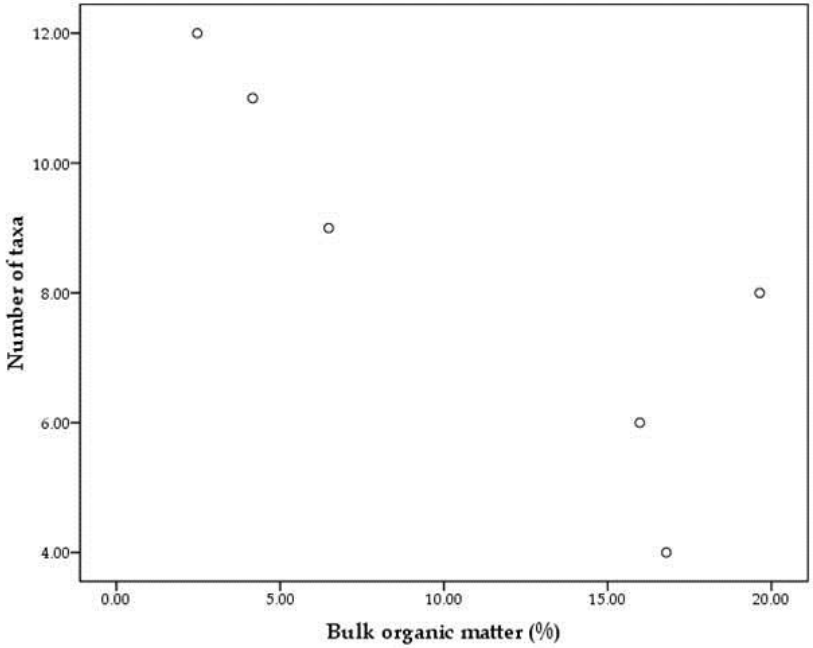


Figure 13. There was a significant negative correlation between the mean number of testate taxa and bulk organic matter content in mull humus type soil (n=6)

4.2.5. Differences between testate amoebae composition in litter and soil

The LF-Index was calculated to estimate the developmental stage of terrestrial testate amoebae communities in litter and soil (Table 7). All terrestrial samples from fresh organic matter (FOM) and soil organic matter (SOM) showed negative LF-values theoretically indicating dominance of filose testate amoebae (r-strategists) and undeveloped communities (Bonnet 1976). The mean LF-index of samples taken from FOM (LF= -0.65 ± 0.25) was not significantly different than those of SOM (LF= -0.43 ± 0.36) ($t_{2,12}$= -1.254, p = 0.139), although there was a trend of a higher abundance of testates classified as K-strategists in SOM (Figure 14). According to the assumptions of the LF-Index the most underdeveloped terrestrial testate community was that of early successional *Betula pendula* woodland. Conversely, the index values suggested mature *Pinus sylvestris* woodland SOM contained the highest proportion of lobose testate amoebae (K strategists) and hence assumed to be the most developed terrestrial testate community. These results however were highly variable suggesting relatively small-scale variability in the proportions of lobose to filose testates in both fresh and soil organic matter between different terrestrial microhabitats.

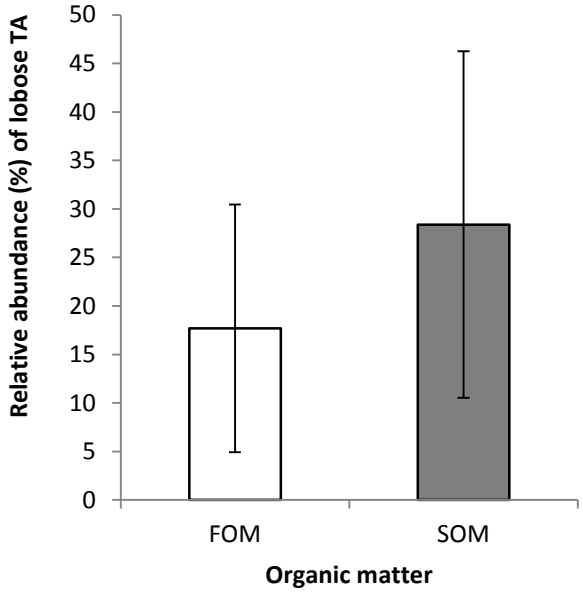


Figure 14. Mean relative abundance (%) ±SD of lobose testate amoebae in terrestrial microhabitats

The highest proportion of stained tests was in fresh organic matter associated with early successional *Betula pendula* woodland (Table 7). Using these data (Table 7) the Pearson Correlation Coefficient was used to test the null hypothesis that there was no relationship between the proportion of filose testate amoebae and the proportion of alive tests (n=14). The null hypothesis was rejected as there was a significant correlation ($r = 0.698$, $p = 0.005$) between filose and stained testates inferred to be alive at the time of sampling (Figure 15). A qualitative observation in the microscopical analysis of this study was that filose testate amoebae preferentially stained better than lobose testate amoebae. These results raise questions regarding the usefulness of using the proportion of live testates as an indicator of the condition of an ecosystem.

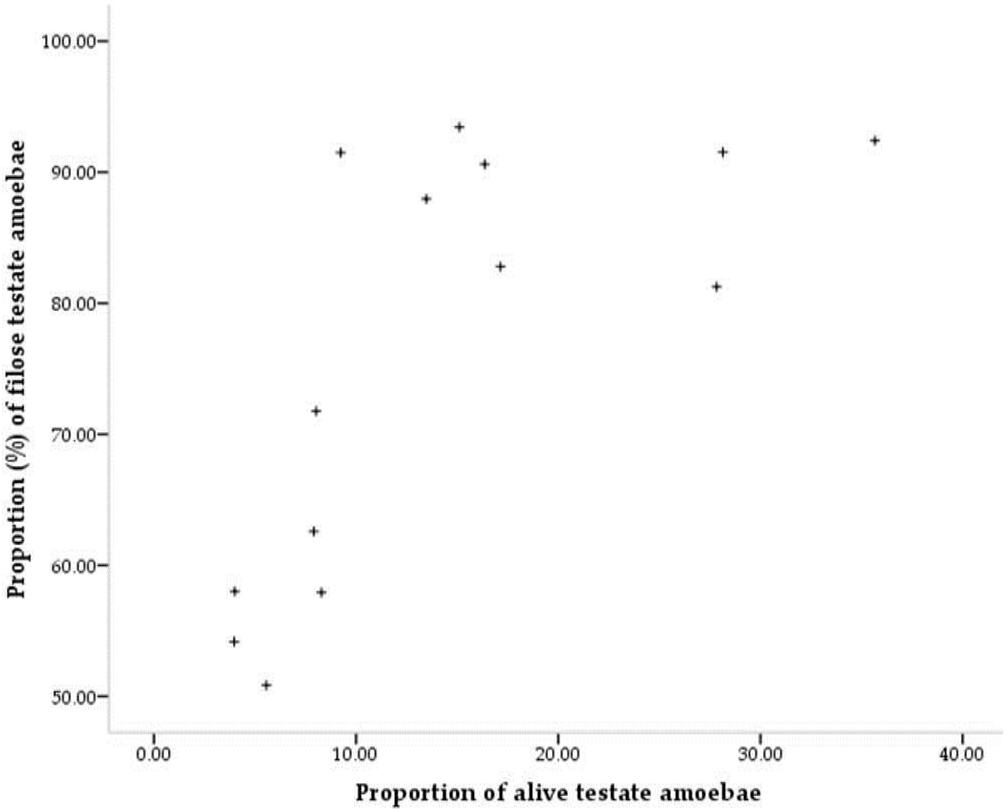


Figure 15. There was a significant correlation between the proportion (%) of filose testate amoebae and the proportion of tests that were stained and presumed to be alive at the time of sampling

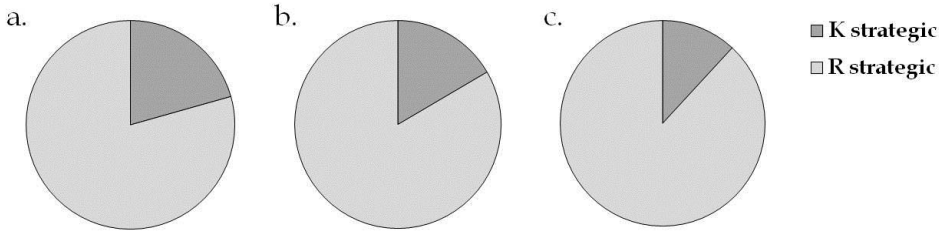
Table 7. Summary of testate amoebae life strategies and indicative health in terrestrial microhabitats. LF index values of - 1 indicate undeveloped communities whereas values of + 1 indicate developed, stabilized communities. The higher proportions of alive testates are potentially indicative of a more healthy community.

	Lobose	Filose	Total	Lobose testates (%)	LF-Index	Alive	Dead	Total	Alive testates (%)
Betula pendula early succession FOM	29	353	382	7.59	-0.85	137	245	382	35.86
Betula pendula early succession SOM	26	371	397	6.55	-0.87	60	337	397	15.11
Mature *Pinus sylvestris* FOM	199	274	473	42.07	-0.16	40	443	483	8.28
Mature *Pinus sylvestris* SOM	175	181	356	49.16	-0.02	21	357	378	5.56
Poaceae FOM grazed wet meadow	25	270	295	8.47	-0.83	83	212	295	28.14
Poaceae SOM grazed wet meadow	21	29	50	42.00	-0.16	2	48	50	4.00
* *Bryophyte* FOM open vegetation	65	313	378	17.20	-0.66	84	406	490	17.14
**Bryophyte* SOM open vegetation	59	150	209	28.23	-0.44	40	458	498	8.03
*Semi open *Typha latifolia, Iris pseudacorus* FOM	80	347	427	18.74	-0.63	140	363	503	27.83
*Semi open *Typha latifolia, Iris pseudacorus* SOM	45	434	479	9.39	-0.81	81	414	495	16.36
*Thick shrub *Alnus glutinosa* FOM	26	190	216	12.04	-0.76	31	199	230	13.48
*Thick shrub *Alnus glutinosa* SOM	67	721	788	8.50	-0.83	74	727	801	9.24
Rhododendron ponticum SOM	171	286	457	37.42	-0.25	38	443	481	7.90
Area cleared of *Rhododendron ponticum* SOM	176	208	384	45.83	-0.08	16	387	403	3.97

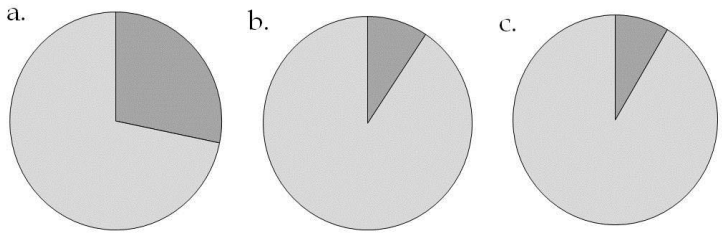
* Samples taken from a man-made island within a shallow lake

4.2.6 Ratio of testate amoebae r-K strategists on an island in a shallow lake

The relative proportions of filose (r-strategists) and lobose (K-strategists) testate amoebae were calculated in fresh and soil organic matter for three stages of terrestrial plant succession to determine the ratio of r-K strategists. Successional stages were a. open vegetation composed of *bryophytes*, b. semi-open vegetation composed of *Iris pseudacorus* and *Typha latifolia* and c. thick shrub *Alnus glutinosa*. A similar trend was observed in both fresh (Figures 16 a – c) and soil organic matter (Figures 17 a – c). The largest proportions of K-strategists were found in open, early successional vegetation and the relative proportion of K-strategists decreased in later plant successional stages. In fact, the largest proportion of R-strategists, considered to be early colonisers, were found in the fresh organic matter associated with thick shrub, *Alnus glutinosa* (88.13%) and soil organic matter (91.50%). These preliminary findings could indicate that soil protists, particularly testate amoebae, may not follow the same processes of the textbook plant succession. Another explanation could be that the presumption of filose testates being r-strategic and lobose K-strategic is incorrect and over simplified.



Figures 16 a – c. The relative proportion (%) of R – K strategists in fresh organic matter in: a) open vegetation (n=3), b) semi-open vegetation (n=3) and c) thick shrub (n=3)



Figures 17 a – c. The relative proportion (%) of R – K strategists in soil organic matter in: a) open vegetation (n=3), b) semi-open vegetation (n=3) and c) thick shrub (n=3)

4.3 Microbial diversity and distribution in freshwater environments

4.3.1 Differences in microbial diversity among freshwater microhabitats

In contrast with terrestrial microhabitats, there was a general trend of higher diatom diversity, compared with testate amoebae, in all aquatic microhabitats (Figures 18, 19 a-d). The mean number of diatom taxa per aquatic sample (n=45) was 22.6 ± 0.8 (SE) compared with testates (n=51) which was 8.6 ± 0.4 (SE). With the exception of the Scrape, a greater number of diatom and testate taxa was observed in sediment samples taken from areas of open water compared with areas dominated by invasive aquatic macrophyte *Crassula helmsii* and monocultures of native aquatic vegetation *Menyanthes trifoliata* and *Phragmites australis*. Sediment samples taken from the benthos of Twin lake were most diverse in terms of the number of testate and diatom taxa. Conversely, the Simpson diversity index which in addition to the number of taxa also takes into account variance of the species abundance distribution was highest in microhabitats dominated by *Menyanthes trifoliata* for both testates and diatoms. All four lakes were different in terms of sediment organic matter content and pH (Table 8). Furthermore, sediment organic matter content was highly variable within a single quadrat for some microhabitats, in particular, Mere End and End Lake *Crassula helmsii*.

Table 8. Summary of environmental variables for different lakes and microhabitats/vegetation zones within lakes

	Bulk organic matter (%)	**pH ***
Mere End Lake		
Open water	17.85 ± 14.76	8.16
Phragmites australis	2.70 ± 0.88	
Menyanthes trifoliata	3.81 ± 2.62	
Crassula helmsii	25.45 ± 27.10	
End Lake		
Open water	27.63 ± 8.74	7.8
Mixed vegetation community	70.51 ± 4.78	
Menyanthes trifoliata	36.82 ± 24.79	
Crassula helmsii	34.19 ± 25.78	

Twin Lake		
Open water	32.70 ± 4.15	
Menyanthes trifoliata	0.90 ± 0.36	7.91
Crassula helmsii	2.40 ± 1.18	
Scrape		
Open water	23.34 ± 0.99	
Phragmites australis	86.98 ± 10.93	7.2
Menyanthes trifoliata	8.85 ± 0.86	
Drainage ditch	54.75 ± 21.92	6.41

* indicative pH measurement

Analysis of Variance (ANOVA) was used to test the null hypothesis that there was no difference in mean testate diversity: taxon richness, Simpsons, Shannon and Margalef indexes, among aquatic microhabitats. There was a significant difference (P=<0.001) in testate amoebae diversity among aquatic microhabitats (n=13) for all diversity measures (Table 9).

Table 9. ANOVA table of differences in mean testate diversity in aquatic microhabitats (n=13)

Diversity	DF	Sum of squares	Mean square	F ratio	P value
Number of taxa					
Between groups	12	299.71	24.98	5.036	<0.001
Within groups	38	188.44	4.96		
Total	50	488.16			
Simpson					
Between groups	12	2.165	0.18	9.673	<0.001
Within groups	38	0.709	0.019		
Total	50	2.874			
Shannon					
Between groups	12	12.966	1.081	8.965	<0.001
Within groups	38	4.58	0.121		
Total	50	17.546			
Margalef					
Between groups	12	14.959	1.247	5.737	<0.001
Within groups	38	8.257	0.217		
Total	50	23.216			

Similarly, there was a significant difference between the mean Shannon and Margalef indexes of diatom diversity among aquatic microhabitats (n=15) (Table 10) and the Simpson index ($K_{14, 30}$= 30.873, P=0.006). In contrast, there was not a statistically significant difference in the number of diatom taxa ($K_{14, 30}$= 23.393, P=0.054) among aquatic microhabitats.

Table 10. ANOVA table of differences in mean diatom diversity in aquatic microhabitats (n=15)

Diversity	DF	Sum of squares	Mean square	F ratio	P value
Shannon					
Between groups	14	2.806	0.200	6.125	<0.001
Within groups	30	0.982	0.033		
Total	44	3.788			
Margalef					
Between groups	14	21.196	1.514	3.804	0.001
Within groups	30	11.941	0.398		
Total	44	33.137			

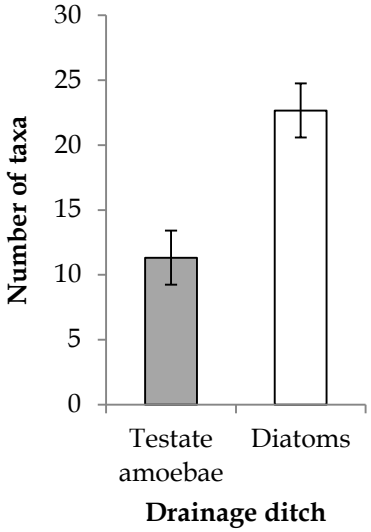
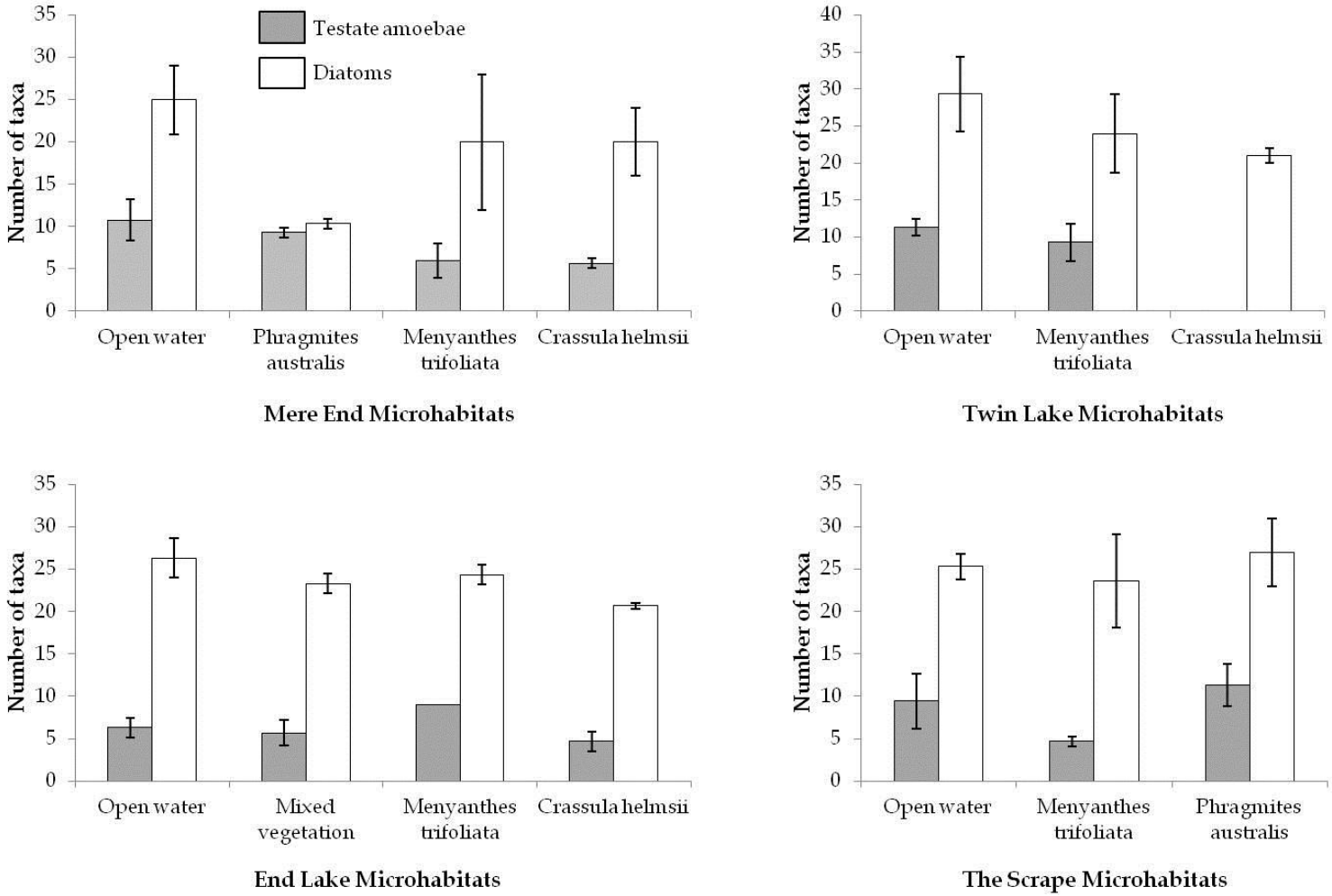


Figure 18. The mean (±SD) number of testate amoebae and diatom taxa in ditch sediment



Figures 19 a – d. The mean (±SD) number of testate amoebae and diatom taxa found in the sediment of four lakes at Mere Sands Wood Nature Reserve

A Two-way Analysis of Variance was performed to test the null hypothesis that there was no significant difference in diatom taxon richness among lakes (n = 4) and substrate type (n = 5). The null hypothesis was rejected as there was a statistically significant difference in diatom taxon richness among lakes (F = 3.577, p = 0.022) and substrate type (F = 4.755, p = 0.003) (Table 11). A similar trend was observed for testate amoebae (Table 12) among lakes (F = 4.241, p = 0.012) and substrate (F = 6.105, p = 0.001). The lakes studied were very different in terms of their morphology (Figures 2 a–d) and organic matter content (Table 8). These results suggest environmental variability between and within lakes and substrate type potentially influences testate amoebae and diatom taxon richness. Furthermore, the relative proportions of lobose versus filose testate amoebae and live testates also appeared to be influenced by different lakes and substrates (Table 13).

Table 11. Two way ANOVA table of differences in diatom taxon richness between lakes (n = 4) and substrate type n = 5)

Taxon richness	DF	Sum of squares	Mean square	F ratio	P value
Corrected model	7	542.27	77.47	3.875	0.003
Intercept	1	15709.92	15709.92	785.78	<0.001
Lake	3	214.51	71.51	3.577	0.022
Substrate	4	380.29	95.07	4.755	0.003
Error	40	799.71	19.99		
Total	48	27057.00			
Corrected total	47	1341.98			

Table 12. Two way ANOVA table of differences in testate taxon richness between lakes (n = 4) and substrate type (n = 5)

Taxon richness	DF	Sum of squares	Mean square	F ratio	P value
Corrected model	11	276.035	25.094	5.025	<0.001
Intercept	1	2073.17	2073.17	415.15	<0.001
Lake	3	63.53	21.18	4.241	0.012
Substrate	4	121.94	30.49	6.105	0.001
Error	36	179.78	4.994		
Total	48	3873.00			
Corrected total	47	455.81			

Table 13. Summary of testate amoebae life strategies and indicative health in aquatic microhabitats. LF index values of - 1 indicate undeveloped communities whereas values of + 1 indicate developed, stabilized communities. The higher proportions of live testates are potentially indicative of a more healthy community.

	Lobose	Filose	Total	Lobose testates (%)	LF-Index	Alive	Dead	Total	Proportion stained %
Mere End *Crassula helmsii*	69	15	84	82.14	0.64	9	99	108	8.33
Mere End *Menyanthes trifoliata*	43	14	57	75.44	0.51	10	81	91	10.99
Mere End open water	137	248	385	35.58	-0.29	57	1015	1072	5.32
Mere End *Phragmites australis*	82	21	103	79.61	0.59	72	78	150	48.00
The Hollow open water	92	12	104	88.46	0.77	2	142	144	1.39
The Hollow *Menyanthes trifoliata*	86	52	138	62.32	0.25	7	148	155	4.52
Scrape *Menyanthes trifoliata*	18	0	18	100.00	1.00	34	334	368	9.24
Scrape *Phragmites australis*	83	348	431	19.26	-0.61	53	500	553	9.58
Scrape open water	391	144	535	73.08	0.46	300	809	1109	27.05
End Lake *Crassula helmsii*	24	2	26	92.31	0.85	8	106	114	7.02
End Lake mixed vegetation community	24	3	27	88.89	0.78	16	139	155	10.32
End Lake open water	56	2	58	96.55	0.93	12	368	380	3.16
Drainage ditch	223	25	248	89.92	0.80	11	259	270	4.07

4.3.2 Correlations between species richness and diversity of testate amoebae and diatoms

The Pearson's correlation coefficient was used in SPSS to test the null hypothesis that there was no significant relationship between species richness and the diversity of testate amoebae and diatoms in the aquatic microhabitats (n=14). Diversity summary statistics for number of taxa, Simpson, Shannon and Margalef indexes of diversity are shown in Appendix 4. There was no correlation between the number of taxa ($r = 0.294$, $p = 0.308$), Simpson ($r = -0.053$, $p = 0.857$), Shannon ($r = 0.028$, $p = 0.925$) and Margalef ($r = 0.099$, $p = 0.735$) indexes of diversity. These results suggest that the species richness and diversity of one microbial group cannot be used as a proxy to explain another.

4.3.3 Correlations between species richness, diversity and environmental variables

Surprisingly, there were no statistically significant relationships ($p>0.05$) found between the number of taxa and diversity of testate amoebae and environmental conditions including bulk organic matter content (%) of the lake sediment and pH. In contrast, there were statistically significant correlations between sediment bulk OM and diatom species richness ($r = 0.651$, $p = 0.012$) (Figure 20), Simpson's index of diversity ($r = 0.578$, $p = 0.030$), Shannon's index of diversity ($r = 0.634$, $p = 0.015$), and the Margalef index of diversity ($r = 0.539$, $p = 0.047$). These results suggest higher diatom taxon richness is found with increasing sediment organic matter. There any many possible explanations discussed for this relationship between the number of diatom taxa and sediment bulk organic matter (Figure 20). Significant negative correlations were found between the Shannon index of diatom diversity and pH ($r = -0.578$, $p = 0.030$) and Margalef index of diatom diversity and pH ($r = -0.663$, $p = 0.010$).

4.3.4 Correlations between species richness and diversity indices

The Pearson's Correlation co-efficient showed consistency between the number of taxa and all diversity indices in aquatic microhabitats (n=14). In other words, there was a

relationship between the number of taxa and the evenness of testate and diatom communities among terrestrial and aquatic habitats. There was a strong positive correlation between species richness and the Shannon index of diversity for testate amoebae ($r = 0.711$, $p = 0.004$) and diatoms ($r = 0.819$, $p = <0.001$). There were also statistically significant relationships between the Shannon and Simpsons index of diversity for testates ($r= 0.990$, $p = <0.001$) and diatoms ($r = 0.924$, $p = <0.001$) and the Margalef index of diversity ($p = <0.05$). However, these results may be expected given that there is some auto-correlation between the variables.

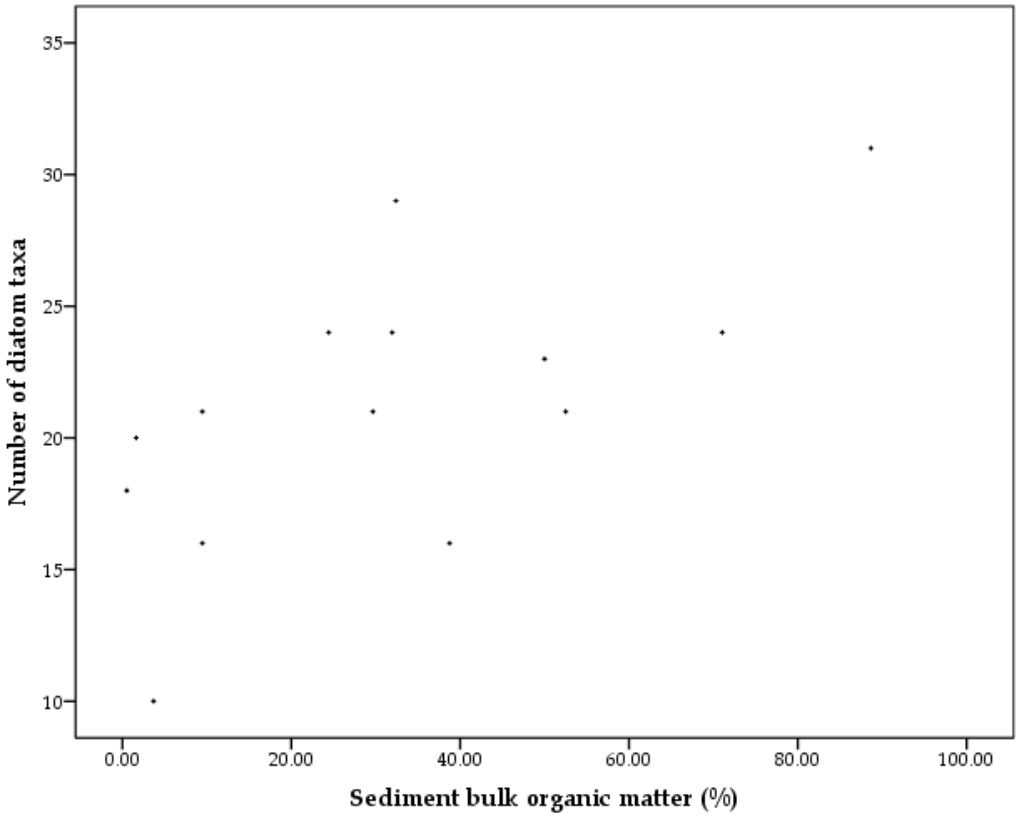


Figure 20. There was a significant correlation between diatom species richness and lake sediment bulk organic matter content (%) in the aquatic samples (n=14).

CHAPTER FIVE

DISCUSSION

5.1 Overview of Biodiversity at Mere Sands Wood – a wider context

5.1.1 A more balanced perspective of biodiversity?

The first aim of this study was to determine and quantify microbial diversity on a single 43 hectare nature reserve and to compare microbial species richness with previously studied macroscopic organisms. Before the present study, a lack of biological records for microbial species from representative microhabitats prevented this comparison. Local biodiversity conservation has previously focused on macroscopic organisms; yet, some scientists in the UK are beginning to realise the true value of incorporating microbial diversity into nature conservation surveys (Esteban and Finlay 2010). On a global scale, one of the biggest collaborative challenges faced by ecologists and conservation biologists is to determine the number of species living on earth (Costello *et al* 2013). Despite an appreciation that microorganisms dominate global biodiversity (Cairns 1993, Nee 2004, Wilkinson 2007), visible macroscopic organisms have dominated biodiversity estimates locally (Corbet 2011), nationally (Dolman *et al* 2012) and internationally (Costello *et al* 2013).

Macroscopic organisms comprised the largest proportion of taxon richness (78.66%) at Mere Sands Wood (MSW) Nature Reserve (Figure 6). This probably represents a bias in taxon richness estimates, possibly a consequence of under sampling more difficult taxonomic groups, as opposed to providing a true estimation of biodiversity. Biological records for sites managed for nature conservation are often biased towards their nature conservation interest, often referred to as 'features'. By definition, the term 'feature' usually refers to a visible, often appealing attribute. For instance, MSW is nationally recognised for over-wintering birds and important numbers of winter wildfowl populations which are perhaps the main wildlife interest or feature and this is reflected in the larger proportion of existing biological records for macroscopic organisms. As in comparable studies (Dolman *et al* 2012), this study utilised

a considerable body of biological records created by specialist and amateur naturalists and members of the public, often referred to as 'Citizen Science' (Cooper *et al* 2007).

MSW has an impressive, extensive biological database for many biological groups, perhaps the most impressive are the macro-fungi records which comprises a relatively large proportion of taxonomic composition (25.46%) after vascular plants (31.34%). In contrast with other studies (Corbet 2010, Dolman et al 2012), a higher proportion of macro-fungi records was observed for MSW, probably because of the many records created by the late Jane Ingham, an expert mycologist (L. Beaton *pers comms*) who had a keen interest in the fungi at MSW. Professor of Mycology Lynne Boddy at Cardiff University (2013) discusses the important contribution and fungal discoveries made by specialists and skilled amateurs and enthusiasts. Geml (2011) argues fungi are probably the least understood of the eukaryotes which is surprising given the huge diversity of fungi, their crucial roles in ecosystems, their potential for extensive dispersal by humans, for example with agricultural and forestry products, their importance to public health, their valued roles in diverse human societies and the threats posed by global change. Considering their importance, Boddy (2013) believes that it is worrying that retiring taxonomists are not being replaced and that fungal biology is not compulsory in many Biology degrees.

Many of the biological records of macroscopic organisms at MSW have been created by specialist and amateur naturalists, which can inevitably increase the number of records collected. Nevertheless, long-term monitoring may not always be feasible because of unstandardized survey methodology. The development of nature conservation priorities and strategies rely upon an understanding of the species present (taxon or species richness) and estimates of their population sizes for monitoring, however, often the most species rich groups are also the most difficult to observe and quantify, which is reflected in the relatively small number of biological records for insects at MSW. Insects comprise a substantial proportion of terrestrial biodiversity and long-term data from the Rothamsted insect survey has highlighted the effects of insect declines on other groups such as insectivorous birds and bats (Conrad et al 2006). Long-term monitoring provides baseline data to evaluate change in ecosystem structure and function which can occur in response to management interventions. (Lindenmayer and Likens 2009). For long-term monitoring schemes to be effective the

challenge is to try to balance scientific rigour, driven by clear questions (Lindenmayer and Likens 2009), with convenience for volunteer participation.

5.1.2 Qualitative observations of testate amoebae, diatoms and macro fungi

Some protists, for instance testate amoebae, are known to encounter fungi in all terrestrial ecosystems (Vohnik *et al* 2012) and some species are known to obtain their supply of energy and nutrients from fungi in terrestrial soils (Wilkinson and Mitchell 2010). They interact with fungi through mycophagy and decomposition of the shells (Crotty *et al* 2012), and some species, such as *Trigonopyxis arcula* are known fungal specialists (Wilkinson and Mitchell 2010, Vohnik *et al* 2012). This raises the question of whether there was likely to be any similarity between species richness of testate amoebae and macro fungi. Biological records of macro fungi at MSW existed for some terrestrial habitats (Figure 7), with the exception of a small number of records from marginal aquatic environments, in particular fungi associated with *Phragmites australis*. This is not to say that freshwater environments at MSW do not support fungi as they do have a role in the ecology of freshwaters (Moss 1998). The lack of data for freshwater environments is more likely a consequence of sampling bias since it is easier to determine the presence of fungi in terrestrial environments compared with their aquatic counterparts. The qualitative data (Figure 7) suggests that further research is needed to establish whether a statistical relationship exists between species richness of protists and macro fungi. One of the limitations with this method was that it was not possible to accurately determine whether the fungi records were from the same locality as the microbial sampling points (Figure 4) and a timing limitation in that the data were collected at different times.

Qualitative observations of macro fungi associated with *Pinus sylvestris* and testate amoebae suggest further research into potential interactions would be useful. Culture-based microcosm studies (Vohnik *et al* 2011) have shown that saprotrophic microfungi (fungi that live and feed on dead organic matter) may affect the composition and distribution of TA communities in *Pinus* litter through the engagement of several direct and indirect interactions. However, such culture-based experiments can be less ecologically informative compared with field-based studies because some taxa are difficult to culture.

5.2 The potential for surrogate measures in microbial diversity surveys

The formulation of conservation strategies requires an understanding of biodiversity patterns but severe data constraints often make surrogate measures necessary (Lamoreux *et al* 2006). Considering the severe lack of quantitative data for eukaryotic microorganisms surrogate measures may be necessary to add knowledge about their diversity and distribution. For example, can the diversity of one microbial group be used as a proxy for others (J. Fisher *pers comms*)? Vertebrates are frequently used to represent all biodiversity; however, correlation analysis of different animal groups in Britain has shown little overlap (Prendergast *et al* 1993), suggesting species-rich areas may not coincide for different taxa. In other words, a species-rich area for one group of plants or animals may not necessarily be species-rich for another. Lamoreux *et al* (2006) on the other hand found that patterns of species richness amongst terrestrial vertebrates were broadly concordant. In the present study there was no correlation between diatom and testate amoebae taxon richness, Simpsons, Shannon and Margalef indexes of diversity in both terrestrial and aquatic habitats, suggesting that one biotic group of eukaryotic microorganisms cannot be used as a proxy for other groups. Other studies (Allen *et al* 1999) have found a low degree of concordance among microscopic and macroscopic assemblage richness measures in lakes, supporting the findings in the present study. However, in terms of consistency between species richness and diversity indices, this study suggests taxon richness, the quickest and simplest measure of diversity produced results that were largely congruent with diversity indices, which in addition to taxon richness also take into account the evenness of the assemblages (Magurran 2004). Although, these results may be a consequence of auto-correlation as the species-richness component forms part of the diversity index.

Surrogate species approaches used in nature conservation and management, for instance, flagship, umbrella and keystone (Simberloff 1998) and indicator species (Lawton *et al* 1998, Melzer 1999, Briers and Biggs 2003) are often criticised because land conserved for one handful of species does not necessarily provide protection for many other species (Favreau *et al* 2006). In Britain, Prendergast *et al* (1993) found species-rich areas did not coincide for different taxa and many rare species did not occur in the most species-rich areas. Moss (2000) believes that the approaches used in nature conservation rest on arguments that

all species must be maintained for the system to function, arguing that structural influence is more a function of the physical form and productivity of organisms, rather than their richness or diversity.

5.2.3 Can genus richness be used as a surrogate for species richness?

This small-scale study aimed to critically appraise the ways in which free-living eukaryote species could potentially be included in local biodiversity conservation surveys. This relatively small-scale study highlighted the potential increase in biodiversity estimates by incorporating microorganisms into nature conservation surveys (Figure 6), although, actually quantifying microbial diversity to species level using light microscopy may be a difficult and time-consuming task for a non-specialist. Testate amoebae and diatoms are ecologically widespread, diverse and abundant groups of morphologically distinctive, shelled protists potentially making them useful microbial candidates for nature conservation surveys. Both groups can be counted directly under the microscope with the possibility of producing population size data comparable to that of macroscopic organisms (Wilkinson and Mitchell 2010, Wilkinson *et al* 2012). Yet, biodiversity research and ecology relies on the accurate identification of species and on reproducible species counts, requirements which Boenigk *et al* (2012) and others (Mann 1999, Schlegel and Meisterfeld 2003) argue is not always met. For example, diatom taxonomy has developed from one part of the phenotype, the valve, and differences in valve morphology are used to separate taxa. For a non-specialist though this is often difficult to determine using light microcopy, especially with some taxa, such as those within the *Achnanthidium minutissimum* complex (see Potapova and Hamilton 2007).

Generic surrogacy may therefore greatly simplify biodiversity surveys for some microbial groups and there is evidence for diatoms (Kelly and Whitton 1995, Smol and Stoermer 2010) and testate amoebae (Wilkinson and Davis 2000), that generic level indices perform nearly as well as species level indices. This raised the question whether higher taxon categories could be used as surrogates for taxon richness for more rapid microbial biodiversity surveys. Many studies have generally found higher taxon richness to be a good predictor of species richness for a variety of macroscopic groups and regions (Williams and

Gaston 1994). Although, examining the relationship between species and genus richness in ant faunas, Anderson (1995) found that the relationship was seriously confounded by differences among habitats and biogeographic regions and by sampling intensity and area, deeming genus richness an unreliable surrogacy for species richness. Limited evidence from the present study suggests a good starting point for eukaryotic microorganisms could be to identify to genus level as there were significant correlations between taxon and genus richness for both groups in aquatic and terrestrial microhabitats (Table 5). Obviously, this would drastically underestimate the true potential microbial diversity, but it may pave the way for more frequent surveys. In addition, further research investigating potential relationships between genus level data and environmental variables are needed, as some studies (Growns 1999, Wunsam *et al* 2022) have found diatom genus level data to be better correlated with environmental variables than species level data.

May (1994) suggests for practical reasons biodiversity may be better quantified at lower or higher levels from genes to ecosystems. Currently, most ecological and palaeoecological work relies on the definition of a species which is crucial for conservation biology. Recently, environmental DNA (eDNA) (Thomsen *et al* 2012), focusing on the level of genes, is suggested to be a useful monitoring tool for a wide range of taxonomic groups. The problem with the sole use of eDNA though is that the organism would not be visually observed in its environment. However, combined with other expertise this may be a promising tool. The challenge is how to combine ecological and molecular data into a meaningful sense for understanding biodiversity and loss of biodiversity at the ecosystem level.

5.3 Terrestrial environments

5.3.1 Insights into potential factors influencing microbial diversity and distribution

This study identified 46 testate amoebae and 52 diatom morphospecies in litter and soils in a 42 hectare nature reserve. Other studies have found that soils contain a high diversity of Protists. For instance, in an intensive study of just 1 ha of upland grassland in Southern Scotland, Esteban *et al* (2006) found 91 testate taxa in 150 samples, and overall 365 species of Protozoa were identified. In the present study there were statistically significant differences in species richness of testate amoebae and diatoms among different terrestrial microhabitats (Figures 10 a – d). With the exception of *Poaceae*, there was a greater diversity of testate amoebae compared with diatom taxa in most terrestrial microhabitats. The striking similarity in diatom and testate taxon richness in soils associated with *Poaceae* provoked further questions into the potential links described between testate amoebae, diatoms and grasses and their relevance in the silica cycle (Wilkinson 2008, Wilkinson and Mitchell 2010). *Poaceae* fresh organic matter produced a high proportion (91.53%) of terrestrial Euglyphida (including: *Corythion*, *Euglypha*, *Tracheuglypha* and *Trinema*). These testates with filose pseudopodia (Table 7) are composed of siliceous idiosomes which Wilkinson and Mitchell (2010) argue could potentially have increased the rate of silica mineralisation in soils, especially grassland soils, with direct implications for nutrient availability to diatoms.

In some open terrestrial microhabitats, for instance, in soils below *bryophyte* communities, diatoms were found to be exceedingly diverse and abundant (Figure 21). Overall, fifty six diatom taxa were recorded in terrestrial soils and litter which is a significant proportion (44.44%) of the overall number of diatom taxa recorded. Other studies have found high diversity of diatoms in soils, for instance, van Kerckvoorde *et al* (2000) recorded 81 diatom taxa from 30 soil samples in Greenland and Moravcova *et al* (2010) recently identified 163 taxa from 108 soil samples from Antarctic regions. Diatoms are photosynthesising algae and are sensitive to pH, therefore it's not surprising that they were scarce or absent in some terrestrial habitats, particularly in acidic mor humus soils with high organic matter found in coniferous woodland and soil associated with *Rhododendron ponticum,* both habitats have a dark understory with little light penetration for photosynthesis. The

slight increase in diatom species richness in areas cleared of *R. ponticum* (Figure 10d) demonstrates the positive effect of management for some microbial groups in terrestrial soils by increasing light availability for pioneer species, such as: *Cocconeis spp, Eunotia spp, Pinnularia spp* to colonise. Temporal factors could be a further reason for the decrease in the number of diatom taxa compared with testates. For instance, other studies of diatoms in soils (see Zancan *et al* 2006) have found diatoms most abundant in March, Heger *et al* (2011) sampled soil diatoms in April and Lund (1946) suggests there is a maximum in spring and early summer correlated with physical conditions and competition from macrophytes. The present study did not account for temporal variation in diversity and distribution which would be a useful aspect of future research. Despite problems highlighted with the taxonomy of soil diatoms, the findings from the present study suggest diatoms in soils should not be overlooked and should form the focus of future studies investigating eukaryotic microbial diversity in terrestrial soils.

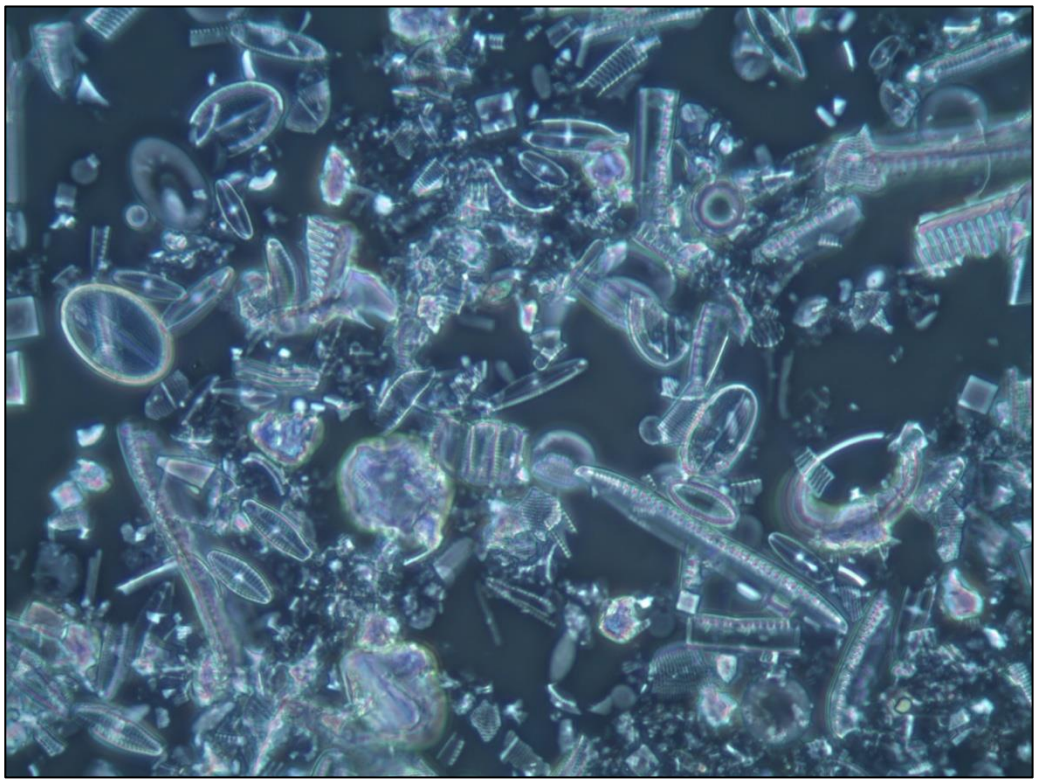


Figure 21. Microcosmos - diatom assemblages in soil underneath *Bryophytes,* demonstrates the potential diversity and abundance of diatoms in some terrestrial environments.
(Photograph taken in phase contrast x1000 magnification under oil immersion)

5.3.2 The influence of humus forms

In contrast with diatoms, testate amoebae were especially common and diversity was relatively high in habitats with mor humus soil. There was a significant relationship between the number of testate taxa and bulk organic matter in mor humus soils associated with *Pinus sylvestris* and *Rhododendron ponticum* (Figure 12). Bulk organic matter percentage was highest in mor humus soils (Table 6) and Wilkinson and Mitchell (2010) suggest testates are often the dominant microorganism, and may have a particularly important role in nutrient cycling, in such soils, which have recently been the focus of extensive recent research (see Vohnik *et al* 2009, Vohnik *et al* 2011, Vohnik *et al* 2012). The ericaceous shrub *Rhododendron ponticum* was introduced to MSW for game cover and is described as a conservation management problem on the reserve and in many sites in Britain (Cross, 1975; Dehen-Schmutz *et al* 2004). Practical conservation management at MSW involves the removal and burning of Rhododendron in affected sites and this practice was occurring at the time of sampling. Studies in north-west England found a smaller number of testate taxa in Rhododendron affected soils compared with non-Rhododendron soils (Sutton and Wilkinson, 2007). Surprisingly, the results of this study found the reverse as the effect of *R. ponticum* management did not seem to have a positive effect on testate taxon richness, although the increased variation in testate taxon richness in areas cleared of *R. ponticum* suggests this may be an artefact of sampling and microscopical analysis as opposed to an indication of the effect of Rhododendron and its management. Concerning data for testate amoebae life strategies (Table 7), the LF index suggested an increase in lobose testate amoebae and hence indicated more stable testate communities in mor humus soils compared with mull humus soils, with the exception of soil organic matter associated with *Poaceae*. On the other hand, the highest relative proportion of live tests, suggested as being an indication the health of an ecosystem (Scott *et al* 2001), was found in fresh organic matter associated with mull humus soils. Although, caution should be applied to these results because filose testate amoebae preferentially stained better than lobose testate amoebae, there was a statistically significant relationship between the proportion of filose testates and stained tests (Figure 15). For future studies using the proportion of stained tests as an indication of the condition of an ecosystem it may be better

to exclude some lobose testate amoebae which do not preferentially stain, for instance, genus *Difflugia*.

5.3.3 Vertical distribution in soils

This study suggested fresh organic matter was a biologically active zone of the soil profile. With the exception of two terrestrial microhabitats sampled (Figure 11) higher testate taxon richness was observed in fresh organic matter compared with soil organic matter (Figures 10 a-d). The greater number of diatom taxa in soil organic matter may be the result of diatoms being washed down the soil profile by rain. An increase in nematode abundance was found in fresh organic matter (67%) and seventy four percent of individuals in the leaf litter were alive at the time of sampling (Figure 22). Soil nematodes have evolved life strategies which enable them to respond to adverse environmental conditions and Treonis and Wall (2005) found a correlation between nematode coiling and soil moisture content, salinity and water potential. Interestingly, this study reflected microsite variation in soil properties occurring at the scale of the nematode worm which could suggest small-scale variation in soil microbial assemblages and perhaps explains the large variation in testate and diatom species richness in some terrestrial microhabitats observed in the present study. Further, like nematode worms, diatoms have also evolved adaptations to withstand survival in the soil environment and Lund (1946) argues the smallness of soil diatoms could be an adaptation to enable diatoms use the water films surrounding the soil particles. Other studies (Foissner 1987) have illustrated graphically that testates are most abundant in the upper soil layers and Bardgett (2005) argues the often overlooked litter horizon may be the most biologically active and functionally important zone of the soil profile. The present study provides circumstantial evidence of the benefit of incorporating the litter horizon in future ecological studies of microbial diversity and distribution.

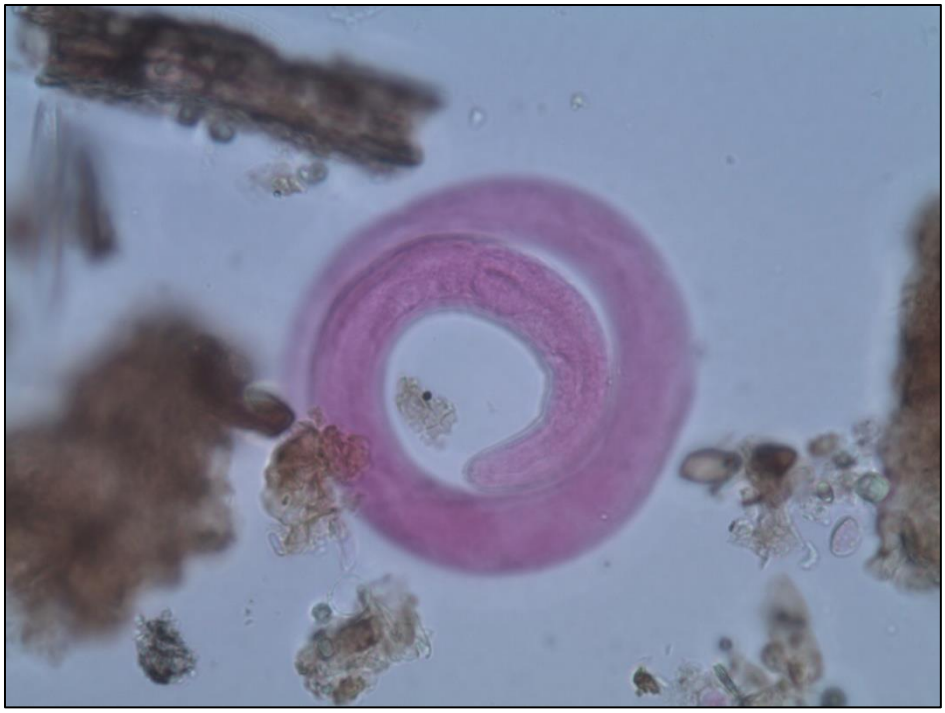


Figure 22. Stained nematode worm detected in the leaf litter of open *Bryophyte* vegetation. The coiled morphology of the organism photographed suggests it has employed anhydrobiotic survival strategy (Treonis and Wall 2005)
(Photograph: x1000 magnification under oil immersion)

5.3.4 Testate amoebae community assembly

Preliminary qualitative findings of the present study suggest testate amoebae do not follow same successional patterns observed in classical plant ecology. In plant-based succession early successional species are replaced with other species over time and the number of species generally declines in later successional stages. Other soil protists, for example, diatoms are cited as showing no evidence of succession of species during the year (Lund 1946), although for diatoms a better term could be 'cyclic periodicity' and not succession. In the present study, small-sized r-strategists (for instance, *Euglypha spp., Trinema spp.*) considered as pioneers (Wanner *et al* 2008) continued to be found in later successional plant communities (Figures 16 and 17 a-c) and the number of testate taxa increased with plant succession (Table 3). Some species, for instance lobose testates *Arcella Catinus* and *Cyclopyxis kahli* considered as K-strategic were not detected in later plant succession. The LF index has been used as a rough and ready way of assigning ecological strategies to testates to estimate the developmental stage of a testate amoebae community (Mattheeussen *et al* 2005, Vincke *et*

al 2006). The results should be treated with caution though as it is not fully clear whether this idea proposed by Bonnet (1976) was originally based on the assumptions that filose testates are r-strategic and lobose K-strategic. Based on these assumptions, the present study suggested more developed communities in later plant succession, for instance, an increase in the abundance of lobose testates in mature *Pinus sylvestris* soils and litter compared with early successional *Betula pendula* (Table 7). Nevertheless, the results of this study correspond with ideas suggested by Smith *et al* (2008) in that later colonists (i.e. lobose testates) occur in addition to, and not instead of pioneers. A similar trend has been observed in terms of the vertical distribution of testates in the soil profile and other studies (Vincke *et al* 2006) found no changes in the amounts of tests belonging to either lobose or filose testate amoebae. This index is based on the assumption that testate amoebae with filose pseudopodia are r-strategic, while those with lobose pseudopodia follow a more K-based strategy. Further research is needed to determine what makes a testate species an r/K strategist and whether there is a continuum between the two strategies, as in some groups of macroscopic organisms, such as fish (Grime and Pierce 2012).

Molecular studies (Heger *et al* 2012) are finding Euglyphids (filose species) smaller than 5μm, which will probably not be detected using light microscopy. Therefore, the proportion of filose testate amoebae used in this analysis may be under-estimated. Furthermore, molecular evidence is finding numerous unknown soil Euglyphids (D. Wilkinson *pers comms*), often referred to as 'cryptic' diversity. A similar situation is probably the case for diatoms as the majority of soil diatoms belong to small-sized species. Studies of diatoms in garden soils (Lund 1946) have found no evidence for succession in diatom communities. For testate amoebae, no species replacement was observed in the present study which is a crucial aspect of most successional theories. These results support the work of Wanner *et al* (2008) that suggest the more flexible concept of 'community assembly' is more appropriate for soil protozoa and should be considered as opposed to 'succession. Additionally, considering the potential for cryptic diversity in Euglyphids, the LF index should be used with caution, particularly in studies using light microscopy.

5.3.5 Potential effects of animal disturbances

The results (Table 3) suggested testate and diatom taxon and genus richness and nematode abundance (Appendix 5, Table 1) was comparatively high in soils and litter on a man-made island within a shallow lake. The presence of certain taxa, particularly *Quadrulella symmetrica,* seemed to indicate higher nutrients in some microhabitats on this island. In the absence of an experimental study with a control site, it was not possible to fully ascertain whether the sampled microhabitats were influenced by animal disturbances. Although it is quite likely they have been influenced over time as the island is heavily used by wintering birds for breeding, feeding and roosting (L. Beaton *pers comms*). Studies have shown that the colonies of birds and mammals exert a large impact on the physical, chemical and biological nature of the nearby environment, especially in the breeding season. In a study investigating the ecology of Protozoa in Chinstrap Penguin (*Pygoscelis Antarctica*), the late Humphrey Smith, a key figure in the ecology and biogeography of Antarctic Protozoa, found three guano-specific species of Protozoa (Smith 1973). More recently, preliminary research into the effects of perturbed soils influenced by the Wandering albatross (*Diomedea exulans*) found lower diversity and evenness in testate communities (Vincke *et al* 2007) and soil diatom species assemblages that seemed to indicate the impact of animal perturbations (Moravcova *et al* 2010).

A further influence of birds is through dispersal mechanisms, providing important ecosystem services. For instance, Suthers (1985) found ground-feeding migratory songbirds to distribute propagules of slime moulds, amoebae, spores and macrocysts which survived passage through the gut. Importantly and more relevant to the present study, waterbirds also act as vectors of passive dispersal of seeds and invertebrates which can have both positive and negative effects on ecosystems. For instance, Green and Elmberg (2013) highlight the vital role waterbirds play in maintaining connectivity among communities in isolated aquatic systems, although, they are also vectors for invasive species of plants and invertebrates. The effects of animal perturbations on the diversity and distribution of protists is an area worthy of further investigation in soil protist ecology studies (Wilkinson *et al* 2012). Long-term enclosure experiments could be a useful way of determining the effects of animal perturbations on microbial communities.

5.4 Aquatic environments

5.4.1 Insights into potential factors influencing microbial diversity and distribution

This microscopical analysis detected a greater number of diatom taxa in the benthos of the aquatic microhabitats sampled, compared with testate amoebae (Figure 19 a – d). The highest number of taxa was found in sediment collected from the deeper, open water benthic zone, with few or no submerged macrophytes. However, this could be an overestimate as it was not possible to ascertain whether the species were alive at the time of sampling or had been imported by the force of gravity from the littoral zone (Moss 1998). Nevertheless, this study highlighted that sampling the benthic zone gives an overall indication of what's occurring in the lake over a longer timescale and may be useful in descriptive studies and when time is limited. The type of organic material in the sediment seemed to be a factor in microbial diversity and distribution as sampling sediment beneath aquatic macrophytes and littoral vegetation produced different results in terms of taxon richness. Taxon richness was generally lower in sediment beneath aquatic monocultures, for instance, *Crassula helmsii* and *Phragmites australis*. Twin Lake, the largest lake in terms of surface area but the shallowest lake was found to be higher in taxon richness of both testate amoebae and diatoms compared with the other lakes sampled. Whilst many of the diatom taxa were sediment-associated species, for instance diatoms in the genera *Fragilaria*, many of the organisms detected in the sediment were epiphytic, for instance, *Achnanthes spp*. which are generally plant-associated taxa (Figure 9). It is difficult to separate plant remains from lake sediment. Furthermore, when sampling the sediment the community composition detected may be the result of abiotic factors, for instance, wave wash or finer sediments being washed down from the littoral zone, as opposed to what was living in the sediment at that time. The results (Figure 20) suggest diatom taxon richness is greater with higher sediment organic matter which may be explained because finer organic matter containing diatoms had been washed down the lake profile. Burbidge and Schroder-Adams (1998) hypothesised that the main factor controlling the distribution of testate populations was the type of organic material in the sediment.

All living organisms need a supply of energy and clearly resource availability is a limiting factor in their survival along with biotic interactions such as predation and competition. Han *et al* (2011) suggest trophy, a higher abundance of food, could be the main reason for the patchy year-to-year distribution of several *Difflugia* testate amoebae in an oligo-mesotrophic reservoir in Southern China, although unlike other studies (Barker *et al* 2010), clear diel vertical migration was not observed. However, over a longer timescale this study provided evidence for a yearly recurrence of a pelagic-benthic cycle in the *Difflugia* investigated; suggesting predator evasion and physiological effects may be involved. Whilst some species in this genus are intrinsically benthic, Han *et al* (2011) found interpreting the year to year differences in planktonic peak height was compounded by patchiness and local conditions, findings which are fairly congruent with Barker *et al* (2010). In contrast, in macrophyte-rich and open water areas of shallow lakes where the nutrient input from the sediment is generally higher than in deeper lakes (Sondergaard *et al* 1990) the relatively substantial litter layer underlying macrophyte beds may result in increased rates of deoxygenation and reduced conditions at the sediment water interface (Barker *et al* 2010).

5.4.2 Potential effects of invasive species

In the lakes studied, microbial taxon richness and diversity was lowest below homogenous monocultures such as *Phragmites australis* and slightly lower under *Crassula helmsii* (Figures 19 a-d). *Crassula helmsii* is a conservation management problem in Europe resulting in the displacement of native aquatic vegetation (Hussner 2008). The reasons for its competitive ability are not fully understood, although, one suggested explanation for its ecological success may be the possession of a regulated Crassulacean Acid Metabolism (CAM), which allows aquatic macrophytes to take up CO_2 in the night in addition to the daytime (Klavson and Maberley 2010). CAM is considered a luxury consumption mechanism as opposed to an adaptation to low availability of resources, requiring complex patterns of internal resource allocation (Grime and Pierce 2012). *Crassula helmsii* shows many traits of a stress tolerant/competitor species, for instance, it remains green throughout the winter with both submerged and emerged shoots, shows a high regeneration capacity and has the ability to form new shoots from single nodes outcompeting native aquatic macrophytes (Hussner

2008). Although the effects of *C. helmsii* on microbial communities are not fully clear, these results raise many interesting questions regarding the potential effects of non-native, invasive plants on microbial diversity and distribution in aquatic environments and the potential for using eukaryotic microorganisms as indicators of ecosystem health. Approximately three million pounds per year are spent eradicating C. helmsii (Oreska and Aldridge 2011), so this is certainly an area worthy of further research. Clearly, there are many different biotic and abiotic factors controlling microbial populations and communities, for instance, genetic differences amongst individuals, environmental gradients and stochastic events, thus, it's far too simple to infer cause and effect of differences in testate amoebae and diatom diversity in the freshwater microhabitats studied.

5.4.3 The influence of sediment organic matter

In the present study, sediment bulk organic matter differed markedly between lakes and microhabitats (Table 10) at the individual replicate scale; indicative pH was variable to a lesser extent. All lakes are very different in terms of shape, surface area, volume and depth (Figures 2 a-d). The relationship between bulk sediment organic matter and diatom taxon richness (Figure 20) can perhaps be explained by the relative abundance of sediment associated taxa in the genus *Navicula*, *Fragilaria* and *Pinnularia* (Figure 9). In the open water microhabitats, sediment bulk organic matter was highest in the benthos of Twin Lake, the largest lake in terms of surface area and the shallowest. This lake is considered the most important lake for wetland birds and at the centre there is a large man-made island. The highest proportions of planktonic diatoms: *Cyclotella* spp. and *Tabellaria* spp. were detected in Twin Lake compared with other lakes studied.

During sampling in September 2011, bottom anoxia was observed in one of the lakes, the Scrape (Figure 1c). Anoxia is a natural event (Marotta *et al* 2012), but is commonly intensified by human induced organic inputs and other inputs such as animal manure, with implications for biodiversity and greenhouse gas emissions. The end product of degradation of organic matter in anoxic lakes is methane (CH_4) produced mainly, but not exclusively by archaea. This study provided evidence of adaptations of organisms to anoxic, low oxygen conditions. A qualitative observation was that the sizes of diatoms found in the sediment of

the Scrape were comparatively larger compared with the same species in other lakes. The classic testate species of low oxygen conditions in freshwater is *Paulinella chromatophora.* It occurs as part of a characteristic community of organisms adapted to low oxygen conditions rich in organic compounds, often with a low pH (Melkonian and Mollenhauer 2005). Surprisingly, this species was not detected in the anoxic sediment of the Scrape with the lowest pH, but, it was observed in the benthos of Twin Lake and in sediment associated with *Menyanthes trifoliata* in End Lake, which were both similar in terms of bulk organic matter (Table 10). This species is currently being used as a model organism in molecular research to infer early steps in the evolution of photosynthetic organelles (Nowack *et al* 2011). Further research on its ecology in freshwaters would be useful. In addition, further research into the pelagic-benthic cycle of *Difflugia* in lakes would be useful to further investigate the response of this genus to anoxic conditions (Han *et al* 2011).

5.4.4 Lake trophic status and condition

Qualitative observations during sampling suggested the deepest lake Mere End Lake (Figure 2b), which is up to 4.5m in depth in some areas, may have been more nutrient-rich with characteristics of a eutrophic lake. Algal blooms observed indicated the high nutrient status of this lake. Mere End also supported an unknown organism (Figures 24 a-b) in high relative abundance in the lake sediment. There is some evidence (Coesel 2001) to suggest that this may be a planktonic form of desmid. Coesel (2001) reports that in alkaline, open water habitats, as eutrophication increased, there was a decrease in desmid diversity, and an increase in the proportion of planktonic forms. In contrast, Twin Lake which is the largest lake in terms of surface area and the shallowest of all four lakes sampled (<1m in some areas) was considered to be most representative of a lake apparently lower in nutrients. The results of this study found a lower mean taxon richness with apparently increasing nutrient conditions (Figures 20 a-b), in contrast, other studies (Leira *et al* 2009) have found nutrient-rich lakes more taxonomically diverse.

Neville *et al* (2010) used the Shannon Index as an indication of the relative 'health' of a testate community in lakes in Canada. Following the same set of principles, The Shannon Index of diversity suggested the diatom assemblage in Twin Lake open water sediment were

said to be in a more favourable condition (Table 3b), with intermediate conditions for testate amoebae (Table 3a), compared with unfavourable conditions for the testate assemblages in the more nutrient-rich Mere End (Table 1a). However, the appropriateness of setting arbitrary boundaries to the Shannon Index values and using these as an indication of the condition or 'health' is speculated. The first question asked is 'what is health?' Further, some regions such as the Arctic and Antarctic are not usually associated with high biodiversity (Wilkinson *et al* 2012), highlighting that the boundaries used by Neville et al (2010), and indeed the present study, are not comparative globally. The results of the LF Index for the open water, benthic samples suggested Twin Lake, with a larger proportion of lobose testates; particularly Difflugia spp., was a more developed, stable community, compared with the Mere End open water sediment (Table 15). Overall, the results of the present study suggest less nutrient-rich lakes tend to contain more developed, stable testate amoebae communities and diverse diatom communities compared with more nutrient-rich lakes, although further extensive research would be needed to support this hypothesis. In the absence of physicochemical variables (nitrate, ammonia, phosphate and chlorophyll a content), it was not possible to fully ascertain the trophic status of the lakes in the present study, which should be considered in future studies.

Interestingly, the presence and relative abundance of retort-shaped testates was markedly higher in the more nutrient-rich Mere End Lake, suggesting some genera, for example, *Cyphoderia* and *Campascus* may be able to tolerate higher nutrient conditions. The basic shape of these genera is similar although taxonomic research (Nicholls 2003) has shown that the structures of the tests are different. *Campascus* has a homogenous chitinoid membrane with scattered sand (exogenous) particles and an oral aperture whilst *Cyphoderia* is covered with endogenously produced scales and lacks the oral membrane. Therefore an alternative suggestion for their notable, almost exclusive presence in Mere End Lake may be the availability of shell building material as opposed to lake trophic status. Molecular data (Heger *et al* 2010) are confirming the presence of a hidden diversity in *Cyphoderia* which cannot be detected using light microscopy; thus, it's quite plausible that the taxon richness estimates in the present study perhaps underestimate the genetic diversity.

5.4.5 Lakes as complex, heterogeneous systems

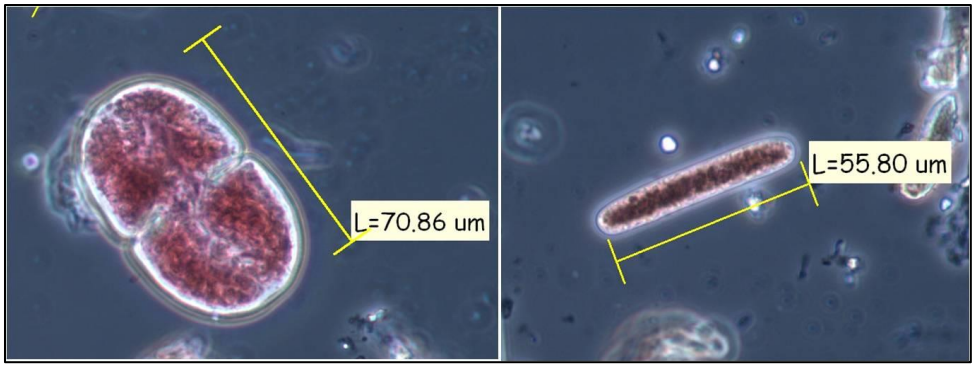
This study showed evidence of variability and patchiness in microbial diversity within a $1m^2$ area (Figures 19 a-d). Species composition and relative abundance of the different diatom and testate genera varied between aquatic microhabitats. Lakes are highly heterogeneous, complex biological systems with different organisms associated with different substrates such as rocks, sediments and plants (Moss 2010), therefore, it's not surprising to find differences in taxon richness and diversity among all lakes and microhabitats studied. However, the variability of these results suggest that taxon richness, coupled with the relative abundance and distribution, often referred to as the 'evenness' of the community, is different for testate amoebae and diatoms at the microhabitat scale. Other studies (Fisher and Dunbar 2007) have found as much variation in benthic diatom species in samples centimetres apart. Patchy distributions have also been observed for testate amoebae in aquatic environments (Han *et al* 2011). Arguably, the most convincing evidence for this micro-scale patchy phenomenon in shallow lakes comes from an exploratory study (Barker *et al* 2010) which found distinct heterogeneities in the three-dimensional vertical distributions of water chemistry and planktonic organisms on centimetre to decimetre scales over a diurnal cycle, rejecting their null hypothesis of uniformity of variables in both macrophyte dominated and open water sites. This study provides strong evidence for temporal changes in vertical gradients of physico-chemical variables, available nutrients and biotic interactions.

The present study did not account for temporal changes in nutrient availability and physico-chemical variability of the lakes studied. In future studies, additional quantitative data for physico-chemical variables over short and longer time-scales would be useful for performing principal components analysis (PCA) and redundancy analysis (RDA) between certain groups and taxa (Kent 2012) at the micro-scale (Barker *et al* 2010)).

5.5 Ecological hotspots

5.5.1 The terrestrial-aquatic interface and potential for dispersal

The previous chapters have considered terrestrial and aquatic habitats as separate entities. However, at MSW there are ecotones between terrestrial and aquatic habitats and the potential for dispersal between some terrestrial and aquatic habitats. It has been realised for some time that rates and reactions of biogeochemical processes, for instance the carbon (C) and nitrogen (N) cycles are often enhanced at the terrestrial-aquatic interface, referred to as 'hotspots' (McClain *et al* 2003). This raises many interesting questions and may have implications for the results of the present study. For instance, the high diversity of testate amoebae and diatoms found in soils and litter on the island within Twin Lake may be a result of this ecotone or bird activity. Furthermore, the presence of testate amoebae species *Quadrulella symmetrica,* one of the few arcellinid genera building its shell from self-secreted siliceous elements (Kosakyan *et al* 2012) indicated nutrient enrichment in the island soils. Was its presence due to nutrient input from animal manure or an increase in nutrients at the terrestrial-aquatic interface? Strikingly, these island soils also contained the highest abundance of nematodes, for example, 89.38% of nematodes recorded in terrestrial habitats were detected from soil and litter on this island and a comparatively high diversity and abundance of desmids (Figures 23 a and b). Rønn *et al* (2012) hypothesise for other protists whether these are fundamentally aquatic organisms, visiting a terrestrial world.



Figures 23 a and b. Desmids present in litter and soil close to the edge of an island within a shallow lake

5.6 Wider significance and directions for future research

5.6.1 Global change, biodiversity and biogeographies

Climate change and biodiversity loss are two global environmental problems for which human behaviour is a significant driver (Vitousek *et al* 1997, Moss *et al* 2009). Human disturbance and degradation of ecosystems, for example, wetland loss, eutrophication of waterbodies, soil degradation, and introductions of exotic and invasive species have created an urgent need to describe earth's biota and determine the spatial distribution and biogeographies of eukaryotic organisms. Besides wind, other possible means of dispersal include spores being carried by migratory animals (see Suthers 1985), driftwood and drifting sea ice and atmospheric currents (Bovrov and Wetterich 2012). Isolated geographical areas, such as the Antarctic and Arctic, provide the opportunity to study biogeographies and diversification in micro and macroscopic organisms which are sensitive to global change. For instance, recent molecular research (Souffreau *et al* 2013) found Antarctic lineages of diatoms *Pinnularia borealis* and *Hantzshia amphioxus* indicating niche differentiation with temperature preferences.

This study has provided baseline data which can be used and built upon to explore unresolved and under-researched questions of microbial biodiversity and biogeography. Importantly, palaeoecological reconstructions rely on baseline data for modern forms and knowledge of their ecology and Scott *et al* (2001) argue that knowledge of the ecology of modern forms could prove invaluable for the palaeoecological reconstructions of many ancient freshwater and marine deposits. However, taxonomic problems in modern faunas directly impact on the analysis of fossil samples (Charman 1999). It is therefore important to address taxonomic problems in one way or another, especially as ecological ideas such as extinction, speciation and endemism rely upon fossil data (Louys *et al* (2012).

5.6.2 Landscape systems ecology – micro to macro scale

While this study has examined testate amoebae and diatoms in terrestrial and freshwater habitats separately, there is clearly a continuum between the two. Cotterill *et al* (2008) highlight that the integrity of soil ecosystems is founded on their processing of nutrients and matter across microscopic and mesoscopic scales. For instance, erosion of terrestrial ecosystems impacts on aquatic systems by the transportation of finer sediments which are further transported by the process of wave wash. Therefore, it was not possible in this study to be fully certain that the testate and diatom community composition was indicative of the living fraction among the different habitats. Different protists have equally important roles in aquatic and terrestrial ecosystems. Protists are eukaryotes and are the ancestors of plants and animals. Importantly, throughout geological time microorganisms have and continue to be responsible for major global biogeochemical cycles, such as photosynthesis, decomposition and nutrient cycling, which are crucial for the functioning of all ecosystems (Wilkinson 2008, Cotterill *et al* 2008, Heger *et al* 2011). It is therefore important for ecologists to think how organisms experience the environment from their own perspective, as opposed to scales which are appropriate to us (Barker *et al* 2010).

Eukaryotic microbial diversity and distribution in the present study was patchy and rather random with micro-scale variation in microbial communities, a pattern observed in other studies (Fisher and Dunbar 2007, Barker *et al* 2010). This poses a major challenge in understanding microbial biodiversity patterns whilst incorporating findings into the context of the wider environment. In addition, human activities add to this complexity. For instance, MSW is largely an artificial ecosystem and human disturbance is necessary to halt natural succession and maintain habitat heterogeneity for associated plants and animals, yet, the effects of management on microbial diversity and distribution are not fully understood. The man-made island at MSW is composed of mixed of layers of sand and peat from the area; this human influence raises questions proposed by Foissner (2011) about the role of testate amoebae cysts and human introductions. Furthermore, Wilkinson (2010) and Perrigo (2012) highlight the potential for human activities to lead to the artificial dispersal of organisms, for example, by the simple means of 'mud on boots' or on a larger scale by the introduction of soils and fungi. Indeed, sampling for the present study probably encouraged the spread of

invasive aquatic plant *Crassula helmsii*! Clearly, the role of humans cannot be ignored in diversity and distribution at the micro and meso scale respectively.

5.6.3 Taxonomic uncertainty - reconciling environmental and molecular data?

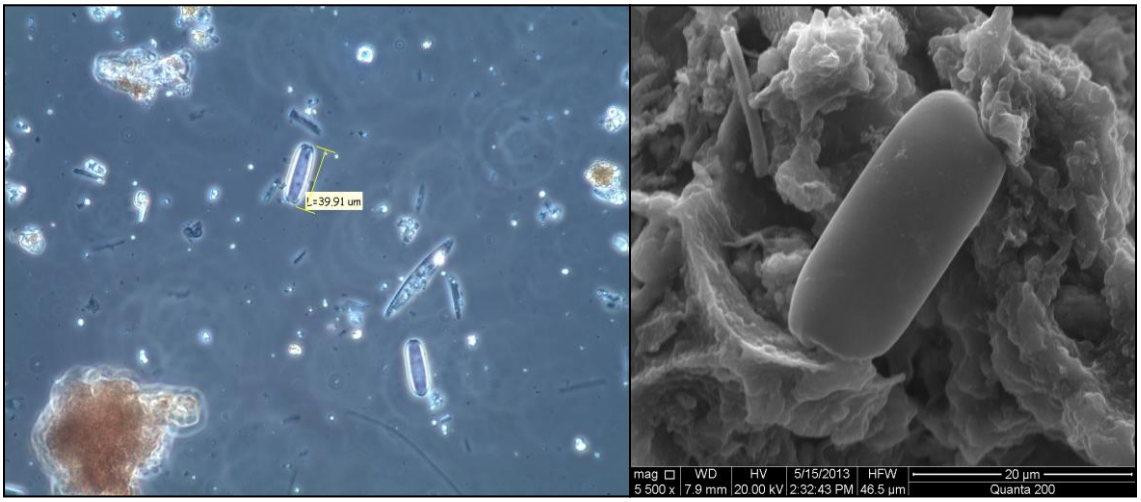
Whilst testate amoebae and diatoms do make useful model organisms in traditional taxonomy studies based on morphology, the present study and Heger *et al* (2011) highlight that it is often time consuming and difficult to identify some species using light microscopy. For instance, soil diatoms in the present study were found to be smaller and sparser than their aquatic forms, and detection amongst soil particles was often difficult using light microscopy. Schuttler (1986) highlights similar difficulties in trying to obtain an inventory and relative species composition of soil diatom assemblages. Furthermore, much of the taxonomy for diatoms is based on the aquatic forms (Krammer and Lange-Bertalot 1999) yet the striae in diatoms, which is one of the features in identification were more faint for soil diatoms which also made identification more difficult. The observations of British soil diatoms produced by Lund (1946) were a useful aid in the identification of soil diatoms, although subsequent changes in diatom taxonomy confused this matter further!

Adding to the complexity of using 'morphospecies' as units of measurement of eukaryotic diversity and distribution, this study highlighted that some microbes are similar to macroscopic organisms, such as vascular plants, in that there is variability in morphology between individuals of the same species, often creating taxonomic confusion. Some (Smith *et al* 2008) suggest intra-specific variation can be dependent on environmental variables such as moisture or availability of shell building material, others (Charman 1999) suggest genetic composition, temperature, food supply or trophic level. It's therefore important to ask the question whether these are new species or different varieties or forms. The term 'splitters' has been used to describe those taxonomists who erect new species previously considered as varieties or forms and Mazei and Warren (2012) argue future studies should be based on a combination of morphometric, ultrastructural (SEM), environmental and molecular data to characterise species complexes, such as the diatom *Achnanthes minutissimum* complex in diatoms (see Potapova and Hamilton 2007) and *Difflugia* testate amoebae (see Mazei and Warren 2012). Previous morphometric studies (Ogden and Hedley 1980) were described on the basis of a relatively small number of individuals, (for instance, as low as n = 1) which cannot account for intra-specific variability resulting from a unique combination of ecological and environmental factors. Furthermore, the taxonomic problems described for modern faunas directly impacts on the analysis of fossil samples leading to differing species richness

estimates for different genera, for instance, estimates of species richness in testate amoebae genus *Difflugia* (Charman 1999).

Molecular studies are not only providing more accurate taxonomy, they also provide information on microbial evolution (Melkonian and Mollenhauer 2005). Testate amoebae ancestors were most likely marine, suggesting a switch to terrestrial and freshwater forms sometime during their evolution (Smith *et al* 2008). Recent molecular phlyogenetic analyses within the *Euglyphida* testate amoebae demonstrated that freshwater and marine species segregate into different subclades, indicating that transitions between these environments occur only rarely in the course of evolution (Heger *et al* 2010). Both the molecular and morphological data suggest that the diversity of *Cyphoderiidae* (Appendix 2, Figures 9 a-e) is strongly underestimated and the molecular data revealed the existence of cryptic species within this family of *Euglyphids*. The morphospecies *Cyphoderia ampulla* was represented by five different phylotypes distributed throughout two distinct freshwater subclades and is therefore a polyphyletic taxon. The phylotypes are characterised by distinct arrangements of the scales revealed by scanning electron microscopy (SEM). Interestingly, molecular studies suggest *C. ampulla* phylotypes were isolated from distinct ecological habitats such as underground water of a sandy beach. This morphospecies was almost exclusively detected in the deepest lake at MSW, Mere End, which is the only lake connected to drainage systems. The diatom *Meridion circulare* was exclusively found in the connecting drainage ditch where Mere End outflows. Interestingly, in a similar study describing the aquatic testate communities in flooded sand/gravel quarry lakes in Bulgaria, similar to MSW, Serafimov *et al* (1995) detected three *Cyphoderia* species. These authors are taxonomists though and so tended to be 'splitters' rather than 'lumpers'! Nevertheless, the molecular data still suggests the total diversity of genus *Cyphoderia* and *Euglyphida* as a whole is much higher than currently recognised (Lara *et al* unpublished) and it's likely that the current estimation of eukaryotic microbial diversity (Costello *et al* 2013) is greatly underestimated (D. Wilkinson *pers comms*). Both intensive ecological (Esteban *et al* 2006) and molecular (Heger *et al* 2010) data suggest the diversity of eukaryotic microorganisms at MSW presented here is a vast underestimation.

SEM was used in the present study to determine a mystery organism present in high abundance in testate samples in most of the aquatic microhabitats (Figures 24 a,b). Using photographs taken at x400 magnification using light microscopy several scientists could not come to an agreement of the higher taxonomy of this organism. Some argued it was a testate amoebae morphotype such as *Archerella/Amphitrema flavum*; others argued desmids namely *Tetmemorus brebissonii*, and others thought it to be a penate diatom in girdle view such as *Nizschia debilis*. Indeed, this organism remains unidentified to this day which highlights the lack of specialists covering the vast field in the study of environmental microbiology. It may be further worrying if alpha-taxonomists are themselves threatened by extinction (see Cotterill *et al* 2008 and Costello *et al* 2013 for contrasting views), which raises the question whether we as humans will ever produce accurate inventories of global microbial diversity!



Figures 24 a and b. Unidentified microscopic organisms abundant in aquatic samples. SEM image (right) light microscopy image (left)

Recent advances in Environmental DNA (eDNA) has been shown to accurately quantify species richness and relative abundance for macroscopic organisms in a mesocosm experiment (Thomsen *et al* 2012). Although, further experimental research is needed to systematically compare protocols (Lodge *et al* 2012), combined with expertise from different disciplines, this may be a potentially useful tool in the future conservation of terrestrial and freshwater environments, albeit with the disadvantage of not being able to observe the organisms present.

5.6.4 Evolutionary life strategies in Protists

By investigating the diversity and distribution of eukaryotic microorganisms on a single nature reserve, useful insights have been gained into the potential of studying evolutionary life strategies in Protists. The present study has identified the presence of dominant taxa, such as *Trinema spp* of testate amoebae and *Fragilaria spp* diatoms in high relative abundance in almost all microhabitats. These are small sized species with a higher potential for dispersal and high productivity, considered as r-strategic (Smith et al 2008). The LF index proposed by Bonnet (1976) suggests strategies existing for protists, and the literature perhaps suggests that later studies (Mattheeussen *et al* 2005) may have equated Bonnet's index to r-K strategies for testate amoebae. The present study suggests further research is needed to validate this assumption.

Qualitative observations of Nematodes in this study demonstrated potential trade-offs. Nematodes form part of the microbial community in soils and they can be divided into groups, for instance: microbivores, bacterivores, cytotrophs, mixotrophs, omnitrophs and fungivores (Crotty *et al* 2012) based on their mouthparts which differ according to feeding habits (Small 1987). The present study highlighted that small microscopic animals have evolved strategies to survive stress and disturbance, for instance, nematodes using anhydrobiotic survival strategy (Figure 22), which is said to play a significant role in the widespread distribution and success of these small metazoans (Treonis and Wall (2005).

With the exception of phytoplankton, knowledge and understanding of evolutionary life strategies in protists is a major gap in 21st century ecological research (D. Wilkinson *pers comms*), a subject in which higher plants have dominated. The classic plant strategy postulated by Grime (1977) recognised the influence of competition, stress and disturbance shaping the structure of plant communities. The CSR model: competitor (C), stress tolerator (S) and ruderal (R) plant strategies has been a useful idea in nature conservation management for over three decades. Grime and Pierce (2012) have recently extended their ideas in a thought provoking book linking evolution and ecology and searching for evidence of C, S and R strategists in other biota including plants, animals, bacteria, archaea, viruses, they also extend their ideas to humans! Intriguingly though, they do not apply any of their evolutionary strategy ideas to any protists at all! In their new publication they define stress

as the limitation of biomass production and disturbance as the destruction of standing biomass. It's suggested (Ponge 2003) that the diversity of terrestrial humus forms can be attributed to the different patterns (strategies) for the capture and use of resources by ecosystems in ascending order of biodiversity and bioavailability. In other words, biodiversity depends on bioavailability and different organisms interact throughout the development of the end product. This study found relationships between humus forms and the number of testate taxa. This concept of humus forms warrants further attention as such ecosystem strategies may be useful in explaining biodiversity and the stability and productivity of ecosystems, and may be useful in nature conservation management.

Developing understanding of evolutionary life strategies in protists, in part, relies upon knowledge of an organism's ecology. In the first instance, why does this organism or this group of organisms inhabit the place they do? Further, evolutionary life strategies rely upon knowledge of ecological ideas such as extinction, speciation, endemism, ideas which often rely upon fossil data (Louys *et al* 2012). Future research should focus on strategy at the community level, and onwards to essential ecosystem processes, using both modern and fossil forms. This study has been an effective starting point to begin to investigate many unanswered questions in freshwater and terrestrial ecology.

CHAPTER SIX

CONCLUSION

The present study has shown how incorporating just two groups of 'shelled' eukaryotic microorganisms can considerably extend knowledge of biodiversity on a single nature reserve. Other intensive ecological studies and molecular research have provided evidence that the diversity of eukaryotic microorganisms presented in this study is greatly underestimated. Nature conservation priorities and strategies rely upon knowledge of species present and their population sizes; however, because species concepts in protists are unclear, the use of surrogacy measures and/or the development of parataxonomic methods may be necessary. This study has provided further evidence that for some microbial groups, such as testate amoebae and diatoms, generic level indices perform nearly as well as species level indices, and generic surrogacy has the potential for more rapid biodiversity surveys. This is important as given the vital role of eukaryotic microorganisms in the structure and function of all ecosystems; ecologists are faced with the challenge of quantifying biodiversity at the micro and meso scale.

Using a nested design, this study has shown that different terrestrial and freshwater microhabitats support different assemblages of microbial communities at small spatial scales. Lower microbial diversity was observed in lake sediment associated with an invasive aquatic plant, *Crassula helmsii* and aquatic monocultures generally. In terrestrial environments, future studies should consider sampling fresh and soil organic matter as the often overlooked litter horizon was found to be a biologically active zone of the soil profile. The relationship between humus forms and microbial diversity and distribution warrants further attention as this may be potentially useful in exploring questions relating to biodiversity and ecosystem function. The lack of correlation between both taxon richness and the diversity of testate amoebae and diatoms in both terrestrial and aquatic habitats provided further evidence that one biotic group cannot be used as a proxy for others. This highlights the complexity in studying the structure of microbial communities and future studies should consider temporal, as well as spatial factors.

Microorganisms play a fundamental role in the ecology of macroscopic organisms. However, as the majority of eukaryotic microorganisms have yet to be described, we are still some way from fully understanding the suite of abiotic and biotic factors influencing microbial diversity and distribution in terrestrial and aquatic habitats. Importantly, in view of the transformations humans have made to ecosystems, future research into evolutionary life strategies in protists may be a useful idea in understanding essential ecosystem processes and also for nature conservation management. Preliminary findings from microbial species in the present study recognised the potential influence of competition, stress and disturbance in shaping the structure of eukaryotic microbial communities. Thus, providing further evidence that ecological differences between macro and microscopic organisms may not be as prevalent as previously thought.

What is clear from the present study is that understanding the diversity and distribution of eukaryotic microorganisms locally, regionally and globally requires a holistic approach, encompassing expertise from different disciplines and embracing technological advances. There is a huge gap of potential, unanswered questions about the diversity and distribution of the 'microcosmos', a visually appealing space!

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References

Adl, M. S. & Gupta, V. S. (2006). Protists in soil ecology and forest nutrient cycling. *Canadian Journal of Forest Research*, **36**, 1805-1817

Adl, S. M., Simpson, A. G., Lane, C. E., Lukeš, J., Bass, D., Bowser, S. S. & Spiegel, F. W. (2012). The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology*, **59**, 429-514

Agapow, P. M., Bininda-Emonds, O. R., Crandall, K. A., Gittleman, J. L., Mace, G. M., Marshall, J. C. & Purvis, A. (2004). The impact of species concept on biodiversity studies. *The Quarterly Review of Biology*, **79**, 161-179

Allen, A. P., Whittier, T. R., Kaufmann, P. R., Larsen, D. P., O'Connor, R. J., Hughes, R. M. & Paulsen, S. G. (1999). Concordance of taxonomic richness patterns across multiple assemblages in lakes of the northeastern United States. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 739-747

Anderson, A.N. (1995). Measuring more of biodiversity: Genus richness as a surrogate for species richness in Australian ant faunas. *Biological Conservation*, **73**, 39-43

Anderson, J. M. (2009). Why should we care about soil fauna? *Pesquisa Agropecuária Brasileira*, **44**, 835–842

Avery, B. W. & Bascomb, C. L. (1982). *Soil survey laboratory methods.* Technical Monograph No. 6. Soil Survey, Harpenden

Bardgett, R.D., Usher, M.B. & Hopkins, D.W. (2005). *Biological Diversity and Function in Soils.* Cambridge University Press, Cambridge

Bardgett, R.D. (2005). *The Biology of Soil*. Oxford University Press, Oxford

Barker, T., Irfanullah, H.M.D. & Moss, B. (2010). Micro-scale structure in the chemistry and biology of a shallow lake. *Freshwater Biology*, **55**, 1145-1163

Battarbee, R. W., Jones, V. J., Flower, R. J., Cameron, N. G., Bennion, H., Carvalho, L., & Juggins, S. (2001). Diatoms. *In* "Tracking Environmental Change Using Lake Sediments." (J. P. Smol, J. B. Birks, and W. M. Last, Eds.), pp. 155-202. Terrestrial, Algal, and Siliceous Indicators. Kluwer Academic Publishing, Dordrecht.

Briers, R. A., & Biggs, J. (2003). Indicator taxa for the conservation of pond invertebrate diversity. *Aquatic Conservation: Marine and freshwater ecosystems*, **13**, 323-330.

Boenigk, J., Ereshefsky, M., Hoef-Emden, K., Mallet, J. & Bass, D. (2012). Concepts in protistology: Species definitions and boundaries. *European Journal of Protistology,* **48**, 96--102

Bonnet, L. (1976). Le peuplement thécamoebien édaphique de la Coted'Ivoire. Sols de la region de Lamto. *Protistologica,* **4**, 539-554

Bennion, H., Johnes, P., Ferrier, R., Phillips, G. & Haworth, E. (2005). A comparison of diatom phosphorus transfer functions and export coefficient models as tools for reconstructing lake nutrient histories. *Freshwater Biology*, **50,** 1651–1670

Bennion, H., Battarbee, R. W., Sayer, C. D., Simpson, G. L. & Davidson, T.A. (2011). Defining reference conditions and restoration targets for lake ecosystems using palaeolimnology: a synthesis. *Journal of Paleolimnology,* **45**, 533-544

Boddy, L. (2013). Spotlight on Mycology. *The Biologist,* 60, 36-37

Boltovskoy, E. (1963). The littoral foraminiferal biocoenosis of Puerto Desado (Patagonia, Argentina). *Contribution to the Cushman Foundation for Foraminiferal Research*, **14**, 58-70

Booth, R.K., Lamentowicz, M. & Charman, D.J. (2010). Preparation and analysis of testate amoebae in peatland palaeoenvironmental studies. *Mires and Peat*, **7**, 1–7

Bovrov, A.A. & Wetterich, S. (2012). Testate amoebae of arctic tundra landscapes. *Protistology*, **7**, 51-58

Bradshaw, E.G., Nielson, A.B. & Anderson, N.J. (2006). Using diatoms to assess the impacts of prehistoric, pre-industrial and modern land-use on Danish lakes. *Regional Environmental Change*, **6**, 17-24

Burbidge, S. M., & Schröder-Adams, C. J. (1998). Thecamoebians in Lake Winnipeg: a tool for Holocene paleolimnology. *Journal of Paleolimnology*, **19**, 309-328

Cairns, J. (1993). Can microbial species with a cosmopolitan distribution become extinct. *Speculations in Science and Technology*, **16**, 69-73

Charman, D.J. (1999). Testate Amoebae and the Fossil Record: Issues in Biodiversity. *Journal of Biogeography*, **26**, 89-96

Charman, D.J., Hendon D. &Woodland, W.A. (2000). *The identification of Testate amoebae in Peats.* Quaternary Research Association Technical Guide No 9. London

Charman, D.J. (2001). Biostratigraphic andpalaeoenvironmental applications of testate amoebae. *Quaternary Science Reviews*, **20,** 1753-1764

Chepurnov, V.A., Mann, D.G., Sabbe, K. & Vyerman, W. (2004). Experimental studies on sexual reproduction. *International Review of Cytology*, **237**, 91-154

Chiverall, R.C., Plater, A.J. & Thomas, G.S.P. (2004). *The Quaternary of the Isle of Man and North West England Field Guide.* Quaternary Research Association. London

Clarke, K.J. (2003). *Guide to the Identification of Soil Protozoa – Testate amoebae.* Freshwater Biological Association, Cumbria

Clifford, H. T. & Stephenson, W. (1975). *An introduction to numerical classification*. Academic Press, New York

Coesel, P.F.M. (2001). A method for quantifying conservation value in lentic freshwater habitats using desmids as indicator organisms. *Biodiversity and Conservation*, **10**, 177-187

Conrad, K.F., Warren, M.S., Fox, R., Parsons, M.S. & Wolwod, I.P. (2006). Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. *Biological Conservation*, **132**, 279-291

Cooper, C.B., Dickinson, J., Phillips, T. & Bonney, R. (2007). Citizen science as a tool for conservation in residential ecosystems. *Ecology and Society*, **12**, 11

Corbet, G. (2011). Life on the links – a perspective in biodiversity, *British Wildlife*, **23**, 104-109

Costello, M.J., May, R.M. & Stork, N.E. (2013). Can we name Earth's species before they go extinct? *Science* **339**, 413-416

Cotterill, F.P.D., Al-Rasheid, K. & Foissner, W. (2008). Conservation of Protists: is it needed at all? *Biodiversity Conservation*, **17,** 427-443

Cross J. R. (1975) *Rhododendron ponticum* L. *Journal of Ecology,* **63**, 345-364

Crotty, F. V., Adl, S. M., Blackshaw, R. P. & Murray P. J. (2012). Using stable isotopes to differentiate trophic feeding channels within soil food webs. *Journal of Eukaryotic Microbiology*. **59,** 520–526

Davis, S.R. & Wilkinson, D.M. (2004). The conservation management value of testate amoebae as restoration indicators: speculations based on two damaged raised mires in northwest England. *The Holocene*, **14**, 135-143

Dehen-Schmutz, K., Perring, C. & Williamson M. (2004). Controlling *Rhododendron ponticum* in the British Isles: an economic analysis. *Journal of Environmental Management,* **70,** 323-332

Dodson, S.I., Arnott, S.E. & Cottingham, K.L. (2000). The relationship in lake communities between primary productivity and species richness. *Ecology* **81**, 2662-2679

Dolman, P.M., Panter, C.J. & Mossman, H.L. (2012). The biodiversity audit approach challenges regional priorities and identifies a mismatch in conservation. *Journal of Applied Ecology,* **49**, 986-997

Esteban, G. F., Clarke, K. J., Olmo, J. L. & Finlay B. J. (2006). Soil protozoa – An intensive study of population dynamics and community structure in an upland grassland. *Applied Soil Ecology,* **33,** 137–151

Esteban, G.F. & Finlay, B.J. (2010). Conservation work is incomplete without cryptic biodiversity. *Nature*, 463, 293

Favreau, J. M., Drew, C. A., Hess, G. R., Rubino, M. J., Koch, F. H., & Eschelbach, K. A. (2006). Recommendations for assessing the effectiveness of surrogate species approaches. *Biodiversity & Conservation*, **15**, 3949-3969

Finlay, B.J. (2002). Global dispersal of free-living microbial eukaryote species. *Science*, **296**, 1061-1063

Finlay, B.J., Esteban, G.F., & Fenchel, T. (2004). Protist diversity is different? *Protist* **155,** 15–22

Fisher, J. & Dunbar, M. (2007). Towards a representative periphytic diatom sample. *Hydrology and Earth System Sciences,* **11**, 399-407

Fisher, J., Deflandre-Vlandas, A., Coste, M., Delmas, F. & Jarvie, H.P. (2010). Assemblage grouping of European benthic diatoms as indicators of trophic status of rivers. *Fundamental and Applied Limnology*, **176/2**, 89-100

Foissner, W. (1987). Soil protozoa: Fundamental problems, ecological significance, adaptations in Ciliates and Testaceans, bioindicators, and guide to the literature. *Progress in Protistology***, 2**, 69-212

Foissner, W. (2006). Biogeography and dispersal of micro-organisms: a review emphasising protists. *Acta Protozoologica*, **45**, 111-136

Foissner, W. (2011). Dispersal of Protists: the role of cysts and human introductions. In: Fontaneto, D. (ed). Cambridge University Press, Cambridge

Fontaneto, D. (2011). *Biogeography of Microscopic Organisms*. Cambridge University Press, Cambridge

Fournier, B., Malysheva, E., Mazei, Y., Moretti, M., & Mitchell, E. A.D. (2012). Toward the use of testate amoeba functional traits as indicator of floodplain restoration success. *European Journal of Soil Biology*, **49**, 85-91

French, C.N. & Moore, P.D. (1986). Deforestation, *Cannabis* cultivation and Schwingmoor formation at Cors Llyn (Llyn Mire), Central Wales. *New Phytologist*, **102,** 469-482

Gaines, S. D., & Denny, M. W. (1993). The largest, smallest, highest, lowest, longest, and shortest: extremes in ecology. *Ecology*, **74**, 1677-1692

Gamito, S. (2010). Caution is needed when applying Margalef Diversity Index. *Ecological Indicators*, **10**, 550-551

Gardi, C., Montanarella, L., Arrouays, D., Bispo, A., Lemanceau, P., Jolivet, C., Mulder, C., Ranjard, L., Rombke, J., Rutgers, M. & Menta, C. (2009). Soil biodiversity monitoring in Europe: ongoing activities and challenges. *European Journal of Soil Science*. **60,** 807–819

Geml, J. (2011) Coalescent analyses reveal contrasting patterns of intercontinental gene flow in arctic-alpine and boreal-temperate fungi. In: Fontaneto, D. (ed) Biogeography of Microscopic Organisms. Cambridge University Press, Cambridge

Gilbert, D., Mitchell, E. A. D., Amblard, C., Bourdier, G., Francez, A.J. (2003). Population dynamics and food preferences of the testate amoebae *Nebela tincta-major-bohemica-collaris* complex (Protozoa) in *Sphagnum* peatland. *Acta Protozoologica*. **42,** 99–104

Gotelli, N.J. & Colwell, R.K. (2001). Quantifying biodiversity: procedures and pitfalls in the measurements and comparison of species richness, *Ecology Letters*, **4**, 379-391

Green, A. J. & Elmberg, J. (2013). Ecosystem services provided by waterbirds. Biological Reviews, doi: 10.1111/brv.12045

Grime, J.P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist,* **111**, 1169-1194

Grime, J.P. & Pierce, S. (2012). *The Evolutionary Strategies that Shape Ecosystems.* Wiley-Blackwell, Oxford

Growns, I. (1999). Is genus or species level identification of periphytic diatoms required to determine the impacts of river regulation? *Journal of Applied Phycology,* **11**, 273-283

Hagen, J.B. (2012). Five Kingdoms, More or Less: Robert Whittaker and the broad classification of organisms. *Bioscience*, **62**, 67-74

Hale, W.G. & Coney, A. (2005). *Martin Mere: Lancashire's Lost Lake.* Liverpool University Press, Liverpool

Hambler, C., Henderson, P.A.& Speight, M.R. (2011). Extinction rates, extinction-prone habitats, and indicator groups in Britain and at larger scales. *Biological Conservation*, **144**, 713–721

Hammer, O., Harper, D.A.T. & Ryan, P.D. (2001). *PAST – Paleontological statistics.* University of Oslo, Norway

Han, B., Wang, T., Xu, L. & Dumont, H.J. (2011). Dynamics in space and time of four testate amoebae (*Difflugia spp*.) co-existing in the zooplankton of a reservoir in southern China. *European Journal of Protistology,* **47**, 224-230

Heal, O.W. (1964). Observations on the seasonal and spatial distribution of testaceans (Protozoa: Rhizopoda) in *Sphagnum*. *Journal of Animal Ecology*, **33**, 395–412

Heger, T.J., Straub F. & Mitchell E.A.D. (2011). Impact of farming practices on soil diatoms and testate amoebae: A Pilot study in the DOK-trial at Therwil, Switzerland. *European Journal of Soil Biology*, **49**, 31-36

Heger, T.J., Mitchell, E.A.D., Todorov, M., Golemansky, V., Lara, E., Leander, B.S., Pawlowski.J (2010) Molecular phylogeny of euglyphid testate amoebae (Cercozoa: Euglyphida) suggests transitions between marine supralittoral and freshwater/terrestrial environments are infrequent. Molecular Phylogenetics and Evolution, 55, 113–122

Heger, T.J., Booth, R.K., Sullivan, M.E., Wilkinson, D.W., Warner, B.G., Asada, T., Mazei, Y., Meisterfeld, R. & Mitchell, E.A.D. (2011). Rediscovery of *Nebela ansata* (Amoebozoa: Arcellinida) in Eastern North America: biogeographical implications. *Journal of Biogeography*, **38**, 1-10

Hesse, P. R. (1971). *Textbook of soil chemical analysis*. John Murray, London

Hussner, A. (2008). Growth and photosynthesis of four invasive aquatic plant species in Europe. *Weed Research*, **49**, 506-515

http://www.iucnredlist.org/
[Accessed 3 March 2013]

James, C., Fisher, J. & Moss, B. (2003). Nitrogen-limited lakes: the Shropshire and Cheshire Meres? *Archiv fur Hydrobiologie,* **158**, 249-266

Jassey, V.E.J, Shimano, S., Dupuy, C., Toussaint, M.L. & Gilbert, D. (2012). Characterizing the feeding habits of the testate amoebae *Hyalosphenia papilio* and *Nebela tincta* along a narrow fen-bog gradient using digestive vacuole content and 13^{C} and 15^{N} isotopic analyses. *Protist,* **163**, 451- 464

Jenny, H. (1946). Arrangement of Soil Series and Types According to Functions of Soil-Forming Factors, *Soil Science*, **61**, 375-392

Kelly, M. G., & Whitton, B. A. (1995). The trophic diatom index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, *7*, 433-444

Kelly, M., King, L., & Ní Chatháin, B. (2009). The conceptual basis of ecological-status assessments using diatoms. *Environment: Proceedings of the Royal Irish Academy*, **109**, 175-189

Kent, M. (2012). *Vegetation Description and Data Analysis: A practical approach.* Wiley-Blackwell, Chichester

Klasven, S.K. & Maberley, S. (2010). Effect of light and CO2 on inorganic carbon uptake in the invasive aquatic CAM plant *Crassula helmsii*. *Functional Plant Biology*, **37**, 737-747

Kosakyan, A., Heger, T.J., Leander, B.S., Todorov, M., Mitchel, E.A.D. & Lara, E. (2012). COI Barcoding of Nebelid Testate Amoebae (Amoebozoa: Arcellinida): Extensive Cryptic Diversity and Redefinition of the Hyalospheniidae Schultze. *Protist*, **163**, 415-434

Krammer K. & Lange-Bertalot H. (1999). *Subwasserflora von Mitteleuropa: Baccillarophycear. Volumes 1-4.* Spektrum Akademischer Verlag GmbH Heidelberg, Berlin, Germany

Krebs, C.J. (1999). *Ecological Methodology.* Benjamin/Cummings, California

Lahr, D. J., Parfrey, L. W., Mitchell, E. A., Katz, L. A., & Lara, E. (2011). The chastity of amoebae: re-evaluating evidence for sex in amoeboid organisms. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 2081-2090.

Lamentowicz, M. & Mitchell, E.A.D. (2005). The Ecology of Testate Amoebae (Protists) in Sphagnum in North-Western Poland in Relation to Peatland Ecology. *Microbial Ecology*, **50**, 48-63

Lamoreux, J. F., Morrison, J. C., Ricketts, T. H., Olson, D. M., Dinerstein, E., McKnight, M. W., & Shugart, H. H. (2005). Global tests of biodiversity concordance and the importance of endemism. *Nature*, **440**, 212-214

Lara, E., Roussel-Delif, L., Fournier, B., Wilkinson, D.M. & Mitchell, E.A.D. (2013). Only one ecology? Euglyphid testate amoebae, the protists that bridge microbial and "macrobial" community ecology. In review

Lawton, J. H., Bignell, D. E., Bolton, B., Bloemers, G. F., Eggleton, P., Hammond, P. M., & Watt, A. D. (1998). Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. *Nature*, **391**, 72-76

Ledeganck, P., Nijs, I. And Beyens, L. (2003). Plant functional group diversity promotes soil protist diversity, *Protist*, **154**, 239-249

Levin, S.A. (1992). The problem of pattern and scales in ecology. *Ecology*, **73**, 1943–1967

McClain. M. E., Boyer, E.W., Dent, L., Gergel, S.E., Grimm, N.B., Groffman.P.M., Hart. S.C., Harvey. J.W., Johnston.C.A., Mayorga.E., McDowell.W.H. & Pinay.G. (2003). Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic Ecosystems. *Ecosystems*, **6**, 301-312

Leira, M., Chen, G., Dalton, C., Irvine, K. & Taylor, D. (2009), Patterns in freshwater diatom taxonomic distinctness along an eutrophication gradient. *Freshwater Biology*, **54**, 1–14.

Lindenmayer, B.D. & Likens, G.E. (2009) Adaptive monitoring: a new paradigm for long-term research and monitoring. *Trends in Ecology and Evolution,* **24**, 482-486

Lodge, D.M., Turner, C.R., Jerde, C.L., Barnes, M.A., Chadderton, L., Egan, S.P., Feder, J.L., Mahon, A.R. & Pfrender, M.E. (2012). Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology*, **21,** 2555–2558

Lousier, J. D. (1974). Response of soil testacea to soil moisture fluctuations. *Soil Biology and Biochemistry*, **6**, 235-239

Louys, J., Wilkinson, D.M. & Bishop, L.C. (2012). Ecology needs a paleontological perspective. In: Paleontology in Ecology and Conservation, Springer Earth System Sciences, 23-38

Lund J. W. G. (1946). Observations of soil algae. The ecology, size and taxonomy of British soil diatoms. *New Phytologist,* **45,** 56–110

Magurran, A.E. (2004). *Measuring Biological Diversity.* Blackwell Science Ltd, Oxford

Mann, D. G. (1999). The species concept in diatoms. *Phycologia*, **38**, 437-495

Marcel, G.A., Heijden, V. & Horton, T.R. (2009). Socialism in the soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*. **97**, 1139-1150

Margulis, L., D. Chase. & R, Guerrero. 1986. Microbial communities. *BioScience,* **36**,160-170

Margulis, L., & Schwartz, K. V. (1998). *Five kingdoms: an illustrated guide to the phyla of life on earth.* W.H. Freeman, New York

Marotta, H., Fontes, M.L.S.& Petrucio, M.M. (2012). Natural events of anoxia and low respiration index in oligotrophic lakes of the Atlantic Tropical Forest. *Biogeosciences*, **9**, 2879-2887

Mattheeussen, R., Ledeganck, P., Vicke, S., Van de Vijver, B., Nijs, I. & Beyens, L. (2005). Habitats selection of aquatic testate amoebae communities on Quqertarsuaq (Disko Island), West Greenland. *Acta Protozoologica*, **44**, 253-263

May, R.M. (1994). Conceptual aspects of the quantification of the extent of biological diversity. *Philosophical Transactions Royal Society,* **345**, 13-20

Mazei, Y.A. & Tsyganov, A.N. (2006). *Freshwater Testate Amoebae.* KMK Publisher, Moscow [in Russian]

Mazei, Y. & Warren, A. (2012). A survey of the testate amoeba genus *Difflugia* Leclerc, 1815: based on specimens in the E. Penard and C.G. Ogden collections of the Natural History Museum, London. Part 1: Species with shells that are pointed aborally and/or have aboreal protuberances. *Protistology*, **3**, 121-171

Melkonian, M., & Mollenhauer, D. (2005). Robert Lauterborn (1869–1952) and his *Paulinella chromatophora*. *Protist*, **156**, 253-262

Melzer A. (1999). Aquatic macrophytes as tools for lake management. *Hydrobiologia* **396**, 181-190

Ming, M., Meng, X. & Wang, E. (2007). Evaluation of four digestive methods for extracting diatoms. *Forensic Science International*, **170**, 29-34

Mitchell, E.A.D., Charman, D.J., Warner, B.G. (2008). Testate amoebae analysis in ecological and paleoecological studies of wetlands: past present and future. *Biodiversity and Conservation,* **17**, 2115-2137

Moravcova, A., Beyens, L. & Van de Vijver, B. (2010). Diatom communities in soils influenced by the wandering albatross (Diomedea exulans). *Polar Biology*, **33**, 241-255

Moss, B. (1998). *The Ecology of Freshwaters: Man and Medium, Past to Future.* Blackwell Science, London

Moss, B. (2000). Biodiversity in fresh waters – an issue of species preservation or system functioning? *Environmental Conservation*, **27**, 1-4

Moss, B., Hering, D., Green, A. J., Aidoud, A., Becares, E., Beklioglu, M. & Weyhenmeyer, G. A. (2009). Climate change and the future of freshwater biodiversity in Europe: a primer for policy-makers. *Freshwater Reviews*, **2**, 103-130.

Moss, B. (2010). *Ecology of Freshwaters: A View for the Twenty-First Century.* J. Wiley & Sons, Chichester

Nee S. (2004) More than meets the eye. *Nature* **429,** 804–805

Neville, L.A., Christie, D.G., McCarthy, F.M.G. & MacKinnon, M.D. (2010). Biogeographic variation in Thecamoebian (Testate amoeba) assemblages in lakes within various vegetation zones of Alberta, Canada. *International Journal of Biodiversity and Conservation*, **2**, 215-224

Nicholls, K. H. (2003). Form variation in *Campascus minutus* and a description of Campascus simcoei sp. n.(Testaceafilosea, Psammonobiotidae). *European journal of protistology*, **39**, 103-112.

Nowack, E. C., Vogel, H., Groth, M., Grossman, A. R., Melkonian, M., & Glöckner, G. (2011). Endosymbiotic gene transfer and transcriptional regulation of transferred genes in *Paulinella chromatophora*. *Molecular biology and evolution*, **28**, 407-422

Oreska, M.P.J. & Aldridge, D.C. (2011). Estimating the financial costs of freshwater invasive species in Great Britain: a standardised approach to invasive species costing. *Biological Invasions*, **13**, 305-319

Ogden, C.G. & Hedley, R.H. (1980). *An Atlas of Freshwater Testate Amoebae*. British Museum of Natural History and Oxford University Press, London and Oxford

Payne, R.J. & Mitchell, E.A.D. (2009). How many is enough? Determining optimal count totals for ecological and palaeoecological studies of testate amoebae. *Journal of Palaeolimnology*, **42**, 483-495

Payne, R. (2009). The standard preparation method for testate amoebae leads to selective loss of the smallest taxa. *Quaternary Newsletter*, **119**, 16-20

Perrigo, A.L., Romeralo, M., Baldauf, S.L. (2012). What's on your boots: an investigation into the role we play in protist dispersal. *Journal of Biogeography*, **39**, 998-1003

Ponge, J.-F. (2003). Humus forms in terrestrial ecosystems: a framework to diversity. *Soil Biology and Biochemistry*, **35**, 935–945

Potapova, M., & Hamilton, P. B. (2007). Morphological and ecological variation within the Achnanthidium minutissimum (Bacillariophyceae) species complex1. *Journal of Phycology*, **43**, 561-575

Prendergast, J. R., Quinn, R. M., Lawton, J. H., Eversham, B. C. & Gibbons, D. W. (1993). Rare species, the coincidence of diversity hotspots and conservation strategies. *Nature,* **365**, 335–-337

Roe, H. M. (2012). Testate amoebae (thecamoebians) as water quality indicators in lakes: development of a modern distributional dataset from the Greater Toronto Area. *Quaternary International*, **279**, 410-411

Roe, H.M., Patterson, R.T. & Swindles, G.T. (2010). Controls on the contemporary distribution of lake thecamoebians (testate amoebae) within the Greater Toronto Area and their potential as water quality indicators. *Journal of Paleolimnology*, **43**, 123-140

Rønn R., Vestergård M. & Ekelund F. (2012) Interactions between bacteria, protozoa and nematodes in soil. *Acta Protozoologica,* **51,** 223–235

Round, F.E., Crawford, R.M.& Mann, D.G. (1990). The Diatoms: Biology and Morphology of the Genera. Cambridge University Press, Cambridge

Serafimov, B.L. , Golemansky, V.G. & Todorov, M.T. (1995).Testate taxocenoses (Rhizopoda, Testacea) in two quarry lakes of Sofia district. Acta Zoologica Bulgarica, **48**, 23-33

Schlegel, M. & Meisterfeld, R. (2003). The species problem in protozoa revisited. European Journal Protistology, **39**, 349–355

Schuttler, P. L. (1986). Concentrating of soil diatoms for assemblage description. *Soil Biology and Biochemistry,* **4,** 389–394

Scott, D.B., Medioli, F.S. & Schafer, C.T. (2001). Monitoring in Coastal Environments Using Foraminifera and Thecamoebian Indicators. Cambridge University Press, Cambridge

Simberloff, D. (1998). Flagships, umbrellas, and keystones: is single-species management passé in the landscape era? *Biological conservation*, **83**, 247-257

Simpson, E. H. (1949). Measurement of diversity. *Nature*, **163**, 688

Small, R.W. (1987). A review of the prey of predatory soil nematodes. *Pedobiologica*, **30**, 179-206

Smith, H.G. (1973). The Ecology of Protozoa in Chinstrap Penguin Guano. *British Antarctic Survey*, **35**, 33-50

Smith, H.G., Bobrov, A. & Lara, E. (2008). Diversity and biogeography of testate amoebae. *Biodiversity Conservation*, **17**, 329-343

Smol, J.P. & Stoermer, E.F. (2010). The Diatoms: Applications for the Environmental and Earth Sciences. Cambridge University Press. New York

Souffreau, C., Vanormelingen, P., Van de Vijver, B., Isheva, T., Verleyen, E., Sabbe, K., & Vyverman, W. (2013). Molecular Evidence for Distinct Antarctic Lineages in the Cosmopolitan Terrestrial Diatoms *Pinnularia borealis* and *Hantzschia amphioxys*. *Protist*, **164**, 101-115

Søndergaard, M., E. Jeppesen, E. Mortensen, E. Dall, P. Kristensen & O. Sortkjær, O. (1990). Phytoplankton biomass reduction after planktivorous fish reduction in a shallow, eutrophic lake: A combined effect of reduced internal P-loading and increased zooplankton grazing. *Hydrobiologia,* **200/201**, 229–240.

Suthers, H.B. (1985). Ground-feeding songbirds as cellular slime mold distribution vectors, *Oecologica*, **65**, 526-530

Sutton, C.A. & Wilkinson, D.M. (2007). The effects of *Rhododendron* on Testate amoebae communities in woodland soils in North West England. *Acta Protozoologica*, **46**, 333-338

Swindles, G.T., Roe, H.M., Charman, D.J. & Sansum, P.A. (2009). Environmental controls on peatland testate amoebae (Protozoa: Rhizopoda) in the North of Ireland: implications for paleoclimate studies. *Journal of Paleolimnology*, **42,** 123-140

Szanser, M., Ilieva-Makulec, K., Kajak, A., Gorska, E., Kusinska, A., Kisiel, M., Olejniczak, I., Russel, S., Sieminiak, D. & Wojewoda, D. (2011). Impact of litter species diversity on decomposition processes and communities of soil organisms. *Soil Biology and Biochemistry*, **43**, 9–19

Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., Orlando, L. & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, **21**, 2565 - 2573

Trappeniers, K., van Kerckvoorde,A., Chardez, D., Nijs, I. & Beyens, L. (1999). Ecology of testate amoebae communities from aquatic habitats in the Zackenberg area (Northeast Greenland). *Polar Biology*, **22**, 271-278

Treonis, A. M., & Wall, D. H. (2005). Soil nematodes and desiccation survival in the extreme arid environment of the Antarctic Dry Valleys. *Integrative and Comparative Biology*, **45**, 741-750

Van Kerckvoorde A., Trappeniers K., Nijs I., Beyens L. (2000). Terrestrial soil diatom assemblages from different vegetation types in Zackenberg (Northeast Greenland). *Polar Biology,* **23,** 392–400

Vincke, S. Vijver, B., Nijs, I. & Beyens, L. (2006). Changes in the testatacean community structure along small soil profiles. *Acta Protozoologica*, **45**, 395-406

Vincke, S., Van de Vijver, B., Ledeganck, P., Nijs, I. & Beyens, L. (2007). Testacean communities in perturbed soils: the influence of the wandering albatross. *Polar Biology*, **30**, 395-406

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, **277**, 494-499

Vohnik, M., Burdikova, Z., Vyhnal, A. &Koukol, O. (2011). Interactions between testate amoebae and saprotrophic microfungi in a Scots pine litter microcosm. *Microbial Ecology*, **61**, 660-668

Vohník M., Burdíková Z. & Wilkinson D. M. (2012). Testate amoebae communities in the rhizosphere of *Rhododendron ponticum* (Ericaceae) in an evergreen broadleaf forest in Southern Spain. *Acta Protozoologica*. **51,** 259–269

Vohnik, M., Burdikova, Z., Albrechtova, J. & Vosatka, M (2009) Testate amoebae (Arcellinisa and Euglyphida) vs. Ericoid mycorrhizal and DSE fungi: a possible novel interaction in the mycorrhizosphere of ericaceous plants? *Microbial Ecology*, **57**, 203-214

Vyverman, W., Verleyen, E., Sabbe, K., Vanhoutte, K., Sterken, M., Hodgson, D.A., Mann, D.G., Juggins, S., Van de Vijver, B., Jones, V., Flower, R., Roberts, D., Chepurnov, V.A., Kilroy, C., Vanormelingen, P. & De Wever, A. (2007). Historical processes constrain patterns in global diatom diversity. *Ecology,* **88**, 1924—1931

Wanner, M., Elmer, M., Kazda, M. & Xylander, W.E.R. (2008). Community assembly of terrestrial testate amoebae: How is the very first beginning characterized? *Microbial Ecology*, **56**, 43–54

Wunsam, S., Cattaneo, A., & Bourassa, N. (2002). Comparing diatom species, genera and size in biomonitoring: a case study from streams in the Laurentians (Quebec, Canada). *Freshwater Biology*, **47**, 325-340

Wilkinson, D. M., & Davis, S. R. (2000). Rapid assessments of microbial biodiversity using relationships between genus and species richness. Studies on testate amoebae. *Acta Protozoologica*, **39**, 23-26

Wilkinson D.M. (2001). What is the upper size limit for cosmopolitan distribution in free-living microorganisms? *Journal of Biogeography,* **28**, 285-291

Wilkinson, D.M. (2007). Do we need to worry about the conservation of microorganisms? In: Midgley, M. (2007) Earthy realism; the meaning of Gaia. *Imprint Academic*, Exeter

Wilkinson, D.M. (2008). Testate amoebae and nutrient cycling: peering into the black box of soil ecology. *Trends in Ecology and Evolution***, 23**, 596-599

Wilkinson D.M. (2010). Have we underestimated the importance of humans in the biogeography of free-living terrestrial microorganisms? *Journal of Biogeography*, **37**, 393-397

Wilkinson, D.M. & Mitchell, E.A.D. (2010) Testate amoebae and nutrient cycling with particular reference to soils. *Geomicrobiology*, **27**, 520-533

Wilkinson, D.M., Creevy, A.L. & Valentine, J. (2012). The past, present and future of soil protist ecology. *Acta Protozoologica,* **51**, 189-199

Williams, P. H., & Gaston, K. J. (1994). Measuring more of biodiversity: can higher-taxon richness predict wholesale species richness? *Biological Conservation*, **67**, 211-217

Withers, P. J. A. & Haygarth, P. M. (2007), Agriculture, phosphorus and eutrophication: a European perspective. *Soil Use and Management,* **23,** 1–4

Yang, J., Smith, H.G., Sherratt, T.N.& Wilkinson, D.M. (2010). Is there a size limit for cosmopolitan distribution in free-living microorganisms? A biogeographical analysis of testate amoebae from polar areas. *Environmental Microbiology*, **59**, 635-645

Zancan, S., Trevisan, R. & Paoletti, M. G. (2006). Soil algae composition under different agro-ecosystems in North Eastern Italy. *Agriculture. Ecosystems and Environment.* **112,** 1–12

APPENDIX 1. Diatom and Testate amoebae taxon list

Diatom taxa

Achanthes deliculata
Achnanthes cf exigua
Achnanthes cf grana
Achanthes cf helvetica
Achnanthes laevis
Achanthes lanceolata
Achnanthes minutissima var inconspicua
Achnanthes minutissima var minutissima
Achnathes cf oblongella
Achnanthes cf. thermalis
Amphora libyca
Amphora cf montana
Amphora ovalis
Amphora pediculus
Caloneis spp
Cocconeis pediculus
Cocconeis placentula var euglypta
Cocconeis placentula var lineata
Cocconeis placentula var placentula
Cyclotella spp.
Cyclotella cf meneghiniana
Cyclotella radiosa
Cymatopleura solea
Cymbella spp.
Cymbella amphicephala
Cymbella cistula
Cymbella cymbiformis
Cymbella microcephala
Cymbella minuta
Cymbella naviculiformis
Cymbella proxima
Cymbella silesiaca
Cymbella cf solea
Diploneis spp
Diploneis elliptica
Diploneis interupta
Epithemia adnata
Epithemia argus
Epithemia cf smithii

Testate taxa

Arcella spp.
Arcella artocrea
Arcella bathystoma
Arcella catinus
Arcella discoides
Arcella cf hemispherica
Arcella vulgaris
Assulina muscorum
Assulina scandinavica
Assulina seminulum
Bullinaria indica
Campascus minutus
Centropyxis aculeata
Centropyxis aerophilla
Centropyxis cassis
Centropyxis constricta
Centropyxis discoides
Centropyxis ecornis
Centropyxis minuta
Centropyxis platystoma
Centropyxis sylvatica
Corythion dubium
Corythion pulchellum
Corythion trinema type
Cyphoderia ampulla
Cyphoderia cf ampulla virtae
Cyclopyxis arcelloides type
Cyclopyxis eurystoma
Cyclopyxis kahli
Cryptodifflugia oviformis
Cucurtibella morph
Difflugia spp.
Difflugia lanceolata
Difflugia cf lithophila
Difflugia lucida type
Difflugia oblonga
Difflugia pristis type A
Difflugia pristis type B
Difflugia cf proteiformis

Epithemia sorex
Epithemia turgida var granulata
Eunotia bilunaris
Eunotia formica
Eunotia implicata
Eunotia intermedia
Eunotia paludosa
Fragilaria spp
Fragilaria brevistriata
Fragilaria capucina
Fragilaria capucina var mesolepta
Fragilaria cf. capucina var amphicephala
Fragilaria construens var venter
Fragilaria elliptica
Fragilaria exigua
Fragilaria parasitica var parasitica
Fragilaria parasitica var subconstricta
Fragilaria pinnata var pinata
Gomphonema spp
Gomphonema acuminatum
Gomphonema olivaceum
Gomphonema parvullum
Gomphonema truncatum
Gyrosigma acuminatum
Hantzschia spp
Hantzschia amphioxus
Hantzschia virgata
Meridion circulare var constrictum
Navicula spp.
Navicula capitata
Navicula cf clementis
Navicula cinca
Navicula cryptocephala
Navicula cuspidata
Navicula cf gibbula
Navicula cf halophila
Navicula ignota
Navicula cf laevissima
Navicula minima
Navicula cf pseudoscutiformis
Navicula radiosa
Navicula recens
Navicula seminulum
Difflugia pulex
Difflugia unknown A
Difflugia unknown B
Difflugia cf urceolata
Euglypha acanthora
Euglypha cf ciliata
Euglypha compressa
Euglypha cristata
Euglypha laevis
Euglypha rotunda
Euglypha strigosa
Euglypha tuberculata type
Euglypha spp
Heleopera petricola
Heleopera rosea
Heleopera sphagni
Heleopera sylvatica
Hyalosphenia ovalis
Hyalosphenia papillo
Hyalosphenia subflava
Lesquereusia cf modesta
Nebela bigibosa
Nebela collaris
Nebela dentistoma
Nebela griseola
Nebela millitaris
Nebela tincta
Nebela tubulata
Paulinella chromatophora
Placocysta spinosa
Plagiopyxis callida
Phryganella acropodia
Pontigulasia cf compressa
Pseudodifflugia gracilis
Quadrulella symmetrica
Retort shaped testate A
Sphenoderia fissirostris
Sphenoderia lenta
Sphenoderia cf macrolepis
Tracheuglypha dentata
Trigonopyxis arcula
Trinema complanatum
Trinema lineare

Navicula cf silevicensis
Navicula cf tenelloides
Navicula trivialis
Neidium cf ampliatum
Neidium cf iridis
Nitzschia amphibia
Nitzschia cf constricta
Nitzschia dissipata var media
Nitzschia filiformis
Nitzschia inconspicua
Nitzschia cf palea
Nitzschia spp.
Pinnularia spp.
Pinnularia appendicula (sylvatica)
Pinnularia borealis
Pinnularia cf brandetti
Pinnularia esox
Pinnularia cf legumen
Pinnularia lundii
Pinnularia major
Pinnularia microstauron
Pinnularia nobilis
Pinnularia streptoraphe
Pinnularia subcapitata
Pinnularia viridis
Rhopalodia gibba
Rhopalodia gibba var minuta
Rhopalodia gibba cf var parallela
Stauroneis spp.
Stauroneis anceps
Stauroneis cf javanica
Stauroneis kriegerii
Stauroneis lapidicola
Stauroneis muriella
Stauroneis cf nobilis
Stauroneis cf phoenicenteron
Stauroneis cf truncata
Surirella spp
Surirella cf amphioxys
Surirella cf bifrons
Surirella brebissonii
Surirella ovalis
Surirella splendida
Trinema enchelys

Tabellaria flocculosa

APPENDIX 2. Photomicrographs of Testate amoebae and Diatoms found at Mere Sands Wood in North West England

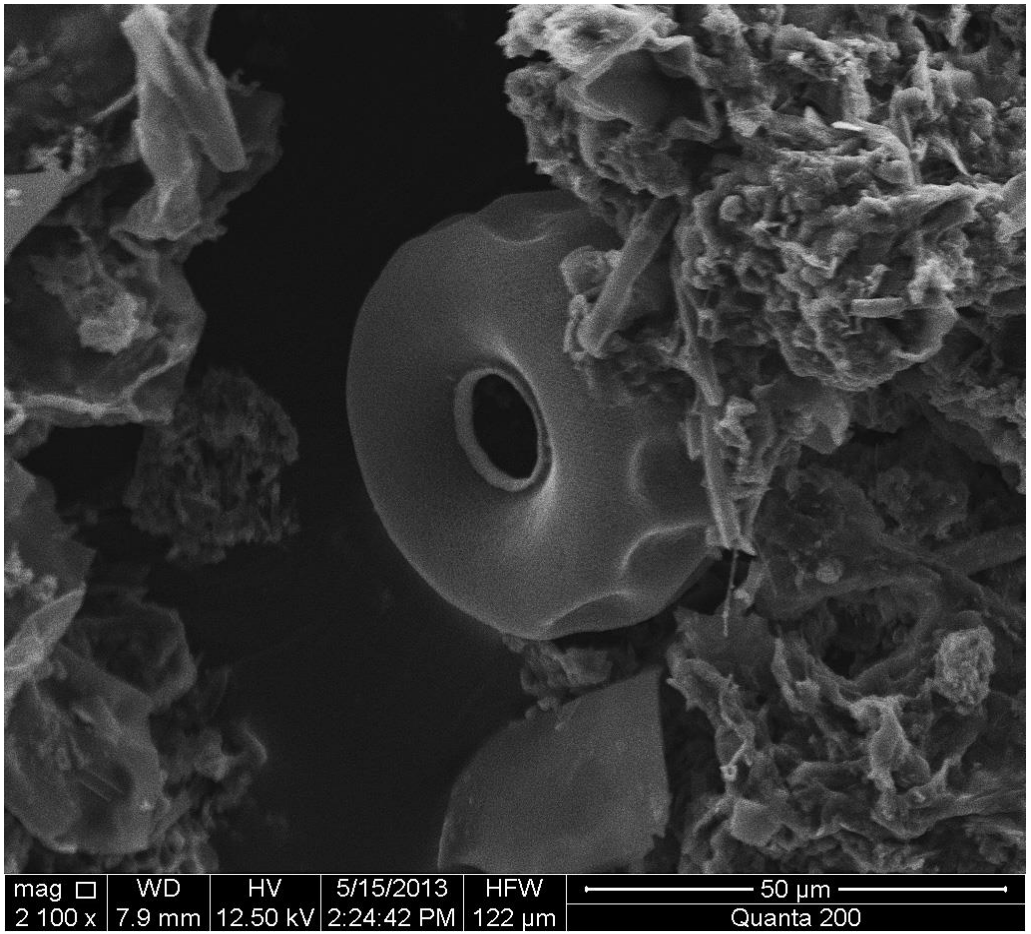
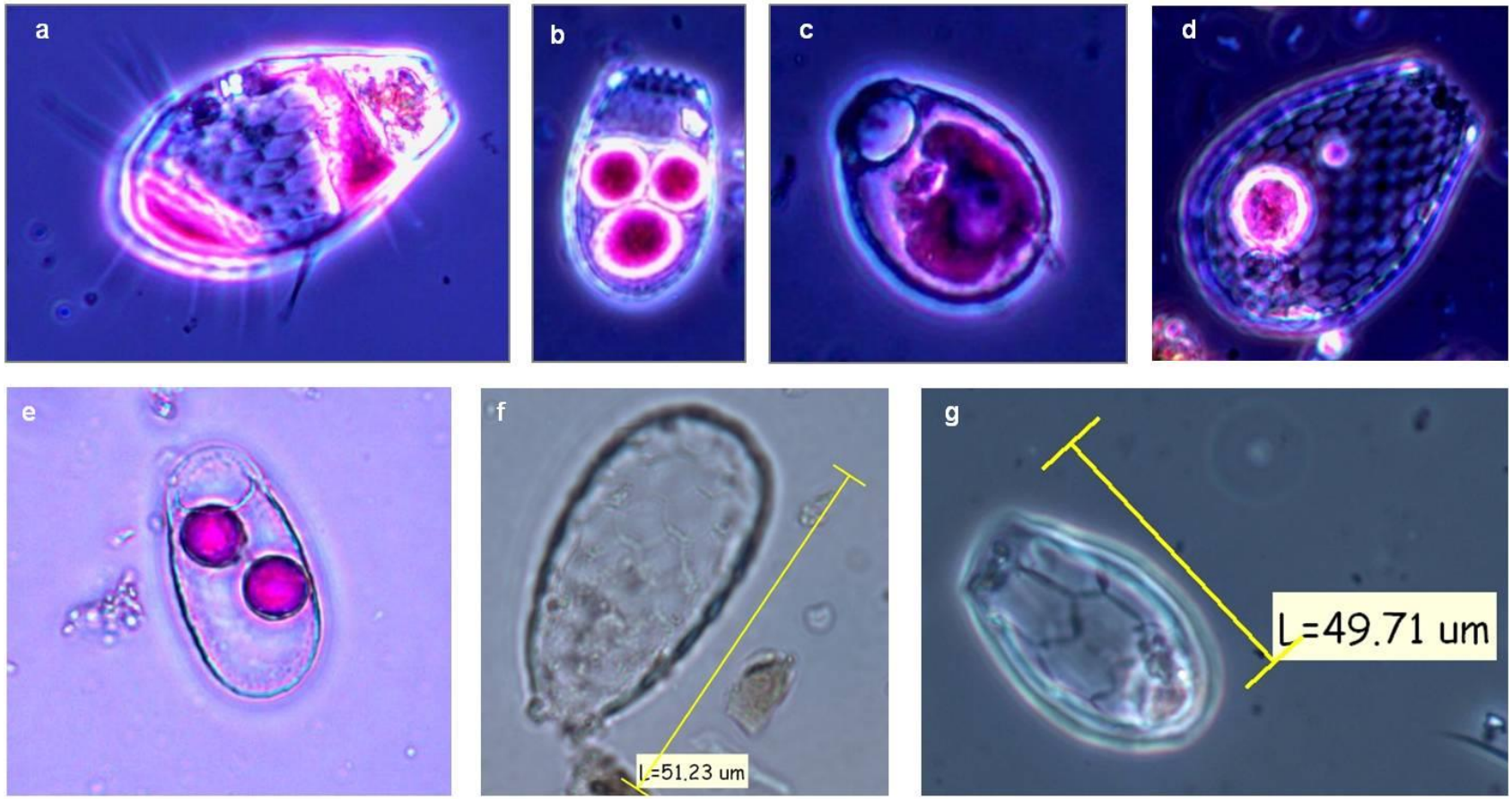
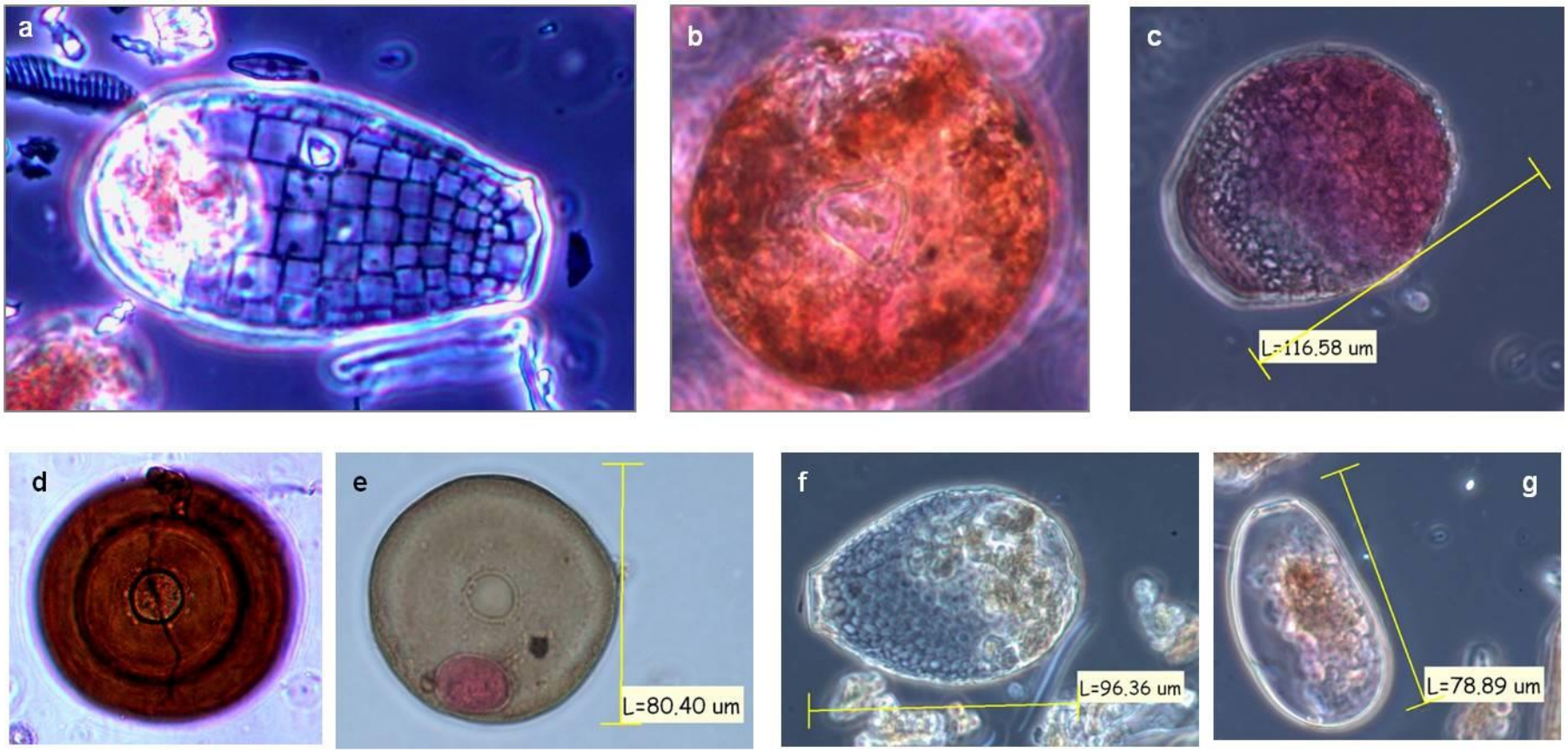


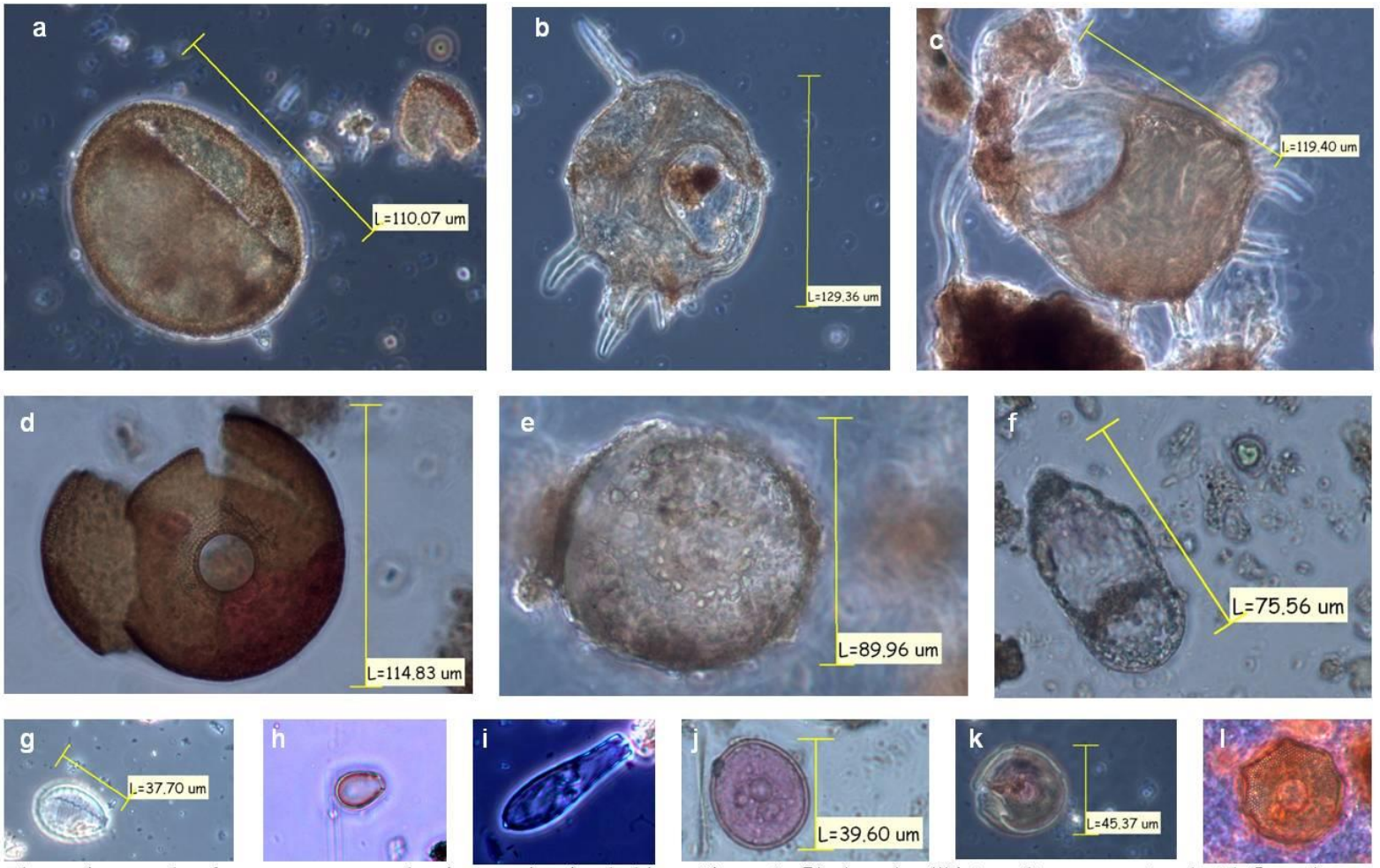
Figure 1. SEM image of *Arcella bathystoma* found in the anoxic sediment beneath the Scrape.



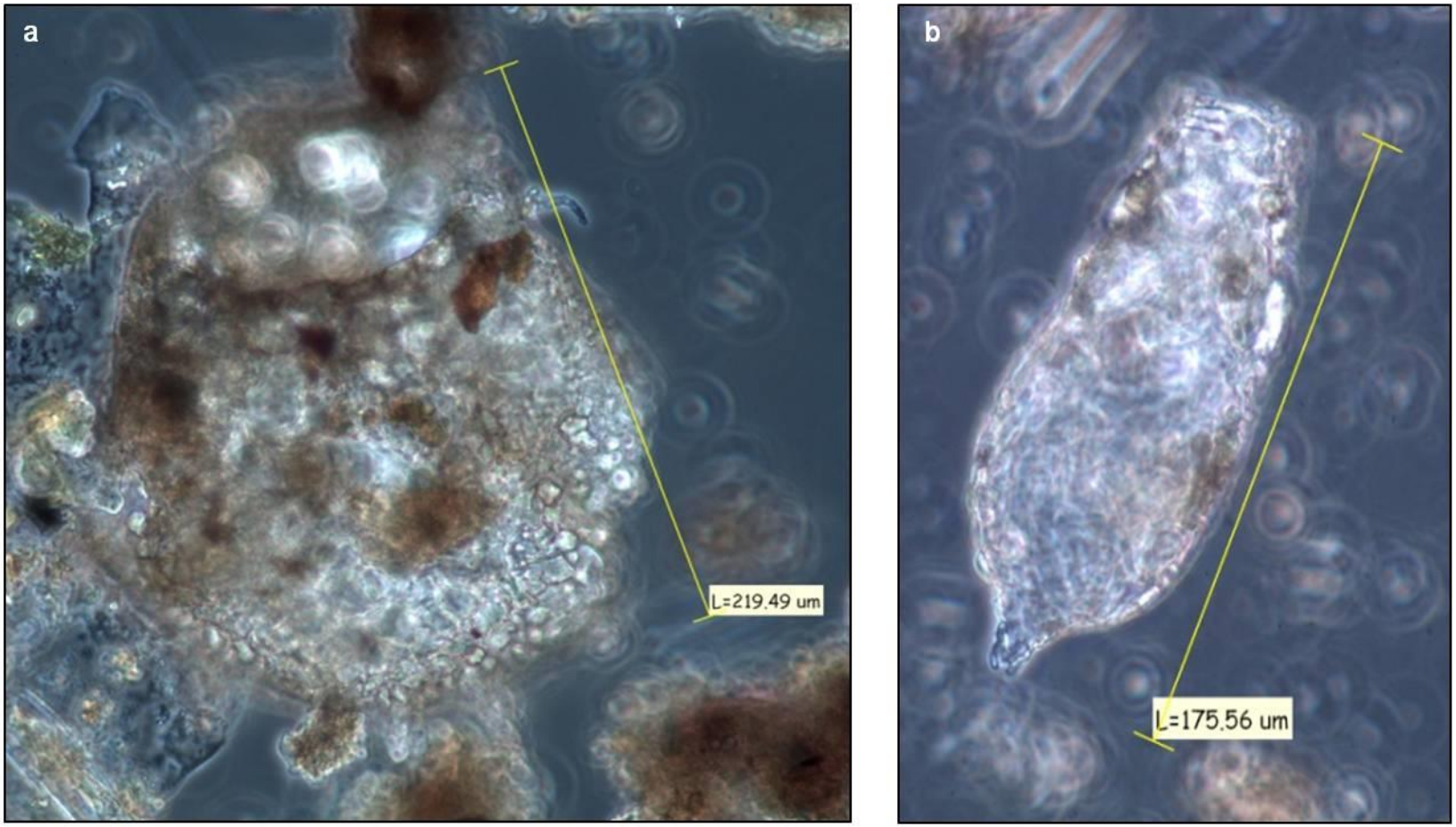
Photomicrographs of some terrestrial filose testate amoebae: Figures 2a. *Euglypha compressa* in Bryophyte litter on an island in a shallow lake, **b.** *Euglypha rotunda* found in litter associated with *Betula pendula*, **c.** *Corythion dubium* found in *Betula pendula* litter, **d.** *Euglypha tuberculata* type (sensu Charman and Hendon) Bryophyte soils, **e.** *Trinema enchelys* found in litter associated with *Betula pendula*, **f.** *Tracheuglypha dentata* in Bryophyte litter, **g.** *Sphenoderia fissirostris* found in soil associated with *Alnus glutinosa* on an island within a shallow lake



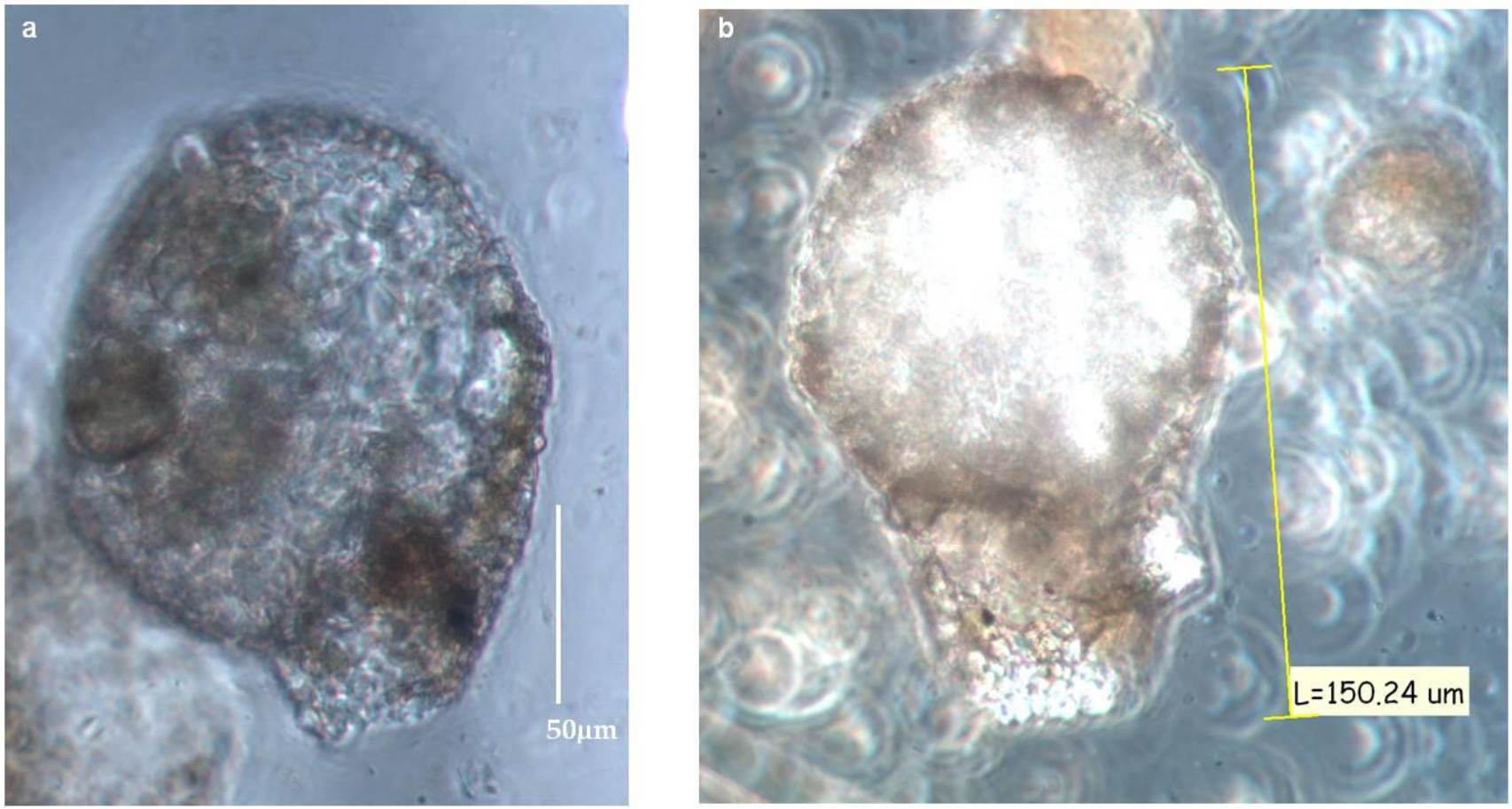
Photomicrographs of some terrestrial lobose testate amoebae: Figures 3a. *Quadrulella symmetrica* in Bryophyte soils on an island in a shallow lake, **b.** *Trigonopyxis arcula* found in soil associated with *Pinus sylvestris*, **c.** *Heleopera sphagni* found in litter associated with *Iris pseudacorus and Typha latifolia* on an island within a shallow lake, **d.** *Arcella catinus* found in soil associated with *Iris pseudacorus and Typha latifolia* on an island within a shallow lake, **e.** *Arcella discoides* found in soil associated with *Iris pseudacorus and Typha latifolia* on an island within a shallow lake, **f.** *Nebela tincta* in litter associated with *Alnus glutinosa*, **g.** *Hyalosphenia subflava* found in soil associated with *Iris pseudacorus and Typha latifolia* on an island within a shallow lake



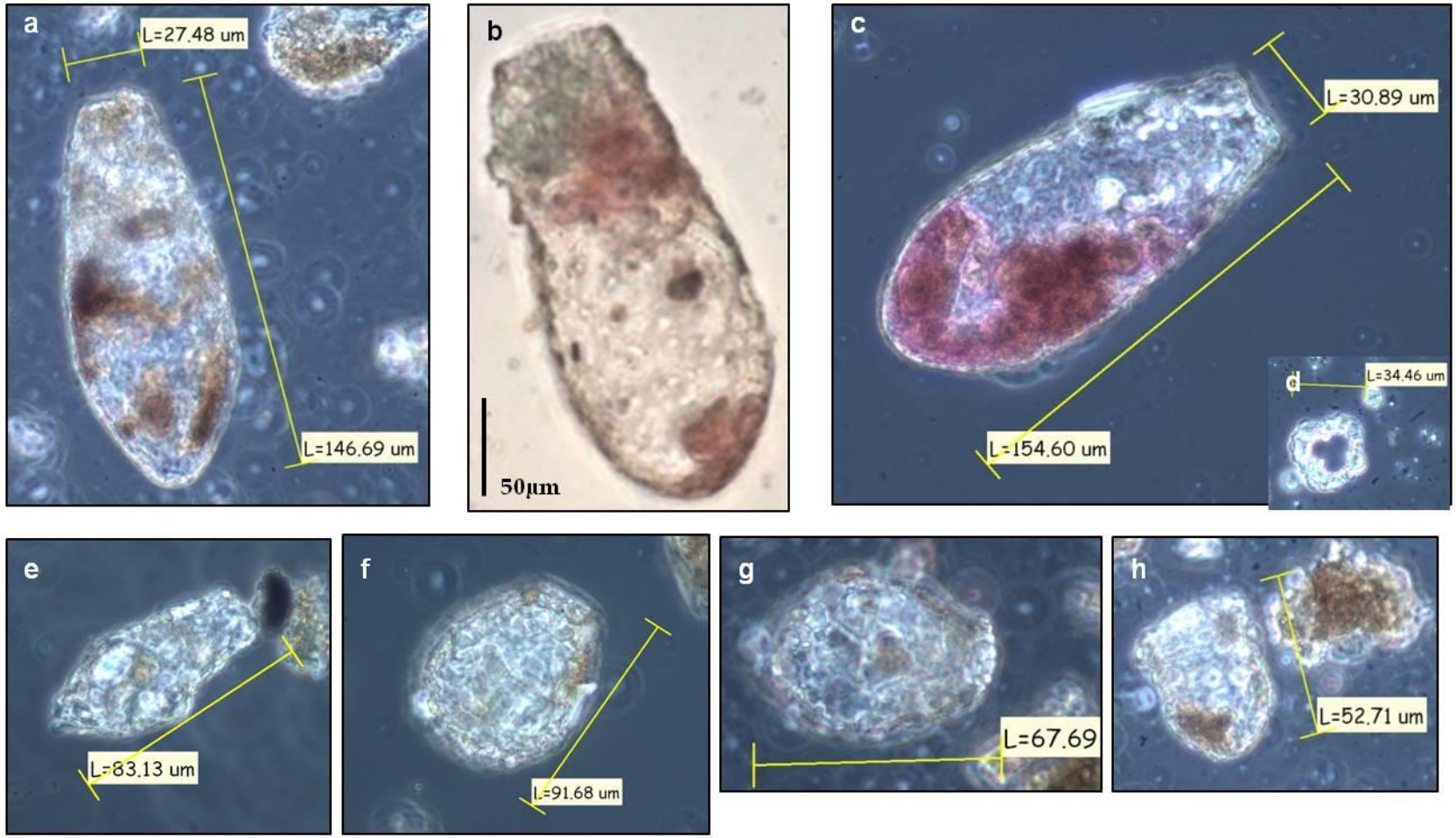
Photomicrographs of some testate amoebae in aquatic microhabitats: **Figure 4a.** *Plagiopyxis callida* in sediment associated with *Crassula helmsii*, **b**. *Centropyxis aculeata* in End Lake sediment, **c.** *Centropyxis* spp. in sediment of a mixed vegetation community, **d**. *Arcella vulgaris* in End Lake sediment associated with mixed vegetation community, **e**. *Cyclopyxis kahli*, **f**. *Centropyxis platystoma* in Twin Lake sediment associated with *Menyanthes trifoliata*, **g**. *Paulinella chromatophora* in End Lake sediment associated with *Menyanthes trifoliata*, **h.** *Cryptodifflugia oviformis*, **i**. *Euglypha cristata* Mere End sediment, **j**. *Arcella hemispherica*, **k**. *Arcella* spp, **l**. *Arcella bathystoma* in ditch sediment



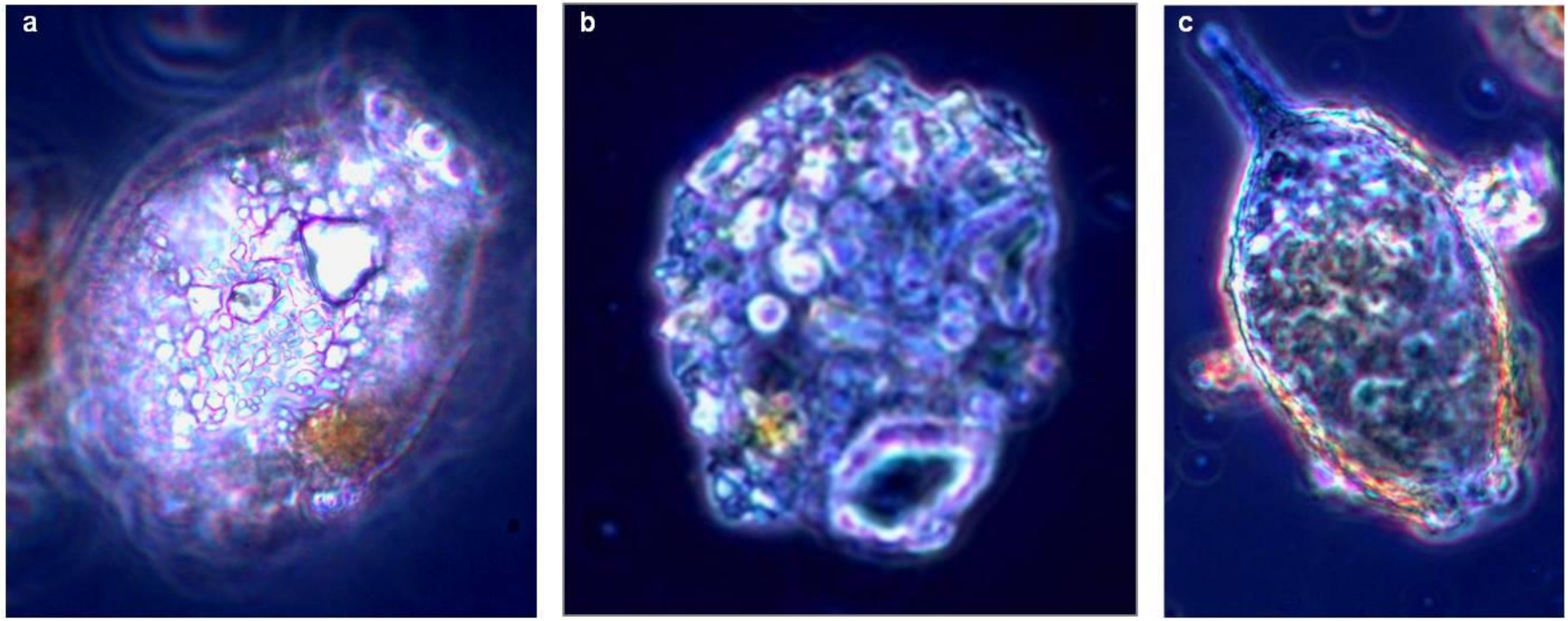
Photomicrographs of some large lobose testate amoebae in aquatic microhabitats: Figure 5a. *Difflugia* cf *urceolata* with blunt spines in Mere End detritus, **b.** *Difflugia* cf *acuminata* found in End Lake detritus beneath *Crassula helmsii*



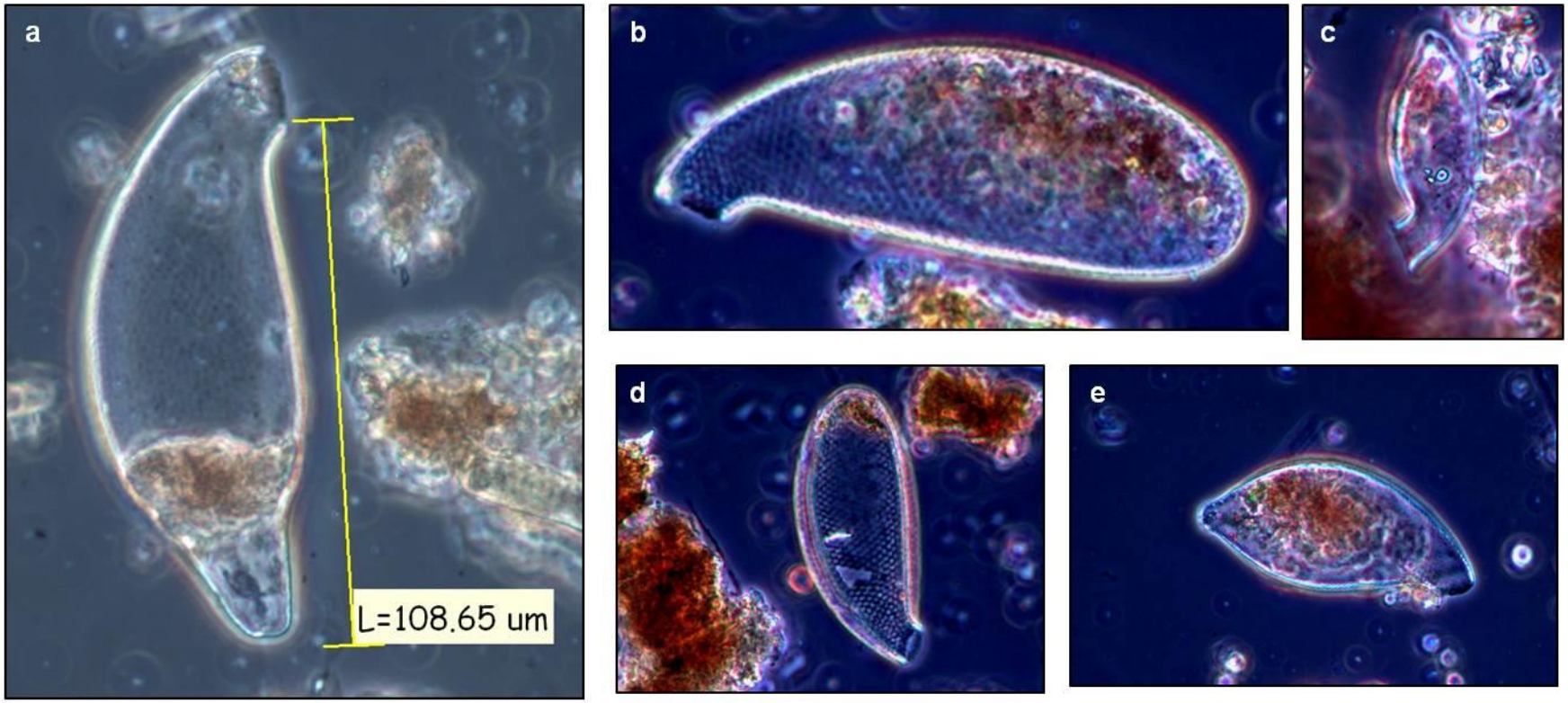
Photomicrographs of some large, lobose aquatic testate amoebae, Figure 6a. *Lesquereusia* cf *modesta*, **b.** *Pontigulasia* cf *compressa* found in Twin Lake detritus



Photomicrographs of some lobose aquatic testate amoebae, Figure 7a. *Difflugia lanceolata* Twin Lake detritus, **b**. *Difflugia oblonga* type End Lake mixed vegetation detritus, **c.** Stained *Difflugia lanceolata* End Lake detritus, **d**. *Difflugia* cf *gramen* aperture Twin Lake detritus, **e.** *Difflugia* morphotype Twin Lake detritus, **f.** *Difflugia* cf *lithophila* Mere End detritus, **g.** *Difflugia lucida* type sensu Charman et al, **h.** *Difflugia pristis* type sensu Charman et al



Photomicrographs of some aquatic lobose testate amoebae, Figure 8a. *Cucurbitella* morphotype Mere End detritus, **b**. *Cucurbitella* morphotype Mere End detritus, **c**. *Difflugia* cf *proteiformis* Mere End detritus,



Photomicrographs of some retort shaped testate amoebae found in Mere End lake sediment, Figure 9a. *Cyphoderia ampulla virtae*, **b.** *Cyphoderia ampulla*, **c.** *Campascus minutus* with disc shaped collar, **d.** *Cyphoderia ampulla*, **e.** *Cyphoderia* morphotype cf *trochus*

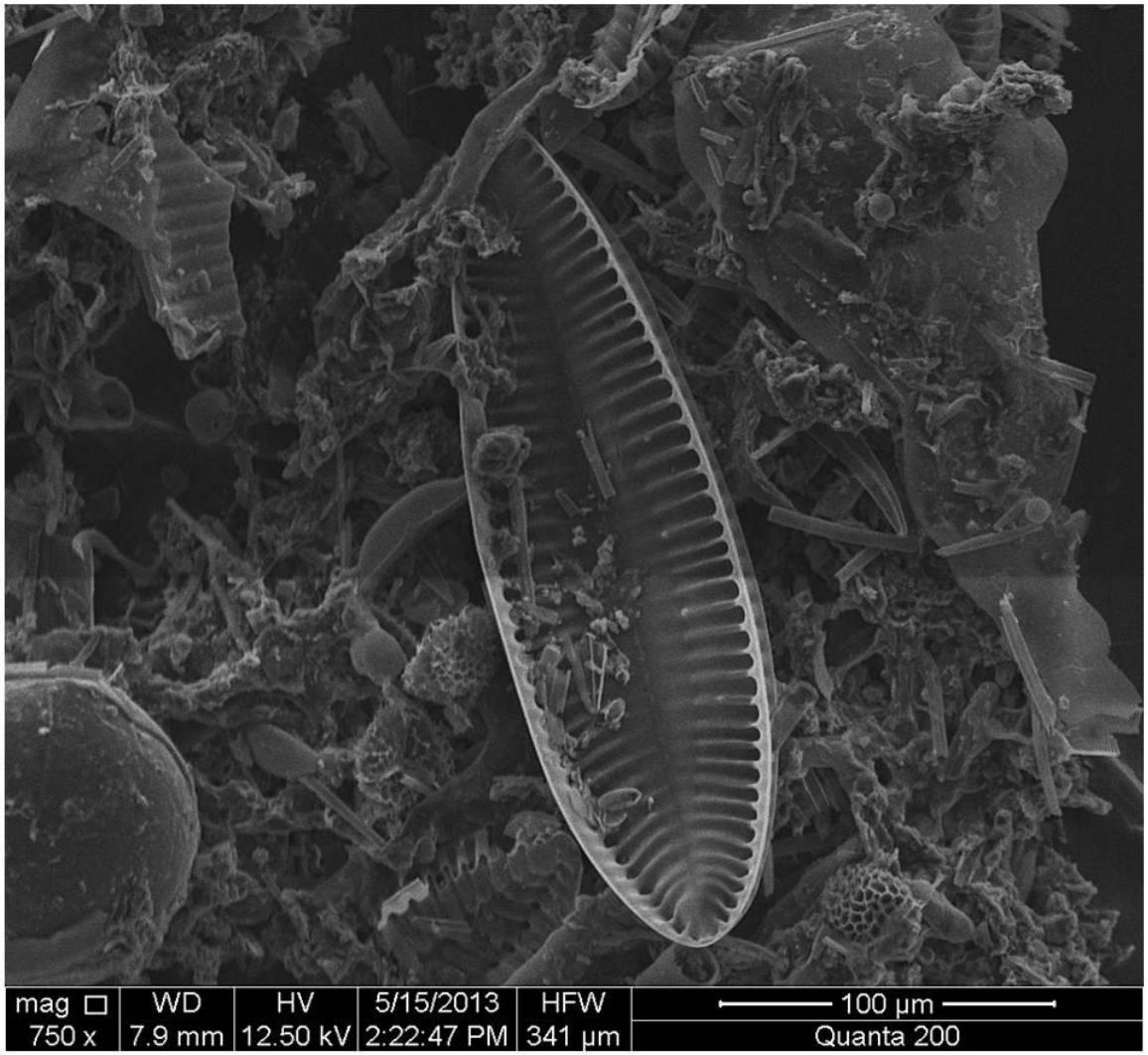


Figure 10. SEM image of *Surirella splendida* found in the anoxic sediment beneath the Scrape. Decaying frustules and possibly fungi hyphae? in the background – part of the ecosystem!

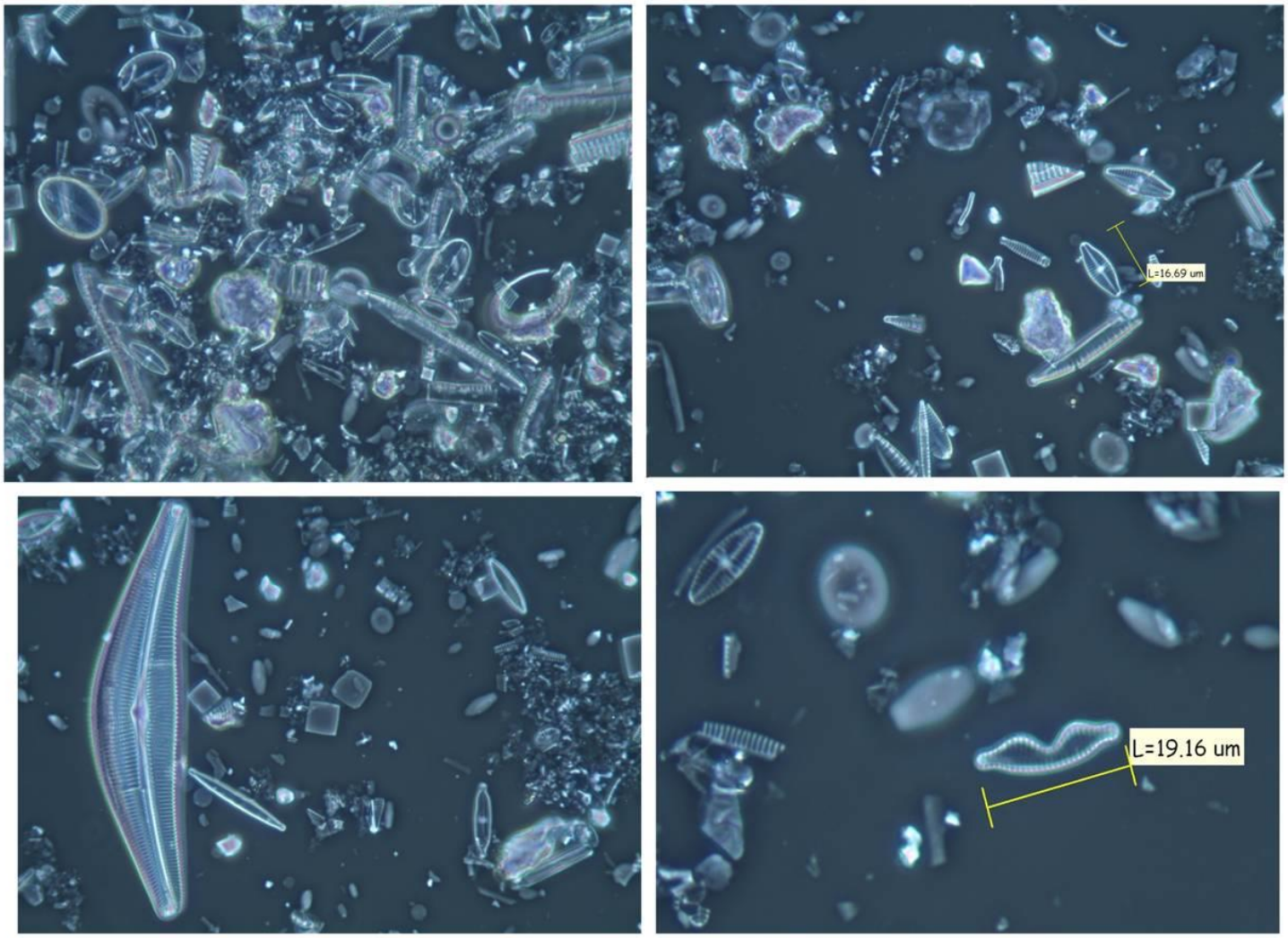


Figure 11. Diatoms in soil organic matter beneath a Bryophyte community on an island in a shallow lake.. An ecological hotspot

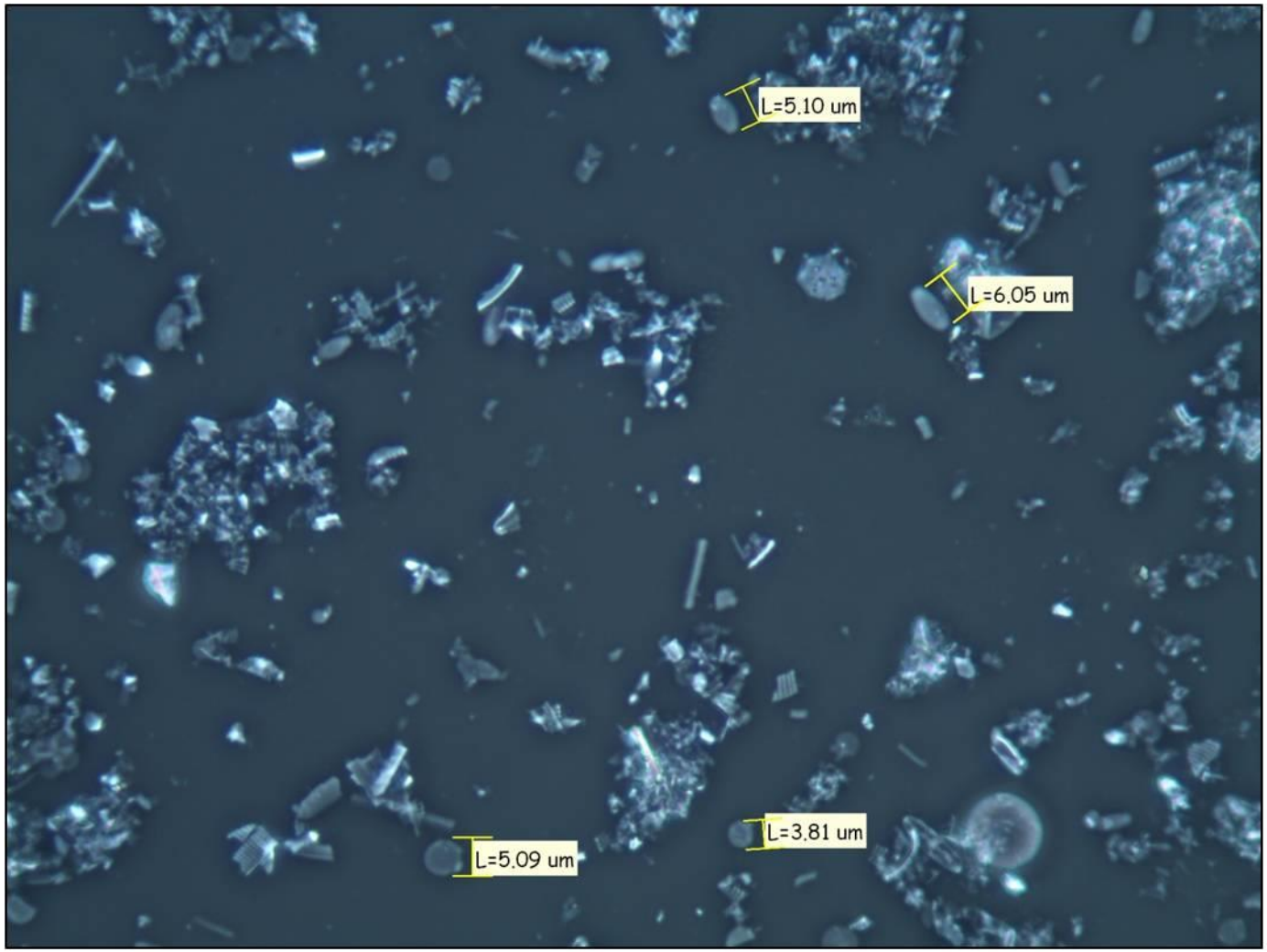


Figure 12. The smallness of soil diatoms could be an adaptation to the soil microenvironment (Lund 1946)

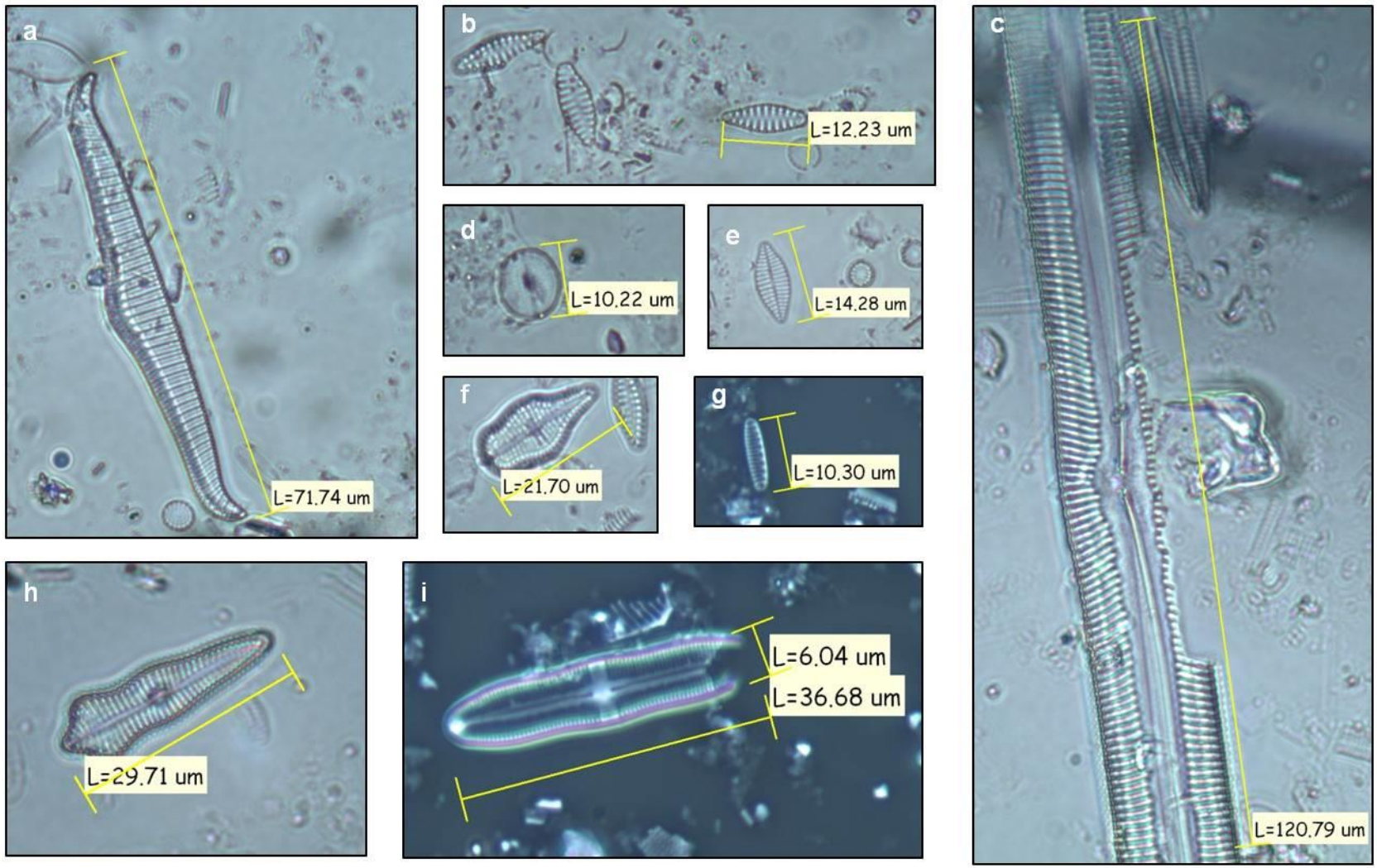
Figures 13 a – f. Diatoms in soil (left to right): *Cocconeis placentula* found in soils in an area cleared of *Rhododendron ponticum*, *Cocconeis* cf *pediculus* in soils beneath Bryophyte community, *Achnanthes* morph cf *thermalis*, *Navicula cinca* with *Navicula seminulum* in background, *Pinnularia viridis*, *Stauroneis anceps*

Figures 14 a - f. Diatoms in soil (left to right): *Pinnularia borealis* and *Stauroneis muriella* found in sandy soil beneath early successional stands of *Betula pendula*, *Gomphonema* and *Pinnularia* morphotypes in grassland soils, *Hantzshia amphioxys* in grassland litter, *Pinnularia viridis* in grassland soil

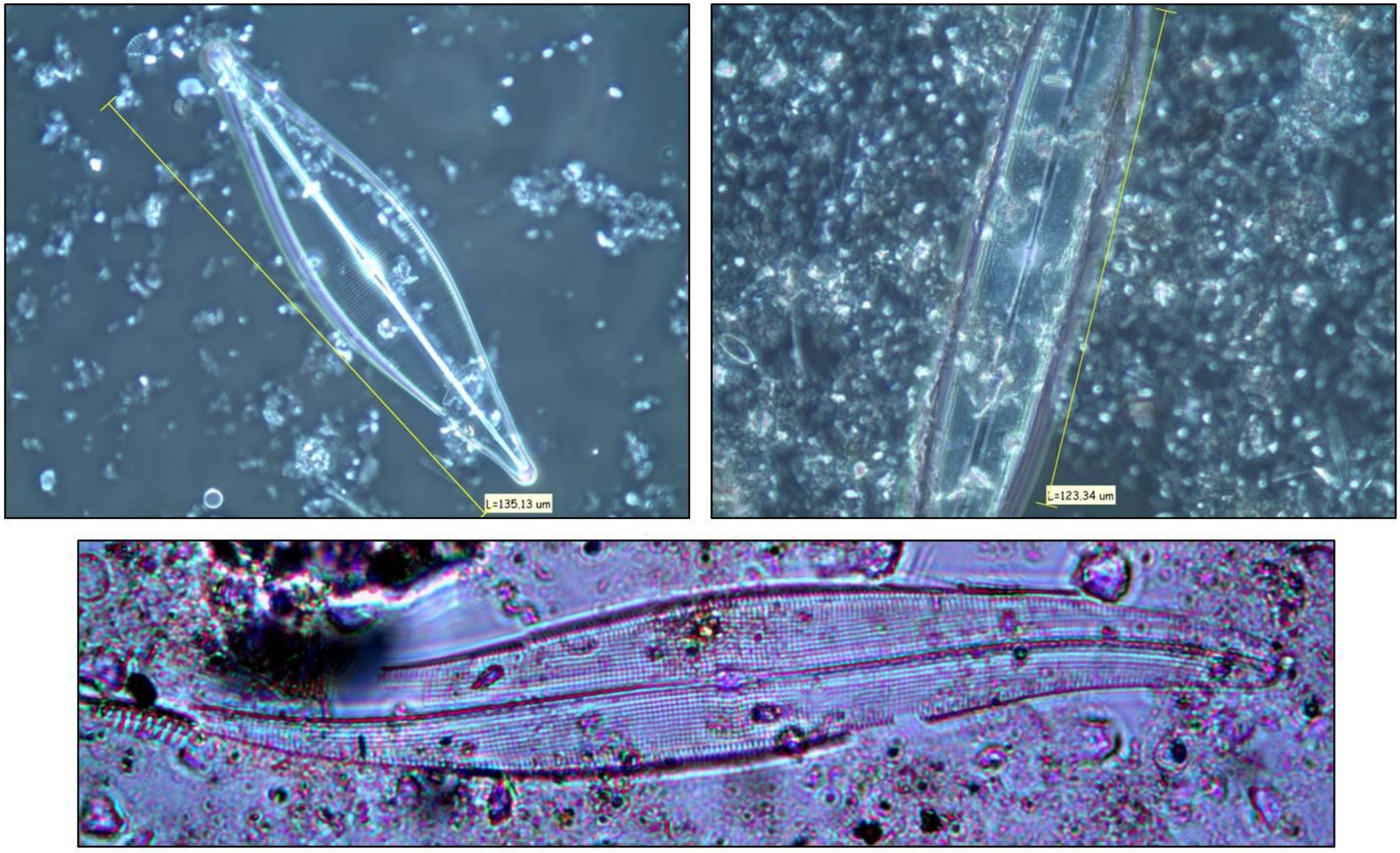
Figures 15 a – f Diatoms in drainage ditches (left to right): *Cymatopleura solea, Fragilaria capucina, Stauroneis javanica, Gomphonema truncatum, Navicula cryptocephala, Navicula slesvivensis*

Figures 16 a - f Diatoms in drainage ditches (left to right): *Surirella ovalis, Surirella brebissonii, Pinnularia microstauron, Achnanthes oblongella, Surirella amphioxys, Meridion circulare*

Figures 17 a - g Diatoms in End Lake: **a**. *Cymbella helmckei* found in sediment associated with *Typha latifolia* and *Iris pseudacorus*, **b**. *Eunotia bilunaris*, **c.** *Diploneis elliptica*, **d**. *Amphora pediculus*, **e**. *Cymbella silesiaca*, **f**. *Cymbella cistula*, **g.** *Rhopalodia gibba*, **h**. *Eunotia implicata*, **g.** *Cymbella cymbiformis*



Figures 18 a – i. Diatoms in Mere End Lake: **a**. *Rhopalodia gibba*, **b**. *Fragilaria pinnata var pinata*, **c**. *Pinnularia esox*, **d**. *Navicula* cf *pseudoscutiformis*, **e**. *Achnanthes deliculata*, **f**. *Gomphonema truncatum*, **g**. *Nitschia inconspicua* in *Crassula* sediment, **h.** *Gomphonema acuminatum*, **i**. *Caloneis* cf *silicula*



Figures 19 a - c Diatoms in the Scrape: **a.** *Navicula cuspidata*, **b.** *Neidium* spp, **c.** *Gyrosigma acuminatum*

Figures 20 a - f Diatoms in the Scrape: **a**. *Epithemia sorex* (left), *Pinnularia lundii* (right), **b**. *Cymbella* spp (left), *Cymatopleura* spp (right), **c**. *Tabellaria floccuosa* (left), *Achanthes* spp (right), **d**. *Epithemia sorex*, **e**. *Gomphonema truncatum*, **f**. *Surirella splendida*

APPENDIX 3. Terrestrial taxon richness and diversity summary statistics

Table 1. Characteristics of the Testate and Diatom assemblages (mean values and standard deviations) in litter and soil associated with early successional *Betula pendula* woodland

	FOM		SOM	
	Testates	**Diatoms**	**Testates**	**Diatoms**
Number of taxa	15.00 ± 1.00	0.33 ± 0.577	10.67 ± 1.528	3.00 ± 1.732
Simpsons diversity index	0.802 ± 0.0867	-	0.777 ± 0.077	0.520 ± 0.193
Shannons diversity index	2.104 ± 0.320	-	1.807 ± 0.312	0.878 ± 0.484

Table 2. Characteristics of the Testate and Diatom assemblages (mean values and standard deviations) in litter and soil associated with mature *Pinus sylvestris* woodland

	FOM		SOM	
	Testates	**Diatoms**	**Testates**	**Diatoms**
Number of taxa	13.33 ± 2.309	-	12.67 ± 3.512	-
Simpsons diversity index	0.841 ± 0.046	-	0.860 ± 0.051	-
Shannons diversity index	2.149 ± 0.166	-	2.201 ± 0.332	-

Table 3. Characteristics of the Testate and Diatom assemblages (mean values and standard deviations) in litter and soil associated with *Poaceae*

	FOM		SOM	
	Testates	**Diatoms**	**Testates**	**Diatoms**
Number of taxa	10.67 ± 1.528	3.00 ± 3.464	6.00 ± 2.00	6.00 ± 2.00
Simpsons diversity index	0.797 ± 0.0364	0.271 ± 0.469	0.781 ± 0.055	0.778 ± 0.060
Shannons diversity index	1.873 ± 0.135	0.598 ± 1.036	1.627 ± 0.278	1.621 ± 0.310

Table 4. Characteristics of the Testate and Diatom assemblages (mean values and standard deviations) in soil associated with *Rhododendron ponticum*and the effects of management

	Dominated by *R.ponticum*		**Area cleared of *R.ponticum***	
	Testates	**Diatoms**	**Testates**	**Diatoms**
Number of taxa	19.67 ± 0.577	-	14.33 ± 2.887	2.33 ± 1.528
Simpsons diversity index	0.923 ± 0.004	-	0.883 ± 0.009	0.417 ± 0.382
Shannons diversity index	2.719 ± 0.042	-	2.338 ± 0.137	0.693 ± 0.693

Table 5a. Characteristics of the Testate assemblages (mean values and standard deviations) in litter and soil under different stages of vegetation succession on an island in a shallow lake

	Open vegetation		**Semi-open vegetation**		**Thick shrub**	
	FOM	**SOM**	**FOM**	**SOM**	**FOM**	**SOM**
Number of taxa	14.00 ± 1.732	14.33 ± 2.517	18.33 ± 4.041	15.00 ± 1.00	13.67 ±7.506	19.00 ± 3.464
Simpsons diversity index	0.854 ± 0.010	0.854 ± 0.048	0.854 ± 0.038	0.787 ± 0.017	0.824 ± 0.040	0.720 ± 0.142
Shannons diversity index	2.170 ± 0.058	2.242 ± 0.231	2.288 ± 0.308	1.971 ± 0.122	2.030 ± 0.357	1.847 ± 0.567

Table 5b. Characteristics of the Diatom assemblages (mean values and standard deviations) in litter and soil under different stages of vegetation succession on an island in a shallow lake

	Open vegetation		**Semi-open vegetation**		**Thick shrub**	
	FOM	**SOM**	**FOM**	**SOM**	**FOM**	**SOM**
Number of taxa	14.00 ± 2.00	27.00 ± 3.606				
Simpsons diversity index	0.870 ± 0.032	0.882 ± 0.046				
Shannons diversity index	2.281 ± 0.233	2.667 ± 0.294				

APPENDIX 4. Aquatic taxon richness and diversity summary statistics

Table 1a. Characteristics of the Testate assemblages (mean values and standard deviations) at Mere End lake

	Open water	***Phragmites australis***	***Menyanthes trifoliata***	***Crassula helmsii***
Number of taxa	10.78 ± 2.438	9.33 ± 0.577	6.00 ± 2.00	5.67 ± 0.577
Simpsons diversity index	0.573 ± 0.184	0.794 ± 0.049	0.751 ± 0.057	0.716 ± 0.051
Shannons diversity index	1.397 ± 0.475	1.876 ± 0.128	1.531 ± 0.269	1.430 ± 0.116

Table 1b. Characteristics of the Diatom assemblages (mean values and standard deviations) at Mere End lake

	Open water	***Phragmites australis***	***Menyanthes trifoliata***	***Crassula helmsii***
Number of taxa	25.00 ± 4.062	10.33 ± 0.577	20.00 ± 8.00	20.00 ± 4.00
Simpsons diversity index	0.837 ± 0.049	0.758 ± 0.073	0.841 ± 0.025	0.819 ± 0.006
Shannons diversity index	2.275 ± 0.241	1.743 ± 0.266	2.261 ± 0.223	2.121 ± 0.036

Table 2a. Characteristics of the Testate assemblages (mean values and standard deviations) at End lake

	Open water	**Mixed vegetation**	***Menyanthes trifoliata***	***Crassula helmsii***
Number of taxa	6.33 ± 1.155	5.67 ± 1.528	9	4.67 ± 1.155
Simpsons diversity index	0.269 ± 0.023	0.339 ± 0.176	0.1551	0.466 ± 0.182
Shannons diversity index	0.588 ± 0.078	0.770 ± 0.377	0.4466	0.911 ± 0.311

Table 2b. Characteristics of the Diatom assemblages (mean values and standard deviations) at End lake

	Open water	**Mixed vegetation**	***Menyanthes trifoliata***	***Crassula helmsii***
Number of taxa	26.33 ± 2.309	23.33 ± 1.155	24.33 ± 1.155	20.67 ± 0.333
Simpsons diversity index	0.804 ± 0.065	0.888 ± 0.013	0.893 ± 0.005	0.882 ± 0.006
Shannons diversity index	2.358 ± 0.262	2.599 ± 0.035	2.575 ± 0.046	2.414 ± 0.043

Table 3a. Characteristics of the Testate assemblages (mean values and standard deviations) at Twin Lake

	Open water	***Menyanthes trifoliata***
Number of taxa	11.33 ± 1.155	9.33 ± 2.517
Simpsons diversity index	0.814 ± 0.124	0.830 ± 0.061
Shannons diversity index	2.081 ± 0.346	1.995 ± 0.307

Table 3b. Characteristics of the Diatom assemblages (mean values and standard deviations) in Twin Lake

	Open water	***Menyanthes trifoliata***	***Crassula helmsii***
Number of taxa	29.33 ± 5.033	24.00 ±5.292	21.00 ± 1.00
Simpsons diversity index	0.881 ± 0.030	0.859 ±0.048	0.819 ± 0.010
Shannons diversity index	2.597 ± 0.177	2.360 ± 0.290	2.167 ± 0.077

Table 4a. Characteristics of the Testate assemblages (mean values and standard deviations) in the Scrape

	Open water	***Menyanthes trifoliata***	***Phragmites australis***
Number of taxa	9.44 ± 3.245	4.67 ± 0.577	11.33 ± 2.517
Simpsons diversity index	0.628 ± 0.176	0.097 ± 0.039	0.794 ± 0.038
Shannons diversity index	1.416 ± 0.440	0.255 ± 0.090	1.836 ± 0.131

Table 4b. Characteristics of the Diatom assemblages (mean values and standard deviations) in the Scrape

	Open water	***Menyanthes trifoliata***	***Phragmites australis***
Number of taxa	25.33 ± 1.528	23.67 ± 5.508	27.00 ± 4.00
Simpsons diversity index	0.836 ± 0.051	0.798 ± 0.039	0.821 ± 0.041
Shannons diversity index	2.398 ± 0.205	2.070 ± 0.253	2.43 ± 0.235

Table 5. Characteristics of the Testate and Diatom assemblages (mean values and standard deviations) in the drainage ditch

	Testate amoebae	**Diatoms**
Number of taxa	11.33 ± 2.082	22.67 ± 2.887
Simpsons diversity index	0.842 ± 0.022	0.923 ± 0.007
Shannons diversity index	2.080 ± 0.158	2.776 ± 0.076

APPENDIX 5

Table 1. Terrestrial testate genera relative abundance tables

	B FOM	B SOM	CW FOM	CW SOM	G FOM	G SOM	RH C SOM	RH SOM	IS 1 FOM	IS 1 SOM	IS 2 FOM	IS 2 SOM	IS 3 FOM	IS 3 SOM	∑	Rel Ab (%)
	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3		
Unknown			10	22					89	37	56	4	3		221	3.87
Arcella	3	1			11			2	13		6	10	1	1	48	0.84
Assulina	35	1	24	25				49							134	2.34
Centropyxis	16		17	77	4		15	34	16	24	22	9	10	23	267	4.67
Corythion	144	7	9	28	8	1	20	62	12	30	17	20	12	17	387	6.77
Cryptodifflugia			37	3		10						1	2		53	0.93
Cyclopyxis	5	13		11	1	10	51	6	4		19	2	1	11	134	2.34
Difflugia						1			6						7	0.12
Euglypha	107	68	68	21	77	6	72	67	137	199	151	173	87	267	1500	26.24
Heleopera	5	10	48		9		11	20	25	17	19	10	8	19	201	3.52
Hyalosphenia			43	45			26	38				1		1	154	2.69
Nebela			22	19			2	36			3		1	6	89	1.56
Phryganella		2	19				58	12		7					98	1.71
Quadrulella									1	11	10	12	2	4	40	0.7
Sphenoderia						1				7	6	4	3	17	38	0.66
Tracheuglypha	1	7			27	3			5	7	12	9	13	7	91	1.59
Trigonopyxis			13	19			14	23			1			2	72	1.26
Trinema	67	282	173	107	158	18	120	108	159	136	161	228	75	390	2182	38.17
															5716	
Nematodes	9	3	0	0	29	0	0	0	28	3	95	92	101	26	386	
Un-id	17	18	29	28	14	5	14	24	23	23	20	12	11	13	251	

List of abbreviations:

B	Early successional *Betula pendula* woodland
CW	Coniferous woodland
G	Grassland
RH C	*Rhododendron* cleared area
RH	*Rhododendron ponticum*
IS 1	Island in a shallow lake open vegetation
IS 2	Island in a shallow lake semi-open vegetation
IS 3	Island in a shallow lake thick shrub
FOM	Fresh organic matter
SOM	Soil organic matter

APPENDIX 5

Table 2. Terrestrial diatom genera relative abundance table

	B FOM	B SOM	G FOM	G SOM	RH C FOM	RH C SOM	IS 1 FOM	IS 1 SOM	CW FOM	CW SOM	∑	Rel ab (%)
	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3		
Achnanthes					1		99	177			277	18.44
Amphora								1			1	0.07
Cocconeis					2	1	2	46			51	3.4
Cyclotella		1						2			3	0.2
Cymbella								1			1	0.07
Eunotia						2		7			9	0.6
Fragilaria	1					1	42	115			159	10.59
Gomphonema							1	2			3	0.2
Hantzshia		3	7	14		2	74	85			185	12.32
Navicula		3	6	25			86	348			468	31.16
Nitzschia		7		2			87	86			182	12.12
Pinnularia		18	8	31		1	18	19			95	6.32
Rhopalodia							2				2	0.13
Stauroneis		6	2	2			9	47			66	4.39
Un-id penate VV	1	4		2			114	69	1		191	
Un-id penate GV		4	3	1			23	70			101	
Un-id centric VV								2			2	
Un-id centric GV								1			1	
Broken penate	1	40	7	27	2	14	129	324			544	

APPENDIX 6

Table 1. Aquatic testate genera relative abundance table

	EL MV	EL CH	EL OW	EL MT	∑	ME MT	ME CH	ME PA	ME OW	∑	S OW	S PA	S MT	∑	TL OW	TL MT	∑	DD	∑	Rel ab (%)
	n=3	n=3	n=3	n=1)		n=3	n=3	n=3	n=9		n=9	n=3	n=3		n=3	n=3		n=3		
Unknown	122	84	319	114	639	13	21	44	642	720	576	97	341	1014	12	9	21	33	2427	52.22
Arcella	6	1	6	1	14	1		11	7	19	17			17	1	1	2	25	77	1.66
Campascus								1	22	23									23	0.49
Centropyxis	6	3	2	2	13	5	1	1	42	49	35	2	8	45	10	26	36	6	149	3.21
Corythion	1				1	14	12	11	70	107		44		44		1	1		153	3.29
Cryptodifflugua	9	16	42	2	69		10	55	18	83	51	79	2	81	3	20	23		256	5.51
Cucurbitella									6	6									6	0.13
Cyclopyxis				1	1	1				1	6		1	7				16	25	0.54
Cyphoderia									61	61				51		3	3		115	2.47
Difflugia	3	2	5		10	33	57	15	61	166	268		3	271	72	39	111	170	728	15.66
Euglypha	2				2				16	16	1	38		39		11	11		68	1.46
Heleopera		1			1	1	1			2		1		1					4	0.09
Hyalosphenia				1	1	2			1	3	13		4	17	3		3	6	30	0.65
Lesquereusia															1		1		1	0.02
Nebela			1		1										1		1		2	0.04
Paulinella				2	2										2		2		4	0.09
Plagiopyxis		1			1														1	0.02
Pontigulasia															1		1		1	0.02
Pseudodifflugia		2			2						132			132				11	145	3.12
Quadrulella									2	2									2	0.04
Sphenoderia									3	3					9		9		12	0.26
Tracheuglypha			1		1				1	1		25		25	1	2	3		30	0.65
Trinema			1		1		3	9	75	87	11	241		252		35	35	14	389	8.37
∑	149	110	377	123	759	70	105	147	1027	1349	1110	527	359	1996	116	147	263	281	**4648**	
Un-id	6	4	3	1		9	3	3	45		0	21	9		10	4				

List of abbreviations: EL - End Lake, ME – Mere End, S – Scrape, TL – Twin Lake, DD – drainage ditches, MV –mixed vegetation community, CH – *Crassula helmsii,* PA – *Phragmites australis*, OW – open water, MT – *Menyanthes trifoliata*

APPENDIX 6

Table 2. Aquatic diatom genera relative abundance table

	EL MV	EL CH	EL OW	EL MT	∑	ME MT	ME CH	ME PA	ME OW	∑	S OW	S PA	S MT	∑	TL OW	TL CH	TL MT	∑	DD	Rel ab	Rel ab (%)
	n=3	n=3	n=3	n=3		n=3	n=3	n=3	n=9		n=3	n=3	n=3		n=3	n=3	n=3		n=3		
Achnanthes	44	148	19	29	240	50	240	54	566	910	24	355	226	605	102	616	458	1176	90	3021	22.10
Amphora	64	160	28	49	301	12	1		3	16	2	21		23	16	21	21	58		398	2.91
Cocconeis	149	147	270	145	711	205	329	21	388	943	40	71	48	159	41	20	93	154	5	1972	14.43
Cymbella	109	34	77	40	260	6	19	41	94	160	73	20	8	101	12	3	6	21	16	558	4.08
Gomphonema	33	32	26	245	336	10	9	2	53	74	2	123	6	131	6	8	11	25	12	578	4.23
Eunotia	32	42	27	18	119	45	17	7	43	112		35	7	42	4		1	5	39	317	2.32
Epithemia	26	31	29	29	115	4	4	1	20	29	12	7	17	36	9	3	11	23		203	1.49
Rhopalodia	3	1	9	10	23	2	1		7	10	1	1	8	10	1	10	15	26		69	0.50
Fragilaria	79	248	56	324	707	206	65	7	1452	1730	372	70	555	997	243	274	208	725	61	4220	30.87
Navicula	29	77	56	82	244	96	104	1	355	556	69	110	81	260	42	53	31	126	46	1232	9.01
Nitschia	10	12	5	31	58	3	2	1	40	46	5	36	17	58	19	25	30	74	63	299	2.19
Pinnularia	5	1			6		3		12	15	1		8	9	3			3	23	56	0.41
Stauroneis		1		1	2	3		1	3	7		8		8	1			1	4	22	0.16
Diploneis			5		5					0	1	1		2						7	0.05
Gyrosigma						4	2	1	17	24	10		3	13	6		2	8		45	0.33
Surirella						1				1	1		11	12	3			3	80	96	0.70
Cymatopleura											8		9	17	1			1		18	0.13
Caloneis							1			1										1	0.00
Neidium									1	1	1		9	10	1			1	1	13	0.10
Meridion									1	1									9	10	0.07
Hantzschia												1		1					4	5	0.04
Cyclotella						14	15	1	23	53		7	1	8	193	40	124	357		418	3.06
Tabellaria			1		1	15	19	4	64	102	3	2	2	7					1	111	0.81
∑	583	934	608	1003	**3128**	676	831	142	3142	**4791**	625	868	1016	**2509**	703	1073	1011	**2787**	**454**	13669	
Un-id penate VV	28	42	40	26		40	59	3	204		52	99	62		110	93	193		90		
Un-id penate GV	69	78	69	99		40	8		333		23	62	34		140	18	21		60		
Un-id centric VV		1						2			26		1		8	8	5				
Un-id centric GV			41			8	2				51		2		176	8	11				
Broken centric															5						
Broken penate	417	223	411	144		203	216	64	537		230	243	106		157	39	50		270		

APPENDIX 7 - Table 1. Presence/absence data for testate amoebae in fresh and soil organic matter

	Island open vegetation *Bryophytes*	**Island Semi-open vegetation**	**Island Thick shrub**	***Betula pendula* woodland**	***Pinus sylvestris* woodland**	**Grassland *Poaceae***	***Rhododendron ponticum***	***R. ponticum* cleared**
Arcella spp.				*		*		
Arcella artocrea				*				
Arcella bathystoma							*	
Arcella catinus	*	*						
Arcella discoides	*	*	*			*		
Arcella vulgaris							*	*
Assulina muscorum				*	*		*	*
Assulina scandinavica				*	*			*
Assulina seminulum				*	*		*	*
Bullinaria indica								*
Centropyxis aculeata								*
Centropyxis aerophilla					*	*	*	*
Centropyxis cassis	*	*	*				*	
Centropyxis minuta		*	*					
Centropyxis platystoma	*	*	*	*	*		*	
Centropyxis sylvatica				*				
Corythion dubium	*	*	*	*	*		*	*
Corythion pulchellum	*	*	*	*	*	*	*	*
Corythion trinema type		*	*					*
Cyclopyxis arcelloides type		*	*	*	*	*	*	*
Cyclopyxis eurystoma				*		*	*	*
Cyclopyxis kahli	*	*		*			*	*
Cryptodifflugia oviformis		*	*		*	*		

Difflugia spp.						*		
Difflugia pulex	*							
Euglypha acanothora			*				*	*
Euglypha cf ciliata			*				*	*
Euglypha compressa	*	*	*	*		*	*	*
Euglypha cristata	*	*	*	*				
Euglypha laevis	*	*	*	*		*	*	*
Euglypha rotunda	*	*	*	*	*	*	*	*
Euglypha strigosa		*	*	*	*		*	*
Euglypha tuberculata type	*	*	*	*	*	*	*	*
Euglypha spp		*	*	*		*		
Heleopera petricola	*	*	*			*	*	
Heleopera rosea							*	
Heleopera sphagni	*	*	*	*	*		*	*
Heleopera sylvatica	*		*	*	*	*	*	*
Hyalosphenia ovalis								*
Hyalosphenia papillo							*	
Hyalosphenia subflava	*	*	*		*		*	*
Nebela bigibosa							*	
Nebela collaris							*	*
Nebela dentistoma							*	*
Nebela griseola							*	
Nebela millitaris			*				*	*
Nebela tincta		*	*		*		*	*
Nebela tubulata							*	
Placocysta spinosa								*
Phryganella acropodia	*			*	*		*	*
Quadrulella symmetrica	*	*	*					

Sphenoderia fissirostris	*	*	*			*	*	*
Sphenoderia lenta							*	*
Tracheuglypha dentata	*	*	*	*		*	*	*
Trigonopyxis arcula		*	*		*		*	*
Trinema complanatum		*	*	*	*		*	*
Trinema lineare	*	*	*	*	*	*	*	*
Trinema enchelys	*	*	*	*	*	*	*	*

Appendix 7 - Table 2. Presence/absence data for diatoms in fresh and soil organic matter

	Island open vegetation ***Bryophytes***	***Betula pendula*** **woodland**	***Pinus sylvestris*** **woodland**	**Grassland** ***Poaceae***	***Rhododendron ponticum***	***R. ponticum*** **cleared**
Achnanthes spp.	*					
Achanthes lanceolata	*					
Achnanthes minutissima var inconspicua	*					
Achnanthes minutissima var minutissima	*					*
Achnanthes cf. thermalis	*					
Amphora cf montana	*					
Cocconeis pediculus	*					
Cocconeis placentula var lineata	*					*
Cocconeis placentula var placentula	*					
Cyclotella spp.	*					
Cyclotella radiosa		*				
Cymbella proxima	*					
Eunotia spp						*
Eunotia implicata	*					
Eunotia paludosa	*					
Fragilaria spp	*					
Fragilaria brevistriata	*					
Fragilaria capucina	*	*				*
Fragilaria construens	*					
Fragilaria elliptica	*					
Fragilaria parasitica var parasitica	*					
Fragilaria pinnata var pinata	*					

Gomphonema spp	*						
Gomphonema olivaceum	*						
Gomphonema parvullum	*						
Hantzschia spp	*						
Hantzschia amphioxus	*				*		*
Hantzschia virgata	*	*					
Navicula spp.	*						
Navicula capitata	*						
Navicula cincta	*						
Navicula cf gibbula					*		
Navicula cf halophila	*						
Navicula ignota					*		
Navicula cf laevissima		*					
Navicula minima	*				*		
Navicula seminulum	*						
Navicula cf tenelloides	*						
Nitzschia dissipata var media	*						
Nitzschia inconspicua	*						
Nitzschia cf palea					*		
Nitzschia spp.	*	*					
Pinnularia spp.	*				*		
Pinnularia appendicula (sylvatica)	*						
Pinnularia borealis	*	*					
Pinnularia microstauron					*		
Pinnularia subrostrata							*
Pinnularia viridis	*				*		
Rhopalodia gibba cf var parallela	*						
Stauroneis spp.	*						

Stauroneis anceps	*					
Stauroneis kriegerii	*					
Stauroneis muriella		*				
Stauroneis cf truncata				*		

Appendix 8 - Table 1. Presence/absence data for testate amoebae in aquatic microhabitats

	Mere End Lake				End Lake				The Scrape			Twin Lake		Drainage ditch
	OW	PA	MT	CH	OW	MV	MT	CH	OW	PA	MT	OW	MT	
Arcella spp.	*		*		*	*			*					*
Arcella bathystoma														*
Arcella catinus		*												
Arcella discoides	*	*			*	*		*	*				*	
Arcella cf hemispherica						*						*		
Arcella vulgaris	*				*	*	*							
Campascus minutus	*	*												
Centropyxis aculeata	*	*	*	*	*	*	*	*	*	*	*	*	*	
Centropyxis aerophilla	*													
Centropyxis cassis	*								*				*	
Centropyxis constricta	*								*					*
Centropyxis discoides	*								*					
Centropyxis ecornis						*	*	*	*		*			
Centropyxis minuta									*			*		
Centropyxis platystoma	*								*				*	*
Corythion dubium		*	*	*						*				
Corythion pulchellum	*									*				
Corythion trinema type	*					*				*			*	
Cyphoderia ampulla	*												*	
Cyphoderia cf ampulla virtae	*													
Cyclopyxis arcelloides type			*											
Cyclopyxis eurystoma														*
Cyclopyxis kahli							*		*		*			*

Cryptodifflugia oviformis	*	*		*	*	*	*	*	*	*	*	*	*		
Cucurtibella morph	*														
Difflugia spp.	*	*		*		*		*	*		*	*	*		*
Difflugia cg gramen												*			
Difflugia lanceolata		*			*				*			*			
Difflugia cf lithophila	*														
Difflugia lucida type	*	*							*			*			*
Difflugia oblonga type	*	*	*	*					*		*				*
Difflugia pristis type round neck			*	*					*			*			*
Difflugia pristis type wide neck	*											*	*		
Difflugia cf proteiformis	*								*						
Difflugia pulex	*		*	*				*	*			*	*		*
Difflugia unknown A												*			
Difflugia unknown B												*			
Difflugia cf urceolata	*					*									
Euglypha cristata	*														
Euglypha laevis										*					
Euglypha rotunda										*			*		
Euglypha strigosa									*	*					
Euglypha tuberculata type	*					*				*					
Euglypha spp										*					
Heleopera rosea			*												
Heleopera sphagni				*				*		*					
Hyalosphenia subflava	*		*				*		*		*	*			*
Lesquereusia cf modesta												*			
Nebela spp.					*							*			

Paulinella chromatophora							*					*		
Plagiopyxis callida								*						
Pontigulasia cf compressa												*		
Pseudodifflugia gracilis								*	*					*
Quadrulella symmetrica	*													
Retort shaped testate A	*													
Sphenoderia lenta	*											*		
Sphenoderia cf macrolepis	*													
Tracheuglypha dentata	*				*					*		*	*	
Trinema lineare	*	*		*					*	*			*	
Trinema enchelys	*	*			*				*	*			*	*

Appendix 8 - Table 2. Presence/absence data for diatoms in aquatic microhabitats

	Mere End Lake				End Lake				The Scrape			Twin Lake			Drainage ditch
	OW	PA	MT	CH	OW	MV	MT	CH	OW	PA	MT	OW	MT	CH	
Achanthes deliculata	*							*				*	*	*	
Achnanthes cf exigua						*									
Achnanthes cf grana												*	*	*	
Achanthes cf helvetica	*														
Achnanthes laevis					*				*		*				*
Achanthes lanceolata	*								*	*		*	*	*	*
Achnanthes minutissima var inconspicua	*		*	*	*	*				*					
Achnanthes minutissima var minutissima	*	*	*	*	*	*	*	*	*	*	*	*	*		*
Achnathes cf oblongella												*			*
Amphora libyca	*		*		*	*							*		
Amphora ovalis	*			*			*		*			*			
Amphora pediculus			*		*	*	*	*		*		*	*	*	
Caloneis spp				*											
Cocconeis pediculus					*							*			
Cocconeis placentula var euglypta				*		*			*			*			
Cocconeis placentula var lineata	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cocconeis placentula var placentula		*			*				*						
Cyclotella spp.		*													
Cyclotella cf meneghiniana			*								*	*	*	*	
Cyclotella radiosa	*		*	*						*		*	*	*	
Cymatopleura solea									*		*	*			
Cymbella spp.			*	*	*	*			*						
Cymbella amphicephala	*								*		*		*		

Cymbella cistula	*			*	*					*		*					
Cymbella cymbiformis	*			*	*	*	*	*									
Cymbella cf helmckei						*											
Cymbella microcephala	*	*	*	*	*	*	*	*	*	*	*						*
Cymbella minuta					*				*								
Cymbella naviculiformis												*					*
Cymbella cf prostrata											*						
Cymbella silesiaca	*		*	*	*	*	*					*	*	*			
Cymbella cf solea												*					
Diploneis spp									*								
Diploneis elliptica					*												
Diploneis interupta										*							
Epithemia adnata	*	*	*	*	*	*	*	*	*	*	*	*	*	*			
Epithemia argus									*								
Epithemia cf smithii						*											
Epithemia sorex	*				*		*	*	*	*	*	*	*				
Epithemia turgida var granulata						*											
Eunotia bilunaris	*		*		*	*											*
Eunotia exigua								*									*
Eunotia formica	*					*	*										
Eunotia implicata	*	*	*	*	*	*	*	*		*	*						
Eunotia intermedia	*		*	*	*	*	*	*		*	*	*	*				*
Fragilaria spp			*		*		*		*			*	*	*			
Fragilaria brevistriata	*				*	*	*	*	*	*	*	*	*	*			
Fragilaria capucina	*	*	*		*	*	*	*	*	*	*	*	*	*			*
Fragilaria capucina var mesolepta									*								

Fragilaria cf. capucina var amphicephala				*												
Fragilaria construens var venter	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
Fragilaria elliptica		*		*					*							
Fragilaria exigua				*												
Fragilaria parasitica var parasitica												*				
Fragilaria parasitica var subconstricta	*		*	*					*				*	*		*
Fragilaria pinnata var pinata	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*
Gomphonema spp					*	*	*	*		*	*	*	*	*		*
Gomphonema acuminatum	*			*	*					*						*
Gomphonema angustatum						*							*			
Gomphonema cf olivaceum									*							
Gomphonema parvullum	*	*	*	*	*	*	*	*		*		*		*		*
Gomphonema truncatum	*		*	*	*	*	*		*	*	*	*	*			
Gyrosigma acuminatum	*	*	*						*		*	*	*			
Hantzschia spp																*
Hantzschia virgata										*						
Meridion circulare var constrictum	*															*
Navicula spp.		*		*	*	*	*			*	*	*	*	*		*
Navicula capitata							*			*	*	*	*			*
Navicula cf clementis														*		*
Navicula cincta			*		*	*			*	*	*	*	*	*		
Navicula construens										*	*	*	*			
Navicula cryptocephala	*				*	*	*	*	*	*	*			*		*
Navicula cuspidata	*								*			*				
Navicula minima	*		*		*		*	*	*	*						
Navicula cf pseudoscutiformis	*															

Navicula radiosa	*		*		*	*	*	*	*	*	*	*	*				
Navicula recens									*								
Navicula seminulum				*													
Navicula cf silevicensis																*	
Navicula trivialis									*								
Neidium spp.												*					
Neidium cf ampliatum	*								*		*					*	
Neidium cf iridis											*						
Nitzschia amphibia	*						*	*	*	*							
Nitzschia cf constricta																*	
Nitzschia filiformis																*	
Nitzschia inconspicua			*	*		*	*		*	*	*	*	*				
Nitzschia spp.		*			*	*	*	*	*	*	*	*	*	*		*	
Pinnularia spp.				*		*										*	
Pinnularia cf brandetti																*	
Pinnularia esox	*																
Pinnularia cf legumen	*																
Pinnularia lundii	*			*													
Pinnularia major											*						
Pinnularia microstauron	*										*					*	
Pinnularia cf *streptoraphe*									*								
Pinnularia subcapitata				*													
Pinnularia viridis	*							*				*				*	
Rhopalodia gibba	*		*	*	*	*	*	*		*	*	*	*	*			
Rhopalodia gibba var minuta									*								
Stauroneis spp.							*			*	*	*					

Stauroneis anceps	*											
Stauroneis cf javanica												*
Stauroneis kriegerii			*									
Stauroneis cf nobilis		*										
Stauroneis cf phoenicenteron	*					*						
Surirella spp			*									
Surirella cf amphioxys												*
Surirella cf bifrons										*		
Surirella brebissonii												*
Surirella ovalis												*
Surirella splendida							*		*			
Tabellaria flocculosa	*	*	*	*	*		*	*	*			*

MANUSCRIPT

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