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**Volcanic impacts on peatland microbial communities: a tephropalaeoecological**

**hypothesis-test.**

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ABSTRACT

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Volcanic eruptions affect peatlands around the world, depositing volcanic ash (tephra)

and a variety of chemicals including compounds of sulphur. These volcanic impacts

may be important for many reasons, in particular sulphur deposition has been shown

to suppress peatland methane flux, potentially reinforcing climatic cooling.

Experiments have shown that sulphur deposition also forces changes in testate

amoeba communities, potentially relating to the reduced methane flux. Large volcanic

eruptions in regions with extensive peatlands are relatively rare so it is difficult to

assess the extent to which volcanic eruptions affect peatland microbial communities;

palaeoecological analyses across tephra layers provide a means to resolve this

uncertainty. In this study testate amoebae were analysed across multiple monoliths

from a peatland in southern Alaska containing two tephras, probably representing the

1883 eruption of Augustine Volcano and a 20th Century eruption of Redoubt Volcano.

Results showed relatively distinct and often statistically significant changes in testate

amoeba community coincident with tephra layers which largely matched the response

found in experimental studies of sulphur deposition. The results suggest volcanic

impacts on peatland microbial communities which might relate to changes in methane

flux.

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KEYWORDS:  Mires, Wetlands, Tephra, Volcanoes, Protozoa, Carbon balance, Methane

INTRODUCTION

Many peatland areas lie within reach of the products of volcanic eruptions. Visible or microscopic layers of volcanic ash (tephra) have been found in peatlands around the world (Dugmore 1989, Pilcher et al. 2005, Hang et al. 2006, Lowe 2008) and volcanic gases may be even more widely dispersed (Camuffo & Enzi 1995, Grattan et al. 1998). The possible impact of these volcanic products has been addressed by only a few studies but there is reason to think that ecological affects might be relatively severe (Payne and Blackford 2005; Hotes et al. 2004, 2010). Particularly important may be impacts on peatland gas flux. Studies of sulphate pollution have shown a suppression of methane flux, believed to relate to a shift in competitive dominance from methanogenic archaea to sulphate reducing bacteria (Gauci and Chapman 2006). As volcanic eruptions produce vast quantities of sulphate it has been modelled that this effect might contribute to a halving of wetland methane flux following a large eruption (Stevenson et al. 2003; Gauci et al. 2005; 2008) and some support for this hypothesis is provided by ice core evidence (Gauci et al. 2008). On a molecule for molecule basis methane is 21 times more powerful than $CO_2$ as a greenhouse gas so changes in methane production have the potential to affect global climate, reinforcing aerosol-forced cooling. So far there is no direct evidence for the inferred microbial competitive shift which is believed to be the cause of the methane suppression. It can be hypothesised that a major change in prokaryote community structure might produce consequent changes in higher microbial groups. The dominant eukaryotic microbial group in peatlands is the testate amoebae, a group of heterotrophic and mixotrophic protists characterised by a solid shell. To see whether sulphate deposition might produce changes in testate amoebae which might be mediated by the shift to sulphate reducing bacteria Payne et al. (2010) investigated the communities of sodium sulphate treated plots for which long-lasting methane suppression had been previously demonstrated (Gauci et al. 2005). Results showed statistically significant changes in community composition with a reduced abundance of small bacterivorous taxa. This result has subsequently been replicated in plots treated with a one-off application of sulphuric acid (Payne 2010). These studies

1    demonstrate changes in microbial community structure in sulphur-treated peatlands.

2    The fact that the largest and most consistent changes are apparent in bacterivorous

3    taxa appears to support the hypothesis of a sulphate-induced change in prokaryote

4    communities, although other explanations for the changes cannot be excluded on this

5    evidence.

6        Experimental studies of this nature are inevitably limited by the uncertainty

7    and limitations of the investigated scenarios. An alternative approach is to use the

8    palaeoecological record to investigate the response to a real volcanic event. Tephra

9    layers preserved in peat allow a volcanic event to be precisely positioned in the

10    stratigraphic sequence. Palaeoecological studies across tephra layers allow the

11    potential to investigate volcanic impacts, an approach termed tephropalynology for

12    studies based on pollen and spores (Lowe and Hunt 2001, Edwards et al. 2004) or

13    tephropalaeoecology for studies based on other micro- or macrofossils (Payne and

14    Blackford 2008a). As testate amoebae are widely preserved in peatlands

15    tephropalaeoecological analyses of testate amoebae provides the potential to

16    demonstrate the impact of a real volcanogenic sulphate deposition event on peatland

17    microbial communities. Due to the sample preparation approach a previous study of

18    this nature had limited ability to identify the compositional changes which have been

19    experimentally identified (Payne and Blackford 2008a; Payne 2009).

20        Here we apply a palaeoecological approach to test the hypothesis that a

21    volcanic event may cause a change in microbial communities which is detectable in

22    the testate amoeba stratigraphic record. A general problem with

23    tephropalaeoecological studies is the difficulty of separating volcanic impact from

24    non-volcanic variability. If changes coincident with tephra-deposition are apparent it

25    is difficult to exclude the possibility of impacts due to broadly-synchronous non-

26    volcanic processes (whether autogenic or allogenic). However, the case for a volcanic

27    impact is much enhanced if consistent palaeoecological changes coincident with

28    tephra deposition can be shown in multiple profiles from the same site and for several

29    tephras (*cf.* Caseldine et al. 1998). This study investigates the tephropalaeoecological

30    record of multiple profiles from a peatland in southern Alaska with the specific aims

31    to:

32    1. Determine if any impacts on the taxa identified as sensitive to sulphur deposition

33        can be identified.

2. Determine if different volcanic events produce similar testate amoeba responses in the same site.

3. Determine if the same tephra layer is associated with similar impacts in profiles from different positions within the same site.

SITE and METHODS

The site (informally termed 'Sterling Moss') is a kettle-hole peatland located in the Kenai Lowlands to the west of the Kenai Peninsula in south-central Alaska, east of the town of Sterling (60°31'N, 150°31'W: Fig. 1). The vegetation of the site is *Sphagnum*-dominated and also includes *Empetrum nigrum, Eriophorum* spp. and *Rubus chamaemorus*. On the basis of topography and vegetation the peatland appears oligotrophic but not ombrotrophic, as for other Kenai peatlands. Results from an initial monolith (extracted in summer 2003) have been reported by Payne and Blackford (2008a, 2008b). A further six monoliths were extracted along a transect of the centre of the site in July 2007 (Fig. 1). All sampling locations have similar vegetation. Monolith blocks, approximately 300 mm deep and 100 mm broad, were removed by cutting down from the peat surface. Stratigraphy was recorded in the field and blocks wrapped in plastic for transport back to the laboratory.

*Tephra analysis*

Tephra layers were macroscopically visible in three monoliths (ST4, ST5, ST6) as a (sometimes discontinuous) thin layer of grey, silt-sized shards, but no tephras were visible in the other two monoliths. To precisely locate tephra layers and produce concentration profiles, samples were prepared by ashing and tephra shards counted under the microscope (Pilcher and Hall, 1992). Samples of either 5mm or 10mm vertical thickness were extracted through the length of the monolith, dried at 110ºC and incinerated at 550ºC; weights pre- and post-incineration were used to calculate loss on ignition (LOI). The residue was washed in 10% HCl, centrifuged, and prepared samples stored in water. A *Lycopodium* spore innoculum was added to allow semi-quantitative estimates of tephra shard concentration (Stockmarr 1971; Caseldine et al., 1998). The prepared sample was mixed with a drop of glycerol on a microscope slide and examined at 400X magnification. The number of tephra shards

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1  coinciding with at least 50 *Lycopodium* spores was counted. Geochemical analyses

2  were used to determine the provenance of the visible tephra layers in three monoliths:

3  the tephras at 11cm in profile ST4, 16cm in ST5 and 14cm in ST6. Samples were

4  prepared by acid digestion following the methods of Persson (1971) and Dugmore

5  (1989) and thin-sections prepared (Dugmore et al. 1995; Swindles et al. 2010).

6  Samples were analysed by wavelength dispersive electron probe microanalysis

7  (EPMA) using the Cameca SX100 microprobe at the School of Geosciences,

8  University of Edinburgh. Analytical conditions were a 20 kV accelerating voltage and

9  4 nA beam current, the beam was rastered over a 10x10 μm grid to minimise sodium

10  mobilisation. Analyses with totals <95% (Hunt and Hill 1993) and minor geochemical

11  populations were removed prior to data analysis. Similarity to geochemical data from

12  a large number of previous Alaskan tephra studies (see Payne & Blackford 2008b for

13  full list) was tested using standard similarity coefficients and SIMAN coefficients

14  (Borchardt et al. 1972). The SIMAN coefficient is calculated as:

15

16  $$SIMAN_{(A,B)} = \frac{\sum_{i=1}^{n} R_i g_i}{\sum_{i=1}^{n} g_i} \qquad g_i = 1 - \sqrt{\frac{(\sigma_{iA}/X_{iA})^2 + (\sigma_{iB}/X_{iB})^2}{E}}$$

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18

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21  where $X_{iA}$ = concentration of element *i* in sample *A*, $R_i = X_{iA}/X_{iB}$, if $X_{iA} < X_{iB}$ and $R_i =$

22  $X_{iB}/X_{iA}$, if $X_{iB} < X_{iA}$ (i.e. the standard similarity coefficient), $\sigma_{iA}$= 1 standard deviation

23  for $X_{iA,}$ and $E$ = the relative error due to the detection limit, set here at 0.7 to exclude

24  only MnO from analysis. A SIMAN coefficient of 1 would indicate identical

25  composition, however given sample heterogeneity this is extremely unlikely, a

26  SIMAN coefficient of 0.95 is taken to indicate correlation (Payne and Blackford

27  2008b).

28

29  *Testate amoeba analysis*

30

31       Samples for testate amoeba analysis were removed at resolutions varying

32  between 2.5 and 10mm vertical-thickness with higher resolution sampling used for the

33  region directly enclosing the tephra; cores were frozen to allow fine peat-cutting. To

1   assess very fine-scale horizontal variability in amoeba response, samples from two

2   sequences on opposite sides of the ST4 monolith were removed (approximately 80mm

3   apart, designated ST4A and ST4B). Samples were prepared using a method based on

4   wet-sieving, similar to that of Hendon & Charman (1997). Peat sub-samples were

5   removed from the core and boiled in 50 ml deionised water. Samples were sieved at

6   250μm but fine sieving was not used as this is liable to lead to loss of the smallest

7   tests and many of the smaller taxa are particularly relevant to this study (Payne 2009;

8   Payne et al. 2009; Payne 2010). Samples were stored in water, slides prepared by

9   mixing with glycerol and examined at 400X magnification. 150 tests per sample were

10  counted, taxonomy used a range of guides with groupings similar to those of Charman

11  et al. (2000); full details are given by Payne et al. (2006). The test count required to

12  identify each additional taxon was recorded to give information on how successful the

13  count total is at identifying taxa (Warner 1990; Woodland et al. 1998; Payne and

14  Mitchell 2009).

15

16  *Data analysis*

17

18      The transfer function of Payne et al. (2006) was used to derive estimates of

19  depth to water table (termed testate amoeba-inferred depth to water table: TI-DWT)

20  and pH (testate amoeba-inferred pH: TI-pH) in $C^2$ vers 1.5 (Juggins 2003). This

21  transfer function is preferred over an alternative transfer function (Markel et al. 2010)

22  due to assured taxonomic consistency (*cf*. Payne et al. 2010), less severe

23  autocorrelation problems (Payne, Telford et al. unpublished data) and the inclusion of

24  surface samples from the same site. As the samples used to derive the transfer

25  function were prepared using fine-filtering the data may not be entirely comparable to

26  that presented here (Payne 2009). To account for this methodological difference

27  transfer function inferences were calculated with the smallest taxa (including

28  *Euglypha rotunda* type*, Corythion dubium* and *Trinema* type) excluded from both the

29  training set and palaeoecological data, this somewhat reduces the effective count but

30  is unlikely to significantly affect model performance (Payne & Mitchell 2009).

31      Volcanic impacts on the testate amoeba data were tested using a constrained-

32  ordination approach. Loss on ignition (LOI) was used as an explanatory variable to

33  represent volcanic impact based on the observations that tephra constitutes the

34  overwhelming majority of inorganic material (Fig. 2) and non-tephra material shows

6

1    limited variability through the profiles. Alternative approaches would be to use the

2    tephra concentration data (e.g. Barker 2000, Eastwood et al. 2002) or a conceptual

3    volcanic-impact model based on exponential decay (Lotter & Birks 1993; Birks &

4    Lotter 1994; Payne & Blackford 2008a). LOI was preferred to tephra concentration

5    data due to the higher resolution while the stratigraphic positioning of a volcanic-

6    impact model is somewhat complicated given the very high resolution. In addition to

7    LOI before/after nominal variables were included for both tephra layers to determine

8    whether lasting impacts are apparent. Species data was reduced in resolution by

9    combining adjacent samples to match that of the LOI data where required and was

10   square root-transformed. Compositional gradient lengths determined using detrended

11   canonical correspondence analysis (DCCA) were short (<1.4 standard deviations) so

12   redundancy analysis (RDA) was selected as the most appropriate direct gradient

13   analysis technique. Depth was partialled out as a surrogate for time to separate the

14   effect of longer-term processes such as climatic change, the catotelm-acrotelm

15   transition and peatland development. Four sets of analyses were conducted using

16   combinations of LOI with one or both before/after variables. Stratigraphically-

17   constrained Monte Carlo permutation tests (999 permutations: Birks 1998) were used

18   to test the null hypothesis of no relationship between explanatory variables and

19   species data. All ordination analyses were conducted using CANOCO vers 4.53 (Ter

20   Braak & Smilauer 1997-2004).

21

22   RESULTS

23

24   *Tephrostratigraphy*

25        There is a good deal of geochemical similarity between tephras ST4-11 and

26   ST6-14, but tephra ST5-16 is distinctly different (Table 1; Appendix 1). Similarity

27   and SIMAN coefficients for ST5-16 show the greatest geochemical similarity with

28   analyses of Augustine 1883 tephra; coefficients generally exceed the 0.95 criterion

29   although there are some differences for several oxides (Table 1). Tephra from this

30   eruption has been found at a similar depth in a nearby Kenai peatland suggesting

31   general equivalency in age (Payne and Blackford 2008b). The ST4-11 and ST6-14

32   tephras show greatest similarity to analyses of Redoubt tephra. Averages of both

33   datasets plot very close to analyses of Redoubt tephra by Begét et al. (1994) and

34   Riehle (1985)(Fig. 3). Similarity coefficients and SIMAN coefficients show greatest

7

1    similarity to Redoubt tephras dated c.350 BP and over 500 BP (Table 1). However,

2    these values are generally less than the commonly applied 0.95 criterion and there are

3    notable differences in $K_2O$ (Table 1, Appendix 1). Given the stratigraphic position

4    and probable identification of Augustine 1883 tephra at 16cm depth in ST5 the 1902

5    or 1966 Redoubt eruptions are probable sources, with tephra from the 1902 eruption

6    previously found in nearby Skilak Lake (Begét et al. 1994). Neither of these three

7    tephras are the same as the ST12 tephra studied by Payne and Blackford (2008a & b).

8    Although no more than one tephra was macroscopically visible in any monolith two

9    separate tephras are detectable in monoliths ST2, ST3 and ST4b and in ST5 a third

10   minor tephra also appears to be present. A lower tephra is likely to lie below the

11   analysed section in monoliths ST4a and ST6. The hypothesised relation of these

12   tephras is shown in Fig. 4 although there is uncertainty in some of these links.

13

14   *Testate amoeba counts*

15       Figure 5 shows the impact of increasing count on number of testate amoeba

16   taxa identified for 92 samples. Although the rate of increase slows with increasing

17   count, a plateau is not reached. New taxa are identified with counts over 130 in 41 of

18   the samples. It is clear that in these samples a count of 150 is insufficient to identify

19   all taxa and consequently little weight should be attached to the presence or absence

20   of minor taxa. This result emphasises the point made by Payne & Mitchell (2009) that

21   while counts of less than 150 may be adequate to characterise the amoeba

22   composition of a sample, a count of considerably more than 150 would be required to

23   be sure of identifying all taxa (*cf*. Wall et al. 2009).

24

25   *Testate amoeba community and transfer function inferences*

26       The testate amoeba community of the analysed sections shows some

27   variability between monoliths, mirroring that found in surface samples from this site

28   (Payne et al. 2006). Common taxa include *Hyalosphenia papilio, Archerella flavum,*

29   *Nebela militaris, Trinema lineare* and *Heleopera sphagni*, all comparatively common

30   in oligotrophic peatlands. Transfer function inferences show only very limited

31   changes in TI-DWT or TI-pH through the profiles with few exceeding the boot-

32   strapped error estimates, untangling pH and DWT reconstructions is difficult as the

33   two are correlated in the training set. Among the more distinct changes are indications

1     of a shift to more acidic conditions above the upper tephra in ST2, ST5 and -less

2     distinctly- ST4b and some indication of a general drying trend in ST5, ST3 and ST4b.

3

4     *Multivariate analysis*

5         The ordination analyses show that LOI explains a significant proportion of

6     species variance in many of the profiles (Table 2). In two of the profiles a large and

7     significant proportion of the variance is explained when using the simplest test with

8     LOI as sole explanatory variable (ST4b and ST5). With the addition of before/after

9     variables for all tephras there is also a significant relationship in profile ST3 and up to

10    a third of variance is explained. Where more than one tephra is present similar

11    variance is explained by LOI and the before/after variable for each tephra suggesting

12    similar impacts. Using the approach of Lotter & Birks (1993) significant variance can

13    be explained by both tephras in one further profile (ST2). It is important to note that in

14    harmonising the species and LOI data some temporal resolution has been lost and this

15    may well be an important cause of the lack of a significant relationship in some of the

16    analyses. Taken overall the results suggest an association between loss on ignition and

17    testate amoeba compositional change, which as LOI is dominated by tephra provides

18    evidence for a volcanic impact on the testate amoebae of these sites. There is evidence

19    for both an immediate short-term impact (shown by the LOI trough) and a lasting

20    longer-term impact (the before/after variables).

21

22    *Species changes*

23

24         Changes in testate amoeba community coincident with the tephra layers are

25    apparent in many of the stratigraphic diagrams (Fig. 6), summarised in Table 3. Many

26    changes appear consistent between tephras. The relative abundance of *Trinema*

27    *lineare* is reduced coincident with tephras ST2-15.5, ST2-21.5, ST3-16.5, ST4a-11.5,

28    ST5-17.5 and ST6-14. *Heleopera sphagni* increases coincident with ST2-15.5, ST2-

29    21.5, ST4a-11.5 and ST4b-11 (this species appears to decrease coincident with tephra

30    ST4b-14.5 but these analyses are lower resolution). *Difflugia pulex* type, *Difflugia*

31    *pristis* type, *Cryptodifflugia oviformis* and *Phryganella acropodia* type all increase

32    across two tephras. The only taxon to clearly show opposing changes in different

33    profiles is *Archerella flavum* which increases across three tephras but shows a distinct

34    decline across tephra ST4b-11. There is no obvious difference between species

responding to tephras suggested to represent Augustine 1883 or Redoubt tephra. Profiles ST4a and ST4b are from opposite sides of the same monolith however there are some differences in both amoeba community and apparent tephra response between the two profiles.

A methodological complication is that as the high resolution samples were extracted and analysed subsequent to the initial samples they are slightly horizontally displaced. Although the high resolution samples were rarely more than c.20mm from the initial coarser resolution samples this offset (or even subsequent decomposition of tests) might explain some instances where there appear to be very abrupt changes between the low- and high-resolution sections of the records, visible for instance in *Trinema lineare* in ST6 and *Heleopera sphagni* in ST2. In the case of ST5 there is also an offset between the position of the tephra peak (determined on the low-resolution samples) and the loss on ignition trough (determined on the high-resolution samples).

The species showing the most distinct change is *Trinema lineare* which has a small translucent test. A more prosaic alternative to a volcanic impact might be that it is simply difficult to locate the tests amid the numerous tephra shards and individuals are overlooked. To account for this possibility some of these samples were re-counted with very close attention to locating these tests; little difference in abundance was found.

*Sulphur indicator species*

Previous studies have investigated the response of testate amoebae to sulphur in experimental studies of peatlands. Payne et al. (2010) studied the affect of sodium sulphate application in a peatland in eastern Scotland and Payne (2010) studied the response to sulphuric acid application in a peatland in western Scotland. Both studies identified sulphur application as capable of leading to compositional change in testate amoeba communities. To aid application of the experimental results to the palaeoecological record these data were reanalysed to identify indicator species using the IndVal technique (Dufrêne and Legendre 1997) with 999 permutations (Table 4). Three species were consistently identified as indicators of non-sulphur treated plots: *Corythion dubium*, *Euglypha rotunda* type and *Trinema lineare*. There is no consistency in species indicative of sulphur-treated plots, the best indicators are from

1   the sulphuric acid experiment (including *Hyalosphenia subflava* and *Difflugia pristis*

2   type) but these should be treated with greater caution due to the more limited scale of

3   this study.

4        Comparing these experimental results to the palaeoecological results in this

5   study there is a good deal of similarity. The species most consistently reduced in

6   abundance coincident with tephra layers (*T. lineare*) is also deleteriously impacted by

7   sulphur-treatment. *D. pristis* type and *H. petricola* are both increased by sulphur-

8   treatment and increase coincident with tephra layers.  There are some differences in

9   species response but these are generally minor, for instance *E. rotunda* type slightly

10  increases coincident with tephra ST4b-11 but is consistently reduced in relative

11  abundance by experimental sulphur deposition. Several species apparently responding

12  to volcanic impacts are not identified in the experimental studies but most are rare or

13  absent in the experimental sites (e.g. *H. papilio*).

14

15  DISCUSSION

16

17  *Evidence for volcanic impacts*

18

19       The results in this study show good evidence for volcanic impacts on testate

20  amoeba communities- there are relatively distinct changes apparent in the

21  stratigraphic diagrams and the ordination results show many to be statistically

22  significant. The changes are characterised by a relatively distinct species response

23  with increased *Heleopera sphagni* and *Difflugia* spp. and particularly, reduced

24  *Trinema lineare*. As discussed above these changes are consistent with experimentally

25  demonstrated testate amoeba response to sulphur and, importantly, no major

26  palaeoecological changes oppose the experimental results. The testate amoebae

27  changes last around 10-20mm. Assessing the duration of impacts on testate amoebae

28  from a short-lived environmental change is difficult because amoebae will be living at

29  a range of depths in the peat on deposition, however given peat accumulation rates it

30  is probable that impacts shown here lasted over a decade. This is consistent with

31  experimental evidence for long-lasting testate amoeba response, probably due to tight

32  cycling of sulphur in acrotelm peats.

33       The testate amoeba response to thin, often cryptic, tephra layers in this study

34  contrasts with that to much thicker layers in other studies. On Deception Island in the

Antarctic Smith (1974, 1985) found *Assulina muscorum, E. rotunda, E. strigosa* and particularly *Corythion dubium* colonising recent tephra layers, this list includes taxa morphologically and functionally similar to taxa declining coincident with tephra in this study. In a palaeoecological record from sub-Antarctic Marion Island Yeloff et al. (2007) found a peak in *Corythion-Trinema* type coincident with a 17cm-thick tephra; the ability of *C. dubium* to colonise the harsh physical environment of new tephra deposits is a likely hypothesis for these observations.

*Alternative explanations*

Despite the similarity in palaeoecological response to experimental studies of sulphur addition there are many other possible ways by which volcanic activity could affect peatland testate amoebae. These possibilities can also be evaluated against the observed changes. Payne and Blackford (2008) suggest a further four general modes of distal volcanic impact on peatlands:

1. An indirect impact through volcanic impacts on climate and weather leading to cooler conditions, potentially a shorter-term increase in precipitation and perhaps lightning. This possibility can probably be excluded as an explanation for the changes observed here given the lack of consistent changes in known hydrophilic or xerophilic taxa and the lack of a distinct TI-DWT response.

2. Physical impacts of tephra including crushing, abrasion, inhibition of gas exchange and photosynthesis in plants with consequent impacts below ground, perhaps through reduced root exudation. This possibility cannot be excluded but given the thin tephra layers experimental studies suggest that major changes are unlikely (Payne & Blackford 2005; Hotes et al. 2004).

3. Impacts on peatland hydrology, such as surface ponding of water due to a formation of a semi-impermeable surface layer of tephra. Given the thinness of the tephra layer this possibility is highly unlikely and the lack of a TI-DWT change suggests this explanation can probably be excluded.

4. Chemical impacts of tephra and tephra leachates. A wide range of chemicals may be adsorbed to the surface of tephra shards or leached in the acidic peatland environment (Pollard et al. 2003; DeVleeschouwer et al. 2008). Chemicals released may include heavy metals like lead and cadmium which have been shown to affect testate amoebae (Nguyen-Viet et al. 2007, 2008).

DeVleeschouwer et al. (2008) have suggested that tephra layers may provide an enclosed space for bacterial growth fed by tephra-derived nutrients, which would in turn provide food for bacterivorous testate amoebae. As there is uncertainty in how these impacts might be manifested in community changes it is difficult to exclude the possibility that such processes might have had a role in producing the observed changes.

Beyond these possible explanations for volcanic impacts leading to testate amoeba community changes another possibility is that the observed changes are due to taphonomy. Tests of many species are rapidly decomposed in soils and form an important component of nutrient cycling (Meisterfeld & Heisterbaum 1986). Idiosome tests are particularly prone to decomposition and (with the exception of *Assulina* spp.) are rarely found below the uppermost peats in palaeoecological records (Swindles and Roe 2007; Mitchell et al. 2008). It is possible that the input of tephra or adsorbed chemicals might modify environmental conditions to become more favourable for the decomposition of sensitive tests. One possible agent is an increase in acidity due to deposition of volcanic acids, although it is uncertain whether any drop in pH would be of sufficient duration and magnitude to produce a detectable change in amoeba relative abundances.

Other changes might occur due to supply of Si. Oligotrophic peatlands are Si-limited and it might be expected that the supply of Si in tephra, although less bioavailable that biogenic Si, would reduce or remove this limitation. Enhanced silica supply and reduced dissolution have been suggested as causes of enhanced diatom concentration coincident with the Laacher See tephra in NE German lakes (De Klerk et al. 2008) and might also explain the presence of idiosome tests in tephra layers on Marion Island (Yeloff et al. 2007). However in our results the most notable change is a *reduced* abundance of an idiosome species (*T. lineare*) and there is certainly no very consistent increase in idiosome tests. The tests which do increase are most commonly of xenosome construction; while it may be that tephra provides material for test construction in these taxa no embedded tephra shards were noted.

To conclude, while it is difficult to exclude other explanations the results here are consistent with what would be expected as a sulphur-impact and this provides a viable hypothesis for the changes detected in this study.

1 *Implications of the results*

2

3       An increasing number of studies are taking a tephropalaeoecological approach

4 both to investigate volcanic impacts and to precisely link multiple palaeoecological

5 records. The presence of tephra layers presents a rare opportunity to use the

6 palaeoecological record to address the environmental impact of a short-lived pollution

7 event and it seems increasingly probable that volcanic impacts on peatlands are

8 represented in the palaeoecological record. Our results emphasise the lesson of

9 Caseldine et al. (1998) that replicated results are required for a more complete

10 understanding of volcanic impacts in the palaeoecological record and their spatial

11 variability.

12       The presence of volcanic impacts suggests that a non-climatic process may be

13 capable of producing changes in a key climatic proxy which could conceivably be

14 mis-interpreted as a climate change. It appears however that any volcanic signal is

15 likely to be short-lived so volcanic forcing of the palaeoecological record is only

16 likely to be a concern for very high-resolution palaeoclimate studies. These results do

17 not show consistent changes in TI-DWT; however this does not necessarily mean that

18 a volcanic impact could not lead to changes in TI-DWT. Many taxa showing apparent

19 changes do have marked hydrological preferences and it is possible that if the

20 community composition was different a TI-DWT change could be produced. Any

21 volcanic impact is likely to be difficult to detect below the acrotelm due to the decay

22 of idiosome tests. Due to these factors it is probable that volcanic impacts are unlikely

23 to be a major issue for routine palaeoclimatology, however studies attempting to use

24 tephra layers to link records between sites (*cf*. Langdon & Barber 2004) should be

25 wary of the possibility.

26      The results presented here provide evidence for volcanic impacts on peatland

27 testate amoebae. Testate amoebae constitute a large proportion of microbial biomass

28 in peatlands (Gilbert et al. 1998a&b) and lie towards the top of the microbial

29 foodweb. These results therefore suggest that there may have been widespread

30 volcanic-induced changes in the microbial community of this peatland. Studies of

31 peatlands exposed to sulphate have shown a reduction in methane flux. Payne (2010)

32 speculated that observed testate amoebae changes might be related to the inferred shift

33 from methanogenic archaea to sulphate reducing bacteria with testate amoeba

34 predation of methanotrophs (recently demonstrated in other protozoa: Murase &

1    Frenzel 2008) seeming a plausible mechanism. While the link between testate amoeba

2    changes and methane is speculative it is possible that the observed changes here may

3    relate to a reduction in methanogenesis. These results therefore provide circumstantial

4    support for the idea that volcanogenic sulphate deposition might lead to methane flux

5    suppression. Methane flux is affected by a wide-range of processes (climatic,

6    physical, chemical and biotic: Dise et al. 1993; Shannon and White 1994; Segers

7    1998) and shows considerable temporal variability, however volcanic flux-

8    suppression may be highly significant, enhancing the climatic impact of large

9    eruptions in regions with widespread peatlands (Gauci et al. 2008). Future work will

10   need to investigate the precise mechanism of sulphate action on testate amoebae to

11   test whether palaeoecological studies of testate amoebae might allow methane flux

12   suppression episodes to be identified. The tight integration of experimental and

13   palaeoecological results is a powerful but little used approach to investigate longer-

14   term environmental change.

15

1

2

3   ACKNOWLEDGEMENTS

4

13

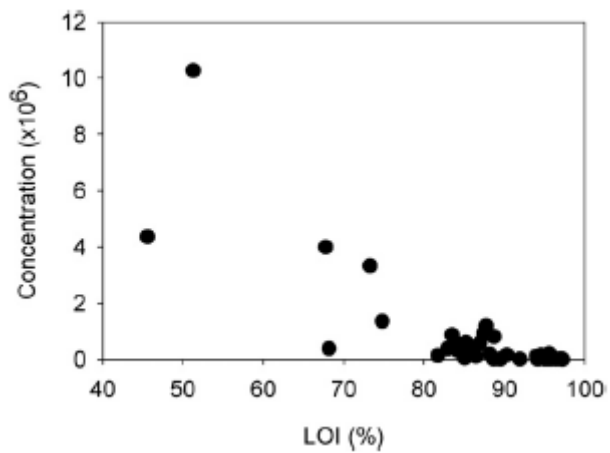1    FIGURES and TABLES

2

3    Figure 1. Sterling Moss site, sampling locations, regional context and adjacent
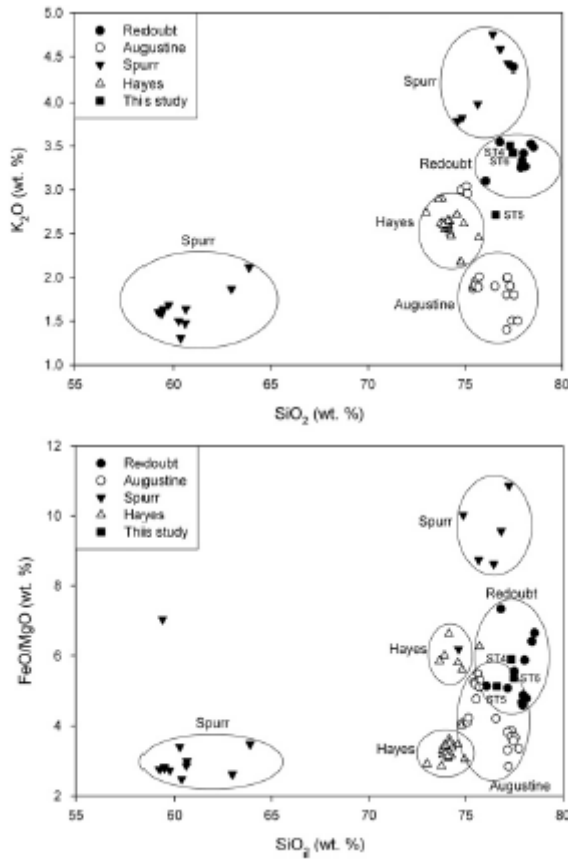
4    volcanoes.
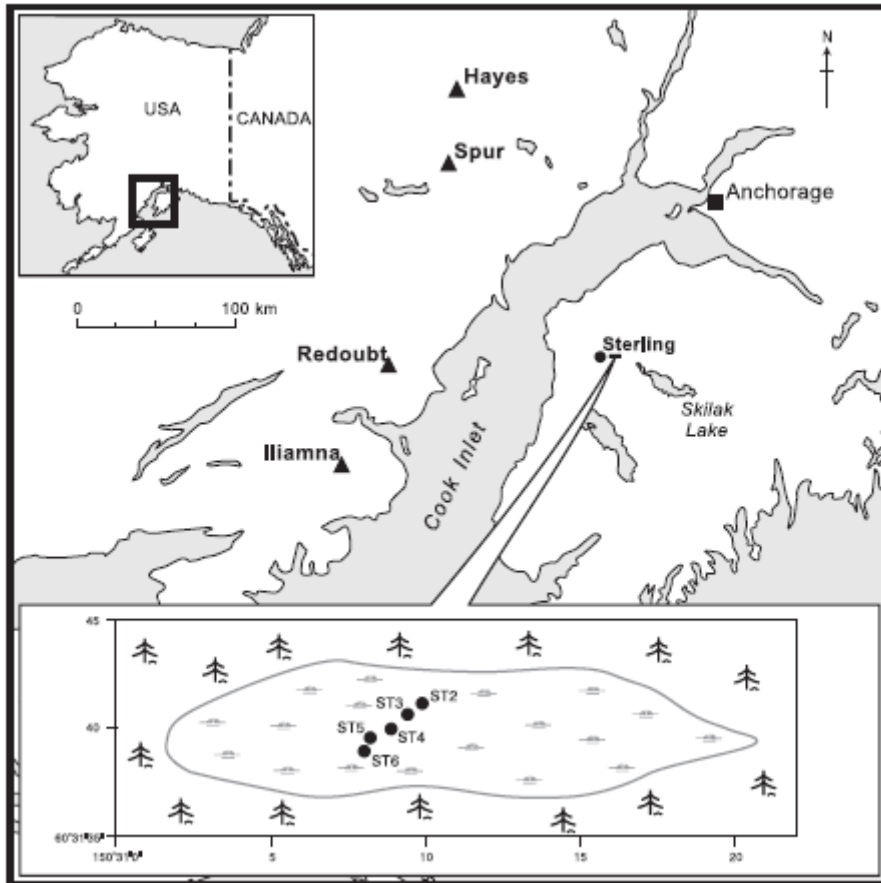


5

6

7    Figure 2. Tephra concentration against loss on ignition (LOI) for selected samples for

8    which both data are available.



9

1    Figure 3. Plots of selected major oxides for tephra layers in this study and comparison

2    data from previous research.



3

4    Figure 4. Testate amoeba inferred depth to water table (TI-DWT) and pH (TI-pH) for

5    six profiles showing boot-strapped standard errors (1000 iterations) and hypothesised

6    correlations between profiles based on tephrostratigraphy.
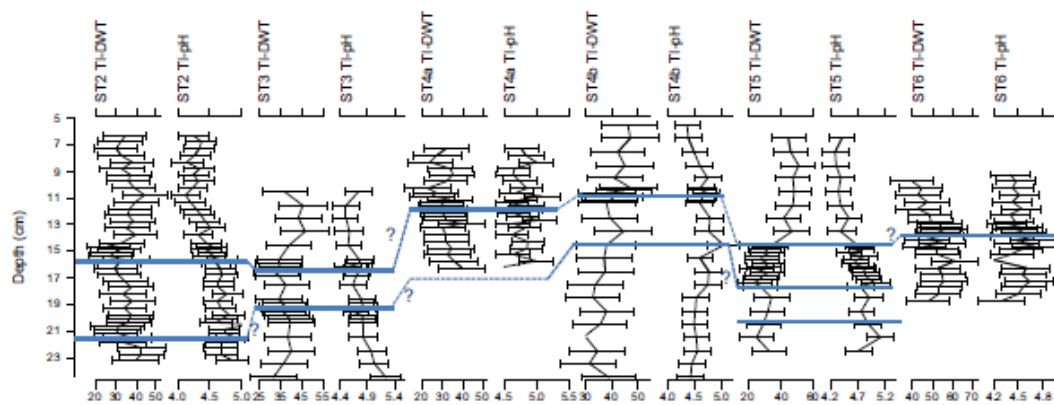


7

8    Figure 5. Impact of increasing test count on number of taxa recorded shown as mean
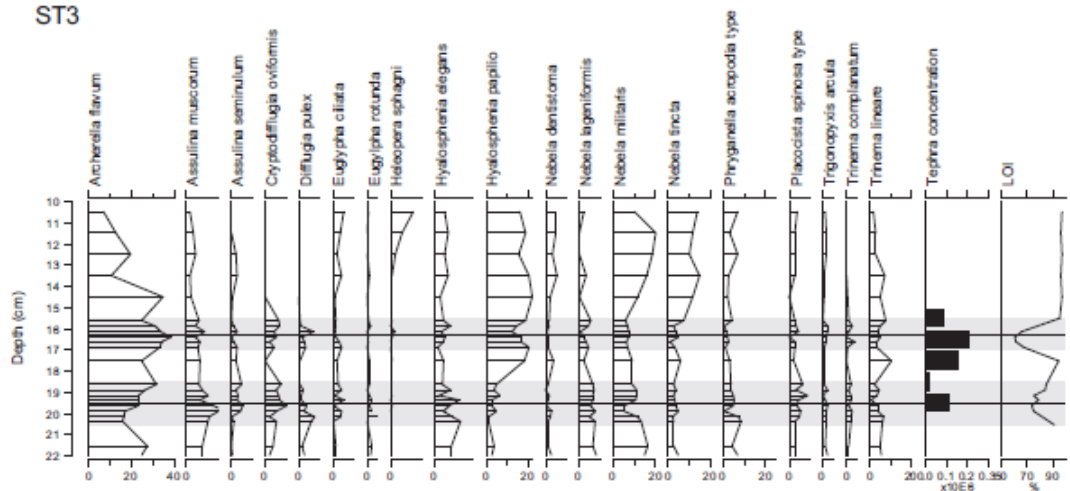
9    and standard deviations.
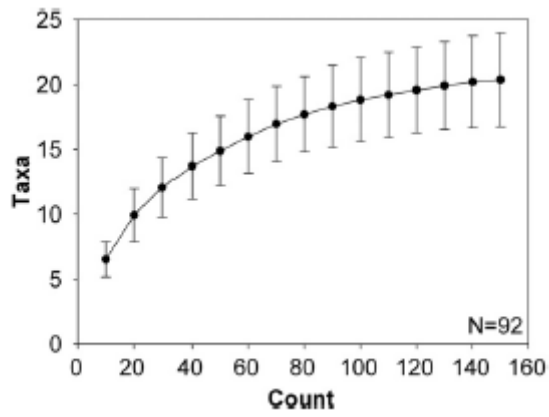
1

2    Figure 6. Testate amoebae diagrams from six profiles of five monoliths, also showing

3    plots of tephra concentration and loss on ignition (LOI). Diagrams show major taxa

4    (>1% total), shaded areas show areas of high resolution sampling and horizontal lines
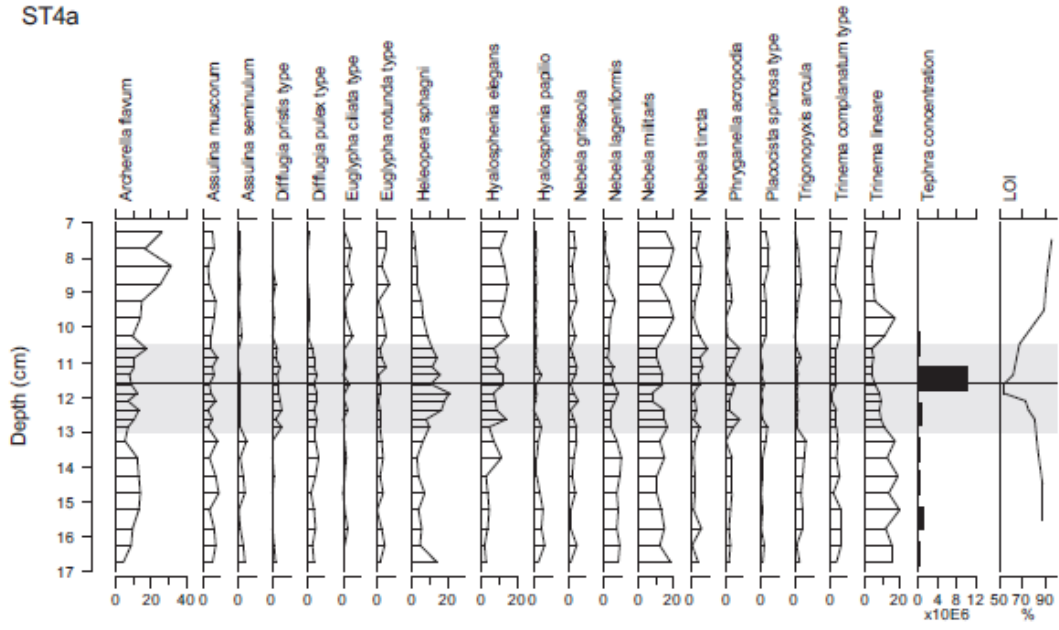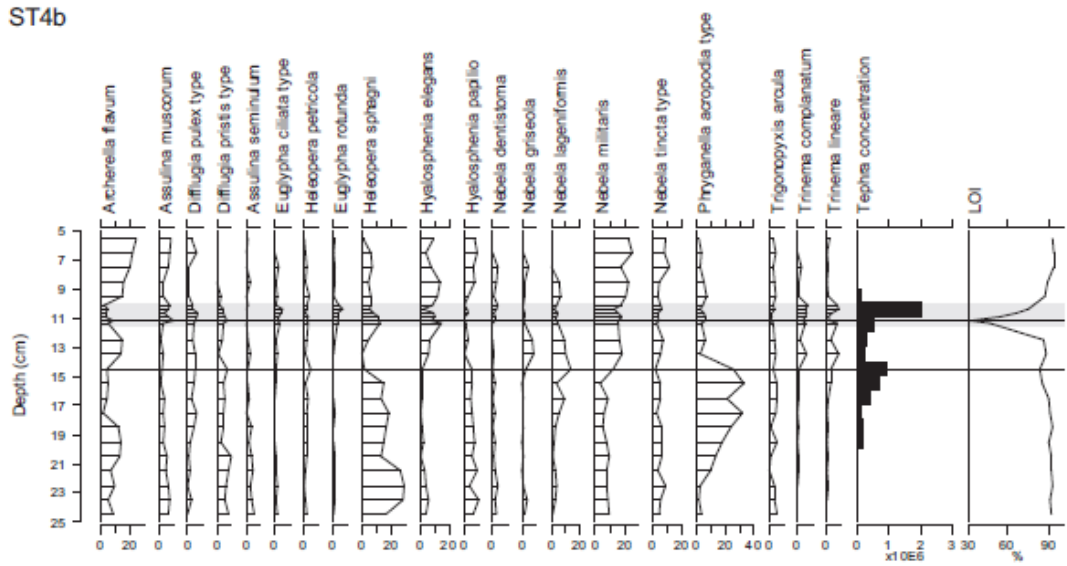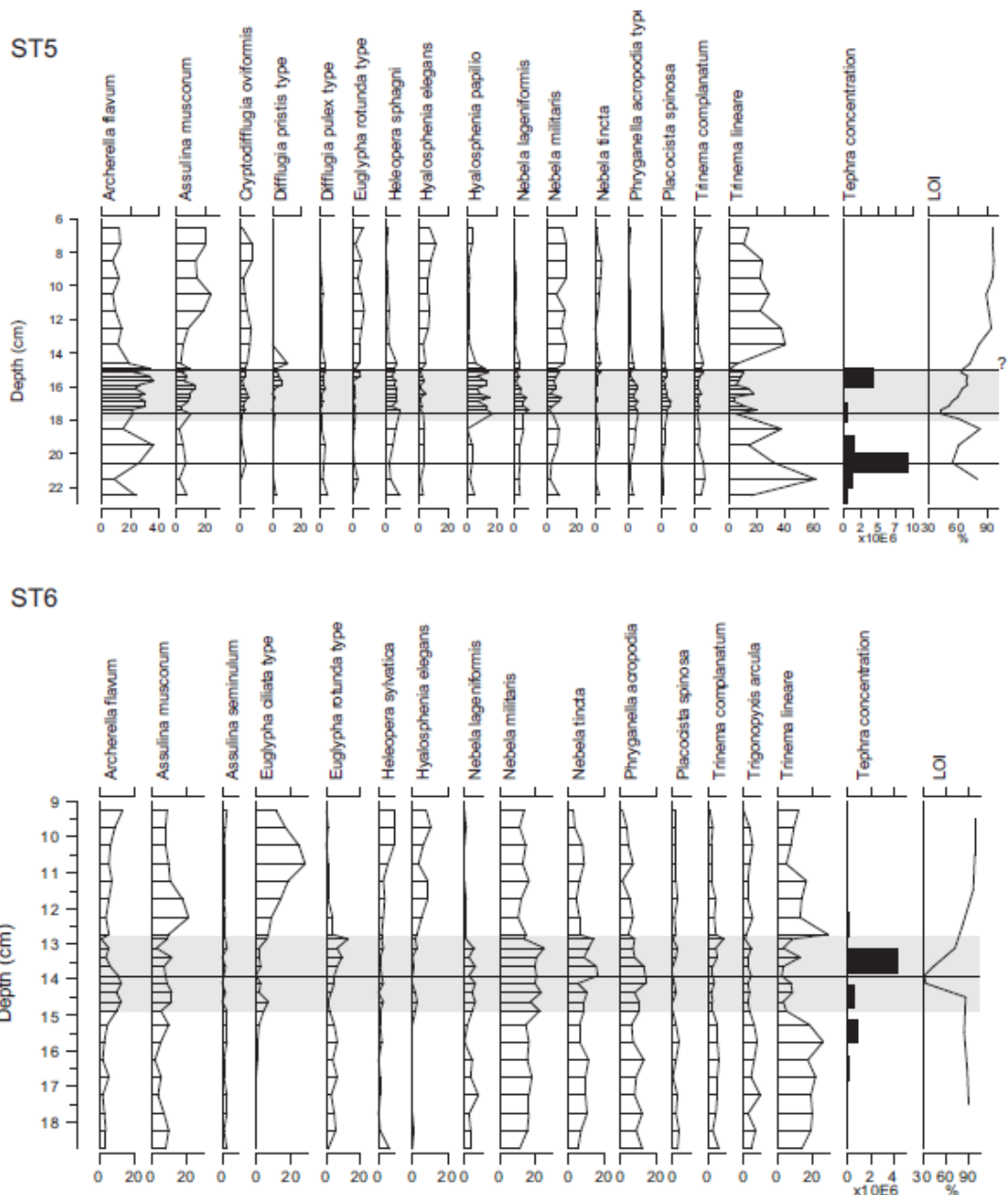
5    show tephra peak.

ST2



ST3

ST4a

ST4b

ST5



ST6
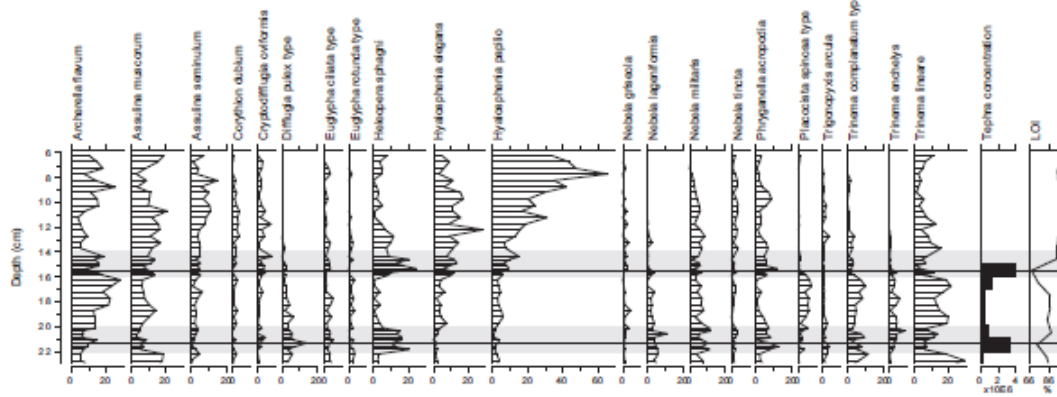
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3    Table 1. EPMA results for three visible tephra layers in this study and most similar

4    comparison data from previous studies. Normalised data is based on 8 major oxides

5    (excluding Manganese and Chlorine, where presented) showing total number of

6    analyses (N). Full (non-normalised) data is presented in Appendix 1. Analyses with

7    totals <95% were excluded following Hunt and Hill (1993). Full details of analytical

8    methodology are given in the methods section of the text.

9

10   Table 2. Results of redundancy analysis (RDA) of log-transformed testate amoeba

11   palaeoecological data across tephra layers in 6 profiles. Showing proportion of

variance explained by loss on ignition (LOI) and before/after nominal variables for upper (B/A-A) and lower (B/A-B) tephras with depth as a co-variable and P-values determined by Monte Carlo permutation tests (999 stratigraphically restricted permutations).

Table 3. Changes in testate amoeba community across tephra layers noted in stratigraphic diagrams (Fig. 6).

Table 4. Taxa responding positively and negatively to treatment with a) sodium sulphate (Payne et al. 2010) and b) sulphuric acid (Payne 2010) in previous experimental studies. Taxa identified using the IndVal technique (Dufrêne and Legendre 1997) with 999 permutations, showing taxa with IV>40 for treated or control plots, significant at P=0.01.

Appendix 1. Electron microprobe data from three tephras. Results as raw percentages, also showing averages of raw and normalized data.

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