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Testate amoebae response to acid deposition in a Scottish peatland.

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ABSTRACT

Peatlands around the world are exposed to anthropogenic or volcanogenic sulphur pollution. Impacts on peatland microbial communities have been inferred from changes in gas flux but have rarely been directly studied. In this study the impacts of sulphuric acid deposition on peatland testate amoebae were investigated by analysis of experimental plots on a Scottish peatland almost seven years after acid treatment. Results showed reduced concentration of live amoebae and changes in community structure which remained significant even when differences in pH were accounted for. Several possible explanations for the impacts can be proposed including taphonomic processes and changes in plant communities. Previous studies have inferred a shift from methanogenic archaea to sulphate reducing bacteria in sulphate-treated peats; it is possible that the impacts detected here might relate to this change, perhaps through testate amoeba predation on methanotrophs.

KEYWORDS: Protists, Mires, Wetlands, Volcanic Impacts, Sulphate deposition, Methanogenesis.

INTRODUCTION

Many peatlands in, or downwind of, industrialised regions have been exposed to acidic sulphur pollution over recent centuries. Impacts have been suggested in terms of changes to the pH (Proctor and Maltby 1998, Skiba *et al.* 1989) and

decomposition rates of peats (Hemond et al. 1980, Sanger et al. 1994), DOC flux (Sanger et al. 1994), methane production (Nedwell and Watson 1995; Watson and Nedwell 1998; Gauci et al. 2002) and the metabolic processes (Ferguson and Lee 1979), growth rate (Ferguson and Lee 1980; Rochefort et al. 1990) and community structure (Tallis 1964; Ferguson and Lee 1980) of peatland plants. The potential scale of such impacts is nowhere more apparent than the Pennine blanket mires of northern England where heavy sulphur-loading combined with other pollutants since the beginning of the industrial revolution has led to the near-total elimination of *Sphagnum* and consequent drastic landscape change (Tallis 1964; Ferguson and Lee 1983; Lee 1998).

Although impacts of sulphur deposition on peatland microbial communities have been inferred from changes in gas flux, few studies have directly investigated microbial community change. This study focuses on testate amoebae, a group of unicellular micro-organisms (protists) which are highly abundant in damp to fully aquatic habitats around the world, and particularly in peatlands. Testate amoebae are increasingly being recognised as an important component of many ecosystems by virtue of their high abundance (up to 30% of microbial biomass in peatlands: Mitchell et al. 2003) and rapid turnover (e.g. Aoki et al. 2007). As testate amoebae lie towards the top of the microbial foodweb and as a group have broad feeding preferences (Gilbert et al. 2000) it is likely that testate amoebae will be sensitive to changed abundance and community structure in many groups at lower trophic levels.

Recent studies have highlighted the sensitivity of testate amoebae to pollution, including deposition of heavy metals (Patterson et al. 1996; Reinhardt et al. 1998; Nguyen-Viet et al. 2007; 2008), nutrients (Gilbert et al. 1998a&b, Mitchell 2004), and atmospheric pollutants (Nguyen-Viet et al. 2004, Balik 1991). This work suggests both the potential of testate amoebae for biomonitoring and also that pollution, both anthropogenic and natural, may complicate the use of testate amoebae as indicators of other variables.

Given the potential impacts of sulphate deposition on both the abiotic and biotic environment of testate amoebae in peatlands it seems probable that sulphate deposition would lead to changes in abundance and community structure. There is some evidence from field surveys for a relationship between testate amoeba communities and sulphate concentrations. Opravilova and Hajek (2006) and Mitchell et al. (2000b) found sulphate to explain a statistically significant proportion of

variance in testate amoeba communities. Swindles et al. (2009) looking at peatlands in northern Ireland and Lamentowicz et al. (2008) looking at a peatland in Poland found sulphate was not a significant environmental variable. In the Polish study this result might be explained by limited variance as only one site was considered, in the northern Irish study the result might be explained by low sulphate concentrations in relatively unpolluted peatlands. Other possible evidence for a relationship between SO_4 and testate amoebae comes from an association with Sr, which is correlated with SO_4 in separate analyses, in an Israeli wetland (Payne et al in press).

In an experimental approach Payne et al. (2009) investigated the testate amoeba communities of experimental plots in a Scottish peatland subject to sulphate deposition. Sodium sulphate was applied for a period of 18 months and 25 samples extracted from each of six plots (three treated and three control) after more than ten years. Results showed statistically significant differences between treated and control plots, particularly characterised by reduced abundance of small bacterivorous taxa (*Euglypha rotunda* type, *Corythion dubium*, *Trinema lineare* and *Trinema complanatum*). Also apparent was a reduced concentration of live amoebae and proportion of tests occupied by living amoebae.

In the pre-industrial era the heaviest sulphate loadings on peatlands would have derived from volcanic eruptions. Such impacts are little-considered but the historical record shows extremely severe impacts of volcanic acid-loading on plant communities even at great distance from volcanic sources (Grattan and Charman 1994; Grattan and Gilbertson 1994; Grattan and Pyatt 1999) and the presence of (crypto)tephra deposits preserved in peatlands around the world testifies to the large areas of peatlands which are within reach of volcanic products. Some palaeoecological records have shown testate amoeba community changes coincident with tephra deposition which might represent a response to volcanogenic sulphate deposition (Dwyer and Mitchell 1997; Payne and Blackford 2008). Although contemporary volcanic sulphate emissions contribute a minority of total sulphur emissions these still constitute a major supply of sulphur to peatlands in many regions (Langmann and Graf 2003).

The experimental study of Payne et al. (2009) may not be a good analogue for the impacts of volcanic sulphate on peatlands. Sulphate was applied over a period of eighteen months and although it is possible for volcanic eruptions to produce extended periods of sulphate deposition (as for the well-documented 1783-4 eruption

of Laki in Iceland), in most distal regions sulphate deposition episodes will be much briefer lasting a matter of hours, days or weeks. Furthermore, the sulphate was applied as sodium sulphate. In real volcanic eruptions much of the sulphur deposited is likely to be as sulphuric acid. By applying only sodium sulphate the previous study did not simulate plant mortality and morbidity, which may well result from volcanic acid deposition and would be likely to affect microbial communities. The use of sodium sulphate also makes it difficult to entirely exclude the possibility that impacts arose from the application of sodium rather than sulphate.

This study aims to determine the impact of sulphuric acid deposition on peatland testate amoeba communities with a particular focus on the possible response to volcanogenic pollution events. The study uses previously established experimental plots on a Scottish peatland, comparing the testate amoeba communities of a plot treated with sulphuric acid with control plots.

SITE and METHODS

Experiments were conducted on the Moss of Achnacree, a large raised bog in Argyll and Bute, western Scotland (UK Grid Reference NM9134). Peat deposits cover around 7 Km^2 and average 1.9m depth, the area has a cool temperate climate with an annual rainfall of around 1500mm. Major plant species of the site include *Calluna vulgaris, Eriophorum vaginatum, Cladonia portentosa* and various *Sphagnum* species (including S *capillifolium, S. magellanicum* and *S. papillosum*). The site has been subject to some peripheral peat-cutting and areas of the site have been drained for agriculture. Experiments were conducted in an uncut area towards the west of the main peat area, approximately 100m from South Ledaig Farm (Fig. 1).

A sequence of fourteen, 1x1m plots was established on the site and plots subjected to deposition of acids and/or volcanic tephra in May 2002. Experiments were designed to approximate possible acid deposition in Scotland following the 2310 ± 20 BCE (Pilcher et al. 1995) eruption of Hekla in Iceland (Hekla-4), which has been implicated in major vegetation change (Blackford et al. 1992). Scenarios were derived by extrapolating the scenario of Grattan and Gilbertson (1994) to the highest levels of tephra deposition noted in northern Scotland (see Payne and Blackford 2005 for details). The plots were re-visited at regular intervals over the subsequent two years and observations of plant communities and measurements of various environmental

parameters undertaken (Payne and Blackford 2005, Payne et al. 2005). Drastic impacts on plants were noted with most plants killed in the heaviest treated plots, but the cover-estimates were insufficiently precise to allow small abundance changes to be monitored (Payne and Blackford 2005). For selected plots testate amoeba communities were also analysed through the experimental period but no consistent changes were noted. Reasons for this lack of detectable response probably include an insufficient sampling density to account for the high spatial variability of testate amoeba communities and an inadequate time period given that most of the tests counted were probably accumulated prior to the experimental period (Payne and Blackford 2005). This study attempts to account for these problems by analysing the amoeba communities of experimental plots almost seven years after acid deposition and using a much higher sampling intensity.

At the end of the main study period in 2004, the experimental infrastructure was removed in accordance with an agreement with the then landowner. The site was revisited in April 2009, almost seven years after establishment of the experiments. Of the three plots with the heaviest sulphuric acid treatment (0.7 mol m^{-2}) only one could be positioned with sufficient accuracy. This plot (MAC11) was not subject to tephra deposition. The impacts of the experimental treatment on plant communities could still be readily determined with more bare ground than surrounding areas, no *Sphagnum* and only immature *Calluna vulgaris* (see Table 1 for species composition). 20 samples of approximately 2x2x5 cm of surface peat were extracted from across the experimental plot and returned to the laboratory. At each sampling spot depth to water table (DWT) was determined by making a small hole and measuring DWT after at least an hour for the water table to equilibrate. Plant species in the immediate vicinity of the sampling location were also recorded. A further 10 samples were extracted from one of the control plots established in the original study (MAC2) and treated with only deionised water. This plot is 8m from MAC11 while testate amoeba communities have been shown to exhibit spatial variability on a very fine scale (Mitchell at al. 2000a). To account for this, 20 further samples were extracted from an additional $1m^2$ area (here termed MAC30) situated 1 m N of MAC11. This area was not a control plot in the previous studies so has not been subject to the disturbance in previous sampling that plots MAC11 and MAC2 have experienced. All plots are situated on hummocks and in most cases surface peat samples consisted of relatively dense, humified peat.

In the laboratory approximately 1 cm^3 of the upper 1 cm of the samples (regardless of surface vegetation) was removed and volume measured by displacement in deionised water. Samples were made up to 30ml with deionised water and pH measured after approximately two hours. Preparations for testate amoebae followed a method based on that of Hendon and Charman (1997) but without the use of back-sieving as recommended by Payne (2009). Samples were allowed to soak for 48 hours before being stirred to disaggregate the peat matrix but were not boiled to avoid killing live amoebae. Samples were subsequently sieved at 300μm and a *Lycopodium* innoculum added (Stockmarr 1971). The <300 μm fraction was retained and stored in water, samples were refrigerated until analysis. Slides were prepared by mixing a drop of the prepared sample with glycerol and examined at 400X magnification. 100 tests per sample were identified and counted (*cf*. Payne and Mitchell 2008) and tests with visible cytoplasm (termed 'live individuals' although truly live individuals could not be distinguished from tests with dead but undecayed cellular contents) differentiated from empty (dead) tests. Taxonomy follows Charman et al. (2000) except where modified by Payne et al. (2009).

Differences in amoeba concentration, proportion of occupied tests, species richness, diversity (Shannon's 'H') and environmental variables between the treated and control plots were tested using Mann-Whitney tests in PAST ver. 1.84 (Hammer et al. 2001). An initial test of the difference between the testate amoeba community of the treated and control plots used Analysis of Similarity (ANOSIM: Clarke 1993) with a Bray-Curtis distance measure. Subsequently the multivariate data was investigated using ordination techniques in Canoco vers. 4.53 (Ter Braak and Šmilauer 1997-2004). Species data were Hellinger distance transformed (Rao 1995; Legendre and Gallagher 2001) and taxa present in four or fewer samples were removed from the dataset. Initially the data structure was investigated using Principal Components Analysis (PCA); subsequently Redundancy Analysis (RDA) was used to test the significance of a nominal variable for experimental treatment. pH and DWT were introduced as co-variables to allow their influence to be accounted for. Significance was assessed using Monte Carlo permutation tests (999 permutations). These analyses were each applied to data based on percentages of all tests, percentages of live individuals, concentrations of all tests and concentrations of live individuals. As an additional exploration of the data structure and differences between

plots the percentage total tests data was subjected to cluster analysis using the Paired Group Method with a Bray-Curtis similarity matrix in PAST ver. 1.84.

As a test of testate amoeba community changes since the end of the previous studies, the amoeba community of plot MAC11 in 2009 was compared to previous analyses from six intervals between 2002 and 2004, beginning one month after treatment and continuing to 24 months after treatment (Payne and Blackford 2005). Due to the probable issues with intra-plot spatial variability in amoeba communities these samples were treated as a single group, ignoring any changes within that period. Only data based on percentage of total tests was used for these analyses. Taxonomic harmonisation was carried out and minor taxa eliminated from the dataset. Difference between the two datasets was tested using RDA, as above, including a nominal variable 'Age' for sampling period.

RESULTS

Twenty eight testate amoebae taxa (plus the rotifer *Habrotrochoa angusticollis*, which was included in calculations) were encountered in the 50 samples, of which the most abundant were *Assulina muscorum* (21.9% of total tests), *Nebela tincta* type (20.9%), *Corythion dubium* (9.4%) and *Phryganella acropodia* type (9.3%). Some differences in amoeba community between treated and untreated plots are relatively clear even in the overall abundance data (Table 2) including greater abundances of *Difflugia pristis* type, *Hyalosphenia subflava* and *Trigonopyxis arcula* in the treated plot and greater abundance of *Corythion dubium* in the control plots. There are also differences in abundance of some taxa between the two control plots (notably *Heleopera rosea*).

Mann-Whitney tests showed significant differences between treated and control plots for amoeba concentrations (whether based on total individuals or only on live individuals) and percentage of occupied tests ($P<0.001$). While the overall test concentration was higher in the treated plot, the concentration of live amoebae and the proportion of occupied tests were greater in the control plots. There was a significant difference in pH between the treated and control plots ($P<0.001$) with lower values in

the treated plot (Fig. 2), there were no significant differences in species richness or diversity between the plots.

Principal components analysis shows very clear differences between the treated and control samples. For data based on percentage of all tests axis one effectively divides the samples into two groups with very little overlap (Fig. 3), other datasets give similar results. There is good coincidence between the two sets of control samples (MAC2 and MAC30) with MAC2 samples having a slight tendency to higher scores on axis two. *Trinema lineare*, *Euglypha rotunda* type and *Corythion dubium* are positively correlated with pH and negatively correlated with the experimental treatment. *Hyalosphenia subflava*, *Difflugia pristis* type, *Trigonopyxis arcula*, *Heleopera petricola* and *Pseudodifflugia fulva* type are positively correlated with the experimental treatment and negatively correlated with pH. Post-hoc Mann-Whitney tests showed significant ($P<0.05$) difference in abundance (% all tests) between treated and control plots for all these taxa except *P.fulva* type.

In Redundancy Analyses the treatment nominal variable explained a significant proportion of variance with all datasets (Table 3). With 'treatment' the sole environmental variable in the analysis up to 18.4% of variance was explained ($P=0.001$). Both pH and DWT were also significant environmental variables but DWT lost significance when pH was partialled out, showing co-variance between the two. Consequently, only pH was used as a co-variable when testing the effect of the experimental treatment. With pH partialled out the treatment nominal variable explained between 4.8 and 6.7% of variance ($P=0.001$). More variance was explained using concentration data than percentage data, suggesting that there are absolute, not just relative changes in abundance. That strong relationships are apparent when using data based only on living individuals is slightly surprising given that counts were low (mean=10 individuals). ANOSIM shows statistically significant differences between treated and control samples using all data sets ($P<0.001$), R_{ANOSIM} varies between 0.29 and 0.45. Cluster analysis results show a general correspondence of identified groupings to treated and control plots but also quite marked differences among the samples of the treated plot with two samples clearly differentiated from all others (Table 4).

There is a significant difference between the 2009 and 2002-2004 testate amoeba community of plot MAC11, a nominal variable explains 24% of variance ($P=0.001$). Some of the differences between treated and control plots noted in the

analysis of 2009 data seem to be matched by changes over the period since previous analysis (Fig. 4). So, *Euglypha rotunda* type and *Corythion dubium* [the *Corythion-Trinema* type recorded in 2002-4 probably only represents *C. dubium*] are reduced in abundance both relative to the control plots and to the 2002-4 data. Similarly, *Difflugia pristis* type is much increased in abundance relative to the control plots and 2002-4 data. These changes could be taken as indicating a continuing impact of the experimental treatment in the period 2004-2009. However other changes are in marked contrast to the differences to the control plots, most notably *Hyalosphenia subflava* which in 2009 was more abundant in the treated than control plots, but much less abundant than in 2002-4. It is recommended that these results are treated with particular caution due to: 1. The small sample size of the 2002-2004 dataset. 2. The difference in sample preparation methods, with fine-sieving used in 2002-4 and likely to lead to underestimation of the abundance of the smallest taxa (Payne 2009), 3. The lack of data on concentrations and differentiation of live from dead individuals in the 2002-4 data, 4. The probability of changes occurring within the 2002-4 period. 5. The impact of non-treatment variables, particularly climatic variability over the intervening period.

DISCUSSION

It is important to recognise the limited scale of this experiment. Although the sampling intensity is relatively high there is no replication at plot scale as only one of the treated plots could be accurately located. Complications due to prior differences between plots cannot be ruled out and comparisons between plots may be complicated if accumulation rates differ so the samples represent differing time periods. Results should be treated with caution and further studies will be desirable to replicate the findings presented here. Furthermore, the extent to which the experimental scenario used here is an accurate representation of reality is also open to question (see discussion in Payne and Blackford 2005), these results should probably be viewed as indicating the nature of the testate amoebae response, but not necessarily the scale of the response.

However, with caveats stated, this study does provide interesting results. The difference between acid-treated and control plots emerges very strongly in the analyses. The unconstrained ordination plot shows a near-perfect divide between

treated and untreated samples along axis one and the constrained ordination shows that a treatment nominal variable explains a significant, and sizeable, proportion of variance with all datasets. Despite the limitations of the experimental design the initial similarity between the treated and control plots, the distinctiveness of the changes and the similarities of the results with the experiment of Payne et al. (2009, discussed below) strongly suggest that the differences between treated and control plots are due to the experimental additions and not to any prior differences.

The univariate data analyses show a statistically significant difference in both concentration of tests and proportion of occupied tests. However, while the proportion of occupied tests and concentration of live amoebae are less in the treated than control plots, the overall concentration of tests is greater in the treated than control plots. This presents a curious dichotomy, suggesting a less active amoeba community but higher concentrations of tests. As total test concentrations are dependent on the degree of decomposition of the peat matrix the explanation for this result might be that surface peat in the treated plots has decomposed more than in control plots, increasing apparent test concentration. Although enhanced decomposition is a conceivable impact of sulphuric-acid treatment this was not suggested by alkali-extraction determined humification of near-surface peats in 2002-4 (Payne and Blackford 2005). The reduced abundance of live testate amoebae here parallels response to nutrient and CO_2 enrichment in peatlands (Gilbert et al. 1998a&b, Mitchell et al. 2003, Mitchell 2004) and H_2SO_4 treatment in a simulated stream system (Costan and Planas 1986). It appears that a wide variety of environmental perturbations may lead to a reduced abundance of testate amoebae.

In both this study and that of Payne et al. (2009) the same three taxa are strongly negatively associated with the treatment: *Corythion dubium*, *Trinema lineare* and *Euglypha rotunda* type. By contrast there is little agreement in the taxa which respond positively. In this study the strongest positive response to sulphuric acid deposition was in *Difflugia pristis* type, *Hyalosphenia subflava* and *Trigonopyxis arcula*. In the experiments of Payne et al. (2009) the taxa showing strongest positive association with sodium sulphate treatment were *Hyalosphenia papilio, Arcella arenaria* type and *Cryptodifflugia oviformis*. Of these taxa *A. arenaria* type was absent and both *H. papilio* and *C. oviformis* were minor occurrences in this study (0.06% and 0.9% respectively). Of the taxa showing a positive response in this study, two (*D. pristis* type and *H. subflava*) were not found at all by Payne et al. (2009) and

the third (*T. arcula*) was a very minor presence, accounting for only 0.04% of total tests. The difference in detected response may therefore relate to initial differences in community composition between the sites.

The three testate amoeba taxa which are deleteriously affected by the experimental treatment (*E. rotunda* type, *C. dubium* and *T. lineare*) form a coherent ecological group. All three taxa are small, with idiosome tests and are believed to be largely or exclusively bacterivorous (Gilbert et al. 2000). There is relatively little information on the autecology of *T. arcula, H. subflava* and *D. pristis* type. The compilation of Gilbert et al. (2000) suggests *T. arcula* feeds on fungi and organic material. There is no information on the feeding preferences of *D. pristis* type and *H. subflava* but other species of *Difflugia* and *Hyalosphenia* have broad feeding preferences ranging from cyanobacteria to micro-metazoa. All of these three taxa are generally considered typical of dry conditions and are frequently found in hummocks. However, differences in wetness cannot explain the differences in abundance of these taxa observed here, there is no significant difference in DWT between plots MAC11 and MAC2 ($P=0.8$) while there are very significant differences in abundance of all these taxa ($P<0.005$). Curiously, the increased abundance of *D. pristis* type in these experiments is in marked contrast with the experiment of Costan and Planas (1986) where acidification with H_2SO_4 in a lotic system reduced *D. pristis* concentrations by an order of magnitude. However it should be noted that the difficulties in testate amoeba taxonomy, particularly in the genus *Difflugia*, are such that it is impossible to be certain that these are the same taxa in both studies, particularly given the difference in environment.

Several possible explanations can be proposed for the mode of impact of the experimental treatment on testate amoebae. The simplest possibility for reduced concentration of live amoebae and preferential loss of some taxa would be that they are unable to cope with acid-stress, possibly through H^+ interference with enzyme or membrane function. Costan and Planas (1986) speculate that acid-shock may perturb the osmotic regulation mechanism of testate amoebae leading to mortality. Over the initial two-years of the experiment there was no overall trend of increased acidity. pH values of samples from the treated plot here are lower than the control plots, but the pH of the treated plot is not highly acidic by the standard of ombrotrophic peatlands (even given the dilute measurement solutions). In the redundancy analysis ‘treatment’

explained variance independent of pH differences so acidification alone cannot explain the changes observed.

It is notable that the taxa most reduced in abundance have idiosome tests while the taxa most increased in abundance have secretion or xenosome tests. One possible explanation for this result could be decomposition of idiosome tests in a more acidic environment. Swindles and Roe (2007) and Payne (2007) have experimentally demonstrated the dissolution of such tests in strong mineral acids, and these tests are also disproportionately lost from the palaeoecological record (Mitchell et al. 2008). However, in this study reduced abundance of *E. rotunda*, *T. lineare* and *C. dubium* was also apparent when only considering live amoebae. Unless lower pH conditions somehow reduce the bioavailability of Si for test construction this counts against a taphonomic explanation for the changes.

Impacts on testate amoeba communities might be related to impacts on plant communities. Over the 2002-4 study period Payne and Blackford (2005) noted near-total plant mortality in plot MAC11 with no new growth noted until a year after acid treatment and differences still apparent when these samples were extracted seven years later. Although the relationships between plant and testate amoeba communities are under-researched the two are likely to be closely linked. In field surveys plant community composition explains variance in testate amoeba communities even when other major controls are accounted for (e.g. Payne and Mitchell 2007). Important mechanisms of plant influence on testate amoeba communities may include litter chemistry (Sutton and Wilkinson 2007); root exudates and the provision of physical niches (for instance the smallest taxa might be able to enter *Sphagnum* hyalocysts). Recent research by Vohník et al. (2009) has even suggested a possible impact of plant communities on testate amoeba taphonomy with mycorrhizal fungi associated with *Rhododendron* spp. using testate amoeba tests (particularly *Centropyxidae* and *Trigonopyxidae*) as a nutrient source. How plant community change would manifest itself on testate amoeba communities is uncertain. A related possibility is that enhanced supply of dead plant material might boost the abundance of testate amoebae which either directly feed on organic matter, or feed on lower micro-organisms that do. That *D. pristis type*, *T. arcula* and *H. subflava* are all associated with drier conditions and peat hummocks might suggest they could be associated with aerobic decomposition. *T. arcula* has been observed to directly feed on organic matter while

Hyalosphenia subflava is associated with drained peatlands where aerobic decomposition is active, which might support this idea (Tolonen 1986).

That the same taxa are deleteriously affected by H_2SO_4 deposition in this study as by Na_2SO_4 deposition in the study of Payne et al. (2009) suggests that the cause of this change is most likely the input of SO_4^{2-} rather than Na^+ or H^+ (either directly or indirectly). Recent studies have shown a reduction in methane efflux in sulphate-exposed peatlands (Dise and Verry 2001; Gauci et al. 2002). The mechanism for this change is believed to be sulphate reducing bacteria (SRB) out-competing methanogenic archaea (MA) for electron donors as using sulphate as an electron acceptor is a more energetically favourable pathway. A limited pulse of sulphate may produce a prolonged impact on methane production due to recycling of sulphur in the upper peat (Wieder et al. 1990; Gauci et al. 2005). In sulphate-treated plots on the Moidach More site studied by Payne et al. (2009) methane efflux suppression simultaneous with sulphate reduction has been demonstrated (Gauci et al. 2002; Gauci and Chapman 2006). While these processes were not directly investigated in the previous study of these experimental plots the distinctive odour of H_2S was noted during core extraction from plots subject to the heaviest H_2SO_4-treatment but not in any of the control plots during 2002-4 (Payne and Blackford 2005). It therefore appears that in this site too sulphate reduction has been stimulated. That the testate amoeba taxa most deleteriously affected in both studies are bacterivores indicates that the reduced abundance of these taxa may be due to a change in their food source. The changes in testate amoeba community in both studies may well be linked to the putative MA-SRB shift. The link between these prokaryotes and testate amoebae -if any exists- is unlikely to be direct predation as in theory anaerobic bacteria and archaea should not co-exist with aerobic protists (although the potential influence of hydrological variability and testate amoeba motility are uncertain). One possible mechanism linking the two groups could be testate amoeba predation of methanotrophs, recently demonstrated in naked amoebae and flagellates (Murase and Frenzel 2008).

The possible mode of impact of the experimental treatment on testate amoeba communities cannot be conclusively determined on the basis of this evidence alone. Several explanations are possible, however, the similarity in response to the study of Payne et al. (2009) does suggest a common forcing, and this common forcing could well relate to the putative MA-SRB shift.

That peatland testate amoebae respond to sulphate deposition appears increasingly clear. This suggests that testate amoebae might have a role as bioindicators, potentially allowing monitoring of both the effects of sulphate pollution on peatland microbial communities and subsequent recovery. The preservation of tests in peats may allow such processes to be studied over longer time-frames. However, this is likely to be complicated by selective test decomposition and the dominant control of hydrology. It would be of particular interest if a known testate amoeba response could be firmly tied to a MA-SRB shift as the preservation of tests in peats might then allow this response to be detected in palaeoecological sequences. Detecting any sulphate-signal in the palaeoecological record is likely to be difficult in practise and may not be possible other than where outside evidence (for instance the presence of a tephra layer or historical information on the occurrence of sulphate pollution) suggest the possibility. The increasing number of environmental variables suggested to be controls on testate amoebae communities urge against a simplistic view of palaeoecological data solely in terms of hydrological change. Non-hydrological controls are likely to be particularly important in peatlands exposed to air pollution over recent centuries.

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FIGURES

Figure 1. Location map of Moss of Achnacree site and relative position of treated plots within the experimental area.

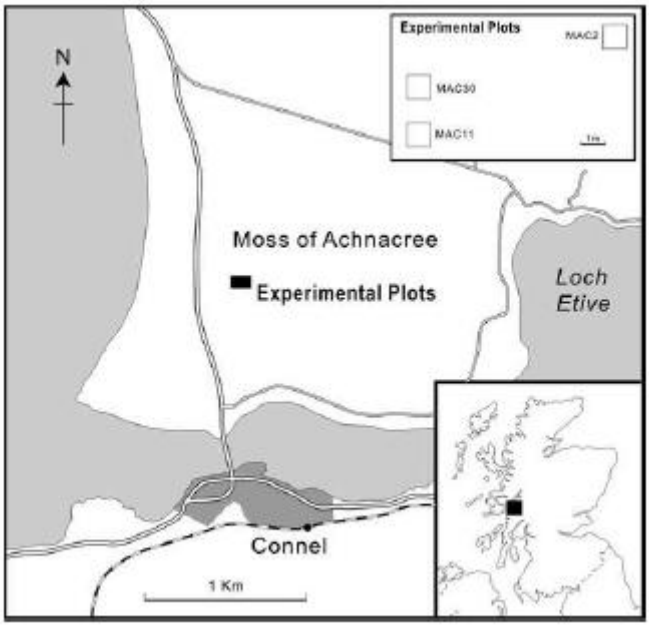


Figure 2. Environmental data for the three experimental plots, showing pH of peat suspension in water and depth to water table at time of sampling. Box plots show median (central line), first and third quartiles (grey box), tenth and ninetieth percentiles ('whiskers') and all outliers (dots).

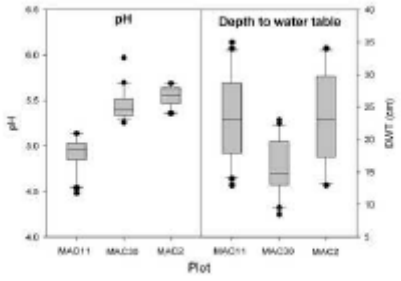


Figure 3. Principal components analysis of Hellinger-transformed testate amoebae data (percentages of all tests, excluding taxa $n \leq 4$) for samples from experimental plots. Filled circles show MAC11 samples (acid treated); triangles show MAC2 samples (control) and squares show MAC30 samples (additional control). Species codes:- AMUSC: *Assulina muscorum*, ASEM: *Assulina seminulum*, BIND: *Bullinularia indica*, CAERO: *Centropyxis aerophila* type, CDUB: *Corythion dubium*, DPRIS: *Difflugia pristis* type, EROT: *Euglypha rotunda* type, HPETR: *Heleopera*

petricola, HSUBF: *Hyalosphenia subflava*, NTINC: *Nebela tincta* type, PACRO: *Phryganella acropodia* type, PFUL: *Pseudodifflugia* type, TARC: *Trigonopyxis arcula*, TLIN: *Trinema lineare*.

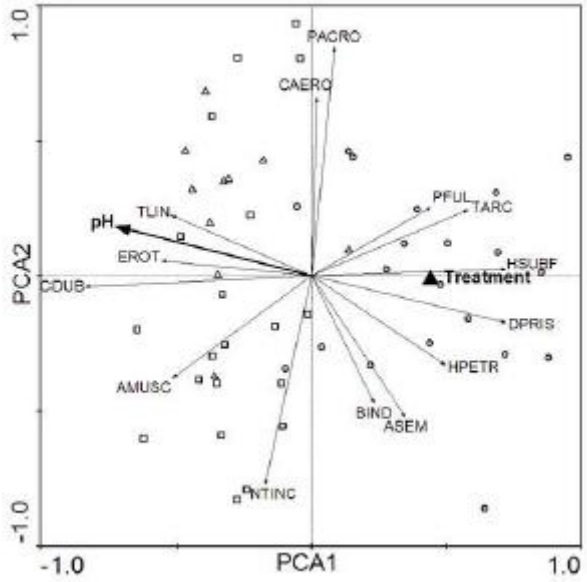
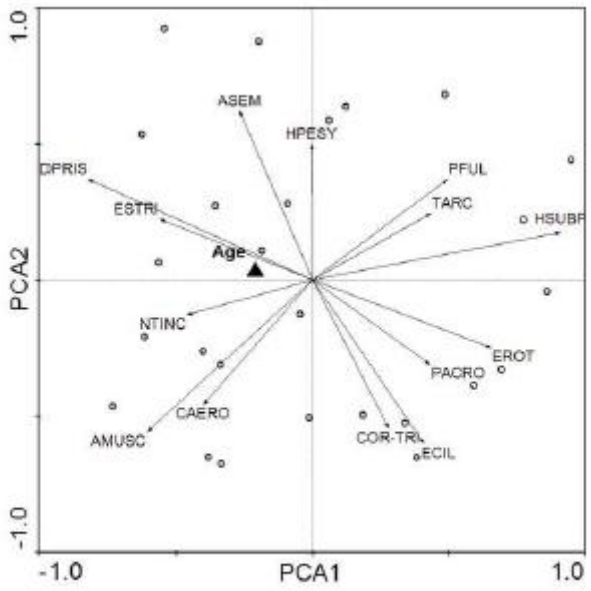


Figure 4. Principal components analysis of Hellinger-transformed testate amoeba data comparing samples from plot MAC11 in 2009 (filled circles) to samples from the same plot extracted between 2002 and 2004 (unfilled circles). 'Age' is a nominal variable for the 2009 samples. Species codes as for Fig.3 and Table 2 with the exception of 'COR-TRI' which shows a *Corythion-Trinema* type (following Charman et al. 2000 but probably only representing *C. dubium* here) and 'HPESY' which shows a grouped *Heleopera petricola- Heleopera sylvatica* type.



TABLES

Table 1. Plant species of the three experimental plots at the time of sampling.

Table 2. Relative abundance of major taxa[1] (over 1% total tests) in three plots: MAC11 (sulphuric acid treated), MAC2 (control) and MAC30 (additional control), see text for full details of experimental set-up. Also showing relative abundance of living individuals by taxon (in parentheses) and taxon abbreviations used in Figs. 3 and 4. Data for living individuals is based on small counts and should be treated with caution.

Table 3. Redundancy analysis of testate amoeba data showing percentage variance explained and P-values of these relationships assessed by Monte Carlo permutation tests (999 permutations).

Table 4. Results of cluster analysis (Paired Group Method using a Bray-Curtis distance measure) on % total tests data showing groups identified at the third level of division.

1 Table 1. Plant species of the three experimental plots at the time of sampling.
2

Plot	**No. Samples**	**Plant species present**
MAC11	20	*Calluna vulgaris, Eriophorum vaginatum, Aulacomnium palustre, Hypnum cupressiforme, Cladonia portentosa, Carex* (undiff.).
MAC2	10	*Calluna vulgaris, Eriophorum vaginatum, Aulacomnium palustre, Hypnum cupressiforme, Cladonia portentosa, Carex* (undiff.), *Sphagnum* (undiff.), *Odontoschisma sphagni.*
MAC30	20	*Calluna vulgaris, Eriophorum vaginatum, Aulacomnium palustre, Hypnum cupressiforme, Cladonia portentosa, Sphagnum* (undiff.), *Odontoschisma sphagni.*

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Table 2. Relative abundance of major taxa[1] (over 1% total tests) in three plots: MAC11 (sulphuric acid treated), MAC2 (control) and MAC30 (additional control), see text for full details of experimental set-up. Also showing relative abundance of living individuals by taxon (in parentheses) and taxon abbreviations used in Figs. 3 and 4. Data for living individuals is based on small counts and should be treated with caution.

Taxon	**Abbreviation**	**Relative abundance all tests in plot (relative abundance living individuals):**		
		MAC11 (%)	**MAC2 (%)**	**MAC30 (%)**
Assulina muscorum Greef 1888	AMUSC	19.2 (10.1)	18.8 (3.9)	26.5 (10.8)
Assulina seminulum (Ehrenberg 1848)	ASEM	3.8 (0.0)	0.7 (1.0)	1.4 (0.4)
Centropyxis aerophila Deflandre 1929 type	CAERO	4.6 (1.3)	3.4 (2.5)	4.4 (1.3)
Corythion dubium Taranek 1881	CDUB	3.3 (3.1)	17.4 (13.6)	11.3 (9.6)
Difflugia pristis Penard 1902 type	DPRIS	10.1 (17.3)	1.4 (0.0)	0.3 (0.0)
Euglypha rotunda Wailes 1911 type	EROT	0.6 (0.0)	1.2 (1.8)	1.8 (1.1)
Euglypha strigosa (Ehrenberg 1872)	ESTRI	4.5 (3.2)	9.6 (10.0)	5.9 (8.3)
Heleopera petricola Leidy 1879	HPETR	8.8 (7.3)	4.0 (7.5)	4.8 (3.2)
Heleopera rosea Penard 1890	HROS	0.4 (0.5)	3.4 (2.8)	0.9 (1.6)
Hyalosphenia subflava Cash and Hopkinson 1909	HSUBF	4.9 (6.6)	0.7 (1.1)	0.3 (0.0)
Nebela militaris Penard 1890	NMILI	3.2 (4.7)	3.5 (5.2)	1.6 (0.9)
Nebela tincta (Leidy 1879) type	NTINC	18.0 (30.0)	16.8 (34.5)	26.5 (57.2)
Phryganella acropodia (Hertwig & Lesser 1874) type	PACRO	9.3 (6.7)	11.7 (0.7)	7.3 (0.9)
Pseudodifflugia fulva Penard 1901 type	PFUL	2.7 (5.2)	0.5 (0.0)	0.5 (0.8)
Trigonopyxis arcula (Leidy 1879)	TARC	4.0 (2.8)	0.8 (0.0)	1.0 (0.0)

[1]Minor taxa are: *Bullinularia indica, Centropyxis aculeata, Cryptodifflugia oviformis, Difflugia minutissima* type, *Euglypha ciliata, Euglypha cristata, Hyalosphenia papilio, Nebela flabellum, Nebela tubulosa, Placocista spinosa, Sphenoderia fissirostris, Trinema complanatum,* and *Trinema lineare*, plus *Habrotrochoa angusticollis*.

Table 3. Redundancy analysis of testate amoeba data showing percentage variance explained and P-values of these relationships assessed by Monte Carlo permutation tests (999 permutations).

Dataset	Explanatory variable	Co-variable	% variance explained	P-value
All tests (%)	Treatment	-	17.9	0.001
	Treatment	pH	4.8	0.001
All tests (concentration)	Treatment	-	18.4	0.001
	Treatment	pH	6.7	0.001
Live amoebae (%)	Treatment	-	14	0.001
	Treatment	pH	5.2	0.001
Live amoebae (concentration)	Treatment	-	13.3	0.001
	Treatment	pH	5.3	0.001

Table 4. Results of cluster analysis (Paired Group Method using a Bray-Curtis distance measure) on % total tests data showing groups identified at the third level of division.

Group	Samples
1	MAC11 (1 sample)
2	MAC11 (1 sample)
3	MAC11 (13 samples), MAC2 (1 sample)
4	MAC30 (20 samples), MAC2 (9 samples), MAC11 (5 samples)