Monitoring Peatland Damage and Restoration Using Testate Amoebae as Indicator Organisms

by

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Abstract

This thesis has examined the response of testate amoebae communities to restoration at three peatland sites in the UK. It builds upon the use of testate amoebae analysis as a palaeoenvironmental tool by exploring the hypothesis that testate amoebae respond to hydrological conditions in restored mires. Previous research has found that testate amoebae act as good proxies for hydrological condition of intact mires and past conditions but little has been done on their reaction to conditions at damaged sites in the UK.

The research aimed to further the understanding of testate amoebae ecology including seasonal variability of communities, a poorly understood area. The secondary aim was to assess the potential for using testate amoebae as biological indicators of peatland damage and restoration. These aims were achieved through studies at three sites covering a range of damage commonly affecting UK sites. The experiments entailed repeated monitoring of a ditch blocking experiment at Coom Rigg Moss which has been affected by peripheral forestry drains, a study of forest removal treatments at Flanders Moss and a study of rewetted cutover surfaces at Fenn's and Whixall Mosses.

The results were analysed using a variety of statistical and multivariate methods. Data on water table and moisture conditions were also analysed and the results of the two were compared. The results showed that testate amoebae communities responded to hydrological conditions but depth to water table was not always the primary factor affecting community composition.

The results of the research indicate that testate amoebae analysis does have potential as a tool for monitoring peatland restoration but further research is necessary to fully understand the factors affecting distributions.

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Acknowledgements

AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

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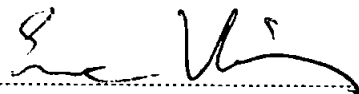
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CHAPTER ONE: Introduction

1.0 Introduction

Peatland environments have been the subject of exploitation for a variety of purposes over many centuries (Williams, 1990). The major threats can be divided into agriculture, forestry and peat extraction. In recent years, the focus on peatlands has shifted from environments to be exploited to important conservation resources (Joosten & Clark, 2002). Successive legislation has improved protection for peatlands, but damage is widespread and the total area of peat worldwide has diminished rapidly over the last century.

In the UK, peatland exploitation increased dramatically after the Second World War, as the nation strove towards self-sufficiency in food production. Increased land areas under both agricultural and timber production greatly affected the integrity of the peatland resource. During the 1980s changes in both forestry and agricultural policy had a positive effect on peatland conservation. The latest EU nature conservation legislation has improved the future of peatlands further by stating that any raised bogs designated as Special Areas of Conservation (SACs) must be actively laying down peat (O'Connell, 1999). This marks a change from simply conserving sites in their present condition, to a proactive stance encouraging restoration of damaged peatlands.

This project studies testate amoebae communities in degraded mires in an attempt to improve the understanding of their responses to modern ecological conditions and particularly, their response to restoration. This is set against a background of a preliminary assessment of the potential for monitoring restoration of UK peatlands using testate amoebae as indicator organisms. Testate amoebae are used extensively as indicators of hydrological conditions in palaeoenvironmental studies and work on their relationships

with vegetation and water chemistry has also been important in recent years (e.g. Balik, 1996; Charman & Warner, 1992; Mitchell *et al.*, 2000; Mitchell *et al.*, 2001; Tolonen, 1986; Warner, 1990; Warner & Charman, 1994). Their applicability to reconstructing past moisture conditions in Britain has been proven by detailed studies by Woodland (1996) and Hendon (1998). Modern studies of testate amoebae have been carried out primarily on intact mires as they are thought to provide the best proxies of past conditions. To further develop knowledge of their modern ecology, studies at damaged locations are required. Transfer of palaeoecological methods to modern ecology has some precedence, notably the use of diatoms as indicators of lake acidification (Battarbee 1984; Battarbee & Charles 1987). It is expected that testate amoebae will provide a useful indication of changing hydrological conditions and resultant impacts on the ecological condition of mires. This could potentially form the basis of a methodology which will help in monitoring restoration of damaged peatlands and a tool for environmental managers.

The sites studied for this PhD are all degraded peatlands that have recently undergone restoration. Coom Rigg Moss in Northumberland, northern England, has been damaged by peripheral drainage and forestry activity. Forest Enterprise has received European funding for damming and monitoring work at the site. Flanders Moss in Stirling, Scotland was planted with exotic conifers in the 1970s. The Forestry Commission have removed these trees and set up an experiment to determine the best method for restoring afforested mires. Fenn's and Whixall Mosses in Shropshire and Clywd on the English / Welsh border have been extensively degraded by peat extraction. Since management control was passed to English Nature and Countryside Council for Wales, large-scale commercial extraction has ceased and restoration has become a priority. These sites are used as representative

examples for the main types of damaged peatlands undergoing restoration management in the UK.

1.1 Aims of the PhD

The main aims of this PhD are:

1) to develop knowledge of testate amoebae ecology in degraded peatlands including responses to management activity, seasonal variability.

2) to consider the potential for the development of a new monitoring technique for peatland restoration using testate amoebae as indicators of change.

1.2 Hypotheses

Although this PhD largely follows the inductive scientific method, developing hypotheses rather than testing them specifically, the process also tests the hypothesis that testate amoebae respond to hydrological differences on damaged and restored mires.

1.3 Thesis Objectives

The six objectives of the PhD were:

1) to examine the response of testate amoebae to damming at a site with a damaged water table.

2) to examine the response of testate amoebae communities responses to forest removal techniques on a previously afforested mire.

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3) to study testate amoebae communities on a cutover mire surface which is undergoing restoration.

4) to develop further understanding of testate amoebae responses to hydrological conditions in degraded mires.

5) to develop an understanding of seasonal variability in testate amoebae communities.

6) to consider the potential for developing a simplified methodology based on testate amoebae analysis which will provide an indication of ecological conditions for practical use on peatland sites.

1.4 Structure of the Thesis

The thesis is composed of eight chapters. This chapter has introduced the thesis, outlined aims and objectives of the work and provided a brief description of the three experimental sites. Chapter 2 is a review of the literature relating to peatland environments and their management, the subject of environmental monitoring and finally, testate amoebae. Chapter 3 outlines methodological experiments carried out at Coom Rigg Moss and presents results and discussion of the implications of these experiments for the rest of the investigation. Chapters 4, 5 and 6 are the result chapters for Coom Rigg Moss, Flanders Moss, and Fenn's and Whixall Mosses respectively. Each chapter comprises an introduction to the site, outline of experimental work and methodologies, results, discussion and conclusions. Chapter 7 is a general discussion of results from all

experiments and implications for developing testate amoebae analysis as management tool. Chapter 8 is the conclusion chapter.

CHAPTER TWO: Literature Review

2.0 Introduction

This chapter includes a review of the literature on peatland environments and their management, experimental restoration of peatlands and ecological monitoring. There is also a review of literature relating to testate amoebae, their ecology and palaeoenvironmental applications of testate amoebae analysis. The literature is reviewed in relation to the research objectives of this PhD.

2.1 Peatlands

2.1.1 Introduction to Wetlands

Peatlands, for classification purposes, should be included in the wider ecosystem group of wetlands. The definition for a wetland, as agreed at the 1971 United Nations Convention on Wetlands of International Importance (normally referred to as the Ramsar Convention) is:

> **"Areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary with water that is static or flowing, fresh, brackish, or salt including areas of marine water, the depth of which at low tide does not exceed 10 metres."**
>
> (Mitsch & Gosselink, 1993: p. 24)

Included within this definition are peatlands. These are waterlogged environments where the soil is composed of decomposed and decomposing plant remains which led to the formation of peat. This study will concentrate on peatland ecosystems and in particular, ombrotrophic raised mires. Terminology in wetland science is an issue of some debate (Wheeler & Proctor, 2000). However, some terms are relatively simple to define and will be used throughout this piece of work. An ombrotrophic peatland is commonly referred to as a 'bog' and relies solely on precipitation for its water and nutrient input creating nutrient poor conditions and controlling the area within which these peatland types occur. These

contrast with minerotrophic peatlands usually called 'fens'. These are commonly fed by either groundwater or rivers in addition to precipitation and have higher nutrient levels than bogs. The distribution of fens is therefore wider than the bogs as the latter have more precise climatic requirements.

Since the agricultural and industrial revolutions, wetlands, including peatlands have been seen as wastelands that can be increased in value through drainage or agricultural improvement (Brooks and Stoneman, 1997a). This opinion has led to a change in human use of wetlands from sustainable harvesting of wetland products such as fish and reeds, to wholesale destruction or degradation. The Somerset Levels in south west England are an example of peatland which has been extensively altered in the past through peat removal and drainage to create improved pasture for cattle grazing and for arable land. There is now very little raised bog remaining, and the ecological nature of the area has been completely transformed. The wet meadows which have superseded the bog are also of conservation value, but these are being degraded by agricultural practice also (Kirkham, 1996). In the Flow Country, northern Scotland, peatlands have also been altered due to the lack of value attached to them. Tax incentives for forestry were offered by the UK government during the 1980s, which resulted in the afforestation of 67,000 hectares of blanket bog and the subsequent loss of conservation value (Everett, 1999). These tax incentives were removed in 1988 and conservation guidelines have been integrated into UK forestry policy which should prevent further planting on lowland bogs (RSPB & Plantlife, 1993).

As a result of drainage and improvement for agriculture, afforestation and peat extraction, only 6% of the original extent of Britain's raised bogs remain in a 'natural' state (RSPB & Plantlife, 1993). This amounts to 6,000 hectares of original raised bog habitat. The total

land area covered by peat in Britain and Ireland is about 2.5 million hectares or 8% of the land surface (Taylor, 1983). The area of blanket bog in the UK is estimated to be 1.3 million hectares, one tenth of the world's total. This gives an added responsibility to conserve them (Lindsay, 1987).

2.1.2 Peat Formation and Properties

Peat growth occurs mainly in the temperate boreal zones and particularly between latitudes 50-70° North (Proctor, 1995), due to the specific climatic requirements of growth. Peat growth tends to be accentuated in oceanic areas, where summer temperatures are lower and summer precipitation is higher. Estimates of precise climatic conditions vary from a minimum annual rainfall of 775mm and mean June/July temperatures of 17°C (Casparie, 1993), to between 800 and 900mm annual rainfall (O'Connell, 1999) and then to 1000mm, at least 160 rain days per year and mean summer temperatures of 15°C (Brooks and Stoneman, 1997a). Despite variation in figures for precipitation requirements, most authors agree that peat growth can only occur where precipitation exceeds evapotranspiration (Lindsay, 1995). Although the following comments apply to all ombrotrophic mires, this discussion will be mainly limited to the UK.

Peat accumulates in mires because environmental conditions and in particular waterlogging, impede the decay of plant material (Clymo, 1984a). It is formed by the semi-decomposed remains of the plants which grew on its surface (Clymo *et al.*, 1998). In northern hemisphere acid mires (see below), these are predominantly *Sphagnum* remains, although some vascular plants are also important components of peat (Wallén, 1986). Most peat systems have two distinct layers called the acrotelm (or upper layer) and catotelm (or lower layer) as illustrated in figure 2.1.
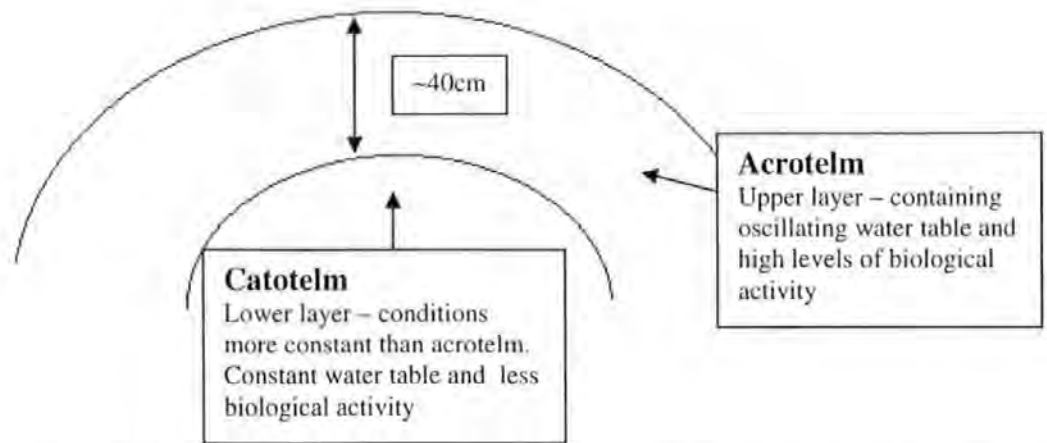
Figure 2.1: Pictorial representation of the peat layers of an ombrotrophic mire (descriptions from Ingram, 1978)

The acrotelm differs from the catotelm in that it has a variable water table and water content, and contains large amounts of peat-forming aerobic bacteria and micro-organisms (Ingram, 1978). The functions of the acrotelm are to support the growth of bog plants, to produce organic matter which then enters the catotelm and forms peat and to feed the catotelm with water (Wheeler and Shaw, 1995). Bragg (1995) referred to the acrotelm as a "header tank" for the catotelm. Where peat has been harvested for fuel or horticultural purposes, the acrotelm is often completely absent.

All mires are minerogenous in origin but they can change to ombrotrophic mires when the *Sphagnum* thickens so that groundwater is excluded (Nykänen *et al.*, 1997). As stated earlier, ombrotrophic peatlands rely solely on precipitation for water supply. When this supply is interrupted or reduced, ecological changes can occur. However bogs have their own methods of self-regulation and when precipitation falls below optimum levels, growth of *Sphagna*, and therefore peat, stops. This in turn reduces evaporation and the bog is able to ration available water resources (Casparie, 1993). This mechanism allows bogs to survive intact during dry periods. When the water table falls below a critical depth, e.g.

40cm below the peat surface for ombrotrophic mires, formation of the bog ceases (Ferland & Rochefort, 1997). The maintenance of a high water table is particularly important for mires, as peat will decompose if exposed to air (Rowell, 1988).

The chemistry of ombrotrophic bogs is important for many reasons. Hydrological isolation from the surrounding water table results in nutrient poor conditions, as the only nutrient source is rainwater. The poor nutrient status of bogs exerts a huge influence on the plants which grow on them, creating conditions of extreme nutrient stress. Plants that have adapted to growth under conditions of low nutrient supply therefore have a competitive advantage in these ecosystems (Moore & Bellamy, 1974).

The second defining element of the chemistry of a bog, which is very important for defining the habitat, is its acidity. The pH of an ombrotrophic bog generally lies between 3.2 and 4.0 (Clymo, 1984b). This is lower than the average pH of precipitation, (which is around 5.6) the only source of water to an ombrotrophic bog. Given that the pH of soil has a major bearing on nutrient availability (Parkinson, 1995); it could be considered to be the most important factor in bog chemistry. Figure 2 illustrates the relationship between pH, nutrient availability and activity of micro-organisms.
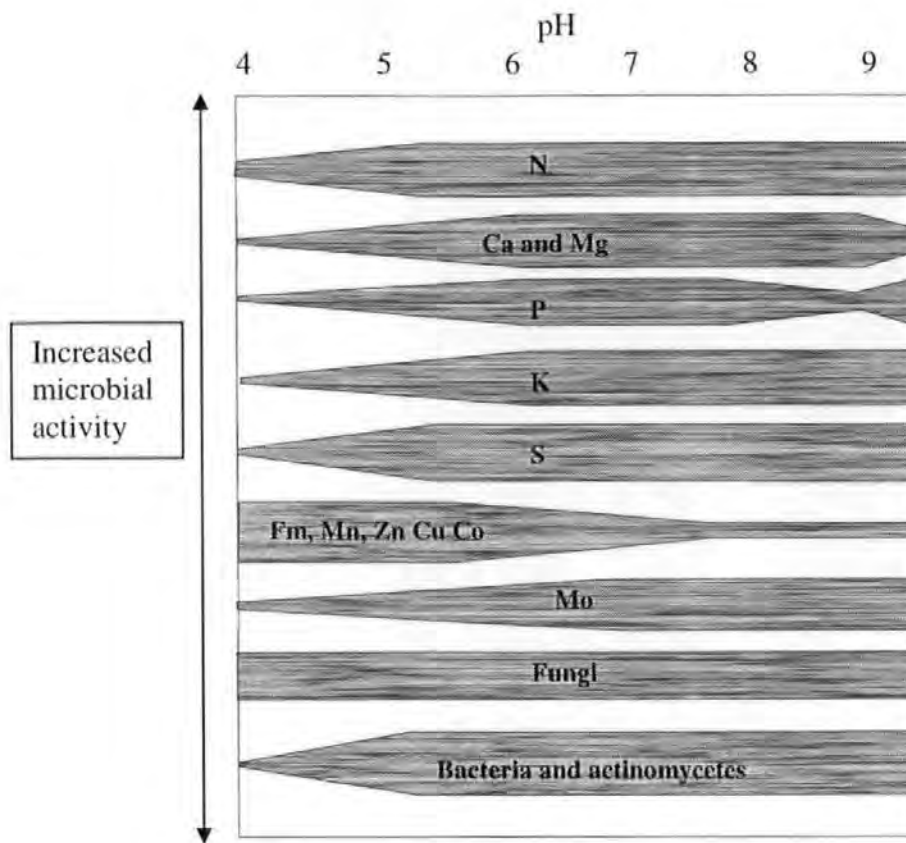
Figure 2.2: Relationship between soil pH, nutrient availability and microbial activity. (From Parkinson, 1995)

2.1.3 Flora

The vegetation of a bog is distinctive and unique to that particular environment. Vegetation patterns are influenced by the physical properties of the bog environment. The combination of acid soils, low nutrient availability, low temperatures and waterlogged conditions create an extremely stressed environment for plant growth. Most plants which are able to grow in the harsh bog environment are acidophilous (acid-loving) (Lindsay, 1995). Very few groups of plants are able to exploit these conditions. However, the most successful are the *Sphagnum* mosses. It has even been speculated that the acid conditions described above are created by *Sphagnum*, as part of its competitive strategy (van Breemen, 1995). This is reinforced by Andrus (1986) who states that *Sphagnum* is able to direct succession through acidification and paludification.

Sphagnum is able to exploit each part of the bog environment. The pattern of pools, lawns and hummocks characteristic of raised bogs provides, on a micro-scale, ecological niches for the various *Sphagnum* species (O'Connell, 1999). Hummock-forming species include *Sphagnum capillifolium* and *S. papillosum*, while the pools are colonised by *S. cuspidatum*, and species such as *S. recurvum* grow on the lawns (Watson, 1981).

Although *Sphagnum* spp. are usually the dominant species on the bogs, define the vegetation community (Hill, 1992), and are also largely responsible for peat formation, vascular plants account for a large proportion of bog flora. A limited number of species are able to tolerate the harsh environmental conditions on a bog, but these must also be able to cope with the upward growth habit of *Sphagnum* (Heathwaite *et al.*, 1993a). For other plants to survive the bog environment, they must have developed a mechanism for adapting to low nutrient supply, acid conditions, waterlogged soils and low temperatures.

Vascular plants tend to occur in large numbers where the surface of the bog has been disturbed or where water levels are low (Lindsay, 1995). Ericaceous species including heathers - *Calluna vulgaris* and *Erica tetralix*, crowberry *Empetrum nigrum* Bog Rosemary *Andromeda polifolia* and Cranberry *Vaccinium oxycoccus* have developed adaptations that allow them to survive on ombrotrophic bogs. They frequently colonise the drier hummocks and are able to withstand waterlogging because of their shallow rooting systems. They deal with the lack of nutrients by being evergreen and maximising the length of photosynthesis during the year (O'Connell, 1999). The sedge family is generally associated with wet conditions growing in a range of environments from wet meadows to salt marshes. This makes wet peatlands ideal environments for them to exploit. Sedges commonly occurring on bogs include cottongrasses *Eriophorum vaginatum* and *E. angustifolium*, Deergrass

Trichophorum cespitosum, White Beak-sedge *Rhynchospora alba*, Carnation sedge *Carex panicea* and Black Bog-Rush *Schoenus nigricans* (O'Connell, 1999). Another plant group which grows on bogs is the Lilaceae family which includes the bog-asphodel *Narthecium ossifragum*.

2.1.4 Fauna

Peatlands, in common with most wetlands support an important bird population. This is perhaps the most widely accessible element of the ecology of a bog. Amongst the birds feeding and often breeding in the peatlands of the Flow Country in Scotland are Arctic skuas *Stercorarius parasiticus*, greenshanks *Tringa nebularia*, dunlins *Calidris alpina* and golden plovers *Pluvialis apricaria* (Everett, 1999). Raptors using peatlands as hunting grounds include the hen harrier *Circus cyaneus* and merlin *Falco columarius* (Heathwaite *et al.*, 1993a). Bird populations are particularly threatened by changes in land use on peatlands (Whitfield, 1997) a study in Canada found that afforestation affected bird numbers because of changes in the structure of the vegetation (Lachance *et al.*, 2005). The impact on bird populations will amplified where planting of non native species, such as those grown for commercial forestry in the UK, takes place.

The invertebrate populations of peatlands are also of particular interest. Dragonflies are particularly successful at exploiting the complex mixture of aquatic and terrestrial environments provided by mires. Peatlands are home to some of the rarest of Britains' dragonfly fauna including *Coenagrion hastulatum* and *Somotochlora arctica*, two rare species which inhabit bogs in the Scottish highlands (Brooks, 1997). Beetles are also important components of the fauna of ombrotrophic mires with several rare species being restricted to peatlands (Heathwaite *et al.*, 1993a)

The microfauna of peatlands is also of interest. Very little is known of how microorganisms contribute to ecological processes within peatlands, however, although they are thought to have a major role in growth of peat. Taxa of particular conservation interest are only found in 'healthy' mires, the Tardigrades are one group of organisms which are particularly uncommon at damaged sites (Hingley, 1993).

2.2 Conservation of Peatlands?

2.2.1 Conservation

Peatlands until recently were seen as wasteland of no value unless they were improved for agriculture or harvested. They are now, slowly, being recognised as highly diverse ecosystems, which play an important role in global environmental systems and maintaining biodiversity.

Since the 1992 United Nations Conference on Environment and Development, often referred to as the Rio Earth Summit, biodiversity has become a popular issue. The reduction of biodiversity has been widely seen as a negative result of expansion of human impact on ecosystems. The peatlands of the UK are important centres of biodiversity, not just nationally, but on a global scale (Adams, 1996). Although they cover around 8% of the land surface in the UK and Ireland, the total area which is intact or undamaged is very low. The global extent of oceanic raised bogs, which are in a condition worthy of conservation, is 46,522 ha and 51% of this occurs in Ireland (O'Connell, 1999). Between 10 and 14% of the global total of blanket bog is found in the UK (Lindsay, 1987). According to Ratcliffe (1977), rarity should be a factor in assessing whether or not to conserve a particular piece of land. On these grounds alone, peatlands merit conservation.

The peatlands of the UK and Ireland are different to those of North America, Scandinavia and Russia. Whilst the basic composition is similar throughout these areas and peat is (almost) always formed by the decomposing remains of *Sphagnum*, there is ecological variability between them. The peatlands of Fennoscandia, the aapa mires (Lindsay, 1992), are unique to that area and it is therefore the responsibility of those countries to conserve their heritage. The blanket mire type present in Britain and Ireland has been recognised as important, as it develops specifically under highly oceanic climatic conditions and, as such, is probably unique (Gore, 1983). These nations have a global responsibility to conserve this peatland type. Although ombrogenous raised bogs are globally widespread, in Britain they are unusual, in common with the blanket mire, in being floristically and structurally oceanic (Lindsay, 1995).

In terms of genetic biodiversity, peatlands are extremely important. The harsh environmental conditions mean that plants living in bogs have to adapt significantly in order to survive. This adds to the genepool in Britain. The carnivorous sundew family (*Drosera spp.*) is one example of a plant which has specific adaptations enabling it to survive in bogs (Lindsay, 1995).

2.2.2 Carbon Cycling and Storage

Northern peatlands contain a significant store of carbon, with as much as 455 petagrams (Pg) estimated to have accumulated since the last glaciation (Gorham, 1991). This accounts for around 30% of the total carbon stored in soils (Melillo *et al.*, 1996) or 12 % of current human emissions (Moore, 2002). Carbon is accumulated as peat grows and the store of semi-decomposed material becomes a carbon store. Estimated accumulation rates are

around 0.076 Pg C / yr^{-1} (Clymo, 1984a). An estimate of the amount of carbon held in peatlands globally is 700 Pg, the same amount as is held in the earth's atmosphere (Pearce, 1994). As soil and vegetation processes are important in fluxes of greenhouse gases, any change in land use can affect emissions (Nykänen et al., 1997).

As well as being a sink of carbon, peatlands are also a source. They release methane (CH_4) into the atmosphere, as a result of the anaerobic breakdown of plant material in the catotelm. Emissions of CH_4 from boreal peatlands are estimated to be 19.5 x 10^{12} g CH_4 year^{-1} (Bubier, 1995). CH_4 is another gas which contributes to the greenhouse effect, although it is less important than CO_2. Studies have shown that a drop in water levels increases CH_4 emissions (Roulet et al., 1992; Bubier, 1995; Daulat & Clymo, 1998). Therefore any drying out of peatlands as a result of changing climate will lead to increases in methane emissions. Any rise in temperature will also increase the amount of CH_4 released into the atmosphere (Daulat & Clymo, 1998).

When mires are drained for agriculture or harvested, their carbon store is destroyed. If drainage lowers the average water table by 20 cm, annual carbon release increases by 100% (Silvola et al., 1996). Changes in water table between 0 and 30 cm below ground level are more significant than those between 30 and 60 cm (Nykänen et al., 1997). Globally, peat harvesting may be releasing 0.242-0.268 Pg Carbon Dioxide (CO_2) into the atmosphere each year (Immirzi & Maltby, 1992). As CO_2 is a major contributor to the greenhouse effect, this reinforces the argument for the conservation of peatlands.

The contemporary issue of global warming therefore provides further incentive to conserve peatlands. As peatlands are a large store of terrestrial carbon and their destruction through

drainage or harvesting releases the greenhouse gases CO_2 and CH_4, the need to conserve them is even more important. The precise impact of global warming on terrestrial ecosystems is unknown, and complex feedback mechanisms could trigger change in any direction (Woodwell *et al.,* 1998). Nevertheless, peatlands should be conserved as a precautionary measure.

2.2.3 Palaeoecology and Archaeology

Bogs have been described as 'witnesses to the past which rarely speak until questioned' (Joosten, 1995: p.381). This adds a further incentive for the conservation of peatlands: the valuable archive of both environmental change and human evolution contained within them. The waterlogged anaerobic conditions of peat are ideal for the preservation of organic material (Coles, 1990). A wealth of archaeological finds have been recovered from peatlands. Some of the best examples are from the Somerset Levels in southwest England. Prehistoric trackways such as the Sweet Track (dated to 3800 BC) have been discovered and have allowed archaeologists to gain insight into the lives of our ancestors (Coles & Coles 1986). Drainage and reclamation of peatlands destroys their stratigraphic record decreasing their value to archaeologists and paleoecologists (Meade, 1992).

The slow growth of peatlands (around 0.16-0.8mm yr^{-1} (Moore, 1990)) and incomplete decomposition of plant material make bogs ideal places for paleoecological study. The preservation of plant and animal remains ranging from pollen to beetles has allowed the reconstruction of environmental conditions at the time of deposition. This has provided insight into past climatic change, and has also been an important tool in the prediction of future changes. The loss of peatlands results in the destruction of this valuable resource and even if restoration of vegetation is possible, restoration of this scientific archive is

impossible. This also allows a more thorough understanding of past biodiversity under different climatic scenarios (Barber, 1993), of particular interest in the light of predicted climate change.

2.3 Exploitation of and Threats to Peatlands

2.3.1 Peat Extraction

Most mires in the British Isles have been affected by peat extraction at some time in the past. Peat is harvested for two major uses, as fuel and horticultural products. Extraction for fuel has a long history. On the Somerset Levels there is evidence for peat use during Roman times as fuel in salt production (Rippon, 1996). In Germany, there is also evidence that peat was used by the inhabitants of the river Weser over 2000 years ago (Göttlich *et al.*, 1993). Evidence of turbary (peat cutting) increases from the thirteenth century onwards because of a shortage of fuelwood supplies in Europe (Williams, 1990).

Peat extraction is potentially the most damaging activity taking place on bogs. Typically the acrotelm which is essential for the formation of peat is completely removed. This leaves a bare surface which is extremely unlikely to regenerate without some kind of human intervention (Ferland & Rochefort, 1997). The recent paleoecological record is also destroyed.

Methods of extraction have evolved since the times of hand cutting for fuel. In Ireland, mechanical extraction by Bord na Móna (the Irish Peat Board) provides 4.5 million tonnes of peat each year for five peat-fired power stations, in 1991, this accounted for 15% of the nation's electricity supply (Feehan & O'Donovan, 1996) dropping to 7% in 2001 (Moore,

2002). Other countries, exploiting their peat reserves for electricity production include Canada, Russia and Finland.

In the UK, peat extraction for horticulture is considered to be the major threat to remaining areas of intact bog (Money, 1995). Annual peat production in the UK is currently 1.76 million m³ (Bather & Miller, 1991) affecting 5400 ha (Temple-Heald & Shaw, 1995). The majority of this is extracted under license from sites of nature conservation value. The recent popularisation of gardening through television programmes the lack of an outspoken public figure opposing the use of peat in gardens and the lack of good advice at point of sale (Doar, 1997) have almost certainly caused a further increase in demand for peat despite the wider availability of peat free alternatives. The largest peat extraction sites in the UK are Thorne Waste and Hatfield Moors. These are both Sites of Special Scientific Interest (SSSIs). However, this statutory nature conservation legislation gives no protection from extraction to sites where mineral planning consent has already been granted (RSNC, 1990). The situation regarding peat extraction in the UK has improved in recent years, notably with the purchase of mineral rights at Thorne and Hatfield Moors by English Nature in 2002 and the subsequent cessation of extraction in 2004.

The extent of peat extraction in Northern Ireland is even greater than in the rest of the UK and 78% of lowland bogs and 46% of blanket bogs have been affected by peat cutting in the past (Cruickshank *et al.*, 1995). The study by Cruickshank *et al.* (1995) emphasised the social benefits of peat cutting in Northern Ireland but stated that, in terms of the local economy, the peat industry contributes very little and that the major economic benefits were to the consumer in the form of cheap fuel.

Aside from the direct impact of peat cutting destroying the vegetated surface, it can also indirectly threaten surrounding uncut peat through drainage (Coles, 1995). This has been a problem at many sites, including Flanders Moss in Stirling, Scotland, and should be taken into consideration when planning restoration works.

2.3.2 Water Abstraction

Although a comparatively minor problem, water abstraction is currently affecting several peatland SSSIs including Redgrave and Lopham Fens in Suffolk and Taw Marsh in Devon (English Nature, 1999). Lowering of water table through abstraction will, as with drainage, result in drying of peat and a loss of conservation value and carbon storage potential. The major source of impact is water abstraction for public water supply. With the projected impacts of global warming on the UK, this could become an increasingly important threat in the future.

2.3.3 Agriculture

Agriculture has had an extremely damaging effect on peatlands. The major impacts are that of drainage for agricultural improvement, overgrazing and nutrient enrichment via runoff from adjacent farmland.

Drainage is potentially the most damaging agricultural activity on a peat bog. It is also the most common response of farmers to an area of boggy land. The physical effects of drainage on peat are primarily the result of shrinkage and alteration of the stability of the acrotelm by lowering the water table into the catotelm. This eventually accelerates decomposition of the peat (Heathwaite *et al.*, 1993b). An example of the impact of

drainage is that of Holme Fen in Cambridgeshire, where ground level has been lowered by 4m since 1854 (Lindsay *et al.*, 1988).

On agricultural land, drainage is essential if the quality of grazing is to be raised and potential threat to livestock (drowning, sinking) is to be removed (Lindsay *et al.*, 1988). In the past, drainage has affected large areas of wetlands and peatlands. It is estimated that it accounts for the majority of the 1,606,000km² of wetlands drained worldwide prior to 1985 (Williams, 1990).

Drainage should not be considered to be a modern impact on wetlands. There is evidence from the Somerset Levels for drainage occurring as far back as the Roman period (Williams, 1970). Since the Second World War however, drainage has intensified. Areas which had been considered to have no agricultural value were drained and ploughed during the war in the battle for self-sufficiency (Fisher, 1993). This drive to convert land to agricultural use continued until recently and until the early 1990s, grants were available from the Ministry of Agriculture for drainage of wetlands for conversion to agriculture. These grants were phased out as overproduction became an issue in the European Union but not before the conservation value of vast areas of land had been irreversibly damaged.

Grazing can positively affect the flora of raised bogs; where scrub and trees are invading, light grazing can be a good form of control (Burgess *et al.*, 1995). Where stocking densities increase, however, the beneficial effect can be outweighed by the negative impact on vegetation composition. Succession can be directed away from a plant community with particular conservation interest to one considered to be less valuable (Welch, 1997). Using the National Vegetation Classification (NVC) codes (Rodwell, 1995), Figure 2.3 shows the

possible successional stages that can be followed on an overgrazed mire. Overgrazing has been a particular problem on Shetland where almost all common grazing land is blanket bog (Birnie & Hulme, 1990). Recent changes to the way agricultural subsidies are paid are expected to reduce stocking densities in upland areas, headage payments which have frequently been blamed for increasing stock densities in the uplands have been replaced with area payments.
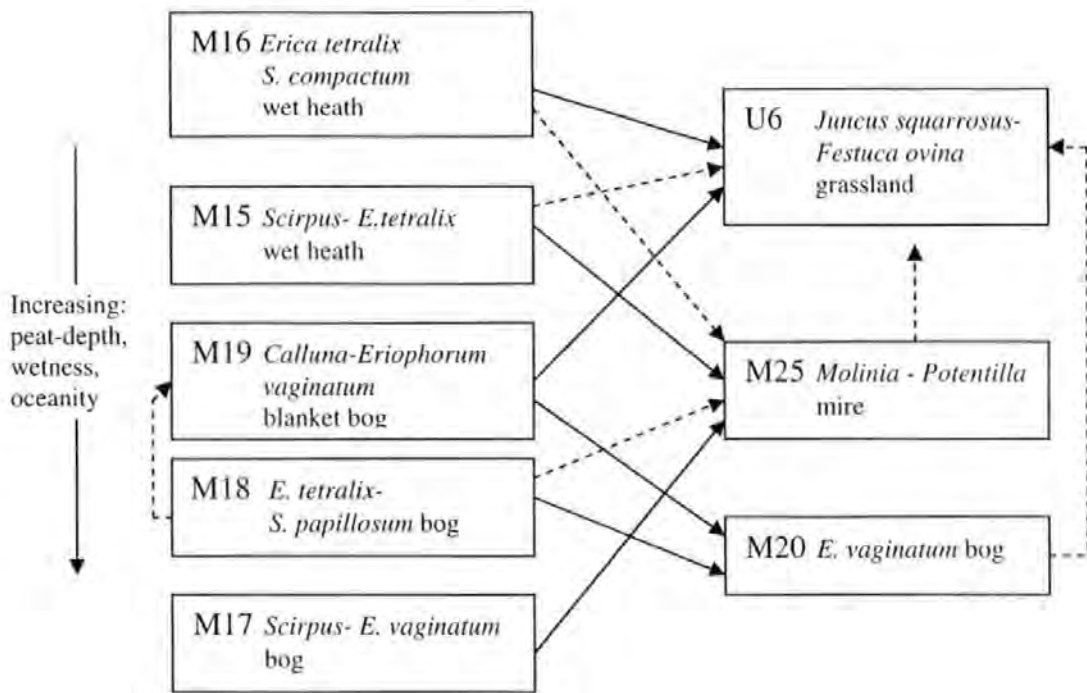
Figure 2.3: Vegetation succession on an overgrazed mire. Solid lines show usual successions, dashed lines show occasional successions (From Welch, 1997: 179).

Other problems associated with high stocking densities are those of erosion of the peat surface (Birnie, 1993) and nutrient enrichment. The latter is particularly problematic where food supplements are given or where animals are brought to richer grasslands at night. Both of these problems can have an indirect effect on vegetation composition.

Nutrient enrichment can also be a problem on peatlands which are surrounded by intensively farmed land. Agricultural runoff of fertilisers can cause localised enrichment, altering the flora significantly. This can also be the result of runoff from domestic sewage outlets, a problem at Fenns and Whixall Mosses in Shropshire and Clwyd (Brooks and Stoneman, 1997a).

2.3.4 Forestry

The expansion of forestry in Great Britain since establishment of the Forestry Comission in 1919 has had an enormous impact on the landscape and ecology of the uplands (Usher & Thompson, 1988). Large areas of peatland in the uplands, especialllly the Flow Country in Scotland, have been affected by forestry. Research conducted in the 1950s concluded that certain types of peat could, with a little preparation, be successfully planted (Zehetmayr, 1954). Areas recommended for planting by Zehetmayr (1954) were mostly in Scotland. However in England, Kielder Forest in Northumberland was recommended and in Wales, Beddgelert Forest in Caernarvonshire and Cloecaenog Forest, Clywd. Since then, advances in technology have allowed planting on deeper peats, thus greatly expanding the area of peatland subject to afforestation (Peterken, 1996).

Preparations for tree planting on peat involve drainage and ploughing. Drainage is necessary to increase timber production from an otherwise low productivity land type (Vasander *et al.*, 1997). The negative effects of drainage have been outlined above. The change in land use from light grazing to forest cover has a major impact on vegetation cover. The ground layer will be transformed from typical mire vegetation types to something more representative of a forest floor. Further problems associated with afforestation of peatlands are that, aside from physical damage to the surface, chemicals

must be applied in order to ensure successful establishment of trees. These include fertilisers, which will alter the nutrient poor status of mires. This may have further effects on vegetation composition on both the surface to be planted and on surrounding peatland. Aerial application of pesticides during the forestry cycle is particularly damaging, as the chemicals cannot be restricted to the forestry area and also have an impact on surrounding peatlands.

Afforestation has affected large areas of blanket bog in northern Scotland (Everett, 1999). Government policy during the 1980s was to give tax allowances to landowners who invested in forestry. This policy was reversed as a result of environmental concerns and the fact that the plantations offered low returns on investment due to the difficult environmental conditions for tree growth (Nykänen *et al.*, 1997). Despite the cessation of planting, this policy will have a long-lasting impact on the ecology of blanket mires in Scotland and the landscape of the uplands in Britain.

The impact of forestry on bird populations was one of the most contentious issues associated with the afforestation of the Flow Country in Caithness and Sutherland. By providing large areas where predators could shelter the effect was to reduce the nesting area of birds, driving them further away from forestry plantations (Avery & Leslie, 1990). This was in addition to the impact of pesticides on the food chain.

A final problem associated with afforestation of peatlands is the impact it has on carbon cycling and storage. The role of peatlands in the global carbon cycle has already been discussed. The impacts of forestry preparations on carbon storage are likely to be severe. The effect of drainage on carbon storage has already been outlined; a 20cm drop in water

table can lead to a 100% increase in annual carbon release (Silvola *et al.*, 1996). Preparation for planting, in addition to damaging the peat surface, is likely to have an impact on CO_2, CH_4 and NO_2 fluxes from peats. As all of these gases contribute to the enhanced greenhouse effect, this is an additional concern attached to afforestation of peatlands.

2.3.5 Pollution

Atmospheric pollution is a particular threat to the low nutrient status of ombrotrophic peatlands. The recent increase in *Molinia caerulea* Purple-moor grass on blanket mires in the west of Britain has been attributed in part to atmospheric nitrogen pollution (Chambers *et al.*, 1999). Research on ombrotrophic bogs in Ireland found that additions of Nitrogen increased growth of *Molinia caerulea*, especially under moisture stress (Tomassen *et al.*, 2004). The problem of atmospheric nitrate pollution will have the greatest impact closest to emission sources and may result in the loss of key species, and eventually degradation and erosion (Moore, 2002).

2.4 Management of Peatlands

2.4.1 Introduction

There are very few ecosystems in the world which have escaped some form of human management. The peatlands of Britain are no exception and many would not persist in their present state were it not for human intervention. Large areas of peatland have been managed for agriculture in the past but have since been withdrawn from agricultural production (O'Carroll & Farrell, 1993). Few peatlands are in an ideal ecological condition primarily due to a combination of past exploitation and a lack of appropriate management.

Management of individual peatlands will vary depending upon the aims, objectives and priorities of the group responsible. Management for agriculture, as outlined above, depends primarily on continued drainage. Conservation of peatlands for wading bird populations is at the opposite end of the management spectrum and depends upon the maintenance of high water tables and open pools.

Knowledge of best practice for management of peatlands of high conservation value is relatively rare (Rowell, 1988). That is not to say they are unmanaged, rather that traditional management ceased some time ago on many sites and detailed records of methods were not kept. The major objective of management in most cases is the maintenance of water levels. High water levels, as was previously stated, are essential to continued peat growth. As the only source of water to ombrotrophic mires is rainwater, little can be done to increase inflow. Therefore control over outflow is the primary method of manipulating water levels. Figure 2.4 summarises the water budget in an ombrotrophic bog.

Precipitation
(Climate change)

Evapotranspiration
(Tree/scrub encroachment climate change, grazing)

Mire Water Table

Run off
(drainage, peatcutting-peripheral and central)

Groundwater
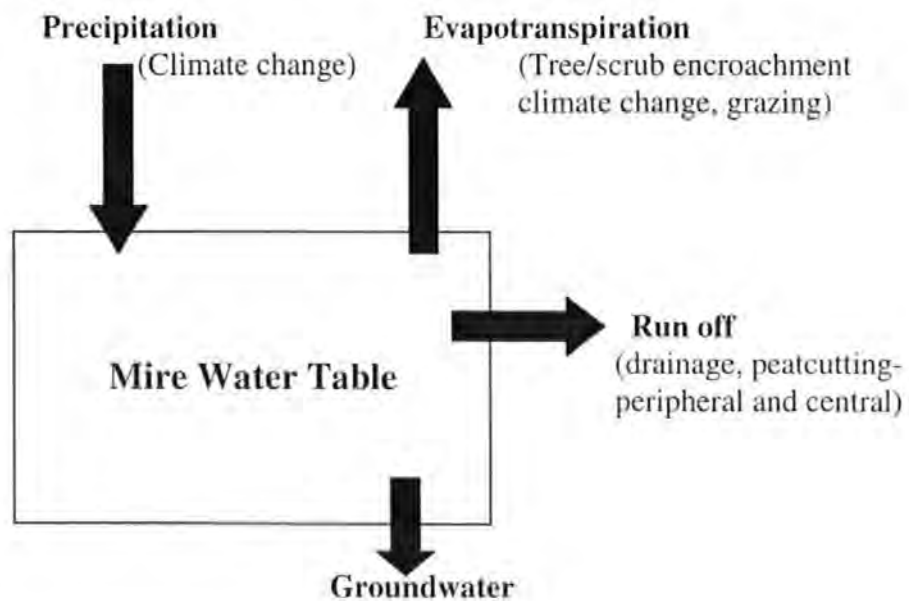
Figure 2.4: Sources and losses of water on an ombrotrophic mire showing threats to the system

Where water levels on peatlands have fallen below their optimum, desiccation of mosses is inevitable. As *Sphagnum* spp. are able to hold ten to twenty-five times their dry weight in water, they are capable of tolerating extended periods of sub-optimal water levels (Andrus, 1986). The management priority on peatlands which are drying out is to raise the water table (Brooks and Stoneman, 1997a). As there are many reasons for lowering water tables, there are also many solutions. Changes in climatic conditions are perhaps the most damaging and are also outside the realm of management control. In the light of predicted climatic change, as a result of the enhanced greenhouse effect, it may not be possible to restore mires where climate is the sole cause of damage.

Where the problem is caused by excess drainage, it is essential to block drains if the water table is to be returned to optimum levels. Ditches within the site can be blocked using a variety of materials; peat dams are cheap and can be very effective as long as the peat used is highly humified and has not been subject to oxidation, while other options include plywood and plastic dams (Brooks and Stoneman, 1997a). Peripheral drainage within the hydrological unit of the bog should be treated in a similar way to internal drainage. Where part of a bog has been cut for peat, the traditional domed shape of raised mires is often destroyed; this can lead to a shedding water table (Coles, 1995). The solution for this is usually the construction of an impermeable layer or bund, around the edge of the mire (Brooks and Stoneman, 1997a) (Figure 2.5). As with drains, the most commonly used material is peat; however, other materials such as clays and plastics are also used.
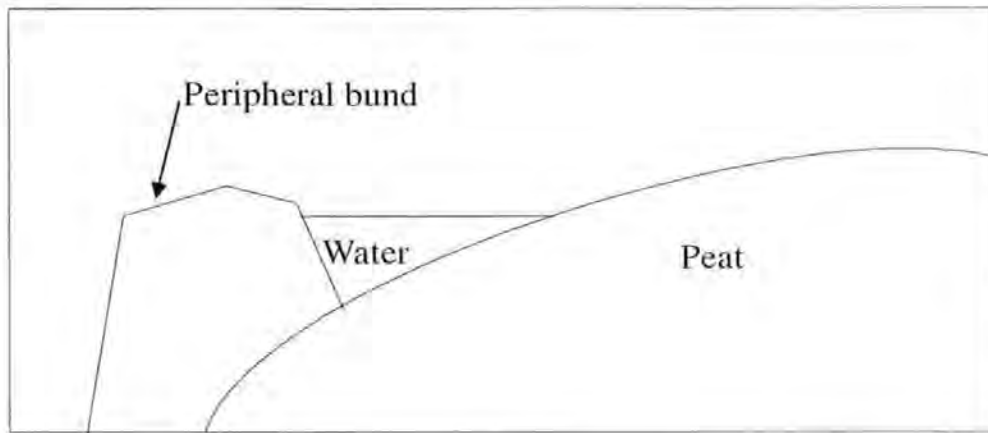
Figure 2.5: A bund used to maintain water levels on a mire (Adapted from Brooks and Stoneman, 1997a)

As the UK is home to one of the largest areas of treeless blanket bog in the world, we have a particular responsibility to conserve this resource (Lindsay, 1987). On a global scale, treeless blanket bog is exceptionally rare and is mainly restricted to oceanic coasts between latitudes 45° and 65°.

This habitat can be irreversibly destroyed by a drop in water levels, which allows trees which are usually excluded by the waterlogged conditions, to invade. Birch *Betula* spp. is particularly problematic; its rapid growth rate makes it a particularly effective invader where drier conditions prevail. The issue of trees on bogs is rather contentious, paleoenvironmental evidence proves that around 4000 years BP, Scots pine *Pinus sylvestris* grew on peat bogs in Scotland (Gear & Huntley, 1991). Whether this was the result of climatic warming providing conditions suitable for colonisation or simply that these areas had reached a climax community is debatable (Ingram, 1995; Chambers, 1997). The actual extent of tree cover has also been questioned. *Pinus* pollen is known to disperse over large areas (Moore *et al.*, 1991); therefore the presence of pollen does not necessarily indicate presence of trees. The characteristics of natural pine forests on British peatlands compared to plantations or North American or northern European pine forests are an important

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consideration. The British forests would have been cyclical, emerging during dryer time periods; multi-aged and would likely have been composed of scattered trees rather than dense forest cover (Peterken, 1996).

It has been widely accepted that the mild oceanic British climate is the reason for climax communities on our mires being treeless (Brooks and Stoneman, 1997a). The major problem associated with scrub encroachment is further drying of the bog through increased evapotranspiration (Rowell, 1988). In these situations, raising water levels can often prompt die back of the invading scrub, however, where trees are well established, further action may be necessary to ensure removal. It is essential that this be carried out sensitively to avoid damaging the surface of the bog. If tree removal is to proceed, it is essential that the underlying hydrological problems be addressed to ensure that removal is permanent.

Pulling is possible where seedlings are young and small; however, where there are large numbers, other methods are often preferable to prevent surface damage (Rowell, 1988). The other options are to kill the trees with chemicals, with obvious implications on nature reserves, however, in the case of birch, regeneration from cut stumps is a major problem therefore the use of chemicals is often unavoidable (Marrs, 1985). Guidelines have been set out on the use of herbicides on nature reserves (Cooke, 1985); these recommend the use of chemicals in the treatment of birch and other woody species but also make recommendations for avoiding damage to desirable species. Another solution is to ring bark the unwanted trees and leave them to die *in situ*. There are some concerns that where trees are left to decompose on site they may cause localised eutrophication both through decomposition and by providing a perch from which birds may continually defecate

(Rowell, 1988). However, this is often more desirable than the damage to the surface which would, inevitably, be caused by dragging trees across the bog.

In some situations, where tree cover is at extremely high levels, felled trees have been removed by airlift with a helicopter, as was carried out at Flanders Moss NNR in Scotland (Brooks and Stoneman, 1997a). The obvious drawback of this is the high cost of hiring a helicopter and the large amount of work which must be carried out at one time, a particular problem where there is a shortage of available labour.

2.4.2 Restoration Projects

Due to a combination of lack of appropriate management and past exploitation, many areas of peatland are in a less than optimum state. Studies of abandoned peat cuttings have shown that natural regeneration rarely produces communities typical of undisturbed peatlands (Money, 1995) therefore, if rehabilitation is to occur, positive management is necessary (Wind-Mulder et al., 1996). Damage caused by peat harvesting need not be permanent as areas which have had their live layer of Sphagnum removed can revegetate within 5 years (Campeau & Rochefort, 1996). It should be noted however that biodiversity and biomass of a revegetated peatland is unlikely to be as rich as that of an undisturbed one. A recent emphasis on restoration ecology has led to the development of techniques for restoring peatlands. The problem of revegetating bare peat surfaces has received particular attention (Ferland & Rochefort, 1997; Grosvernier et al., 1997; Robert et al., 1999). This is a particularly difficult task as environmental conditions on bare peat surfaces are extremely harsh and are not conducive to plant survival.

Hydrological stability is of ultimate importance to the survival of peatlands, however, there is a complex relationship between the hydrology of a bog and its vegetation. The

vegetation is dependent upon the maintenance of optimum hydrological conditions but it also plays a role in maintaining these conditions (Heathwaite, 1994). *Sphagna* are able to hold water in their cells and utilise this in dry conditions, thus also allowing the plants a role in the maintenance of a bogs hydrology. Moss cover on an undamaged site is well adapted to sustaining water levels (Price, 1996) but where cover has been lost, the self sustaining mechanisms are lost also.

Although hydrology is the defining physical characteristic of a bog, surface temperature is probably the most difficult obstacle for plant growth. Owing to the dark colour of the peat, sunlight is absorbed and huge fluctuations in surface temperature can occur. Temperatures of up to 60°C have been measured 1cm below the surface of bare peat (Buttler *et al.*, 1998). This will cause desiccation of any plants (including *Sphagna*) trying to colonise the surface.

Restoration of peatlands usually concentrates on reestablishment of vegetation. As *Sphagnum* spp. are the most important components of an active peat bog, for restoration to be successful, suitable conditions for *Sphagnum* establishment must be created (Buttler *et al.*, 1998). The first stage of restoration usually involves raising water levels, as regeneration of peat forming mosses requires a high and stable water table (LaRose *et al.*, 1997). To do this, the simplest solution is to block drainage ditches. These are common on post-harvested peatlands as the process of harvesting involves draining the area first to allow access to mechanical harvesting equipment.

Once water levels have been raised successfully, the next stage is *Sphagnum* regeneration. There are two approaches which can be taken here. Firstly, natural regeneration may be more successful where there is source material on site; however, it is likely to be a long

process. Where only part of the acrotelm has been removed, natural regeneration is likely to be relatively successful. This is because there is a source of undecomposed plant material for regeneration. In contrast, where the entire acrotelm has been removed, regeneration is much more difficult due to a lack of source material (Grosvernier *et al.*, 1997).

In the case of a totally removed acrotelm over a large area, it is often necessary to use an alternative approach involving some method of *Sphagnum* reintroduction if vegetation cover is to be achieved relatively quickly. Experiments have been carried out on the efficacy of introducing *Sphagnum* fragments or 'diaspores' to areas of bog to hasten revegetation (Ferland & Rochefort, 1997; Grosvernier *et al.*, 1997). It has been found that introducing *Sphagnum* diaspores can produce excellent results but that different species have variable success rates. For example *Sphagnum fallax* has proved to be highly successful in the Jura Alps in Switzerland (Grosvernier *et al.*, 1997).

Different species are able to colonise different niches on a bog including bare peat surfaces. There is evidence to suggest that some preparation of bare surfaces can enhance regeneration. The creation of artificial hummock-hollow topography has been shown to enhance *Sphagnum* growth in the depressions compared with flat surfaces, as a result of increased humidity, shelter from wind and reduced surface temperatures (Ferland & Rochefort, 1997; Campeau *et al.* 2004).

It has been found that the provision of shade and protection from the wind can improve regeneration rates of *Sphagnum*. Conditions provided by pioneer species such as *Eriophorum vaginatum* can counterbalance an unstable water table by providing a suitable

microclimate (Grosvernier *et al.*, 1995; Ferland & Rochefort, 1997). More recently, concerns have been raised about the use of *Eriophorum vaginatum* as a nurse plant as establishment of moss species was more closely associated with hydrologic characteristics (Lavoie *et al.* 2005). Providing ecological conditions for colonisation by higher plants should be considered part of a successful revegetation programme whilst bearing in mind the concerns raised by Lavoie *et al.* (2005). The provision of artificial shade has also been shown to improve *Sphagnum* survival rates with plastic membrane widely used in regeneration trials (Grosvernier *et al.*, 1995; Campeau & Rochefort, 1996). Straw has also been found to provide an effective mulch for protecting *Sphagnum* from desiccation (LaRose *et al.*, 1997) although again, more recent research has raised concerns that decomposition of organic mulches may impede recovery of the carbon sink facility (Waddington *et al.* 2003). Experimental restoration at Waun Fignen Felen, a badly eroded site in the Brecon Beacons in Wales, is employing straw mulch and jute netting to stabilise the surface and aid regeneration.

A final consideration when attempting restoration of bare peat surfaces is the peat chemistry. Although *Sphagna* are widely acknowledged to have low nutrient requirements, addition of artificial fertilisers has proved beneficial in the early stages of regeneration (Rochefort *et al.*, 1995). Fertilisation also encourages growth of vascular plants which will then further encourage *Sphagnum* growth through the provision of shade. pH is also important for the reestablishment of vegetation as certain species of *Sphagnum* can be encouraged by slightly acidic conditions (pH *c.*5.3; Grosvernier *et al.*, 1997), well below the norm for an ombrotrophic bog (pH 3.2-4.0 (Clymo, 1984b)). The result of restoration where conditions described above are provided may be a poor fen type environment which can then proceed through succession to ombrotrophic mire.

The above section has concentrated on restoration of bare peat surfaces. Rehabilitation of peatlands which have been damaged by overgrazing, drainage and forestry activity is also a major priority. These issues have been covered in the discussion of management issues on peatlands. As no two bogs are the same, it is not possible to lay down guidelines on how to restore a bog; individual ecosystem characteristics must be used in identifying restoration methods (Pfadenhauer & Klötzli, 1996). The time scales of regeneration make measurement of ultimate success difficult. As peat grows at around 1mm yr^{-1} (Clymo, 1983), and may be harvested at the rate of 500mm yr^{-1}, vegetation recovery can be slow also, with some plants needing time to be migrate into the area and some needing significant hydrological improvements before successful reestablishment is possible. Restoration of individual mires to pre-damaged conditions on a human life span is impossible. A measure of success is needed, therefore, which can identify early stages of peat development and make forecasts of recovery based on this. Testate amoebae may offer some potential as an early indicator of success of restoration at any given site. This study will investigate the possible application of these organisms to monitoring peatlands.

2.4.3 Conservation Legislation

In the UK there are several tiers of conservation legislation available to protect sites from damage. These range from national legislation, to European and International. The most widespread of these is the Site of Special Scientific Interest (SSSI) designation. This was introduced in the 1949 *National Parks and Access to the Countryside Act* and was updated in the 1981/1985 *Wildlife and Countryside Acts* as a conservation designation to allow protection of sites for their scientific interest (DETR, 1998). Existing legislation was made more robust with the introduction of the Countryside and Rights of Way Act (CRoW)

which came into force in 2001. The above pieces of legislation have provided legal protection for some of the best examples of peatlands in the UK. An important element of the CRoW act is that public bodies now have a duty to further the conservation SSSIs (English Nature 2003). The emphasis at sites affected by extraction is now on rehabilitation.

SSSIs were intended to form part of a network of wildlife sites of each habitat type. They are designated by the government conservation agencies (Countryside Council for Wales CCW, English Nature EN and Scottish Natural Heritage SNH). They are usually privately owned and managed although there is management plans are often agreed between the landowner and the relevant conservation agency. The National Nature Reserve (NNR) designation affords a higher degree of protection to a site. NNRs are usually owned and managed by one of the government conservation agencies which allows for more positive management to take place.

The European Habitats Directive was adopted in 1992 as a way of providing a common standard for nature conservation across the EC (Hopkins, 1995). There are two sides to this legislation, the Special Areas of Conservation (SACs) which aim to conserve habitats, and the Special Protection Areas (SPAs), legislation for protecting birds. The application of SACs to raised bogs is of particular significance as the legislation states that any raised bog proposed as a SAC has to be managed to ensure that it actively continues to lay down peat (O'Connell, 1999). As there are many raised bogs within the UK which are not actively laying down peat but are of significant conservation value, if a reasonable area of land is to be conserved, these will need to be included in the network of SACs. This has implications

for the management of raised bogs and techniques must be developed which will encourage renewed growth of peat on these sites.

The major international conservation legislation affecting peatlands is the Ramsar Convention. This allows the designation of wetlands of international importance for the conservation of birds. Although peatlands do not provide habitats for a rich diversity of birds, some support internationally important populations of wading birds. During 1999, the Flow Country in northern Scotland was designated as a Ramsar site and an SPA for the internationally important populations of golden plovers, dunlin and greenshank (Everett, 1999).

Non governmental organisations (NGOs) also play a major role in peatland conservation in the UK. Forsinard in the Scottish Highlands is an area of afforested blanket bog owned by the RSPB. Management and restoration are being carried out by the RSPB. Plantlife, another conservation organisation, own and manage several peatland sites including the Munsary peatlands which are managed in liaison with both the local community and the statutory conservation body Scottish Natural Heritage.

Despite the many tiers of conservation legislation available, peatlands continue to be threatened by agriculture and development. A recent agreement with English Nature and peat producers has removed the threat of peat extraction on lowland raised bogs, Thorne and Hatfield Moor is one site where peat extraction has ceased as a result of this agreement.

2.5 Rationale for Ecological Monitoring

The study and description of natural areas is an integral part of ecology. Monitoring is often used as a word to describe all types of study, but it is important to distinguish this term from other similar terms (Hellawell, 1991):

1. Survey: a study where a set of qualitative or quantitative measurements are made with no preconceptions of what the findings ought to be. Carried out only once

2. Surveillance: a programme of surveys which add the dimension of time in the study of a variable

3. Monitoring: intermittent surveillance carried out in order to study the degree of correspondence or otherwise with a predetermined standard.

All three levels of observation outlined above are important to environmental management. Surveys can be used to establish baseline ecological conditions and from that point devise appropriate management techniques. They can also be used to establish the numbers of species or the distribution of a particular habitat type (e.g. The Peatland Survey of Northern Scotland (Lindsay *et al.*, 1987)).

Surveillance can be used where resources are few or monitoring is not practical; it allows the detection of change over long time periods. In essence it is surveying followed by the repetition of surveys at a later date. It can be used to observe changes although the causes of change are unlikely to be fully understood. An example of surveillance is the work carried out by Chapman and Rose (1991) at Coom Rigg Moss, which was in essence a

repetition of earlier survey work carried out by Chapman (1964a, 1964b, 1965). It resulted in the observation of changes in vegetation over time and, although it was not possible to identify specific causes of change, theories were put forward (Chapman & Rose, 1991), which subsequently could be addressed by the use of monitoring.

Monitoring of the vegetation and physical characteristics of a site allows comparison and correlation with management techniques and also the comparison of site characteristics with the health of a particular species. It is an important tool for an environmental manager as it can be used to detect changes within a site which may affect its conservation value at an early stage. A common deficiency of environmental management is the failure to apply the results of monitoring to future management (Sutherland, 1996).

Monitoring is carried out for a variety of purposes. It is used to identify baseline ecological conditions on a site, which is particularly important for targeting management activity at specific problems. It is also used as a method of monitoring management efficacy (Johnson, 1997). In the case of restoration of damaged peatlands, an experimental process, monitoring is essential so that methods which prove effective can be replicated on other sites (Wheeler & Shaw, 1995). The results of monitoring on nature reserves can be used to convey information to the public about the importance and effectiveness of restoration and also about the general ecology of the site (Johnson, 1997).

The most common variable to be monitored on peatlands is hydrology. As access is often a problem on peatlands and water tables vary greatly over time, many problems arise from the desire to monitor. There is technology available for constant automated monitoring of water levels; however it is prohibitively expensive for most conservation sites. A system

was devised by Bragg *et al.* (1994) which allowed the maximum and minimum water levels to be monitored between readings. These Water Level Rain Gauges (WaLRaGs) are cheap and are therefore used on many sites in the UK where hydrological monitoring is deemed necessary. More high-tech solutions are being tested for peatland monitoring; work by Harris *et al.* (2005) found that analysis of remotely sensed images was effective for assessing near-surface moisture stress in *Sphagnum*.

Monitoring should be considered to be an integral part of management; however, a cautionary note must be added. Large amounts of information are gathered on the ecology of nature reserves often without consideration of its importance or application. Where high costs are involved, planning is important to ensure the right information is gathered and that there is a practical use for that information.

2.6 Testate Amoebae

2.6.1 Introduction

Testate amoebae are unicellular organisms composed of a cytoplasm cell enclosed in a shell or test. They occur in great numbers in damp environments, particularly peats, soils and lakes. The composition of their shells facilitates long-term preservation and enables the major application of testate amoebae analysis to date – palaeoenvironmental reconstruction.

2.6.2 Biology and Systemics

Testate amoebae are protozoans biologically classified within the Subkingdom Protista, Superclass Rhizopoda and the Subclass Testacealobosea (Committee on Systematics and Evolution of the Society of Protozoologists, CSESP, 1980). They are variously referred to

as arcellaceans, thecamoebae, rhizopods and testaceans as well as testate amoebae. This work will use the term testate amoebae throughout.

They are a component of the soil inhabiting microfauna and range in size from 5-250μm (Sleigh, 1973). They are closely related to the naked amoebae, ciliates and other protozoa (Stout & Heal, 1967) but they differ from other members of the Superclass Rhizopoda in the ability to produce their own shell or test (Corbett, 1973).

Tests are formed either from organic material secreted by the amoebae, which can be smooth material or pre-formed siliceous plates, or from various particles found in their habitat including diatoms, silica particles and fungal hyphae (Warner, 1988). The former are referred to as autogenous and the latter xenogenous or agglutinated. The test provides protection against desiccation to the cytoplasm (Charman *et al.*, 2000). The purpose of encystment is to enable the testate amoeba to survive harsh conditions including desiccation and freezing (Charman *et al.*, 2000). There are two methods of encystment depending on the severity of the conditions. A wrinkled membrane or pellicle can cover the cyst very rapidly (a matter of minutes) (Bonnet, 1964) or a true cyst can be formed and the opening of the test sealed with a plug of mucus (Stout & Heal, 1967).

Reproduction in testate amoebae is primarily by asexual fission where the mother test produces a daughter test through nuclear division. This process observed under laboratory conditions took approximately forty-five minutes (Ogden, 1981). In clonal cultures in laboratories however, sexual reproduction has been observed. Schönborn and Peshcke (1990) observed sexual reproduction in laboratory populations of *Assulina* and meiosis was observed during electron microscopy studies of *Arcella vulgaris* (Mignot & Raikov, 1992).

Reproduction and indeed species distribution is thought by some to be dependent upon the availability of test building materials (Louisier, 1982; Tolonen, 1986). There have been incidences of two species dependent upon mineral particles for their shell construction – *Difflugia* spp. and *Heleopera* spp. were found reproducing in culture conditions where test building materials are not available (Louisier, 1984). These species created their shells in the same way as autogenous species.

Reproduction rates in testate amoebae have been found to vary according to habitat and species. Schönborn (1992) found rates of between 9 and 27 generations per annum in moss whilst rates found by Heal (1964) were less than 10 generations per annum depending upon species. Other authors cite population doubling rates rather than generations per annum. Ogden (1981) quotes doubling rates for *Euglypha* spp. between 2.3 and 4.4 days and for *Assulina muscorum* 2.3-2.9 days. Louisier (1984) came up with a similar figure for population doubling.

The seasonality of testate amoebae reproduction is largely unknown. A study of testate amoebae in lakes indicated that reproduction was correlated with temperature with peaks in testate amoebae numbers occurring in July (Hunt & Ming Chein 1983). In studies of peatlands Heal (1964) found that population numbers peaked between May and July. Earlier workers found contradictory evidence, e.g. Cutler *et al.* (1922) found higher populations in autumn and lower numbers in summer.

2.6.3 Taxonomy

Much of the early taxonomic work on testate amoebae was based on the living specimens and therefore the soft parts such as the nuclei and pseudopodia were important in identification (Ellison & Ogden, 1987). Today, shell morphology in testate amoebae is the principal feature used to differentiate species (Heal, 1963; Tolonen *et al.*, 1992; Bobrov *et al.*, 1995). This is the result of previous applications of testate amoebae analysis. They have been used as hydrological indicators in palaeoenvironmental studies and, as it is only the test which is preserved, this has become the primary method of identification.

The need to refer to several monographs for identification was a particular problem identified by Tolonen (1986) which has been addressed by the development of a comprehensive key for species of ombrotrophic peats by Charman *et al.* (2000). The quality of the identification keys in separate monographs is variable with different keys focussing on different taxonomic groups (Woodland, 1996).

Testate amoebae can generally be identified to species level under high-powered light microscopy. Shell features including construction material, colour, size, shape and ornamentation are the most commonly used in identification (e.g. Corbett, 1973; Tolonen, 1986; Ellison & Ogden, 1987). Whilst these features are effective in the identification of the majority of species there are problems in some cases. Each of the features described above as important in identification can be problematic in at least one species or genus of testate amoeba. Difficulties in separating similar taxa were described by Heal (1962), who noted that *Amphitrema stenostoma* had been confused with *A. wrightanium* and that *Heleopera petricola* was confused with *H. sylvatica*.

Shell ornamentation can be either patterns occurring on the surface of the test or other features such as spines. Individuals of the same species can exhibit differing characteristics leading to misidentification, e.g. species with spines appearing without spines (Bobrov *et al.*, 1999). This has been a problem in this project with some level of confusion between *Euglypha tuberculata* and *Euglypha strigosa*. Identification of species of *Nebela* with similar test morphology can be complicated by multiple surface patterns (Bobrov *et al.*, 1995).

Other morphological features commonly used in the identification of disc shaped species are the apertures and psuedostomes. Variability of the pseudostome has been observed in the species *Trigonopyxis arcula* (Bobrov *et al.*, 1995) (Figure 2.7). This level of variability had led to this species being separated by Hoogenraad & de Groot (1948) into *T. arcula* and *T. microstoma* (Charman, 1997). This variability almost certainly affects other testate amoebae identified by their apertures such as *Bullinularia indica*, *Centropyxis* spp. and *Cyclopyxis* spp. (Woodland, 1996).
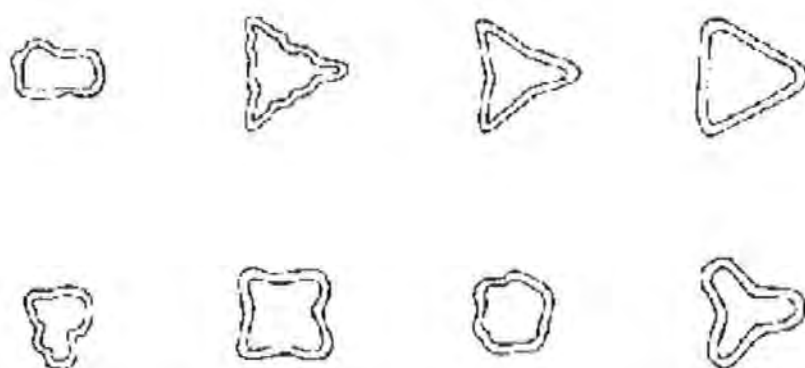
Figure 2.7: Pseudostome shapes in *Trigonopyxis arcula sensu lato* identifed by Bobrov *et al.* (1995: 121)

Colour of tests has proved particularly difficult when a stain is used. For example, the use of safranine for staining samples results in a pink tinge in all individuals. The species of

Heleopera are most easily misidentified as a result of this. *Heleopera rosea* is distinguished from other species of *Heleopera* by its wine coloured test (Charman *et al.*, 2000) but when stained with safranine, it becomes indistinguishable. This has not been an issue in this work as safranine staining is now thought unnecessary (Woodland 1996). Rose Bengal staining is used in this study to separate living and dead specimens however, despite its resulting pink colour; this has not been found to hinder identification.

The test building materials can also cause confusion. Corbett (1973) noted that *Nebela* spp. can produce forms made out of secretion rather than the characteristic mosaic of plates. This could lead to their misidentification as *Hyalopshenia* spp., a point that has been noted in this study. Bobrov *et al.* (1995) found nine surface pattens in *N. militaris* ranging from the detailed mosiac of plates to a pattern almost devoid of plates (Figure 2.8). This was thought to lead to possible confusions with *H. papilio*.
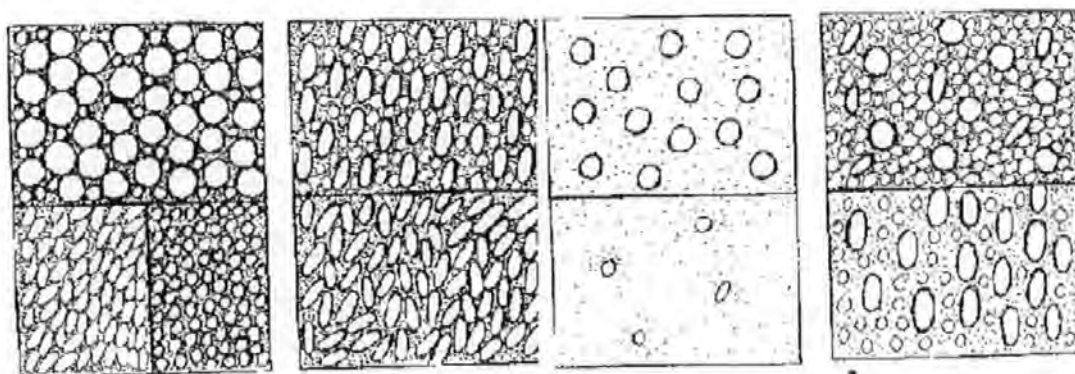
Figure 2.8: Surface pattern variability observed in *N. militaris* (From Bobrov *et al.*, (1995: 122))

Species of *Corythion* and *Trinema* cannot be consistently separated under normal light microscopy and distinguishing features are not always visible using electron microscopy

(Charman *et al.*, 2000). This could be problematic if the species are found to have different ecological tolerance ranges.

Specific examples of identification problems are described in table 2.1 adapted from Woodland (1996).

Taxa	Confused with?	Other Comments
Assulina muscorum	*A. semimulum* Also possibly *Placocista spinosa* where spines are damaged in preparation.	Polymorphism in *A. muscorum* affecting colour and size of test – ranges from russet brown to colourless (Schönborn & Peschke, 1990); usually brown but occasionally colourless (Ogden, 1981)
Arcella artocrea	*A. gibbosa* (Tolonen *et al.*, 1992)	
Arcella discoides	*A. megastoma* and *A. polypora* (Warner, 1987)	See below
Arcella rotunda aplanata	*A. discoides scutelliformis* (Tolonen, 1986); *A. vulgaris*	Systematics of *Arcella* poorly understood. Intermediate forms difficult to identify (Tolonen, 1986).
Centropyxis aculeata	*C. hirsuta* (Tolonen, 1986)	Regarded by Heal (1962) as a species complex.
Corythion dubium	*Trinema lineare* (Tolonen, 1986)	Plates diagnostic, but very small test size (18-35µm – Ogden & Hedley, 1980)
Centropyxis aerophila type	*C. cassis; C. constricta; C. ecornis* and *C. platystoma* (Warner, 1987).	Conglomerate group, which may contain a number of separate species with specific niches (Tolonen, 1986; Warner & Chmielewski, 1992).
Euglypha strigosa- type	*E. compressa, E. ciliata* (Warner, 1987).	Where spines are damaged, missing or obscured identification becomes difficult (Bobrov *et al.*, 1999)
Phyrganella hemispherica	*Centropyxis eurystoma* (Tolonen, 1986); *Difflugia globulosa* (Tolonen, 1986); *Cyclopyxis arcelloides* (Meisterfeld, 1979)	Also referred to as *Phygranella acropodia* (Warner, 1987)

Table 2.1: Peatland testate amoebae with disputed taxonomy (Updated from Woodland, 1996)

Further confusions in taxonomy are caused by adaptations of testate amoebae to environmental conditions. Wanner and Meisterfeld (1994) found that shell characteristics

are affected by environmental factors such as chemical composition of the substrate, temperature and food composition. These factors primarily affected the size of shell, theoretically leading to problems in identification. Bobrov *et al.* (1995) advised caution in relying upon shell morphology for differentiating between varieties or species of *Trigonopyxis arcula sensu lato* and recommended that *T. arcula* should be treated as one polymorphic complex instead of two or more species. Morphological differences between northern and southern hemisphere populations were a particular issue.

That most authors comment on the difficulties encountered in precise taxonomic identification of testate amoebae is testament to the complications encountered. The continuing work on taxonomy and the production of simplified and amalgamated keys will hopefully reduce this problem in the future. As test size is also related to habitat selection (Woodland, 1996), it is an area which offers great potential for bioindication (Wanner, 1999)

The taxonomic classification in the key produced by Charman *et al.* (2000) will be used for identification throughout this project.

2.6.4 Ecology

a) Habitat Preferences

The major limitation on testate amoebae is moisture availability. They are essentially aquatic animals living in films of water around *Sphagnum* leaves and soil particles. Their specific habitat requirements are not known in detail, but moisture requirements and to some extent pH have been widely explored in recent years. Other influences on their distribution are temperature, oxygen availability and light.

Testate amoebae are found in a wide range of habitats from freshwater and forest floor to peat bogs, damp soils and, occasionally, saltmarshes (Tolonen, 1986; Warner, 1988; Charman *et al.*, 1998) although they are best represented in forest litters and zonal peats (Stout & Heal, 1967). The chief limiting environmental factor in their distribution is moisture availability (Tolonen, 1986). However, their ability to protect themselves from desiccation during dry periods by encysting allows them to survive where there is instability in the moisture regime (Warner, 1988). In extreme cases, this ability to encyst allows them to live in dry conditions for up to half the year (Tolonen, 1986).

Testate amoebae are highly abundant in soils and populations of up to $16 \times 10^6 \, m^{-2}$ have been found in peatland environments (Heal, 1962). This figure is a mean of all species with lowest population concentrations coming from *Nebela tincta* ($2.1 \times 10^6/m^2$) and highest from *Amphitrema flavum* ($20 \times 10^6/m^2$). Other authors have found lower concentrations e.g. Schönborn (1962) reported a range of $0.3-2.8 \times 10^6 \, m^{-2}$ depending on water content. Within coniferous forest soils, variations in population densities are found between the needle and the humus layer (Schönborn, 1986), higher densities were seen in the needles and lower in the humus. Abundances between 7000 and 23 000 individuals per gram dry weight were quoted for spruce soils by Wanner (1991) whilst numbers in orchards and deciduous forests were ten times lower. Salt marsh figures are given as up to 65 600 individuals per cm^3 (Charman *et al.*, 1998).

b) Hydrology

Different species of testate amoebae have differing moisture requirements. The first attempt to characterise taxa hydrologically was by Harnisch (1927). This classification based on European mires recognised four hydrological associations:

"I. Forest moss type. Consisting of various species of *Difflugia*, *Centropyxis*, *Arcella*, *Assulina*, *Euglypha*, *Nebela*, *Corythion* and *Trinema*. This community is typical of non-bog-forming Sphagna in forests, heaths and along lake shores.

II. *Hyalosphenia* type. Containing *Hyalopshenia elegans* and *H. papilio* in addition to the species present in Type I. This community occurs in all Zwischenmoors (middle-aged mires), mature Hochmoors (raised mires) and marginal areas.

III. *Amphitrema flavum* type. Containing the species of Type II, plus *A. flavum*. It is the most widely distributed in Hochmoors.

IV. *Amphitrema wrightanium* type. Containing *A. wrightanium* in addition to the species in Type III. It is not very common, being restricted to well-developed Hochmoors and is always the most diverse assemblage."

(Harnisch, 1927; pp.348-349 (translated in Tolonen, 1986))

Later classification was produced by Bartos (1940) who proposed four classes distinguished by qualitative moisture affinity:

Hydrophilous – inhabiting submerged mosses

Hygrophilous – needing moist conditions

Xerophilous – living in dry conditions

Eurytopic – with no clear preference

Table 2.2 shows the species falling in these classes.

Hydrophilous	Hygrophilous	Xerophilous	Eurytopic
Arcella discoides	Arcella rotundata	Arcella arenaria	Assulina muscorum
A. vulgaris	A. arenaria	Bullinularia indica	Centropyxis erystoma
Centropyxis cassis	A. catinus	Centropyxis aerophila	C. aerophila sylvatica
Difflugia acuminata	Assulina semimulum	Phyrangella	Euglypha ciliata
Nebela vitrea	Bullinularia indica	hemispherica	Nebela collaris
	Centropyxis aculeata	Trigonopyxis arcula	
	C. aerophila sphagnicola		
	C. cassis		
	C. platystoma		
	Corythion dubium		
	Euglypha strigosa		
	Heleopera petricola		
	Hyalosphenia elegans		
	Nebela collaris		
	N. lageniformis		
	N. militaris		
	N. tubulosa		
	Pontigulasia spiralis		
	Quadrulella symmetrica		
	Trinema enchelys		

Table 2.2: Classification of peatland testate amoebae by Bartos (1940) (from Woodland, 1996: p.42)

This classification was updated by De Graaf (1956), following differences found in moisture requirements within the hygrophilous group, which was split in to α-hygrophilous

and β-hygrophilous whilst the eurytopic group was dropped. Some however, still consider this group to be relevant (Mitchell *et al.*, 1999). Schönborn (1962) made the first attempt to produce a semi-quantitative classification of testate amoebae moisture requirements. This was done by relating peatland moisture content classes devised by Jung (1936) to testate amoebae populations. Figure 2.9 illustrates the horizontal distribution devised.

Key to species

1	*Trigonopyxis arcula*	9.	*N. militaris*
2	*Centropyxis orbicularis*	10	*Assulina muscorum*
3	*Phyrangella hemispherica*	11	*Amphitrema flavum*
4	*Bullinularia indica*	12	*Heleopera sphagni*
5	*Euglypha compressa*	13	*Nebela marginata*
6	*Corythion dubium*	14	*Difflgia globulosa*
7	*Nebela collaris*	15	*Nebela carinata*
8.	*N. minor*		
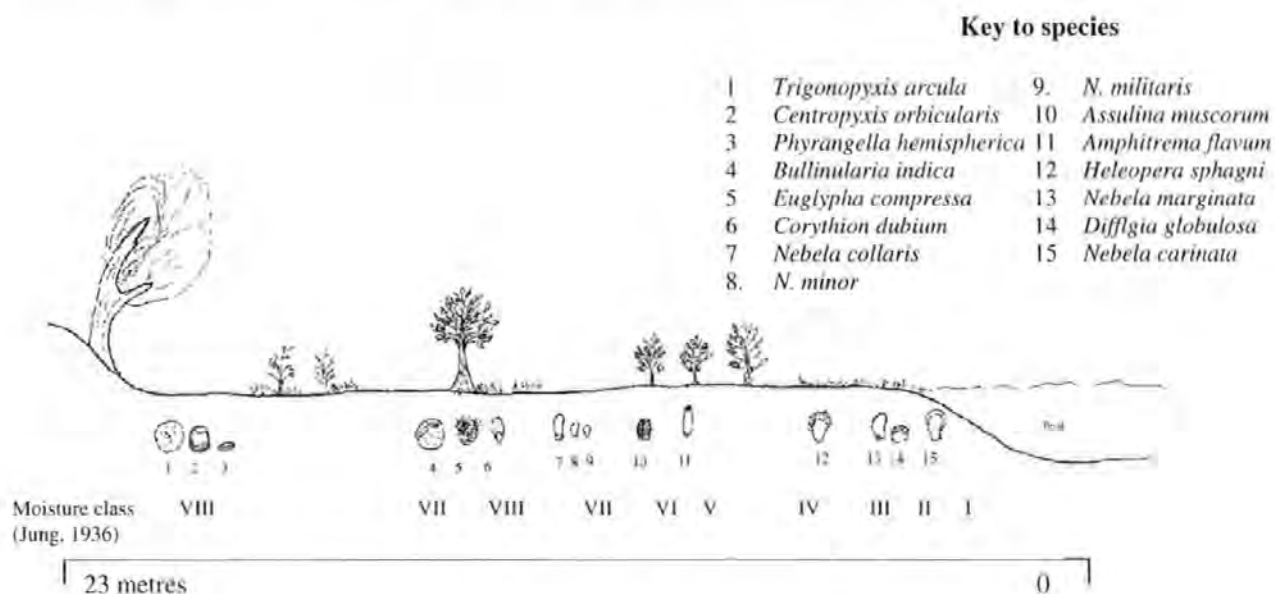
Figure 2.9: Horizontal distribution of testate amoebae in a forested mire (after Schönborn, 1962)

More recently, researchers in Europe (Tolonen *et al.*, 1992; 1994; Woodland, 1996; Lamentowicz & Mitchell, 2005), North America (Charman & Warner, 1992; 1997) and in New Zealand (Charman, 1997) have attempted to produce quantitative moisture requirements for testate amoebae. Extensive studies of testate amoebae communities in British peatlands allowed Woodland (1996), through the development of transfer functions, to produce a tool for hydrological reconstruction. Mitchell *et al.* (1999) took this further and produced in depth analyses of the influence of water table looking at depth to water table, % water in fresh moss samples, total porosity and water content after one hours

drainage. This allows more ecological information to be derived from testate amoebae communities.

The dependence of testate amoebae on water to live is a limiting factor not only in habitat preference but also in size of individuals. The spaces between leaves of *Sphagnum* usually measure up to 300µm in diameter (Corbett, 1973) therefore limiting growth forms of testate amoebae. Heal (1963) postulated that moisture availability restricted the size of individuals. More recent work on variability and polymorphism in testate amoebae has reinforced this theory. Wanner (1999) found that terrestrial species of the same genus are smaller than freshwater species indicating that moisture availabilty was a controlling factor on size. Mitchell *et al.* (1999) also found pore size to be a factor influencing distribution.

2.6.5 Microdistribution & Movement

Testate amoebae distribution on individual *Sphagnum* stems has been widely commented upon and investigated. Quantitative investigations found 71.3% of individuals of *Hyalopshenia papilio* living in the head of the *Sphagnum* plant and 22.8% between 2 and 4 cm from the top (Heal, 1962). Schönborn (1963) found vertical distribution of living testate amoebae was related to the moisture gradient (Figure 2.10). Vertical distributions in moss were also recognised by Beyens (1984), he attributed the paucity of species in the uppermost layers with the absence of food and particles for shell building. More recently, research by Mitchell & Gilbert (2004) into vertical distributions and nitrogen deposition found that species diversity increased with depth. Work on Michigan peatlands also found vertical variation in species assemblages on *Sphagnum* stems (Booth, 2002).
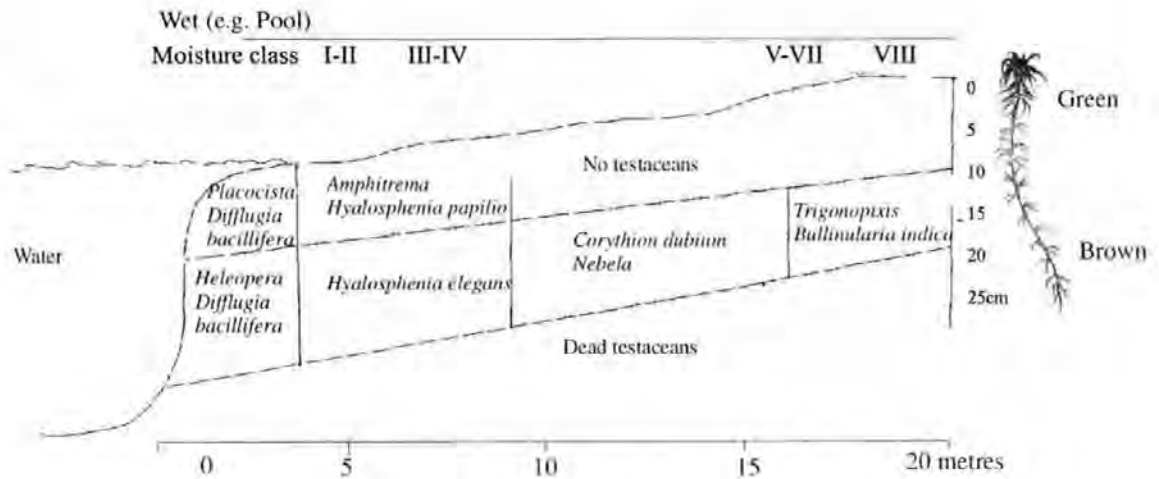
Figure 2.10: Vertical distribution of testate amoebae on a *Sphagnum* stem (after Schönborn, 1963)

The microtopography of a habitat has been found to influence distribution of testate amoebae. The hummock-hollow pattern of a mire surface is reflected in the testate amoebae community (Mitchell *et al.*, 1999). Communities on the moss – soil microecotone in spruce and beech forests and the mesoecotone between a meadow and spruce forest were observed by Balík (1996a, 1996b). He found that there was a conspicuous transition between the moss carpet and the humic soil horizon in the spruce forest including changes in eudominant species. Abundance and biomass of testate amoebae decreased from the moss carpet to the soil. The ecotonal effect between the beech forest and moss was present but less pronounced (Balík 1996a). Studies on the mesoecotone were less successful showing less marked changes (Balík 1996b).

On a larger scale, differences between vegetation and testate amoebae communities in five European bogs were examined by Mitchell *et al.* (2000). The results showed that differences between the vegetation of the sites were much greater than differences in testate

amoebae communities. Testate amoebae, like other free living microbes, appeared to have a cosmopolitan distribution (Finlay, 2002), a theory that has been widely questioned in the past with people preferring to think of the existence of northern and southern populations (Hoogenraad & de Groot, 1979; Warner, 1988; Woodland, 1996). This cosmopolitan nature may be a pan-European distribution rather than global, there is some debate over the distribution of some key taxa, *Apodera (Nebela) vas* (Certes) has been found in samples from the southern hemisphere but is unknown in the northern hemisphere (Mitchell & Meisterfeld, 2005). Mitchell *et al.* (2000) also found that the testate amoebae communities appeared to represent the pollution gradient found across Europe.

The transport and therefore colonisation of testate amoebae is a subject which has been largely unexplored. Pseudopodia, the protoplasmic projections used in feeding are also used for moving around (Woodland, 1996). It has also been suggested that wider dispersal is effected by birds (Charman *et al.*, 2000) or mites (Chardez, 1960). Water transport was also considered to be a factor influencing distribution.

2.6.6 Water Chemistry

Various studies have found that pH influences the distribution of testate amoebae (Heal, 1961; Mitchell *et al.*, 2000; Lamentowicz & Mitchell, 2005). In detailed analyses though, it has been seen as secondary to moisture availability (Charman & Warner, 1992; 1997; Vincke *et al.*, 2004). Corbett (1973) suggested that it might affect microdistribution where acid hummocks emerge from otherwise alkaline flushes. In studies in the Jura mountains, Mitchell *et al.* (1999) found that *Difflugia tuberculata* was unable to reproduce where the pH was less than 4.5 suggesting that some, if not all, taxa could be useful as indicators of pH. Heal (1961; 1964) found that species had distinct preferences for either bog or fen sites

and that fens (pH>5) had greater species diversity than bogs. Beyens (1986) also found a positive relationship between species diversity and alkalinity.

Experiments on the effects of artificial enrichment have shown that increased availability of nutrients has a negative impact on the testate amoebae communities in nutrient-poor, acid peatlands (Gilbert et al., 1998). These decreases in numbers could have been the result of increased populations of other microorganisms increasing competition for space, light, moisture and food. Tolonen et al. (1994) found that the Carbon/Nitrogen ratio in peat was an important control on species distribution. Woodland (1996) found insufficient evidence to extend this to British peatlands.

Carbon dioxide (CO_2) and oxygen (O_2) tensions have been found to influence testate amoebae populations. They have been found to be less tolerant of high CO_2 and low O_2 tensions than other protozoa (Stout & Heal, 1967).

2.6.7 Other Influences

Many of the factors influencing testate amoebae taxonomy may also influence distribution. Stout & Heal (1967) suggested availability of test building materials is important in distribution whilst pore space was a factor affecting species composition.

Pollution was indicated as a possible controlling factor on testate amoebae communities by Mitchell et al. (2000).

Balík (1991) found that pollution from road traffic around Warsaw had an impact on testate amoebae populations lowering abundance, number of species and species diversity at more polluted sites. More recent research into the possibility of using testate amoebae as

bioindicators of atmospheric pollution found that species richness was lower in polluted zones but that densities were unaffected (Nguyen-Viet *et al.*, 2004).

The above factors can only be considered to be minor influences on testate amoebae populations with the major factors affecting distribution being hydrology and pH.

2.6.8 Palaeoecology

Testate amoebae analysis, as a technique for environmental indication was developed within palaeoecology. Over the last ten years it has become a firmly established technique for reconstructing hydrological change in past environments. The majority of the work has been carried out in the Northern Hemisphere, particularly in Canada (Charman & Warner, 1992, 1997; Warner & Charman, 1994) Britain (Woodland, 1996; Hendon, 1998; Woodland *et al.*, 1998) and northern Europe (Tolonen, 1992, 1994; Mitchell *et al.*, 1999, 2000). Some preliminary work has been carried out in New Zealand (Charman, 1997) with the aim of developing effective methodologies and transfer functions.

Although this PhD focuses primarily on modern ecological preferences of testate amoebae, the results will also be relevant to palaeoecology. The work on diatom responses to lake acidification have provided further insight into diatom ecology which has proved useful for their use as palaeoecological proxies. Testate amoebae's hydrological preferences are currently linked to depth to water table. Seasonal patterns and variation between different peat surfaces may prove useful in understanding fossil communities.

2.6.9 Potential as Bioindicators

Bioindicators are organisms that are used for qualitative and or quantitative characterisation of environmental factors (Foissner, 1999). The potential for palaeoecological tools to be developed into modern environmental indicators has been established with the use of diatoms as indicators of acidification of lakes (Battarbee & Charles, 1987). The potential for using testate amoebae and other soil protozoa as biological indicators of environmental quality has been examined by Foissner (1997, 1999). Other work has indicated that testate amoebae have potential as bioindicators although morphological changes are seen to be more important than species distribution, abundance and community composition (Wanner, 1995).

In a comparative study of agricultural systems, testate amoebae were found to be highly sensitive bioindicators in agroecosystems as their populations were greatly reduced by intensive agricultural practices (Foissner, 1997). A later paper by Foissner (1999) indicated that testate amoebae were useful indicator organisms in many terrestrial environments because they are more easily counted than other protozoans and because of their high biomass and abundances.

This study will investigate the potential for developing the bioindicator role of testate amoebae as a system for monitoring peatlands. It seeks to develop the knowledge of the ecological requirements in the context of modern degraded mires.

2.7 Summary

This chapter has outlined the conservation importance of British peatlands both in terms of British ecology and as a part of the global resource. It has also considered the importance

of peatlands in the global carbon cycle. They are the most important terrestrial store of CO_2 and therefore of great importance in terms of global climates and as a factor in future global environmental change – climatic warming in particular. The issue of peatland restoration has been considered and also the types of restoration currently being researched and implemented. Management and monitoring problems in peatlands were examined.

The literature on testate amoebae has been reviewed including aspects of their biology, ecology, taxonomy and uses in palaeoecology. The potential of testate amoebae as biological indicators was also considered.

The following chapters will draw upon the literature cited here to help understand the modern ecology of testate amoebae in damaged peatlands in the UK. This chapter provides the background to the modern pressures on peatlands, to restoration work being carried out at the study sites and the potential problems. The possibility of using testate amoebae as a tool for monitoring damage and restoration is considered.

CHAPTER THREE: Coom Rigg Moss Part 1: Methodological Studies

3.0 Introduction

As a prelude to the study at Coom Rigg Moss, fully described in Chapter 4, a test of the sampling strategy employed there was carried out. The aim of this experiment was to test the sampling strategy, provide an indication of its effectiveness, and thus assist in the establishment of experiments at other locations.

The main experiment at Coom Rigg Moss involved monthly sampling from the same locations over a period of 24 months to examine the response of testate amoebae populations to ditch blocking. Small sampling plots were established to minimise microtopographical variability. Sample sizes were also small (single stems of *Sphagnum*) to minimise disturbance of the sample plots. It was important to ensure that both sample sizes and plot sizes would produce robust and comparable results. A small experiment was set up to test whether the testate amoebae assemblage from a single stem of *Sphagnum* was generally representative of the community in a 25 by 25cm area of bog. Four trial plots each comprising an individual species of *Sphagnum* were sampled on the 10th July 2000. The plots were selected to cover the major species of *Sphagnum* sampled in the main experiment at the site. Figure 3.1 shows the location of the sampling plots on the bog. The plots of *S. magellanicum* (*SM*), *S. papillosum* (*SP*) and *S. capillifolium* (*SC*) were all located next to Forestry Commission plot D. *S. tenellum* (*ST*) was sampled from the north side of the bog next to plot A as this was the only location where there was a large enough continuous area of this species. Water table data were not collected from the individual plots; however, the layout means that the data from the long term monitoring plots can give a general idea of water level within these trial plots. Percentage moisture data were calculated from wet and dry weights of samples.
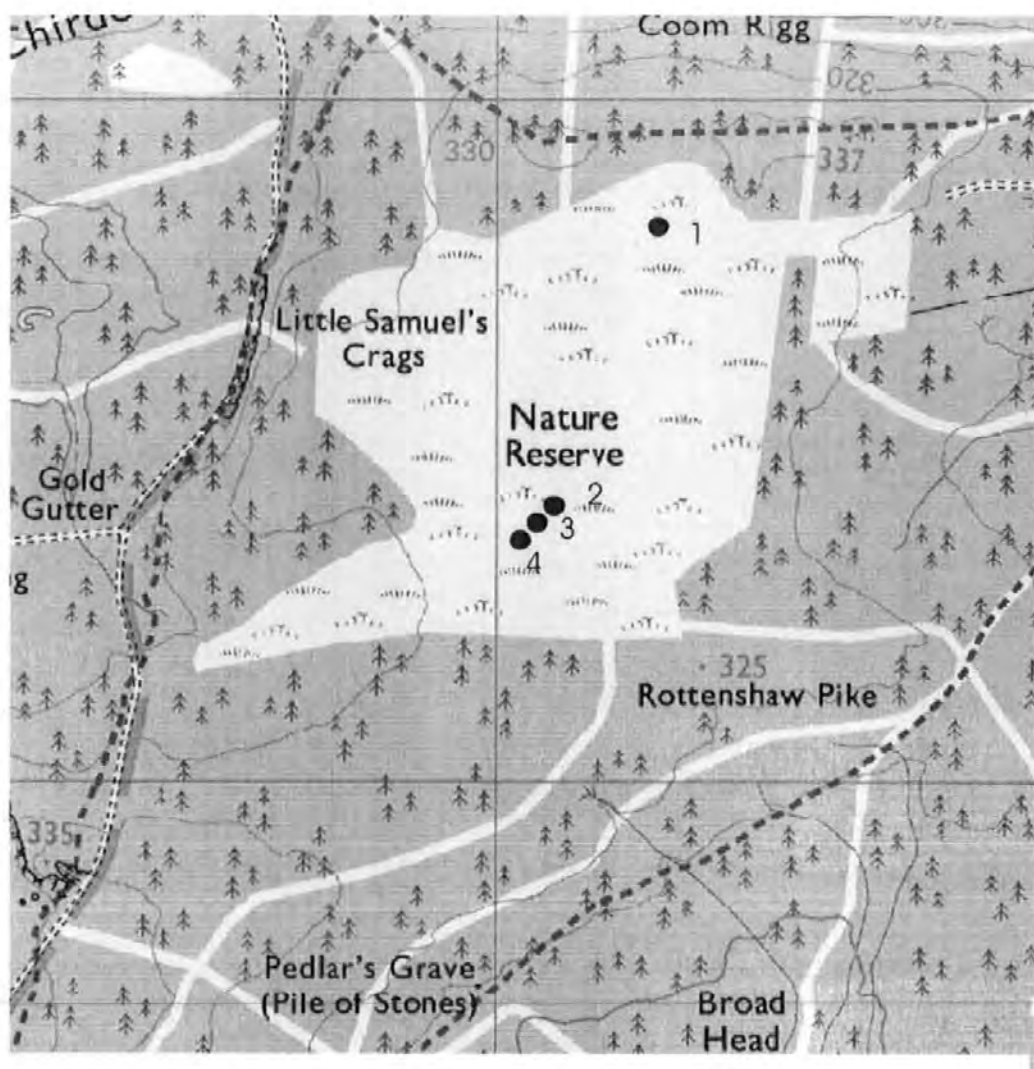
Scale 1:10000
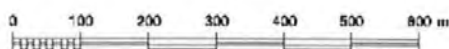
Sample plot ●
1 = *Sphagnum tenellum*
2 = *Sphagnum magellanicum*
3 = *Sphagnum capillifolium*
4 = *Sphagnum papillosum*

Coom Rigg hydrological unit

Figure 3.1: Map of Coom Rigg Moss showing locations of sampling plots.

Each of the four plots was 25 by 25cm – replicating the layout of the long-term monitoring plots. Ten individual *Sphagnum* stems were sampled randomly from each plot. The results of this sub-study should allow quantification of potential methodological errors.

3.1 Sample preparation

Samples were taken to the laboratory in Plymouth for processing. Each stem was weighed to record a wet weight, placed into a sterilin tube and covered by a Rose Bengal and alcohol solution. Rose Bengal is a dye effective on live material and the process allows living and dead individuals to be separated. The exotic marker spore *Lycopodium clavatum* was added at this stage. One tablet (~12,500 spores) was added to each sample. This is a standard palaeoecological method for deriving concentrations and allowing comparisons between samples (Stockmarr, 1971).

The samples were left in the Rose Bengal stain overnight and then sieved, through a 300µm sieve and back sieved (15µm) as recommended by Hendon and Charman (1997). Samples were rinsed with distilled water in the process of sieving to remove excess stain. Samples were then centrifuged and mixed with glycerol jelly to make microscope slides.

Counts of testate amoebae were made at x100 magnification with x400 used for further identification. Identification was aided by the use of the key in Charman *et al.* (2000), with reference to other monographs where necessary. At least 150 individuals in each sample were counted unless there were too few tests present. For single stems, the small sample sizes meant that sometimes there was very little living material. Counting therefore continued until either two slides had been completed and at least 500 *Lycopodium* spores counted or until 250 individuals had been counted.

Other microscopic organisms were often found on slides, including nematodes, mites and rotifers, and were identified where possible using keys in Hingley (1993). As for testate amoebae, where identification was not possible, photographs were taken using image analysis, and stored for further reference. These organisms were not always included in analyses but data may be useful for future work.

Counts were made of testate amoebae populations on each stem and the 'other' organisms found were also counted. The results were expected to show that there were more differences between plots than within them. This in turn would confirm that potential changes in testate amoebae communities in the permanent plots at Coom Rigg Moss were the result of changing hydrological conditions rather than natural variability of the sample plots.

3.2 Results

3.2.1 Physical characteristics of the plots

The four species of *Sphagnum* vary both in physical form and in growing habit. *Sphagnum papillosum* and *S. magellanicum* are hummock-forming species; individual plants are large or 'swollen' with large leaves and stems (Hill, 1992). *S. capillifolium* also forms hummocks but has much finer leaves and stems. *S. tenellum* is more likely to grow in wet lawns and has fine leaves and stems compared with the other species (Watson, 1981; Hill, 1992). Figure 3.2 illustrates these physical differences in growth form.
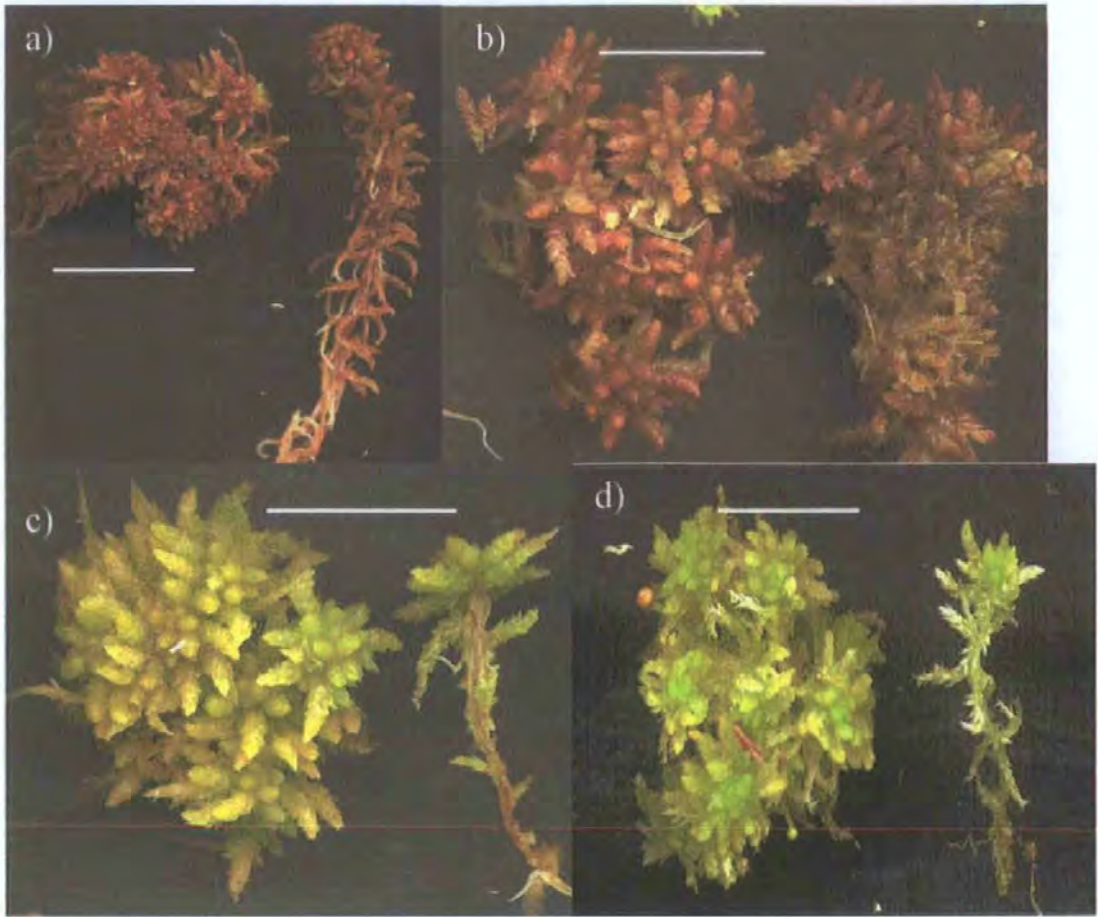
Figure 3.2: Images of *Sphagnum* species showing the differences in size of stems, leaves and capitula. a) *S. capillifolium* b) *S. magellanicum*, c) *S. papillosum* d) *S. tenellum*. White scale line in images represents ~ 1cm.

3.2.2 Moisture content

Although water table measurements were not taken within the individual plots, moisture content of the individual samples was measured. Table 3.1 shows the means and standard deviations for each sample plot. *S. magellanicum* had the highest moisture content and *S. tenellum* the lowest. *S. tenellum* appears to be the most variable as it has the highest standard deviation. To test whether the moisture variation between plots was greater than within them, non-parametric analysis of variance was used. A non-parametric test was used as the data were not normally distributed. Kruskal-Wallis tests showed that there were more differences between samples than within them, ($p = 0.00$). Figure 3.3 is the dotplot of moisture content from the MINITAB output. *Post-hoc* testing is recommended

to interpret a positive result from analysis of variance. Dytham (2003) suggests using the Mann-Whitney test on each pair of samples as a non-parametric alternative to the least significant difference (LSD) test.

```
                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level        N      Mean    StDev   ---------+---------+---------+-------
SC          10    92.931    0.404   (--*--)
SM          10    98.435    0.796                                 (--*--)
SP           9    94.812    0.519              (--*--)
ST          10    94.780    1.576              (--*--)
                                    ---------+---------+---------+-------
Pooled StDev =     0.952                   94.0      96.0      98.0
```
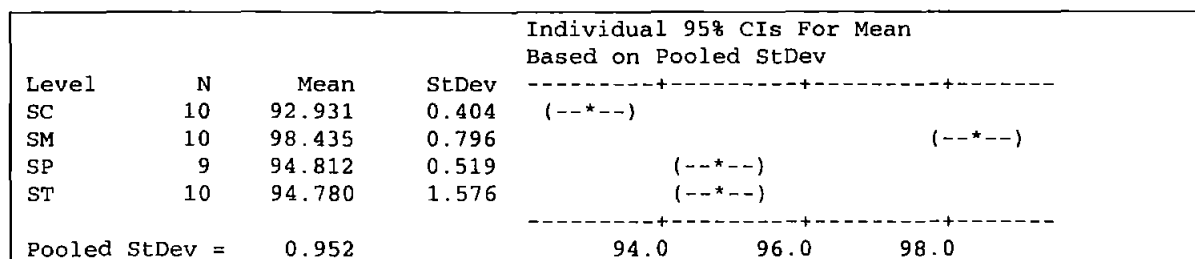
Figure 3.3: Dotplot of moisture content.

% Moisture	S. capillifolium	S. magellanicum	S. papillosum	S. tenellum
Mean	92.93	98.44	98.81	94.78
St Dev	0.40	0.80	0.52	1.58
Median	93.00	98.59	94.83	94.49

Table 3.1: Mean and median % moisture with standard deviations for each plot, indicated by the *Sphagnum* species sampled.

Table 3.2 shows the results for the Mann-Whitney tests on the pairs of samples. The only pair of samples which are not statistically different are *S. papillosum* and *S. tenellum*. The differences between all other pairs of samples are statistically significant. The sample plot with the highest moisture content is *S. magellanicum* and with the lowest moisture content, *S. tenellum*. There is more difference in moisture content between sample plots than within them.

	S. capillifolium	S. magellanicum	S. papillosum	S. tenellum
S. capillifolium (1st sample)	-	p = 0.00 ** (less than)	p = 0.00 ** (less than)	p = 0.00 ** (less than)
S. magellanicum (1st sample)	p = 0.00 ** (greater than)	-	p = 0.00 ** (greater than)	p = 0.00 ** (greater than)
S. papillosum (1st sample)	p = 0.00 ** (greater than)	p = 0.00 ** (less than)	-	p = 0.22 (greater than)
S. tenellum (1st sample)	p = 0.00 ** (greater than)	p = 0.00 ** (less than)	p = 0.22 (less than)	-

Table 3.2: Results for Mann-Whitney tests used for *post-hoc* testing on moisture content of sample groups. (= significant at 99%)**

3.2.3 Testate amoebae data

Testate amoebae data was analysed in several sections. Firstly, a comparison of numbers of taxa was made to see whether one plot has higher numbers of taxa than the others. Raw numbers were then examined to see whether there was any variability in the numbers of testate amoebae found in the four species of *Sphagnum*. Species composition was compared to see whether there were distinct patterns within sample plots. Statistical analyses were then carried out on concentrations, percentages and percentage live data. Finally, relationships were explored using multivariate analysis techniques.

3.2.4 Numbers of taxa

The species variability within sample plots can be assessed by looking at numbers of taxa found in each plot (Table 3.3). The highest total number of taxa was found in the *S. papillosum* sample plot where mean per stem was also highest. The lowest taxa total was in *S. magellanicum* although the lowest stem mean was in *S. capillifolium*.

	S. capillifolium	*S. magellanicum*	*S. papillosum*	*S. tenellum*
Total taxa per plot	17	16	24	23
Mean per sample	7.60	10.20	12.56	10.3
StDev	2.88	1.75	2.44	3.33
Median	7.5	10	12	10.5

Table 3.3: Number of taxa by plot with mean and medians per stem and standard deviations.

To test whether the differences in numbers of taxa were statistically significant, a Kruskal-Wallis test was carried out. The test produced a significantly significant result with a p value of 0.00 (adjusted for ties) showing that there is more variation in number of taxa between sample plots than within the sample plots. *Post-hoc* testing was carried out using Mann-Whitney tests on each pair of samples as for the moisture data. This showed that all pairs were significantly different except for *S. magellanicum* and *S. tenellum*. Where

relevant, results for this section are adjusted for tied data, which means that observations with identical values are taken into account in the analysis.

	S. capillifolium	S. magellanicum	S. papillosum	S. tenellum
S. capillifolium (1st sample)	-	p = 0.03 * (less than)	p = 0.00 ** (less than)	p = 0.04 * (less than)
S. magellanicum (1st sample)	p = 0.02 * (greater than)	-	p = 0.02 * (less than)	p = 1.00 (not equal)
S. papillosum (1st sample)	p = 0.00 ** (greater than)	p = 0.02 * (greater than)	-	p = 0.04 * (greater than)
S. tenellum (1st sample)	p = 0.04 * (greater than)	p = 1.00 (not equal)	p = 0.04 * (less than)	-

Table 3.4: Results for Mann-Whitney tests used for *post-hoc* testing on numbers of taxa by sample plot. (* = significant at 95%, ** = significant at 99%)

3.2.5 Differences between testate amoebae populations

a) Raw Numbers

To make further comparison in total numbers counted between samples, concentrations and live percentages were compared. The aim was to count a minimum of 100 testate amoebae from each sample. This minimum was not achieved for 9 samples from the 40. The numbers counted in the individual stems and in different species of *Sphagnum* varied greatly as is illustrated in Table 3.5. Highest counts were made in the *S. magellanicum* plots and the lowest in the *S. tenellum* plot which also had the most samples with counts of fewer than 100 testate amoebae.

	ST	SP	SM	SC
1	51	64	127	203
2	109	107	209	30
3	15	105	113	108
4	81	101	180	103
5	108	110	121	10
6	13	140	158	134
7	160	-	112	121
8	114	158	205	291
9	102	109	209	214
10	23	86	209	106
Mean	77.60	108.89	163.30	132.00
SD	49.90	27.39	42.90	84.70
Median	91.5	107.0	169.0	114.5

Table 3.5: Numbers of testate amoebae counted by sample with mean and medians and standard deviations per sample plot.

Again, a Kruskal-Wallis test was carried out on the data. The p value was 0.00 which means that the numbers counted were significantly different between *Sphagnum* species. The results from *post-hoc* testing are shown in Table 3.6. *S. magellanicum* had significantly higher numbers of testate amoebae than *S. papillosum* and *S. tenellum*. The other samples were not significantly different. Figures are adjusted for ties where relevant.

	S. capillifolium	S. magellanicum	S. papillosum	S. tenellum
S. capillifolium (1st sample)	-	p = 0.10 (less than)	p = 0.27 (greater than)	p = 0.11 (greater than)
S. magellanicum (1st sample)	p = 0.10 (greater than)	-	p = 0.0 ** (greater than)	p = 0.00 ** (greater than)
S. papillosum (1st sample)	p = 0.27 (less than)	p = 0.00 ** (lessthan)	-	p = 0.20 (greater than)
S. tenellum (1st sample)	p = 0.11 (greater than)	p = 0.00 ** (less than)	p = 0.20 (less than)	-

Table 3.6: Results for Mann-Whitney tests used for *post-hoc* testing on numbers of testate amoebae by sample plot. (= significant at 99%)**

b) Concentration Data

Concentration data was calculated for each sample using the formula below:

$$\text{Total concentration per gram dry weight} = \left(\left(\frac{1}{\text{Sample dry weight}} \right) \times \left(\frac{\text{Total testate amoebae per stem}}{\text{Total lycopodium per stem}} \right) \right) \times 12,500$$

To see whether high numbers of individual testate amoebae on stems translated into high concentrations of testate amoebae, the above analysis was repeated on concentration data. Logged concentrations were used to minimise noise in the dataset. The highest concentrations were found in *S. magellanicum* as with total counts and the lowest in *S. papillosum*. Lowest total concentrations were found in *S. tenellum* with *S. papillosum* coming a close second. Kruskal-Wallis testing was carried out on logged concentrations. The result of the test was significant at the 99% confidence interval ($p = 0.00$).

	S. capillifolium	*S. magellanicum*	*S. papillosum*	*S. tenellum*
Mean	4.571	4.879	4.143	4.203
S.D.	0.147	0.068	0.051	0.123
Median	4.711	4.887	4.151	4.265

Table 3.7: Logged concentrations by plot with mean and medians per stem (no/g dry weight) and standard deviations.

Post-hoc testing showed that there were significant differences between all pairs of samples except for *S. papillosum* and *S. tenellum*. This contrasts with numbers counted, where the only difference was between *S. magellanicum* and *S. tenellum* and *S. papillosum*.

	S. capillifolium	S. magellanicum	S. papillosum	S. tenellum
S. capillifolium (1st sample)	-	p = 0.04 * (less than)	p = 0.02 * (greater than)	p = 0.03 * (greater than)
S. magellanicum (1st sample)	p = 0.04 * (greater than)	-	p = 0.00 ** (greater than)	p = 0.00 ** (greater than)
S. papillosum (1st sample)	p = 0.03 * (less than)	p = 0.00 ** (lessthan)	-	p = 0.14 (less than)
S. tenellum (1st sample)	p = 0.03 * (less than)	p = 0.00 ** (less than)	p = 0.14 (greater than)	-

Table 3.8: Results for Mann-Whitney tests used for *post-hoc* testing on logged concentrations of testate amoebae by sample plot. (* = significant at 95%, ** = significant at 99%).

c) Percentage live

The percentage of living testate amoebae has been calculated for each individual taxon but also, an overall live percentage has been calculated for the individual stems. This has been used as it is thought to be a measure of population turnover. This may be useful when tracking restoration projects. The Kruskall-Wallis test was carried out on percentage live but the result was not significant (p = 0.141) so *post-hoc* testing was not performed. This analysis suggests that the variation in population turnover was greater within sample plots than between them

3.2.6 Species Composition of the Sample Plots

Concentrations and percentages of testate amoebae and other organisms were calculated from the raw counts. Percentage live data was also calculated. Some descriptive analysis has been carried out. Due to the large amounts of data generated in these counts, summary tables of percentages have been made for each sample set (Table 3.9). Only those taxa which made up more than 5 percent of the population in one or more set of samples have been included in this table. Percentages have been used as they are more easily compared than concentrations which were highly variable between samples. Testate amoebae taxa

are arranged by hydrological preference. A frequency value has been calculated along the lines of phytosociological classifications (Clements, 1916), based on the number of samples in which a species is present. An explanation of classifications is shown below.

i.   species present in 1-2 of 10 samples

ii. species present in 3-4 of 10 samples

iii. species present in 5-6 of 10 samples

iv. species present in 7-8 of 10 samples

v.   species present in 9-10 of 10 samples

A range, value and mean are shown alongside frequency. This is shown as a percentage (Table 3.9). This table allows the level of similarity to be assessed qualitatively. Where samples are most consistent, frequency values will be high and ranges low. Total number of taxa found in each sample plot has also been included in this table.

Testate amoebae		ST	SP	SM	SC
Euglypha strigosa	Mean	4.89	5.96	9.14	11.27
	StDev	3.96	5.75	5.64	17.76
	Range	0-13	0-20	2-20	0-54
	Frequency				
Euglypha tuberculata	Mean	0.85	5.06	2.66	3.21
	StDev	1.17	7.45	3.56	4.97
	Range	0-3	0-24	0-11	0-14
	Frequency				
Amphitrema flavum	Mean	0.00	0.53	0.13	47.20
	StDev	0.00	0.87	0.29	45.48
	Range	-	0-2	0-1	0-95
	Frequency	-			
Nebela tincta	Mean	20.21	24.82	44.11	12.42
	StDev	11.80	7.75	17.24	12.42
	Range	8-43	16-36	19-65	2-40
	Frequency				
Assulina semimulum	Mean	7.64	0.42	0.00	0.00
	StDev	6.92	0.96	0.00	0.00
	Range	0-23	0-3	-	-
	Frequency			-	-
Assulina muscorum	Mean	14.76	1.36	5.18	4.04
	StDev	13.14	1.78	7.21	4.06
	Range	0-41	0-5	0-21	0-10
	Frequency				
Heleopera sphagni	Mean	17.14	2.68	6.12	0.00
	StDev	13.06	3.86	5.76	0.00
	Range	5-45	0-10	1-18	-
	Frequency				-
Corythion -Trinema typ	Mean	8.62	31.24	3.53	13.95
	StDev	8.60	14.27	6.16	16.38
	Range	0-25	6-48	0-21	1-43
	Frequency				
Nebela flabellulum	Mean	6.51	6.69	6.24	2.60
	StDev	9.40	2.78	7.73	5.14
	Range	0-16	2-10	0-23	0-14
	Frequency				
Heleopera petricola	Mean	10.81	12.02	17.30	2.68
	StDev	8.61	7.04	11.28	4.40
	Range	0-27	1-25	2-41	0-15
	Frequency				

Table 3.9: Summary of testate amoebae counts showing mean percentages, range and constancy. Only taxa present at >5% in at least one sample plot are shown. Constancy values i to v represented by shading – lightest to darkest. ST = *Sphagnum tenellum*, SP = *S. papillosum*, SM = *S. magellanicum*, SC = *S. capillifolium*.

From this table dominant taxa can be identified. For the purpose of these descriptions, dominant taxa are those present at higher than 10% of the total population. Those taxa making up between 5 and 10 % are considered to be important components of the

population but less dominant. The samples of *Sphagnum tenellum* are dominated by the testate amoebae *Assulina muscorum*, *Nebela tincta*, and *Heleopera sphagni*. *S. papillosum* samples are dominated by *Heleopera petricola*, *Nebela tincta*, and *Corythion-Trinema* type. *S. magellanicum* is dominated by *Heleopera petricola* and *Nebela tincta*. The samples of *S. capillifolium* form two distinct groups: one dominated almost entirely by *Amphitrema flavum* to the exclusion of other testate amoebae species; the second dominated by *Euglypha strigosa*, *Nebela tincta* and *Corythion-Trinema* type. The reasons for the divisions within this set of samples are unknown, as there was no obvious physical variability in the sample plot.

The highest numbers of taxa were found in the *S. papillosum* and *S. magellanicum* samples and the lowest in the *S. capillifolium* and *S. tenellum* samples. These species of *Sphagnum* are very different in structure. *Sphagnum papillosum* and *S. magellanicum* have large leaves and stems and *Sphagnum capillifolium* and *S. tenellum* is much finer with small leaves and stem (Plate 3.1). Testate amoebae live in the films of water between leaves and it is thought that a larger leaf size provides greater living space thus supporting larger populations (Mitchell *et al.*, 2003).

The large number of other organisms occurring in the samples have been counted and, where possible, identified. As there were so many unidentified organisms, the data have not been presented in tabular format; however, it appears that some of these organisms could be important in the characterisation of sample set. Most sample sets have high frequencies of Bdelloid rotifer species. Nematodes also seem to be important components of all sample sets. Analyses of the datasets were carried out both including and excluding these organisms, so that some idea of their importance as a means of characterising sample

groups could be gained. Only the mites *Ceratoppa bipilis*, *Hermannia gibba* and the rotifer *Habtrocha angusticollis* were identified to species level.

All of the sample sets have some variability within them, mostly in the presence of different species in individual samples. These species are generally present at low concentrations or make up a low percentage of the total testate amoebae population.

3.2.7 Multivariate Analysis

The analysis of data in the previous section suggested strong differences between sample plots. Moisture content of samples varied much more between sample plots than within individual samples. Numbers of testate amoebae found appeared to be dependent on the sample plot and species composition was highly variable between plots.

To further investigate these patterns, the next stage was to carry out multivariate statistical analyses on the species composition of the samples. Similarity testing and cluster analysis using PC-Ord were performed to see whether distinct groups could be identified and whether divisions would form along the lines of the sampling sets. This section is divided into three sub-sections, analysis of concentration data, percentage data and percentage live data.

a) Concentration data

Cluster analysis was performed on the concentration data. Data were analysed using relative, or squared, euclidean distances to calculate similarity coefficients and Ward's method or minimum variance clustering, the methods recommended by Kent & Coker (1992). The dendrogram resulting from these analyses is shown in Figure 3.4. Sample

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*SP*7 was excluded from the analysis as *Lycopodium* spores were not added in the processing stage and therefore concentrations could not be calculated.

This dendrogram shows the samples falling into four distinct groups:

1. *Sphagnum tenellum* and *S. papillosum.* This group includes all *ST* except *ST* 8. All *SP* samples and *SC* 2, 3, 4 and 5.

2. *S. magellanicum* (a). This group is dominated by *SM* samples and also contains *SC* 1 and *ST* 8

3. *S. magellanicum* (b). Comprises the remaining *SM* samples (2, 6 and 10)

4. *S. capillifolium* 6-10

The most distinctive group is composed of the *S. capillifolium* samples with the high counts of *Amphitrema flavum*. *SM* samples are distinct from *SP* and *ST* samples which are grouped together with *SC* 2-5. The overlap between *SP* and *ST* can probably be attributed to the similar moisture characteristics.

To gain a greater understanding of the groups Detrended Correspondence Analysis (DCA) was performed using the program CANOCO (Ter Braak & Šmilauer, 1998). Group four was excluded from this analysis as it is so distinctive and thus may obscure the patterns in the rest of the data.

Figure 3.4: Dendrogram showing cluster analysis of concentration data including all organisms
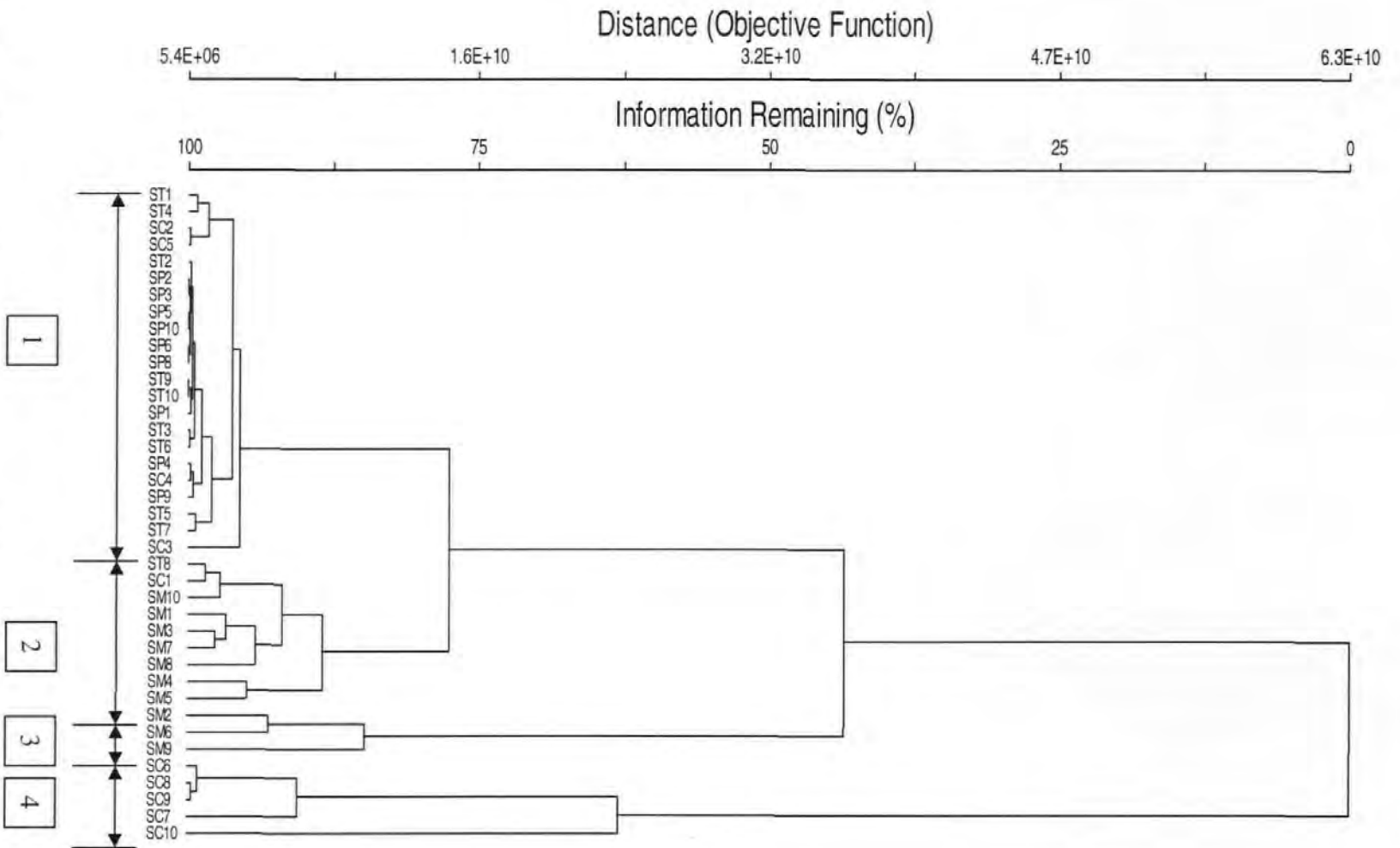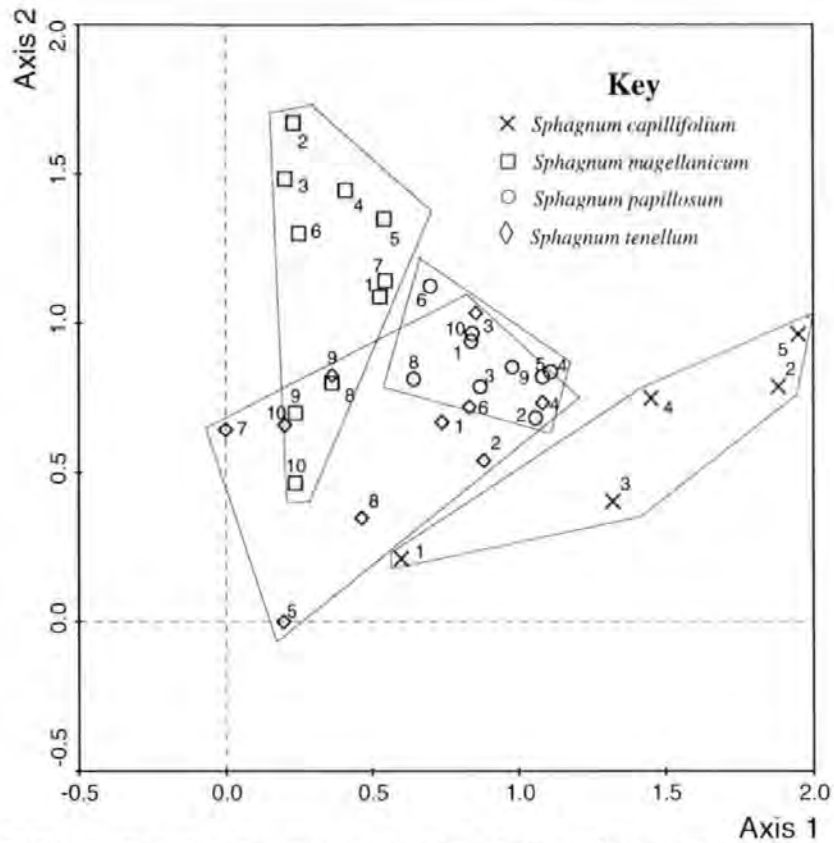
Figure 3.5: DCA ordination on concentration data (all organisms). Polygons have been drawn to show groups and overlap. Numbers refer to sample number (Table 3.5).

Figure 3.5 shows the DCA ordination for concentration data including all organisms corresponding with the data used in figure 3.4 but excludes samples *SC* 6-10. It shows that there is close association between sample plots, the large amount of overlap between *SP* and *ST* samples seen in the cluster analysis is also clear in this graph. The *SC* group is widely dispersed on the first axis. Samples with low counts, from the *ST* plot (3, 4 and 6) overlap more with *SP* samples than those with high counts. *SC* 2 and 5 also had low counts and are at the extreme of this widely dispersed group.

Figure 3.6 shows the dendrogram produced using only testate amoebae concentration data. Divisions between groups are the same as for the complete dataset (Figure 3.5) but there are some minor differences in *SP/ST* group.
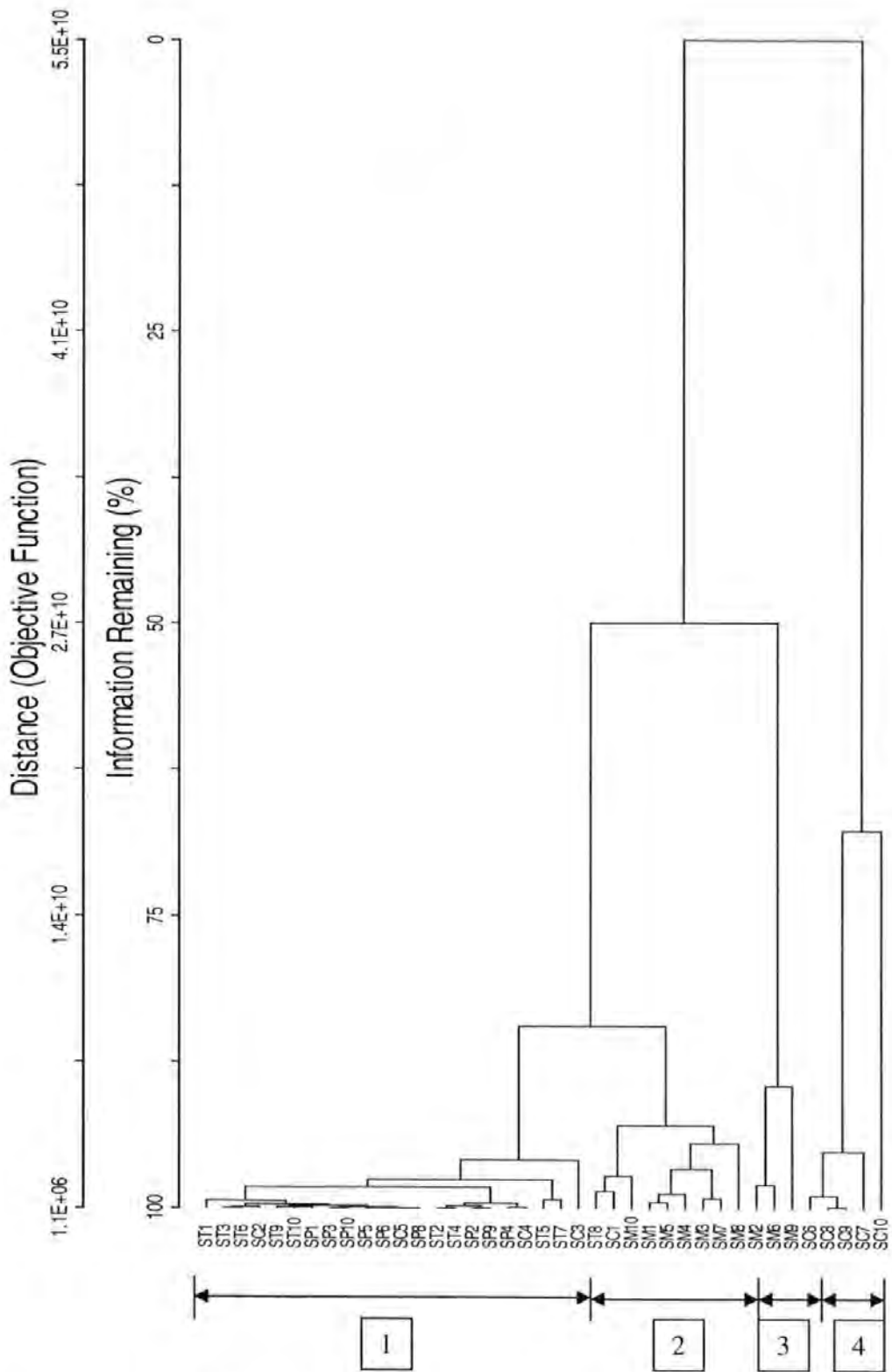
Figure 3.6: Dendrogram showing cluster analysis based on testate amoebae concentration data only.

Figure 3.7 shows the DCA output for this dataset. It shows more distinct groups than Figure 3.5 – the majority of *SM* samples are closely bunched with only *ST10*, which had a low testate amoebae count, falling inside the *SM* group. *ST* samples are not as tightly clustered, perhaps reflecting the greater variability in moisture content, but appear to be more closely related to each other than to the samples from other groups. *SP* samples are closely clustered and distinct from other groups. The *SC* group is, although widely dispersed on the second axis, closely positioned in terms of the first axis. *SC* 5, which is positioned away from the other *SC* samples, has a count of only 10 testate amoebae.
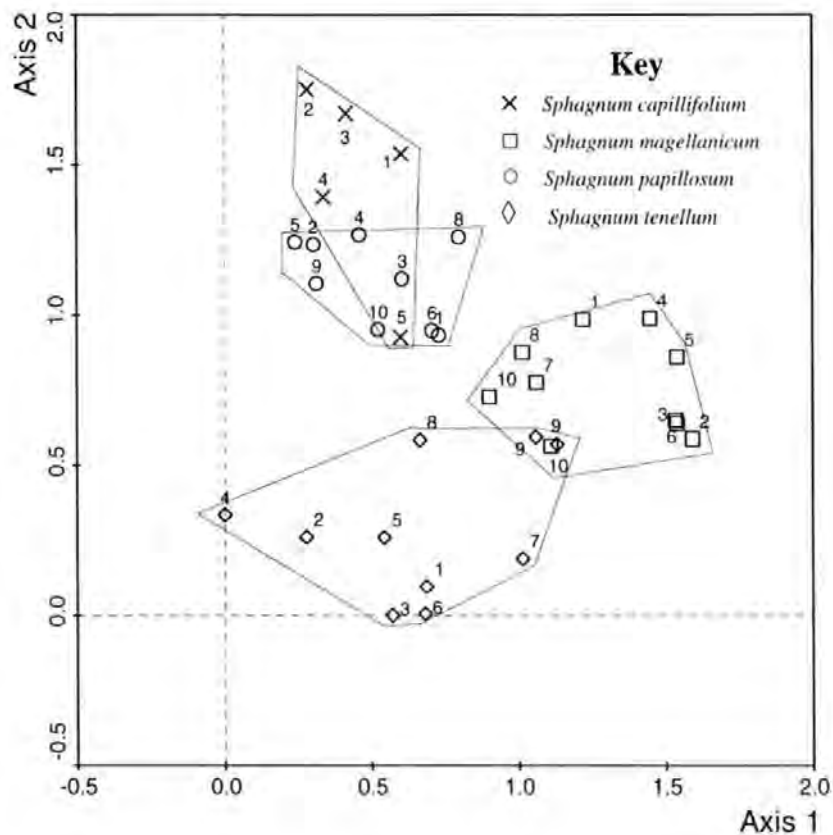
Figure 3.7: DCA on testate amoebae concentration data. (See Fig. 3.6 for further explanation).

CCA was performed on testate amoebae concentrations, moisture data and stem length. Stem length showed no relationship with species composition. However moisture content was more significant. The output including % moisture is shown in Figure 3.8. This ordination includes the *Amphitrema flavum* dominated *S. capillifolium* samples. All

samples appear to be strongly constrained by moisture content. *SM* samples are the most distinct. This agrees with the results of the Kruskal-Wallis test which showed that *SM* samples had high moisture content (Table 3.1, Figure 3.3). *SP* samples had the highest moisture content; a further, unmeasured, factor must influence the species composition of these samples as they are more closely aligned with the drier samples. The differences between the two groups of SC samples are illustrated very clearly here on axis 2 indicating that they are not a function of % moisture.
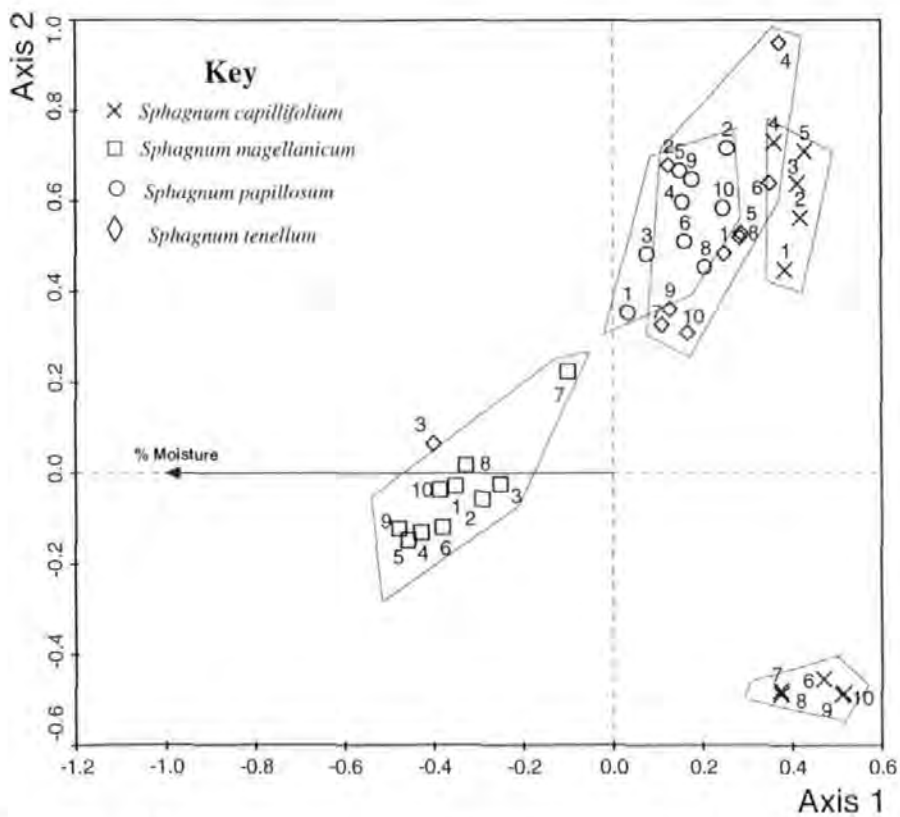
Figure 3.8: CCA of testate amoebae concentrations and moisture content. (See Fig. 3.5 for further explanation).

b) Percentage Data

Analyses were also carried out on percentage data. Cluster analysis and ordination was used as for the concentration data. Figure 3.9 shows the dendrogram produced from the testate amoebae percentage data. Percentage data were calculated as a proportion of the total number of testate amoebae
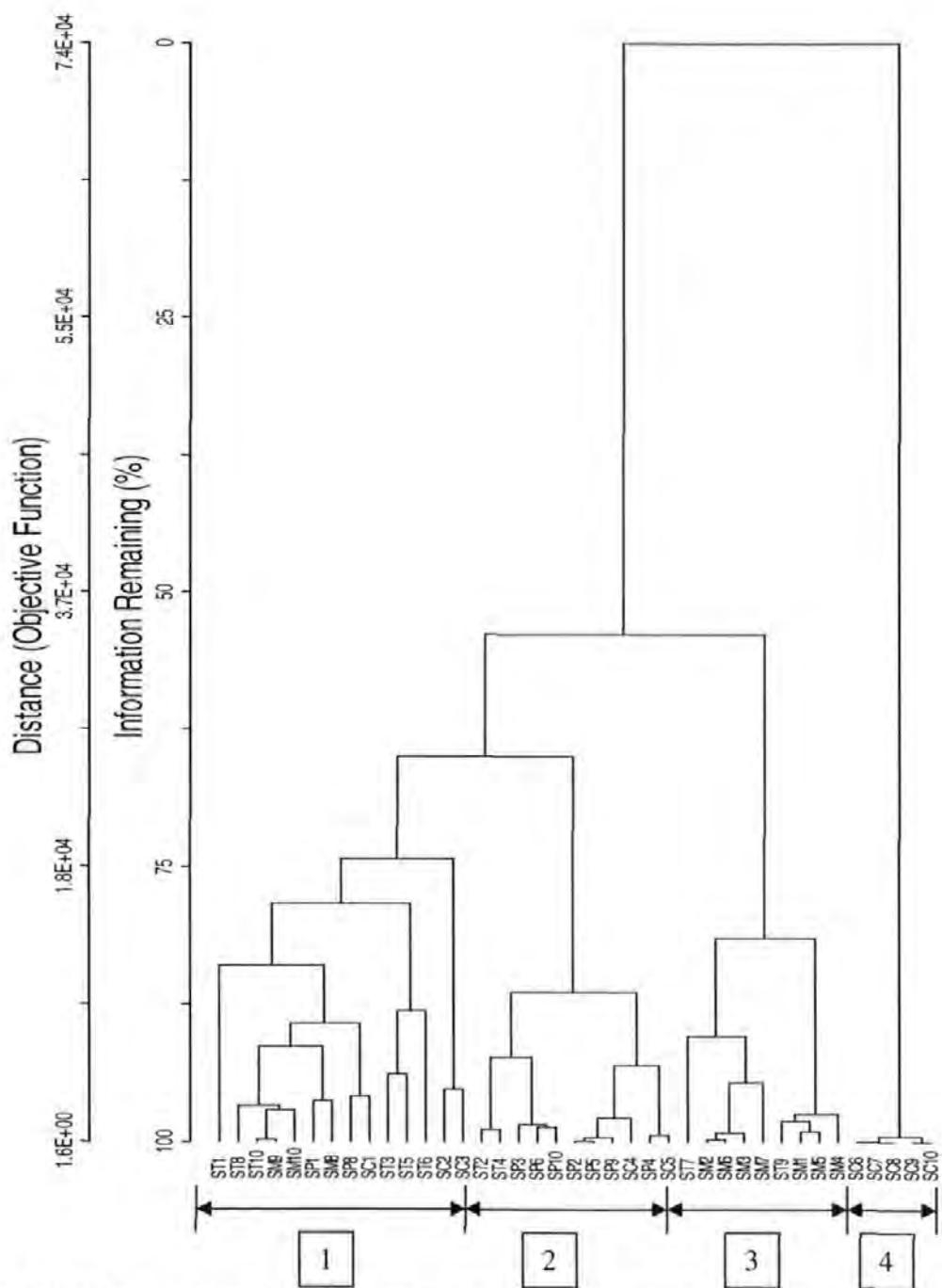
Figure 3.9: Dendrogram showing cluster analysis based on percentage data (testate amoebae only)

The groups tended to form around the sample plots, although there was some overlap. There was more difference between sample plots than in the concentration analyses and there was a division between *SP* and *ST* samples. Four groups were formed.

1. *Sphagnum tenellum.* This group is dominated by *ST* samples (*ST* 1, 3, 5, 6, 8 and 10). *SP* 1 and 8, *SM* 8, 9 and 10 and *SC* 1, 2 and 3 were also in this group.

2. *S. papillosum.* This group is dominated by *SP* samples (2, 3, 4, 5, 6, 9 and 10) and also contains *ST* 2 and 4 and *SC* 4 and 5.

3. *S. magellanicum.* This group comprises mainly *SM* samples (1, 2, 3, 4, 5, 6 and 7) and also contains the remaining *ST* samples (7 and 9).

4. *S. capillifolium* 6-10.

When the analysis was repeated including all organisms, six groups formed and there was less coherence between *ST* samples (dengrogram not shown).

Ordination using percentage data produced similar results (Figure 3.10). Whilst the plots are still fairly well defined, there is much more overlap than within the groups formed by concentration data. Sample groups are more widely spaced. All *SC* samples fall within the *SP* samples. The dispersal of *ST* samples visible in the dendrogram is not obvious from the cluster analysis.
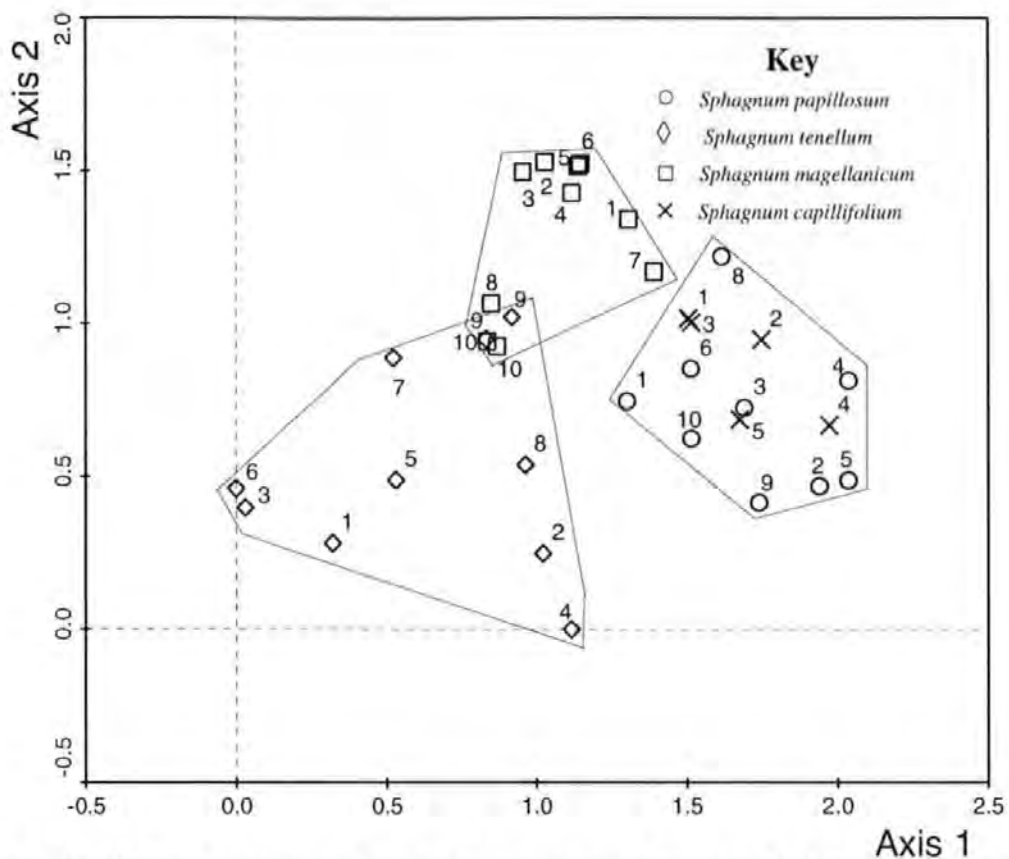
Figure 3.10: DCA of percentage data (all organisms). Samples *SC* 6-10 excluded from analysis for clarity. (See Fig. 3.4 for further explanation).
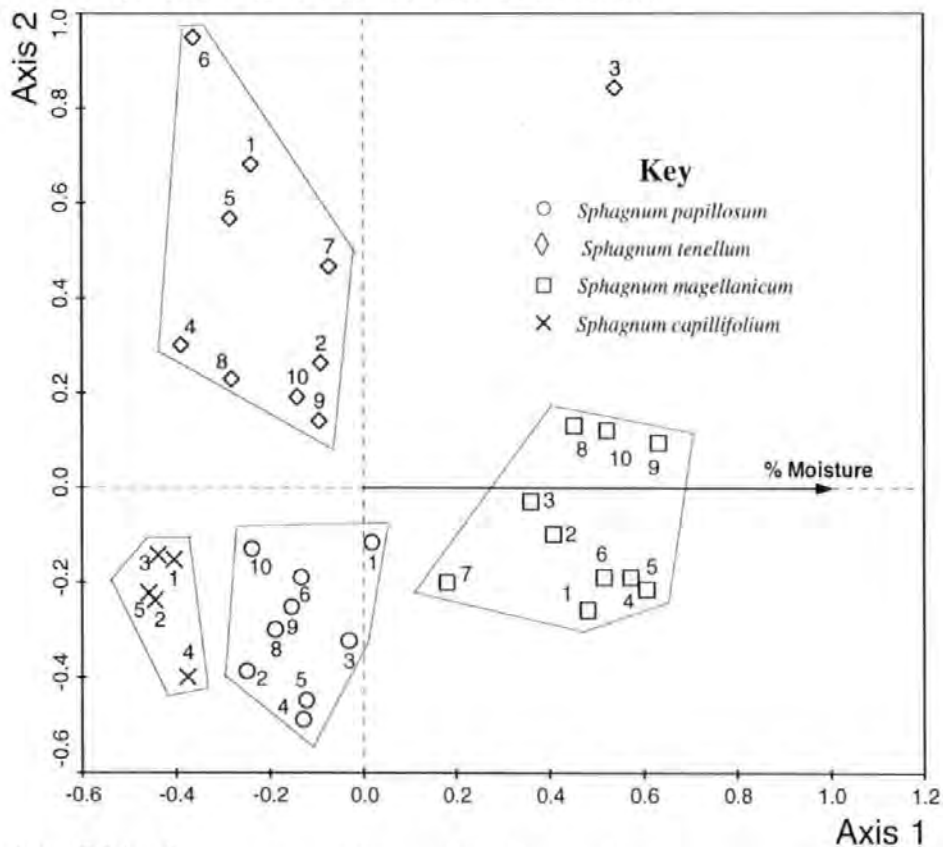
Figure 3.11: CCA of percentage data and moisture data (testate amoebae only). (See Fig. 3.4 for further explanation).

The CCA of percentage data using only testate amoebae results showed strong grouping of sample plots. Samples from *SP* and *SC* plots are separated by % moisture but *ST* and *SP* are separated by some other factor. The *SM* plot is distinguished from the other plots by % moisture. *ST* 3 is separated from its other *ST* samples by % moisture; it appears that its moisture content is more similar to the *SM* plot. The low count has probably affected the ordination of this sample.

c) *Percentage Live Data*

The percentage of live testate amoebae in each sample is thought to give some indication of population turnover. Where percentages are high, reproduction levels exceed mortality rates. Cluster analysis showed that there were large differences between groups and a large amount of overlap between sample plots (Figure 3.12). Ordination of percentage live data showed more overlap between sample groups than analyses of concentration data or percentage data although the *SC* and *SM* samples are relatively distinct (Figure 3.13). The lack difference in percentage live in these samples could be because the structure of the sample plots were broadly similar and thus differences between locations were minimal or it could be a facet of seasonality. It may be that in July, there is little difference in reproduction rates between taxa. Polygons have not been drawn around sample groups on this ordination because of the large amount of overlap but the locations which were most distinctive were *SP* and *SM*
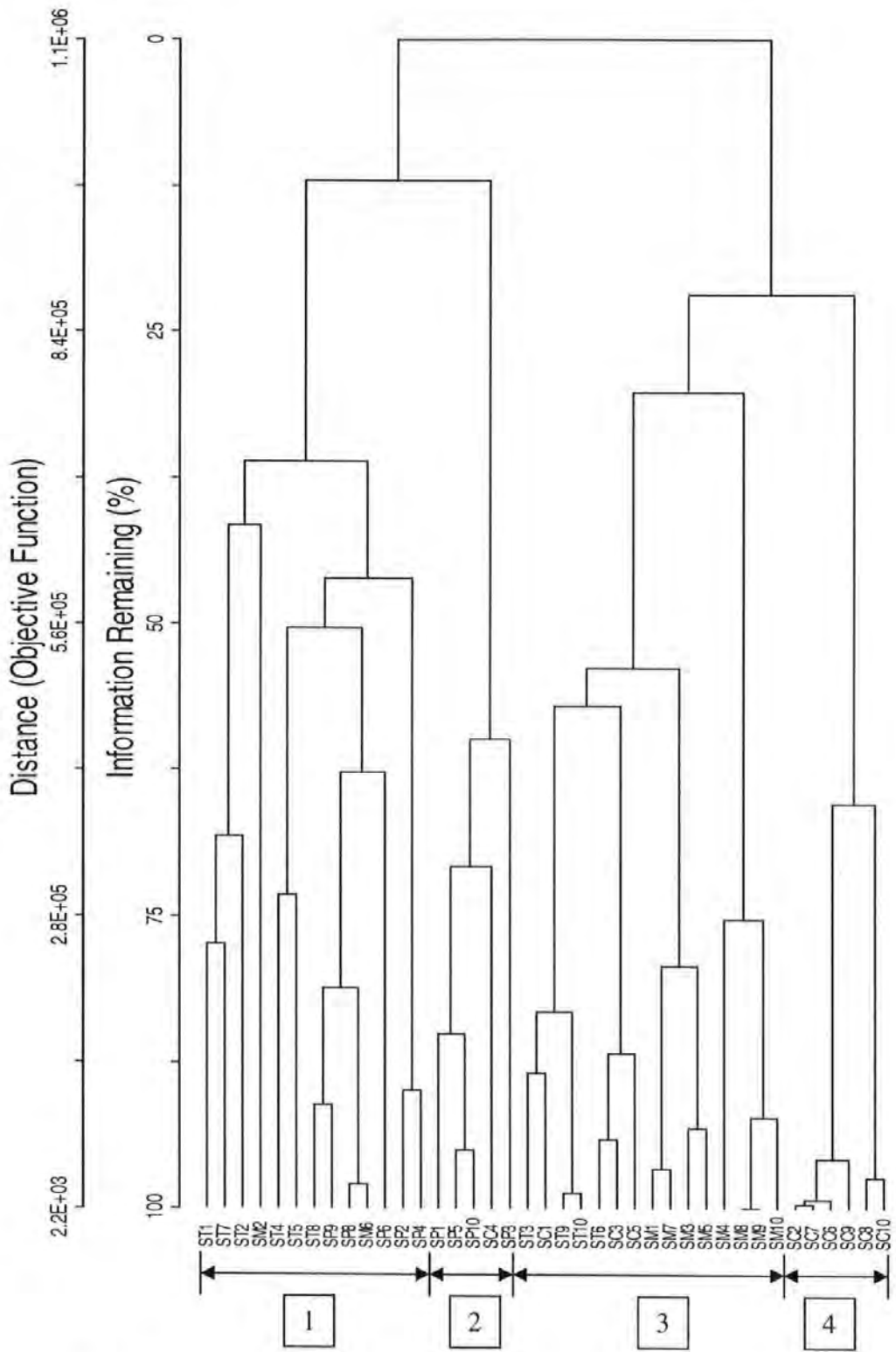
Figure 3.12: Dendrogram showing cluster analysis of percentage live data (testate amoebae only).
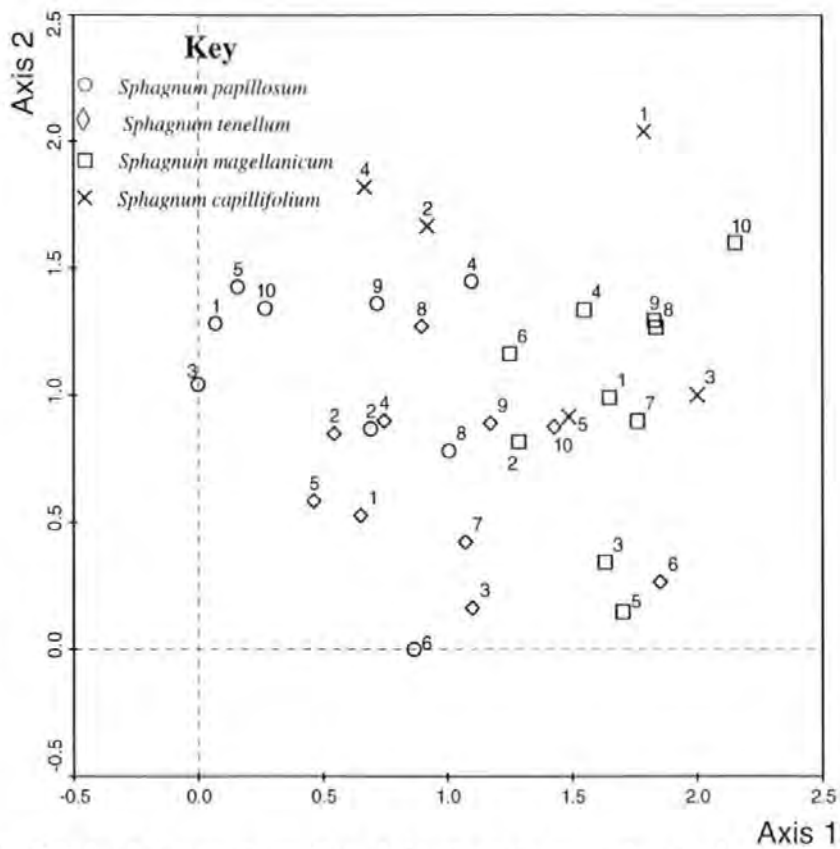
Figure 3.13: DCA of percentage live data (testate amoebae only). (See Fig. 3.5 for further explanation).
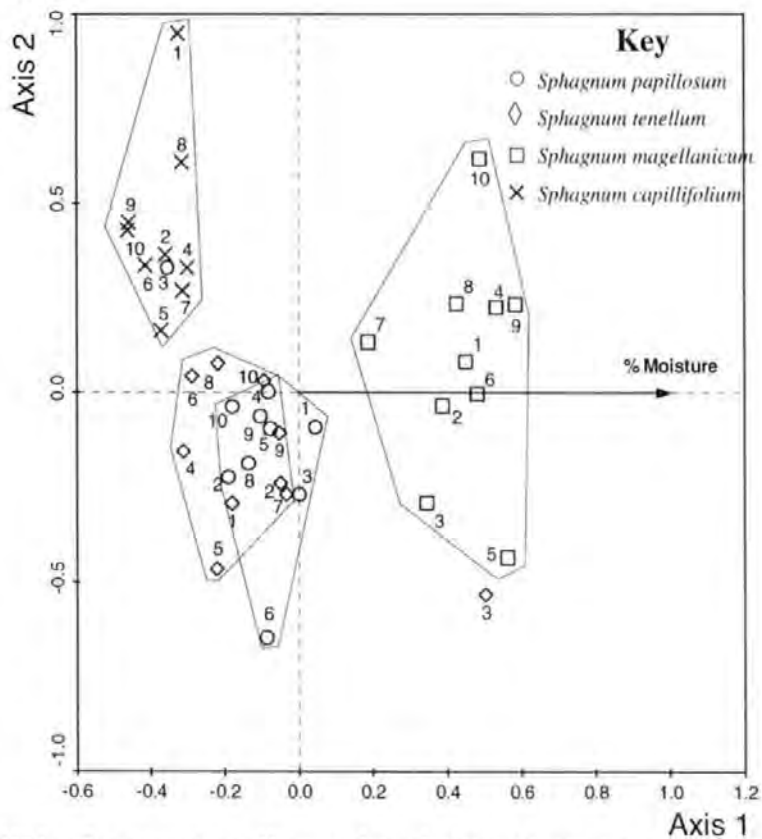
Figure 3.14: CCA of percentage live and moisture (testate amoebae only). (See Fig. 3.5 for further explanation).

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When the environmental variable of % moisture is included in analysis (Figure 3.14), the *SM* and *SC* samples become more distinctive because of the differences in moisture content of the two groups (Table 3.1, Figure 3.3). *SM* and *SP* groups appear to be separated by moisture although the figures in table 3.1 show that this is not the case. The *SP* and *ST* groups overlap the most and *ST* 3 (with low testate count) is not aligned with other *ST* samples. *SC* samples are more strongly grouped than in other analyses suggesting that percentage live is not a good indication of differences in species composition.

3.3 Discussion

The aims of this experiment were, firstly, to establish whether the small sample sizes necessary to avoid damaging the site would produce counts of testate amoebae sufficiently high as to confirm the reliability of the sampling method used experimental design of the ditch-blocking monitoring at Coom Rigg Moss. The second aim was to establish whether counts of testate amoebae from a single stem of *Sphagnum* were representative of the plot. Thirdly, this experiment offered the opportunity to explore the most useful analytical techniques for understanding modern testate amoebae communities. The results are mainly applicable to the experiment at Coom Rigg Moss although they have been considered when establishing experiments at the other sites, and are potentially useful for sampling testate amoebae at other damaged sites.

3.3.1   Sample size

The single stems of *Sphagnum* have proven to be large enough to produce counts of over 100 in most cases. *Sphagnum* species appear to influence the numbers found in each stem although because replicates were not taken, other factors could be affecting communities

e.g. depth to water table. These measurements were not taken in an effort to minimise disturbance of the bog surface, particularly as sample sites were close to the long-term monitoring plots. Further study sampling replicate *Sphagnum* species may help to confirm associations with testate amoebae community assemblages. Although counts of over 100 testate amoebae were achieved from the majority of samples, the time input was unacceptably high in some samples.

3.3.2 *Were counts representative of plots?*

Counts of between 50 and 100 testate amoebae were generally aligned with the rest of the sample group in the multivariate analyses indicating that, although not ideal, they are still valid in analyses. Counts below 50 frequently lie either on the edges of groups or away from the other samples. Despite these issues, each analysis has shown that the samples from the individual plots are more alike than samples from the entire dataset. The most valuable analyses in this study were made using concentration data and percentage data. Analyses of percentage live data were not particularly effective, suggesting that this may not be a useful measure of testate amoebae communities from locations with minimal ecological differences.

There is one major concern arising as a result of this work. The results from the *Sphagnum capillifolium* sample set show a major division. Two groups were formed; *Euglypha strigosa, Nebela tincta* and *Corythion – Trinema* type were dominant in samples *SC*1-5 and *SC*6-10 were dominated by extremely high numbers of *Amphitrema flavum* to the exclusion of other taxa. This raises a major question about how characteristic a single stem of *Sphagnum* is of the 25cm by 25cm plot from which it has been taken. However, the fact that the individual samples within the two groups were so similar indicates that there is not

a major problem with the sampling method. It is possible that *A. flavum* is an extremely competitive species which excludes all other testate amoebae from its immediate area, either through predation or feeding competition. Further research is necessary to prove this. The difference cannot be attributed to processing methods as all samples were treated identically. The need for good record keeping is reinforced by the results from the *SC* group as a clear record of sample layout could have allayed concerns arising from this sample set. As a precaution, any unusually high counts of *A. flavum* in the regular monitoring from Coom Rigg Moss will be treated with caution.

There were differences in numbers of taxa found in each sample plot with the highest number in *S. papillosum* samples and the lowest in *S. capillifolium*. This could be connected to variety of spaces available and thickness of water films, it is probably that *S. papillosum* provides a range of small and large niches and *S. capillifolium* provides only small niches as a result of their different leaf structures (Figure 3.2).

The efficacy and reliability of the sampling method appears to have been confirmed by these studies. Small amounts of variability within the sample groups did not affect replicability and, although there is overlap between the groups, this was to be expected as samples were taken on the same day from the same area of the same site, i.e. mostly from plot D in the long-term restoration experiment, so ecological conditions should have been broadly similar. The *Sphagnum tenellum* samples were taken from the north side of the bog near to the edge where conditions may have been drier whilst the other 3 groups were taken from an area near to the centre. This has not had a huge effect on variation between samples. The species of *Sphagnum* seems more important than the location of the sample plot although, as indicated earlier, further study would be needed to confirm this.

The analyses using percentage data have been included in this section for purposes of comparison. The concentration data were more effective as a means of classifying groups. Groups are more robust when concentration data are used, compared to when percentage data are analysed. Concentration data should therefore be used in analysis wherever possible. The possible reason for the differences between the two ways of analysing data is that with the percentage data, the varying numbers of individuals of each species affect the percentages. This 'compositional effect' does not affect concentration data.

The other organisms observed have not all been identified but have been included in some analyses to assess their use as indicators of conditions. Analyses including these data have been compared with those where testate amoebae only were used. The groups formed in the cluster analysis were more distinctive, although the CANOCO output using only testate amoebae showed less overlap of groups. This indicates that some of the other organisms are useful in distinguishing between samples but that perhaps the large number of individual occurrences causes some confusion.

Some of these organisms have been identified at least to family but others are still unidentified. Efforts will be made to identify those individuals which appear to be useful indicators when further analyses have been completed.

Another issue to consider is the size of testate amoebae counts. Although the counts of between 50 and 100 individuals were well aligned with other group members, counts of less than 50 were often outliers in multivariate analyses, low counts are acknowledged as a potential problem when taking single stem samples. The results of these stem tests show

that there is more variability between sampling plots than within them. In analysis, the clear majority of samples formed groups around plots and few individual samples lay outside of these groups.

3.4 Conclusions

This experiment was designed to test whether it was possible to achieve counts of over 100 testate amoebae from single stems of *Sphagnum* and to assess whether communities were representative of the 25 by 25 cm sample plots. The results from this would confirm the reliability of the methodology applied in the long-term experiments at Coom Rigg Moss and provide guidance for sampling at other locations.

The results showed that single stems were more representative of the 25 by 25cm squares used for sampling than of the wider area being studied. The implications of this for the project are that the methodology in place is effective and will produce reliable and replicable results.

Although counts of over 100 testate amoebae were achieved in most samples, some required a greater counting effort. Where possible, sampling at other sites should be planned to allow larger sample sizes, avoid low counts and make counting more efficient.

In terms of analysis, concentrations as opposed to percentage live of testate amoebae produced more conclusive results in analysis of variance. In multivariate analyses where differences species composition were analysed, percentage data were slightly more effective for classifying groups in this experiment.

The 'Other Organisms' found in these samples were not random occurrences but important elements of the ecology of the site. Their usefulness in the classification of samples was not confirmed in the analyses. They were rarely identified to species level and therefore further study of the ecology of taxonomic groups may be able to identify key organisms which are important in peatland ecology.

This chapter has also explored the methods used for analyses. Analysis of variance carried out on numbers of testate amoebae have been useful. Multivariate techniques have been effective in exploring the testate amoebae community composition data.

CHAPTER FOUR: Coom Rigg Moss Part 2: The impact of ditch blocking

4.0 Introduction

Many peatlands have been damaged indirectly by management activities in the surrounding area. Peat extraction and drainage for forestry and agriculture in the area adjacent to bogs can result in increased lateral and downward water loss through the peat (Schouwenaars, 1995). Afforestation can change both the traditional management of unplanted sites by reducing grazing and burning (Anderson *et al.*, 1995) and may impact upon water table through increased interception and evapotranspiration and increased runoff into peripheral drains (Smith & Charman, 1988). These activities do not always directly affect the site but can have an indirect impact on the hydrological unit. The Forest Enterprise in Northumberland has carried out restoration work on unplanted peatlands within Kielder Forest which have been affected by drainage of the surrounding land. These have provided an ideal site for monitoring the impact of hydrological restoration.

4.1 Coom Rigg Moss

Coom Rigg Moss is a National Nature Reserve (NNR) designated in 1960 (Burlton, 1996). Since 1987 an inter-agency approach to management has been developed to ensure effectiveness. The agencies involved are English Nature, The Northumberland Wildlife Trust, the Forest Enterprise, Newcastle University and the National Park Authority (Burlton, 1997). It is situated in the county of Northumberland (and within Northumberland National Park) in Kielder Forest, 40km north-east of Carlisle (Figure 4.1) and has a long recorded history with extensive studies in the 1950s and 1960s (Chapman, 1964a, b, 1965). It is one of the Irthinghead mires Site of Special Scientific Interest (part of a larger group of mires - the Border Upland Natural Area (Merricks, 1995)) first notified in 1969, classified in the *Nature Conservation Review* as a 1* or top quality site (Ratcliffe,

1977) and renotified under the revised legislation in 1983. It is 39.6 hectares in area and has been described as a series of raised mires joined together by blanket bog (Chapman, 1964 a & b).

Scale 1:200000
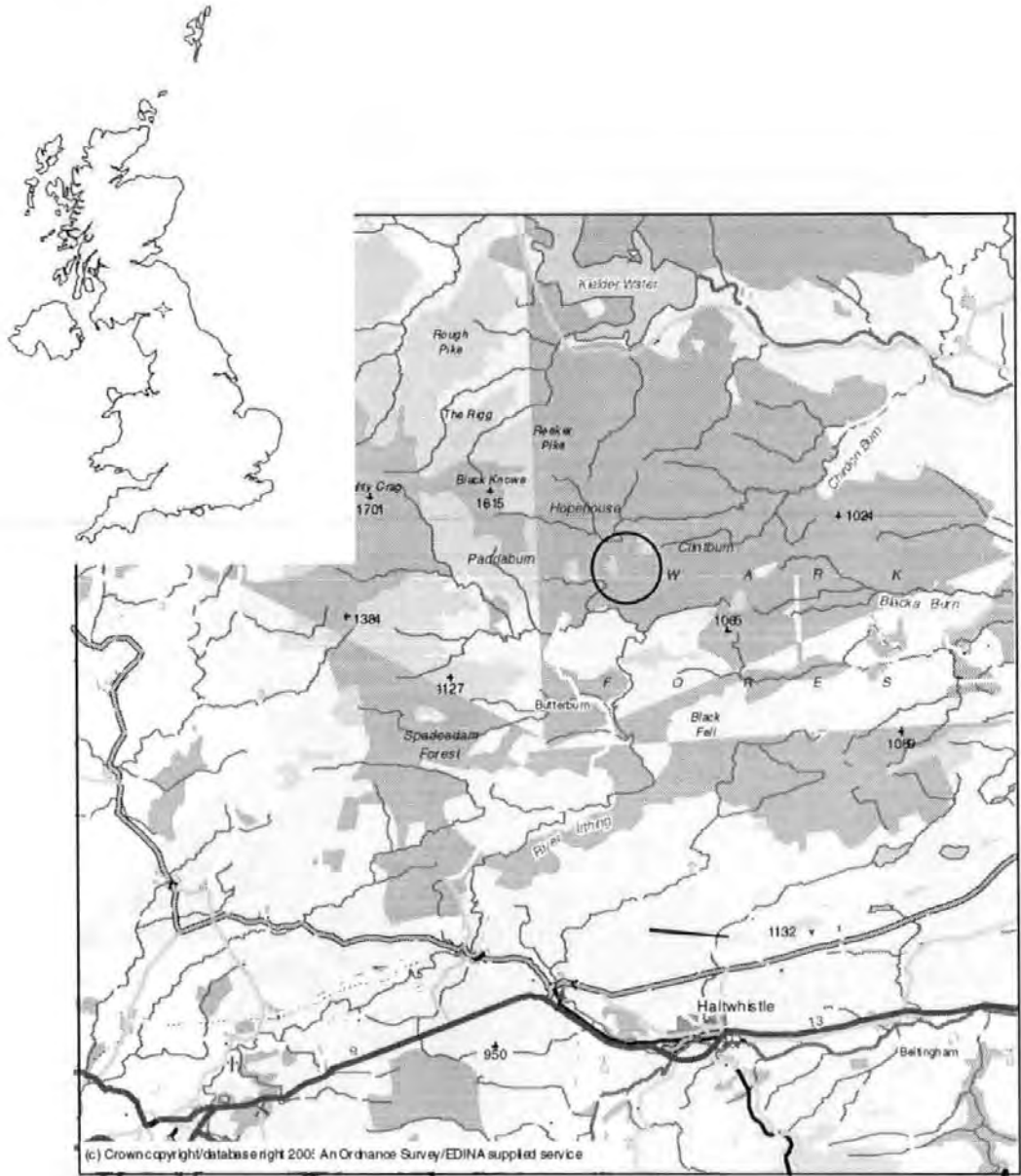
Figure 4.1: Location of Coom Rigg Moss within Kielder Forest

A further tier of conservation legislation has been added recently; in 1995 the Border Mires were proposed as a Special Area of Conservation (SAC). This provides statutory

recognition of their status as an important peatland area at a European level. International recognition of their importance is evident in the Border Mires designation as a Ramsar site. This relates to the internationally important position of the mires as a breeding ground for bird populations.

The geology of the area consists of a series of shales, sandstone, bands of limestone and coal seams (Merricks, 1995). Average annual rainfall for the area is 1270mm and monthly evapotranspiration never exceeds precipitation (Merricks, 1995). The combination of the geology and hydrology of the area have enabled the growth of peat at Coom Rigg Moss.

Vegetation at the site is typical of a raised bog and also of the Border Mires (Burlton, 1996). The major NVC type on the mire is M18a *Erica tetralix-Sphagnum papillosum* (*Sphagnum magellanicum-Andromeda polifolia* sub community) raised and blanket mire, with smaller areas of NVC type M2b *Sphagnum cuspidatum / recurvum* bog pool community and M19 *Calluna vulgaris-Eriophorum vaginatum* blanket mire (Rodwell, 1991). The dominant *Sphagna* and most abundant peat-forming species are *Sphagnum papillosum* and *S. magellanicum* with *S. tenellum* and *S. capillifolium* growing at lower cover percentages in wetter areas. The common vascular plants in the Border Mires are *Andromeda polifolia* bog rosemary *Calluna vulgaris* heather, *Drosera rotundifolia* round leaved sundew *Erica tetralix* cross-leaved heath, *Eriophorum vaginatum* and *E. angustifolium* Hare's tail and common cotton-grasses, *Narthecium ossifragum* bog asphodel and *Vaccinium oxycoccus* cranberry (Smith *et al.*, 1995). Typical bog vegetation is distributed across the deeper central peats, with plant communities more typical of moorlands growing on the peripheral shallow peats.

Surrounding Coom Rigg Moss are forestry plantations dominated by *Picea sitchensis* sitka spruce and *Pinus contorta* lodgepole pine. The total area of Kielder Forest plantation is 50 000ha and is the largest contiguous management unit of the Forest Enterprise (Burlton, 1997). The area was planted between 1925 and 1960 with the majority of planting occurring between 1946 and 1960 (Smith & Charman, 1988). The mires within the forest survived mainly because they were too wet to drain for successful timber growth (Smith & Charman, 1988). Peripheral drainage ditches have affected many of the peatlands within Kielder Forest as drainage of the afforested land was a prerequisite for successful tree growth. Most of the mires were left intact with little afforestation of the peat body although sites such as Paddaburn Moss have been largely planted (Smith *et al.* 1995).

Where the central mire unit has remained unplanted, the surrounding land use has frequently had a negative impact; these are sites where planting extended onto the mire edges and into their hydrological units. Figure 4.2 shows the hydrological unit of Coom Rigg Moss and the proximity of the forestry plantation, sampling locations are also shown on this map. Figure 4.3 shows the proximity of forestry to Coom Rigg Moss. At some sites in the Border Mires, drainage ditches extend onto the mire surfaces, sites were prepared for planting but abandoned when it was realised that tree growth would not be economically viable.
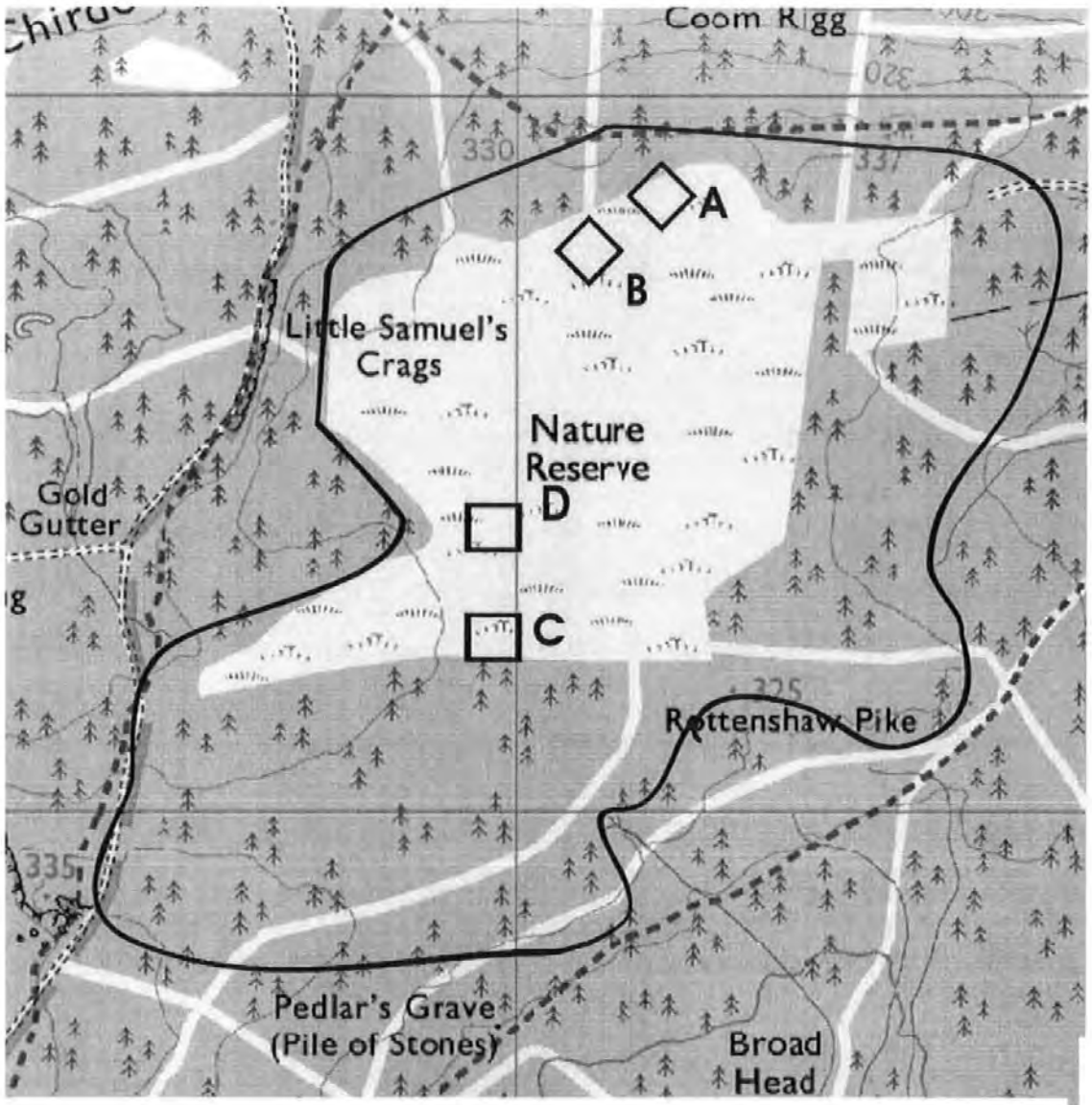
Scale 1:10000
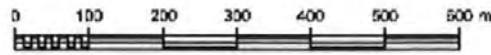
Forestry Commission Sample plots

Coom Rigg Moss hydrological unit

Figure 4.2: Coom Rigg Moss hydrological unit, forestry plantation and sample plots.

Figure 4.3: Coom Rigg Moss with afforestation visible in the background

Current conservation management of the mires focuses on the impact of the surrounding forestry plantations; natural regeneration of Sitka Spruce is kept under control through pulling of seedlings (Burlton, 1996). Recent research suggested that vegetation changes on the Border Mires have been caused by changing climatic conditions over the twentieth century (Hendon & Charman, 2004). This, combined with research that concluded a reduction in grazing pressure has altered the vegetation on the periphery of Butterburn Flow, another site in Kielder Forest (Smith *et al.*, 2003), indicates that a combination of factors have affected vegetation at Coom Rigg Moss.

4.2 Methods

4.2.1 Outline of restoration work: Responses to Ditch Blocking at Coom Rigg

The focus of the major study at this site is the impact of restoration work. The three major issues in peatland restoration were discussed in the introduction to the thesis. This site falls into the 'restoration of hydrological conditions' category. Peripheral drains, part of forestry preparations at the site, were blocked in February 2001 as part of the European Union funded Border Mires LIFE project. Forest Enterprise (FE) established two pairs of sampling plots (A and B; C and D) each containing four WALRAGs (Water Level Rain

Gauges) (Bragg, *et al.,* 1994) for monitoring hydrology at the site (Figure 4.2 shows the layout of these monitoring plots). Within these areas two plots were set up close to the site of ditch blocking (A and C) and two control plots some distance from the ditch (B and D). Each monitoring area contained four sampling plots where a WALRAG was installed – 16 in total.

Hydrological measurements were taken at least quarterly with more frequent visits in the summer months. WALRAGs give maximum and minimum water levels between visits as well as the water level on the day the measurement is taken. Using WALRAGs as monitoring tools means that more detail on the water table is available and that less frequent monitoring will still provide a good hydrological record. Hydrological monitoring began in January 2000 and continued until August 2002. The measurements were taken for a year before ditch blocking in order to establish baseline hydrological conditions and allow hydrological response to ditch blocking to be understood.

4.2.2 Testate Amoebae sampling

Sampling for testate amoebae began in January 2000 when hydrological monitoring started, and continued until August 2002. The methodology was tested in the experiment described in Chapter 3. Sample plots were placed next to the FE WALRAGs (Figure 4.4). Single stems of moss were collected from these plots by John Parkin of FE throughout the monitoring period. The aim was, as with the hydrological monitoring, to establish baseline ecological conditions in the testate amoebae communities over the first year and then to monitor their response to ditch blocking operations during the following months.

Figure 4.4: A Walrag with a testate amoebae monitoring plot in the foreground (located by white plastic markers)

The sampling plan on Coom Rigg (Figure 4.2) follows monitoring designed by the Forest Enterprise. Testate amoebae monitoring plots were established close to each of the 16 WALRAGs. These are 25 by 25 cm squares of uniform vegetation composed (ideally) of one species of *Sphagnum*. The plots were chosen to be close enough to the WALRAG to

have comparable ecological conditions but not so close that they were affected by any draw down created by the WALRAG. Each 25 cm plot covers a single hummock or a flatter lawn area, as the aim was to monitor as uniform an area as possible. The plots were marked with plastic corner pieces and poles to make them easy to locate and to prevent damage from trampling when water table measurements were taken.

A single stem of *Sphagnum* was collected from each plot whenever the Forest Enterprise took water table measurements. The purpose of sampling only one stem per site each time was to inflict minimal damage on the plots. It was thought that if larger samples were taken, the microtopography of the plots would be altered over the course of the two-year monitoring period, possibly altering the microhabitat for the testate amoebae. This may have resulted in the monitoring picking up changes made by sampling rather than the results of hydrological changes.

Samples were taken and sent to Plymouth where they were processed as outlined in Chapter 3. Microscope slides were produced and counts were carried out using techniques already described.

4.3 Results

Monitoring of hydrology and testate amoebae populations at Coom Rigg Moss began early in 2000. The ditches in plots A and C were blocked in the spring of 2001. Repeated monitoring continued until August 2002. Baseline conditions for the site were therefore available and pre- and post- damming comparisons have been made.

4.3.1 Hydrological measurements

The aim of the restoration was to improve the hydrological conditions at the site. Overall, there was very little change in hydrology as a result of the restoration over the monitoring period. Table 4.1 shows the mean pre- and post- damming minima from WALRAG measurements. Plots A and C are adjacent to ditches and plots B and D are the corresponding control sites. The control sites were wetter than the sites close to the ditches in the period before damming, and plot B had the wettest conditions overall. Water table minima and maxima are shown to illustrate the extent of change at Coom Rigg. At a peatland site, it is usually the driest conditions that have the greatest impact on vegetation composition as bog plants are less able to tolerate drought than excessively wet conditions. There appears to be very little change in water table in any of the plots, with the exception of plot C where the water table rose by around 3 cm in minimum, maximum and mean values. The figures show that there was very little change in plots A and B but water tables rose slightly in plot D. The increase in was greatest in plot C.

	Plot A Mean (cm)	Plot B Mean (cm)	Plot C Mean (cm)	Plot D Mean (cm)
Pre-damming (minimum)	17.57	10.82	19.36	15.43
Post-damming (minimum)	17.54	10.87	16.46	15.15
Pre-damming (maximum)	4.46	1.32	4.21	3.43
Post-damming (maximum)	4.78	1.75	1.77	2.29
Pre-damming (mean)	11.02	6.07	11.79	9.43
Post-damming (mean)	11.30	6.38	9.12	8.72

Table 4.1: Water table minima, maxima and means for each treatment plot. Data are the means of four WALRAGs per plot for pre- and post- damming periods.

Figures 4.5 to 4.8 illustrate the changes in water table over the monitoring period; 4.5 and

4.7 show minima whilst 4.6 and 4.8 show maxima. Figures 4.5 and 4.6 show that, whilst

water table fluctuated in plots A and B, there was little overall directional change over the

monitoring period. The difference in water level between the two treatment plots remained

constant. In plots C and D (Figures 4.7 and 4.8), a steady rise in water table is observed,

water levels increase more in plot C than in plot D. The overall increase is more visible in

the maxima plot (Figure 4.8).
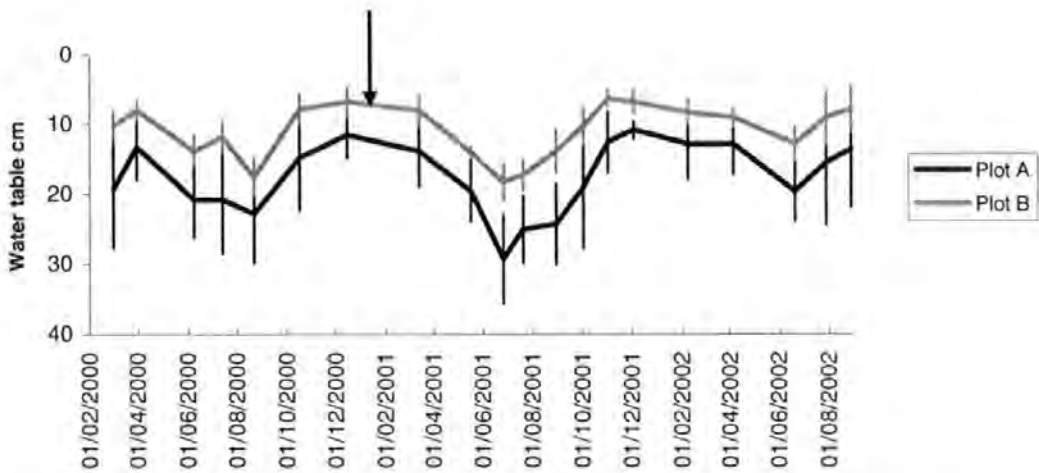
Figure 4.5: Water table measurements – mean values for treatments A and B, expressed as the mean minimum measurements from WALRAGs and standard deviations (error bars). Each value is the mean of four WALRAGs. Arrow marks approximate time of damming.
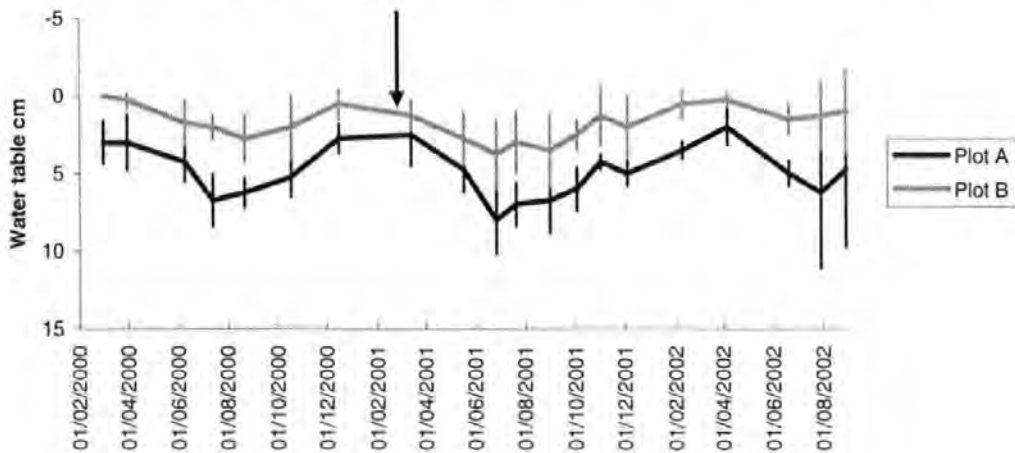
Figure 4.6: Water table measurements – mean values for treatments A and B, maximum measurements (See Figure 4.5 caption for further explanation).
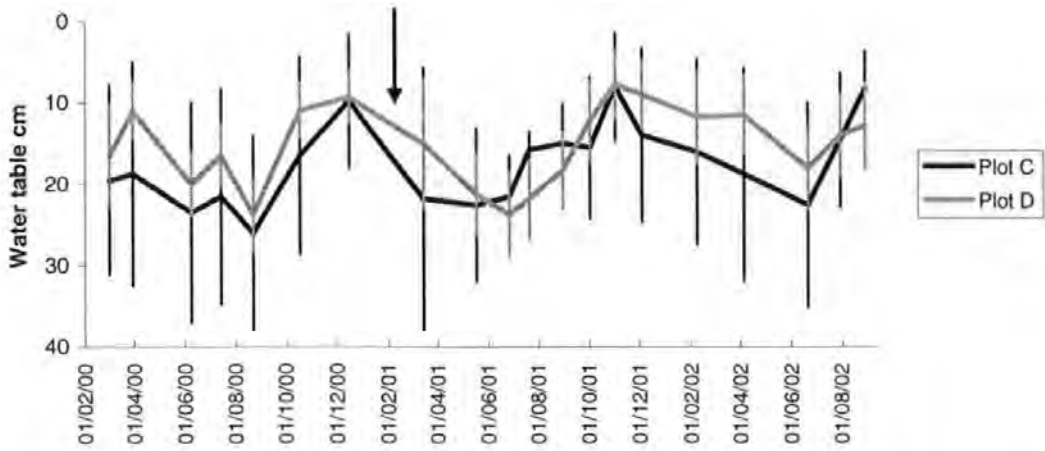
Figure 4.7: Water table measurements – mean values for treatments C and D, minimum measurements (See Figure 4.5 caption for further explanation).
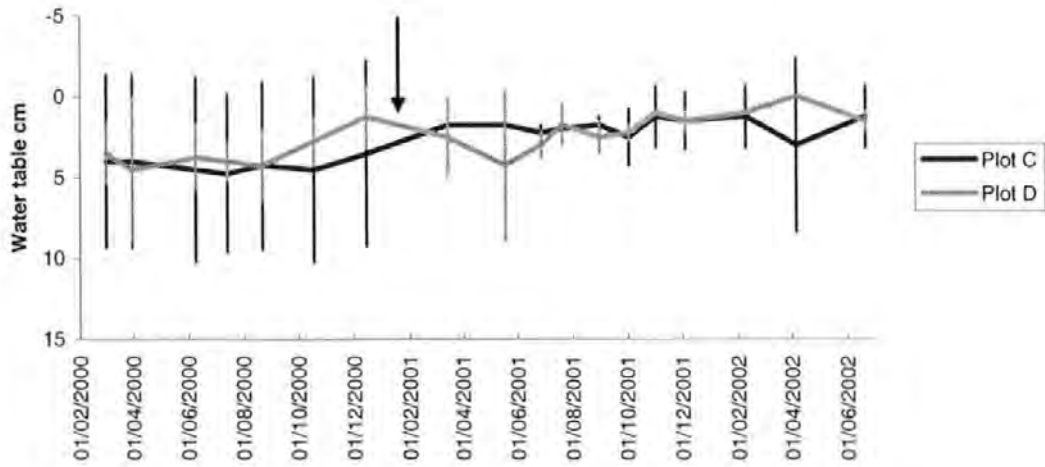
Figure 4.8: Water table measurements – mean values for treatments C and D, maximum measurements (See Figure 4.5 caption for further explanation)

Mann-Whitney tests to test for differences in the water table showed that minimum water table in plot A was significantly higher than plot B (p = 0.00). There was no significant difference in minimum water table between plots C and D (p = 0.09). Maximum water

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table in plot A was also significantly higher than in plot B ($p = 0.00$). There was no significant difference in maximum water table between plots C and D ($p = 0.92$).

Further analysis was necessary to fully understand the results of water table monitoring. A second analysis was carried out where the pre-damming average was subtracted from post-damming measurements to see whether the change in water levels were clearer. Mann-Whitney tests on these data showed significant differences on changes from day and maximum measurements in plots C and D ($p = 0.000$ and $p = 0.003$ respectively) over the monitoring period, the change was greater in plot C with a median of 2.46 cm compared with 1.18 cm in plot D. There were no significant differences in changes of minimum depth to water table in plots C and D ($p = 0.218$).

The above analyses, carried out on plots A and B, showed no significant differences in water table changes (min $p = 0.505$, day $p = 0.918$, max $p = 0.758$). Water tables remained stable before and after damming.

A comparison of water levels before and after damming, using Mann-Whitney tests, showed a significant difference in water levels (day measurements) in plot C – post-damming measurements were significantly less than pre-damming with a probability of 0.001 (i.e. the water table was closer to surface after damming).

None of the other sample plots had any significant differences in water table before or after damming.

4.3.2 Moisture content

Another facet of the hydrology of the bog is moisture levels. Changes in moisture levels at the site can be examined by looking at the moisture content of samples. The individual plots were combined to give mean moisture content for each treatment; these values are shown in Table 4.2 and were plotted against time (Figures 4.9 and 4.10). Overall, treatment A has the lowest moisture content, moisture levels dropped in all treatments and treatment C had the highest moisture levels both before and after damming.

	Treatment A	Treatment B	Treatment C	Treatment D
Pre-damming	90.74	93.18	93.41	91.36
Post-damming	84.42	90.19	91.22	87.56

Table: 4.2: Moisture content (expressed as a percentage) by treatment (mean for all samples) before and after damming. Treatments A & C are dammed plots, B & D are undammed.
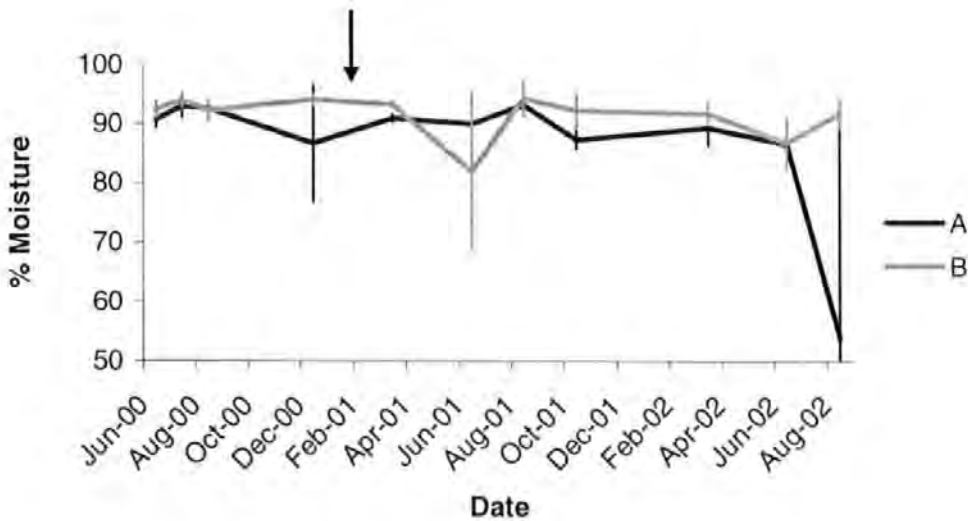
Figure 4.9: Moisture content of treatments A (dammed) and B (control) plotted over time. (Treatment means with error bars showing standard deviations). Arrow marks approximate time of damming.
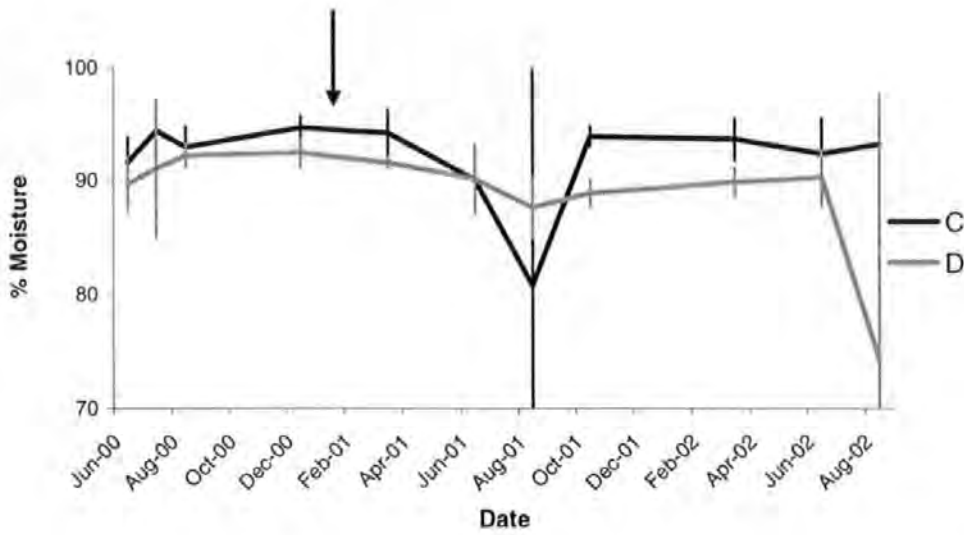
Figure 4.10: Moisture content of treatments C (dammed) and D (control) plotted over time. (See Figure 4.9 caption for further explanation).

Mann-Whitney tests on pre- and post- damming moisture content showed that moisture levels in treatment A were significantly lower than those in plot B both before and after damming (p = 0.022 (pre-), p = 0.006(post-)) and that in treatment C, moisture levels were significantly higher than treatment D before and after damming (p = 0.006 (pre), p = 0.000). Moisture content is much less stable than depth to water table and may be more sensitive to particularly dry or wet periods.

4.3.3 Testate amoebae responses

Samples for testate amoebae analyses were collected alongside hydrological measurements. Sixteen samples were collected each month, four from each of the four monitoring plots. However, due to the sheer numbers involved, samples were only counted at around three month intervals. Samples from all months were prepared and archived in case it proved necessary to count samples from more closely spaced intervals. Three months have been used for annual comparisons – March, June and August. Samples from these months were counted in 2000, 2001 and 2002 to allow annual comparisons

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from immediately before, immediately after and almost two years after ditch blocking took place.

The testate amoebae response to damming is more complex than the water table response, partly because of the very large range of variables that can be used. There are several aspects to examine. This section begins with comparisons of numbers of taxa found in the plots followed by some examination of raw numbers of testate amoebae. Species composition pre- and post- damming will be then be compared. Some statistical analyses on concentration and percentage data from the site were carried out. Finally, relationships between testate amoebae populations and environmental variables were explored using multivariate techniques.

Both living and dead tests were counted in samples from Coom Rigg, which allowed concentration and percentage live data to be calculated from samples. Calculation methods for these data are outlined in chapter 3. The tables below show a summary of the data for each sample plot arranged by month. Table 4.3 is a summary showing the total number of taxa found in each treatment by month. Plot C had the highest number of taxa and plot A had the lowest. The number of taxa did not change after ditches were dammed.

	Plot					
Date	**A**	**B**	**C**	**D**	**Mean**	**S.D.**
Jan-00	18	13	23	20	18.50	4.20
01-Mar-00	12	12	18	13	13.75	2.87
30-Mar-00	13	18	21	17	17.25	3.30
Jun-00	13	14	18	17	15.50	2.38
Jul-00	19	19	19	15	18.00	2.00
Aug-00	13	17	20	15	16.25	2.99
Dec-00	13	13	20	15	15.25	3.30
Mar-01	17	16	18	9	15.00	4.08
Jun-01	7	16	25	16	16.00	7.35
Aug-01	14	18	22	8	15.50	5.97
Oct-01	19	19	18	19	18.75	0.50
Mar-02	6	9	21	4	10.00	7.62
Jun-02	15	19	22	16	18.00	3.16
Aug-02	18	18	21	18	18.75	1.50
Mean	14.07	15.79	20.43	14.43		
S.D.	4.05	3.12	2.14	4.52		

Table 4.3: Number of taxa found in each treatment plot by month with means and standard deviations for plot and month.

Table 4.4 shows the mean total numbers of testate amoebae found by sample plot and month for samples taken in 2000 (pre-damming). Percentage live is expressed as the mean for the four samples from each plot, as is concentration. Table 4.5 is the same figures for 2001 and 2002 (post-damming). Counts from plot C were consistently higher than other plots. Plots A and D had the lowest counts. There is no obvious pattern in percentage live and concentrations were highly variable in all plots.

Mar-00	A	B	C	D
Total (Mean)	33.3	85.5	121.5	48.5
Total (Range)	4-48	34-127	45-217	31-74
% Live	53.0	52.8	34.2	49.3
Concentration (Mean)	15080	69265	151713	17892

Jun-00	A	B	C	D
Total (Mean)	32.3	96.8	86.0	74.0
Total (Range)	12-55	27-138	19-135	14-129
% Live	44.6	51.2	35.2	41.4
Concentration (Mean)	56584	34976	68933	22378

Jul-00	A	B	C	D
Total (Mean)	96.8	88.5	124.0	90.3
Total (Range)	40-189	18-118	107-135	25-118
% Live	81.4	79.7	60.4	76.6
Concentration (Mean)	21504	17916	53459	33832

Aug-00	A	B	C	D
Total (Mean)	79.8	88.0	161.3	82.8
Total (Range)	2-248	63-105	114-210	10-110
% Live	69.9	48.7	39.6	59.1
Concentration (Mean)	43681	21412	43022	35972

Dec-00	A	B	C	D
Total (Mean)	24.3	58.0	97.5	50.0
Total (Range)	0-96	3-115	15-142	1-192
% Live	42.0	64.9	56.0	82.1
Concentration (Mean)	4481	12302	36877	30708

Table 4.4: Pre-damming testate amoebae results. Showing total counted by location (mean of four samples), range counted, number of taxa found within each monitoring plot, mean % live and concentrations (individuals per gram dry weight).

Mar-01	A	B	C	D
Total (Mean)	67.3	60.0	89.3	64.0
Total (Range)	4-114	3-108	30-122	8-117
% Live	79.7	86.3	64.8	78.5
Concentration (Mean)	28801	22910	36356	57378

Jun-01	A	B	C	D
Total (Mean)	6.5	92.3	115.8	39.5
Total (Range)	6-8	21-122	106-126	0-80
% Live	69.8	41.1	39.5	31.5
Concentration (Mean)	17848	15277	41390	11218

Aug-01	A	B	C	D
Total (Mean)	33.3	26.5	94.5	11.0
Total (Range)	1-119	7-54	13-150	7-18
% Live	48.0	58.9	49.0	28.3
Concentration (Mean)	22517	37416	36008	20096

Oct-01	A	B	C	D
Total (Mean)	63.8	69.5	89.5	28.5
Total (Range)	1-113	6-150	24-119	4-61
% Live	76.8	53.0	43.9	65.3
Concentration (Mean)	20576	13131	32822	21511

Mar-02	A	B	C	D
Total (Mean)	25.0	8.8	82.5	1.8
Total (Range)	5-74	2-22	26-130	1-4
% Live	62.2	66.3	44.4	68.8
Concentration (Mean)	25003	9820	48395	10550

Jun-02	A	B	C	D
Total (Mean)	34.8	42.8	141.8	32.8
Total (Range)	21-55	4-95	33-237	17-56
% Live	62.7	46.8	20.0	51.4
Concentration (Mean)	19820	15820	87433	23425

Aug-02	A	B	C	D
Total (Mean)	42.8	102.3	95.5	4.0
Total (Range)	2-94	70-124	26-133	1-7
% Live	43.2	44.9	26.8	62.9
Concentration (Mean)	30368	49670	51559	7051

Table 4.5: Post-damming testate amoebae results. (See Table 4.4 caption for further explanation).

Tables 4.6 a-d are summaries of the species data by sample plot (percentage composition). Taxa are arranged by hydrological preference with wetter indicators at the top and drier indicators at the bottom (based on data from Charman *et al.* 2000). There is very little obvious variation in species assemblages between plots with most locations dominated by *Nebela tincta, Assulina muscorum* and *Corythion – Trinema* type.

Over the monitoring period, assemblages remained fairly constant, although there were some subtle changes in the minor taxa. In a comparison of plot A (adjacent to dammed ditch), August samples from 2000, 2001 and 2002, *Bullinularia indica*, a taxon commonly found on dry hummocks increased, whilst *Arcella catinus*, more commonly found in wetter conditions, decreased. *Arcella catinus* was present in March 2000 in plot A, but disappeared over the monitoring period. In plot B (the control for plot A), *Difflugia pristis*, a taxon found in wet conditions, increased in the August 2001 and 2002 samples. *Difflugia pristis* increases in numbers in plot C (dammed) from June 2001 and by August 2002 is a major component of the testate amoebae population. *Nebela marginata*, another wetter indicator, also increases in percentages in plot C, whilst *Heleopera sphagni*, a drier indicator, decreases slowly after June 2001. *Nebela flabellulum* (a dry indicator) also shows a decrease.

	Jan-00	Mar-00	Mar-00	Jun-00	Jul-00	Aug-00	Dec-00	Mar-01	Jun-01	Aug-01	Oct-01	Apr-02	Jul-02	Aug-02
Arcella catinus type	21.65	10.96	7.41	15.36	1.36	15.23	3.13	1.75	8.33	0.63	13.08	0.00	10.64	4.23
	0-55	0-25	0-30	0-33	0-5	0-50	0-13	0-7	0-17	0-3	0-49	-	0-38	0-17
Euglypha strigosa	19.31	7.87	24.21	2.99	3.52	1.57	2.34	0.93	0.00	0.42	8.63	0.00	0.91	3.15
	2-41	0-29	0-50	0-8	0-7	0-3	0-9	0-3	-	0-2	0-27	-	0-4	0-10
Euglypha tuberculata	0.00	0.00	0.00	4.95	3.22	2.22	0.26	1.76	0.00	1.26	2.08	1.79	0.00	0.00
	-	-	-	0-17	0-6	0-9	0-1	0-4	-	0-5	0-5	0-7	-	-
Nebela tincta	20.72	31.74	23.46	22.78	37.02	28.02	3.39	45.18	29.17	26.51	37.48	38.91	27.00	21.92
	2-41	4-100	12-30	10-44	29-35	0-55	0-14	35-50	0-50	0-50	3-100	1-71	10-51	0-50
Assulina semimulum	2.84	2.81	0.00	2.50	1.34	3.02	0.00	1.19	8.33	4.20	3.32	0.00	3.05	4.46
	0-8	0-8	-	0-10	0-3	0-12	-	0-5	0-33	0-17	0-13	-	0-9	0-12
Assulina muscorum	8.50	23.18	28.47	16.70	19.99	33.54	4.43	15.70	22.92	20.14	17.95	12.14	34.28	17.61
	2-12	0-58	14-53	3-36	8-40	2-50	0-18	9-25	0-50	0-44	0-49	0-20	6-76	0-50
Corythion -Trinema type	3.38	7.14	11.27	7.35	13.22	0.00	25.52	18.33	23.96	13.13	4.91	45.04	4.01	13.03
	0-10	0-29	0-35.29	0-17	3-27	-	0-100	1-26	0-67	0-50	0-10	14-97	0-13	0-50
Heleopera petricola	13.22	0.72	0.00	1.56	3.24	4.13	4.95	6.97	0.00	2.78	2.99	0.00	8.78	3.34
	0-32	0-3	-	0-6	0-6	0-17	0-20	0-18	-	0-11	0-7	-	0-19	0-12
Bullinularia indica	0.23	1.09	0.46	4.17	1.44	1.98	0.26	0.88	0.00	25.63	2.58	0.00	3.23	15.27
	0-1	0-4	0-2	0-17	0-4	0-8	0-1	0-4	-	0-100	0-7	-	0-13	0-60
* Hyalopshenia ovalis	1.46	9.78	0.46	12.85	0.00	6.75	0.00	0.22	0.00	0.00	0.66	0.34	1.36	4.76
	0-4	0-39	0-2	0-43	-	0-27	-	0-1	-	-	0-3	0-1	0-5	0-16

Wet

Dry

Table 4.6a: Summary of testate amoebae data from treatment A; (species means expressed as percentage of total testate amoebae, range and constancy – light to dark shading representing constancy of 1 to 4). Blank cells indicate absence. (* insufficient data for water table but considered to be a species of wet pools or damp minerotrophic conditions, **insufficient data for quantitative measure but dry hummocks and drained mires.) Only taxa occurring at >5% are included.

Wet

Dry

Table 4.6b: Summary of testate amoebae data from treatment B; (See Table 4.6a caption for further explanation)

	Jan-00	Mar-00	Apr-00	Jun-00	Jul-00	Aug-00	Dec-00	Mar-01	Jun-01	Aug-01	Oct-01	Apr-02	Jul-02	Aug-02
Euglypha strigosa	9.28	2.63	7.87	3.48	3.56	2.62	1.54	1.62	0.41	1.27	3.06	0.00	0.78	0.23
	0-24	0-9	5-12	0-11	0-7	2-3	0-5	0-6	0-2	0-3	0-10	-	0-3	0-1
Euglypha tuberculata	0.00	2.84	0.00	4.89	1.33	1.59	6.15	2.42	2.25	2.25	0.67	0.00	0.65	0.23
	-	0-11	-	4-8	0-3	0-6	0-17	0-7	0-9	0-7	0-3	-	0-2	0-1
Difflugia pristis	0.00	0.00	0.00	0.00	1.13	0.00	0.00	0.00	3.51	18.12	1.06	5.40	1.04	19.44
	-	-	-	-	0-2	-	-	-	0-8	0-48	0-3	0-13	0-3	3-58
Nebela tincta	22.51	42.43	33.20	33.51	15.59	40.35	21.00	26.85	39.05	24.10	21.21	19.13	22.87	7.16
	20-25	0-75	13-52	15-50	3-34	19-64	7-33	0-45	28-45	6-43	10-35	0-33	0-34	0-20
Nebela marginata	0.00	0.00	0.00	0.00	12.31	0.00	11.47	11.01	8.76	6.23	8.02	37.50	0.00	8.47
	-	-	-	-	0-21	-	0-35	0-32	2-18	0-14	0-16	0-100	-	2-18
Assulina semimulum	14.28	0.35	0.20	2.24	0.45	0.80	0.46	9.28	2.07	1.27	2.50	0.00	3.40	0.69
	0-38	0-1	0-1	0-6	0-1	0-2	0-2	0-33	0-8	0-3	0-10	-	0-7	0-3
Assulina muscorum	6.92	30.65	17.39	16.46	19.70	24.77	16.60	5.90	11.68	4.03	12.13	12.59	17.20	38.37
	0-21	0-100	6-35	2-56	3-50	1-51	0-50	0-13	2-19	0-14	0-21	0-33	0-38	1-65
Heleopera sphagni	7.63	0.43	3.15	11.47	2.89	4.26	0.93	8.33	1.10	0.93	2.11	0.00	3.29	1.53
	0-20	0-1	0-13	0-39	0-11	0-15	0-4	0-33	0-2	0-4	0-7	-	0-12	0-4
Nebela flabellulum	11.88	2.89	10.34	5.50	3.62	1.02	3.30	0.94	6.54	3.86	0.72	3.13	1.95	0.95
	0-40	0-9	3-29	0-17	0-14	0-2	0-10	0-3	0-13	0-7	0-2	0-13	0-8	0-3
Heleopera petricola	19.18	12.81	12.93	17.59	16.32	15.68	4.94	3.31	12.92	4.84	16.35	4.26	17.75	4.02
	11-29	0-27	3-21	8-35	9-33	6-21	0-10	0-9	3-21	0-14	0-33	0-13	0-50	0-9
Corythion -Trinema type	2.63	0.25	2.09	1.30	18.73	2.89	31.04	18.86	7.11	21.06	15.62	7.39	0.53	8.19
	0-6	0-1	1-3	0-2	8-37	2-3	0-67	7-33	5-10	7-29	4-33	0-25	0-2	0-19

Table 4.6c: Summary of testate amoebae data from treatment C; (See Table 4.6a caption for further explanation)

Wet

	Jan-00	Mar-00	Mar-00	Jun-00	Jul-00	Aug-00	Dec-00	Mar-01	Jun-01	Aug-01	Oct-01	Apr-02	Jul-02	Aug-02
Hyalosphenia elegans	0.38	0.00	0.21	0.00	5.85	0.00	0.00	0.00	0.00	5.08	1.14	0.00	0.00	0.96
	0-1	-	0-1	-	0-10	-	-	-	-	0-12	0-5	-	-	0-3
Euglypha strigosa	9.31	8.55	10.96	4.22	4.22	6.79	5.57	1.67	3.17	2.02	1.74	3.67	2.82	5.48
	0-21	0-23	1-19	2-7		0-16	0-20	1-3	0-8	0-6	0-5	0-15	0-6	0-8
Cyclopyxis arcelloides	0.13	0.88	0.00	0.00	0.00	0.00	0.90	0.00	1.26	1.33	0.00	1.15	9.77	1.20
	0-1	0-4	-	-	-	-	0-4	-	0-2	0-3	-	0-4	0-18	0-4
Euglypha tuberculata	0.00	0.00	1.88	8.67	10.60	3.79	11.85	9.05	6.01	6.35	8.66	10.25	6.23	2.27
	-	-	0-7	1-16	2-25	<1-12	3-33	1-23	0-21	0-13	1-23	0-22	0-17	0-8
Difflugia pristis	0.00	0.00	0.23	0.00	5.38	0.00	1.67	2.87	9.40	5.50	2.71	10.60	26.78	45.25
	-	-	0-1	-	1-13	-	0-3	0-9	1-19	0-14	0-7	0-17	0-81	2-63
Nebela tincta	26.00	38.08	18.20	17.54	15.45	31.34	11.70	16.35	17.19	26.02	12.84	14.83	12.43	2.46
	12-46	9-70	7-31	11-25	7-26	12-75	4-20	4-30	10-25	8-62	9-21	6-30	3-20	0-9
Nebela marginata	0.00	0.00	0.00	0.00	3.85	0.00	9.71	10.57	5.09	10.96	4.49	11.88	0.00	5.85
	-	-	-	-	0-13	-	2-27	0-30	0-19	0-31	0-13	2-38	-	0-14
Assulina semimulum	4.04	0.29	2.57	0.56	1.02	1.66	0.00	0.57	2.40	2.80	1.96	2.92	2.08	2.67
	2-9	0-1	1-8	0-1	0-2	0-3	-	0-2	0-4	0-11	1-4	0-8	0-4	0-8
Assulina muscorum	8.97	2.45	12.10	8.80	8.47	11.26	3.56	9.83	8.38	5.00	9.89	6.03	6.35	8.78
	0-17	0-6	5-19	5-18	1-15	3-22	1-7	4-13	0-14	0-15	7-13	4-9	1-15	5-12
Heleopera sphagni	5.26	9.54	4.46	6.44	3.90	9.35	6.53	7.15	6.99	0.93	4.98	2.88	0.63	0.45
	0-14	0-35	0-15	0-16	0-12	0-30	0-26	0-18	0-18	0-4	0-19	0-8	0-3	0-1
Corythion -Trinema type	1.85	0.00	5.62	2.32	16.55	1.02	12.14	20.34	6.55	10.08	9.30	9.50	0.51	4.94
	0-4	-	0-20	1-5	6-28	0-2	3-33	7-33	5-10	0-14	2-21	2-19	0-1	0-12
Nebela flabellulum	14.60	2.15	2.80	10.54	1.30	2.30	2.68	2.80	2.64	1.87	0.44	0.77	2.55	0.00
	2-23	0-8	0-7	0-25	0-5	0-8	0-10	0-7	0-4	0-7	0-1	0-3	1-6	-
Heleopera petricola	17.08	20.38	22.61	24.35	16.66	23.65	17.44	11.28	20.98	14.31	27.13	15.26	18.44	4.64
	9-32	15-29	5-45	7-36	6-28	2-47	0-35	0-22	10-25	0-29	4-56	0-30	1-40	0-9
Bullinularia indica	1.02	0.59	1.17	0.19	0.00	0.39	2.05	1.11	1.93	0.46	0.89	2.77	2.19	0.00
	0-3	0-2	<1-1	0-1	-	0-1	0-7	0-3	1-3	0-2	0-3	1-5	1-3	-
* Hyalopshenia ovalis	0.25	0.85	1.26	5.78	0.20	2.04	0.00	0.57	2.22	0.17	0.00	0.00	0.20	0.38
	0-1	0-2	0-5	0-12	0-1	<1-6	-	0-2	0-8	0-1	-	-	0-1	0-2
** Heleopera sylvatica	0.50	0.00	3.64	0.00	0.00	0.00	4.77	0.00	0.00	0.93	0.00	0.23	0.00	7.56
	0-2	-	0-9	-	-	-	0-10	-	-	0-4	-	0-1	-	0-20

Dry

Wet ↑

Dry ↓

Table 4.6d: Summary of testate amoebae data from treatment D; (See Table 4.6a caption for further explanation).

	Jan-00	Mar-00	Mar-00	Jun-00	Jul-00	Aug-00	Dec-00	Mar-01	Jun-01	Aug-01	Oct-01	Apr-02	Jul-02	Aug-02
Arcella catinus type	8.04	3.03	7.51	10.53	11.19	15.69	17.58	0.00	0	23.61	8.74	0	1.47	0.00
	0-24	0-11	0-28	0-21	0-40	0-60	0-67	-	-	0-94	0-33	-	0-6	-
Euglypha strigosa	4.83	18.62	10.66	4.50	15.35	5.42	1.04	13.59	1.02	0.00	3.85	0.00	0.74	11.90
	0-9	0-33	2-25	0-17	0-39	0-11	0-4	0-25	0-3	-	0-8	-	0-3	0-33
Cyclopyxis arcelloides	1.35	0.74	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.00	6.25	0.00	0.00	0.00
	0-5	0-3	-	-	0-1	-	-	-	-	-	0-25	-	-	-
Euglypha tuberculata	3.26	0.00	3.74	5.49	10.14	21.54	20.05	4.19	5.67	14.94	6.05	0.00	0.45	0.00
	0-13	-	2-9	0-9	0-17	0-52	0-75	0-13	0-13	0-45	0-15	-	0-2	-
Difflugia pristis	0.00	0.00	0.00	0.00	0.42	0.00	6.51	0.00	1.99	2.27	5.20	0.00	0.00	0.00
	-	-	-	-	0-2	-	0-25	-	0-4	0-9	0-18	-	-	-
Nebela tincta	21.51	27.03	21.37	17.75	12.47	10.14	11.59	21.42	28.11	21.92	22.33	37.50	39.78	20.00
	0-38	0-33	2-48	3-39	6-23	0-21	0-33	0-61	0-43	0-50	8-46	0-100	29-47	0-80
Nebela marginata	0.00	0.00	0.00	0.00	0.65	0.00	0.26	0.00	0.63	1.39	0.41	0.00	0.00	10.71
	-	-	-	-	0-2	-	0-1	-	0-3	0-6	0-2	-	-	0-43
Assulina muscorum	26.41	19.75	16.75	32.55	20.25	12.80	5.47	23.67	8.49	27.76	9.44	25.00	14.42	8.33
	0-75	0-56	8-26	7-50	7-36	0-31	0-22	5-43	0-30	0-50	0-25	0-100	0-50	0-33
Euglypha rotunda type	2.94	0.00	1.94	2.32	3.98	3.24	1.43	5.42	4.90	2.27	10.35	0.00	0.00	3.57
	0-12	-	0-4	0-7	0-13	0-12	0-6	0-15	0-14	0-9	0-23	-	-	0-14
Corythion -Trinema type	3.88	2.80	7.44	0.79	18.49	1.62	27.21	23.96	6.15	5.84	4.67	0.00	2.51	5.00
	0-9	0-6	2-14	0-2	11-33	0-5	0-100	0-43	0-14	0-14	0-13	-	0-6	0-20
Nebela militaris	0.00	0.50	1.19	0.60	0.00	1.83	1.30	0.00	2.27	0.00	3.85	6.25	1.04	8.33
	-	0-2	0-5	0-2	-	0-6	0-5	-	0-6	-	0-10	0-25	0-11	0-33
Heleopera petricola	7.39	12.38	8.29	5.70	5.28	14.43	6.25	1.50	11.21	0.00	5.74	6.25	10.69	7.14
	0-11	0-33	6-12	0-17	0-11	0-35	0-25	0-6	0-29	-	0-23	0-25	0-21	0-29
Hyalopshenia ovalis	2.33	5.56	2.53	13.73	0.00	8.66	0.00	0.00	0.00	0.00	2.62	0.00	18.09	25.00
	0-5	0-22	0-6	0-23	-	0-30	-	-	-	-	0-8	-	0-41	0-100

a) Differences in testate amoebae populations before and after damming

Concentration data were highly variable between samples and seemed unlikely to produce meaningful results. A logarithmic conversion was carried out on the concentration data and analyses performed using this converted dataset.

Mann-Whitney tests on pre- and post- damming logged concentrations showed that testate amoebae populations before damming were significantly higher than post damming at the 95% confidence level (p = 0.0448). This was a comparison including all treatment sites. Comparisons of the individual treatments before and after damming showed that there were significant differences in treatment B (control). Pre-damming logged concentrations were higher than post damming (p = 0.0348). All other treatments showed that there were no significant differences.

Live percentages have been used in this project as an indication of population turnover. The analyses used on concentration data were repeated on live percentages. Mann-Whitney tests on pre- and post- damming percentages showed that there were no significant differences (p = 0.7052).

b) Is the response affected by seasonality?

To further explore the impact of damming on the testate amoebae communities changes over time needed to be examined. The starting point for this was to plot the concentrations over time. Figures 4.11 and 4.12 show the logged concentrations in treatments A and B and C and D respectively with error bars to show standard deviations.
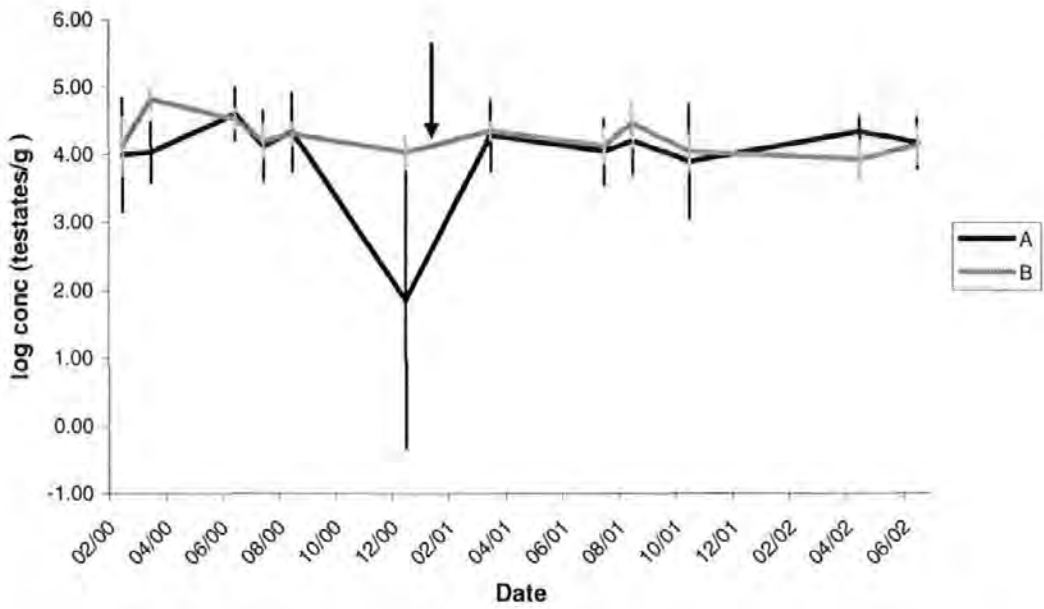
Figure 4.11: Logged concentrations (testates per gram dry weight) plotted against time and standard deviations (error bars) for treatments A and B. Arrow marks approximate time of damming.
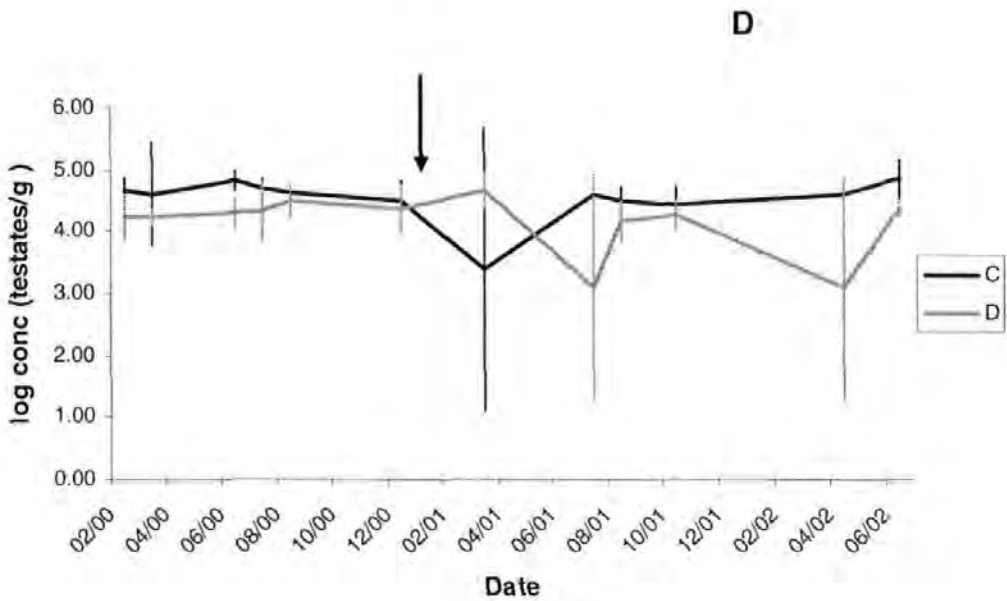
Figure 4.12: Logged concentrations (testates per gram dry weight) for treatments C and D (see caption Figure 4.11 for further explanation).

The concentrations for plot B are more consistent than for plot A. The major dip in concentrations in plot A is from the December 2000 counts. Some of the counts for that

month were, overall, rather low and should, perhaps be discounted from analyses. However, although low counts would be unacceptable for percentage data, they are a reasonable measure of overall concentrations. Differences between treatments are not obvious from looking at the graphs in Figures 4.11 and 4.12 although there does seem to be more variability in the summer months for plot D, especially after damming.
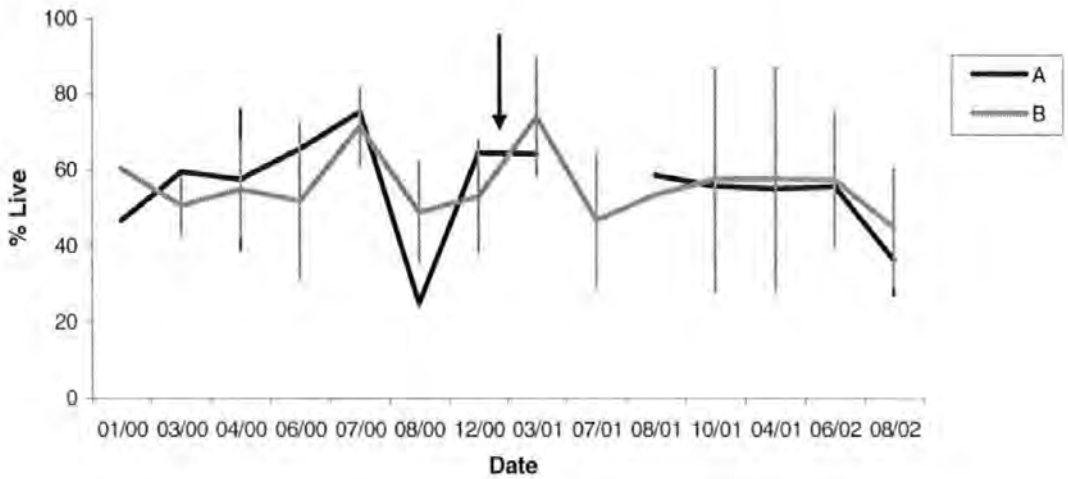
Figure 4.13: Percentage of live testate amoebae plotted against time for treatment plots A and B and standard deviations (error bars). Counts of less than 50 testate amoebae are excluded from plots. Arrow marks approximate time of damming.
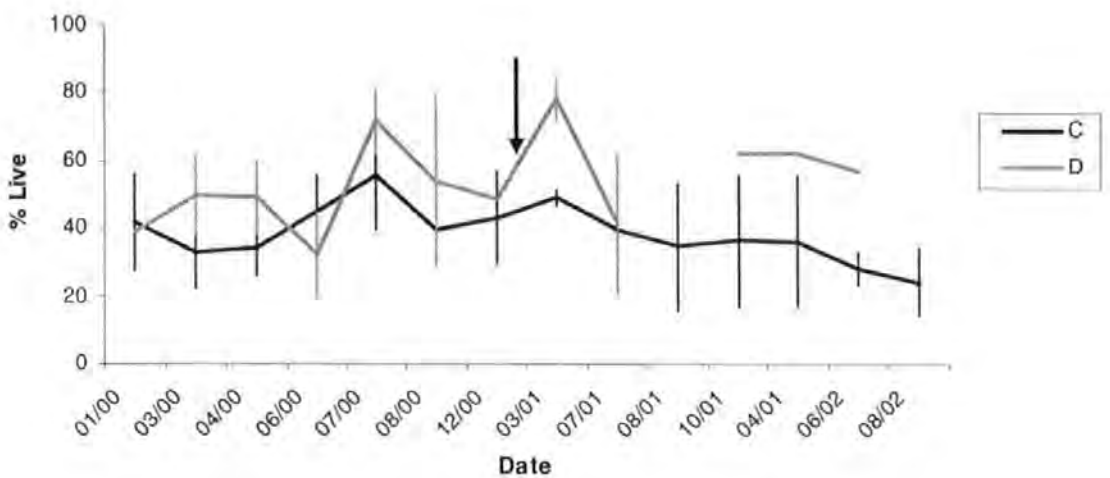
Figure 4.14: Percentage of live testate amoebae plotted against time (treatments C and D); (See Figure 4.13 caption for further explanation).

Figures 4.13 and 4.14 show percentage live data plotted against time in treatments A and B and C and D respectively. The variability in these plots seems much greater than in the plots of logged concentrations indicating that they may not be as useful in analyses.

The output from the above charts is rather inconclusive. Analyses of variance (ANOVAs) may produce statistically robust results and clarify any differences. The first ANOVA was on the data for 2000. As damming was not carried out until early 2001, a comparison of counts for all pre-damming months, should allow any seasonal variability to be seen without being complicated by changes unrelated to seasonality. The output for this analysis is shown in Table 4.7.

Source	d.f.	SS	MS	F	P
Month	5	7.6	1.5	2.83	0.020
Residual (error)	90	48.2	0.5		
Total	95	55.8			

Table 4.7: One-way ANOVA comparing logged concentrations with sampling months (2000).

The results of this analysis show that there was a significant difference (p = 0.02) between logged concentrations (testate amoebae per gram dry weight *Sphagnum*) in the five months sampled in 2000. However, the concentrations for December 2000 were lower than all other months and the standard deviation much greater. Fewer testate amoebae were counted in December. When this month is excluded from the ANOVA, the p value is 0.394, higher than the 95% rejection level. There is no significant seasonal difference in counts from March to August 2000.

A two-way ANOVA on the 2000 data comparing plots (A – dammed, B – control, C – dammed, D – control) and sampling month (again, excluding December) showed a

significant difference between plots (p = 0.006) but not between months (p = 0.338). The mean concentrations in treatment C were higher than the means for the other three treatments. This is shown in the output for a one-way ANOVA of treatments for 2000 in Table 4.8.

Source	d.f.	SS	MS	F	P
Sample plot	3	2.4	0.8	4.65	0.005
Residual (error)	76	13.0	0.2		
Total	79	15.3			

Table 4.8: One-way ANOVA comparing logged concentrations for 2000 with treatment plot

A one-way ANOVA on concentrations for all sampled months in 2001 also showed no significant difference between months (p = 0.706), when treatment plots were analysed, there was no significant difference between plots (Table 4.9). A two-way ANOVA on the data from 2001 showed no significant difference between months (p = 0.702) or treatments (0.897).

Source	d.f.	SS	MS	F	P
Treatment plot	3	0.7	0.2	0.24	0.867
Residual (error)	44	41.4	0.9		
Total	47	42.1			

Table 4.9: One-way ANOVA comparing logged concentrations for 2001 with treatment plot

One-way ANOVAs on the data from 2002 showed no significant difference between months (p = 0.255) but that there was a significant difference between treatments. The minitab output showed that the difference was between treatments C and D with A and B appearing very similar (Table 4.10).

Source	d.f.	SS	MS	F	P
Treatment	3	5.3	1.8	3.81	0.016
Residual (error)	44	20.4	0.5		
Total	47	25.7			

```
                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Level   N    Mean   StDev  ---+---------+---------+---------+------
A      12   4.2684  0.3990            (-------*-------)
B      12   4.2349  0.3963            (-------*-------)
C      12   4.7064  0.2713                      (-------*------)
D      12   3.7671  1.2107  (-------*-------)
                       ---+---------+---------+---------+------
                       3.50      4.00      4.50      5.00
```
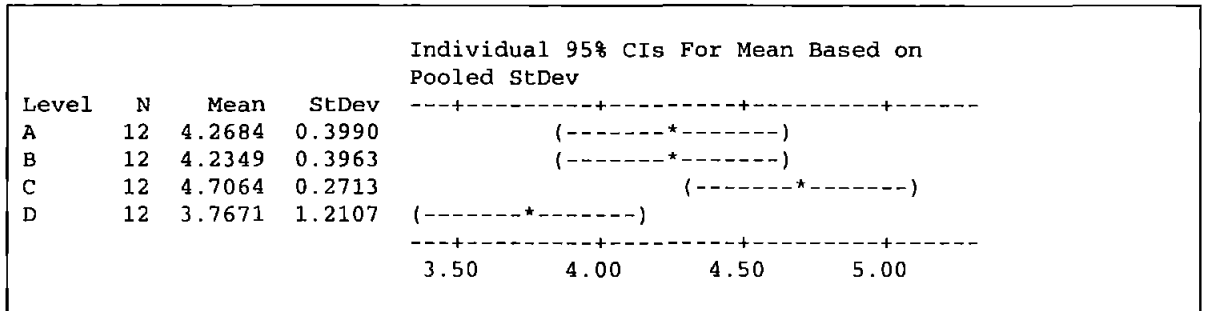
Table 4.10: One-way ANOVA comparing logged concentrations from 2002 with treatment plot and dotplot of means and confidence intervals. Dotplot included showing differences in logged concentrations between paired plots.

c) Month by month response to ditch blocking

As comparing data from all months showed that there were no differences in either logged concentrations or percentage live data from before and after damming, further analysis was necessary to see whether there were differences between specific months before and after damming. ANOVAs were carried out on matching months (pre- and post- damming) to see whether there was more difference between months than between samples.

One-way ANOVAs on logged concentrations for groups of months (comparisons of data from March, June and August) showed that there were no significant differences between years 2000, 2001 and 2002 for any of the months. The p value for June's analysis was 0.067. This suggests that testate amoebae populations may be more sensitive to change in June than other times of year.

A two-way ANOVA on June's data produced a statistically significant result for treatment type (A, B, C, D) and for date. The output is shown in Table 4.11. The treatments were marginally more significant than the date but neither explained a large proportion of the variation.

Source	d.f.	SS	MS	F	P
Treatment	3	4.27	1.42	3.25	0.033
Year	2	2.86	1.43	3.26	0.050
Interaction	6	2.32	0.39	0.88	0.52
Error	36	15.76	0.44		
Total	47	25.21			

Table 4.11: Two-way ANOVA for June logged concentrations (2000, 2001, 2002)

Table 4.12 shows the results of the two-way ANOVA where ditch status (blocked versus unblocked) is compared instead of sample plot. The date is no longer significant but there are significant differences between samples from blocked and unblocked plots, although the proportion of the variation explained is still low.

Source	d.f.	SS	MS	F	P
Ditch status	1	2.15	2.15	4.59	0.038
Year	2	2.86	1.43	3.05	0.058
Interaction	2	0.48	0.24	0.51	0.602
Error	42	19.71	0.47		
Total	47	25.21			

Table 4.12: Two-way ANOVA comparing June logs (2000, 2001, 2002) and ditch blocking status.

d) Changes in species assemblage over time – multivariate analyses

To explore the relationships between species assemblages and sample plot, canonical correspondence analyses were carried out using Canoco (Ter Braak & Šmilauer 1998). The data for the individual samples was combined to give one value for each plot (A, B, C and D) for each sample time. This was to overcome the problem of low counts with some samples, so that a complete set of data was available. Groups of months were analysed together – March, June and August from 2000, 2001 and 2002 to see whether there were

any changes over time. The entire dataset was analysed. Percentage live data and logged concentration data were analysed separately to assess the value of each measure.
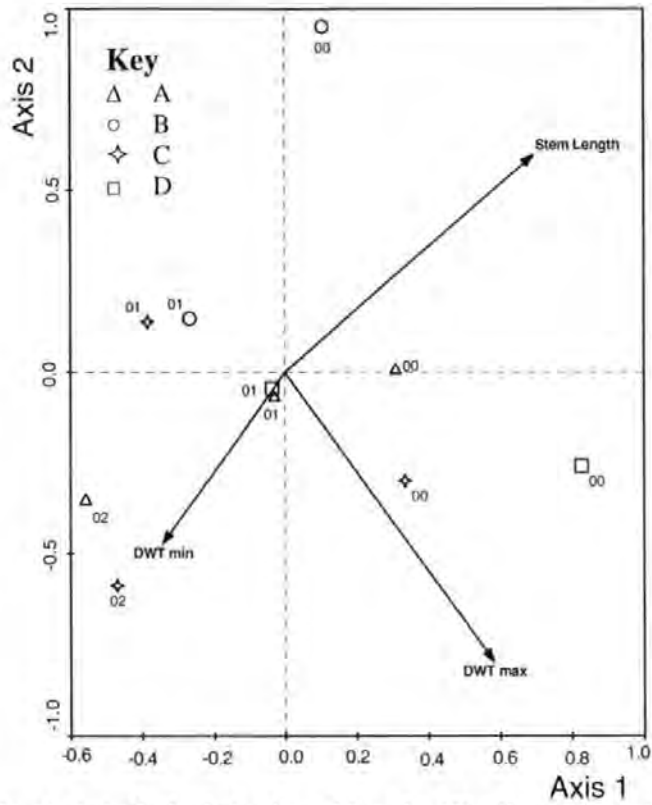
Figure 4.15: CCA of totalled data for March (% live of testate amoebae) with environmental variables. (Numbers represent sampling year)
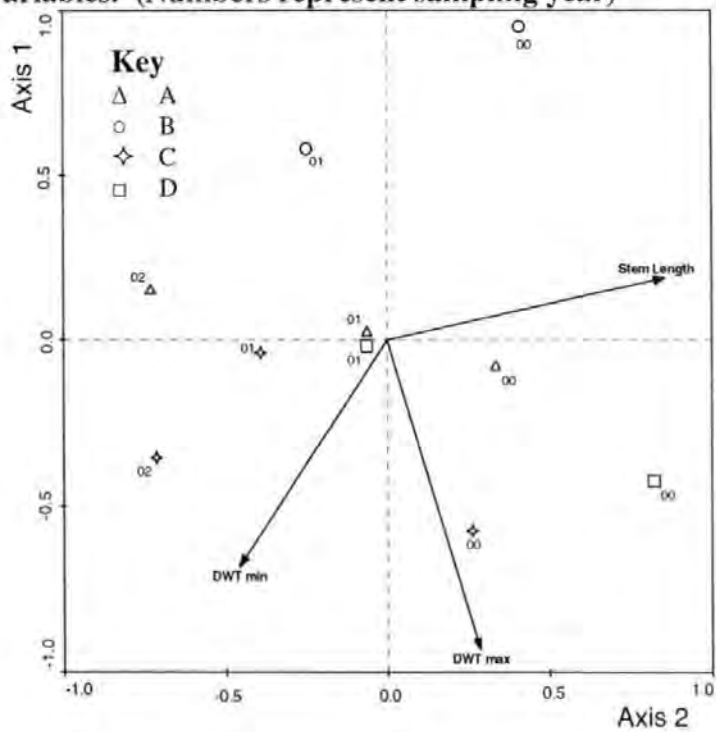
Figure 4.16: CCA of March totalled data (logged concentrations) with environmental variables (numbers represent sampling year).

Figures 4.15 and 4.16 are CCAs of the percentage live data and logged concentrations for March 2000, 2001 and 2002. There are only two values for 2002 because plots B and D did not produce combined counts higher than 50 testate amoebae. Both plots show a separation of samples from each year, with a progression from left to right along axis 1. Monte Carlo tests produced a significant result for the first axis with the logged data (p = 0.01) but not for the percentage data (p = 0.56). In Figure 4.15, samples appear to be grouped by plot along axis 2 to some extent. Moisture data were not available for March 2000 so are not included in these analyses.
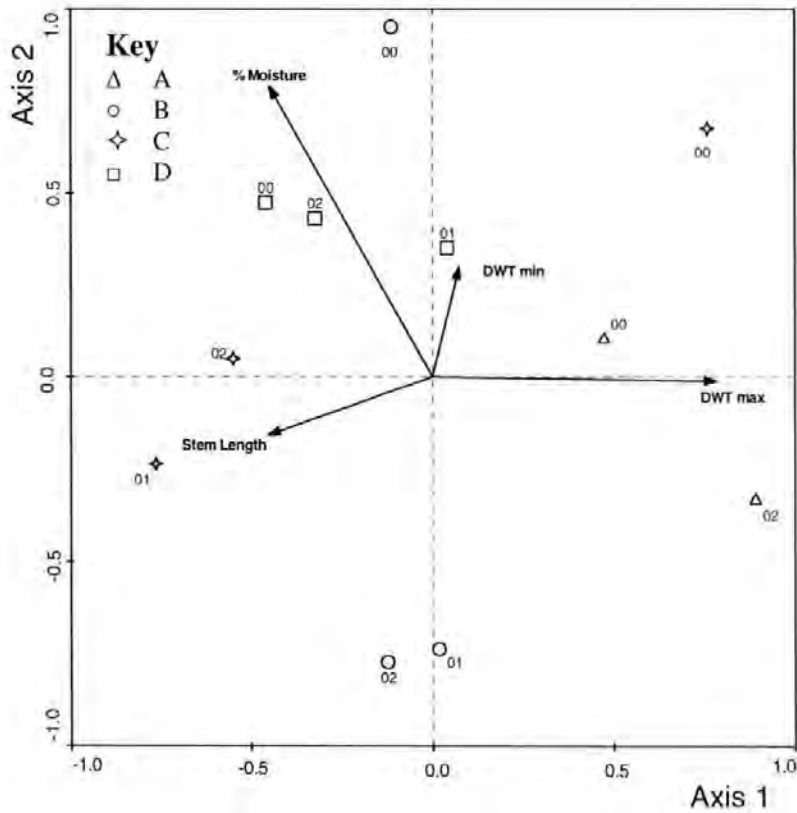
Figure 4.17: CCA of totalled data for June (% live) with environmental variables (numbers represent sampling year).
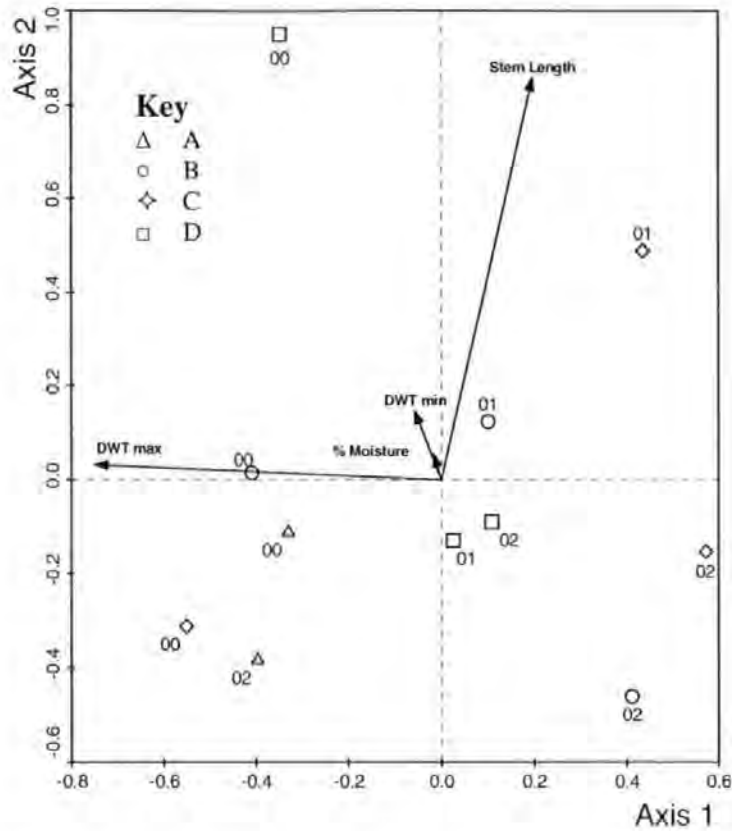
Figure 4.18: CCA of June totalled data (logged concentrations) with environmental variables (numbers represent year).

Figures 4.17 and 4.18 are CCAs for June samples. The pattern seen in the March samples is less distinct here, although there is some separation of years. This is more apparent in the logged sample CCA (Figure 4.17). In Figure 4.18 (% live data), D samples form the most coherent group. Monte Carlo tests did not produce significant results for either analysis (% live axis 1 – p = 0.57, logged concentration axis 1 – p = 0.15). Moisture content was an important variable in the percentage live analysis but was less important to the logged concentration data. Maximum depth to water table (how high water table rose) was more important than minimum.

In Figures 4.19 and 4.20 (August percentage live CCA and logged concentration CCA), there is separation along axis 1 of samples from 2000 (pre-damming) and samples from 2001 and 2002 (post-damming). Monte Carlo results show that the first axis is significant

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in both analyses (% live – p = 0.01, logged concentrations – p = 0.00). Moisture content was more relevant to the logged concentration data than the percentage live data for August samples. This is the reverse of the results for June.
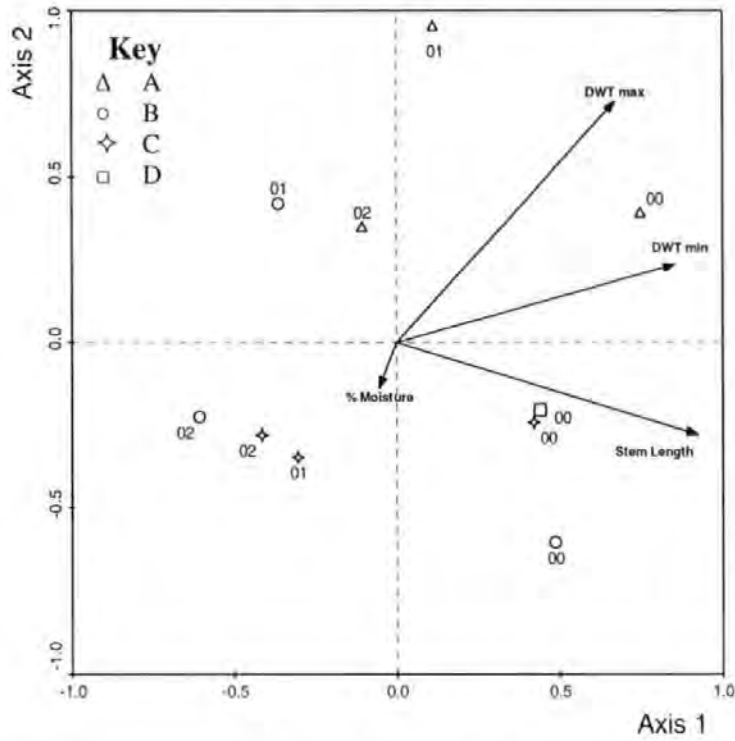
Figure 4.19: CCA of totalled data for August (%) with environmental variables (numbers represent year).
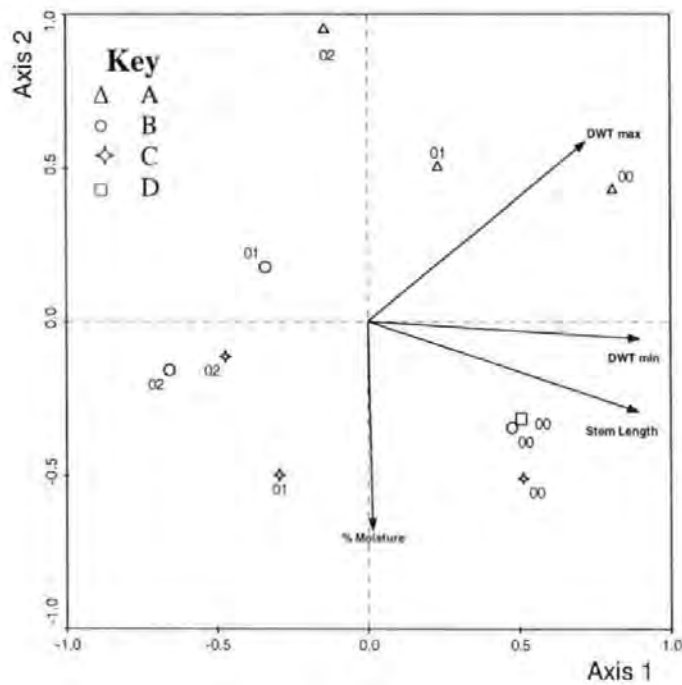
Figure 4.20: CCA of August totalled data (logged concentrations) with environmental variables (numbers represent year).

Figures 4.21 and 4.22 are the CCAs for all samples % live and logged concentrations. In Figure 4.21, although there is no distinct grouping of samples, the A samples tend to be found in the top left, B samples on the right of the graph, towards the bottom and C towards the bottom left. D samples are less coherent. Monte Carlo tests did not produce significant results for either the first axis (p = 0.26) or all axes (p = 0.16).
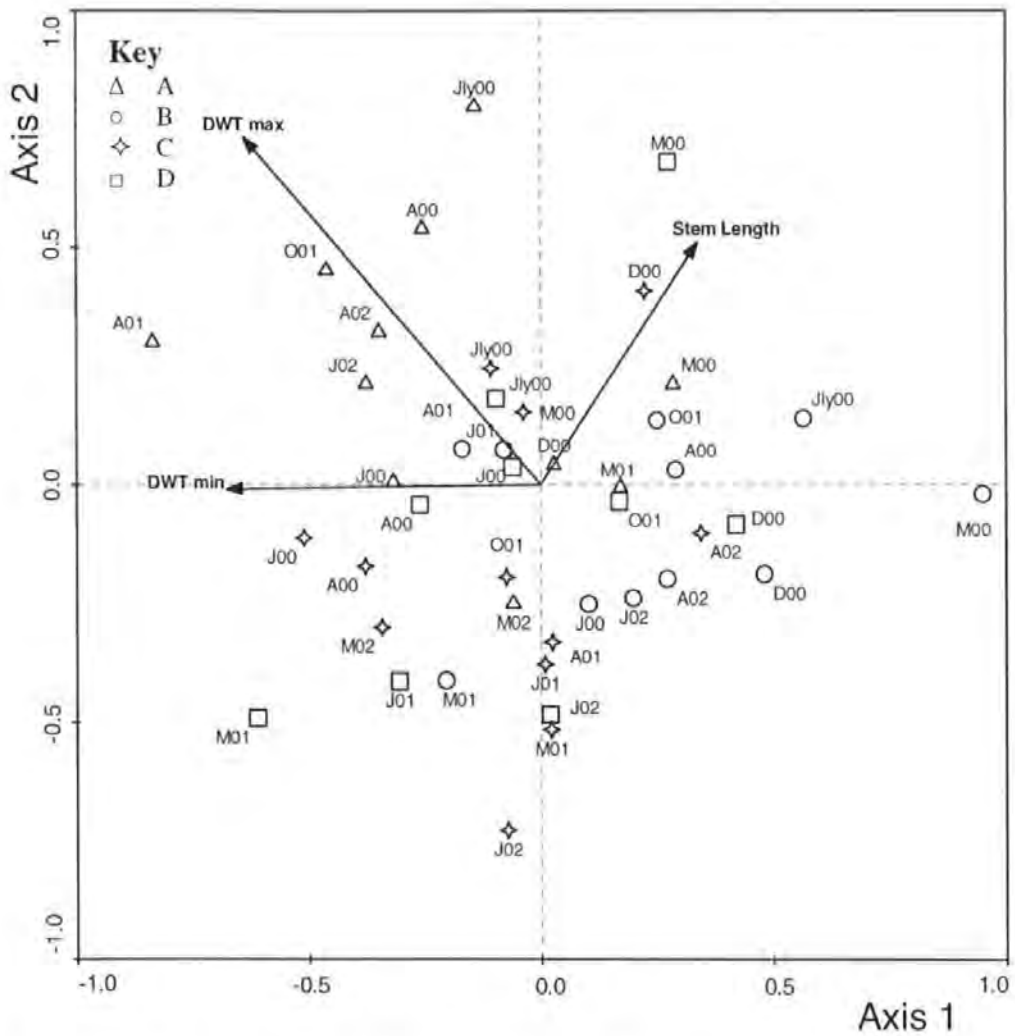
Figure 4.21: CCA of % live for all samples (totalled counts) with environmental variables (numbers represent sampling year, letter indicates sampling month – M = March, J = June, Jly = July, A = August, O = October, D = December).

Samples in Figure 4.22 (logged concentrations) show similar grouping to those in Figure 4.21. Monte Carlo tests again did not produce a significant result for the first axis (p = 0.26), although the eigenvalue for all axes was statistically significant (p = 0.03). In both analyses, there is more coherence between sample locations than sample month. The annual variability seen in the analyses of groups of months is not as clear when the entire dataset is analysed.
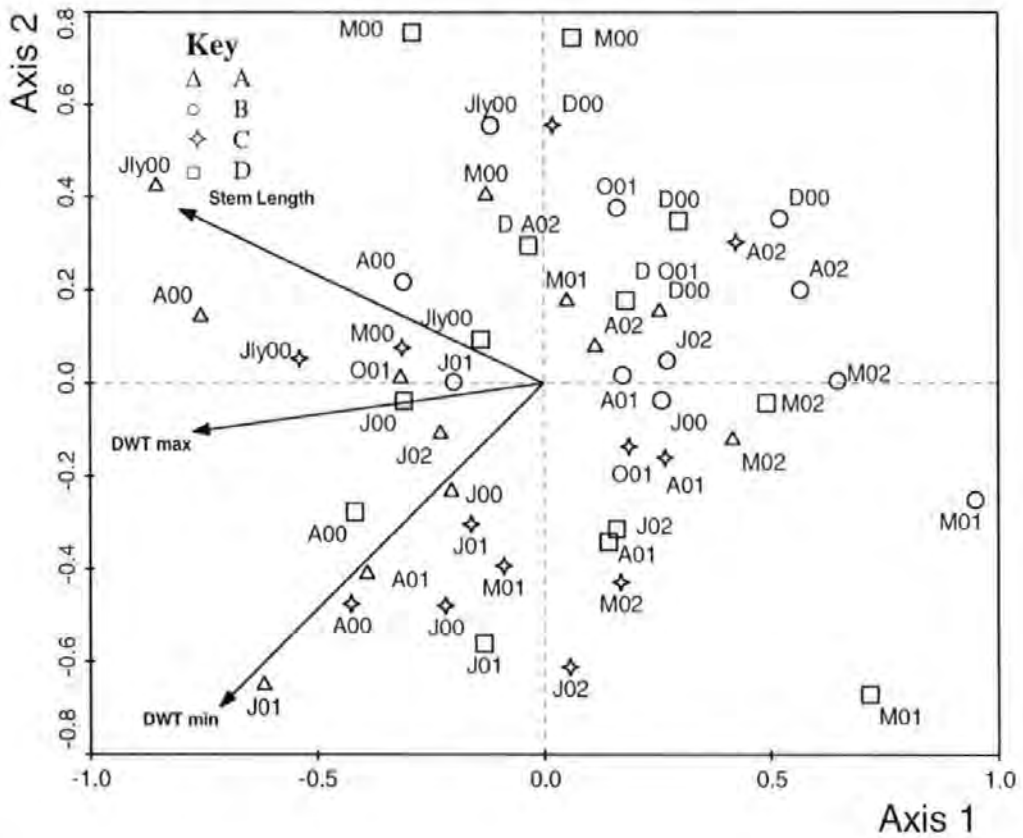
Figure 4.22: CCA of logged concentrations for all samples (totalled counts) with environmental variables (numbers represent sampling year, letter indicates sampling month – M = March, J = June, Jly = July, A = August, O = October, D = December).

4.4 Discussion

This chapter reports the results of a 30 month experiment assessing the response of testate amoebae communities to ditch blocking at Coom Rigg Moss. The bog is thought to have been damaged by drainage and peripheral forestry activity. The site is being restored as part of the Border Mires LIFE project; the aim was to improve hydrological conditions. Locations at the bog have been studied repeatedly both before and after ditch blocking work. Raising water levels on sites affected by peripheral land use is an important issue in restoration of raised bogs, as this is frequently the cause of damage. The inclusion in European legislation of a requirement not only to maintain good condition but also to ensure that damaged sites are restored so that they are actively laying down peat (O'Connell, 1999), means that all sites designated as SACs (Special Area for Conservation) have to be managed in a proactive manner.

4.4.1 Hydrological response

The aim of restoration work at Coom Rigg was to raise water levels across the site. Two peripheral drainage ditches were blocked in order to achieve this aim. Four experimental plots were established to monitor changes and assess the success of restoration. Water table was repeatedly monitored using WALRAGs (measuring minimum and maximum) before and after ditch blocking took place. Pre – restoration water levels ranged from minima of 10.87cm in control plot B to 19.36cm in dammed plot C and maxima of 4.46cm in dammed plot A to 1.32cm in control plot B. These water levels, although not optimal, are still within a healthy range for formation of peat. Some water table measurements from the 1950s showed that the water table was around 10cm below the peat surface (Chapman, 1965). Although not directly comparable with the WALRAG results, this figure gives some indication of the change in hydrological conditions over the last 50 years. The point

at which peat formation ceases is generally when water table falls to more than 40cm below the surface (Schouwenaars, 1993; Ferland and Rochefort 1997).

Despite the relatively high water table, the nature of Coom Rigg has changed dramatically over the past 50 years. Studies by Chapman (1964a, 1964b and 1965) showed a raised bog in relatively good condition with extensive *Sphagnum* carpets. A repeat survey in the 1980s (Chapman & Rose, 1991) found that the vegetation structure had been altered; *Sphagnum* carpets have been replaced by vegetation types dominated by *Eriophorum* spp. *Calluna vulgaris* and *Deschampsia flexuosa*. When interpreting long term changes in hydrology, caution should be exercised, as they are often a consequence of the interaction of a variety of factors (Moore, 2002). There has been some debate as to whether changes at Coom Rigg were induced by cessation of grazing when forests were planted, the impact of afforestation on the hydrology of the site (Chapman & Rose, 1991; Anderson *et al.* 1995), or a combination of the above factors and changing climatic conditions (Hendon & Charman, 2004). As well as being the major component of peat growth, *Sphagnum* also contributes to sustaining a high water table on bogs (Price, 1996). This factor further underlines the need to encourage higher cover of *Sphagnum* at sites where condition is deteriorating. Restoration at this site must focus on minimising that the impact of peripheral forestry activity on the hydrological unit in order to recreate *Sphagnum*-dominated peat-forming vegetation.

The results showed that there was an increase in water levels in plot C (the driest of the monitored plots), where minimum water levels increased by 3cm over the monitoring period. Maximum water table at this location also increased by around 2.5cm. In comparison, maximum water table in the control plot D (paired with plot C) rose by

approximately 1cm. In plots A and B, there was no change in minimum water table and maximum water table fell by around 3mm. This could simply be the result of seasonal variability in rainfall, rather than an indication that this area of the bog is becoming drier.

The comparison of pre- and post-damming water tables suggest that ditch blocking in plot C was effective, as the water table rose slightly and, from looking at the plots of water table against time, it is likely that this upward trend will continue (Figures 4.7 and 4.8). The increase in water levels in plot D could be a secondary effect of dams adjacent to plot C. Given the physical distance between plots C and D, damming at this location should be considered a success as the interior of the bog has benefited from peripheral restoration activity. The lack of response in sample plot A could have several explanations. The most likely of these is that the dams were not adequately watertight. Water could have been leaching from another source or, the damming may have halted deterioration in hydrological conditions which existed prior to restoration work. The fact that there was no change in water table at sample plot B (control for A) suggests that the first explanation is most likely. In a study of a peatland restoration project in Finland, Vasander *et al.* (1992) found raising water levels through blocking drainage ditches problematic as more dams were needed than initially installed to prevent leakage. It is important to remember that restoration monitoring results from Coom Rigg are only available for 18 months after ditch blocking. This is still the early stages of recovery; however some remedial work should be carried out to ensure that water table increases in response to dams in plot A.

4.4.2 Testate amoebae responses

Monitoring of testate amoebae communities at Coom Rigg began twelve months prior to ditch blocking. This has allowed comparisons of pre- and post-damming populations as

well as providing the opportunity to examine seasonal variability. There were several problems with low counts here and the major issue was sample size. Single stem samples were used in order to reduce the impact of monitoring on the sample plots and to allow for high frequency sampling. However, the opportunities provided by repeated sampling must be balanced by the drawbacks of low counts. In retrospect, improved data quality may have been gained by less frequent sampling, using larger sample sizes. The immediate problem has been overcome here by combining samples for some analyses.

The first measure used for analysis was the number of taxa found in each plot. The highest numbers were found in plot C. This is likely to be a product of the consistently higher counts achieved in samples from plot C. When plots and months with low counts were compared with numbers of taxa, there was some correlation. This reinforces the need to achieve high counts in order to produce statistically robust results.

a) Changes in species composition over time

A study of testate amoebae communities at a drained and restored bog site in Finland saw increases in *Assulina semimulum*, *Heleopera petricola* and *Nebela tincta* in response to rising water levels (Jauhiainen, 2002). These species were all present at Coom Rigg both before and after ditch blocking but showed little change in dominance over the monitoring period.

A comparison of species composition over time at the four sampling locations showed that most of the sample locations were dominated by *Assulina muscorum*, *Nebela tincta*, *Corythion-Trinema* type and *Euglypha strigosa*. Most taxa remained fairly constant throughout the monitoring period. Some composition changes were observed in plot C, the

location where water table changed most. *Difflugia pristis* and *Nebela marginata* – both wetter taxa - increased from around June 2001, and *Heleopera sphagni* and *Nebela flabellulum* indicators of slightly drier conditions (Charman *et al.*, 2000), decreased. Other plots had some minor changes but were less consistent changes than in plot C. The water table in plot C was lowest before damming and it increased more than in the other plots. This increase in water table raised the annual mean by over 2cm and has altered conditions enough to affect the testate amoebae community composition. The only other location where the post-damming average had altered was plot D. The change here was less than 1cm and therefore unlikely to have had a dramatic impact on communities.

b) Concentrations

Concentration data were problematic, because the high levels of variability both within and between sampling location made identifying patterns difficult. Some effort to overcome this was made by log transforming the entire dataset. This reduced variability and allowed for statistical analyses. Analysis of the transformed dataset showed that concentrations were significantly higher post-damming but that the only individual plot where concentrations increased was plot B. There was no change in water table in plot B and moisture content was significantly lower after damming. This result may be related to random population changes at this location or other unmeasured environmental parameters. When matching months from 2000, 2001 and 2002 were compared, the only significant result was for the June comparison. This may suggest that communities are more sensitive to conditions in June than at any other time of year. However, when the boxplot for the one-way ANOVA was examined, concentrations increased in 2001 but decreased again in 2002. This suggests that using simple sets of concentration data for analysing responses to small changes in water table is not particularly effective.

c) Percentage live data

ANOVAs on percentage live data showed no significant difference between sample location or sampling date. Again, this suggests that where changes in water table are relatively small, this is not a good measure of testate amoebae response.

d) Multivariate analyses

Canonical correspondence analysis (CCA) was performed on the datasets. Comparisons of matching months from each year were examined first. Both percentage live data and logged concentrations were used to compare their effectiveness. These analyses were made using the combined datasets discussed earlier. The output for most months showed a difference between sample years with samples from 2000 being more closely grouped than those from 2001 and 2002 where some mixing occurred. There was little difference in results from percentage live and concentration datasets suggesting that both measures are equally valid. The results for the March comparison were most clear.

When the entire datasets were analysed, there was less obvious separation between sampling year, with some distinct patterns in sample location indicating that differences between locations were greater than differences between years. Again, both percentage live and logged concentration data produced very similar results.

Each analysis showed that maximum and minimum depth to water table (DWT) exerted a slightly different impact on the samples. The factors varied in importance from month to month, although the arrow for DWT maxima was longer in each ordination, suggesting that it was more important than minima. This is contrary to common understanding of peat growth and functioning of peat systems, where minima are accepted as being more

important although, as the results were not statistically significant, this would need further investigation.

4.5 Conclusions

The major issue with the results from Coom Rigg Moss is that there simply was not a statistically significant change in water table over the monitoring period. There could be several reasons for this but the situation should be investigated to ensure that the management goal of improving hydrological conditions is achieved. This lack of response to damming could be attributed to the climatic influence proposed by Hendon & Charman (2004). It is also possible that variability in precipitation over the monitoring period may have affected restoration success. If rainfall was significantly lower than average, ditch blocking would take longer to alter hydrological conditions. Precipitation records for the area, both before ditch blocking and during the monitoring work, may be useful for further analyses. It appears likely that the ecological changes at Coom Rigg Moss are not simply a result of the adjacent afforestation. Longer term monitoring will be necessary to determine whether these changes are a bar to the restoration of good hydrological condition.

The location where water table altered most has seen some change in testate amoebae community composition, suggesting that they do respond to fairly minor hydrological fluctuations. The most useful analyses here were the community composition tables and CCAs. There seems to be little difference between analysing percentage live data or logged concentrations in this instance.

An important consideration for further sampling is the possibility of taking larger samples to avoid low counts. The reason for taking such small samples at Coom Rigg was to avoid the possibility of damaging plots, and therefore, simply monitoring damage inflicted by sampling. Where a site is monitored less intensely, larger sample sizes should not create this problem.

Overall, the testate amoebae communities have changed very little but, as hydrological conditions have remained fairly constant, this is not surprising. It may be useful to return to this site and carry out repeated monitoring of both hydrology and testate amoebae to see whether the reason for little change occurring was that the site was in very early stages of restoration and, if conditions have improved now, whether testate amoebae have responded to improvements. The availability of a baseline dataset makes this a very interesting monitoring project.

Another consideration arising from the monitoring at Coom Rigg is that some drier taxa of testate amoebae persist even when water table increases, if other ecological conditions allow. An example of this may be *Bullinularia indica* which was present in most samples. This is a species common to forested mires and the surrounding forestry may be a more important factor than hydrology in its continued presence.

CHAPTER FIVE: Forest Harvesting and Peat Bog Restoration at Flanders Moss NNR

5.0 Introduction

One of the major management issues affecting the conservation interest of peatlands in the UK is damage caused by forestry plantations. The damage caused by the afforestation of large areas of Britain's peatlands has been discussed in section 2.3.4. The aims of this project are to develop knowledge of testate amoebae ecology in degraded bogs and to assess the potential for using testate amoebae as bioindicators of peatland restoration. To fulfil these aims, it was necessary to carry out monitoring of the types of damage commonly found in UK peatlands. It was therefore felt that a section of the study should be dedicated to forestry removal. Objective 2 was to compare peatland condition in a restored afforested mire. The experimental restoration work by the Forestry Commission at Flanders Moss provided an opportunity to meet this objective.

5.1 Flanders Moss

Flanders Moss is situated 16km west of Stirling in the Forth valley (Figure 5.1), close to the villages of Kippen and Thornhill in central Scotland. The site is managed largely by Scottish Natural Heritage (SNH) with a smaller area managed by the Scottish Wildlife Trust (SWT). The Moss is the largest area of near-natural lowland raised bog in Britain and is therefore of great conservation importance (Jacobs, 1998). Flanders Moss is a remnant of a much larger series of raised peat bogs which once occupied the Forth valley. Most of this area has been reclaimed for agriculture since the mid-18th century. At this time, peat was seen as a waste product and was dug out of the site and disposed of down the River Forth (Brooks and Stoneman, 1997a).
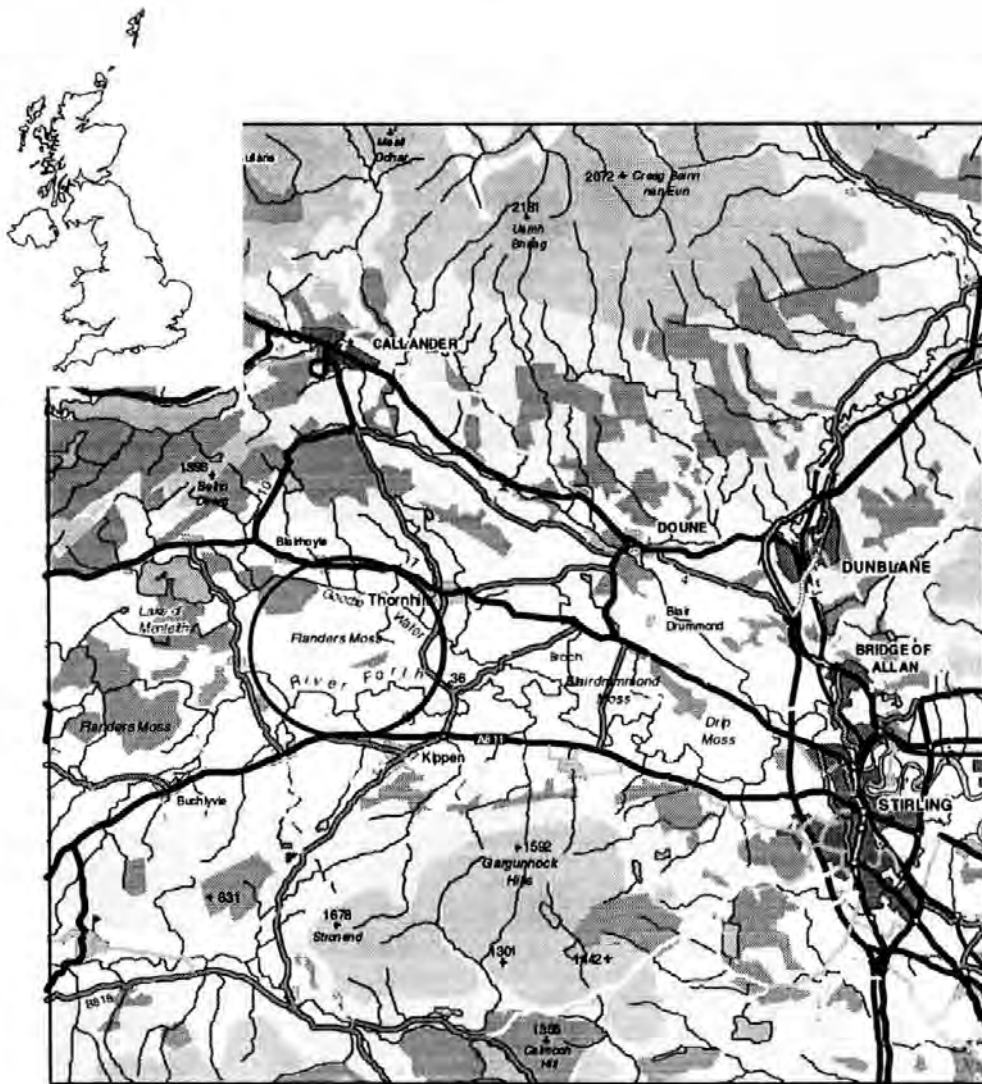
Figure 5.1: Location of Flanders Moss (circled).

The conservation designations applying to the site are Scottish Wildlife Trust reserve, Site of Special Scientific Interest (SSSI), National Nature Reserve (NNR), Special Area of Conservation (SAC), and Geological Conservation Review (GCR) Site. In terms of

legislation, it is therefore highly protected. The area of the site is 859 hectares, of which some 540 ha is active raised bog. The Scottish Wildlife Trust reserve covers 45 hectares of the site (Brooks and Stoneman, 1997a). The area designated as a SAC covers Collymoon Moss, Flanders Moss, Killorn Moss and Shigarton Moss and is 1031 hectares in total. SAC designations are given to the site on the grounds that it is one of the best areas of active raised bog in Britain and that it contains more than 10% of the UK's resource of such habitat (Jacobs, 1998).

The objectives of management at the site are "to maintain and enhance the lowland raised mire habitat with particular regard to its hydrology and physical integrity" (Brooks and Stoneman, 1997a: p.189). The site has many conservation problems, including conflict with surrounding landowners over hydrological management. This is a frequent problem with lowland raised mires, as they are usually surrounded by good quality agricultural land. Further problems on the bog are previous damage from forestry activity, burning, drainage for peat cutting, and birch encroachment.

A study commissioned by the Nature Conservancy Council (NCC) showed that birch woodland is actively colonising the site (Bannister, 1978). This is usually an indirect result of a falling water table and is probably a response to past drainage activity. Tree removal and ditch-blocking have been management priorities at the site in the past. Preparations for peat extraction during the 1980s affected an area of 140 hectares. Vegetation was stripped and drainage ditches put in place (Jacobs, 1998). Peat extraction was never carried out and

vegetation has regenerated in this area, although species composition is still severely limited by reduced water levels.

Vegetation on Flanders Moss varies largely as a result of the management issues outlined above. There is a mosaic of near-natural primary raised bog, wooded primary raised bog, conifer plantations, scrub, fen and rough grassland (Jacobs, 1998). Raised bog vegetation is composed of a discontinuous *Sphagnum* moss carpet dominated by *S. papillosum* and *S. magellanicum* with *S. capillifolium, S. recurvum* and *S. tenellum* occurring less frequently and *S. imbricatum* (Nationally Scarce), *S. fuscum* and *S. molle* occurring rarely. Vascular plants abundant in these areas include *Erica tetralix, Andromeda polifolia, Narthecium ossifragum, Vaccinium oxycoccus, Eriophorum angustifolium* and *Rhyncospora alba*.

Areas of poor fen vegetation at the site are composed of *Carex curta, C. rostrata, Viola palustris, Sculletaria galericulata* scullcap, *Galium palustre, Juncus acutiflorus* and *Sphagnum squarrosum* and *S. fimbriatum*.

5.2 Methods

5.2.1 Description of Restoration Work

One of the major causes of damage to Flanders Moss was a 40 hectare forestry plantation on the eastern edge of the bog. The stand, planted in 1973, was composed of *Picea abies* and *Pinus contorta*. Forest Enterprise managed the plantation. The removal of this plantation was important for the continued conservation interest of the bog. Opinion in nature conservation is that rehabilitation of afforested peatlands should be successful; the bog surface should not be

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as severely damaged as it would be during peat harvesting and there is every possibility that the acrotelm can regenerate (Brooks & Stoneman, 1997b).

The Forestry Commission (FC) removed the trees in 1998 and established an experimental area in 1997 to study the impact of tree removal at Flanders Moss and to test different restoration techniques. A ten-year monitoring programme was set up to study the recovery of the bog after tree removal. This experimental area provides the focus of this study on Flanders Moss. The results from this research will influence future FC policy on tree removal from peat. Figure 5.2 is a photograph of the experimental area from July 2000, when the first samples were taken.

Figure 5.2: Overview of restoration project at Flanders Moss showing plough furrows and dams (circled).

The aim of the FC experimental work was to examine the impact of two damming treatments and three different tree removal treatments on restoration of the bog. Tree removal techniques were combined with damming techniques to produce six treatment types. Each treatment was replicated four times producing twenty-four sample plots. The treatment methods are outlined below and a plan of the site is shown in Figure 5.3

24 F	18 F	12 F/D	6 H
23 H	17 W	11 W	5 W/D
22 H/D	16 W/D	10 H/D	4 W
access track			
21 F/D	15 H/D	9 F	3 F/D
20 W	14 F/D	8 W/D	2 H/D
19 W/D	13 H	7 H	1F

Figure 5.3: Layout of sample plots. See text for description of plots. Individual plot size is 5m by 3m.

There are three tree removal treatments illustrated in Figures 5.4 a-c

W - Whole tree harvest (entire tree removed, no residue left except in brash access mats)

H - Harvest conventionally (trunk removed, 'lop and top' - side branches and top portion of tree left *in situ*)

F - Fell to waste (entire trees felled and left *in situ*)

These are combined with two damming treatments:

U - Dam main drains only (target water level range 0-50cm below surface) (referred to by just the tree removal code)

D - Dam drains and plough furrows (target water level ranges 0-25 cm below surface)

Numbers in the plot labels in Figure 5.3 refer to the FC plot numbers.

Figures 5.4 a-c: Forest removal treatments – W, H and F. Testate amoebae plots are in the foreground of Figures a and b.

The tree removal treatments are designed to determine whether there is a need to change from conventional forestry harvesting methods when trees are being removed for peatland restoration. In recent bog restoration work, where scrub removal has been carried out, a major assumption has been that no part of the tree should be left on site, as it could alter the nutrient status of the bog in the long term (Bacon and Lord, 1996; Brooks and Stoneman, 1997a).

The Forestry Commission monitored hydrology in the experimental area using measurements from four dipwells and one WALRAG in each treatment plot. Water levels were measured

monthly between December 1997 and October 2000. Measurements were also taken to coincide with testate amoebae sampling in January and May 2001. Additional monitoring of peat and water chemistry and vegetation has also been carried out at intervals over the last five years. Intermittent monitoring will continue until 2007.

5.2.2 Testate Amoebae Sampling

As the trees were removed from Flanders Moss three years before the study of the testate amoebae began, it was not possible to assess baseline conditions. The advantage of carrying out monitoring at this site was that restoration was longer established than at Coom Rigg Moss, so ecological improvements should have progressed much further. The key issue was therefore, a comparison between the tree removal and damming methods implemented by the FC. Four sampling dates were set at three month intervals to help fulfil the fifth objective of this PhD study, developing knowledge of seasonal variability in testate amoebae communities as well as studying differences between restoration techniques. Sampling was planned for July and October 2000 and January and April 2001. The final sampling date was delayed until May 2001 by access restrictions as a result of the foot and mouth disease outbreak.

Testate amoebae monitoring plots were placed within each of the Forestry Commission's treatment plots. Twenty-five centimetre square plots were placed in a uniform sward of *Sphagnum* on the level ground between the plough furrows and the ridges (Figure 5.2). They were marked with plastic corner pieces and small poles to minimise the threat of trampling. The sampling method described in Chapter 3 was applied at this site with some adaptations. As a result of the difficulties encountered in achieving counts of over 100 testate amoebae in

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the methodological experiments, and because the plots were only visited four times, larger samples were taken. Samples from Coom Rigg were single stems of *Sphagnum*; at Flanders Moss, small 'plugs' of around 5 stems of *Sphagnum* were taken.

Samples were brought back to Plymouth where they were processed as outlined in Chapter 3. Microscope slides were produced and counts were carried out using techniques described in section 3.1.

5.3 Results

5.3.1 Hydrological measurements

The Forestry Commission took dipwell measurements within the treatment plots every month between December 1997 and October 2000. Further measurements were taken to coincide with testate amoebae sampling in January and May 2001. There were six dipwells in each treatment plot. Mean values were calculated from these to give one reading per plot. The mean readings for each treatment plot are plotted as line graphs in Figures 5.5, 5.6 and 5.7 below. They are grouped by treatment, with dammed and undammed on the same pages for ease of comparison.

The hydrological data shows a fairly uniform water table response across the site. Water levels dropped rapidly in the summer of 1998 when the trees were removed. They then recovered and, overall, have increased from between 20 - 30 cm below the surface before the trees were removed, to 5 and 20 cm below surface afterwards. The target water level for the

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dammed plots was 0 - 25 cm and this has been achieved across the site regardless of hydrological treatment.

(a)

(b)
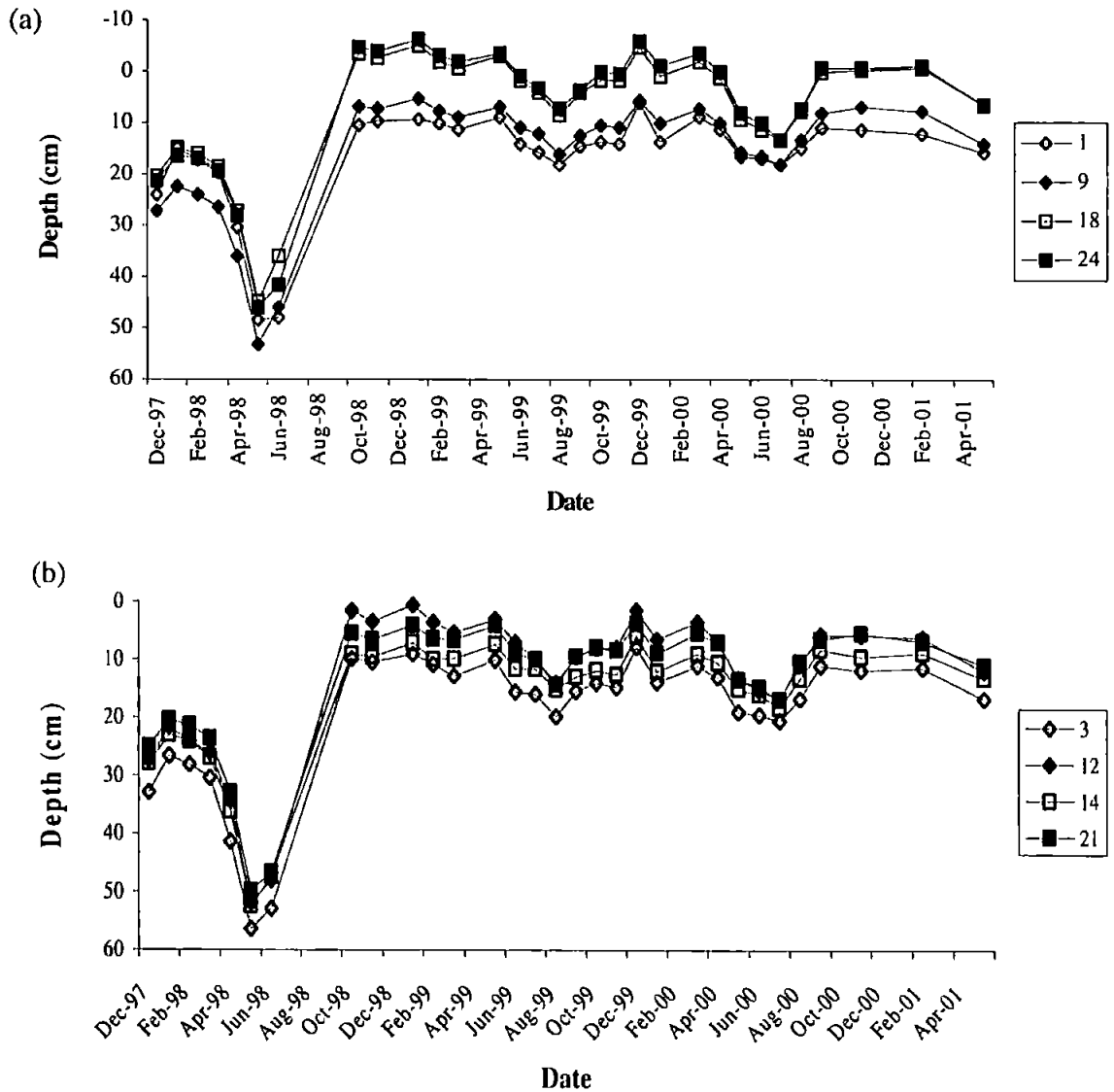
Figure 5.5: Depth to water table (cm) means for 6 dipwells in treatments F (a) and F/D (b) plotted over time. Numbers refer to plots (Figure 5.3). Trees were removed in the summer of 1998. Data from the Forestry Commission.
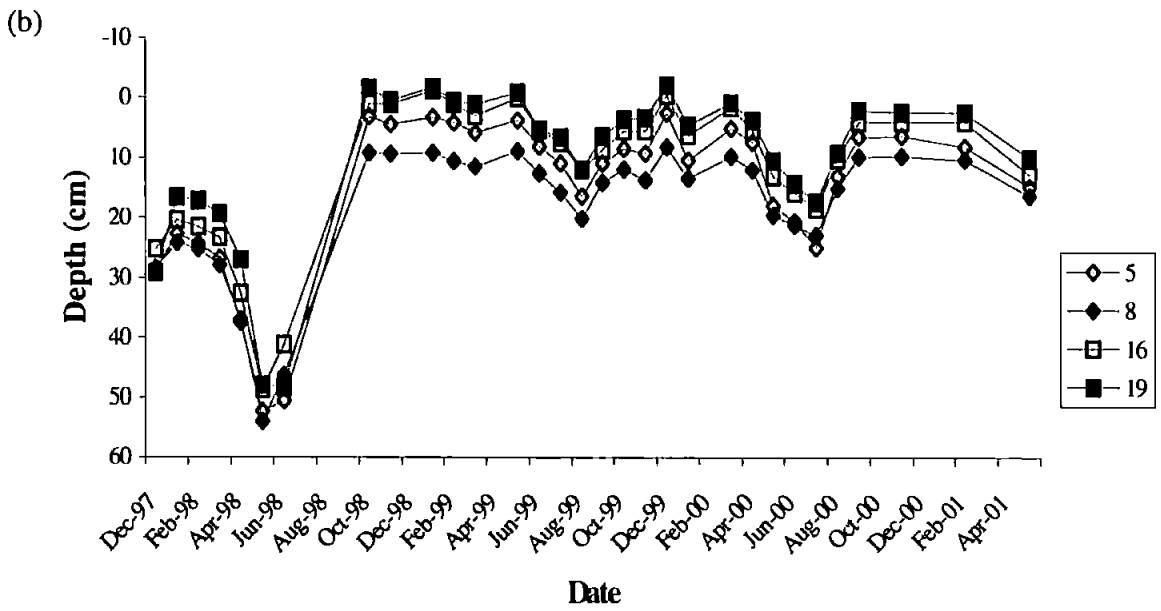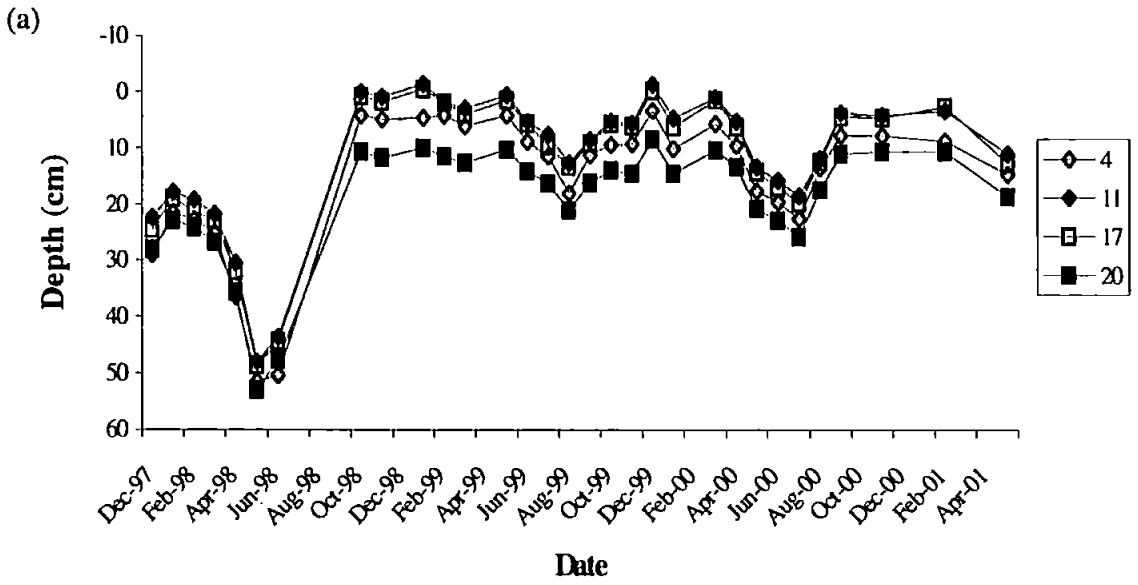
Figure 5.6: Depth to water table (cm) means for 6 dipwells in treatments W (a) and W/D (b) plotted over time. See Figure 5.5 caption for further explanation.
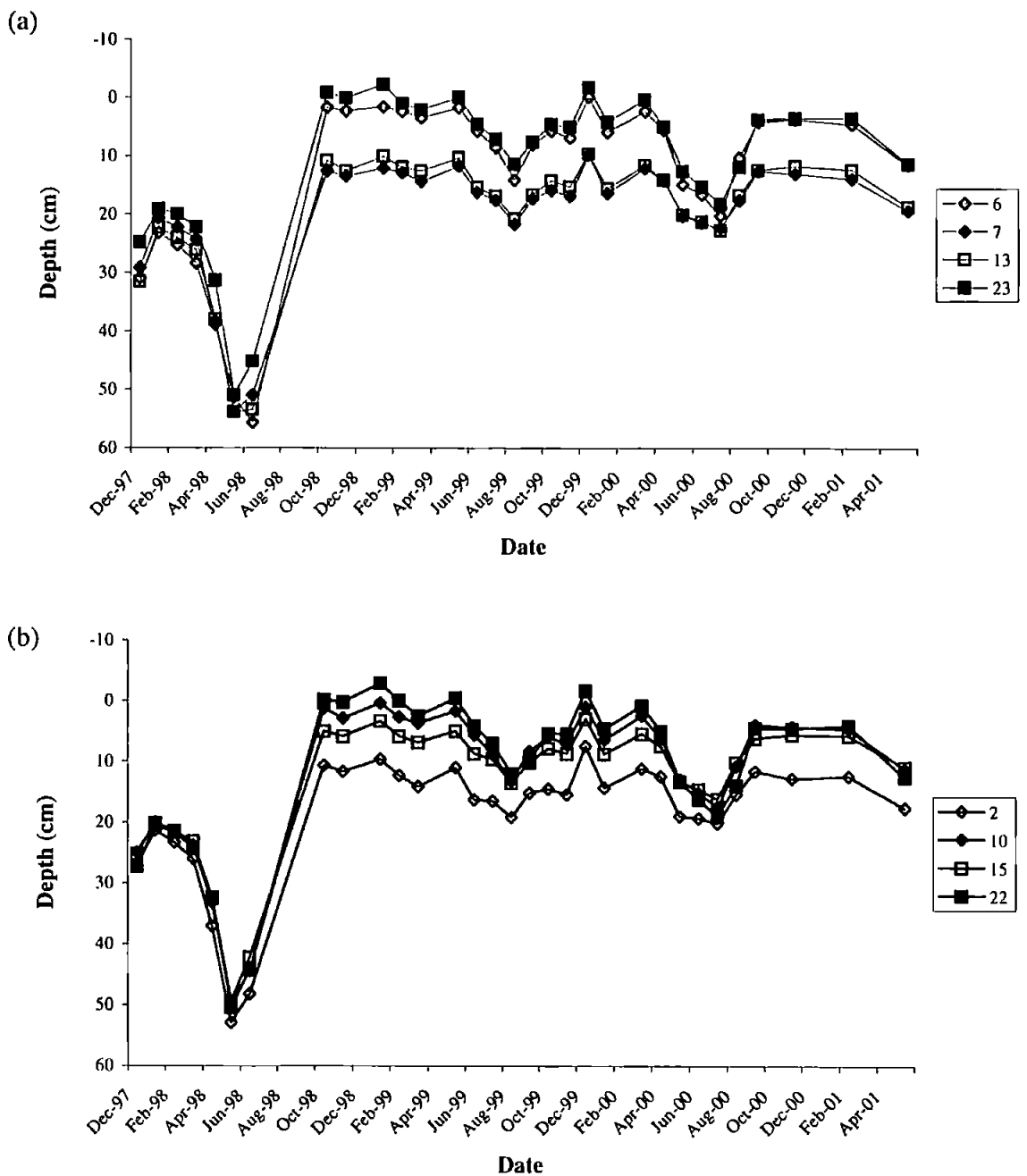
Figure 5.7: Depth to water table (cm) means for 6 dipwells in treatments H (a) and H/D (b) plotted over time. See Figure 4.3 caption for further explanation.

To test the relationships between damming method, tree removal technique and water levels, two-way analyses of variance (ANOVAs) with replication were performed. ANOVA is a statistical tool commonly used to compare within sample variance with between sample

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variance. This analysis was designed to establish whether there was more difference in water levels between damming technique and tree removal treatments. The ANOVAs would also show whether this variance was greater than the variance between sample plots. ANOVAs were carried out on both the entire data set and results from individual months. The statistical output is shown in Tables 5.1a-d and Table 5.2.

Source	d.f.	SS	MS	F	P
Damming	1	0.46	0.46	0.07	0.800
Forestry	2	78.00	39.00	5.57	0.013
Interaction	2	26.82	13.41	1.91	0.176
Residual (Error)	18	126.14	7.01		
Total	23	231.43			

Table 5.1a: Results of two-way ANOVA on July's Dipwell data

Source	d.f.	SS	MS	F	P
Damming	1	0.70	0.70	0.04	0.837
Forestry	2	6.6	3.30	0.20	0.822
Interaction	2	32.5	16.20	0.98	0.394
Residual (Error)	18	297.9	16.5		
Total	23	337.6			

Table 5.1b: Results of two-way ANOVA on October's dipwell data

Source	d.f.	SS	MS	F	P
Damming	1	3.6	3.6	0.18	0.672
Forestry	2	8.5	4.2	0.22	0.808
Interaction	2	35.6	17.2	0.91	0.422
Residual (Error)	18	353.6	19.6		
Total	23	401.2			

Table 5.1c: Results of two-way ANOVA on January's dipwell data

Source	d.f.	SS	MS	F	P
Damming	1	0.0	0.0	0.00	0.985
Forestry	2	24.0	12.0	0.94	0.409
Interaction	2	24.8	12.4	0.97	0.397
Residual (Error)	18	229.4	12.7		
Total	23	278.4			

Table 5.1d: Results of two-way ANOVA on May's dipwell data

Source	d.f.	SS	MS	F	P
Damming	1	1.15	1.15	0.03	0.868
Forestry	2	63.63	31.81	0.77	0.464
Interaction	2	117.71	58.85	1.43	0.244
Residual (Error)	90	3694.94	41.05		
Total	95	3887.43			

Table 5.2: Two-way ANOVA result for forestry treatment and damming status Vs depth to water table.

The data were arranged in two columns to allow a comparison between dammed and undammed treatments. Forestry treatments were grouped together. The analysis shows that for the individual months and for the entire dataset, damming treatments do not have a statistically significant impact on water levels. The forestry treatments do not have a statistically significant impact except in July (p = 0.013). A one-way ANOVA on the dipwell data showed that there was a statistically significant difference in water level between the sampling months. The plot of 95% confidence intervals in Table 5.3, shows that the mean water table for July was the highest of all months (depth to water table greatest). July is therefore the driest month sampled. The impact of forest removal technique on the water table could therefore be enhanced by dry conditions. It will be important to bear this in mind in the context of the other field experiments. Two-way ANOVAs on the entire dataset show that neither forest removal nor damming technique have a statistically significant impact on water table, although when both dammed and undammed pairs are combined, forestry and damming treatment do have a significant impact (p = 0.032) (Table 5.4).

Source	d.f.	SS	MS	F	P
Month	3	2628.9	876.3	64.57	0.000
Residual (Error)	92	1248.5	13.6		
Total	95	3887.43			

```
                                     Individual 95% CIs For Mean
                                     Based on Pooled StDev
Level       N      Mean    StDev     ---------+---------+---------+------
July        24    19.278    3.172                              (--*--)
Oct         24     6.618    3.831     (--*--)
Jan         24     6.847    4.177     (--*--)
May         24    13.389    3.478                  (--*--)
                                     ---------+---------+---------+------
Pooled StDev =    3.684              10.0      15.0      20.0
```

Table 5.3: Results of One way ANOVA - month Vs depth to water table (DWT) and plot of confidence intervals for means.

Source	d.f.	SS	MS	F	P
Month	3	2628.9	876.3	62.66	0.000
Damming & Felling treatment	5	182.5	36.5	2.61	0.032
Interaction	15	59.0	3.9	0.28	0.996
Residual (Error)	72	1007.0	14.0		
Total	95	3887.43			

Table 5.4: Two-way ANOVA output for month and restoration treatment Vs depth to water table (DWT).

Changes in water table at the site appear to be temporal changes and a reaction to damming of the main forestry drains rather than differences between damming intensity or forest removal treatments. The type of forest removal and damming method employed however does not seem to be significant in terms of any impact on hydrology.

5.3.2 Moisture content

Another measure of hydrology is moisture content of samples. This has been used in the descriptions of hydrological response to damming at Coom Rigg Moss (Chapter 4). As the statistical analyses of depth to water table showed no significant differences between

treatments, it seemed sensible to investigate possible differences in moisture content. Analysis of variance carried out on moisture content of samples also show little difference between damming treatments and forest removal treatments. A one-way ANOVA on moisture content and month shows that July had the lowest moisture content of all months. Given that July also had the greatest depth to water table, this result is unsurprising. The output is shown in Table 5.5.

Source	d.f.	SS	MS	*F*	*P*
Month	3	253.36	84.45	28.02	0.000
Residual (Error)	92	277.33	3.01		
Total	95	530.69			

```
                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level         N      Mean    StDev  ------+---------+---------+---------+
July          24    91.056   2.803  (---*----)
October       24    94.326   0.868                        (----*---)
January       24    95.485   1.304                               (----*---)
May           24    93.532   1.322                    (----*---)
                                    ------+---------+---------+---------+
Pooled StDev =      1.736           91.2      92.8      94.4      96.0
```

Table 5.5: Results of One-way ANOVA comparing moisture content and sampling month and plot of confidence intervals for means.

5.3.3 Testate Amoebae responses

Testate amoebae concentrations and also live percentages were calculated as described in chapter 3. Due to the large amount of raw data generated in the counts, summary tables have been produced. Table 5.6 shows that the highest numbers of testate amoebae were found in May and the lowest in January. The highest number of taxa was found in May and the lowest in January. Concentrations vary enormously between samples. This indicates a problem with using concentrations for analyses; further investigations of concentration data from other sites will be needed to assess its usefulness as an analysis tool. Live percentages appear rather

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variable but the highest were found in January and the lowest in May which was the opposite

of the pattern observed for concentration data. Some of this variability may be due to low

counts. Therefore analyses were carried out excluding lower counts to see whether this

affected the results. Counts in January were lower across all treatments, possibly because the

bog was frozen when sampling took place.

July	F	F/D	H	H/D	W	W/D
Total counted (mean)	71.5	71.5	71.8	74.0	121.5	183.5
Total counted (range)	7-188	87-185	2-213	2-203	12-209	74-256
No. taxa	14	20	21	16	21	20
% live (mean)	56.6	56.6	44.6	56.7	42.5	54.0
Concentration (mean (no./g-1dw))	12971	20660	132594	16305	8773	13991

October	F	F/D	H	H/D	W	W/D
Total counted (treatment mean)	131.8	111.0	62.0	53.3	64.3	165.3
Total counted (range)	49-258	12-209	11-192	23-79	51-79	26-299
No. taxa	23	25	17	21	20	21
% live (mean)	56.2	66.0	64.1	49.8	35.0	43.7
Concentration (mean (no./g-1dw))	15468	17345	9171	7407	18895	22941

January	F	F/D	H	H/D	W	W/D
Total counted (treatment mean)	113.5	20.3	55.0	60.8	21.8	102.0
Total counted (range)	4-229	3-59	1-212	4-205	19-35	2-203
No. taxa	22	12	16	22	20	17
% live (mean)	61.9	66.7	83.2	46.9	39.1	51.7
Concentration (mean (no./g-1dw))	21431	11307	9249	28382	2587	18449

May	F	F/D	H	H/D	W	W/D
Total counted (treatment mean)	184.3	144.8	100.5	143.0	141.0	174.3
Total counted (range)	30-244	91-206	27-183	60-245	19-229	11-271
No. taxa	23	24	23	25	29	28
% live (mean)	47.8	36.8	43.3	35.0	22.9	28.4
Concentration (mean (no./g-1dw))	87988	41709	16807	51905	196954	21951

Table 5.6: Summary data of means for all treatments (4 replicates) by month. Showing total number of testate amoebae counted (treatment mean) and range, total number of taxa per treatment, mean percentage live, mean concentrations (testate amoebae per gram dry weight of moss).

Tables 5.7 a-d show a summary of the species data. Means and ranges are shown for

individual species. A constancy score (C) similar to those used in phytosociological

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classification (Clement 1916) has been given to indicate how often each species is present in samples. This is represented by shaded blocks.

Wet ↑

	F	F/D	H	H/D	W	W/D
Arcella discoides	9.33	0.00	15.18	48.03	17.72	0.68
	0-37	-	0-61	0-100	0-40	0-3
		-				
Euglypha strigosa	18.31	24.22	16.55	22.12	13.77	36.06
	1-32	10-37	0-44	0-50	0-25	6-72
Cyclopyxis arcelloides	5.99	1.93	0.47	0.00	1.90	2.46
	0-14	0-4	0-2	-	0-4	0-8
				-		
Euglypha tuberculata	5.81	14.04	3.48	4.37	12.82	7.56
	0-14	0-35	0-13	0-17	5-35	6-9
Difflugia pristis	0.00	0.00	1.64	5.36	0.00	0.50
	-	-	0-7	0-21	-	0-1
	-	-			-	
Nebela tincta	10.19	17.95	14.23	2.97	5.95	11.91
	0-25	1-52	0-32	0-6	1-17	0-33
Euglypha rotunda	0.00	3.43	0.70	1.19	2.38	5.61
	-	0-7	0-3	0-5	0-7	1-12
	-					
Corythion -Trinema type	21.50	12.50	21.65	5.88	20.12	8.72
	3-43	3-26	6-50	0-16	2-50	1-20
Heleopera petricola	2.30	2.81	1.06	0.00	1.08	8.32
	0-7	0-10	0-4	-	0-4	0-31
				-		
Trigonopyxis arcula	2.90	4.57	13.32	2.50	2.96	1.56
	0-9	2-10	0-50	0-10	0-8	0-6
Bullinularia indica	16.65	8.39	0.00	3.08	3.34	8.74
	8-26	1-26	-	0-11	0-7	0-28
			-			

	F	F/D	H	H/D	W	W/D
* Hyalopshenia ovalis	1.34	1.34	0.94	1.51	10.46	1.18
	0-2	0-2	0-4	0-4	<1-32	0-3

Dry ↓

Table 5.7a: July species composition by treatment. (species means expressed as percentage of total testate amoebae, range and constancy – shading indicates constancy score, darkest = 4/4 samples, lightest, 1/4. Blank cells indicate absence. Taxon arranged by hydrological preference data from Charman *et al.* 2000 (*insufficient data for quantitative measure but characteristic of dry hummocks and drained mires). Only taxa occurring at >5% in one or more samples are included.

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	F	F/D	H	H/D	W	W/D
Arcella discoides	0.68	20.08	6.63	1.40	0.44	0.00
	0-2	0-75	0-18	0-4	0-2	-
						-
Euglypha strigosa	5.44	11.98	19.89	11.35	11.96	19.25
	0-16	0-25	0-55	0-18	0-43	0-35
Cyclopyxis arcelloides	4.37	2.07	1.43	6.10	6.42	1.37
	0-6	0-7	0-6	0-13	0-20	0-4
Euglypha tuberculata	4.17	6.57	6.33	6.88	2.64	16.79
	0-9	0-17	0-17	0-21	0-4	2-31
Difflugia pristis	1.16	0.00	0.00	15.22	0.00	0.00
	0-5	-	-	0-61	-	-
		-	-		-	-
Nebela tincta	16.39	26.78	14.62	4.22	28.93	10.54
	0-34	3-52	0-27	0-17	4-81	1-17
Nebela parvula	0.00	0.76	0.00	15.51	1.43	0.11
	-	0-3	-	0-62	0-6	0-<1
	-		-			
Assulina muscorum	2.01	0.42	4.49	3.27	1.07	0.59
	0-7	0-1	0-9	0-12	0-4	0-2
Euglypha rotunda	1.95	5.11	4.27	5.57	5.31	14.71
	0-3	0-13	0-8	0-14	0-18	2-27
Corythion -Trinema type	7.92	3.84	11.07	9.70	8.13	2.33
	0-18	0-8	0-42	3-17	0-16	0-4
Heleopera petricola	2.32	2.81	14.31	0.74	2.06	7.35
	0-5	0-10	0-42	0-3	0-6	0-14
Trigonopyxis arcula	8.01	2.87	0.65	0.32	6.99	6.07
	1-24	0-8	0-3	0-1	0-19	1-16
Nebela collaris	0.00	0.30	0.26	0.00	3.92	5.36
	-	0-1	0-1	-	0-11	0-15
	-			-		
Bullinularia indica	18.79	7.00	9.77	16.31	17.24	10.12
	<1-43	0-27	0-39	0-47	1-63	0-27

Wet ↑

Dry ↓

Table 5.7b: October species composition by treatment. (see Table 5.7a caption for explanation)

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Wet ↑ ... Dry ↓

	F	F/D	H	H/D	W	W/D
Arcella discoides	0.00	4.55	8.33	0.00	1.32	0.00
	-	0-18	0-33	-	0-5	-
	-			-		-
Nebela carinata	8.75	11.36	0.00	0.00	1.32	0.00
	0-25	0-45	-	-	0-5	-
			-	-		-
Arcella catinus type	0.00	0.00	0.00	6.25	2.63	12.50
	-	-	-	0-25	0-11	0-50
	-	-	-			
Euglypha strigosa	5.27	0.00	16.12	6.12	0.00	3.84
	0-13	-	0-33	0-17	-	0-10
			-		-	
Cyclopyxis arcelloides	4.00	3.13	2.48	16.87	11.24	21.08
	0-8	0-12.5	0-10	0-50	0-26	<1-50
Euglypha tuberculata	2.69	0.00	0.12	1.22	5.13	3.50
	0-7	-	0-<1	0-5	3-7	0-14
		-				
Difflugia pristis	16.05	0.00	0.00	2.68	5.26	0.00
	0-64	-	-	0-11	0-21	-
		-	-			-
Nebela tincta	15.21	31.10	2.95	29.22	16.07	15.04
	0-38	0-73	0-12	8-67	0-43	0-56
Assulina muscorum	3.33	8.76	0.24	1.34	2.03	2.00
	0-10	0-33	0-1	0-5	0-5	0-8
Euglypha rotunda	1.20	0.00	6.72	0.98	0.71	11.33
	0-3	-	0-25	0-4	0-3	0-33
		-				
Corythion -Trinema type	5.11	0.00	39.82	4.72	6.20	11.38
	0-14	-	1-100	0-13	0-14	0-46
		-				
Heleopera petricola	0.13	0.00	11.56	1.10	1.32	5.05
	0-1	-	0-25	0-4	0-5	0-19
		-				
Trigonopyxis arcula	2.57	8.76	3.89	2.79	7.99	0.74
	0-9	0-33	0-16	0-7	5-14	0-2
Bullinularia indica	32.08	23.30	5.66	19.15	16.50	10.46
	9-75	0-75	0-23	0-48	0-53	0-33

	F	F/D	H	H/D	W	W/D
* *Hyalopshenia ovalis*	1.05	1.27	0.00	0.49	6.67	0.25
	0-3	0-5	-	0-2	0-21	0-1
			-			

Table 5.7c: January species composition by treatment. (see Table 5.7a caption for explanation)

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	F	F/D	H	H/D	W	W/D
Arcella discoides	0.32	4.52	1.88	9.35	12.07	7.72
	0-1	0-16	0-8	0-33	0-42	0-27
Euglypha strigosa	12.98	7.34	8.41	12.31	9.97	8.75
	1-15	3-11	3-13	1-25	0-31	0-25
Cyclopyxis arcelloides	4.82	5.97	4.69	5.83	2.67	7.09
	0-13	0-11	1-8	0-14	0-8	0-18
Placocista spinosa	11.44	0.27	1.55	0.44	0.70	1.46
	0-45	0-1	0-5	0-1	0-2	0-4
Euglypha tuberculata	6.98	12.52	14.95	16.42	15.32	4.08
	1-21	1-44	2-22	7-41	8-32	0-10
Difflugia pristis	11.92	0.00	1.85	0.13	0.00	3.49
	0-48	-	0-7	0-1	-	0-14
		-			-	
Nebela tincta	10.85	23.55	7.91	5.63	8.16	8.09
	0-36	8-45	0-23	1-10	5-14	0-15
Assulina muscorum	1.13	2.12	5.39	2.91	2.15	2.61
	0-3	<1-3	2-10	<1-8	0-4	<1-9
Euglypha rotunda	8.80	4.52	2.76	6.60	4.86	6.03
	0-20	0-6	2-4	0-19	1-10	4-9
Corythion -Trinema type	18.78	16.28	17.10	11.34	22.10	10.64
	2-35	4-47	4-22	3-21	10-35	3-18
Nebela militaris	0.41	5.00	5.07	9.56	0.93	15.92
	0-1	1-14	0-15	0-28	0-3	0-43
Heleopera petricola	2.59	3.33	2.19	0.33	1.65	13.71
	0-4	<1-6	0-9	0-1	0-5	0-22
Trigonopyxis arcula	1.47	3.60	4.60	3.31	6.79	6.03
	0-4	0-7	1-8	1-5	3-13	0-12
Bullinularia indica	2.17	6.40	14.92	8.68	4.22	2.30
	0-5	0-18	0-39	0-17	0-13	0-7

* *Hyalopshenia ovalis*	1.45	1.34	0.14	1.09	0.23	0.26
	0-6	0-4	0-1	0-3	0-1	0-1

Wet ↑

Dry ↓

Table 5.7d: May species composition by treatment. (see Table 5.7a caption for explanation)

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Two parameters were used to examine testate amoebae communities within the experimental area. Concentrations, calculated using an exotic marker grain, and live percentages.

a) Live Percentages

Live percentages have been used, as they are thought to be indicative of turnover of testate amoebae. In terms of peatland restoration, high turnover of testate amoebae populations could mean changing ecological conditions are either allowing new individuals to move into the area, or, are encouraging an increase in reproductive rates.

Figure 5.8 shows the bar charts of mean live percentages by month for each treatment. Any counts where fewer than 20 testate amoebae were found, have been excluded from these charts. Error bars are included where more than two samples had counts over 20. From these charts it is clear that the live percentages in the fell to waste samples were higher and more consistent than the other two forest removal treatments. Whole tree removal had the lowest percentages, with harvest falling between the two. Live percentages appear to be slightly lower in undammed plots for the harvest and whole tree removal treatments than in the dammed plots.
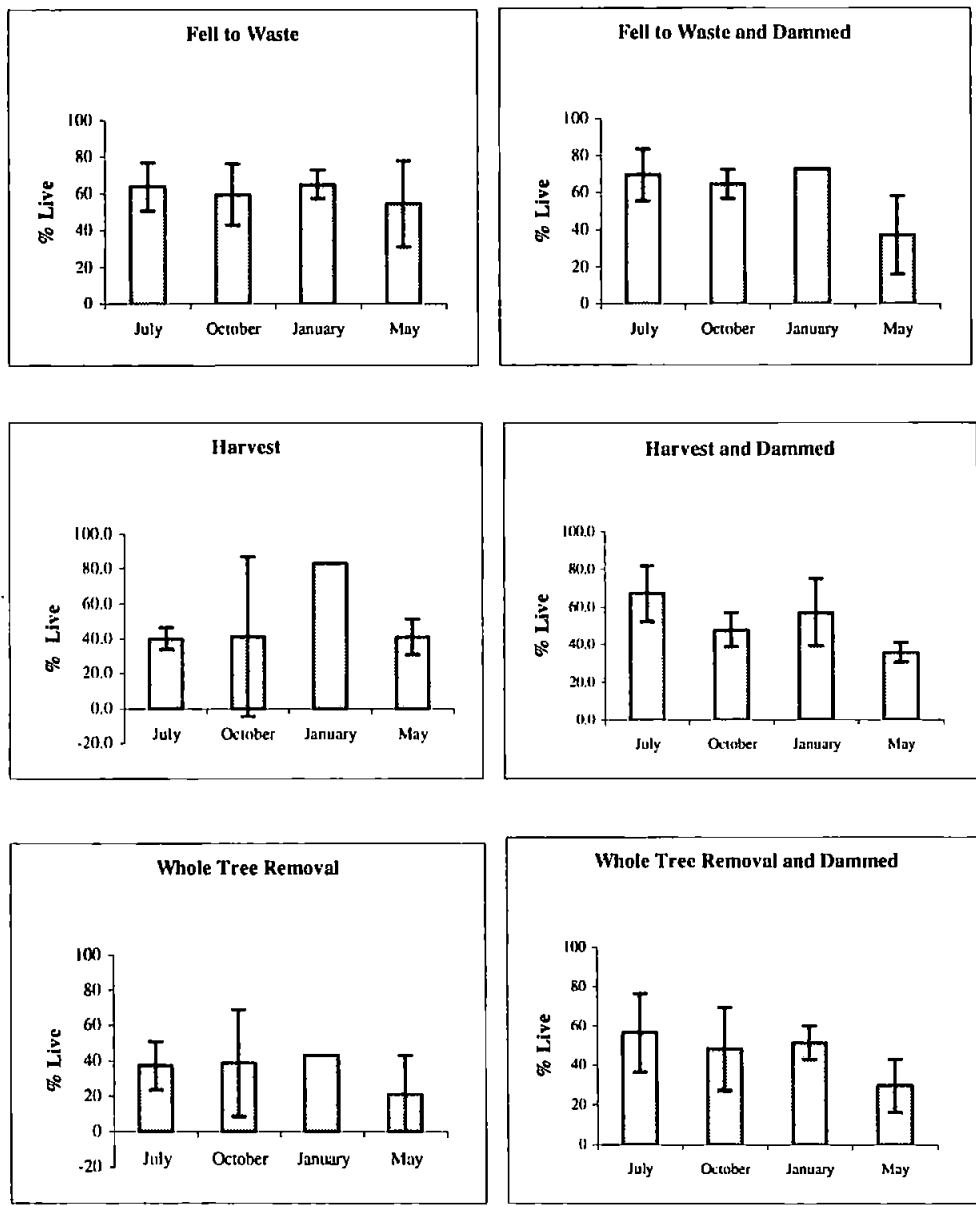
Figure 5.8: Mean % live by month and treatment showing standard deviations (error bars). Samples with counts of less than 50 testate amoebae excluded from analysis. Standard deviations only calculated where there were 2 or more samples with counts >50.

To test the inferences drawn from the graphs, statistical analyses of variance were conducted

on the 'percentage live' for the entire population. These were done in the same way as the

ANOVAs for the hydrological data to allow direct comparison. The results of these are presented in Tables 5.7 a-d and Table 5.8.

Source	d.f.	SS	MS	F	P
Felling	2	1952.97	976.48	4.00	0.036
Damming	1	607.02	607.02	2.49	0.132
Interaction	2	329.78	164.89	0.68	0.521
Residual (Error)	18	4392.07	244.00		
Total	95	7281.83			

Table 5.7a: Two-way ANOVA results for July % live data

Source	d.f.	SS	MS	F	P
Felling	2	852.74	426.37	0.88	0.434
Damming	1	7.37	7.37	0.02	0.903
Interaction	2	220.8	110.40	0.23	0.799
Residual (Error)	18	8764.88	486.938		
Total	23	9845.80			

Table 5.7b: Two-way ANOVA results for October % live data

Source	d.f.	SS	MS	F	P
Felling	2	3126.36	1563.18	5.60	0.013
Damming	1	186.48	186.48	0.67	0.424
Interaction	2	1425.19	712.59	2.55	0.106
Residual (Error)	18	5020.87	278.94		
Total	23	9758.91			

Table 5.7c:Two-way ANOVA results for January % live data

Source	d.f.	SS	MS	F	P
Felling	2	2605.08	1302.54	4.53	0.026
Damming	1	152.51	152.51	0.53	0.476
Interaction	2	610.14	305.07	1.06	0.367
Residual (Error)	18	5179.27	287.74		
Total	23	8547.01			

Table 5.7d: Two-way ANOVA results for May % live data

Source	d.f.	SS	MS	F	P
Felling	2	6872.30	3391.16	8.35	0.000
Damming	1	0.50	0.45	0.00	0.973
Interaction	2	2136.80	1068.38	2.63	0.078
Residual (Error)	90	36549.70	406.11		
Total	95	45469.30			

Table 5.8: Two-way ANOVA result for all months' % live data

Data were arranged, as were the dipwell data, in two columns to allow comparison between dammed and undammed treatments and forestry treatments. The individual months show that there was a significant difference between forestry treatments, except for October. There was no significant difference between damming treatments in any month. The ANOVA for all months confirmed that forestry treatment had a significant impact on the testate amoebae populations but that extra damming did not. The interaction between extra dams and forest removal technique, although not statistically significant, accounted for around 5% of the variation.

The results show that live percentages of testate amoebae are highest in the fell to waste treatment. The harvest treatment has the second highest live percentages and whole tree removal the lowest. This conclusion suggests that live percentages are higher where some tree remains are left on the site. A one-way ANOVA comparing F and H treatments with W was used to test this hypothesis. The output is shown in Table 5.9. The result is significant at more than 99% rejection level. Sample plots containing some tree remains are seen to have increased live percentages of testate amoebae.

Source	d.f.	SS	MS	*F*	*P*
Treatment	1	6462	6462	16.57	0.000
Residual (Error)	94	39007	415		
Total	95	45469			

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level     N    Mean  StDev  ---------+---------+---------+---------+
F and H  64  57.22  20.03                              (------*-----)
W        32  39.82  21.04  (--------*--------)
                          ---------+---------+---------+---------+
                              40.0      48.0      56.0      64.0

Pooled StDev = 20.37
```
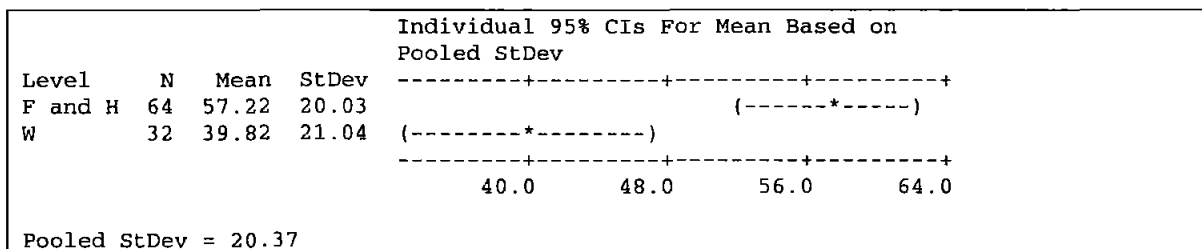
Table 5.9: Results of One-way ANOVA - comparison between plots with tree remains (F and H) and whole tree removal (W) and plot of confidence intervals for means

b) Species Composition

A more subjective, but more detailed, comparison of the treatments can be made by looking at species composition. Tables 5.10a and b show a summary of the major characteristics in testate amoebae populations for each of the treatments. The most common taxa across the whole site are *Bullinularia indica*, *Euglypha strigosa*, *Nebela tincta*, *Corithion – Trinema* type and *Euglypha tuberculata*.

Wet ↑

Species	July	October	January	May
A. discoides				
E. strigosa				
C. arcelloides				
E. tuberculata				
N. tincta				
N. parvula				
E. rotunda				
C-T type				
N. militaris				
B. indica				

Dry ↓

Table 5.10a: Taxa occurring at >5% represented by shaded cells (mean figure for all sample plots by month) – arranged by hydrological preference according to data from Charman *et al.* (2000).

	Species	F	F/D	H	H/D	W	W/D
Wet ↑	A. discoides						
	E. strigosa						
	C. arcelloides						
	E. tuberculata						
	D. pristis						
	N. tincta						
	E. rotunda						
	N. militaris						
	C-T type						
	H. petricola						
Dry	T. arcula						
↓	B. indica						

Table 5.10b: Taxa occurring at >5% represented by shaded cells (mean figure for all sample plots by treatment) – arranged by hydrological preference according to data from Charman *et al.* (2000).

Tables 5.10a and b and 5.12a and b show variations in species composition between months and treatments and in percentage live between months and treatment. The tables show that four species (*B. indica, E. strigosa, N. tincta* and *C-T* type) made up a large portion of the population and were common to all treatments in each sampling month. May had the largest number of other species making up more than 5% of the population and January had the least. Table 5.11 confirms that May also had the highest number of species recorded and January had the lowest. When analysed by treatment, there is little variability in the total number of species recorded. The fell to waste treatments (dammed and undammed), have fewer species representing over 5% of the population and the highest number of species over 5% is found in the dammed whole tree removal treatment (Table 5.10b).

Treatment	July	October	January	May	Mean	SD
F	14	23	22	23	20.50	4.36
F/D	20	25	12	24	20.25	5.91
H	21	17	16	23	19.25	3.30
H/D	16	21	22	25	21.00	3.74
W	21	20	20	29	22.50	4.36
W/D	20	21	17	28	21.50	4.65
Mean	18.67	21.17	18.17	25.33		
SD	1.20	2.71	3.92	2.58		

Table 5.11: Summary of number of taxa counted by month and treatment with means and standard deviations.

Wet ↑ / Dry ↓

Species	July	October	January	May
E. strigosa				
E. tuberculata				
N. tincta				
A. muscorum				
C-T type				
N. militaris				
T. arcula				
B. indica				

Table 5.12a: Taxa where >25% of the individuals were alive when sampled by month represented by shaded cells. Taxa arranged by hydrological preference according to data from Charman et al. (2000).

Wet ↑ / Dry ↓

Species	F	F/D	H	H/D	W	W/D
N. carinata						
E. strigosa						
E. tuberculata						
N. tincta						
A. muscorum						
E. rotunda						
C-T type						
N. militaris						
H. petricola						
T. arcula						
B. indica						
H. ovalis						

Table 5.12b: Taxa where >25% of the individuals were alive when sampled by treatment represented by shaded cells. Taxa arranged by hydrological preference according to data from Charman et al. (2000).

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It is important to consider not only where live percentages were highest but also which taxa were present at highest live percentages as their particular hydrological and ecological requirements are likely to be represented by higher values. Tables 5.12a and 5.12b provide insight into these ecological and hydrological preferences. Again, *B. indica*, *E. strigosa* and *N. tincta* are the most common species across treatment and sampling month. January has the least species at or above 25% live and May the most. There were more species with greater than 25% live in the fell to waste treatments (dammed and undammed) and less in the whole tree removal treatments (dammed and undammed) and in the dammed harvested treatment. *E. strigosa* and *N. tincta* are more indicative of wetter conditions and were more common in samples from locations where tree remains were left than in the whole tree removal treatment. *B. indica* was common across treatments. Although it is indicative of drier conditions, it is also common to forested peatlands, and its ubiquitous presence may be a result of the afforestation at the site. It was also a common taxon at Coom Rigg Moss, which was surrounded by forestry plantations.

c) Multivariate Analyses

Canonical correspondence analysis (CCA) (Ter Braak & Šmilauer, 1998) was used to explore the relationships between environmental variables and total species composition. Each month was analysed separately, comparing testate amoebae communities (concentrations) with water table depth (DWT) and moisture content of the sample. The graphical outputs are shown in Figures 5.9a-d and 5.10.
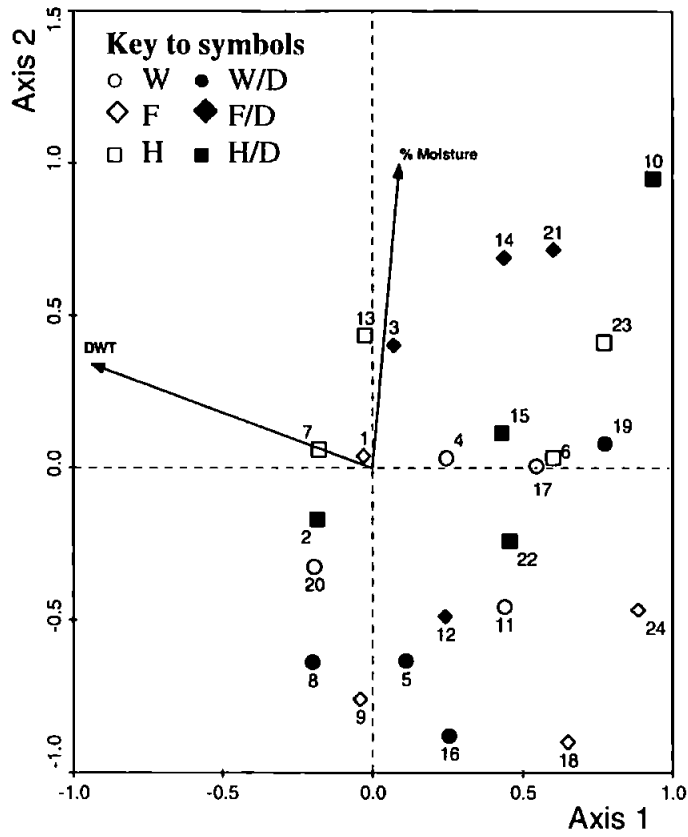
Figure 5.9a: July CCA concentrations and environmental variables
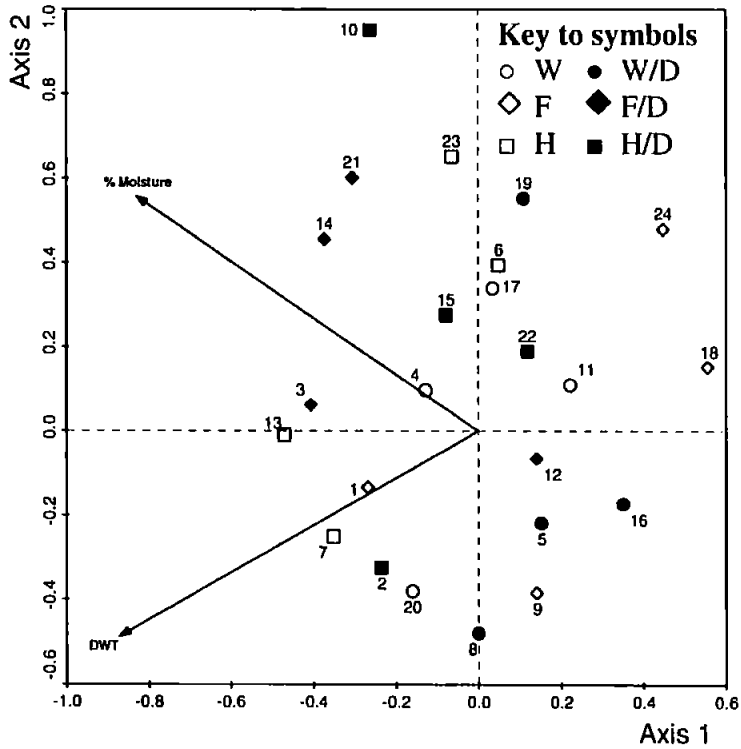
Figure 5.9b: October CCA of concentrations and environmental variables.

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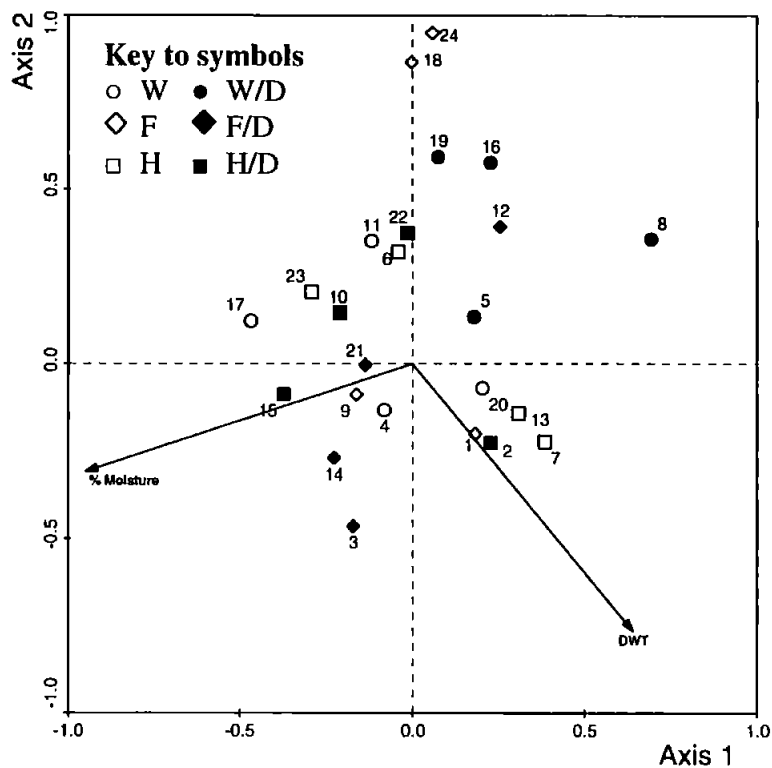
Figure 5.9c: January CCA of concentrations and environmental variables.
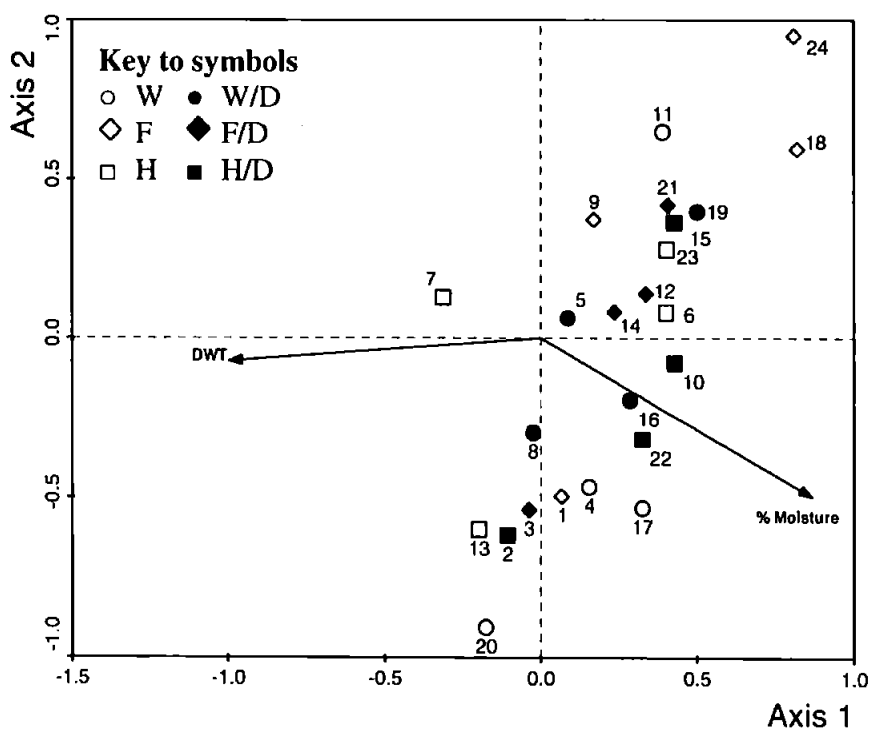
Figure 5.9d: May CCA of concentrations and environmental variables.

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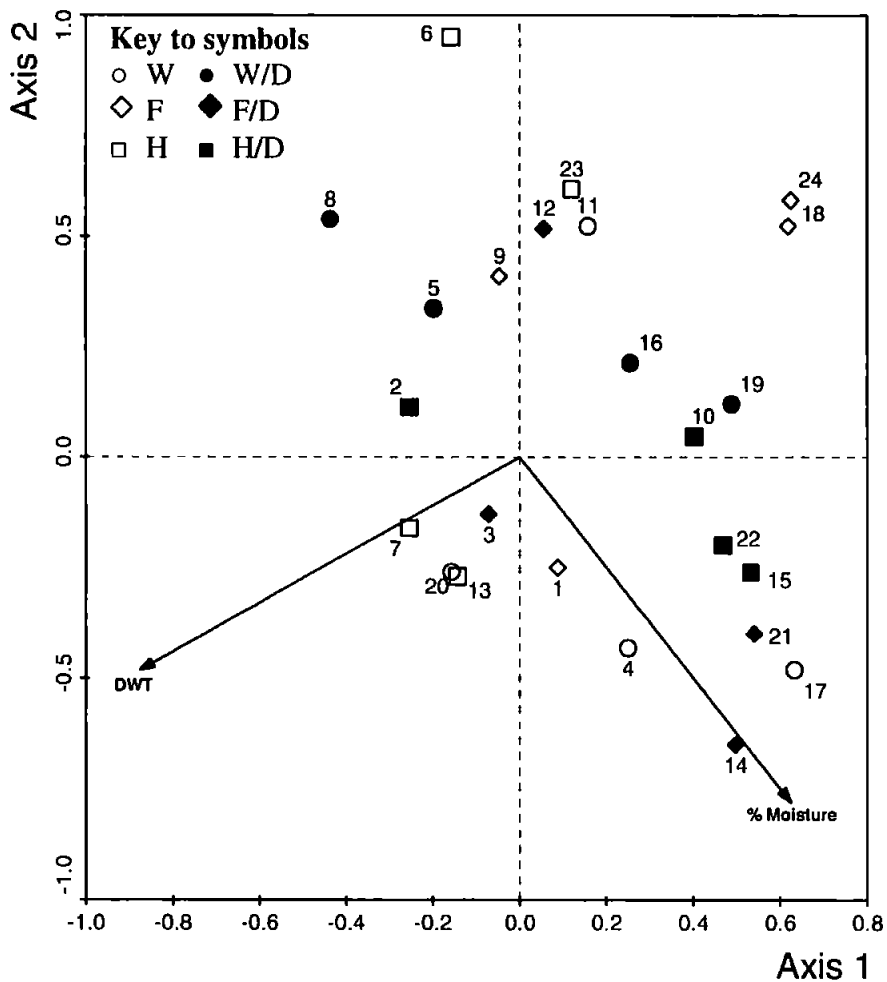
Figure 5.10: Annual means: CCA of concentrations and environmental variables

The above graphs (Figures 5.9a-d and 5.10) show that there is little correspondence within treatments. The pattern emerging is similar for all months (except for January) and for the mean annual count. This analysis is unlikely to be useful in understanding patterns in the data set. Percentage moisture and depth to water table (DWT) do not seem to have a uniform impact on concentrations of testate amoebae.

After analysing the concentration data with CCA, percentage live data was treated in the same way. Again there was little correspondence within the treatments. The layout of the sample sites is similar to the layout of the concentration results. Only the analysis of annual mean percentage live data is shown (Figure 5.11).
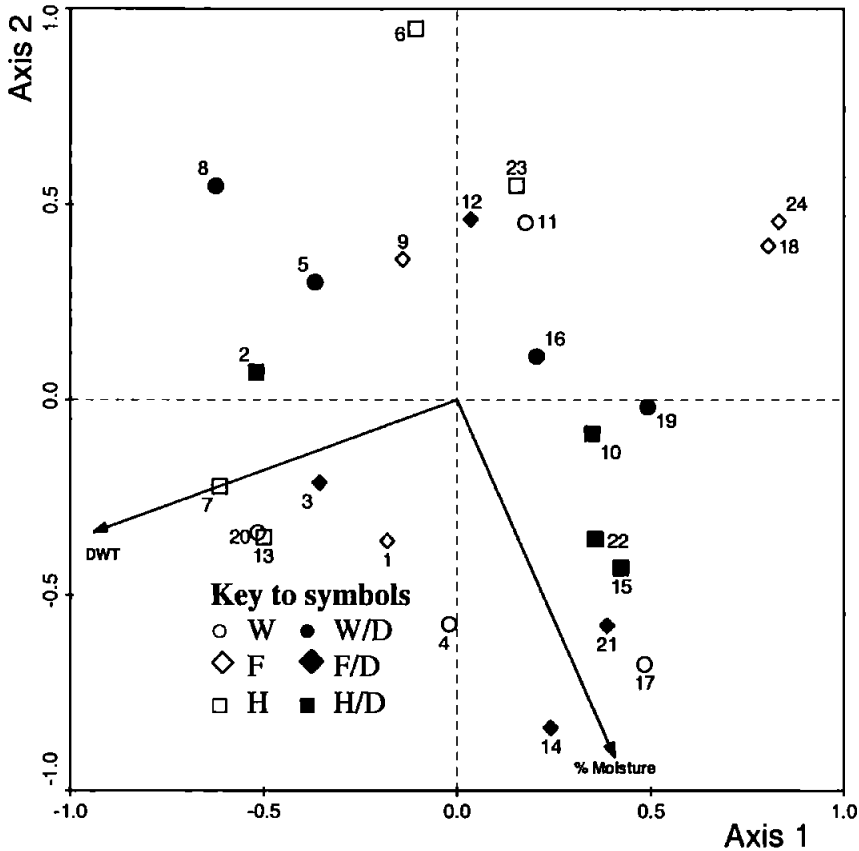
Figure 5.11: Mean annual CCA % live and environmental variables

5.4 Discussion

This experiment has studied testate amoebae populations within a forest removal trial on a small area of raised bog at Flanders Moss NNR. The trial was carried out by the Forestry Commission (FC) and was designed to assess the need for removing all tree waste and for damming of plough furrows when rehabilitating afforested bogs. Previous tree removal work for peatland restoration worked on the assumption that all organic material needed to be

removed from the site to avoid altering the nutrient balance of the bog (Rowell, 1988; Brooks and Stoneman, 1997a). Removing whole trees can be costly and, where traditional forestry harvesting methods are used, cause damage to the bog surface (Brooks and Stoneman, 1997b; Bacon and Lord, 1996) so the FC has produced an experimental layout to allow comparison between whole tree removal, conventional harvest methods and leaving the trees to decompose *in situ*.

5.4.1 Hydrological results

The aim of the FC's damming work was to assess the need for additional dams in plough furrows in bog restoration. The main forestry drains were dammed across the site. The results from the FC's dipwell data show that water levels vary very little between plots, despite the different hydrological and tree removal treatments. Statistical analyses confirm that there is no significant difference between treatments. Variations in hydrological conditions have been temporal rather than spatial. Water levels have increased significantly since the trees were removed and the dams put in place. A rise in water table of 20 to 40 cm is a typical response to felling conifer crops on peatlands (Smith *et al.*, 1995). Prior to ditch-blocking, there were several months when the water table fell more than 40 cm below the surface of the peat. Schouwenaars (1993) defined the lowest point to which water levels could drop during the growing season and still support *Sphagnum* growth as 40cm below the peat surface. Target water levels were 0-25 cm for the dammed plots and 0-50 cm for the undammed. Only one measurement after tree removal (in whole tree removal, undammed, plot number 20, July 2000) showed a water table depth of lower than 25cm below the surface. This illustrates that the hydrological response has been successful across the whole area.

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The most likely reason for the uniformity of hydrological response is that the experimental area was relatively small and there is little difference in ground level across the area. It is probable that all sample plots have benefited from the damming of the plough furrows. Where larger areas of forestry are removed, damming plough furrows in addition to main forestry drains may be shown to be essential for maintaining raised water levels.

5.4.2 Testate amoebae

Despite the fact that no statistically significant differences in hydrological response or moisture content of samples were identified across the site, analysis of the testate amoebae showed that there were some differences between treatment types. Previous research into testate amoebae habitat requirements has shown that hydrology is the principal environmental variable affecting habitat selection (Tolonen, 1986; Warner, 1988; Woodland *et al.*, 1998). Statistical analyses have shown no significant differences in water levels across the experimental site except for during July. Soil chemistry also influences testate amoebae but to a lesser extent. The pH is a key factor affecting community composition (Charman *et al.*, 2000). As Flanders Moss is an ombrotrophic raised bog, hydrological input and therefore soluble chemical input comes from precipitation (Proctor & Maltby, 1998). It is highly unlikely that there would be any variability in chemical composition of rainfall over this experiment. Other factors influencing peat chemistry are the degree of aeration of the peat, and water level (Ross, 1995). Peat chemistry could vary between plots because the tree remains would be expected to release nutrients as they decompose. However, given the small area of the experiment at Flanders Moss, and the minor hydrological differences, chemistry is

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unlikely to vary greatly as groundwater is likely to mix between plots, mobilising soil nutrients and thus reducing the impact of the tree remains. All of these issues make it likely that there are further factors affecting the testate amoebae populations at this site.

In a ditch-blocking experiment at both a fen and a bog site in Finland, Jauhiainen (2002) found that the species that increased in the bog site were *Assulina seminulum, Heleopera petricola* and *Nebela tincta*. At Flanders Moss, *Nebela tincta* was common in all sample plots and had a high (>25%) percentage of live individuals in most treatment methods and during all months except October. *Assulina seminulum* was not common at the site and *Heleopera petricola* was only recorded in higher proportions in the harvested plots (undammed) and the whole tree removal plots (dammed). *Euglypha strigosa* decreased in numbers after restoration in Finland but was common in many plots at Flanders Moss. This may indicate that the site is still in the early stages of recovery.

The feature of the testate amoebae population that shows the largest difference between treatments is the percentage of live individuals. Where there is a high proportion of live individuals, it can be assumed that reproduction and immigration rates are relatively high and that survival rates are high because the number of empty tests being deposited is being exceeded by the number of live individuals in a particular location. Louisier (1982) and Tolonen (1986) suggested that reproduction is dependent upon the availability of test building materials. As testate amoebae respond to hydrological condition, an increase in turnover of those taxa common to wetter sites, would indicate that conditions are returning to those of a healthy bog. Reproduction rates in peat bogs have been estimated at between 9 and 27

generations per annum (Schönborn, 1992). From the data collected here, it appears that the sample plots where some tree remains have been left *in situ* ('fell to waste' and 'harvest'), have higher proportions of live individuals especially of *Euglypha strigosa* and *Nebela tincta* and therefore provide better conditions than the plots where the whole tree has been removed.

This result is contrary to current opinion in restoration of forested mires. Past restoration work at Flanders Moss, where birch scrub has been removed, has worked upon the assumption that, in order to prevent large changes in nutrient status, all plant material should be removed (Brooks and Stoneman, 1997a). Tree remains, if left *in situ* can continue to drop litter, shade out bog plants and allow weed establishment (Bacon and Lord 1996). The rationale behind leaving tree remains on the site was to see whether expensive, and potentially damaging, removal of tree waste is always necessary.

The major division in this experiment seems to be between plots with some tree remains and those where all tree material has been removed. There is little difference between plots where the trees have been felled to waste and those where conventional harvesting methods have been used. One factor that could be encouraging higher reproduction rates in the sample plots with tree remains is the protection of the surface from the elements and subsequent rise in humidity levels. When the trees were removed from the plots subject to whole tree removal, the ground conditions were dramatically altered. Aside from the rise in water table, the protection afforded by tree cover was removed. Wind, with its drying effect, increased and temperatures became more extreme (Anderson *et al.*, 1995). These changes were greatly reduced in the plots where some tree remains were left. Each method has some negative

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impacts. Therefore this experiment will help to assess which conditions are the most important for bog regeneration.

Extensive research into recovery of bare peat surfaces in Canada and in Europe has shown that *Sphagnum* recolonisation rates are better where some shelter is provided (Buttler *et al.*, 1998; Campeau & Rochefort, 1996; Price *et al.*, 1998). Various materials have been tested to provide shelter, ranging from straw mulch (LaRose *et al.*, 1997) to plastic membrane (Grosvernier *et al.*, 1995; Campeau & Rochefort, 1997). Experiments in Canada have compared the impacts of irrigation with the provision of shelter on *Sphagnum* regeneration and found that provision of shelter is more effective (Rochefort & Bastien, 1998) perhaps due to increased surface humidity. An experiment looking at the effect of vascular pioneer species on bog regeneration, found that *Sphagnum* mosses had higher survival rates under two *Eriphorum* species, in this instance, straw mulching was unnecessary (Boudreau & Rochefort, 1998). Other experiments have focused on microtopography, (Ferland & Rochefort, 1997; Heathwaite *et al.*, 1993; Price *et al.*, 1998). Price *et al.* (1998) and Quinty & Rochefort (1997) found that, on ploughed peatlands, twice as many *Sphagnum* diaspores (fragments of *Sphagnum* plants used in restoration work) colonised hollows compared to ridges. Again, this is likely to be due to increased surface humidity. This does not mean that overall, ploughed areas regenerate better than unploughed, because the poorer colonisation of the ridges counters the increases in the furrows.

Both the provision of shelter and a ploughed microtopography can protect the peat surface from high levels of desiccation by the wind and, to some extent, from high levels of solar

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radiation. The reduction of these environmental stresses results in increased humidity at the surface of the bog. Surface humidity appears to be a very important environmental condition for recolonisation of peat by *Sphagnum*, as a humid environment is essential for *Sphagnum* survival and growth (Chopra & Kumra, 1989). This experiment has not measured humidity but it appears that water table and percentage moisture measurements are not accurate proxies. Surface humidity cannot be fully explained by these other measurements. Work on peatland restoration in Canada has shown that water table is less important than surface conditions. Ferland & Rochefort (1997) found that a plentiful supply of water to *Sphagnum* diaspores was not enough; to ensure their survival it was vital to provide the correct humidity conditions at the peat-air interface.

Although the experiment at Flanders Moss investigated regeneration after forestry removal, there are many parallels with regeneration of bare peat surfaces. The site had been extensively drained and ploughed prior to afforestation. Consequently, the surface had been greatly modified from the original raised bog. Forestry plantations on bogs can lead to a large reduction of peat water content and shrinkage of the peat surface within 20 years (Anderson *et al.*, 2000; Pyatt *et al.*, 1992). David and Ledger (1988) stated that the drainage of bogs in preparation for forestry production removed up to 50% of the vegetated surface, depending upon the spacing of the plough furrows. The vegetation of the ground layer within a pine forest differs greatly from that of a raised bog. MacDonald (1928) examined ground vegetation in forestry plantations on peat, when the canopy closed. Ground flora became more similar to forest floor vegetation than peatland, with sparse cover of mosses such as *Hypnum cupressiforme* and increases in *Molinia caerulea*; species more common to forest

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communities. The vegetation changes occur when the canopy closes at 10 to 20 years after planting because of the dense shading effect (Anderson *et al.*, 1995). Restoration of the bog surface is therefore, not simply a case of rewetting, but also regeneration of suitable vegetation. The surface of the bog was also extremely exposed, a characteristic of mined peatlands. The provision of shelter was, therefore, very important at this site. It appears that the experiment, which aimed to assess the need for removal of trees, has demonstrated that, not only is it unnecessary to remove all tree remains, but, it could actually prove beneficial to leave them *in situ*.

It appears that, in the short-term at least, the provision of shelter by the tree remains outweighs any negative impacts on the nutrient status of the bog. This needs careful monitoring. When the samples were taken for testate amoebae analysis, the tree remains were only in the early stages of decomposition. The size of the trees mean that it could be some time before these remains fully decompose. Therefore, continued monitoring of nutrient status is important. In the medium to long-term, the recovering bog could be damaged by increases in soil nutrients. For example, more recent research into restoration of peat surfaces in Canada has suggested that where straw mulches are used, their decomposition impedes recovery, as nutrient enrichment becomes a problem (Waddington *et al.*, 2003).

There is an alternative explanation for the higher percentages of live testate amoebae in the plots with tree remains. It could be that the removal of entire trees has led to greater disturbance of the bog surface than leaving them *in situ* at the time of felling, on subsequent monitoring visits and by grazing animals. In the plots where tree remains have been left,

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central paths have been used for access and other areas have been left undisturbed. To measure the rate of disturbance on whole tree removal plots in future experiments, estimates of compaction and grazing pressure could be made.

5.5 Conclusions

This chapter has worked towards fulfilling objective 2 of the thesis - comparing testate amoebae community responses to different forest removal treatments. Seasonal patterns in testate amoebae communities (objective 5) have also been examined to some extent. The potential for developing a practical monitoring tool for peatland restoration (objective 6) is considered.

The testate amoebae responses are set against the background ecological responses to restoration at the site. The water table of the afforested part of Flanders Moss has increased significantly over the monitoring period and is now well within the target range set by the Forestry Commission. This has been a universal response, as when data from individual monitoring months were analysed, neither forest removal nor damming treatment has any statistically significant impact on depth to water table. The increases in water table at this site have occurred because of the removal of the forestry plantation and the blocking of major forestry drains.

Testate amoebae monitoring is only able to compare forestry removal and damming treatments on four dates in 2000 / 2001 as restoration was already well established when samples were taken. The first finding was that the percentage of live testate amoebae in a sample was

unaffected by damming intensity. This implies that there was little difference between damming treatments, a conclusion supported by the hydrological response.

Perhaps more significantly, the type of tree removal technique practiced did have an impact on percentage live, with the highest percentages of live testate amoebae found in plots where some tree remains had been left *in situ*. As water table data do not show a corresponding response, testate amoebae are displaying a greater sensitivity to environmental conditions at the site. The taxa present in higher live percentages in monitoring plots with tree remains were *E. strigosa* and *N. tincta*, both of which are indicative of wetter bogs with mean annual water table measurements in the UK of around 4-5cm below ground level (Charman *et al.*, 2000). Water table at Flanders Moss was deeper than this and did not vary much between treatments so it appears that a further, unmeasured, factor was affecting testate amoebae communities.

Surface humidity has been found to be an important factor in the restoration of bare peat surfaces in Europe and Canada (e.g. Grosvernier *et al.*, 1995; Campeau *et al.*, 2004) and this is likely to be a factor affecting testate amoebae community composition at Flanders Moss. As taxa with higher water table requirements are surviving in dryer locations than would be expected, it is probable that this is a positive impact. Recovery of peatland communities is being facilitated by the shelter that tree remains provide for the bog surface. Further research into surface humidity and testate amoebae is necessary to provide more explanation of this result.

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CHAPTER SIX: Fenn's and Whixall Mosses – Rewetting a cutover surface

6.0 Introduction

In a study of testate amoebae communities in damaged peatlands, it is important to focus on sites affected by the major causes of damage. Objective 3 of the thesis was to study testate amoebae communities on a restored cutover mire surface. The third experimental site for this project therefore fulfils this objective by focusing on peat extraction, a commercial application which has had a devastating impact on lowland raised mires across the UK (Money, 1995). Peat extraction for fuel and horticultural products has affected most peatland sites in the UK to some degree and it became more intensive and destructive over the last century with the advent of mechanical extraction methods. Restoration or rehabilitation of post-mined sites is extremely difficult as the acrotelm, or living layer at the surface of the mire is removed, creating a hostile environment for peat-forming plants (Johnson, 1997). In addition, the nutrient balance is totally disrupted as the peat begins to decompose, creating nutrient-rich conditions where they were previously nutrient-poor (Berry *et al.*, 1996). This section of the thesis also works towards objective 4 (developing the understanding of testate amoebae responses to hydrological conditions in degraded mires).

6.1 Fenn's and Whixall Mosses

Fenn's and Whixall Mosses straddle the Welsh / English border in Wrexham and Shropshire. They are located near the town of Whitchurch in Shropshire (Figure 6.1). The site has been managed jointly by Countryside Council for Wales (CCW) and English Nature (EN) since 1991. The mire complex which comprises Fenn's, Whixall, Bettisfield, Wem and Cadney mosses is part of the Midlands Meres and Mosses (Sinker, 1962), designated a Site of Special Scientific Interest (SSSI), and is the third largest and most

southerly raised mire in Britain (Berry *et al.*, 1996). Fenn's and Whixall and Bettisfield Mosses were designated a National Nature Reserve in 1994, and are also part of the Midlands Meres and Mosses Ramsar site. Their designation as a Special Area of Conservation (SAC) reinforces the requirement to restore the site to active peat formation (O'Connell, 1999).

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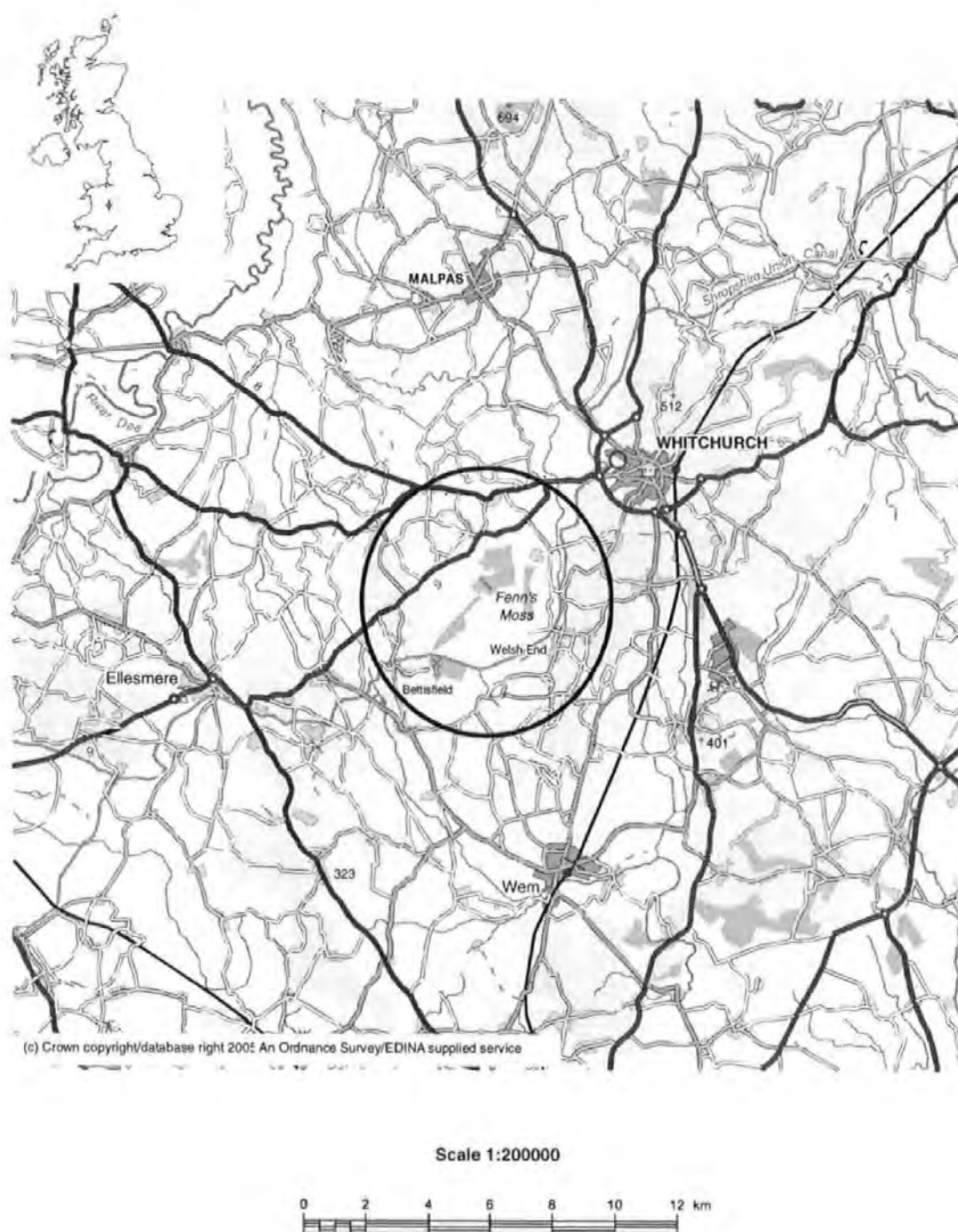
Figure 6.1: Location of Fenn's and Whixall Mosses

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The site has a long history of peat extraction with turbary rights, documented in manorial records, dating back to 1572 (Berry *et al.*, 1996). Handcutting was well established at this site, and progressed to the large-scale exploitation of the late 19th century, which continued until 1990. This has resulted in extensive drainage and water table manipulation and left a patchwork of cut areas, cutting methods and some relatively small uncut sections. Within the cut areas, there are abandoned hand cuts, old commercial hand cut areas and recent mechanically cut commercial cuttings. Despite the current conservation interest in the site, in a survey of the Midland Meres and Mosses, Sinker (1962) described Fenn's and Whixall Mosses as being of little ecological interest because of the damage caused by peat extraction.

Commercial peat extraction at the site ceased in 1990, when mineral extraction rights were passed from Croxden's Horticultural Products to the Countryside Council for Wales (CCW) and English Nature (EN). A condition survey of the site in 1991, categorised remnant vegetation by cutting type. Vegetation at the site varies mostly by cutting history and many plants of high conservation value have survived within uncut areas. There is a spectrum from the uncut areas, where there is typical raised mire vegetation, e.g. Welsh Bettisfield Moss, dominated by bogmosses including *Sphagnum pulchrum* and *Sphagnum magellanicum*, to the mechanised commercial cuttings in the central area of Fenn's Moss, where vegetation is dominated by *Molinia caerulea, Rubus fruticosus, Pteridium aquilinum* and *Betula* spp. with patches of mire species (*Eriophorum* spp. *Erica tetralix*, and *Andromeda polifolia*) (Berry *et al.*, 1996). Despite the large-scale exploitation of the Moss, it remains home to species of high conservation value and its value has been recognised with several UK and international conservation designations (outlined above).

The main conservation management priority at the site has been raising water levels as the first step towards rehabilitating mire vegetation on drained and cut areas. This is one of the first attempts to recreate mire vegetation on a cutover site in the UK (Joy & Pullin, 1999). Experimental work in Canada has shown that *Sphagnum* can recolonise bare peat surfaces (Campeau & Rochefort, 1996; Ferland & Rochefort, 1997). However, the long-term success and, in particular, the possibility of re-establishing active peat formation is extremely uncertain and there are many hurdles to effective restoration. At Fenn's and Whixall there are two additional problems; local concerns about flooding limit how much water levels can be raised and there is potential for nutrient enrichment of the site from surrounding farmland, a long-held concern (Sinker, 1962).

6.2 Methods

6.2.1 Description of restoration work

Restoration of Fenn's and Whixall moss has been an ongoing process since management passed to CCW/EN in 1991. Experimental work here has therefore had to be a comparison of differences between areas currently undergoing restoration management, as there was no opportunity to establish pre-restoration baseline conditions.

The study concentrates on a transect of dipwells which runs across management compartments 1.9 and 3.9 on Fenn's Moss (Figure 6.2) from the old railway line, across Fenn's Moss main drain, to an access track which marks a change in peat harvesting method. The sampled area was harvested by Croxdens Horticultural Products (CHP) between 1989 and 1990 and is a network of old cuttings. Drainage ditches in compartments 1.9 and 3.9 were blocked by CCW and EN in 1991/1992 and 1992/1993/1994 respectively as part of the restoration of Fenn's and Whixall Moss.

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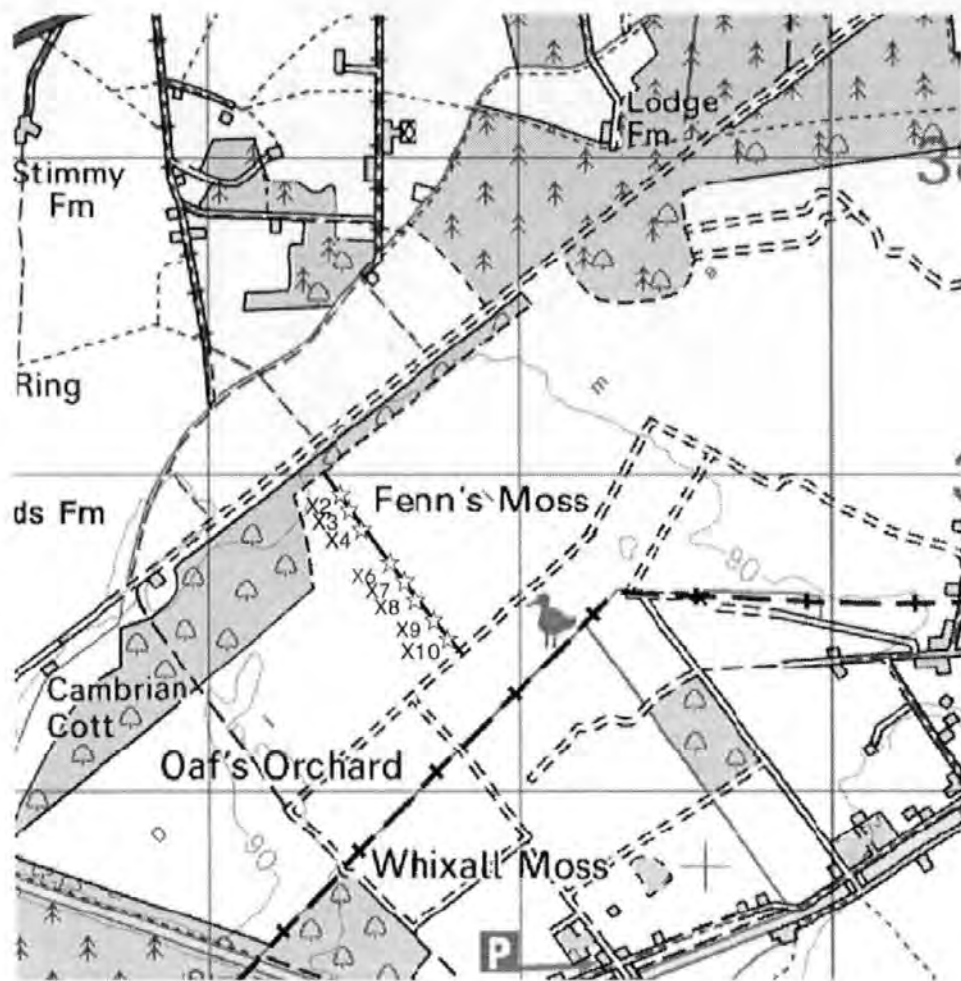
Figure 6.2: Detailed map of Fenn's and Whixall Mosses showing sampling transect and dipwell locations (numbered and marked with stars).

The rationale behind the selection of management compartments 1.9 and 3.9 for monitoring were that the peat harvesting technique is typical of most cutover mire sites in the UK and a large proportion of Fenn's and Whixall has been cut in this manner. There are three variables to consider in the restoration of this site: (1) cutting method (some reference to different cutting types was made in the site description), (2) time since cutting, and (3), hydrological regime. By selecting an area where (1) and (2) were constant, the

aim was to establish the optimal hydrological conditions for restoration through examining testate amoebae communities.

This area was ideal for the experiment, since the cutting method was the same across both management units, and the whole area was cut within 2 years. The same restoration techniques have been used, but the hydrological response to restoration has not been uniform.

6.2.2   Testate amoebae sampling

The mechanised extraction technique used by CHP has left a typical pattern on the surface of the bog with a ditch (1), a cut surface adjoining the ditch (2), a raised area of drier waste peat (3) and then the 'fey' (a local term referring to an older cut surface) (4) (Figures 6.3 and 6.4). This pattern was used as the basis of the sampling at Fenn's and Whixall. One sample was taken from each of the surfaces so that a picture of the testate amoebae communities within each microtopographical area could be developed. Sampling points were chosen next to the dipwells on this transect. The cutting pattern could not be identified at dipwells X1 and X5; therefore, no samples were taken from these points. Figure 6.5 shows typical vegetation at the sampling locations.

The intention was to sample for testate amoebae using a similar method to that described in Chapter 3. However, as there were very few locations with active *Sphagnum* growth, the methodology had to be adapted and other plant material was sampled. Where *Sphagnum* was present in the sample plot, several stems were taken as at Flanders Moss. At other locations, samples of plant litter (i.e. *Molinia* or *Calluna / Erica*) were taken from the base

of the plant. Where vegetation cover was not continuous, peat was scraped from the surface.
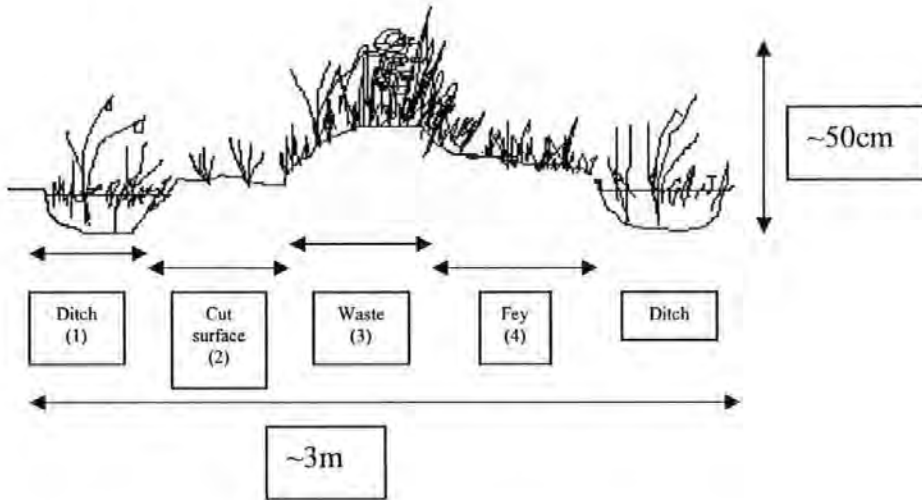
Figure 6.3: Profile of cutting surface showing position of sampling surfaces
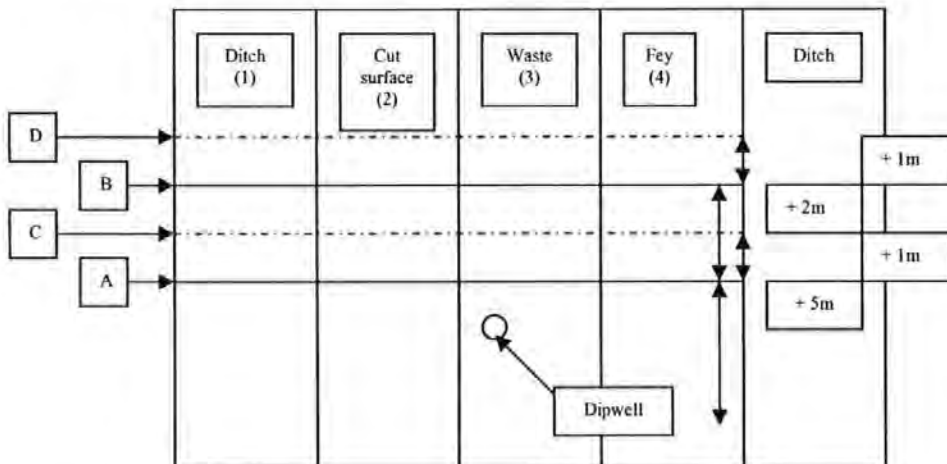
Figure 6.4: Sampling layout. Samples A and B – July, C and D – October.

Figure 6.5: Typical vegetation pattern at sampling locations.

Sampling was carried out in July 2003 and repeated in October 2003. Eight samples were taken from each dipwell point, one from each surface 1m from the dipwell and a replicate 5m from dipwell in July and at 2m and 6m from the dipwells in October. The staggering of distances from dipwells was to avoid the possibility of sampling the same spot twice and thus studying damage inflicted by previous sampling. The sampling methods used at Coom Rigg Moss and Flanders Moss (described in chapter 3) could not be applied at this site because of the variable nature of the substrate. Wherever possible, samples of *Sphagnum* were taken. However, where there was no *Sphagnum* growing; samples of plant litter (a handful of dead plant material from the base of the living plant) or bare peat were taken (a scraping of surface material). Samples were taken back to Plymouth and testate amoebae were extracted using methods outlined in section 3.1. When counting testate amoebae, only live tests were counted as it was necessary to ensure that fossil amoebae which had been exposed by peat extraction were not confused with modern individuals.

A brief examination of the vegetation was made at each of the sampling locations in July and October. The dominant species were identified and percentage cover was estimated to create a more detailed picture of the differences between dipwell sites and of the sampling locations within these sites.

The sampling process resulted in the collection of 128 samples. A subset of these samples was counted to reduce the time spent counting. Dipwell locations were selected on the basis of the vegetation assessment, hydrological data and to maximise the area studied. The locations were classified as dry, intermediate, wet and very wet using data from Gilman (2000).

X2 – dry

X3 – wet

X4 – wet

X6 – wet

X7 – intermediate to dry

X8 – very wet

X9 – very wet

X10 – dry

Dipwell locations X2 and X3 were selected for counting from management compartment 1.9. X2 was the driest location according to the hydrological data and X3 was a wetter site. X3 had standing water when the July samples were taken. Locations X6, X7 and X8 were selected from management compartment 3.9. This selection covered the range of ground conditions. Although X10 was by far the driest location, the ditch was much deeper than at the other dipwells, there was no *Sphagnum* growth and the only available

sample material was algal. It was decided that this was not representative of the cutting pattern in the rest of the area so it would be less useful in terms of understanding the site.

6.3 Results

6.3.1 Vegetation composition

Tables 6.1a-6.1e show the vegetation composition of the dipwell locations where testate amoebae were counted. Samples A1-4 and B1-4 were taken in July 2003 and samples C1-4 and D1-4 in October 2003. Dipwell X2 had the most uniform vegetation across sampling points. The dominant species were *Molinia caerulea* and *Calluna vulgaris*, plants commonly found on moorland and drier peatlands. All other dipwell locations had ditches dominated by *Sphagnum* spp., indicating more constant wet conditions. Hydrological data from dipwell X3 showed that it was a much wetter location than X2. This is supported by the vegetation composition; all surfaces of X3 had a high percentage cover of *Eriophorum* spp., a plant typical of wet, boggy sites (Stace, 1997). Dipwell X6 was a wetter location according to hydrological records (Gilman, 2000); however, the vegetation was more typical of plants with wider water table tolerances. X7 was another dry location. Vegetation at sampling surfaces 1 and 2 was again typical of wetter conditions, with surfaces 3 and 4 being dominated by drier vegetation types and in particular, the presence of *Rubus* spp., indicating much drier conditions (Stace, 1997). Dipwell X8 was a very wet location. The presence of *Sphagnum* spp. and *Eriophorum* spp. on all sampling surfaces indicated a much higher water table. The presence of *Rubus* spp. in the vegetation at D4 probably shows that there was a drier raised area at that spot.

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4
Betula spp.	15	0	5	0	0	0	0	0	0	0	0	5	0	0	0	0
Calluna vulgaris	0	80	25	0	50	70	20	70	30	40	80	5	20	60	80	40
Erica tetralix	0	0	0	60	0	5	0	10	0	0	0	0	0	0	0	0
Molinia caerulea	85	30	80	40	10	40	90	20	90	80	20	90	90	40	30	80
Pteridium aquilinum	0	0	10	0	0	5	5	10	0	0	0	10	0	0	5	0
Rubus spp.	0	0	0	0	0	0	0	5	0	5	0	0	0	0	0	0

Table 6.1a: Vegetation composition X2. A1 to D4 refer to sampling locations (see Figure 6.4). Numbers are percentage cover of each plant species.

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4
Bare peat	0	0	0	15	0	0	0	0	0	80	0	0	0	40	10	0
Calluna vulgaris	0	5	25	0	0	20	25	5	0	20	30	20	0	0	20	30
Erica tetralix	0	0	5	15	0	0	0	0	0	0	0	0	0	0	0	0
Eriophorum angustifolium	0	0	0	0	0	0	0	0	30	20	10	20	30	40	5	10
Eriophorum vaginatum	40	40	5	25	30	25	40	25	0	20	10	30	0	5	40	20
Holcus lanatus	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
Molinia caerulea	0	0	95	80	0	5	15	80	0	0	50	80	0	0	30	40
Moss	0	0	40	0	0	0	5	0	0	0	10	0	0	0	40	0
Sphagnum palustre	0	25	0	0	0	20	0	0	0	0	0	0	0	0	0	0
Sphagnum recurvum	90	0	0	0	80	0	0	0	100	0	0	0	100	20	0	0

Table 6.1b: Vegetation composition dipwell X3 (see Table 6.1a caption for more detail).

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4
Bare peat	0	0	0	0	0	0	0	0	0	50	0	20	0	50	0	10
Calluna vulgaris	0	25	20	20	0	40	20	30	5	30	30	30	0	20	40	25
Erica tetralix	5	25	40	10	5	25	40	10	5	20	15	10	0	0	5	0
Eriophorum angustifolium	0	0	0	0	0	0	0	0	40	5	10	15	25	10	0	0
Eriophorum vaginatum	20	20	0	0	40	10	0	0	0	10	0	0	20	10	0	0
Molinia caerulea	0	10	80	70	10	10	40	60	0	5	60	60	0	0	50	70
Moss	0	0	30	20	0	20	5	0	0	5	0	0	0	10	0	0
Pteridium aquilinum	0	5	0	10	0	10	5	30	0	0	0	10	0	5	0	20
Sphagnum cuspidatum	0	0	0	0	0	0	0	0	20	0	0	0	100	0	0	0
Sphagnum recurvum	80	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0

Table 6.1c: Vegetation composition dipwell X6 (see Table 6.1a caption for more detail).

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4
Bare peat	0	20	0	0	0	0	0	0	0	40	0	0	0	40	0	0
Betula spp.	0	0	0	0	0	5	0	0	0	0	0	0	0	5	0	0
Calluna vulgaris	0	10	10	0	0	10	0	30	0	15	5	30	0	30	0	30
Drosera rotundifolia	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erica tetralix	0	20	20	25	20	15	15	15	0	15	10	10	0	15	10	0
Eriophorum angustifolium	50	10	0	0	40	30	0	0	25	15	0	0	15	10	0	0
Eriophorum vaginatum	20	20	0	0	20	20	0	0	20	30	0	0	30	20	0	0
Molinia caerulea	0	30	40	40	0	0	80	40	0	0	80	50	0	0	80	50
Moss	0	0	15	0	0	20	0	10	0	0	0	0	0	0	0	0
Pteridium aquilinum	0	0	60	60	0	5	20	60	0	0	20	30	0	0	20	25
Rubus spp.	0	0	10	10	0	0	0	5	0	0	5	0	0	0	0	0
Sphagnum cuspidatum	0	0	0	0	0	0	0	0	0	0	0	0	80	0	0	0
Sphagnum recurvum	90	0	1	0	90	0	0	0	100	5	0	0	0	0	0	0

Table 6.1d: Vegetation composition dipwell X7 (see Table 6.1a caption for more detail).

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4
Andromeda polifolia	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
Bare peat	0	40	0	40	0	15	0	25	0	30	0	25	0	30	0	70
Calluna vulgaris	0	5	20	5	0	15	25	20	0	10	20	0	0	10	30	25
Drosera rotundifolia	0	0	5	10	0	5	0	10	0	0	0	0	0	0	0	0
Erica tetralix	0	5	20	20	0	30	10	20	0	15	15	25	0	30	20	15
Eriophorum angustifolium	20	20	40	0	60	30	0	5	30	10	0	20	60	10	0	20
Eriophorum vaginatum	25	10	0	40	25	10	0	5	10	25	30	20	0	40	0	10
Molinia caerulea	0	5	30	20	0	10	60	30	0	0	50	20	0	0	80	20
Moss	0	0	10	0	0	0	20	0	0	0	0	0	0	0	0	0
Dicranella heteromalla	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
Rubus spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Sphagnum cuspidatum	0	0	0	0	0	0	0	0	90	0	0	0	100	0	0	0
Sphagnum recurvum	100	25	20	0	100	25	10	60	0	20	30	0	0	0	0	0

Table 6.1e: Vegetation composition dipwell X8 (see Table 6.1a caption for more detail).

6.3.2 Hydrological results

Dipwell measurements were taken in July and October to coincide with sampling. The data collected by CCW and EN as part of their long-term monitoring was used to support these measurements and to get a more detailed picture of hydrological conditions at the site. Both visits were preceded by the first heavy rainfall after prolonged dry spells. The measurements from sampling days are shown in Tables 6.2 and 6.3. The data do not agree entirely with the hydrological record of Gilman (2000). X2 appears to be a very wet site according to the July and October measurements, despite the long-term record revealing that it is rather dry and X7 also appears to be very wet in October, despite being classified

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as dry. The measurements from the previous and subsequent fortnightly monitoring are included in Tables 6.2 and 6.3. They confirm the suspicion that the measurements taken on the days of testate amoebae sampling are anomalously high. They also show that water levels rose significantly immediately after the heavy rainfall events. This illustrates the dangers of using one-off measurements as an indication of hydrological conditions at a site.

Dipwell	10/07/03 dwt (cm) CCW / EN	July dwt (cm) EV	24/07/03 dwt (cm) CCW / EN	Standing water?
X2	24	2	13.8	No
X3	16.5	4	3.7	Yes
X6	21.7	- 5	8	No
X7	39.1	14	16.1	Yes
X8	3.3	- 8	3.6	Yes

Table 6.2: Water table measurements from July (EV) with data from long-term monitoring (EN/CCW) for previous and subsequent readings. (Negative readings indicate water levels above the surface).

Dipwell	15/10/03 dwt (cm) CCW / EN	October dwt (cm) EV	30/10/03 dwt (cm) CCW / EN	Standing Water?
X2	29.4	- 9	17.2	No
X3	15.1	- 3	4.9	No
X6	20.9	8	-2.6	No
X7	31.2	5	16	No
X8	3.6	- 4	-1.1	No

Table 6.3: Water table measurements from October (EV) with data from long-term monitoring (EN/CCW) for previous and subsequent readings. (Negative readings indicate water levels above the surface).

Plotted dipwell readings from 2002 and 2003 are shown in Figures 6.6 and 6.7. They are consistent with previous data (Gilman, 2000) showing that the wettest site was X8, the two driest were X2 and X7 although the hydrological data from 2002 and 2003 show that X7 is now drier than X2 contrary to previous water table measurements. Water levels range

from 10cm above ground level at the wettest site (X8) to around 40cm below ground level at the driest site (X7).
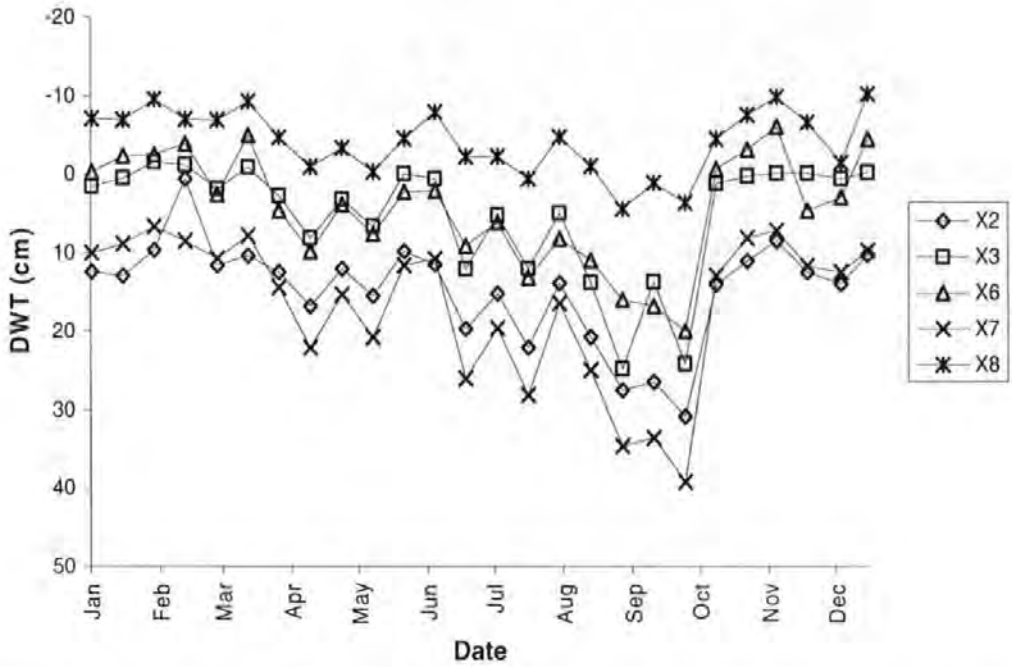
Figure 6.6: Dipwell readings 2002 for sampled sites (fortnightly measurements taken by EN/CCW)
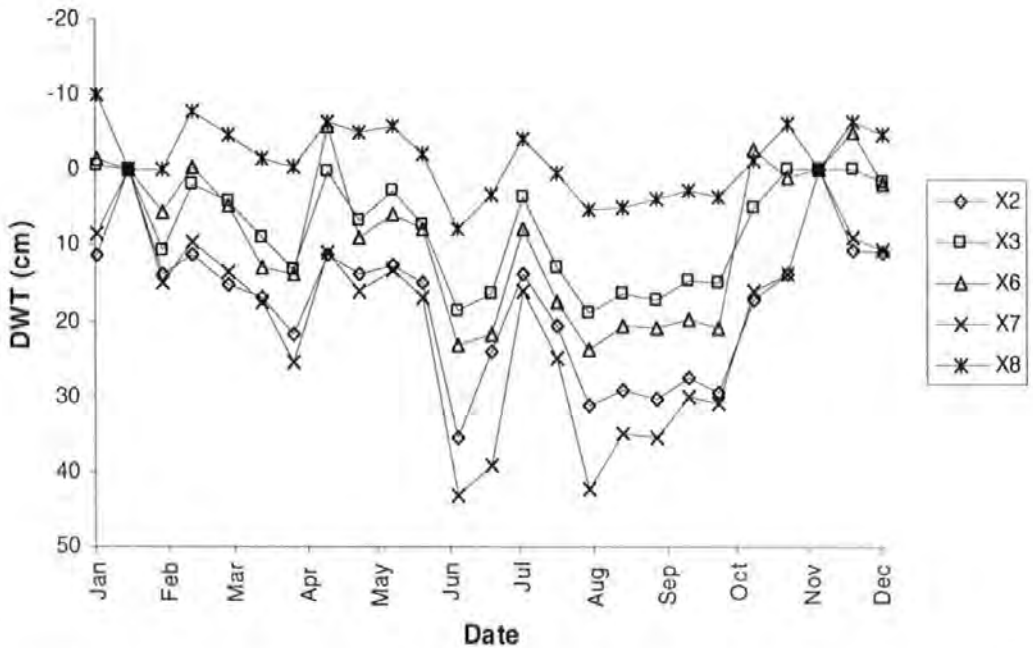
Figure 6.7: Dipwell readings 2003 for sampled sites (fortnightly measurements taken by EN/CCW)

A two-way ANOVA on the hydrological records from 2002 and 2003 and the dipwells and sample date showed that there were statistically significant differences between dipwell location (p = 0.00) and between the date water table measurements were taken (p = 0.00). Water levels were fairly consistent throughout the winter and spring of 2002 but dropped considerably during the summer and remained low until October.

6.3.3 Testate amoebae – raw numbers

Counts of testate amoebae were made from each of the 16 sample spots at the five dipwell locations. Only live tests were counted here as removal of the surface for peat harvesting has exposed old peat surfaces and, therefore, the likelihood of counting fossil tests was high. This meant that percentage live data (as used in analysis of Coom Rigg Moss and Flanders Moss) were not available. The number of taxa found are shown in Table 6.3. The lowest number of taxa was found in the ditch samples (1) and the highest in samples from the waste (3) and fey (4). Dipwell X7 had the lowest number of taxa and X3 the highest.

		Surface					
		1	2	3	4	Mean	St Dev
Dipwell	X2	18	18	21	20	19.25	1.50
	X3	20	22	20	22	21.00	1.15
	X6	13	13	21	19	16.50	4.12
	X7	9	17	17	18	15.25	4.19
	X8	11	20	17	17	16.25	3.77
	Mean	14.20	18.00	19.20	19.20		
	St Dev	4.66	3.39	2.05	1.92		

Table 6.3: Number of species counted by dipwell and sampling surface

Concentration data were calculated by using an exotic marker spore (*Lycopodium*). Table 6.4 shows the number of testate amoebae counted by dipwell and sampling surface. The aim was to count more than 100 tests in each sample and there were only two samples where this was not achieved (X8 A1 and B1). Wet weight concentrations were calculated

because there was some concern about the amount of material removed in the extraction process, creating inconsistent dry weight results.

Dipwell		Sampling Surface			
		1	2	3	4
X2	Total (mean)	171	164	176	173
	Total (range)	155-191	111-217	157-220	143-199
	Conc wet weight	3634	13396	8036	11261
	Conc (range)	3320-3891	3004-24658	5472-10680	3268-25422
X3	Total (mean)	180	114	114	124
	Total (range)	128-245	108-119	107-128	114-135
	Conc wet weight	866	874	1672	2954
	Conc (range)	610-1436	225-2121	873-2035	939-5458
X6	Total (mean)	127	120	143	136
	Total (range)	108-136	111-138	121-176	122-141
	Conc wet weight	608	2565	6236	1587
	Conc (range)	321-1252	460-3654	2178-9303	1274-4643
X7	Total (mean)	185	153	188	195
	Total (range)	120-232	141-168	136-206	158-220
	Conc wet weight	542	2619	7553	5349
	Conc (range)	127-1330	1711-3811	1286-13648	1935-7510
X8	Total (mean)	101	99	144	113
	Total (range)	52-127	46-118	117-173	111-116
	Conc wet weight	192	4783	7484	2688
	Conc (range)	56-378	98-9685	560-19208	1880-4453

Table 6.4: Numbers and concentrations (testate amoebae / g wet weight) of testate amoebae by dipwell and sampling surface.

Statistical analysis was carried out to determine whether there was more difference in testate amoebae concentrations between dipwells than between the sampling surfaces. Analyses of variance (ANOVAs) comparing concentrations at each dipwell showed that there was a statistically significant difference in concentrations at dipwell locations (p = 0.00) (Table 6.5). The statistical output showed that samples from dipwell X2 had much

higher means than X3, X6, X7 and X8 reflecting the contrast in vegetation between X2 and

the other dipwell sites.

Source	d.f.	SS	MS	*F*	*P*
Dipwell	4	5.1E+08	1.3E+08	6.01	0.000
Residual (error)	75	1.6E+09	2.1E+07		
Total	79	2.1E+09			

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                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level   N   Mean   StDev    --+---------+---------+---------+------
2       16  9082   7365                              (------*------)
3       16  1592   1310     (------*-----)
6       16  3100   2768        (------*-----)
7       16  4016   3762          (-----*------)
8       16  3787   5347         (------*-----)
                            --+---------+---------+---------+------
                              0       3500      7000     10500

Pooled StDev = 4612
```
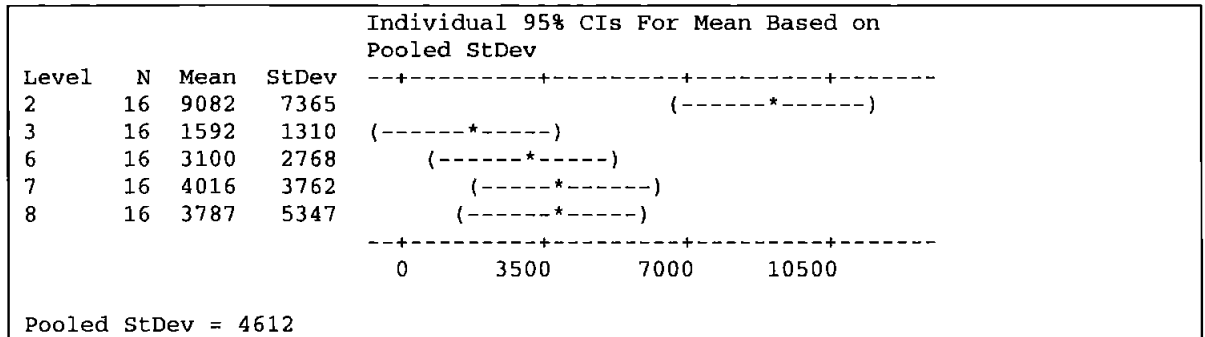
Table 6.5: One-way ANOVA comparing concentrations with dipwell and dotplot showing confidence intervals for means

A one-way ANOVA comparing sampling surface with concentrations showed that there

was a statistically significant difference between surfaces (p=0.011) (Table 6.6). The

mean for surface 1 (ditch) was significantly lower than for the other surfaces, as is clear

from the dotplot of means and confidence intervals. This reflects the stark contrast

between the wet ditch and the other, drier surfaces. A two-way ANOVA on the data set

confirmed that the differences between both dipwells (p = 0.00) and sampling surface (p =

0.00) were significant. Interaction between the two factors was not statistically significant

(p = 0.375). To assess whether there was a difference between July and October samples,

the above ANOVAs were repeated on the data for the two sampling dates. Dipwell was

statistically significant in July (p = 0.038) but not October (p = 0.177). Surface was not

significant in July (p = 0.097) but it was for October (p = 0.037). A two-way ANOVA on

the July dataset showed that the significant differences for the dipwells did not stand up to

further analyses (p = 0.084), surfaces were still not significant (p = 0.136) and nor was

interaction between the two factors (p = 0.988). In a two-way ANOVA on October's

dataset, the dipwells were still not significant again (p = 0.181) and surfaces were just

outside the 95% probability (p = 0.056). Interaction between the two was not significant in

October (p = 0.815).

Source	d.f.	SS	MS	*F*	*P*
Surface	3	2.9E+08	9.5E+07	3.97	0.011
Residual (error)	76	1.8E+09	2.4E+07		
Total	79	2.1E+09			

```
                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N   Mean  StDev   ----+---------+---------+---------+-----
surface 1   20  1169  1327    (--------*-------)
surface 2   20  4848  6375                     (-------*--------)
surface 3   20  6196  4940                        (--------*--------)
surface 4   20  5048  5393                     (--------*--------)
                              ----+---------+---------+---------+-----
                                  0       2500      5000      7500

Pooled StDev = 4896
```
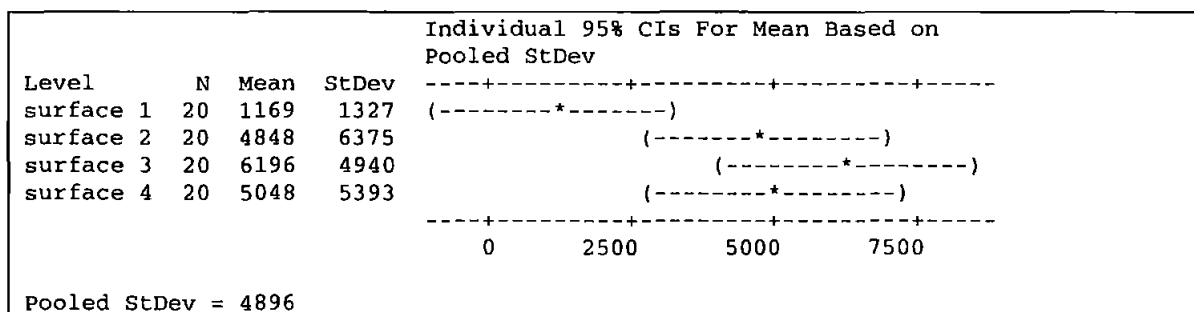
Table 6.6: One-way ANOVA comparing concentrations with surface and dotplot showing confidence intervals for means.

To investigate the impact of sampling surface further and in particular, to assess whether

there were any changes in testate amoebae concentrations in response to the different

hydrological conditions at the dipwells (see Figures 6.6 and 6.7), one-way ANOVAs were

performed on data from individual surfaces. The results appear to show that different

surfaces are more responsive to hydrological differences. Tables 6.7 to 6.10 show the

results of one-way ANOVAs and dotplots of means and confidence intervals. Surfaces 1

(ditch) (Table 6.7) and 2 (cut surface) (Table 6.8) had statistically significant results but

surfaces 3 (waste) (Table 6.9) and 4 (fey) (Table 6.10) were not statistically significant. In

the case of surfaces 1, 2 and 4 (ditch, cut surface and fey), the dot plots show that dipwell 2

is the most different (Tables 6.7, 6.8 and 6.10 respectively) even where changes are not

statistically significant. As the driest dipwell locations were X2 and X7, some more

grouping of samples may have been expected if testate amoebae were simply responding to

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hydrological changes. Given the large contrast in vegetation between dipwell X2 and the other locations, it is possible that testate amoebae are responding to a combination of hydrology and vegetation.

Source	d.f.	SS	MS	F	P
Dipwell	4	3.1E+07	7.8E+06	55.59	0.000
Residual (error)	15	2.1E+06	140908		
Total	19	3.3E+07			

```
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean   StDev  --+---------+---------+---------+-------
2      4  3634.5   236.1                               (--*---)
3      4   866.2   390.7          (--*---)
6      4   608.1   434.3        (--*--)
7      4   542.3   537.8        (---*--)
8      4   191.7   135.3    (---*--)
                           --+---------+---------+---------+-------
                             0      1200      2400      3600

Pooled StDev = 375.4
```

Table 6.7: One-way ANOVA comparing concentrations from surface 1 (ditch) at each of the dipwell locations with dotplot of confidence intervals for means.

Source	d.f.	SS	MS	F	P
Dipwell	4	4E+08	9.9E+07	3.95	0.022
Residual (error)	15	3.8E+08	2.5E+07		
Total	19	7.7E+08			

```
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N   Mean   StDev  -------+---------+---------+---------+--
2      4  13396    9612                    (--------*--------)
3      4    874     857   (-------*--------)
6      4   2565    1448     (--------*--------)
7      4   2619     962     (--------*--------)
8      4   4783    5403       (--------*-------)
                         -------+---------+---------+---------+--
                              0      6000     12000     18000

Pooled StDev = 5007
```

Table 6.8: One-way ANOVA comparing concentrations from surface 2 (cut surface) at each of the dipwell locations with dotplot of confidence intervals for means.

Source	d.f.	SS	MS	F	P
Dipwell	4	109412473	27353118	1.16	0.368
Residual (error)	15	354319841	23621323		
Total	19	463732313			

```
                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Level  N   Mean  StDev  -------+---------+---------+---------+--
2      4   8036   2353             (---------*---------)
3      4   1672    539   (---------*----------)
6      4   6236   3510          (---------*----------)
7      4   7553   5106             (---------*---------)
8      4   7484   8596             (---------*---------)
                       -------+---------+---------+---------+--
                              0      5000     10000     15000

Pooled StDev = 4860
```
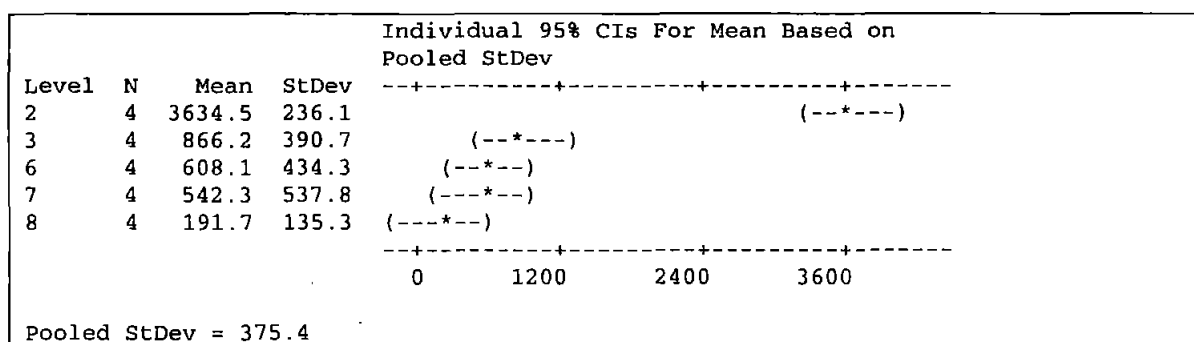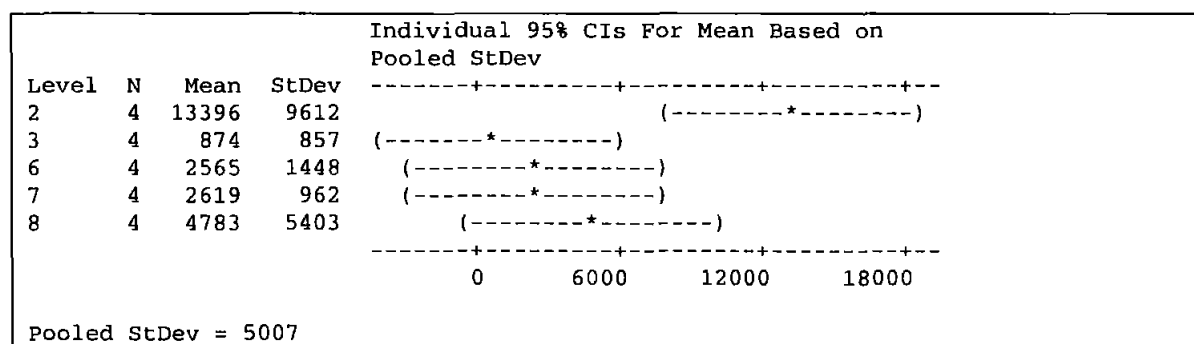
Table 6.9: One-way ANOVA comparing concentrations from surface 3 (waste) at each of the dipwell locations with dotplot of confidence intervals for means.

Source	d.f.	SS	MS	F	P
Dipwell	4	2.1E+08	5.3E+07	2.33	0.104
Residual (error)	15	3.4E+08	2.3E+07		
Total	19	5.5E+08			

```
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean   StDev  -----+---------+---------+---------+----
2      4   11261   10054                 (----------*---------)
3      4    2954    1878   (---------*---------)
6      4    2990    1382   (---------*---------)
7      4    5349    2394       (---------*---------)
8      4    2688    1199   (---------*----------)
                        -----+---------+---------+---------+----
                             0      5000     10000     15000

Pooled StDev = 4768
```
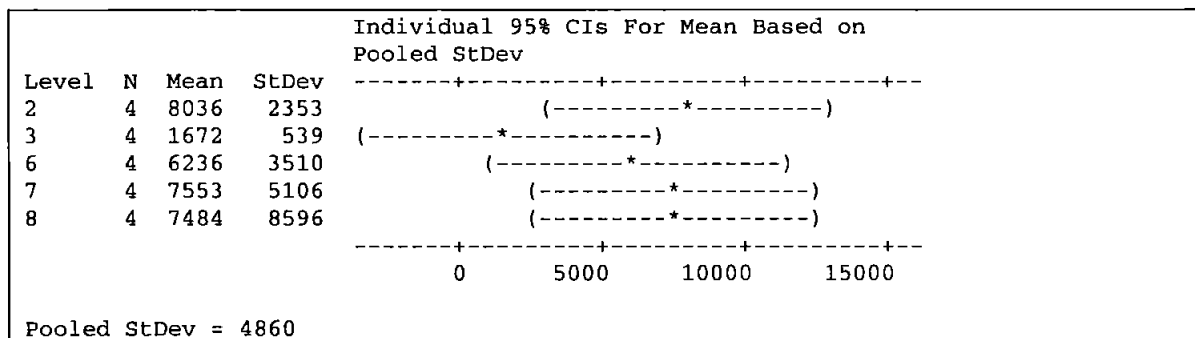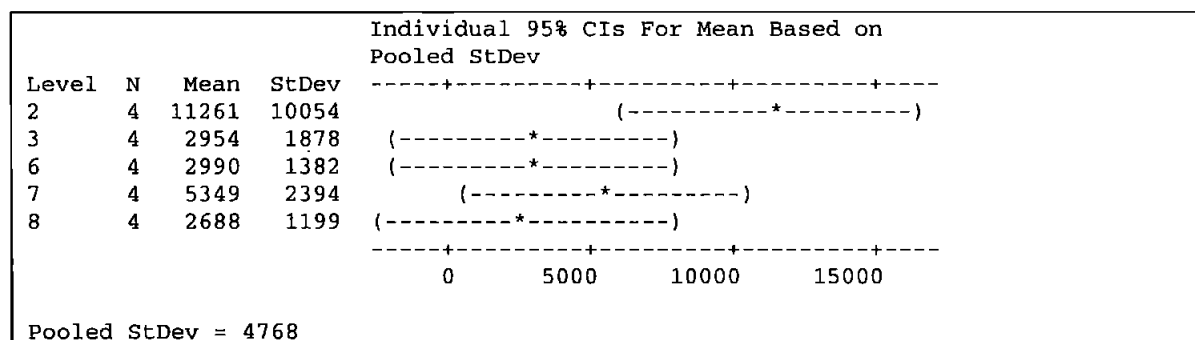
Table 6.10: One-way ANOVA comparing concentrations from surface 4 (fey) at each of the dipwell locations with dotplot of confidence intervals for means.

One-way ANOVAs comparing sampling surfaces within dipwells showed that the surfaces were only significantly different at dipwells X6 and X7 (p = 0.017 and p = 0.023).

There was still rather a large amount of variation in concentrations between samples from the same dipwell and surface (seen in Table 6.4). There appeared to be some influence from the sample material in particular in relation to some of the anomalously high

concentrations within groups of samples. ANOVAs were carried out to see whether sample material had a statistically significant impact on concentrations. The minitab output is shown in Table 6.11. The source material was given a code number from 1 to 5. 1 was *Molinia* litter, 2 *Sphagnum*, 3 other mosses, 4 Ericaceous plant litter and 5 was peat. Categories *Molinia* (1) and Ericaceous plant litter (4) had the highest means. The p value of 0.00 shows that sample material has a significant impact on the number of testate amoebae found per gram wet weight. Mosses, including *Sphagnum*, and peat had much lower numbers of testate amoebae than litter from vascular plants.

Source	d.f.	SS	MS	F	P
Dipwell	4	7.1E+08	1.8E+08	9.47	0.000
Residual (error)	75	1.4E+09	1.9E+07		
Total	79	2.1E+09			

```
                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level        N      Mean    StDev   ---+---------+---------+---------+---
1            26     7417    6821                            (----*----)
2            24      929    1714    (----*----)
3            18     2929    2049        (----*-----)
4             7     8797    2900                       (--------*--------)
5             5     3156    3441    (-----------*-----------)
                                    ---+---------+---------+---------+---
Pooled StDev =              4321    0        3500      7000     10500
```

Table 6.11: One-way ANOVA comparing concentrations per gram wet weight with sample material (Sample material codes: 1 = *Molinia* litter, 2 = *Sphagnum*, 3 = other mosses, 4 = Ericaceous litter, 5 = peat) with dotplot of confidence intervals for means.

6.4.4 Testate amoebae - species composition

Tables 6.12a to 6.12e show a summary of the species data, highlighting, in particular, species which change in composition between sample surfaces. The tables show a mean percentage value for each species (percentage of total testate amoebae), the range, and a shaded cell representing a constancy value. The lightest shading indicates that the species was found in one of the four samples and the darkest, four out of four samples. These figures were drawn from averaged data for all locations. Testate amoebae species are organised according to their UK hydrological indicator values (Charman *et al.*, 2000).

At most dipwells, there is a distinctive pattern, with sampling surface 1 (the ditch) seeing a large increase in *Arcella discoides, Placocista spinosa* and *Difflugia rubescens.* Although not the species with the wettest indicator values, these species have been described as typical of bog pools or very wet and waterlogged conditions (Charman *et al.*, 2000). The other three locations had greater species variation and more species which are indicative of drier conditions. The results seem to correspond well with the vegetation study. Dipwell X2, where vegetation was consistently dominated by *Molinia caerulea* and *Calluna vuglaris*, had the least variation in testate amoebae species across the sampling surfaces and dipwell X8 had the most distinct differences between sample surface 1 (ditch) and surfaces 2-4.

species	1	2	3	4
Arcella discoides	18.5 0-69	18.2 0-73	2.9 0-11	2.9 0-11
Euglypha strigosa	1.05 0-2	1.76 0-6	3.04 2-4	5.30 0-18
Cyclopyxis arcelloides	14.3 2-31	22.5 4-42	14.5 3-36	15.8 6-24
Placocista spinosa	4.8 0-14	1.0 1-2	1.4 0-4	0.9 0-2
Euglypha tuberculata	2.2 1-4	3.6 1-5	5.7 0-16	6.7 3-17
Difflugia pristis	0.3 0-1	0.7 0-2	7.3 0-29	0.3 0-1
Nebela tincta	4.3 0-10	9.2 1-25	8.8 1-16	6.2 1-18
Assulina semimulum	6.3 0-15	2.3 0-5	7.1 0-25	1.5 0-4
Assulina muscorum	14.6 2-33	17.0 6-25	10.9 5-16	17.1 8-30
Corythion-Trinema type	7.4 2-20	6.8 2-15	8.3 2-19	6.0 1-11
Nebela militaris	8.6 2-18	8.1 0-18	15.4 0-35	19.2 0-40
Trigonopyxis arcula	8.3 0-32	3.7 1-8	3.1 1-7	7.1 0-20
Hyalosphenia subflava	4.8 0-19	1.1 0-4	1.5 0-4	7.4 0-26

Wet ↑

Dry ↓

Table 6.12a: Summary of testate amoebae data from dipwell X2 (species means expressed as percentage of total testate amoebae, range and constancy – light to dark shading representing constancy of 1 to 4). Blank cells indicate absence.

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species	1	2	3	4
Arcella discoides	34.7	18.5	12.0	7.6
	8-54	3-43	5-21	0-18
Euglypha strigosa	8.8	14.3	4.7	2.6
	0-22	0-1	0-11	0-6
Cyclopyxis arcelloides	13.7	16.8	4.6	9.3
	1-23	4-30	0-8	0-25
Euglypha tuberculata	3.3	6.0	6.4	5.1
	0-10	0-12	1-13	3-8
Difflugia pristis	5.5	11.5	7.0	8.0
	0-21	0-24	0-16	3-16
Nebela tincta	0.3	0.2	4.3	5.2
	0-1	0-1	0-6	0-17
Difflugia rubescens	12.2	5.3		0.9
	0-27	0-20		0-3
Assulina muscorum	1.7	5.2	5.9	5.9
	0-4	0-13	2-8	2-12
Corythion-Trinema type	3.1	2.6	10.8	11.4
	0-7	0-6	9-14	1-18
Nebela militaris	2.0	0.2	13.3	10.1
	0-5	0-1	4-22	4-22
Euglypha rotunda	2.4	1.1	4.8	2.9
	0-9	0-3	4-6	0-6
Trigonopyxis arcula	2.9	2.4	3.7	2.0
	1-5	0-6	2-7	0-4
Hyalosphenia subflava	1.6	1.5	5.5	1.1
	0-6	0-6	2-10	0-3
Bullinularia indica	1.3	1.3	7.5	1.0
	0-4	0-4	1-11	0-2
Psuedodiffllugia fulva	4.9	5.3	3.3	21.7
	0-18	0-21	0-13	0-62

Wet ↑

Dry ↓

Table 6.12b: Summary of testate amoebae data from dipwell X3. See Table 6.12a for more details.

201

| | | sampling surface | | |
species	1	2	3	4
Arcella discoides	64.6 42-85		0.8 0-2	0.2 0-1
Euglypha strigosa	8.4 1-20	1.1 0-3	2.0 0-7	4.8 1-16
Cyclopyxis arcelloides	16.4 0-55	3.2 1-5	7.0 4-9	9.7 6-12
Euglypha tuberculata	5.2 1-12	19.2 9-29	13.4 9-14	9.2 4-14
Nebela tincta			10.7 2-22	12.8 4-21
Assulina muscorum	0.7 0-3	19.0 11-38	26.8 16-27	13.4 3-23
Corythion-Trinema type	2.7 0-8	13.4 3-30	5.4 4-7	6.7 2-15
Nebela militaris	0.2 0-1		9.3 5-15	2.7 0-6
Trigonopyxis arcula	0.2 0-1	12.7 2-23	8.7 3-21	19.6 9-30
Hyalosphenia subflava		14.2 7-33	3.6 1-7	11.2 3-22
Bullinularia indica		7.3 4-11	2.8 1-4	1.4 0-4
Psuedodifflugia fulva	0.7 0-2	5.9 0-20	2.1 0-4	2.7 2-4

Wet ↑

Dry ↓

Table 6.12c: Summary of testate amoebae data from dipwell X6. See Table 6.12a for more details.

202

| | sampling surface | | | |
species	1	2	3	4
Arcella discoides	55.4	1.7	0.4	
	5-91	0-4	0-1	
Euglupha strigosa		6.9	3.6	5.0
		2-11	0-13	1-15
Cyclopyxis arcelloides	0.3	1.9	14.9	11.8
	0-1	0-3	5-26	3-17
Placocista spinosa	17.2	0.3	1.0	0.1
	3-42	0-1	0-2	0-<1
Euglypha tuberculata	0.7	21.6	4.0	7.3
	0-1	9-46	0-14	0-16
Nebela tincta		0.6	12.1	20.7
		0-1	7-16	5-41
Difflugia rubescens	25.2			
	0-92			
Assulina semimulum			13.2	2.6
			1-32	0-6
Assulina muscorum	0.4	14.2	18.3	11.2
	0-2	7-24	3-30	2-20
Corythion-Trinema type	0.2	2.6	5.6	3.5
	0-1	1-4	1-10	2-8
Nebela militaris		4.8	7.7	8.9
		0-13	0-16	0-30
Trigonopyxis arcula		8.8	9.4	3.2
		3-14	0-23	0-6
Hyalosphenia subflava	0.3	22.4	3.6	16.4
	0-1	10-40	<1-7	3-41
Bullinularia indica		5.638495024	2.6966997	1.25560593
		4-8	0-9	<1-3

Wet ↑

Dry ↓

Table 6.12d: Summary of testate amoebae data from dipwell X7. See Table 6.7a for more details.

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species	sampling surface 1	2	3	4
Arcella discoides	32.0 17-58	4.6 0-15	16.6 1-39	0.2 0-1
Amphitrema wrightanium		0.2 0-1		16.3 1-34
Euglypha strigosa	0.7 0-2	10.6 0-40	2.5 1-4	2.0 0-5
Placocista spinosa	11.5 0-29	0.6 0-2	0.3 0-1	1.8 0-7
Euglypha tuberculata	3.0 0-7	16.0 3-17	6.2 2-11	18.6 18-20
Difflugia pristis	0.8 0-2	12.7 2-28	1.2 0-3	11.8 0-41
Difflugia rubescens	41.6 27-72	3.3 0-13		
Assulina muscorum	1.2 0-4	13.8 4-20	16.4 5-22	22.1 18-30
Corythion-Trinema type	8.3 2-18	13.2 1-28	12.8 1-27	5.3 2-12
Nebela militaris		5.3 0-20	10.6 0-18	0.2 0-1
Bullinularia indica		2.1 0-4	0.7 0-2	2.6 0-5

Wet (arrow upward) ... Dry (arrow downward)

Table 6.12e: Summary of testate amoebae data from dipwell X8. See Table 6.12a for more details.

6.3.5 Multivariate analysis

Canonical correspondence analysis was carried out on concentration data to see if the patterns suggested by the subjective analyses of species composition were statistically significant. All samples were analysed together in the first instance. The CCAs for this are shown in Figures 6.8a-d. Figure 6.8a shows the samples only, categorised by sampling surface, while Figure 6.8b is the same ordination but this time, categorised by dipwell. Figures 6.8c and 6.8d are ordination diagrams of environmental variables and testate

amoebae species respectively. Each of these CCA plots is shown separately for clarity of presentation. In general, there is much more coherence between sampling surface than dipwell. Samples from surface 1 (ditch) show particularly strong separation from other surfaces. The other three surfaces all overlap but surfaces 2 and 3 are slightly more grouped. Samples from surfaces 3 (waste) and 4 (fey) overlap much more than groups 1 and 2. This reflects the similarities in vegetation on these two surfaces. Dipwells are not clearly defined, although samples from X2 are most closely clustered. This reflects the findings from the subjective analyses.

The environmental variables are separated into sample material (nominal variables shown as stars) and plant species identified at the sample locations. Hydrological data are not used in these analyses; as there was only one measurement for each dipwell, depth to water table would only differentiate between dipwell locations and would have overpowered other unmeasured ecological factors. A Monte Carlo test on the dataset gave a p value of 0.034 for the first axis. Axis one of the graph is most strongly related to surface material and is also the only axis with an eigenvalue greater than 0.2 (actual value 0.248), explaining the most variation of all axes (23.6%). Samples are grouped according to both the vegetation found during the sampling visits and the sampling material.

The species plot (Figure 6.8d) showed that samples from surface 1 (ditch) are strongly associated with occurrences of *Nebela griseloa* and *Difflugia rubescens*. These taxa had to be excluded from the plot so that the other taxa could be clearly seen but were positioned at coordinates -0.1 (axis 1) × 4.8 (axis 2) and 2.1 (axis 1) × 3.8 (axis 2) respectively. Surface 2 (cut surface) is associated with *Bullinularia indica, Nebela flabellulum* and

Euglypha tuberculata. Surface 3 (waste) is most strongly aligned with *Nebela tincta,*

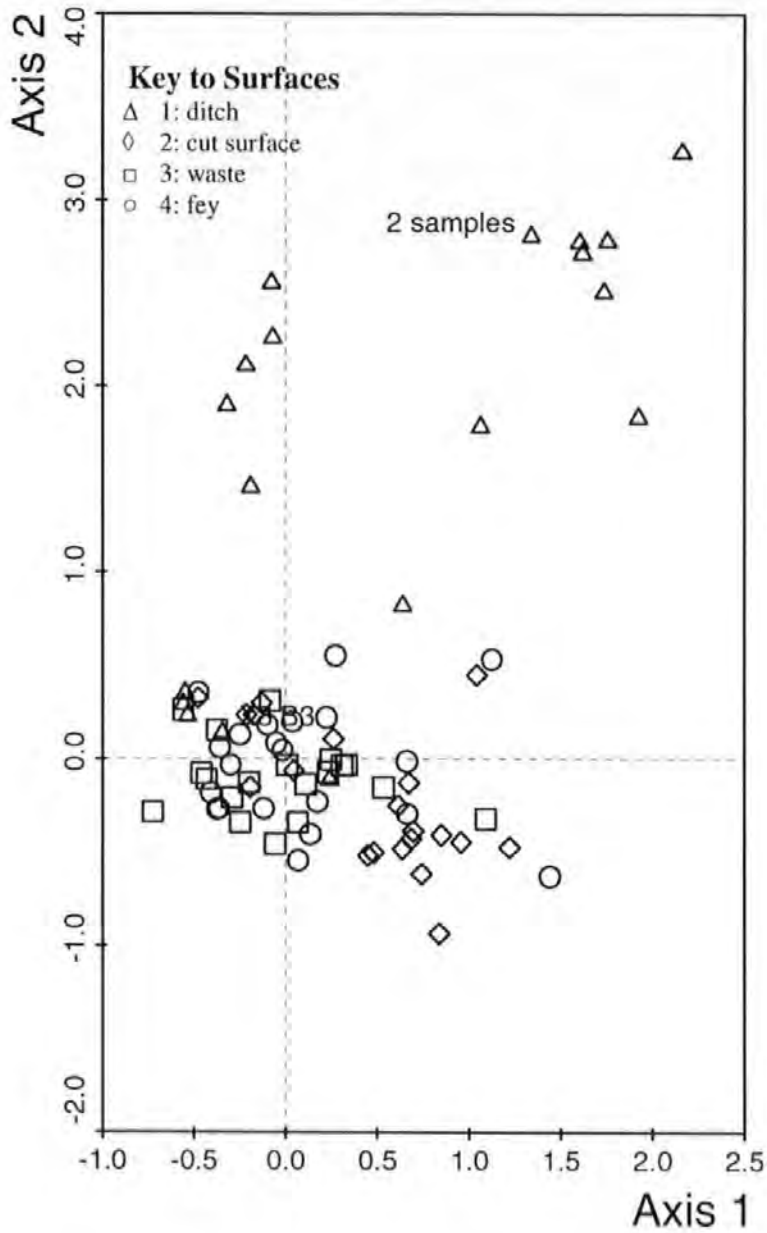*Nebela militaris, Heleopera petricola* and *Cyclopyxis arcelloides.*

Figure 6.8a: CCA of testate amoebae concentrations for all samples. Simple ordination of samples categorised by sampling surface.
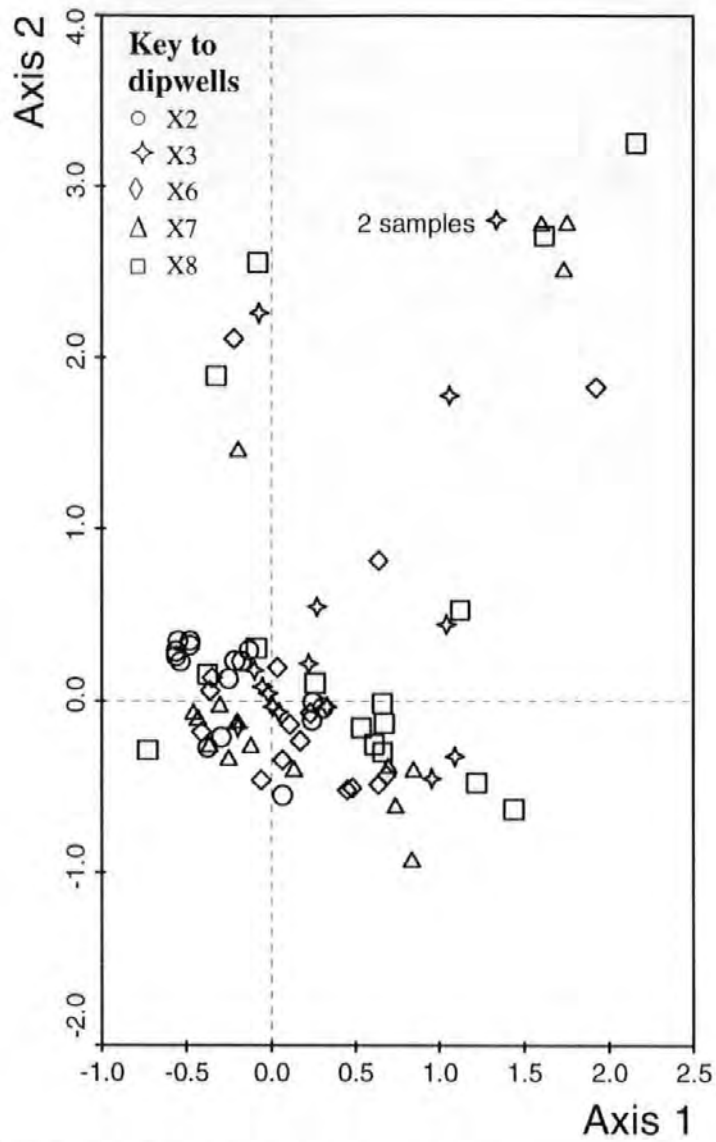
Figure 6.8b: CCA of testate amoebae concentrations for all samples. Simple ordination of samples categorised by dipwell
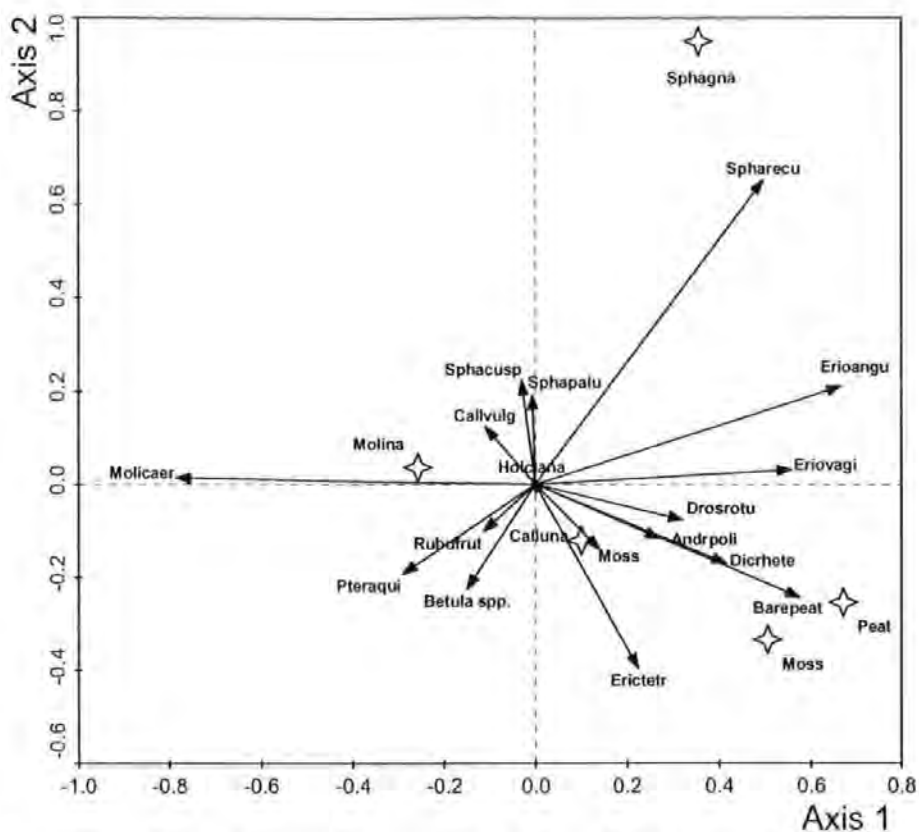
Figure 6.8c: CCA of testate amoebae concentrations for all samples. Ordination plot of environmental variables. Full explanation of species abbreviations in Table 6.8. Stars indicate nominal variables - sample material: Peat, Moss, *Calluna*, *Molinia* and *Sphagna*.

Andrpoli	*Andromeda polifolia*
Barepeat	Bare peat
Betula spp.	*Betula* spp.
Callvulg	*Calluna vulgaris*
Drosrotu	*Drosera rotundifolia*
Dicrhete	*Dicranum heteromalla*
Erictetr	*Erica tetralix*
Erioangu	*Eriophorum angustifolium*
Eriovagi	*Eriophorum vaginatum*
Holclana	*Holcus lanatus*
Molicaer	*Molinia caerulea*
Moss	Moss
Pteraqui	*Pteridium aquilinum*
Rubufrut	*Rubus fruticosis*
Sphacusp	*Sphagnum cuspidatum*
Sphapalu	*Sphagnum palustre*
Spharecu	*Sphagnum recurvum*

Table 6.13: Plant species abbreviations used in ordination diagrams.
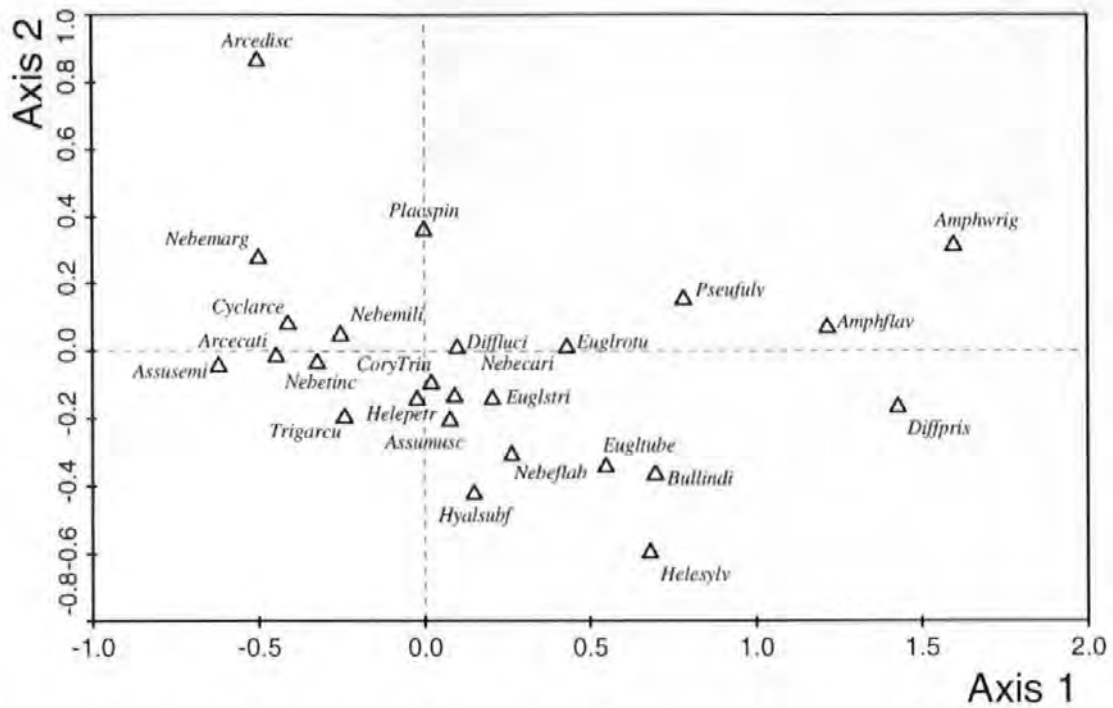
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Figure 6.8d: CCA of all samples. Simple ordination diagram of testate amoebae species. Full explanation of species abbreviations in Table 6.9.

Amphflav	*Amphitrema flavum*
Amphwrig	*Amphitrema wrightanium*
Arcecati	*Arcella catinus* type
Arcedisc	*Arcella discoides*
Assumusc	*Assulina muscorum*
Assusemi	*Assulina semimulum*
Bullindi	*Bullinularia indica*
CoryTrin	*Corythion – Trinema* type
Cyclarce	*Cyclopyxis arcelloides*
Diffluci	*Difflugia lucida*
Diffpris	*Difflugia pristis*
Euglrotu	*Euglypha rotunda*
Euglstri	*Euglypha strigosa*
Eugltube	*Euglypha tuberculata*
Helepetr	*Heleopera petricola*
Helesylv	*Heleopera sylvatica*
Hyalsubf	*Hyalosphenia subflava*
Nebecari	*Nebela carinata*
Nebeflab	*Nebela flabellulum*
Nebemarg	*Nebela marginata*
Nebemili	*Nebela militaris*
Nebetinc	*Nebela tincta*
Placspin	*Placocista spinosa*
Psuefulv	*Pseudodifflugia fulva*
Trigarcu	*Trigonopyxis arcula*

Table 6.14: Testate amoebae species abbreviations used in ordination diagrams.

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To examine whether there were any differences between samples from July and October, the above analyses were repeated for the individual months. The ordination results are shown in Figures 6.9a-d and Figures 6.10a-d respectively. The findings are similar to those for the entire dataset. Dipwell X2 is most distinctive, despite the fact that it lies in the same area as other samples; all of the members of this group are clustered together in July. There is little cohesion between dipwell locations in October.

Important testate amoebae species in July samples from surface 1 (ditch) were *Arcella discoides, Difflugia lucida, Difflugia rubescens, Euglypha strigosa, Placocista spinosa* and *Heleopera sphagni*. For dipwell X2, the important testate amoebae species were *Psuedodifflugia fulva, Heleopera petricola, Nebela flabellulum, N. militaris* and *N. tincta*. In October, the most important species for surface 1 were also *Arcella discoides* and *Placocista spinosa*. *Nebela tincta* was the most important testate amoebae species for surface 3 (waste).

The ordinations for separate months are showing strong alignment of sample material with axis one. In July the eigenvalue for the first axis was 0.450 (explaining 19.2% of variation), this was the only axis with a value over 0.2. A Monte Carlo test showed that the eigenvalue for this axis was statistically significant ($p = 0.002$) October's result also showed that axis one was the only axis with a prominent eigenvalue of 0.354 (explaining 27.3% of variation). The Monte Carlo test did not give a significant result for this axis ($p = 0.522$) however.
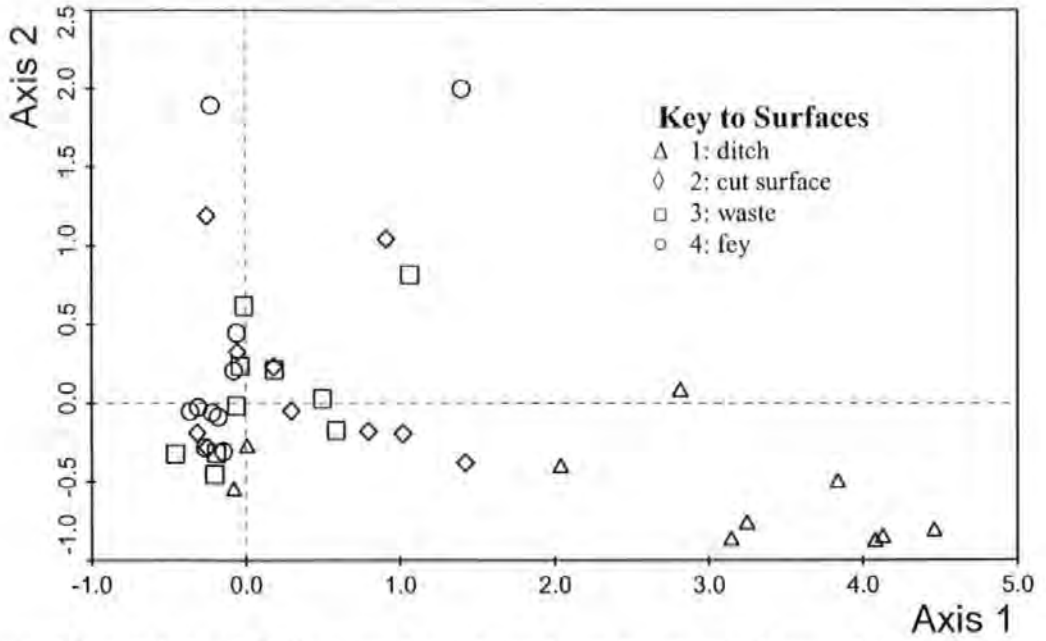
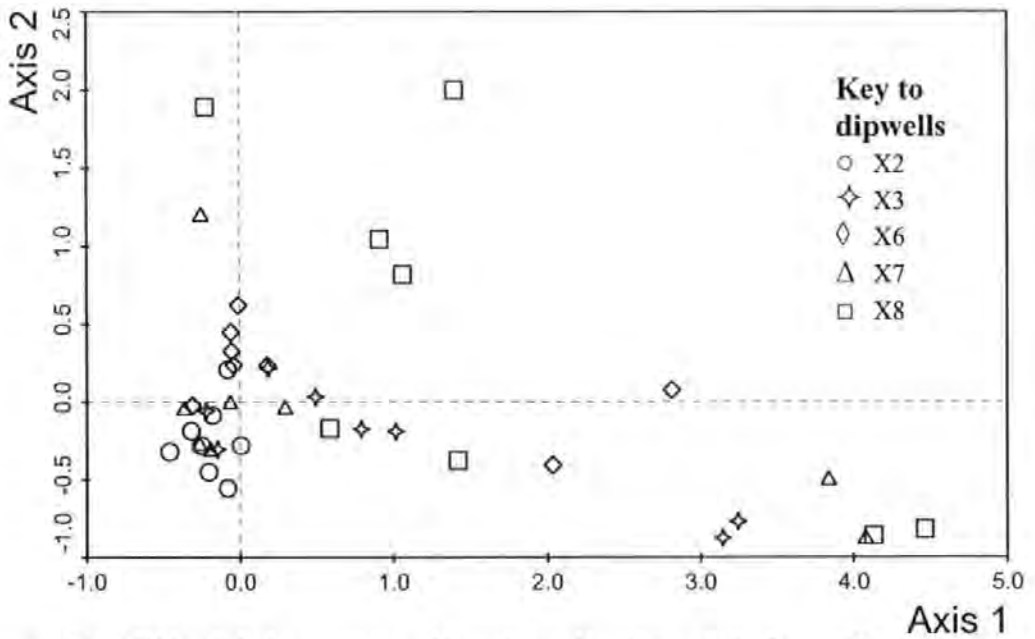**Figure 6.9a: CCA of July samples. Simple ordination of samples categorised by sampling surface.**

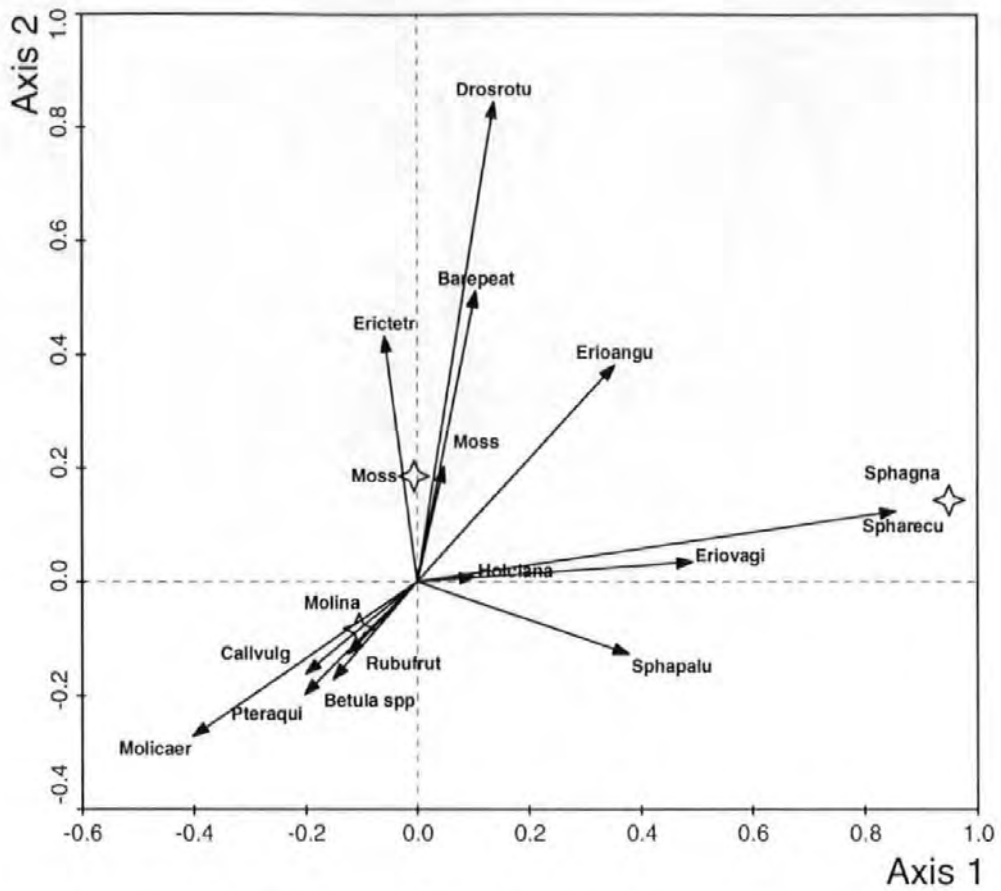**Figure 6.9b: CCA of July samples. Simple ordination plot of samples categorised by dipwell.**

Figure 6.9c: CCA for July samples. Simple plot of environmental variables (Abbreviations Table 6.13).
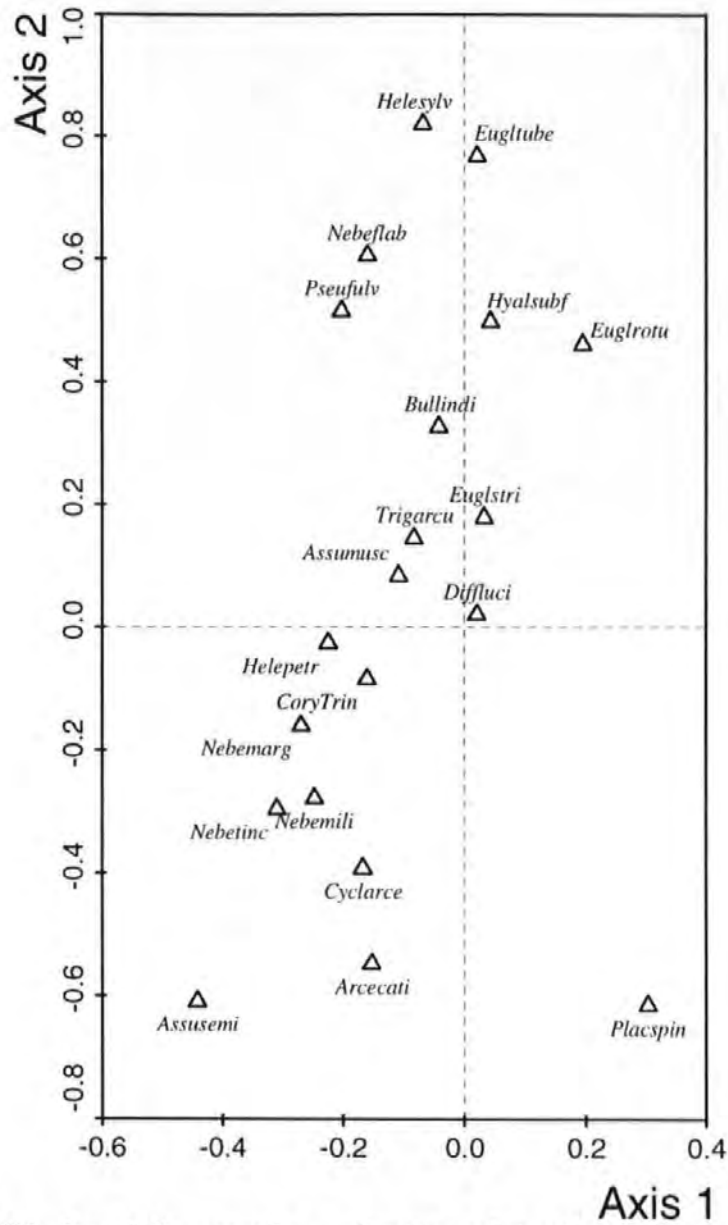
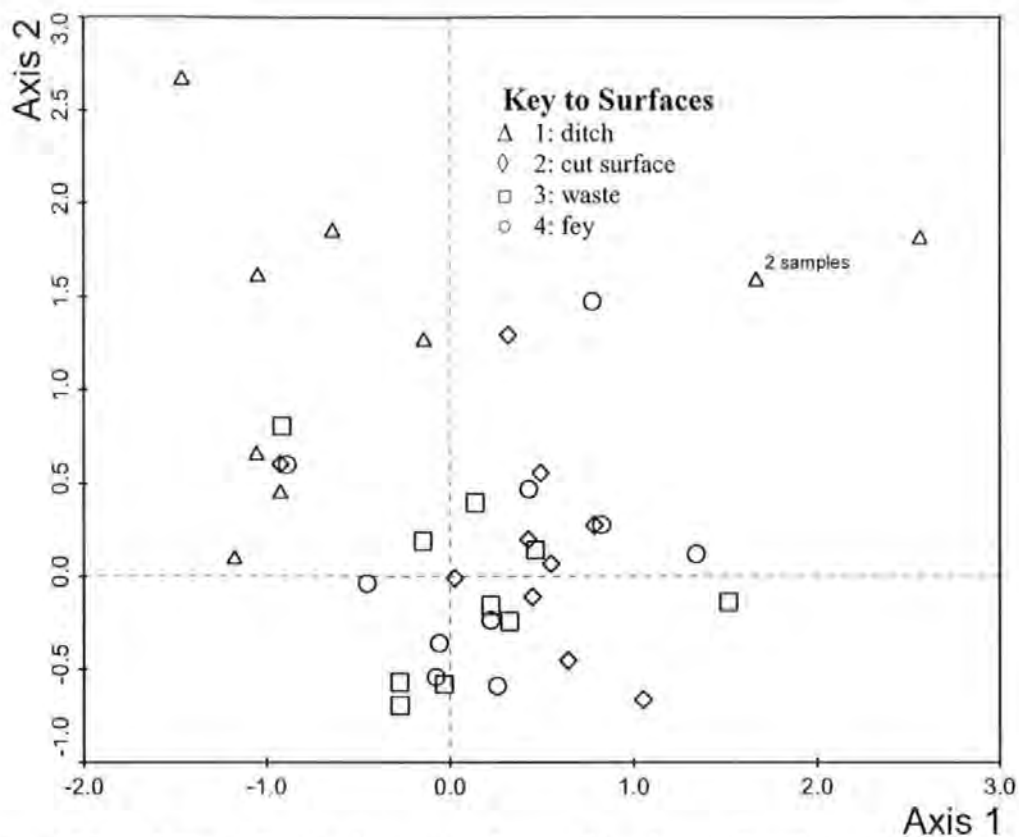**Figure 6.9d: CCA for July samples. Scatter plot of testate amoebae species. (Abbreviations Table 6.14).**

Figure 6.10a: CCA of October samples. Simple ordination plot of samples categorised by sampling surface.
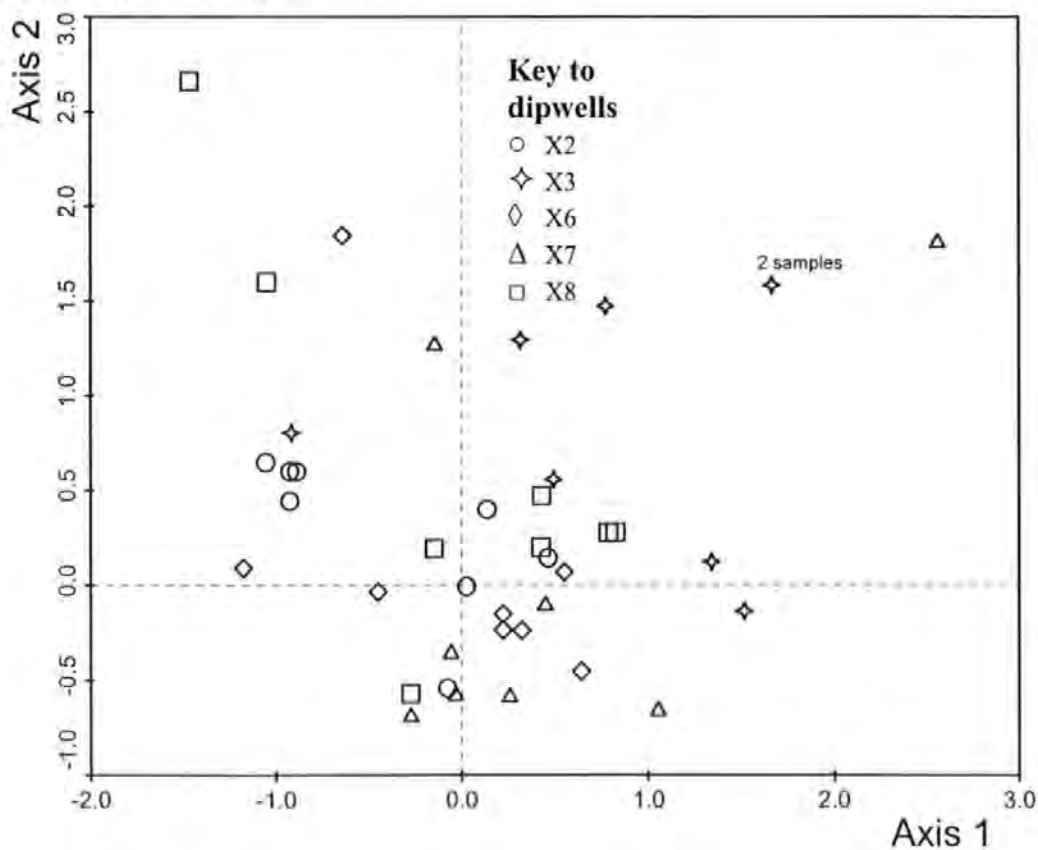

Figure 6.10b: CCA of October samples. Simple ordination plot of samples categorised by dipwell.
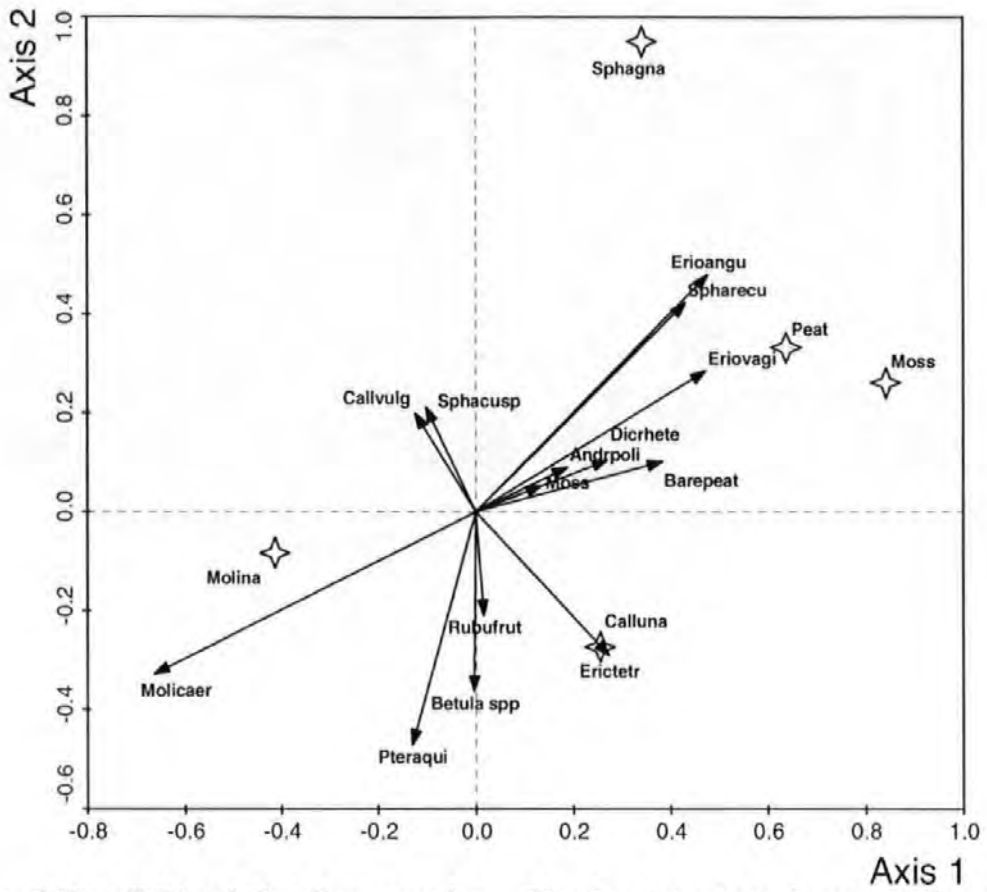
Figure 6.10c: CCA of October samples. Simple plot of environmental variables. (Abbreviations Table 6.13).
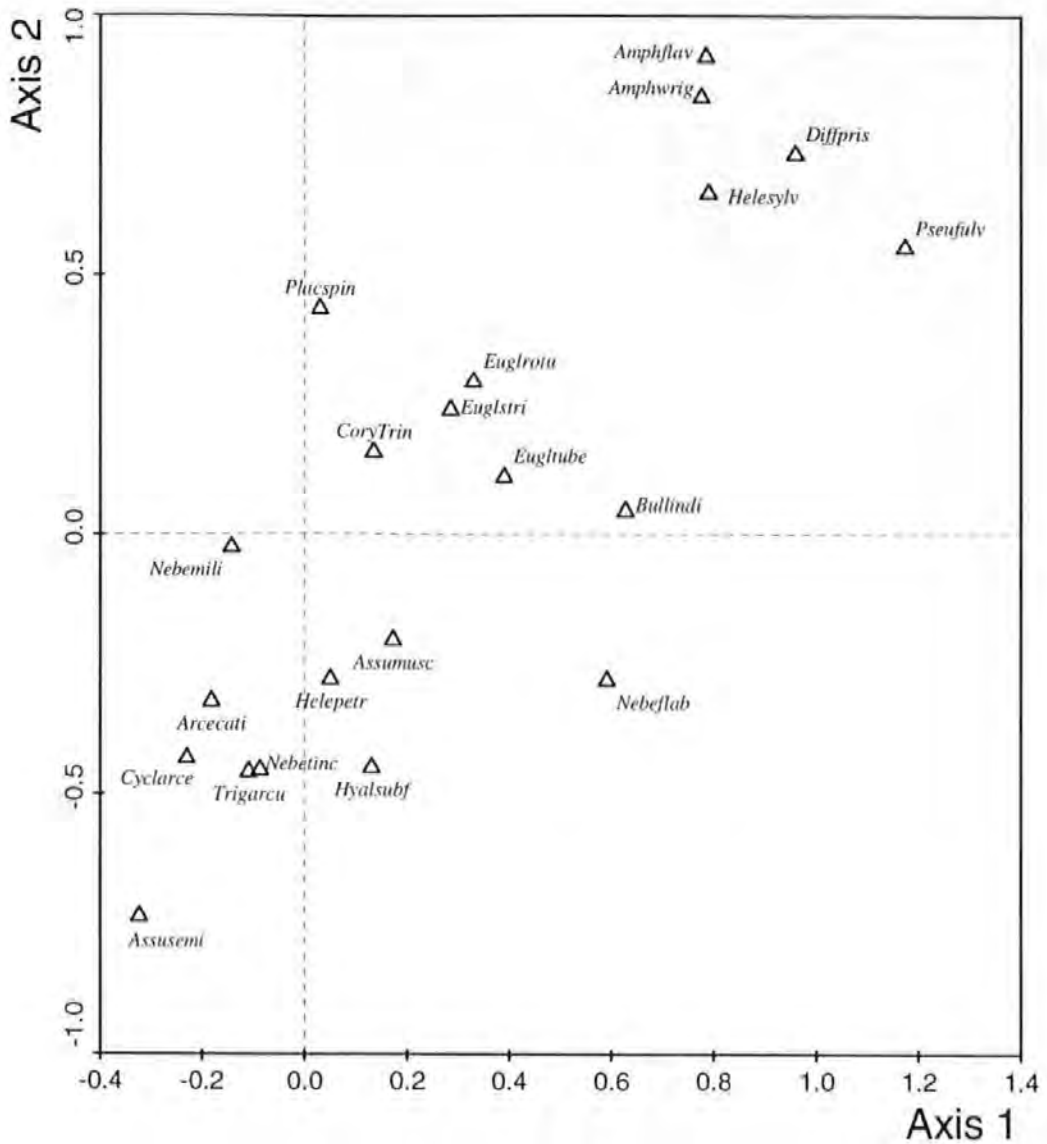
Figure 6.10d: CCA of October samples. Simple ordination of testate amoebae species. (Abbreviations Table 6.14).

Each dipwell location was analysed individually (results from July and October counts were combined). Ordination plots from dipwells X2, X6 and X7 are shown (Figures 6.11a-c). The most distinctive grouping of sample surfaces is found at dipwells X6 and X7 (Figures 6.11b and c). This confirms the species composition results. Sampling surface 1 (ditch) is at the opposite end of the graphs from the other surfaces at all of these dipwells. Dipwell X2 (Figures 6.11a) had less cohesion between sample surfaces. The secondary sample locations (A, B, C and D) were more similar than sample surfaces. This may reflect the fact that there was less surface variability at this location.
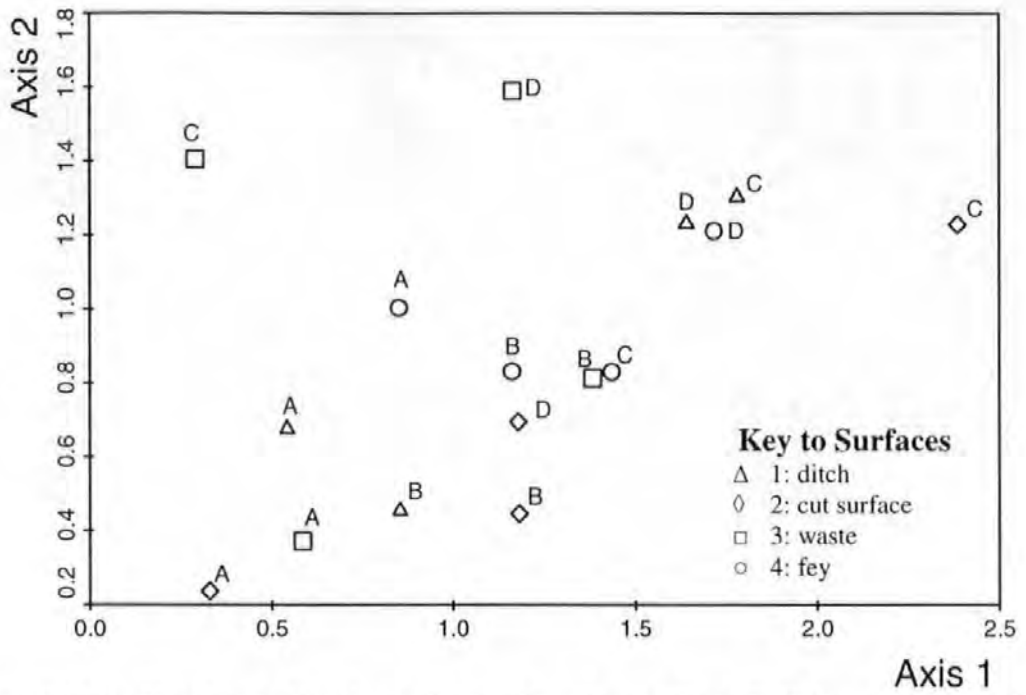
Figure 6.11a: Simple ordination of samples from dipwell X2. Letters indicate secondary location and month sample was collected (see Figure 6.4)
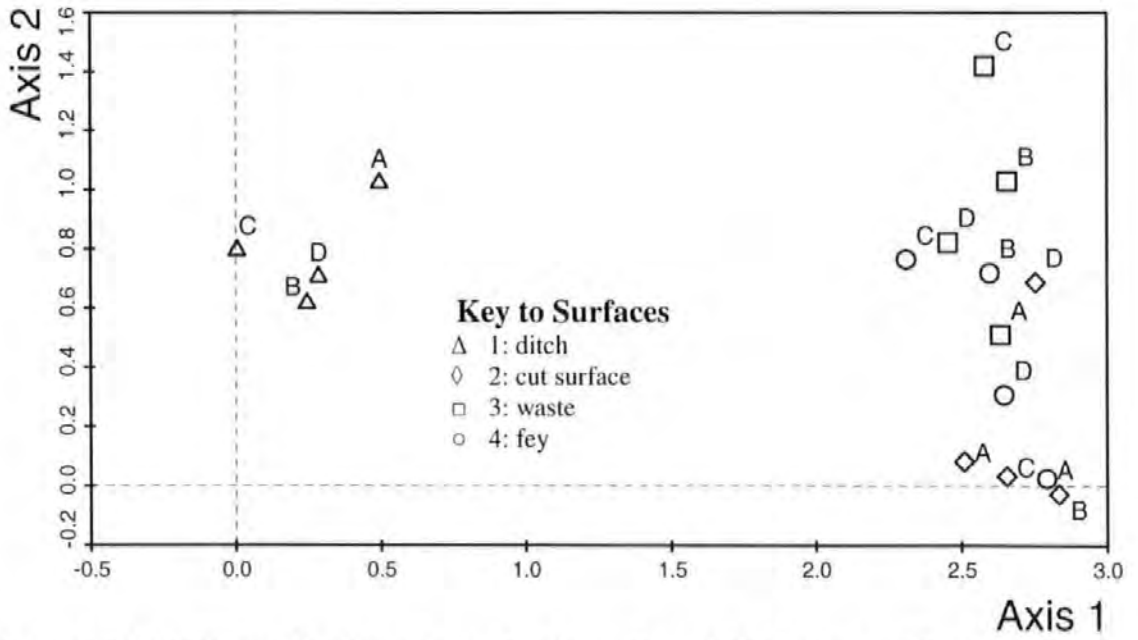

Figure 6.11b: DCA ordination of samples from dipwell X6 letters as Figure 6.11a)
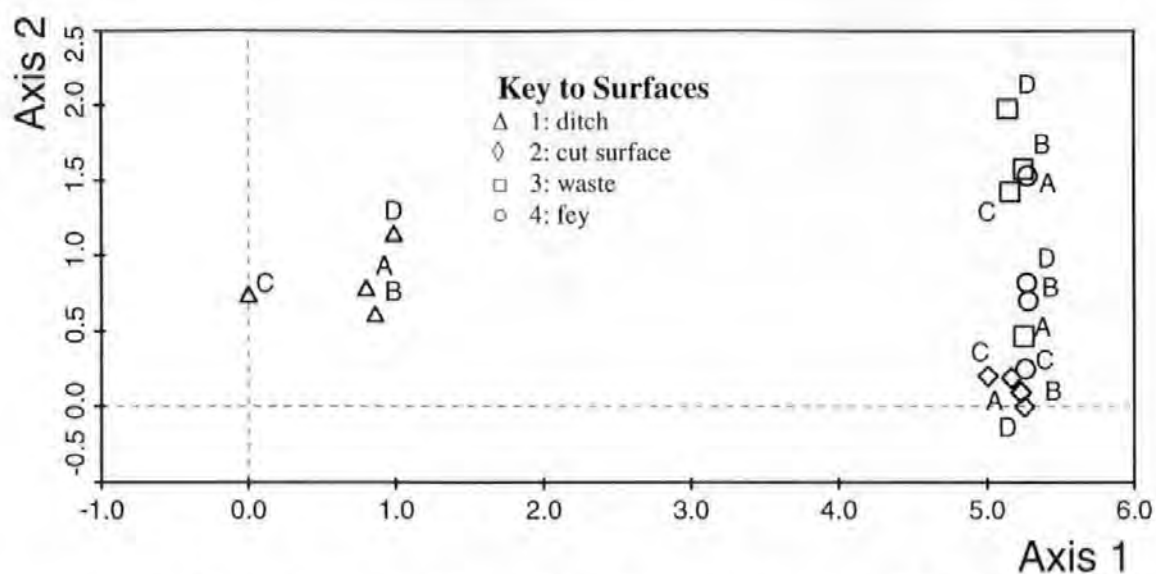
Figure 6.11c: DCA ordination of samples from dipwell X7 (letters as Figure 6.11a)

6.4 Discussion

This section of the thesis is devoted to the response of testate amoebae to hydrological and microtopographical conditions on a restored cutover mire. The testate amoebae populations within two management units on Fenn's and Whixall Mosses were studied to fulfil objective 3 of the PhD. Further understanding of testate amoebae responses to hydrological conditions (objective 5) has also been developed. The area of the bog studied was harvested in the early 1990s by Croxdens Horticultural Products (CHP). The entire growing surface had been removed through mechanical peat extraction. Since management control of the site passed to English Nature and the Countryside Council for Wales (EN & CCW), restoration work has been carried out across the bog. Ditches were blocked in the area studied for this chapter between 1991 and 1993. Restoration of severely degraded mires such as this one is highly experimental and long-term success is uncertain (Joy & Pullin, 1999) although experimental work in Canada has suggested that *Sphagnum* regeneration can occur within 5 years given the correct conditions (Campeau & Rochefort, 1996).

6.4.1 Hydrology and vegetation

Although not the focus of this study, an understanding of the hydrological conditions at sampling points and the vegetation are important, as any variability in testate amoebae community composition should be set against that background. The first stage in regeneration of post-harvested mires is to raise the water table and achieve hydrological stability in order to mimic conditions of an undamaged mire (La Rose *et al.*, 1997). This then provides ecological conditions for *Sphagnum* recolonisation. Results of hydrological surveys from 1993 to 2000 at Fenn's and Whixall Mosses show that water level variability has been subdued in management compartment 1.9 (dipwells X2-4) but there has been no significant increase in winter water levels (Gilman, 2000). Management compartment 3.9 (dipwells X5-10) has seen an increase in winter water levels since damming in 1993/94 but, as an increase was found across the network of dipwells regardless of damming conditions, it has been suggested that this is the result of climatic conditions (Gilman, 2000). The lack of major hydrological improvements suggests that regeneration at this site is a complex process. The areas sampled were close to the edge of the site, which could indicate that the management activities in adjacent areas are hampering rising water tables.

Statistical comparisons of hydrological data from 2002 and 2003 showed that there were significant differences between dipwell locations. As testate amoebae respond to water table depth, significant differences between communities at different dipwell locations would be expected. Despite the above comments on the lack of improvement in water table, there was only one dipwell (X7) where water levels fell more than 40cm below the surface during 2002 and 2003. This is significant, as 40cm is regarded as the lowest point to which the water table can fall and still support *Sphagnum* growth (Schouwenaars, 1993).

As dipwells are located on the driest part of the cutting surface transect, hydrological conditions should be suitable for *Sphagnum* growth across most of this area.

Surface type at Fenn's and Whixall Moss has been studied to see whether testate amoebae communities respond to microtopographical conditions. Peat harvesting has greatly modified the site and the resulting surface topography is distinctive. Previous studies of peatland regeneration have shown that microtopography is very important in reestablishment of *Sphagna* with regeneration occurring more quickly in hollows, where there is some protection from desiccation (Ferland & Rochefort, 1997). The vegetation study from Fenn's and Whixall concurs with this; *Sphagnum* cover is higher in the ditch areas. Another study has shown that pioneer species such as *Eriophorum vaginatum* can improve survival rates for *Sphagnum* (Grosvernier *et al.*, 1995). The presence of *Eriophorum* spp. in many of the wetter areas should therefore be viewed as a positive sign in terms of *Sphagnum* re-establishment.

Vegetation cover is being strongly influenced by the cutting pattern. The micro-topographical variability has left some areas perched above the water table, whilst other areas are fully submerged. This has resulted in very different ecological conditions across small areas. At most locations, the ditch (surface 1) resembles wet hollow or pool vegetation types on a mire. Surfaces 3 and 4 – the waste material and the 'fey' are more like damp grassland and surface 2, the cut surface, often has bare peat remaining. *Sphagnum* is beginning to regenerate on surface 2 at most locations, whilst the presence of other mosses is greater than for any other surface and *Molinia*-dominated communities are less frequent.

6.4.2 Testate amoebae communities

There are large differences in testate amoebae communities within dipwell locations, with the drier surfaces having testate amoebae species indicative of drier conditions and the ditches having species indicative of bog pools. This reinforces the finding that restoration at the site is working in a patchy manner. The species tables show very distinctive divisions in testate amoebae communities, with the greatest differences being between surface 1 (ditch) and the other three surfaces. This is reflective of the distinctive hydrological and vegetation differences. Surface 1 is most similar to a bog pool environment and the testate amoebae communities reflect this. Analyses of testate amoebae communities in the UK, Finland, Canada and New Zealand have consistently found that *Arcella discoides*, found in highest numbers in the ditches, is representative of very wet conditions (Charman *et al.*, 2000). The analyses here indicate that, even where a site has been extensively damaged, the testate amoebae communities are very similar to those of undamaged sites with similar hydrology.

Correspondence analysis was used to establish which variables had the most impact on testate amoebae communities. The results agreed with the conclusion drawn from Tables 6.7a-e that surface is one of the strongest factors affecting testate amoebae communities. There is a large amount of contrast between the wetter pool like surface 1 (ditch) and drier surfaces. Surface 2 (cut surface) is slightly separated from other surfaces in the ordination diagrams. Surfaces 3 and 4 (waste and 'fey') showed most overlap reflecting the very similar vegetation communities.

Surface humidity and temperature are other ecological factors which may be important to testate amoebae communities at a site such as Fenn's and Whixall. The communities in the

ditches were representative of bog pool communities. The cut surface adjacent to the ditch was much less consistent in terms of testate amoebae community composition. Tables 6.7a-e show that at some locations, these communities were more like those in the ditches and at others, they resembled the waste and fey communities. At some dipwells these surfaces had high percentages of bare peat. These locations are likely to have much more variable humidity levels (Ferland & Rochefort, 1997; Campeau *et al.*, 2004) and greater fluctuations in surface humidity (Buttler *et al.*, 1998).

An interesting finding from the testate amoebae analysis was the large amount of variability in concentrations depending on the source sample material. Beyens & Chardez (1984), Tolonen (1986) and Louisier (1982) have all found that availability of test building materials affects reproduction rates in testate amoebae. The highest concentrations at Fenn's and Whixall were found in the litter of vascular plants (*Molinia caerulea* and Ericaceous litter). It could be that these materials provide more test building materials and food sources for testate amoebae through higher rates of decay and mineralization than mosses. This also suggests that higher concentrations of testate amoebae are not necessarily an indicator of a 'healthy' peatland and that species composition is more important than raw numbers if testate amoebae are to be used as bioindicators. Beyens (1984), found that there was a strong vertical distribution of testate amoebae in *Sphagnum*; he attributed the lower numbers found in the upper layers of moss to the availability of food and particles for test building. Balik (1996a and 1986b) found, in contrast to the results here, that biomass of testate amoebae decreased from moss carpet to soil. This could be because he was looking at testate amoebae within the soil horizon, rather than in the litter layers. He also found changes in dominant species along a moss - soil transect

that echo the changes in species composition found here between the ditches and drier, *Molinia*-dominated higher ground.

6.4.3 Methodological issues

It is clear that there are other factors influencing differences in testate amoebae communities between samples aside from those accounted for in the correspondence analyses. The first factor is the pre-restoration conditions, which were assumed to be the same at all locations. It is highly likely that this was not the case; however, in the absence of a baseline survey of pre-restoration testate amoebae communities, this assumption had to be made. The major difference seems to be between management compartments. Compartment 1.9 is closer to the edge of the bog which may explain the slightly drier conditions. The compartments are separated by a ditch between dipwells X4 and X5. This probably means that hydrological conditions are mutually exclusive between management compartments, i.e. changes in one compartment do not necessarily affect the other compartment.

There was no information on moisture content of the samples and this is a second confounding factor. This was because of concerns over quantities of material removed during the processing being uneven due to different sample materials. This information may have helped to explain some of the variation between samples although, given that it was not a major factor at Flanders Moss, it is unlikely. Hydrological data were based only on depth to water table at the dipwells. The dipwell was located on the same surface at each point and, given the topography, hydrological conditions should vary by comparable amounts on each surface. Hydrological measurements at each point were deemed unnecessary, would have proved difficult to obtain, and, given the anomalous readings

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taken on sampling days, may not have been particularly representative. Long-term records of hydrological differences between surfaces would be more useful but are not available.

Another environmental factor, which may have a strong influence on variability in testate amoebae communities at Flanders Moss, is surface humidity. Some estimate of humidity was possible at Flanders Moss by using the presence or absence of tree remains as a guide. This was not possible at Fenn's and Whixall but, given the important role it plays in re-establishment of *Sphagnum* on bare peat surfaces (Rochefort et al., 1995; Grosvernier *et al.*, 1995; Campeau & Rochefort, 1996; LaRose *et al.*, 1997), humidity may be even more important at this site. Surface temperature has not been measured at this site and, given the highly variable vegetation cover, surface temperatures could vary greatly between surfaces. This could have a direct bearing on both *Sphagnum* reestablishment (Buttler *et al.*, 1998) and testate amoebae communities.

6.5 Conclusions

It is clear that there are ecological differences between the cutting surfaces at Fenn's and Whixall Mosses. The primary difference is vegetation cover. There is a range from wet heath on the waste surfaces (3) to bog pool in the ditches (surface 1). Testate amoebae populations were influenced by several factors including surface, depth to water table and sample material. There were obviously further factors for which data were not available.

Surface material had a major influence on concentrations of testate amoebae. This is particularly important, as it indicates that high concentrations do not necessarily indicate optimal ecological conditions for peatlands undergoing restoration and may contribute towards the development of a biological monitoring tool. It also highlights the importance

of keeping records of sample material to ensure that observed differences between samples are not solely the result of different source material. The differences in testate amoebae concentrations between samples from *Sphagnum*, other mosses and surface peat and samples from *Molinia* litter and Ericaceous plant litter were large enough to produce some confusion in the early stages of analysis.

The work here has shown that concentrations of testate amoebae can be used effectively in comparisons of restored surfaces. High counts of live testate amoebae can be achieved when taken from a variety of surfaces and substrates and using Rose Bengal dye allows living testates to be clearly distinguished from dead individuals.

Restoration at Fenn's and Whixall is working in a patchy manner. *Sphagnum* growth is largely confined to the ditches and the species occurring here are more representative of fen communities than ombrotrophic bog. However, the large amount of *Eriophorum angustifolium* and *Eriophorum vaginatum* colonising the wetter spots is a positive sign.

In common with Flanders Moss, surface humidity may be an important factor influencing testate amoebae patterns at Fenn's and Whixall. It may be advisable to measure this in future research into testate amoebae on restored surfaces.

Possible solutions to the lack of bog vegetation on the higher ground surfaces would be to further raise the water levels or to mechanically spread the peat from these areas. Either of these solutions is likely to cause opposition among both local communities and conservationists. There are already concerns about increased flooding of homes, difficulties with access because of flooded roads and the loss of productive agricultural

land as a result of raising of water levels on the bog, any further increases are likely to be difficult to negotiate. Any physical modification of the surface would have to be carefully considered. The size of the harvested areas is such that it would involve a large amount of work to have an impact on the site. Hydrological flow across the site would be modified possibly increasing problems with public perceptions of the conservation work. The balance between restoring the site and maintaining a positive relationship with the surrounding population is a very difficult one.

Most importantly, it should be remembered that restoring a site such as Fenn's and Whixall is a major challenge. Any progression to peatland conditions should be viewed positively.

CHAPTER SEVEN: General discussion

7.0 Introduction

Peatlands are rare habitats which have been extensively damaged and continually threatened by human activity (Williams, 1990). Over the past 20 years, the ecological value of these sites has been recognised and peatland restoration has become a nature conservation priority (e.g. Wheeler & Shaw, 1995; Foss, 1995; Brooks & Stoneman, 1997a). Restoration work is still largely experimental, with the main focus in the UK being to raise water levels through ditch blocking (Brooks & Stoneman, 1997a; Wheeler & Shaw, 1995). Experimental work in Canada and Europe has gone further by attempting to restore active peat growth on cutover bogs with techniques ranging from reintroduction of *Sphagnum* diaspores (Ferland & Rochefort, 1997; Grosvernier *et al.* 1997), to manipulation of surface conditions (Grosvernier *et al.*, 1995; Campeau & Rochefort, 1996; LaRose *et al.*, 1997).

Testate amoebae have been used in palaeoecology as proxies for hydrological condition in studies in western Europe, (eg. Tolonen, 1992, 1994; Woodland *et al.* 1998; Mitchell *et al.*, 1999) North America (Warner & Charman, 1994) and Russia (Bobrov *et al.*, 2004). Because hydrological response is a major focus in monitoring the success of peatland restoration (Brooks & Stoneman, 1997a; Wheeler & Shaw, 1995), testate amoebae analysis has potential as a biological monitoring tool (Wanner, 1999).

The first aim of this PhD was to assess the possibility of using testate amoebae as biological indicators of peatland restoration. To fulfil this aim, three peatland sites were studied as examples of the major causes of peatland damage – drainage and peripheral land use impacts at Coom Rigg Moss, afforestation at Flanders Moss and peat extraction at

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Fenn's and Whixall Mosses. The management at these sites represented the most common restoration issues in the UK – ditch blocking and tree removal. Coom Rigg Moss provided the opportunity for establishing long-term monitoring of restoration and allowed comparison of pre- and post- restoration conditions. Restoration was already underway at Fenn's and Whixall Mosses and Flanders Moss, so investigations at these sites concerned comparisons of post-restoration condition following different treatments over a much longer period of management.

7.1 Methodological Issues

In order to minimise damage to the experimental sites and to ensure that sampling did not change the nature of the monitoring plots, it was important to take the smallest samples possible. This was particularly important at Coom Rigg Moss, where sampling was carried out over a period of two and a half years. A methodological experiment was established on Coom Rigg Moss to assess how representative single stems of *Sphagnum* were of a 25cm by 25cm plot in terms of testate amoebae composition. It was also important to ensure that testate amoebae counts of around 150 individuals and a minimum of 100 could be achieved from such small samples as this is the number recommended to get an accurate representation of species composition (Woodland *et al.* 1998).

The results from chapter three showed that, testate amoebae assemblages from single stems of *Sphagnum* were generally representative of the 25 cm square plot in which they grew. The results confirmed that there was more variance between plots than within them. In multivariate analyses, outliers were generally the counts of less than 50 testate amoebae, counts between 50 and 100 individuals were well aligned with the rest of the group. There were some issues with the amount of time needed to achieve counts from such small

samples. The aim was to count at least 150 individuals counting a maximum of 2 slides; however, numbers were so low in some samples that it was necessary to count four slides to reach 100 testate amoebae. The highest counts came from the *Sphagnum magellanicum* plot and the lowest from *Sphagnum tenellum*. The general recommendation, based on the results of this sub-study, would be to take larger samples wherever possible; however, it may also be advisable to avoid sampling the finer species of *Sphagnum* when small samples are necessary.

The logic behind sampling single stems of *Sphagnum* was that continual monthly monitoring was planned at Coom Rigg Moss and if larger samples were taken, monitoring may have simply detected changes caused by the sampling process. The comments on seasonality in section 7.3 suggest that such frequent monitoring was not necessary and that the three monthly monitoring at Flanders Moss was adequate to ensure that seasonal fluctuations were accounted for. As the amount of time required to achieve a count of over 100 individuals from single stems was often prohibitively long, less frequent, larger samples would have been a more reliable way of achieving high counts. A single stem should be regarded as the bare minimum sample required and it is clearly preferable to use 2-3 stems wherever this can be done without significant damage to the monitoring location.

This sampling strategy was used at Coom Rigg Moss, and was adapted for the study at Flanders Moss. It is effective only where there is active *Sphagnum* growth over most of the bog. At Fenn's and Whixall Mosses, where there was only patch *Sphagnum* cover, major modifications were necessary. Plant litter and the upper 1cm of peat were sampled from areas where there was no *Sphagnum* growth.

7.2 Data analysis and detection of differences.

Large amounts of data are produced by these counts and there are several options for analysis. In the methodology experiments, analysis of variance (ANOVA) was useful for establishing broad patterns in data. Distinctions could be made between locations based on raw numbers of testate amoebae, concentrations, numbers of taxa and percentage live data. ANOVA continued to prove useful for establishing broad patterns in testate amoebae communities at all three experimental sites. Tables showing percentages of the major taxa at each location were made along with frequency scores. These were useful for establishing patterns in important components of the testate amoebae communities. Further analysis was necessary to assess how similar each group of samples was and to include some environmental variables.

The ecological analyses packages CANOCO (Ter Braak & Šmilauer, 1998) and PC-Ord were used to carry out multivariate analyses on data in the methodology experiments (Section 3.2.7). Generally DCAs and CCAs were more useful than cluster analysis. Concentration data were more useful than percentage data because where one taxon was overrepresented in one or more samples, percentage data were much more distorted than concentration data. Percentage live data were useful in the methodological experiments (Section 3.2.7 (b)); however, groups of co-varying taxa were not as well-defined as when percentage or concentration data were used and CCAs on percentage live data from Coom Rigg Moss (main experiment) and Flanders Moss were not particularly effective. Percentage live analyses appeared to be most useful where ecological differences between plots were small and results should have been broadly similar for each group. CCAs on concentration data from Fenn's and Whixall Mosses confirmed patterns found in the tabular analyses.

Percentage live data have been used as an indicator of population turnover. Lower percentages of live testate amoebae may indicate high die-off and higher percentages suggest that, either reproduction rates are higher than mortality rates or immigration rates are high. Both cases would indicate that ecological conditions are suitable for healthy testate amoebae populations.

The restoration experiments are from three very different sites. Each has been damaged by different land use practice, so different methods were needed for both sampling and analysis. Coom Rigg Moss had been affected by peripheral forestry activity and drainage. Some of the drains extended on to the bog and the Forestry Commission received European LIFE funding to carry out restoration work. The long-term nature of the experiment at Coom Rigg Moss meant that only small samples could be taken. The practicalities of this have been investigated in Chapter 3, where the sampling methodology was tested. Analysis at this site was multi-layered; there were comparisons of control and dammed plots, pre- and post- damming comparisons and comparisons of samples taken from different times of year. On the whole, there was little change in hydrology at Coom Rigg Moss. This was reflected in the testate amoebae response. Analysis of both percentage live data and concentration data produced broadly similar results and were equally useful.

At Flanders Moss, restoration work was established some time before sampling. The restoration here was experimental work by the Forestry Commission designed to test various tree removal techniques. The hydrological response to restoration had been very positive but was fairly uniform across the site, with little difference in water levels between the tree removal techniques or damming treatments. The differences in testate amoebae

populations were mainly identified through the percentage live data. Again, preliminary patterns were identified using analysis of variance and differences were followed up with DCAs using CANOCO (Ter Braak & Šmilauer, 1997)

At Fenn's and Whixall Moss, the site had been damaged by peat extraction and restoration work had been underway since the early 1990s, when control over the mineral extraction rights and conservation issues were passed to the Countryside Council for Wales and English Nature. Analysis here focused on testate amoebae concentrations with broad patterns established using analysis of variance but comparisons of testate amoebae community composition proved a very effective method for assessing differences between surfaces and sampling locations.

The results have shown that different elements of counts were useful for analysis at each site. Percentage live was most useful at Flanders Moss and equally as important as concentrations at Coom Rigg. At Fenn's and Whixall it was not possible to separate recently dead tests from fossils because the peat extraction process had exposed old layers of peat.

When planning testate amoebae monitoring on restored peatlands, it is important to identify the potential analysis problems and adapt the methodology accordingly. Without early separation of live and dead tests, much of the analyses would not have worked. If staining had not been used at Fenn's and Whixall Mosses, it would not have been possible to build a true picture of the modern testate amoebae communities and analyses would not have been meaningful in a modern context.

7.3 Seasonality

There is much uncertainty about seasonality of testate amoebae populations in peatland environments. Heal (1964) suggested that peatland populations peaked between May and July consistent with subsequent research from lake environments where numbers were positively correlated with temperature and highest numbers were found in July (Hunt & Ming Chein 1983). Recent research into the ecological preferences of testate amoebae has focused mainly on hydrological associations with some insight into chemistry, as it has largely been carried out as background to palaeoenvironmental reconstructions (Woodland, 1996; Hendon, 1998; Booth, 2002; Schnitzen *et al.*, 2003; Wilmhurst *et al.*, 2003; Vincke *et al.*, 2004). There is very little recent literature on seasonal population dynamics. However Robson *et al.* (2005) noted that there was little seasonal varibility in *Assulina muscorum* populations in Tierra del Fuego despite the differences in UV-B radiation.

The main experiment at Coom Rigg allowed seasonal variability to be investigated. There were no statistically significant patterns within these samples. A plot of logged concentrations over time (Figure 7.1) showed that counts were lower in December and higher in the June and July samples, agreeing to some extent with the research into lakes (Hunt & Ming Chein 1983).
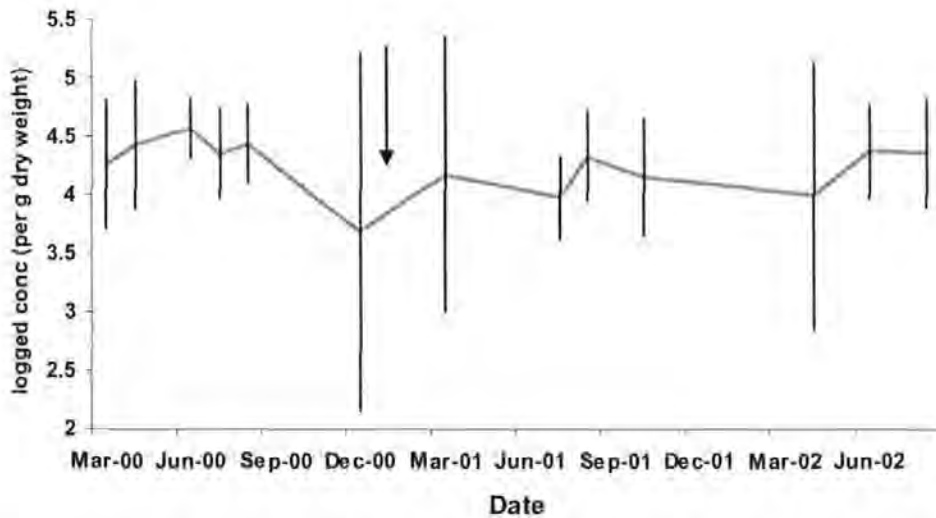
Figure 7.1: **Coom Rigg Moss – Logged concentrations of testate amoebae plotted over time – mean values for each month, with standard deviations (error bars). Arrow indicates approximate time of damming.**
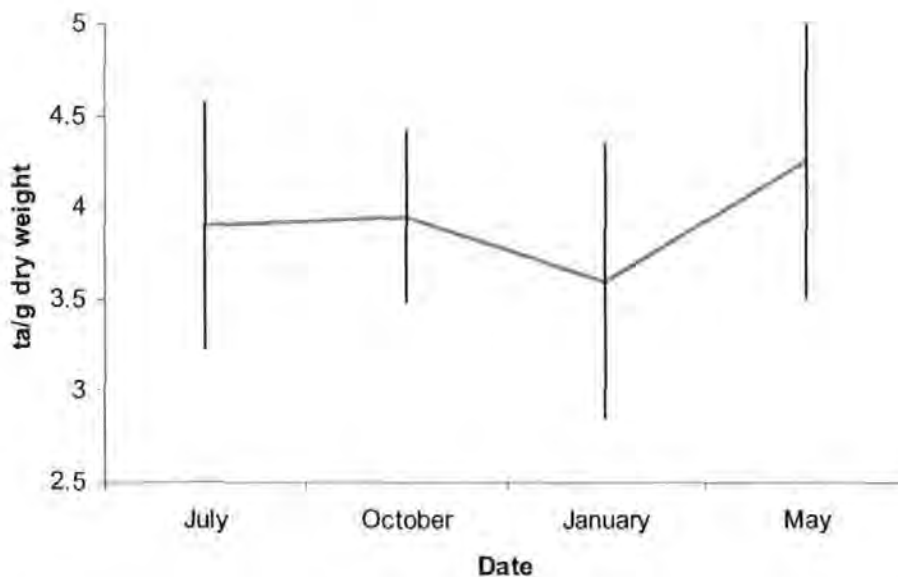
Figure 7.2: Flanders Moss – Mean logged concentrations of testate amoebae plotted over time with error bars showing standard deviations.

Plots of concentrations from Flanders Moss showed a similar pattern with lowest numbers in January and highest in May (Figure 7.2). This pattern is more pronounced in samples from whole tree removal (W) and harvested (H) plots (Figure 7.3), with much less variability in the samples from the fell to waste (F) plots, possibly as a result of the

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mulching effect of the tree remains. Where entire trees have been left *in situ*, most of the ground area of the plots is provided with a canopy of tree waste which could be helping to maintain a much more even surface temperature and protecting the *Sphagnum* from frost. Locations without the mulching effect may have greater variability in conditions in the winter months, when the groundwater and plants are frequently frozen.
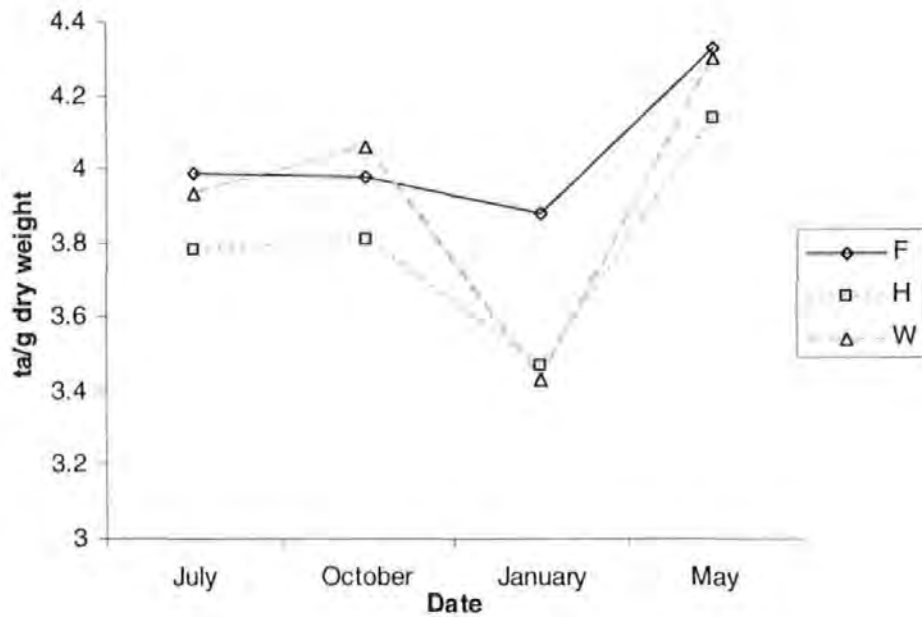

Figure 7.3: Flanders Moss: Logged concentrations of testate amoebae plotted over time by tree removal treatment. (Letters refer to forest removal treatment F = fell to waste, H = harvested, W = whole tree removal, dammed and undammed samples have been combined) Error bars omitted for clarity S.D. close to that in figure 7.2.

Numbers could also have been affected by the smaller sample sizes, the result of a Spearman's Rank Correlation of total number of testate amoebae counted in each sample with sample weight was statistically significant, the data were positively correlated ($p = 0.001$, $r_s = 0.484$). Sampling month had a statistically significant impact on weights (Kruskal-Wallis, $p = 0.001$), the months with the lowest values were July and January. As concentrations from July were not as low as those from January, this suggests that there were further variables affecting numbers of testates in samples. The impact of stem length

on numbers counted was also tested using a Spearman's Rank Correlation. Again the ranks were positively correlated with a correlation coefficient of 0.837 (p = 0.001). To establish whether these correlations were cause and effect relationships, regression analyses were carried out on the ranks. Both factors were statistically significant, with R-Sq (adjusted) values of 22.6% for weights and 61.7% for stem length. As these two factors are strongly positively correlated, (p = 0.001, r_s = 0.417), it is likely that sample weight is influenced by stem length and therefore its relevance is a product of its relationship with stem length. It may not be an important factor on its own.

Figure 7.4 shows the mean number of taxa found at Coom Rigg Moss by month and the total number found each month. There is no clear pattern in this plot, highest total numbers of taxa were found in samples from August 2000, July 2001 and October 2001. It may not be appropriate to use this as an example of seasonality, as the damming in early 2001 may have affected seasonal variability in the subsequent months. However there is no evidence of significant change at this point in time.
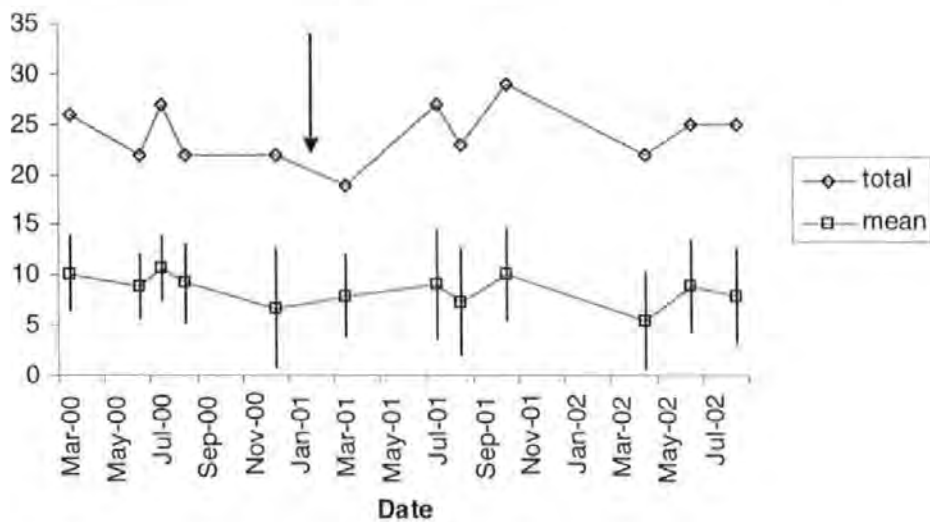

Figure 7.4: Numbers of taxa at Coom Rigg Moss: mean per sample by month with error bars showing standard deviations and total by month. Arrow marks approximate time of damming.

Figure 7.5 is a plot of mean numbers of taxa found by treatment and totals per treatment at Flanders Moss. The highest mean numbers were found in the May samples in each treatment and the lowest in January. This may be an indication of seasonal variation in taxa or it may be related to the size of samples. A correlation of stem lengths with numbers of taxa was carried out to establish this. A Spearman's Rank correlation was carried out as neither dataset was normally distributed. The datasets were positively correlated with an r_s value of 0.206 (p = 0.044). Stem length was obviously affecting taxa numbers but the relatively low r_s value indicates that there are other significant factors involved. It may be that some taxa are migrating further down into the moss layer; however, studies on testate amoebae distribution on *Sphagnum* found that most living individuals were in the upper 5cm (Schönborn, 1963). A study on nitrogen deposition and *Sphagnum* recolonisation in peatlands also concluded that species richness decreases with depth (Mitchell & Gilbert, 2004). Given the patterns seen in Figures 7.3 and 7.5, it is likely that seasonal variability is another factor influencing number of taxa found in samples.
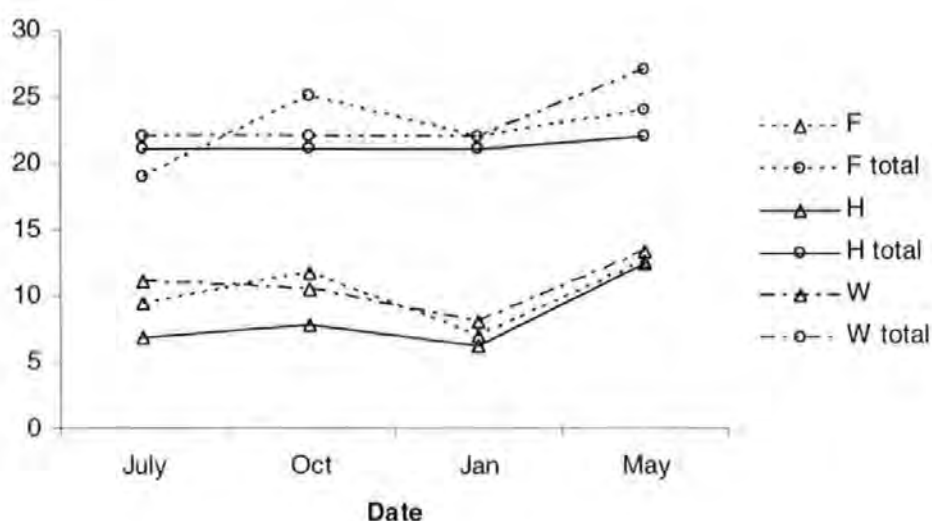
Figure 7.5: Numbers of taxa at Flanders Moss: mean and total by month and by treatment.

A further explanation for the lack of large seasonal fluctuations in numbers of testate amoebae at Coom Rigg Moss and Flanders Moss could be that the oceanic conditions and in particular the mild damp winters which have led to the formation of bogs at these locations do not provide extremes of dry or cold which could have a serious impact on testate amoebae survival. The lake studies (Hunt & Ming Chein, 1983) were carried out in a group of glaciated lakes in New York State, where surface water temperatures were consistently less than 6°C in winter and exceeded 20°C in summer, much stronger seasonal patterns than found in the UK. To observe strong seasonal patterns, it may be necessary to take regular samples from sites in much colder locations such as Ile de la Possession studied by Vincke *et al.* (2004) or the Jura mountains in Switzerland studied by Mitchell *et al.* (1999).

Seasonal patterns could not be observed at Fenn's and Whixall Moss because samples were only taken in two months.

7.4 Testate amoebae responses to hydrology

As research into testate amoebae has established that they respond to hydrological conditions on intact mires in the UK (Woodland, 1996; Hendon, 1998), the assumption was that they would respond to improving hydrological conditions at damaged sites. The sites all have very different water table regimes and management interventions. At Coom Rigg Moss; there was very little hydrological response to damming over the monitoring period and testate amoebae responses also showed very few clear changes. The changes which did occur at Coom Rigg Moss were subtle fluctuations of individual taxa. Two taxa, *Cyclopyxis arcelloides* and *Difflugia pristis*, arrived after damming in samples from plot C, where there was the greatest increase in water table. Research by Wanner and Xylander (2005) found that below-ground microbial communities respond in an additive model to changes in ecological conditions; that is, new species can immigrate into an area without the major shifts in community composition that are common in plant communities. At Coom Rigg Moss, therefore the subtle changes in taxa which have occurred over the monitoring period could be the beginnings of testate amoebae responses to improvements in hydrological conditions.

At Flanders Moss, the water table was relatively high, with negligible differences between sample plots. As restoration work was carried out in 1997, some time before testate amoebae monitoring was established, the hydrology of the site had stabilised and the experiment was a comparison of different treatments. It is not possible to comment on the response of testate amoebae to water table, as there was so little difference between plots.

Fenn's and Whixall Moss was rather different. There were hydrological differences between the five sampling locations and at each location, the four sampling surfaces had

different hydrological conditions. The ditches (1) always had standing water and some submerged vegetation; at the cut surface (2) and on the fey (4), water level was close to the surface and the waste surface (3) was perched above the water table. There were distinct differences in testate amoebae numbers and community composition across the four surfaces, with taxa indicative of wetter conditions occurring in high numbers in the ditches but being absent or very rare on the other surfaces. The transition in testate amoebae communities was less pronounced at the drier locations (X2 and X3).

When the individual surfaces were compared by sampling location, the driest location (X2) had higher concentrations of testate amoebae on each surface, except for the waste (3). From the work at Fenn's and Whixall Moss, it seems that individual taxa can be used effectively to describe hydrological conditions. They may therefore be useful as bioindicators of restoration but some caution must be applied in using the cruder concentration data as a measure of a healthy bog as drier conditions have produced higher numbers. This may also be the case at Coom Rigg Moss, as concentrations decreased at some locations after damming. It is important to remember that, although common in damp conditions, testate amoebae also occur in high numbers in drier environments such as soils (Wanner, 1995; Balík, 1996b; Foissner, 1999), so high concentrations in a peatland are not necessarily indicative of good or improving ecological conditions.

At Flanders Moss, despite the relatively uniform water table, there were differences in testate amoebae communities between plots. The differences were between plots with some tree remains and those where the whole tree had been removed. This appeared to be a product of the mulching effect of tree remains and was considered to be a response to surface humidity conditions. This could prove an important factor in monitoring

restoration, as it has been found that *Sphagnum* re-establishment on bare peat surfaces is much more successful where shelter is provided either by mulches, (Campeau & Rochefort, 1996; Quinty & Rochefort, 1997), companion planting (Grosvernier *et al.*, 1995) or manipulation of microtopography (Campeau *et al.*, 2004). Testate amoebae are not responding directly to water table but to moisture or humidity conditions in their living zone. Water table has been used as a convenient ecological measurement of hydrological status but it may be less meaningful than other measures of hydrology, such as moisture content. This is not to say that water table is not an important factor influencing testate amoebae distributions; it is normally directly related to the other measurements in undamaged peatlands. In sites where vegetation and peat structure have been altered, there may not be a direct relationship between water table depth and moisture content in the testate amoebae living zone. It may be more important when restoring peatlands to measure surface humidity and moisture conditions than simply depth to water table.

7.5 Sample material

When analysing data from Fenn's and Whixall Mosses, it became apparent that the different substrates sampled produced highly variable concentrations of testate amoebae. The highest counts came from the litter of Ericaceous plants and *Molinia caerulea*. In the long-term monitoring experiments at Coom Rigg Moss, *S. tenellum* produced some of the highest counts.

A Kruskal-Wallis test of logged concentrations and *Sphagnum* species at Coom Rigg confirmed that concentrations varied by species (p = 0.00 (adjusted for ties)), and *post-hoc* testing confirmed that *S. tenellum* produced the highest counts. The statistical reliability of this finding is low, as a much higher proportion of samples came from other species of

Sphagnum. In addition, the lowest counts in the methodological experiment at Coom Rigg Moss came from *Sphagnum tenellum* samples. In a study of testate amoebae in Michigan peatlands, Booth (2002) found that testate amoebae taxa and *Sphagnum* species were arranged along similar hydrological gradients. The concentrations in samples from Coom Rigg Moss do not entirely conform to this pattern, probably because the analyses were not comparing equal numbers of samples from each *Sphagnum* species and also because other factors such as size of water films may have an impact on testate amoebae concentrations (Mitchell *et al.*, 2003). At Flanders Moss, almost all samples were *Sphagnum capillifolium* so a comparison of *Sphagnum* species was not possible.

Connections between substrate and testate amoebae communities have been made by several authors (Louisier, 1982; Berger *et al.* 1985; Schönborn, 1986; Mitchell *et al.*, 2004). Most comparisons have been made of testate amoebae populations between different layers in soils or litter, although Mitchell *et al.* (2004) compared testate amoebae communities in mosses at different altitudes. The important factors for establishment of testate amoebae communities in leaf litter were substrate, food supply and availability of test building materials (Louisier, 1982). In a study of ecotonal effect on testate amoebae communities in beech and spruce forest soils, Balík (1996a) found higher abundances and biomass in mosses than in soils. This is contrary to the findings at Fenn's and Whixall Moss in this project (Chapter 6) where concentrations were higher in vascular plant litter than in mosses.

The findings of Louisier (1982) indicate that higher concentrations would be expected in the vascular plant litter than the moss and peat at Fenn's and Whixall because availability of test building materials would be higher as a result of higher decomposition and

mineralization of the organic matter. Also, Schönborn (1986) found that the higher availability of humus particles in humus layers compared to needle layers in coniferous soils favoured different taxa. *Euglypha cilata, Assulina muscoum* and *Corythion dubium* were more common in humus layers and *Centropyxis* spp. and *Trigonopyxis arcula* in needle layers. This was attributed to availability of test building materials.

7.6 Test size and morphological issues

Three testate amoebae taxa were observed to have wider size ranges than quoted in Charman *et al.* (2000). *Nebela tincta* varied in size from around 70μm to around 150μm. To assess the importance of this, when counting, individuals were placed into four size categories, <90μm, 90-110μm, ~125μm and >130μm. *Nebela militaris* specimens were placed into two categories, <75μm and >75μm and *Hyalospenia elegans* was also assigned two categories but was only found in a few samples.

Test size has been suggested as being closely related to habitat conditions (Woodland, 1996). It is unclear whether this would mean that different sized taxa select different habitats or whether individuals within taxa can respond to changes in habitat by growing to different sizes. Bobrov *et al.* (1995) discussed shell morphology and its ecological significance in species of Nebelid and *Trigonopyxis arcula*. Other studies into morphology of testate ameobae (Bobrov & Mazei, 2004; Golemansky & Todorov, 2004) have paid little attention to implications for habitat requirements.

To see whether there was any pattern in *Nebela tincta* test size in samples from Coom Rigg Moss, counts from each monitoring plot were combined to give a total value for each treatment. Percentages of the four size classes were then calculated. Table 7.1 shows a

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summary of these results with shading used to represent bands (see caption Table 7.1). The same process was carried out on sampled from Flanders Moss; samples were grouped by forestry treatment (Table 7.2). Although there are no definitive patterns in these tables, the Coom Rigg samples appear to have higher percentages of larger individuals in the samples with higher moisture content. A similar pattern is seen in the samples from Flanders Moss. The largest individuals (>130µm) rarely comprise more than 50% of all *Nebela tincta* and individuals under 90µm are most common in most samples. Further analysis was necessary to establish whether there were any definitive patterns in size class distribution of testate amoebae. At Fenn's and Whixall Mosses, only two size classes were found (Table 7.3). There was not a discernable pattern in the percentage table.

Sample	% Moisture	<90 µm	90-100 µm	~125µm	>130 µm
C Aug 01	80.80				
B July 01	81.97				
A Oct 01	87.25				
D Oct 01	88.96				
A Apr 02	89.20				
C July 01	90.13				
D July 01	90.47				
A Mar 01	90.98				
D July 00	91.10				
D Mar 01	91.63				
B Oct 01	92.14				
A Dec 00	92.48				
A Aug 01	92.94				
A July 00	93.02				
B Mar 01	93.15				
D Dec 00	93.18				
C Apr 02	93.68				
C Oct 01	93.96				
B July 00	93.98				
B Dec 00	94.08				
B Aug 01	94.19				
C Mar 01	94.27				
C July 00	94.39				
C Dec 00	94.66				

Table 7.1: Summary of *Nebela tincta* test sizes from Coom Rigg Moss– percentages from each treatment plot with moisture content. Clear cells indicate absence of that size class, light to dark shading indicates percentage bands 0-10%, 10-25%, 25-50% and 50-100%. Only locations where combined counts of over 10 individuals were found are included. Samples are ordered by moisture content.

Sample	% Moisture	<90 µm	90-100 µm	~125µm	>130 µm
October F	72.53				
July W	91.91				
May H	93.44				
May F	93.46				
May W	93.65				
October W	94.25				
October H	94.66				
January F	95.59				

Table 7.2: Summary of *Nebela tincta* test sizes from Flanders Moss – percentages from each forestry treatment. Cell shading as for Table 7.1. Samples ordered by moisture content.

Sample	% moisture	<90 µm	90-110 µm
X7 J3	74.40		
X6 J4	75.34		
X7 O4	75.52		
X2 O3	75.96		
X7 O3	76.43		
X7 J4	77.59		
X6 O4	77.84		
X6 O3	79.13		
X6 J3	80.45		
X8 O3	82.21		
X2 J3	82.69		
X2 J4	82.74		
X2 J1	82.96		
X2 J2	82.98		
X3 J4	85.07		
X3 J3	88.55		
X8 A3	91.75		

Table 7.3: Summary of *Nebela tincta* test sizes from Fenn's and Whixall Mosses – percentages from each dipwell and surface by month. Cell shading as Table 7.1. Samples ordered by moisture content.

To establish whether these patterns were significant, these data were plotted in scatter plots for Coom Rigg Moss and Flanders Moss (Figure 7.6 and 7.7). For clarity, the % moisture range in Figure a 7.6 has been restricted to values between 87% and 95% and 91% to 95% in Figure 7.7, as outlying values made the rest of the results difficult to read. Figure 7.8 is the scatter plot for Fenn's and Whixall Mosses. At Coom Rigg Moss and Flanders Moss, size classes and moisture content were unrelated. At Fenn's and Whixall Mosses, the <90µm individuals showed no relationship with moisture. However, the larger individuals

(90-100 µm) had a weak linear relationship with moisture content, suggesting that moisture may be connected to size of *Nebela tincta*.
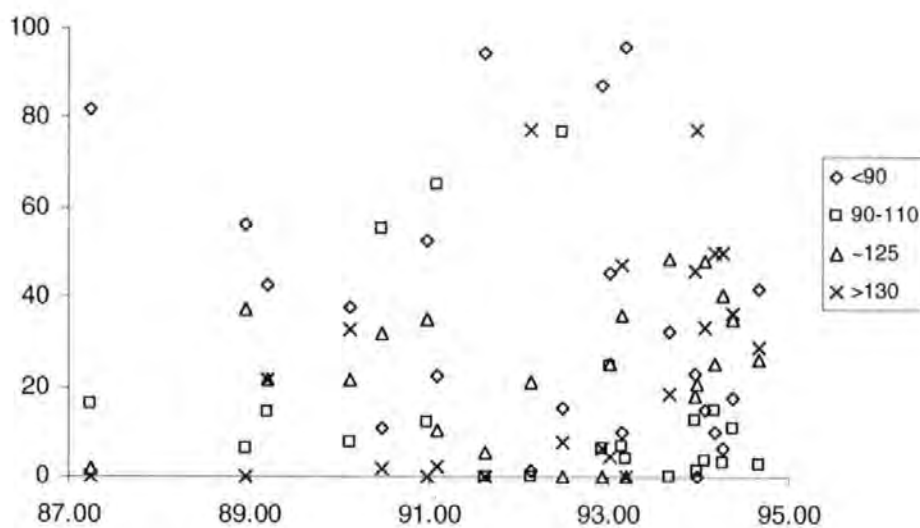

Figure 7.6: Scatter plot of *Nebela tincta* size classes and moisture at Coom Rigg Moss
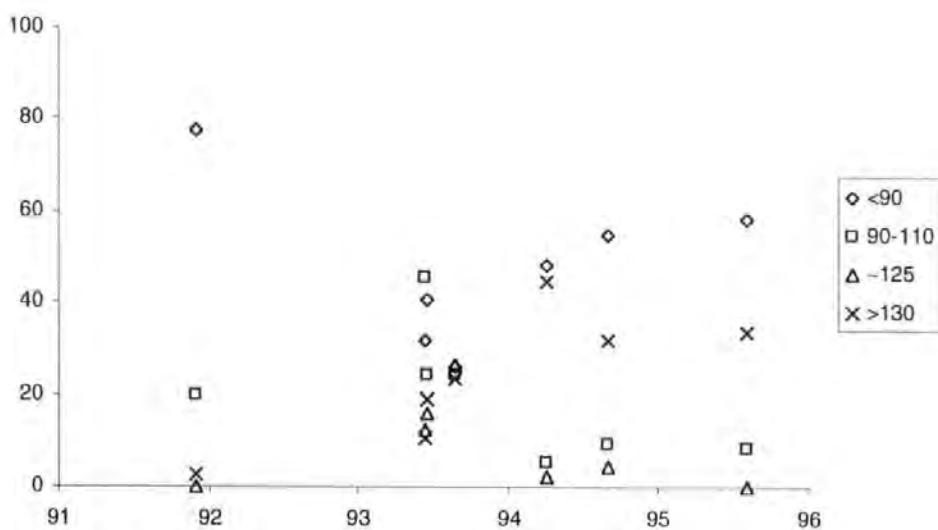

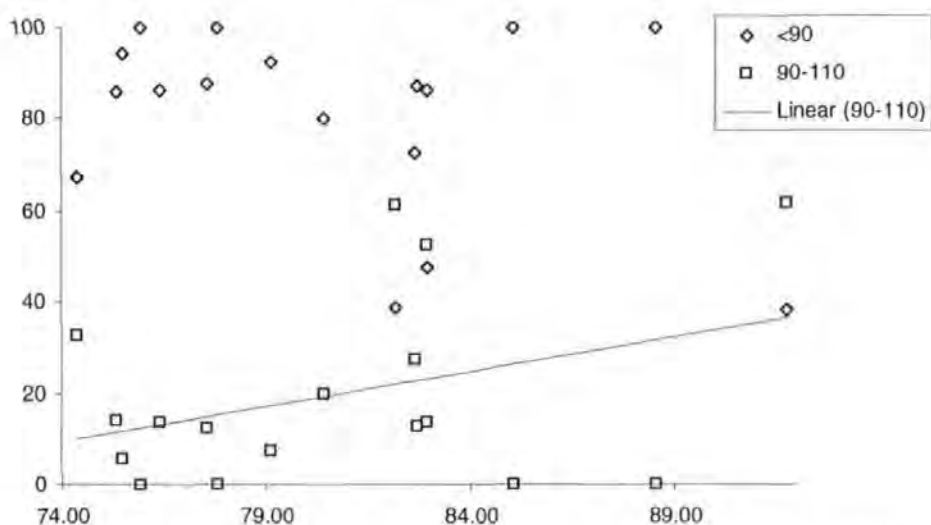**Figure 7.7: Scatter plot of *Nebela tincta* size classes and moisture at Flanders Moss**

Figure 7.8: Scatter plot of *Nebela tincta* size classes and moisture at Fenn's and Whixall Mosses

To explore the possibility that *N. tincta* sizes altered over the monitoring period, samples from each month at Coom Rigg Moss were amalgamated and plotted against time (Figure 7.9). Trendlines are omitted from the plot for clarity. However, there was an increase in the <90μm and ~125μm groups and a decrease in 90-110μm. The >130μm group remained fairly constant. These changes could be a response to damming; a comparison of changes at sampling locations over time showed an increase in the <90μm at the dammed locations and a slight decrease at the undammed locations. This does not relate to increases in water table as there were increases at one dammed and one undammed location and little change at the other locations. The ~125μm group increased at the two locations where water table rose but decreased where there was little change. The proportion of individuals over 130μm decreased at the location where the greatest increase in water table occurred but remained fairly stable at other locations. It is difficult to tell whether these changes are related to hydrological conditions, partly because of the minimal changes at Coom Rigg Moss and partly because there were relatively few samples available for comparison.
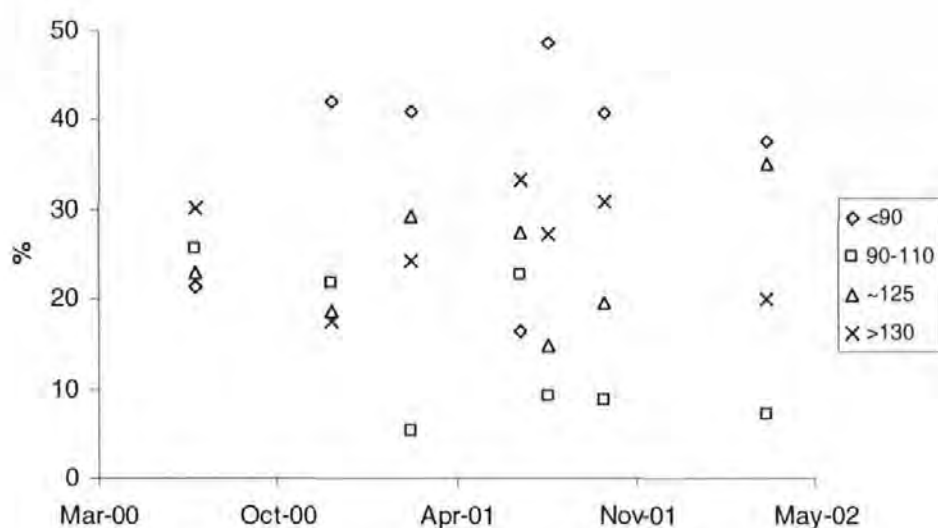
Figure 7.9: Scatter plot of *Nebela tincta* size classes and time at Coom Rigg Moss.

To fully understand patterns, more precise measurements would be needed, from sites with a wider range of hydrological conditions. This may be an interesting area to explore to further the understanding of modern ecology of testate amoebae and may prove useful in the development of a biomonitoring technique.

7.7 Other organisms

A large amount of living material other than testate amoebae, including nematodes, tardigrades and rotifers, was observed in the samples. Wherever possible, these other organisms were identified and counted. They have been included in some analyses in the methodological experiment (Chapter 3). Time did not permit further exploration of the use of these organisms as biomonitors. Initial results in chapter 3 suggested that identification was not always possible and preliminary analyses showed that they were not particularly useful.

Some of these organisms may be helpful in interpreting the success of restoration or providing more detail than testate amoebae alone. Testate amoebae may prey on some of the smaller organisms for food, or indeed, provide a food source for other organisms, their relationship with nematodes is both predator and prey (Yeates & Foissner, 1995). Tardigrades and their eggs, although important elements of the microfauna, do not occur in high enough numbers to permit their use as bioindicators (Jönsson, 2003). Another group of organisms, the Cladocerans, provide an important food source for larvae of some peatland dragonflies (Odonata) (Brooks, 1997); their presence in samples from Coom Rigg Moss could indicate good condition in relation to dragonfly habitat.

Other organisms may also be important for movement of testate amoebae within the bog. Louisier (1982) suggested that metazoans may be responsible for fast colonisation rates by testate amoebae in leaf litter. Chardez (1960) found that testate amoebae were dispersed by mites. The mites *Hermannia gibba* and *Ceratoppa bipilis* and other elements of the microfauna could therefore be important indicators for the potential restoration of testate amoebae communities. Robson *et al.* (2005) found that communities of rotifers and nematodes responded much more slowly to differences in UV-B radiation than testate amoebae suggesting that they may not be useful as biological indicators of restoration.

7.8 General comments

As peatland restoration methods vary depending on starting conditions, type and degree of damage (Pfadenhauer & Klötzli, 1996), it makes sense that monitoring technique and indeed the specific ecological condition being monitored will vary from site to site.

The experiments here have provided some indication that testate amoebae may be useful biological indicators of peatland restoration but the responses at the three sites have been very different. This is largely due to the ecological differences between the sites and provides some indication of the difficulties in developing a monitoring tool to cover such a wide range of conditions. It may be that when developing the tool, it is necessary to provide slightly different methodologies for different types of peatland and different types of damage. The aims of restoration are broadly similar for all categories of damage, to raise water table and provide *Sphagnum* with the appropriate ecological conditions but the way that these aims are achieved has to be adapted in response to starting conditions (Brooks & Stoneman, 1997).

There was some indication that restoration requirements are similar between afforested and cutover sites. Provision of shelter has increased live percentages of testate amoebae at Flanders Moss. However, monitoring these different types of damage has to be slightly different as the issue of avoiding fossil testates is usually only relevant at cutover sites. The optimal location for monitoring condition is an important element for both of these site types as there is a similar pattern of ditches and drier flat areas. If monitoring plots are established in ditches, conditions are likely to be much closer to those of a healthy site than the flat areas (Campeau *et al.*, 2004).

The assumption from this work is that two sites damaged by, for example forestry, will be more comparable than one afforested and one cut site. This has not been tested here, as the objectives of the PhD were to cover the main types of damage and there was not time to study duplicate sites. To confirm this assumption, further research is necessary.

It is clear that monitoring will need to be repeated to ensure that a thorough understanding of condition is achieved. A one-off sample will probably give a reasonable idea of conditions but a population explosion in one or more taxa may be short-lived and could provide an inaccurate picture of the conditions. The frequency and timing of repeat monitoring is complex. The observations of seasonal patterns in testate amoebae populations suggest that there are optimal times of year for monitoring. Late spring to early summer and early autumn may be better than winter or early spring, as the risk of frost is relatively high and this has implications not only for ease of sampling but also testate amoebae numbers. High summer is probably best avoided, as very hot and dry conditions can also affect counts. It is thought that extremes of temperature will result in unrepresentative counts and that some taxa are disproportionately affected. At less damaged sites, where minimal changes in hydrological condition are likely, monitoring may need to continue for longer than at sites where larger scale ecological changes are expected.

CHAPTER EIGHT: Conclusions and recommendations for further research.

8.0 Introduction

The possibility of using testate amoebae as a biomonitoring tool has been raised by several authors (Wanner, 1995; Woodland, 1996; Lamentowicz & Mitchell, 2005). In order to evaluate the practicability of using testate amoebae for biological monitoring on damaged and restored peatlands, it was first necessary to establish that they do respond to hydrological condition at such sites. The research for this PhD has attempted to develop the understanding of modern testate amoebae ecology as background for biological monitoring. The thesis aims were:

1) to develop knowledge of testate amoebae ecology in degraded peatlands including responses to management activity and seasonal variability.

2) to consider the potential for the development of a new monitoring technique for peatland restoration using testate amoebae as indicators of change.

This chapter is a summary of the findings of the research and is divided into four sections. The first section will concentrate on the findings of the research in the context of first aim (section 1.1) summarising the results of the site studies. The potential of testate amoebae analysis as a biological monitoring tool (aim 2, section 1.1) is considered next. The hypothesis that testate amoebae respond to hydrological differences on damaged and restored mires is discussed in the following section. Finally, recommendations for future work are made.

8.1 Modern ecology of testate amoebae

8.1.1 Sampling issues

To use testate amoebae analysis as a monitoring tool on ombtrotrophic mires, it is important to ensure that sampling does not damage the surface of the monitoring sites more than is necessary, and that later monitoring does not simply reflect ecological changes caused by previous sampling. To assess the feasibility of using single stems of *Sphagnum*, the sampling experiment in chapter 3 was carried out. Potential problems with the size of sample had to be quantified to ensure that sufficiently high counts could be achieved and that testate amoebae communities were representative of the wider area. The methodological experiments in chapter 3 set out to achieve this.

The findings of the methodological experiments showed that testate amoebae communities from single stems of *Sphagnum* were generally representative of those in the 25cm plot from which they were taken. In multivariate analysis, samples from the same plots were grouped together and were more similar to each other than to other locations on the bog. The results from chapter 3 also confirmed that it is possible to achieve counts of over 100 testate amoebae from single stems as long as enough time can be devoted to counting.

8.1.2 Response to restoration

The response to three types of restoration management was assessed by the field experiments in this study:

a) Coom Rigg Moss NNR – The hydrology of this site had been damaged by peripheral drainage ditches and by the afforestation of some of the hydrological unit and the surrounding area. Other issues which have been proposed as potential causes of damage

are changes in grazing regime and climatic change. Restoration at this site involved damming forestry ditches

b) Flanders Moss NNR – A small area of this bog was planted with exotic conifers in the 1970s. These trees were removed in 1997 as part of a Forestry Commission experiment testing tree removal technique in the restoration of afforested mires.

c) Fenn's and Whixall Mosses – This site has been intensively harvested for peat in recent centuries; the surface of the bog has been radically altered by this process. In 1991, English Nature and Countryside Council for Wales were given control of the mineral extraction rights and embarked on an extensive restoration programme entailing raising water levels in order to create conditions suitable for *Sphagnum* re-establishment.

Monitoring at Coom Rigg Moss was designed to assess whether testate amoebae communities would react to water table increases; the predicted response to damming. Four monitoring areas were established, two sites adjacent to the peripheral forestry ditches (dammed sites) and two control sites. Water table responded to the ditch blocking at one of the dammed sites and its control; damming did not result in an increase in water table at the other dammed site and water levels at the control remained unchanged. Subtle changes in testate amoebae communities were observed at the dammed location where water table rose. Communities at the control site where water tables increased slightly remained unchanged. The minor changes in communities could be because testate amoebae from drier locations are able to tolerate a broader range of hydrological conditions than those which live in wetter environments and thus have a competitive advantage over taxa migrating into sites in response to increases in water table.

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Alternatively, it could be that water table changes were not of a high enough magnitude to have much impact on testate amoebae community composition. The difference in water table requirements between two taxa from the location with the biggest change in water table at Coom Rigg Moss *Heleopera petricola* (dry indicator, decreased in summer samples) and *Difflugia pristis* (wet indicator, increased overall) is around 3 cm (Charman *et al.* 2000), roughly the increase in water table at this location. Although the magnitude of hydrological change here matches the difference in requirements between these two taxa, the post-damming average water table is deeper than either of these taxa require, indicating that depth to water table is not the only factor affecting community composition.

At Flanders Moss, hydrological differences between the restoration treatments were not statistically significant but testate amoebae communities were. Differences were identified in the percentage live data of the testate amoebae populations. Forestry treatments were associated with statistically significant differences between communities, but there were no differences between communities which could be attributed to the two damming treatments. Two taxa indicative of wetter conditions, *Nebela tincta* and *Euglypha strigosa*, had larger live populations, assumed to indicate higher reproductive rates, at locations where some tree remains were left on the bog surface than at those locations where the whole trees were removed. It was concluded that the tree remains were acting as a mulch, and protecting the surface from extremes of temperature and moisture variation and raised surface humidity. Testate amoebae were more responsive to such surface conditions than water table depth. The implication of this is that depth to water table is not the most important measure of hydrological condition on damaged and restored bogs.

At Fenn's and Whixall Mosses, testate amoebae community composition was influenced by surface conditions; taxa indicative of wetter conditions lived in the ditches and drier taxa lived in the waste peat, which was perched above the water table. This reflected vegetation differences observed at the site. Testate amoebae communities were much more diverse in the drier peat, suggesting that species richness is not a useful indicator of hydrological condition. Comparisons of testate amoebae populations from individual surfaces (e.g. ditches) at dipwells with different hydrological conditions showed that communities responded to hydrology, as measured by water table depth, at this site. An interesting finding at Fenn's and Whixall was that testate amoebae populations in different sample material were highly variable. Concentrations in vascular plant litter were much higher than in *Sphagnum* samples. This may be explained by higher decay and mineralization, resulting in greater availability of test building materials in vascular plant remains than *Sphagnum*.

8.1.3 General findings on testate amoebae ecology

The seasonal variability of testate amoebae communities was low, although there were some indications of higher populations with increased reproduction in spring and late summer. There were problems in achieving high counts from winter samples, especially when the bog surface had been frozen, although these could also be attributed to smaller sample sizes. Samples taken in high summer (August) sometimes had lower numbers of testate amoebae than those taken at other times. The lack of major seasonal fluctuations in testate amoebae numbers could be the result of a mild oceanic climate. To explore this further, samples from locations where there are stronger seasonal trends in temperature would be needed.

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In general, testate amoebae at the three study sites responded to hydrological conditions in broadly similar way to those in undamaged peatlands. The other factor which seemed to have an impact on communities was surface humidity, suggesting that it is important to consider aspects of hydrology other than depth to water table when monitoring peatland restoration. Surface humidity was also found to be an important factor in the restoration of harvested peatlands in Canada (e.g. Ferland & Rochefort, 1997).

8.2 Development of monitoring tool.

The secondary aim of the PhD was to consider the possibility of developing a monitoring tool using testate amoebae as biological indicators of peatland damage and restoration. The results from the research presented here have produced recommendations for further research into developing this tool. There are several issues which have arisen, these are outlined below.

The important considerations for sampling can be divided into sample size, sampling time and sample material.

Sample size was an important consideration when sampling at Coom Rigg Moss, the regular repeated sampling, planned to tie in with hydrological monitoring by the Forestry Commission, meant that small samples of one stem of *Sphagnum* had to be taken to avoid damaging sampling plots. It was possible to achieve counts of over 100 tests from these samples and they were representative of the sample plot from which they were taken. However, they required a large time input to achieve these counts. Wherever possible, it is recommended that larger samples of 3-5 stems of *Sphagnum* (or equivalent) are taken.

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Samples of 3-5 stems were taken from Flanders Moss and consistently higher counts were achieved here with less counting effort.

Sampling time is important when monitoring restoration despite the absence of definitive seasonal patterns in testate amoebae populations. Problems with low counts were greater in samples taken in high summer and winter. Optimal sampling time is probably between March and the end of June or early July and in early autumn. Sampling should be avoided during spells of frost or extended warm dry periods. It is advisable to take at least twice yearly samples to ensure that any seasonal fluctuations are accounted for.

Wherever possible, the same sampling material, including the same species of *Sphagnum*, should be taken from all sampling locations for ease of comparison. If this is not possible, it is important to be aware of potential differences in concentrations.

Repeat sampling from matched locations within the restoration area should be taken as with other ecological and palaeoecological monitoring.

Finally, it is important to adapt sampling strategy and monitoring intensity depending upon the issues specific to each site including mire type, type and level of damage and the stage of restoration. The methodology for monitoring restoration at an afforested site is different to monitoring cutover sites. Different data analysis techniques are also appropriate for different sites. Percentage live was a useful indicator of differences between treatments at Flanders Moss but it can only be used at locations where the peat is uncut. Where harvesting has occurred, mixing between fossil and modern tests could occur and may distort the results. Absolute concentrations are a useful measure but differences between

concentrations from different sample materials must be considered. Log transforming concentration data can help overcome some of these issues.

The main indicators that can be used to monitor the condition of restored peatland surfaces can be summarised as:

- Measures of reproduction rates such as the percentage of living tests present.

- Changes in the assemblage of taxa present, reflecting shifts in overall hydrological status.

- Measures of abundance including concentration (numbers g^{-1} dry weight).

8.3 Hypothesis response

Although the research presented here has been an exercise in assessing responses to a range of management measures, rather than hypothesis testing, the process also tested the hypothesis that testate amoebae respond to hydrological differences on damaged and restored mires. At the locations studied here, it appears that testate amoebae populations are responding to hydrological condition but that this response is not always directly related to depth to water table, and other elements of hydrology are important influences. In particular, the moisture content and humidity in the surface material appears to be most important, and management can have an important influence on these variables, even if water tables remain unchanged.

8.4 Recommendations for future research

The research here has laid the foundations for the development of a tool for monitoring peatland damage and restoration. There are several recommendations for the development of this work and for the application of this research to palaeoecological studies.

- Replicate studies should be carried out at sites undergoing comparable restoration to assess whether testate amoebae responses are similar across similar site types as defined by management problems and mitigation.

- Investigations into size and hydrological status may be useful. Some study of test size in *Nebela tincta* was carried out here but to fully assess responses to hydrology, precise measurements of individuals are required.

- Assessment of the level of taxonomy required to detect changes is needed. If a practical tool for non-specialists is to be developed, it would be easier to be able to define key taxa which are abundant and consistent in restoration projects and to assess whether identification of, e.g. *Euglypa*, to species level, is really necessary to be able to understand condition. This method is not entirely untested; a study by Wilkinson and Davis (2000) examined existing datasets and found a strong correlation between species richness and genera richness. Testing this potential further could prove useful for palaeoecological studies. Interpretation of hydrology may be easier if larger taxonomic groups can be used and may increase use of testate amoebae in environmental reconstructions.

- The issue of hydrological response is important. If testate amoebae are more responsive to moisture and humidity than depth to water table. This will have implications for palaeoenvironmental study. It may also indicate that these hydrological variables are more important in restoration of damaged peatlands and should therefore be given higher priority when site managers are monitoring restoration programmes.

- Further studies of seasonal responses in testate amoebae are required so that a recommendation for optimum sampling time can be made. It is likely that seasonal variability in the UK is limited; however, this may not be the case in more continental climates. Further understanding of seasonal variability may be also useful for palaeoecological studies.

Appendix 1: Index to common names of plant species referred to in the text.

Andromeda polifolia	Bog Rosemary
Betula spp.	Birch
Calluna vulgaris	Ling
Carex curta	White Sedge
Carex panicea	Carnation Sedge
Carex rostrata	Bottle Sedge
Deschampsia flexuosa	Wavy Hair-grass
Drosera rotundifolia	Round leaved sundew
Empetrum nigrum	Crowberry
Erica tetralix	Cross-leaved Heath
Eriophorum angustifolium	Common Cottongrass
Eriophorum vaginatum	Hare's tail Cottongrass
Galium palustre	Common Marsh-bedstraw
Holcus lanatus	Yorkshire-fog
Juncus acutiflorus	Sharp-flowered Rush
Molinia caerulea	Purple Moor-grass
Narthecium ossifragum	Bog Asphodel
Picea abies	Norway Spruce
Picea sitchensis	Sitka spruce
Pinus contorta	Lodgepole pine
Pinus sylvestris	Scots Pine
Pteridium aquilinum	Bracken
Rhynchospora alba	White beak-sedge
Rubus fruticosis.	Bramble
Schoenus nigricans	Black Bog Rush
Sculletaria galericulata	Skullcap
Trichophorum cespitosum	Deergrass
Vaccinium oxycoccus	Cranberry
Viola palustris	Marsh Violet

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