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The Use of Testate Amoebae in Monitoring Peatland Restoration Management: Case Studies from North West England and Ireland

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Abstract. The nature conservation, and wider environmental importance of peatlands, particularly in relation to carbon management, has led to there being a growing interest in attempting to manage degraded peatlands in a way that will restore them to fully functioning peatland ecosystems. Much of this management is concerned with the rewetting of these sites therefore it has become important to monitor the surface wetness of these bogs and if possible compare current wetness with previous, pre-damage, conditions. We present previously unpublished case studies of the use of testate amoebae to monitor bog restoration schemes in N.W. England (Holcroft Moss, Cheshire) and Ireland (Ardagullion Bog, Co. Longford). In addition we summarise the key conservation related conclusions of our previously published work on two other sites in N.W. England – Astley Moss (in the Chat Moss complex of Greater Manchester) and Danes Moss (Cheshire). At Holcroft the record of lead pollution from the peat core allows us to date recent changes in the testate community preserved in a peat core and relate these to both conservation management and other changes in the landscape around the bog in over the last 50 years. Ardagullion Bog provides an illustration of the utility of using multiple peat cores in the testate monitoring of peat bog restoration and illustrates that a bog that has only suffered limited 'damage' is able to be restored to something close to pre damage conditions on a decadal time scale. We also summarise what we see as the main lessons from testate studies of bog restoration – both from the case studies described in this paper and from the wider literature – and discuss the conditions under which testate amoebae may be of particular use in peatland restoration.

Key words: Restoration ecology, raised bog, *Hyalosphenia subflava*, *Archerella*, *Amphitrema*, *Sphagnum*, *Molinia caerulea*.

> 'Ecologists have a duty to inform conservation policy.'
> (Grime and Pierce 2012, p. 120).

INTRODUCTION

Peatlands provide the habitats for a fascinating biota, including 'mosses with an infinite variety of colours...

insect-eating plants and beautiful orchids' (Rydin and Jeglum 2006, p. 1), in addition they play a potentially important role in the global carbon cycle – containing about half as much carbon as the whole atmosphere (Dise 2009). Whilst peatlands represent an important terrestrial carbon sink, disturbance (principally through cutting and draining) releases an estimated 10 million tons of carbon into the atmosphere annually in the UK alone (Worrall *et al*. 2011) and consequently, the protection and restoration of peatlands is the focus of recent policy and practice initiatives (e.g. IUCN UK Peatland Programme, http://www.iucn-uk-peatlandprogramme.org/).

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Globally the area of land under peatlands has been declining and in Europe this has been particularly dramatic, with an estimated 52% of active peatlands lost and in some countries, such as Denmark and the Netherlands, almost all peatlands have gone (Chapman *et al.* 2003). In lowland England the historical decline has also been dramatic with some 37,000 ha of peatland being reduced to less than 500 ha by the start of the 1990's (Lindsay 1993). For example the 'Fens' in eastern England were a very extensive area of wetland based on both silty soils and peats – with the peats being the subject of extensive drainage from the 1600's onwards (Goodwin 1978, Pryor 2010). In North West England – one of the areas considered in this paper – the 2,500 ha raised mire complex of Chat Moss (Fig. 1) was drained from the mid-18th century onwards (Wilkinson and Davis 2005). This area of North West England – from north Cheshire to Southern Cumbria – was the second most extensive area of lowland peat after the Fens, but has been extensively drained for agriculture (Shimwell 1985). In Ireland it is a similar story, with the added driver of a significant use of peat for power generation, which again requires drainage to facilitate peat 'harvesting' (Charman 2002). The raised bogs of the Irish Midlands have been particularly reduced because their peat is extensive, accessible and so economically attractive for large scale extraction (Cabot 1999) and continues to account for c. 5% Ireland's total energy consumption (Howley *et al.* 2009). This widespread and extensive drainage means that there are now many areas of fully or partly drained peatland that could be potentially restored by rewetting and other conservation management.

The nature conservation, and wider environmental importance of peatlands, particularly in relation to carbon management, has led to there being a growing interest in attempting to manage degraded peatlands in a way that will restore them to fully functioning peatland ecosystems (e.g. Lunt *et al.* 2010, Lunn and Burlton 2013). As well as the straightforward nature conservation implications of drainage this is important in the context of the carbon cycle, potentially influencing the fluxes of carbon between bog and atmosphere (e.g. Clymo *et al.* 1998, Urbanová *et al.* 2012, Grand-Clement *et al.* 2013). As much of the past 'damaging' management has been by draining for agriculture, forestry or other land uses, a key aspect of much of peatland restoration has been rewetting the site – via blocking drainage ditches, removing tree cover etc. (Charman 2002, Rydin and Jeglum 2006). This has created a need to monitor changes in bog wetness on these sites and to compare the wetness levels with past values. Currently bog wetness can be measured by using a variety of methods such as water ('dip') wells to measure the depth to water table (e.g. Ferland and Rochefort 1997, Meade 1992; for a brief review see Rydin and Jeglum 2006) or by direct measurements of soil moisture content (e.g. Carroll *et al.* 2011). Testate amoebae ('testates') provide another approach to such monitoring; with the advantage that their remains preserved in peats can allow one to reconstruct changes that happened before monitoring began at a site.

Testates are protists in which the single cell is enclosed within a shell or 'test'. Although polyphyletic – being split between at least two eukaryote 'supergroups' (Adl *et al.* 2012) – they seem to form a reasonably uniform ecological grouping. The morphology of their shells allows them to be identified to a range of different morphospecies, making them a useful group for environmental monitoring as they can be enumerated by direct counting – providing population data as well as just species lists (Wilkinson and Mitchell 2010). In addition the tests are often preserved in peats allowing former testate communities to be established. This preservation potential has led to an interest in using testate amoebae to reconstruct past changes in bog surface wetness (as different taxa have different moisture preferences). This approach has been widely used in Holocene climate reconstruction over the last couple of decades, with attempts to use testates to give quantitative reconstructions of past changes in bog surface wetness (e.g. Woodland *et al.* 1998, Patterson and Kumar 2002, Payne *et al.* 2011). This palaeoecological approach is also potentially relevant to their use in monitoring more recent changes in response to conservation management.

Classically in peat core based palaeoecological studies radiocarbon dating is used to establish a chronology; however, this approach is of limited use for more recent peats from the last few hundred years (Charman 2002). This is unfortunate as it is often these more recent changes that will have the greatest relevance for conservation management, indeed in the context of assessing the effect of management interventions a decadal time scale will usually be more relevant than one of a few hundred years. In order to put such recent changes in bog hydrology into some sort of context the lead content of the peat at Holcroft Moss was measured, as fluctuations in anthropogenic lead can provide an archive of industrialization and landscape modification
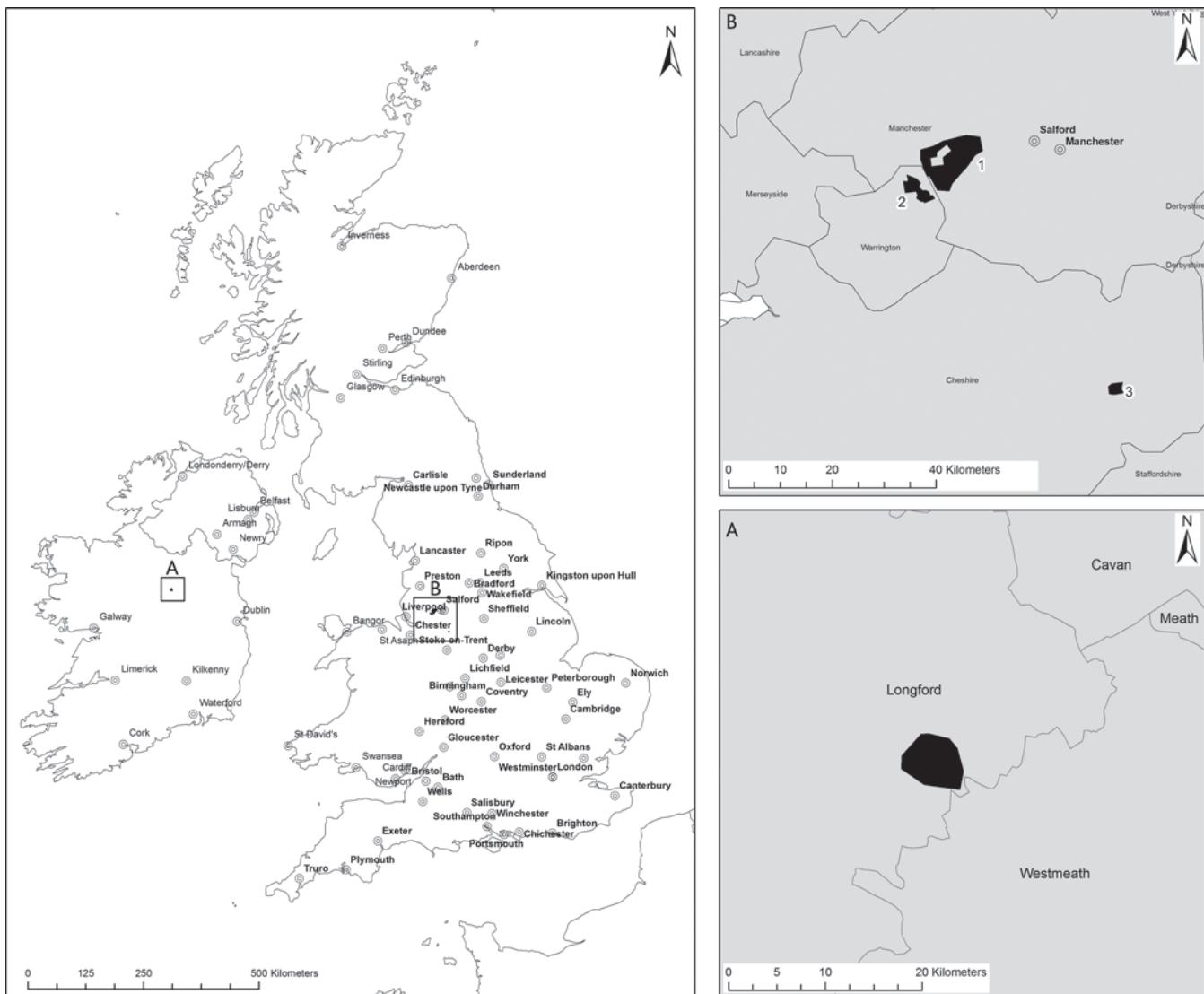
Fig. 1. Left: map of UK and Ireland showing locations of sites mentioned in text. Right Top: NW England, showing 1. Chat Moss (Astley Moss as cut-out), 2. Holcroft Moss and 3. Danes Moss. Right Bottom: Location of Ardagullion Bog, Co. Longford.

during the Holocene, particularly since Roman times (Marx *et al.* 2010, Farmer *et al.* 2009). Experimental work suggests that lead can be reasonably immobile in peat cores – so preserving a record of past pollution (Novak *et al.* 2011). Several such records have demonstrated a dramatic increase in heavy metal pollution since the mid-1800's, coincident with the onset of the Industrial Revolution and increased burning of coal and more recently vehicle exhaust emissions (Farmer *et al.* 2009, Novak *et al.* 2008). The increase in lead from the burning of coal in the UK reached its peak in 1955,

whilst lead from emissions resulting from the addition of tetra alkyl lead to petroleum reached a maximum between 1973–1985, although this has been reported as occurring earlier (as much as 30 years) in heavily industrialized areas (Novak *et al.* 2008). Holcroft Moss has proven to be a good site to apply such an approach, being situated within relatively close proximity to the historically, heavily industrial cities of Manchester and Liverpool, and with the M62 motorway (completed in this area c. 1972) running alongside. So this site provides a good illustration of how informative a peat core

from a site with well dated recent (i.e. 20th century) peats can be. Indeed because in this case lead allows us to apparently identify the early 1970's, our lead curve is much more useful for interpreting the effects of recent management changes that other approaches to dating recent peats, such as SCP's (industrial soot) which would be more useful in identifying the much earlier rise of heavy industry in the area during the 19th century.

Our second main site described in this paper, Ardagullion Bog, Co. Longford, Ireland, provides an example of a multi-core approach to monitoring a peatland. Because extracting environmental data, such as testate amoebae or pollen, from peat cores is very time consuming often only one core is examined from each location – although looking at multiple cores can be very informative (e.g. Turner *et al.* 1993). Our Irish site provides an example of the utility of using multiple cores for monitoring peatland restoration – as does our previous work in North West England also summarized below (Davis and Wilkinson 2004).

As far as we are aware the first use of testate amoebae as indicators of bog wetness in a management context was Warner and Chmielewski's (1992) study of the effects of drainage on forested mire in Canada. They concluded that testates were potentially a useful tool in monitoring the effects of management on bog hydrology. Since this first brief publication a number of more extensive studies have been published using testates as a way of monitoring wetland hydrology – for example Mitchell *et al.* (2000) showed testates to be useful organisms for monitoring bog surface water chemistry as well as hydrology. Several studies have focused on using testates to study the success of restoration management of drained bogs, in both Finland and Britain, where the intension has been to make the sites wetter again (Davis and Wilkinson 2004, Jauhiainen 2002, Vickery 2006) or at sites in the Jura Mountains in Switzerland where natural processes have led to some recovery of abandoned cut-over peatlands (e.g. Buttler *et al.* 1996, Laggoun-Défarge *et al.* 2008). In this paper we describe case studies (the majority of which are previously unpublished) from lowland peat bogs in North West England and Ireland where testate amoebae data are available to help evaluate the success of conservation management schemes intended to rewet previously drained or degraded bogs. As our studies were carried out over a period of 15 years, and for a range of different reasons, there are unsurprisingly differences in approach between them (these are detailed in the Methods section). In addition the nature of this work – the

Irish data being part of a conservation inspired larger survey and the Holcroft Moss data being a 'spin off' from a study focusing on changes over several thousand years – means that it is more characteristic of the type of data likely to be available to help inform conservation management decisions rather than the extremely high resolution (and time consuming) data that may come from a more typical 'academic' research project where the key criteria is suitability for publication in high impact journals rather than the cost effective provision of management related data.

METHODS

Study sites

North West England; Holcroft Moss and the surrounding area

The area of North West England comprising north Cheshire and the southern part of the old county of Lancashire once contained extensive peatland systems. This region has been subject to major programmes of palaeoenvironmental survey (e.g. Hall *et al.* 1995, Leah *et al.* 1997) which found very little post-prehistoric peat surviving and substantial peat loss since the 1960's (reviewed for Chat Moss by Wilkinson and Davis 2005). Two of these bogs have previously been studied from the context of using testate amoebae to inform conservation management; namely Astley Moss (in the Chat Moss complex in Greater Manchester) and Danes Moss, Cheshire (Figs 1 and 2). The results of this work was described by Davis and Wilkinson (2004) and key management related points are also summarised in this paper. Shimwell (1985) describes both Holcroft Moss and Chat Moss as large basin peatlands 'developed over boulder clays and Late Glacial flood gravels' while Danes Moss is smaller and probably associated with a kettle hole (Shimwell 1985) or started as a 'flood plain mire' in a 'broad shallow depression' (Leah *et al.* 1997).

Holcroft Moss (Latitude 53.434889°, Longitude –2.476411°: Figs 1 and 3) is currently a nature reserve and was declared a site of Special Scientific Interest – as defined under UK legislation – in 1991 having been acquired by the Cheshire Wildlife Trust in 1990 and is unusual for the region in having extensive surviving recent peat (Valentine *et al*. unpublished. See also Fig. 4). The palaeoecology of the site was initially investigated by Birks (1965) who presented peat stratigraphy, a pollen diagram of relatively low resolution by modern standards and noted the preservation of testate amoebae within the sequence.

The site is described as degraded lowland raised bog (Natural England pers. comm.) and has a substantial cover of peat-forming vegetation dominated by the cotton grasses *Eriophorum vaginatum* and *E. angustifolium* and purple moor grass *Molinia caerulea* along with a number of bog moss *Sphagnum* species. *M. caerulea* is a dominant plant on many of the areas of drained peatland in the area (Newton 1971). Conservation management at the site has included both restricting water loss, mainly by the use of plastic piling

Fig. 2. Top: The sampling site on Astley bog in 1998 when the samples described in Davis and Wilkinson (2004) were taken. Bottom: Danes Moss in 1999 when the samples described in Davis and Wilkinson (2004) were taken.

Fig. 3. Top: A general view across the current (in 2012) surface of Holcroft Moss from close to our core site. Bottom: The edge of Holcroft Moss (in 2011) showing both the fence designed to keep the sheep used for conservation grazing on the bog and – between the fence and the taller vegetation – the plastic piling sunk into the peat to try and maintain a wetter bog surface.
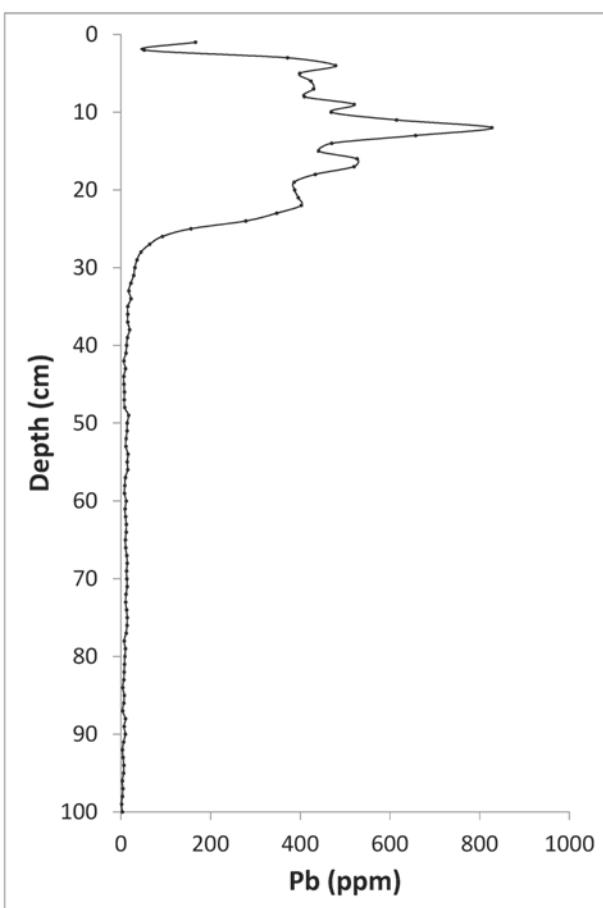
Fig. 4. Lead content (ppm) of the top metre of peat at Holcroft Moss.

surrounding the site which was installed between 1999 and 2001 (Fig. 2), and vegetation management via summer grazing mainly to reduce the dominance of *M. caerulea*. This grazing regime started in 1993 following on from scrub clearance from the bog surface in the early 1990's (Natural England, pers. comm.; Cheshire Wildlife Trust, pers. comm.).

Ardagullion Bog, Co. Longford, Ireland

Our study of Ardagullion Bog, Co. Longford (Latitude 53.727504°, Longitude –7.525126°) (Fig. 1) formed part of a Natura 2000-LIFE funded project focussed on restoration of previously afforested peatlands in the ownership of the state forestry service, Coillte ("Restoring Raised Bog in Ireland" – LIFE04 NAT/IE/000121). A total area of 571.2 ha of midland raised bog was subject to some degree of restoration as part of this project. Ardagullion Bog is partially uncut, preserving long, intact sequences (in many Irish midland bogs upwards of 12 m peat depth is not unusual). Much of the remaining uncut bog is covered with typical Midland raised bog vegetation, including *Calluna vulgaris*, *Vaccinium oxycoccus* and *Eriophorum vaginatum*. The mosses *Sphagnum papillosum*, *S. capillifolium* and *S. magellanicum* are common on the uncut section, with *S. austinii* (formally *S. imbricatum*) found at the centre of the site. Great Sundew *Drosera anglica* is found in pools towards the centre of the bog with *Menyanthes trifoliata* also present in some pools. The cutover sections to the north-west, east and south-east are dominated by *Molinia caerulea*, *Juncus effusus* and *Eriophorum vaginatum* (cf. http://www.npws.ie/media/npwsie/content/images/protectedsites/sitesynopsis/SY002341.pdf).

Vegetation was assessed by pre-restoration survey using a number of fixed location 10 × 10 m quadrats at each site. These were monitored over the life of the project with four surveys at each site quadrat from August 2005 to July 2008. Conifer plantation (largely Sitka Spruce *Picea sitchensis*, Norway Spruce *Picea abies*, Lodgepole Pine *Pinus contorta* with some Scot's Pine *Pinus sylvestris*) was removed by clear-felling and removal of mature trees and felling to waste of young or moribund trees (i.e. leaving a significant quantity of woody material *in situ*), with 'wind rowing' to allow the development of natural bog vegetation between rows of woody debris. Forestry drains were blocked using peat dams while more substantial open drains were blocked using plastic dams.

Field methods

At Holcroft Moss 12 cores were taken in two transects across the length and width of the bog to establish the depth of the peat present. One core of 4.22 m was obtained in March 2011 from the highest part of the raised bog, which also had the greatest depth of peat. The top 20 cm of this core was prepared for analysis of testate amoebae at 2 cm intervals. In addition surface samples were gathered in October 2012 from a relatively intact area of bog and, for contrast, from an eroding path on the bog surface.

For the Irish site sampling took place during Spring 2008; with three 0.5 m cores extracted using a Russian Peat Corer. These cores were taken from areas of contrasting surface condition with the aim of obtaining one sample each from a wetter area, a drier area and an area of intermediate wetness. The taxonomy of vascular plants in this paper follows Stace (2010).

Laboratory methods

Extraction of testate amoebae followed a modified version of the protocol of Charman *et al.* (2000), as summarised below. For the Irish site 1 cm³ samples were measured by displacement in a 10 cm³ measuring cylinder and placed into a tall 150 ml beaker along with 25 ml distilled water and one *Lycopodium* tablet, to quantify testate amoeba concentration – however in this paper all testate data are presented as percentages (however, the *Lycopodium* spore curve is presented in our graphs to give an indication of testate density in the different samples. A greater number of spores counted indicating a lower testate density). Samples were vigorously boiled for 10 minutes (adding more water if necessary), and then washed through a 300 μm sieve and centrifuged at 3,500 rpm for 5 minutes and the supernatant discarded. The pellet was then resuspended and mixed with glycerol in an approximately 1:1 ratio then mounted for microscopic examination. The method for the Holcroft samples differs slightly here, following centrifuging the samples were decanted into microfuge tubes for storage, and one drop of glycerol was added to one drop of sample when mounting on slides, then gently mixed with a cocktail stick, before placing the cover slip ready for counting. For both the British and Irish samples tests were counted us-

ing compound microscopes at × 400 magnification. For all sites, following the work of Payne and Mitchell (2009), 150 individual tests were counted per sample where possible – the total testate sum also including the Bdelloid Rotifer *Habrotrocha angusticollis* based upon the presence of large numbers of this organism in some samples and the inclusion of this organism in many previous ecological studies of testate amoebae. Identification largely relied upon the work of Charman *et al.* (2000) for the Irish samples, while for Holcroft Moss Charman *et al.* (2000) was used in conjunction with other guides – especially Mazei and Tsygonov (2006), Ogden and Hedley (1980), and Clarke (2003). The Holcroft Moss testates were counted by JV and the Irish samples by SRD. The previously published samples from Astley Moss and Danes Moss were processed in a similar manner – see Davis and Wilkinson (2004) and Davis (2001) for details.

For Holcroft Moss lead content was also measured using X-ray fluorescence. Peat samples were oven dried at 40°C then hand ground to a powder using an agate pestle and mortar. Samples were put into XRF pots with spectro certified polyprophylene film and measured in a Bruker S2 ranger.

CASE STUDIES

North West England

Holcroft Moss

The lead results (Fig. 4) confirm that the upper section of the core is undisturbed and, unusually, also allow us to date changes in the last few decades – a time scale particularly relevant to the conservation management of the site. The large increase in lead around 14 cm depth is most likely associated with the opening of the M62 motorway (a large multi-lane road carrying heavy traffic that at the time would have been using leaded petrol) and the subsequent decline in lead is probably due to the phasing out of lead in vehicle fuel in the UK during the 1980's. The initial rise in lead around 28 cm likely relates to the rise of industry in the area during the mid-19ᵗʰ century. These lead data allows us to assign approximate dates to the changes in testate communities in the top 20 cm of peat core.

The most obvious changes in the testate communities in the top 20 cm of peat core (Fig. 5) are an increase in diversity towards the surface and a dramatic decline in taxa considered by Charman *et al.* (2000) to be indicators of a dry bog surface – especially *Hyalosphenia subflava* but also *Diffugia pulex*. The decline in *H. subflava* is especially interesting as (based on lead data) it predates the start of significant conservation management on the site around 1990. This decline is approximately associated with the construction of

the M62 motorway, which caused the deposition of a large amount of excavated spoil on the edge of the site between the more intact bog and the road. Plausibly this has restricted water movement off the site and so increased bog surface wetness. If so it has effectively acted as an accidental 'bund' – these banks of peat or other sediments are often constructed during the restoration of large areas of damaged bogs as a conservation measure to try and keep water on the site (Charman 2002). The increase in *Phryganella acropodia* in zone HM-III (3–9 cm depth) is intriguing. This taxon is thought to mainly feed on fungi, or fungal exudates (Ogden and Pitta 1990, Wilkinson and Mitchell 2010, Vohník *et al*. 2011). Its brief rise and fall could be related with disturbance associated with the start of active conservation management – which may have briefly increased fungal abundance on the bog surface by, for example, increased water levels killing some of the less water tolerant plants. However the largest change, post-motorway construction, to bog management was probably the introduction of the plastic piling only ten years before our sampling – even allowing for the poorly humified nature of the surface peat 9 cm peat 'growth' in a decade seems unlikely, especially for a bog surface with very limited *Sphagnum* (based on our lead data a date of very approximately 1990 would seem more probable for the junction between zones HM-III and HM-IV). A plausible interpretation for these changes around 9 cm is that the bog surface was affected by several very warm years, with dry summers, that occurred around 1989–1990 (Kington 2010) – this slight increase in *H. subflava* at this depth is consistent with this explanation as would be the rise of *P. acropodia* if a drying of the bog surface caused an increase in fungal activity. Alternatively – or in addition – these testates may be responding to the disturbance of the site due to the scrub removal that followed the bog becoming a nature reserve in 1990; for example *P. acropodia* could be feeding on fungi associated with the woody remains of the cleared scrub (Edward Mitchell, pers. comm.).

One complication in the interpretation of these types of data is the possibility that some testates preserve better than others, so taxa may be found at the top of the core but not at greater depth because of the breakdown of the tests within the peat. Based on the very limited studies on the breakdown of tests this is a possible explanation for the restriction of most *Euglypha* tests to the top of the core and to the surface samples (Fig. 6) as Payne (2007) suggests these taxa do not preserve
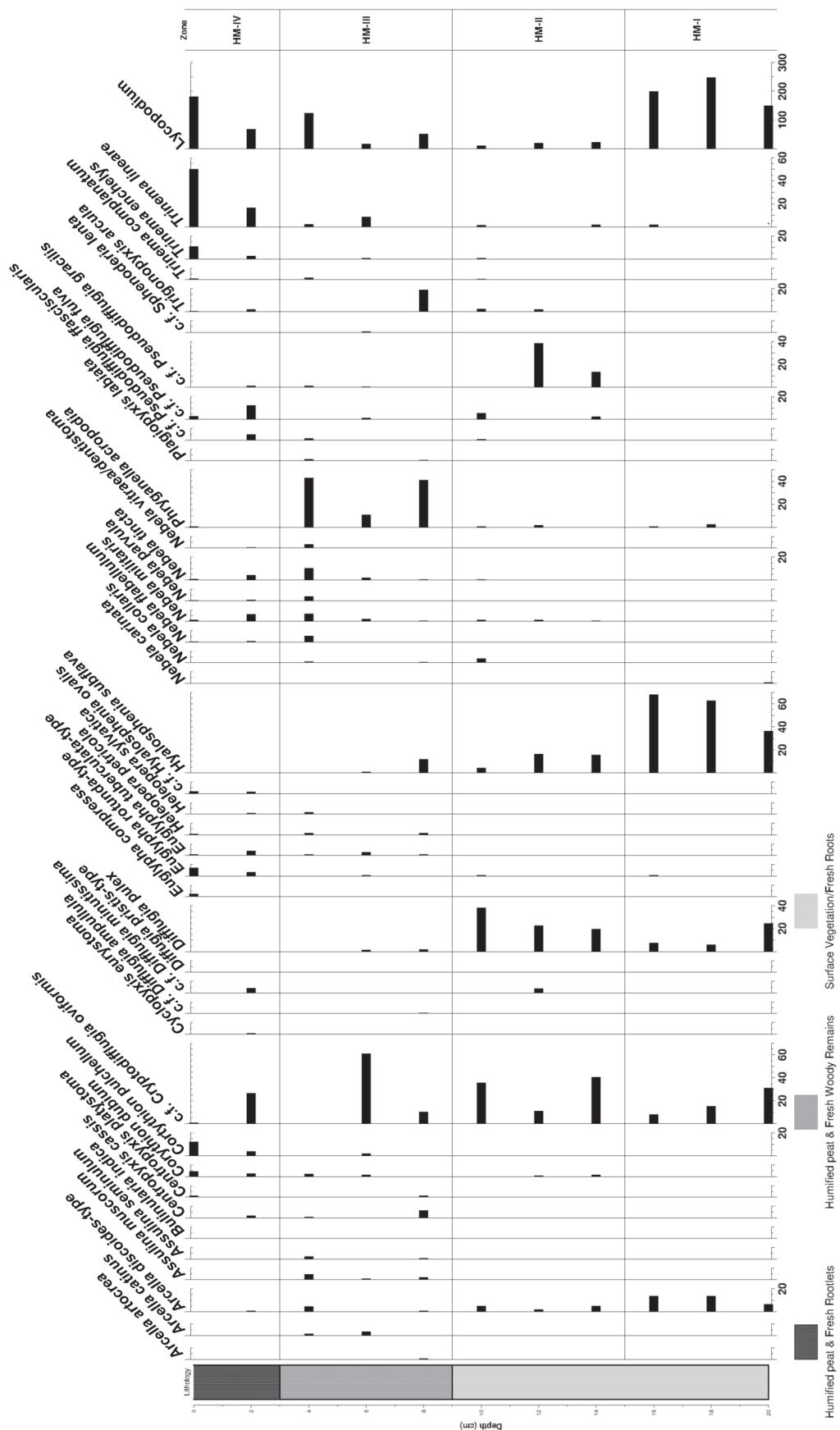
Fig. 5. Testate amoebae from the top 20 cm of Holcroft Moss, data are presented as percentages of the total testates in each level. The diagram has been subdivided into zones (HM I – HMIV) to better aid interpretation. The zones were defined by a combination of simple inspection and multivariate analysis.

well in peats. Although *Trinema lineare* has a similar test construction to *Euglypha* spp. it appears to survive better in peat cores (Mitchell *et al.* 2008) and so differential preservation is unlikely to be the explanation for its presence high in the core and rarity at greater depths. *T. lineare* is usually considered to be a species that prefers relatively dry conditions (Charman *et al.* 2000) but its distribution in the core does not match that of other dry indicators and its significance is not obvious. In summary the testates suggest a dry bog in the historically recent past becoming wetter (and more species-rich in testates) starting around the time of the construction of a major road apparently altering run-off from the bog surface. The surface samples from the 'path' site (Fig. 6B) also show a 'dry' signature – presumably because the absence of vegetation allows the path to dry out in summer more than the surrounding vegetated bog surface.

Other NW England sites

In addition to the studies at Holcroft Moss we (SRD and DMW) have previously studied the testate amoebae of two other bogs in the area – Danes Moss and Astley Moss – both sites under conservation management (Davis 2001; Davis and Wilkinson 2004, 2005). These sites were extensive areas of peat that had a long history of small-scale peat extraction and drainage before management for nature conservation started in the second half of the 20th century. Here we very briefly summarise the key points of this work as it relates to the use of testate amoebae as a monitoring tool in peatland con-

servation. Both of these sites have much less recent peat than Holcroft with only approximately 30 cm of peat from the last 2,000 years – suggesting either very slow peat growth and/or some missing peat due to past land use. At both sites a main core and two subsidiary cores were taken (each core 20 m apart). The subsidiary cores largely followed the patterns of changes in testate taxa shown by the main cores (Davis and Wilkinson 2004).

Danes Moss (Figs 1 and 2) is one of the largest peatlands in northern Cheshire, situated towards the east of the county at the foot of the Pennine Hills (Tallis 1973). In the 1970's Danes Moss had a vegetation dominated by purple moor-grass *Molinia caerulea* tussocks. In 1974, as part of the conservation management of the site, the water level was raised by blocking the outfall and in addition streams bringing in nutrient rich water from surrounding agricultural land were diverted. A vegetation survey in 1987 found *Sphagnum* developing on some of the waterlogged tussocks (Meade 1992). Our peat cores were collected a decade later, in 1999, and the surface samples contained testates associated with wet conditions (especially several species of *Euglypha*) but not taxa that would be considered characteristic of an 'undamaged' raised bog (Davis and Wilkinson 2004). This suggested a partial success for the management – the bog was wetter, but not necessarily approaching its 'pre-damage' condition.

Astley Moss (Figs 1 and 2), is part of the Chat Moss complex of peatlands, and the testates told a similar story. This site has been under conservation management for less time than Danes Moss – becoming a nature re-

A

B
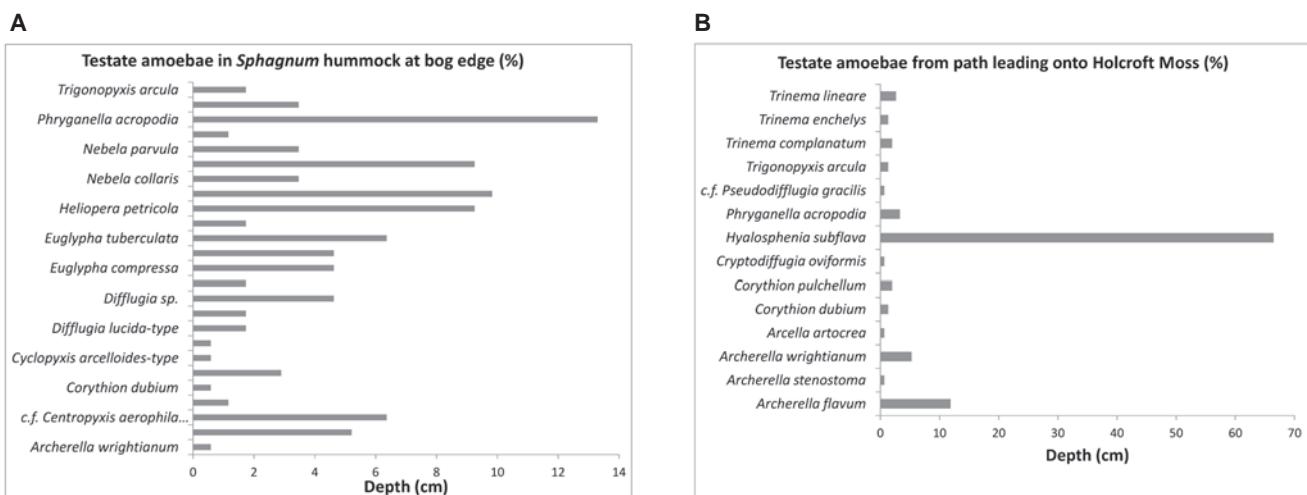
Fig. 6. A: Surface testate samples from a raised 'hummock' site on Holcroft Moss. B: Surface testate samples from bare peat on a path at Holcroft Moss. Note that in the older literature (including all the more accessible identification guides) *Archerella flavum* is refered to as *Amphitrema flavum*.

serve in 1987. As with Danes Moss conservation management included ditch blocking to increase bog surface wetness. The surface samples in our cores (collected in 1998) again showed a wet site (e.g. large numbers of *Arcella discoides*) but not the mix of taxa which would be associated with a relatively natural bog surface. We previously concluded that at Astley 'The current testate amoebae assemblage shows that management has made the site as wet as at any time over the last 2,000 years but has failed to achieve typical raised mire conditions' (Davis and Wilkinson 2005, p. 50). However, at this site there seems to have been significant changes in the testate community around 2,300 years ago associated with natural processes such as climate change and possibly a bog burst event – so it is over 2,000 years since the site had a testate community typical of a British raised bog. Since the publication of our Astley Moss work another hypothetical bog burst event has been invoked to explain similar changes in peat core testate amoebae from Derryville Bog in Ireland (Gearey and Caseldine 2006).

Ireland: Ardagullion Bog, Co. Longford

The three cores taken from this site were intended to sample a range of different bog microforms of differing wetness (Table 1). An uneven microtopography was an important characteristic of many bogs in Ireland and Britain prior to drainage. These microforms range from hummocks (sometimes 50+cm above mean water level) to wet hollows, 'lawns,' and bog pools (Belyea and Clymo 1998). This microtopography provides a range of different microhabitats on the bog surface – for example the testates *Amphitrema stenostoma* and *A. wrightianum* are often associated with bog pools while *Nebela militaris* is more likely to be found in hummocks (Charman *et al.* 2000). At a site like Ardagullion bog which has surviving microtopography clearly multiple cores may be desirable to establish the success of conservation management.

Table 1. Details of the microforms sampled by the three cores from Ardagullion Bog, Ireland.

Site	Core	Brief description
Ardagullion Bog	ARD 1	Dryish hummock
Ardagullion Bog	ARD 2	Wettish hollow
Ardagullion Bog	ARD 3	Between pools; wet

All three cores (Figs 7–9) were broadly comparable and exhibited evidence of rewetting probably connected to the conservation management at the site. Two of the cores (ARD1 and ARD2) include basal zones with high levels of typical 'healthy' bog taxa, taken to represent baseline conditions prior to drainage. Key 'bog' taxa at this site are the species of *Amphitrema* and *Archerella*, all of which are associated with wet conditions often in bog pools (Charman *et al.* 2000). Note that *Archerella flavum* used to be placed in the genus *Amphitrema* – and although a species of wet conditions it is usually found in less wet situations than *Amphitrema wrightianum* (Gomaa *et al.* 2013). In all three cores a middle zone exists in which, while present, these typical bog taxa are part of a broader assemblage including many xenosomic taxa (i.e. taxa that make use of particles from their environment in test construction such as *Difflugia* spp. and *Heleopera* spp.) – possibly suggesting increased mineral input onto site or nutrient enrichment. In all three cores the presence of *Amphitrema* and *Archerella* are at times low and the assemblage in this middle zone is clearly not that of a 'typical' active raised mire. Finally, in all three cores the uppermost zone includes clear and significant evidence of rewetting. In ARD 1 (Fig. 7) and ARD 2 (Fig. 8) this appears to have been particularly effective and achieved a level which has enabled successful recolonisation of *Amphitrema* and *Archerella* and a return to something closely resembling an active raised mire assemblage (albeit with high levels of xenosomic taxa remaining). The fact that these changes are apparent only in the top few centimetres of these cores strongly suggests that they are a response to the recent conservation management. In ARD 3 (Fig. 9) there is some evidence of a similar progression but on a drier scale – suggested by the dominance of *A. muscorum*.

In summary at Ardagullion Bog the recent management by tree felling and drain blocking appears to have been successful in making the site wetter. In two of our three sampling sites this has returned the testate community to one similar to a relatively undamaged bog while at the third site (ARD3) it has probably made the site much wetter, but without creating what might be considered a 'text book' bog testate community. The success at this site may be partly down to the limited nature of past damage. For example the scarcity of *H. subflava* (compared to the NW England sites) suggests the bog surface did not dry out as much in the past.
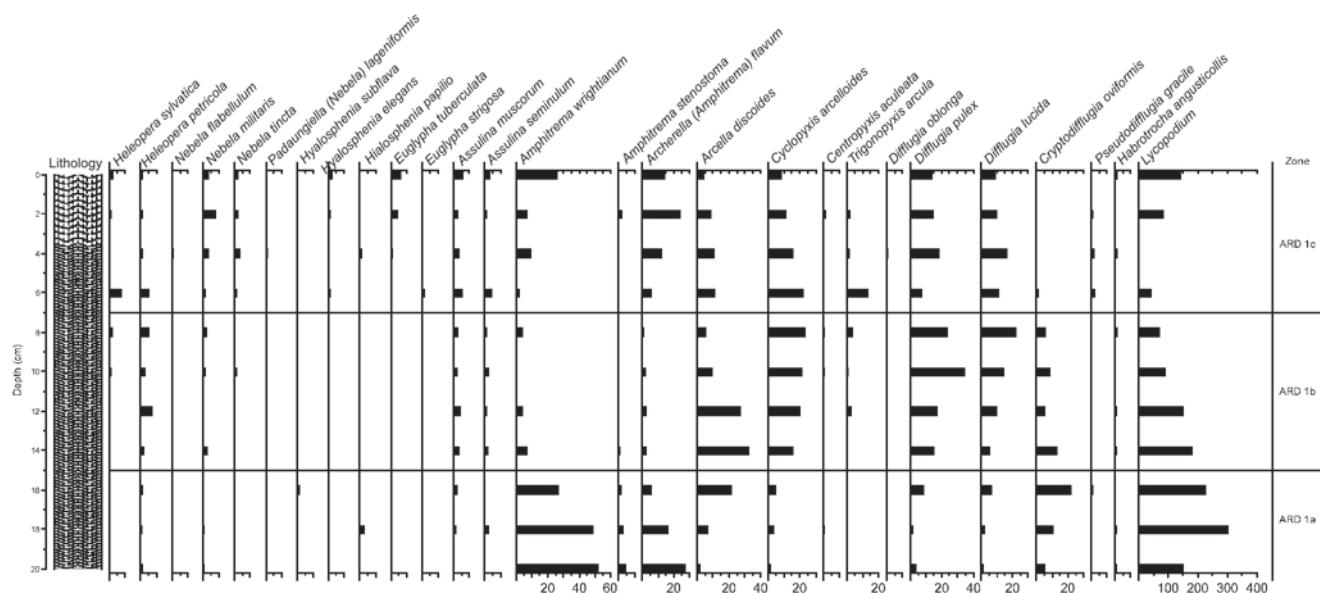
Fig. 7. Core ARD 1 selected percentage testate amoebae diagram, data are presented as percentages of the total testates in each level. The diagram has been subdivided into zones to better aid interpretation. Note that in the older literature (including all the more accessible identification guides) *Archerella flavum* is refered to as *Amphitrema flavum* and *Padaungiolla lageniformis* is called *Nebela lageniformis*.
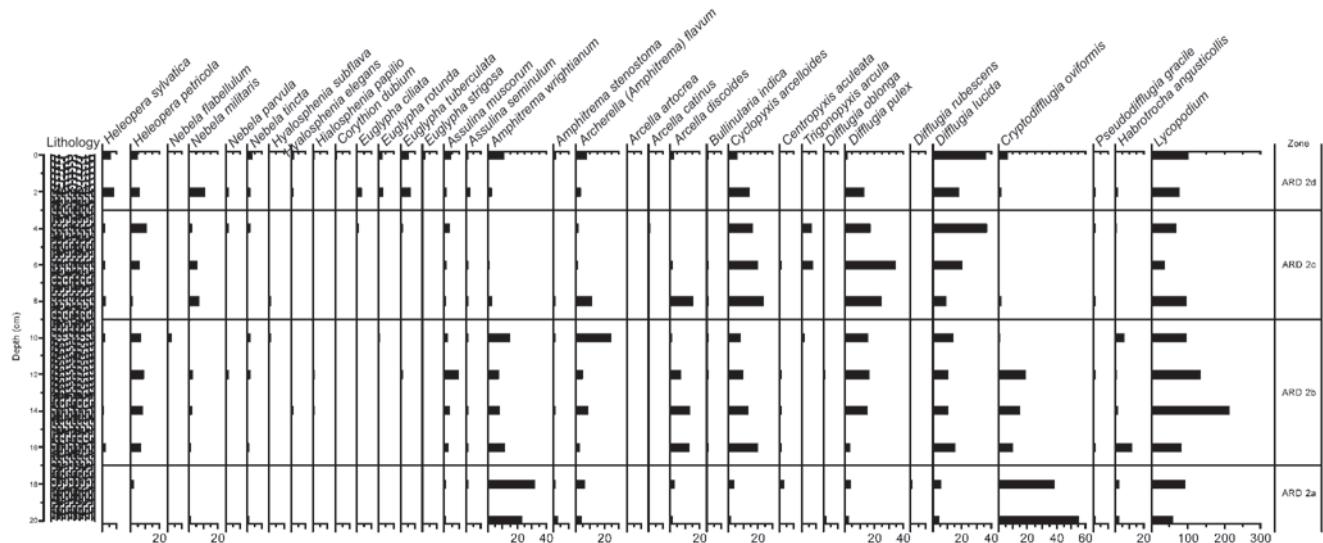
Fig. 8. Core ARD 2 selected percentage testate amoebae diagram, data are presented as percentages of the total testates in each level. The diagram has been subdivided into zones to better aid interpretation. Note that in the older literature (including all the more accessible identification guides) *Archerella flavum* is refered to as *Amphitrema flavum*.
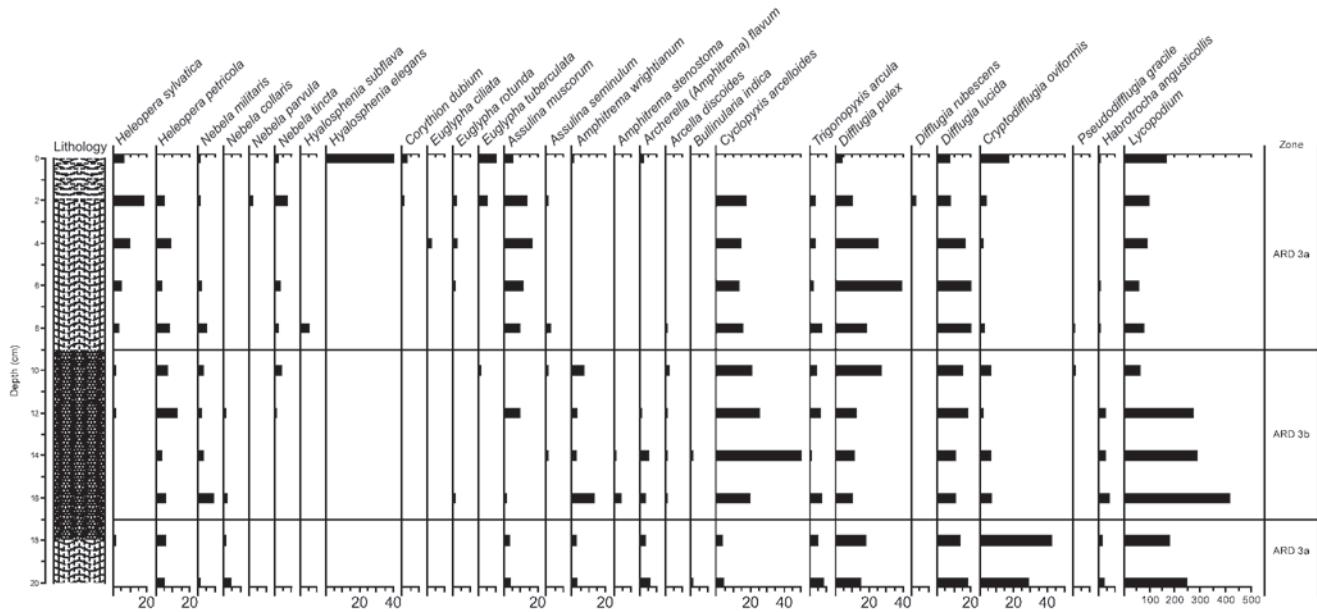
Fig. 9. Core ARD 3 selected percentage testate amoebae diagram, data are presented as percentages of the total testates in each level. The diagram has been subdivided into zones to better aid interpretation. Note that in the older literature (including all the more accessible identification guides) *Archerella flavum* is refered to as *Amphitrema flavum*.

DISCUSSION

Testate amoebae have been used to monitor a range of environmental factors including heavy metal pollution (Nguyen-Viet *et al.* 2007), nitrogen pollution (Payne *et al.* 2012), different types of farming practices (Heger *et al.* 2012) and the success of floodplain restoration schemes (Fournier *et al*. 2012). Several studies have used testate amoebae to monitor the success of bog rewetting through either natural processes following the abandonment of relativity small scale peat extraction (Buttler *et al.* 1996, Laggoun-Défarge *et al.* 2008) or active restoration schemes involving ditch blocking, tree removal etc. (Jauhiainen 2002, Davis and Wilkinson 2004, Vickery 2006). All these studies concluded that testate amoebae were at least of some use in investigating the success of the regeneration of 'damaged' bogs and similar conclusions can be drawn from the case studies described in this paper. However, this does not necessarily mean that testates provide a sensible approach to monitoring peatland restoration schemes. The counting and identification of tests under a microscope

is a relatively slow process and recently several authors investigating non-peat bog systems have questioned if the time consuming nature of this work was matched by an appropriate degree of utility in the results for environmental monitoring purposes (Heger *et al.* 2012, Payne *et al.* 2012). One situation where there may be a great benefit from using testates is when a particular bog is lacking long term monitoring data (e.g. water table data from dip well etc.), or if a bog is too remote to make regular visits to monitor water table feasible. For example in the case of Holcroft Moss, testates have allowed us to identify the likely role of spoil dumping from road construction in altering the hydrology of the site, and for Astley Moss testate data – combined with plant macrofossil data – was able to establish that the site had been hydrologically unstable, and possibly not *Sphagnum* dominated, over the last 2,000 years. This was a result that could potentially inform decisions about the targets for restoration – although in this case it made no difference to the management decisions for this site (Boyce 2000).

If a key advantage of using testates is that peat core data can extend information on bog surface wetness into

the past then it would appear useful if these data could be made more quantitative to compare with the numerical data from current dip wells on a bog surface and other hydrological measurements. A potential approach to doing this is available in the Quaternary palaeoecological literature. In the use of testates from peat cores in reconstructing bog surface wetness as an approach to studying past climate changes it has become common to use statistical relationships (transfer functions) between testate communities and depth of water table to provide quantitative reconstructions (e.g. Woodland *et al.* 1998, Amesbury *et al.* 2008, Amesbury *et al.* 2012). However, so far, these quantitative approaches have not been used in studies whose main focus has been the use of testates to monitor and/or inform bog restoration schemes; as with the present study these have used more qualitative approaches to inferring bog conditions from testate data (Buttler *et al.* 2004, Jauhiainen 2002, Davis and Wilkinson 2004, Laggoun-Défarge *et al.* 2008). One reason for a lack of quantitative approaches may be the feeling that they cannot offer the precision that would be required by conservation managers; for example Booth *et al.* (2010/11) suggest that even with good data, testate based water table reconstructions have a mean error of 6–8 cm. In addition several recent studies have suggested that the construction and use of such statistical relationships between testates and water table are more problematic than has been assumed in the past (Payne *et al*. 2011a, b).

Until our understanding of bog testate community ecology and the correct statistical approaches to use in inferring water table data improves, we believe that the more qualitative approach utilizing key indicator taxa is as robust as any. This is a controversial topic with one referee supporting our approach while another was very firmly of the opinion that these more quantitative approaches should be applied to data of this type. However, non-transfer function approaches also have the advantage of potentially requiring less taxonomic knowledge on the part of the person counting the testates as they can still function even if all the taxa making up the community have not been identified. For example *H. subfava* is a key indicator of a bog surface in poor condition at many sites (for example it still dominates at Holcroft in surface samples from eroded paths on the bog surface which will tend to dry out in summer – Fig. 6B). For 'undamaged' or successfully 'restored' bogs *Archerella flavum* and *Amphitrema wrightianum* appear to be a good indicator of an undamaged *Sphagnum* rich bog (as well as being

at Ardagullion Bog these were common at both Astley Moss, Danes Moss and Holcroft Moss in the past – being present deeper in the peat cores from these sites). In the Jura Mountains in Switzerland *Nebela tincta* was found to be a good indicator of an undamaged peat bog (Laggoun-Défarge *et al.* 2008); however, this species shows a range of variation (Kosakyan *et al.* 2012) and so may potentially contain multiple taxa with different ecological preferences. Potentially a small set of easily recognised testates could make relatively rapid testate amoebae analysis – of either surface samples of older peat – practical for a range of conservation bodies. A similar idea has been suggested for using testates to monitor the effects of invasive *Rhododendron* shrubs on woodland soil where there are some evidence that *Trigonopyxis arcula* can be used as an indicator taxon (Sutton and Wilkinson 2007, Vohník *et al*. 2012). In addition Wilkinson and Davis (2000), in a paper that included some data from the Astley Moss peat core, showed that at least some environmentally useful data can be found in testate data sets even if the taxa are only identified to genus level – something that requires little taxonomic expertise on the part of the person collecting these data. In addition there is also limited data to suggest that a simple ratio of live to empty tests may be informative in monitoring peatland restoration – unfortunately the full details of this work (Vickery 2006) seems to have never been formally published. Clearly there are potentially a range of options available for using a 'quick-and-dirty' approach to monitoring restoration with testates – but further work would be required to fully validate these approaches.

It is apparent that there are two broad approaches to using testate amoebae to monitor bog regeneration illustrated by the case studies in this paper and the small existing literature on the topic. One is to use testates from peat cores to investigate changes in the recent past and/or establish a baseline 'pre-damage' condition from several 10's to a few 1,000's of years ago – this has been the main focus of the case studies in this paper and is discussed extensively above. Alternatively, or in addition, modern surface samples can be used to monitor current conditions on the bog surface – for example the surface samples from Holcroft Moss described in this paper, or those from the Swiss Jura (Laggoun-Défarge *et al.* 2008) or from the work of Vickery (2006) at a number of UK sites. A related example is the use of bog surface testates to monitor the effects of experimental pollution treatments with sulphates (Payne *et al.* 2010). One potential option for the future is to use

DNA data rather than morphological identification under a microscope to census bog surface testates (e.g. Lara 2011). Heger *et al.* (2012) have already suggested this may turn out to be a more time efficient approach when using both testates and diatoms to monitor the state of agricultural soils. Another potential advantage with this approach is that it could allow the use of other protist (or prokaryotic) taxa which are more difficult to identify during direct counts under a microscope. For example Mieczan (2010) showed differences in the distribution of ciliates, as well as testates, on the surface of Polish peat bogs; ciliates are harder to enumerate by direct microscopy than testates and so molecular methods could be potentially useful. However, one of the advantages of testates in monitoring peat bog restoration and management is that 'their analysis does not require expensive equipment or consumables' (Laggoun-Défarge *et al.* 2008, p. 726). This is clearly not the case with molecular approaches – so we consider it likely that microscope based methods will continue to be those most often applied to practical conservation studies (but with molecular methods potentially important in more research focused work on the microbial ecology of restored bogs).

As well as providing site specific management information, testate amoebae data have the potential to inform peat restoration schemes more widely. In this context we conclude by briefly summarising what we see as the key wider lessons from both the case studies described in this paper, and the wider literature of similar work. Firstly these data provide evidence to support the common sense view that sites with the most limited drainage and other modifications will be more easy to restore – either purely through natural succession as at La Chaux d'Abelin the Swiss Jura (Laggoun-Défarge *et al.* 2008) or management interventions as at Ardagullion Bog, Co. Longford (this paper). La Chaux d'Abelin is particularly interesting in showing that in some cases no active management may be required to restore a bog surface (but note the climatically favourable location for peat formation high in the Swiss Jura). For the more seriously 'damaged' sites (e.g. our NW English sites; Astley Moss, Danes Moss, Holcroft Moss) restoration has been successful in making the sites much wetter on a decadal time scale, but not necessarily in returning the testate community to one typical of an undamaged bog – presumably in part because of the scarcity of *Sphagnum* on the current bog surfaces. In this context it is worth reiterating the point that the peat core testate data

suggests the possibility that *Sphagnum* may have been rare at Astley Moss for much of the last 2,000 years so its rarity may not be a product of human disturbance. This raises interesting questions for restoration schemes about how typical is a 'typical bog' and should such an abstract concept always be used as the model to try and match during restoration.

In the context of the more large scale engineering approaches to peatland restoration Holcroft Moss is an especially interesting site, because the lead record allows us to date the changes of the last few decades in the peat core. This strongly suggests that the creation of an unintentional 'bund' from road construction debris made the site wetter (so providing evidence that intentional bunds may be useful on sites that have suffered significant drainage) and also shows that these changes in testate amoebae communities in response to increased bog surface wetness can happen on a sub-decadal scale. So even with the limited number of studies currently available there are some general lessons that can be drawn from testate monitoring and applied to a wider range of peatland restoration schemes.

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