

1 WETL234

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3 **Vertical stratification of testate amoebae in the Elatia Mires, northern Greece:**
4 **Palaeoecological evidence for a wetland response to recent climatic change, or**
5 **autogenic processes?**

6

7 Richard J. Payne^{1,2} and Jacqueline M. Pates³

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9 ¹The Fitch Laboratory, British School at Athens, Odhós Soudhías 52, GR-10676 Athens,
10 Greece.

11 ²Geography, School of Environment and Development, The University of Manchester,
12 Oxford Road, Manchester, M13 9PL, UK.*

13 ³Department of Environmental Science, Lancaster Environment Centre, Lancaster
14 University, Lancaster, LA1 4YQ, UK.

15 * To whom correspondence should be addressed. E-mail: r.j.payne@manchester.ac.uk

16

17 ABSTRACT

18

19 The Elatia Mires of northern Greece are unique ecosystems of high conservation value.

20 The mires are climatically marginal and may be sensitive to changing hydroclimate,

21 while northern Greece has experienced a significant increase in aridity since the late 20th

22 century. To investigate the impact of recent climatic change on the hydrology of the

23 mires, the palaeoecological record was investigated from three near-surface monoliths

1 extracted from two sites. Testate amoebae were analysed as sensitive indicators of
2 hydrology. Results were interpreted using transfer function models to provide
3 quantitative reconstructions of changing water table depth and pH. AMS radiocarbon
4 dates and ^{210}Pb suggest the peats were deposited within the last c.50 years, but do not
5 allow a secure chronology to be established. Results from all three profiles show a
6 distinct shift towards a more xerophilic community particularly noted by increases in
7 *Euglypha* species. Transfer function results infer a distinct lowering of water tables in this
8 period. A hydrological response to recent climate change is a tenable hypothesis to
9 explain this change; however other possible explanations include selective test decay,
10 vertical zonation of living amoebae, ombrotrophication and local hydrological change. It
11 is suggested that a peatland response to climatic change is the most probable hypothesis,
12 showing the sensitivity of marginal peatlands to recent climatic change.

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14 **KEYWORDS:** Mires, Peatlands, Climate Change, Testate Amoebae, Palaeohydrology

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2 INTRODUCTION

3 Climate change may lead to changes in peatland carbon sequestration (Belyea and
4 Malmer, 2004; Lavoie et al., 2005), gas flux (Chapman and Thurlow, 1998; Regina et al.,
5 1999; Keller et al., 2004), plant communities (Weltzin et al., 2003; Robroek et al., 2007)
6 and permafrost melting (Camill, 2005; Turetsky et al., 2007). In many regions of the
7 world impacts on peatland hydrology have been noted (e.g. Klein et al., 2005). The
8 peatlands likely to show the greatest impacts are the most marginal sites in regions which
9 experience the greatest climate change. Such a case may be the Mediterranean region,
10 where peatlands are rare but are often climatically marginal and are likely to be sensitive
11 to comparatively minor climatic changes. Mediterranean peatlands are relatively
12 unimportant in terms of their carbon reserve but are extremely important in terms of
13 regional biodiversity.

14 Meteorological records for northern Greece show a general trend of reduced
15 precipitation over the last c.50 years. Climatic impacts on the hydrology of northern
16 Greece have already been noted and are modelled to increase in severity over coming
17 decades with consequent impacts for human populations (Mimikou, 1993; Mimikou et
18 al., 1999; 2000; Bürger, 2002; Baltas and Mimikou, 2005). These changes may have
19 affected peatlands in the region. Most peatlands in Greece have been heavily impacted by
20 human activity primarily through drainage for agriculture in the 20th Century, and often
21 have low conservation value (Bouzinou et al., 1994; 1997; Christanis, 1996). An
22 exception to this is the Elatia Mires of northern Macedonia where remoteness and Cold
23 War access restrictions have prevented major impacts. These mires are the most

1 oligotrophic peatlands in Greece and are probably the only location in the southern
2 Balkans where many wetland endemics typical of more nutrient-poor conditions may be
3 found. These are the only peatlands in Greece where *Sphagnum* is a permanent presence.
4 Due to their unique nature the Elatia Mires have been a focus of recent conservation
5 attention. This study uses a palaeoecological approach based on testate amoebae analysis
6 to test the hypothesis that the hydrology of the Elatia mires is responding to recent
7 climatic changes.

8

9 SITES and METHODS

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11 The Elatia Mires lie in the Elatia Forest, approximately 70 km north of the city of
12 Drama and 5 km south of the Bulgarian frontier at around 1500m asl. (41°29'N, 24°19'E;
13 Fig. 1). Four small peatlands are situated in clearings within a natural coniferous forest
14 dominated by *Picea abies*, the only such forest in Greece (Papazisimou et al., 2002). A
15 comparatively cool and moist climate combined with impermeable granite bedrocks have
16 allowed peat formation. Peat deposits are shallow, probably not exceeding 1 m of
17 continuous peat, although deeper, buried, peat deposits are also found in one site. The
18 mires have been termed 'transitional' due to their mix of features typical of fens and bogs
19 (Papazisimou et al 2002). Samples were extracted from the two most oligotrophic sites:
20 Dexameni mire (site DE; mean pH 6.5) and Krya Vrissi mire (site KB; mean pH 6.4).
21 Vegetation of the mires includes *Juncus effusus*, *Carex* spp., *Eriophorum latifolium*,
22 *Myosotis* spp., *Geum* spp., *Ranunculus* spp., *Mentha spicata*, *Plagiomnium elatum*,

1 *Sphagnum flexuosum*, *Aulacomnium palustris* and *Climacium dendroides* (Papazisimou et
2 al 2002).

3 Peat monoliths between 16 and 26 cm in length were extracted by cutting down
4 from the peat surface. Two monoliths were removed from central areas of the Krya Vrissi
5 mire and one from Dexameni; denoted KB1, KB2 and DE respectively. Peat stratigraphy
6 was noted and humification recorded on the Von Post scale (Von Post, 1924). Contiguous
7 1cm-deep samples were taken through the length of the monoliths.

8 Testate amoebae analysis was used to reconstruct changes in hydrology. Testate
9 amoebae are shell-forming unicellular microorganisms that are abundant in peatlands and
10 sensitive to peatland hydrology. By analysing the changing community composition
11 down the length of a peat core and interpreting the results with a transfer function model
12 it is possible to reconstruct how mire wetness has varied over time (Charman, 2001;
13 Mitchell et al., 2008). Such reconstructions have been validated by comparison with
14 instrumental data and independent proxy-climatic records (Charman and Hendon, 2000;
15 Charman et al., 2004; Schoning et al., 2005).

16 Peat sub-samples for testate amoebae analysis were boiled in deionised water,
17 filtered at 250 µm and then back-filtered at 15 µm with the 15>250 µm fraction retained
18 (Hendon and Charman, 1997). Slides were made up with glycerol and amoebae identified
19 following the taxonomic scheme described in Payne and Mitchell (2007); a count of 150
20 tests was aimed for. Amoebae diagrams were constructed using C² ver. 1.4 (Juggins,
21 2003) and zoned using optimal sum of squares partitioning (Birks and Gordon, 1985) in
22 ZONE ver. 1.2 (Juggins, 1992). Quantitative environmental reconstruction was carried
23 out using the transfer function models developed by Payne and Mitchell (2007). Depth to

1 water table (DWT) was reconstructed using a maximum likelihood model ($RMSEP_{boot}$
2 1.9cm) and pH using a weighted average model ($RMSEP_{boot}$ 0.4). The reconstructed
3 values are termed testate amoebae inferred depth to water table (TI-DWT) and testate
4 amoebae inferred pH (TI-pH). Bootstrapped error estimation with 1000 cycles was used
5 to provide sample-specific error estimates.

6

7 Chronology

8 Establishing reliable chronologies for recent peat deposits has been a persistent
9 challenge for peatland palaeoecologists (Turetsky et al., 2004) and is particularly
10 problematic for these sites as they are non-ombrotrophic and include unusual plant
11 communities. A search for cryptotephra (following the method of Pilcher and Hall,
12 1992) failed to identify any shards and a search for spheroidal carbonaceous particles
13 (following the method of Rose et al, 1995) failed to find adequate concentrations. Two
14 radiometric methods, ^{210}Pb and ^{14}C analysis were applied to the peat profiles. ^{210}Pb was
15 analysed by assuming equilibrium with its grand-daughter ^{210}Po . Peat samples were
16 dissolved in strong acids with a ^{209}Po yield tracer, plated onto copper disks and activity
17 measured by α -spectrometry. Four samples from towards the base of the monoliths were
18 AMS radiocarbon dated (Goodsite et al, 2001; Goslar et al, 2005). Bulk samples were
19 used due to the absence of *Sphagnum* (Nilsson et al. 2001). Samples were carefully
20 prepared to minimise risk of external contamination and rootlets were picked out.

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1 RESULTS

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3 Testate amoebae

4 Testate amoebae were found through the length of the monoliths but apparent
5 concentrations were low (counting time approximately 6-8 hours per sample). The three
6 testate amoebae diagrams show similar trends (Fig. 2). At the base of the profiles (zones
7 DE-1, KB1-1 and the lower portion of KB2-1) the community composition is noted by
8 abundant *Diffugia* spp., particularly *Diffugia pulex* type. Above this section there is a
9 decline in these taxa leading to a more diverse community noted by *Centropyxis*
10 *aerophila* (zones DE-2, KB1-2 and the upper portion of KB2-1). The next significant
11 change is a marked increase in *Euglypha rotunda*, and in KB1, *Euglypha ciliata* type. The
12 uppermost samples are different from those directly below, recognized as a separate zone
13 in KB2 (zone KB2-3) and DE (zone DE-3).

14 Overlap between the palaeoecological data and the modern training set is very
15 good. Over 99% of all amoebae counted are included in the training set; the total for
16 individual samples does not fall below 97%. Water table reconstructions show a similar
17 pattern between sites (Fig. 3). From the base of the sequences to c.6 cm depth there is low
18 amplitude variability with no obvious similarity between profiles. At 6 cm there is a rapid
19 increase in values that significantly exceeds bootstrapped error estimates, representing a
20 significant lowering of water table in all sites. At the top of the sequence there is a slight
21 decline in TI-DWT values and then a resumed increase in sites KB1 and DE, although
22 there is a continued decline in site KB2. pH reconstructions show gradual lowering of pH

1 values, increasing in rate above c.6 cm. The changes in pH are less pronounced than
2 those in DWT and (particularly in KB2) only marginally exceed the error estimates.

3 The main amoebae community change is a shift in the most abundant taxon to
4 *Euglypha rotunda* from *Centropyxis aerophila* type and *Diffflugia pulex* type. *C.*
5 *aerophila* is generally regarded as typical of moderately wet conditions, while *E. rotunda*
6 is probably most typical of intermediate conditions (Charman et al., 2000). The indicator
7 value of *D. pulex* type is uncertain. The majority of transfer functions to encounter both
8 taxa show *E. rotunda* to have a higher DWT optimum than *C. aerophila* type (or
9 equivalent *C. cassis* type) (Payne et al., 2006; 2007; in press; Woodland et al., 1998;
10 Charman and Warner, 1997; Charman, 1997; Warner and Charman, 1994; Lamentowicz
11 and Mitchell, 2005). The transfer function results therefore agree with the known
12 autecology of the taxa in interpreting this change as a shift to drier conditions.

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14 Chronology

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16 The ^{210}Pb results show no decline in activity with depth and a sequence of peaks
17 and troughs (Fig. 4). It was not possible to determine the “unsupported” component of the
18 ^{210}Pb ; insufficient material was available for direct ^{226}Ra analysis and the base of the
19 unsupported layer was not reached. The lack of a monotonic decrease in activity with
20 depth undermines the attempt to use the method for dating. There are two possible causes
21 of these results; very rapid peat accumulation, as suggested by the radiocarbon dates, and
22 movement of lead within the peat profile given the minerotrophic nature of the sites
23 (MacKenzie et al., 1998).

1 Radiocarbon dates were all returned as post-bomb and calibrated using CaliBomb
2 (<http://calib.qub.ac.uk/CALIBomb/frameset.html>). All dates give multimodal probability
3 distributions ranging from the mid-1950s to post-1995, indicating rapid peat
4 accumulation (Table 1). As the dates were based on bulk samples it is possible that they
5 have been contaminated by modern carbon, perhaps through penetration by sedge roots.
6 However, the samples were prepared carefully to avoid contamination and obvious roots
7 were removed. Systematic differences between dates on bulk samples and selected
8 macrofossils have not been proven (Blaauw et al. 2004). The dates are internally
9 consistent in showing the peats to be late 20th century in age and are not contradicted by a
10 date of 100 ± 40 ¹⁴C yrs BP at 31-36 cm from a neighbouring site (Papazisimou et al.,
11 2002). For two samples from the KB2 monolith, the deeper sample (GdA-1178: 21-
12 24cm) has a highest probability peak more recent than the upper sample (GdA-1016: 13-
13 15cm). However this date also has a subsidiary probability peak at 1957-1958 (10.3%).
14 As there is no stratigraphic reason to suspect a reversal it seems more likely that this
15 older peak is the correct one.

16 Neither the ²¹⁰Pb results nor the radiocarbon dates allow us to establish a secure
17 chronology for these profiles. However both sets of results can be taken to suggest that
18 the sediments are very recent. Most probably these peats have accumulated within the last
19 few decades but it is not possible to be more precise. Nevertheless, there is no reason to
20 suspect disturbance of the stratigraphy and it is still probable that these profiles do
21 preserve a continuous record of testate amoebae changes and may reveal recent
22 hydrological changes in the mires.

23

1 DISCUSSION

2

3 The testate amoebae results show a single large change in amoebae community at
4 around 6 cm depth which can be inferred as a shift to a drier mire surface. This is entirely
5 consistent with the hypothesis of a lowered water table due to the general reduction in
6 precipitation in northern Greece. However simple attribution of the changes to a climatic
7 impact is not possible due to the presence of other factors which could also be responsible
8 for these changes.

9

10 1. Non-climatic hydrological change

11 It seems probable that the Elatia Mires have undergone hydrological change;
12 however as the sites are not ombrotrophic it is possible that this is unrelated to climate. It
13 is possible that processes such as forestry, tectonic or geomorphological change in the
14 wider area could have lead to a change in water input into the mires. Although there is no
15 particular evidence to suggest that this may be the case the hypothesis cannot be
16 discounted on the basis of the data presented here.

17

18 2. Vertical zonation of living amoebae

19 Interpretation of the uppermost testate amoebae assemblages may be complicated
20 if the amoebae are still alive below the surface. Testate amoebae have been noted to
21 exhibit vertical zonation forced by gradients in light, moisture and mineral material for
22 test construction (Heal, 1962; Booth 2002; Mitchell and Gilbert 2004; Mazei et al. 2007).
23 It is not clear that vertical zonation could explain the species changes observed. Taxa

1 with xenosome tests such as *Diffflugia* spp. and *C. aerophila* are commonly observed in
2 lower horizons, probably due to availability of material for test construction. However the
3 typical position of *Euglypha* species varies greatly between studies (Chacharonis 1956;
4 Booth 2002; Mitchell and Gilbert 2004; Mazei and Bobnova 2007). The only study in
5 transitional mires (Mazei and Bobnova 2007) found vertical zonation to be much weaker
6 than in bogs. In these sites alive or encysted amoebae were only noted in the top 2-3 cm.
7 It is probable that vertical zonation in this region could explain the unusual communities
8 in the uppermost samples, but unlikely that vertical zonation is the cause of the major
9 change at 6cm.

10

11 3. Test preservation

12 The taxa which are primarily responsible for the increase in TI-DWT in the upper
13 portions of the sequence (*E.rotunda*, *E.ciliata*, *C.dubium*) all have tests constructed of
14 idiosomes. Such tests (and particularly those of *Euglypha*) may be particularly prone to
15 decomposition in the fossil record (Lousier and Parkinson, 1981; Swindles and Roe,
16 2007; Payne 2007). Selective test loss could have led to inaccurate palaeoenvironmental
17 reconstruction in these sites (Mitchell et al. 2008). A number of strands of evidence
18 suggest this is unlikely to be the major cause of the changes: 1) during microscopy no
19 apparent reduction in overall test concentrations was noted with depth (although
20 concentrations were not enumerated), this might be expected given the high proportion of
21 idiosome tests; 2) no increase in degraded tests was noted with depth; 3) the taxa
22 concerned decline sharply at around 6 cm, however they continue through the rest of the
23 profiles in lower concentrations; 4) other closely related taxa reach their highest

1 concentrations lower in the profiles (e.g. *E.tuberculata* in KB1); 5) the timescale under
2 consideration is very short so differential preservation is perhaps less likely than in
3 longer-term studies; 6) when *Euglypha* spp. are removed from the reconstructions an
4 increase in TI-DWT at 6cm remains (Fig. 3), although this is less marked and there are
5 other changes such as a large drop in TI-DWT at the top of KB1.

6

7 4. Autogenic mire development processes

8 The Elatia mires have been termed ‘transitional’ between fens and bogs; it is
9 possible that the changes in the palaeoecological record are due to ombrotrophication.

10 Ombrotrophication can be expected to lead to major amoebae community changes as fens
11 have distinctly different testate amoebae communities from bogs and nutrient status is an
12 important secondary gradient (Opravilova and Hajek, 2006).

13 Conventionally it has been assumed that an autogenic mechanism drives
14 ombrotrophication; peat gradually accumulates above the water table until it is no longer
15 reliant on groundwater and becomes acidified by leaching and the establishment of
16 *Sphagnum* (Hughes, 2000). This would sit well with the reconstructed increase in TI-
17 DWT and slight decrease in TI-pH. However, it seems unlikely that this autogenic model
18 could explain the suddenness of the amoebae change, the 2cm+ offset between
19 stratigraphic and amoebae community changes and the apparent synchronicity in change
20 between two sites. More recent studies have suggested allogenic forcing of
21 ombrotrophication with a lowered water table leading to the peat surface being separated
22 from groundwater (Hughes, 2000; Hughes and Dumayne-Peaty, 2002; Hughes and

1 Barber, 2003; Hughes et al., 2000). It is therefore also possible that ombrotrophication is
2 occurring, but is driven by a real allogenic change.

3

4 The results presented here are consistent with the hypothesis of a climate change-
5 induced hydrological change; however other explanations cannot be excluded. It is
6 possible that multiple processes have lead to the observed patterns. If the changes are due
7 to climate then this study provides the first evidence for the impacts of recent climate
8 change on Mediterranean peatlands. Climate change is likely to be a key challenge to
9 regional peatland conservation.

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13 **Acknowledgements**

14

15 This study was primarily funded by a BSA MacMillan-Rodewald fellowship to RJP.
16 Radiocarbon dates were funded by grants from the Richard Bradford McConnell Fund
17 and a Gladstone Memorial Prize. Thanks to Kimon Christanis and Stavros Kalaitzidis
18 (University of Patras) for discussion of the Elatia Mires, to Edward Mitchell (Lausanne)
19 for discussion of testate amoebae ecology and taxonomy, and to two anonymous
20 reviewers for comments on a previous draft. The map was prepared by Edward Mitchell.
21 This work was carried out by permission of the Greek Institute of Geology and Mineral
22 Exploration (IGME) and the Forest Service of the Prefecture of Drama.

23

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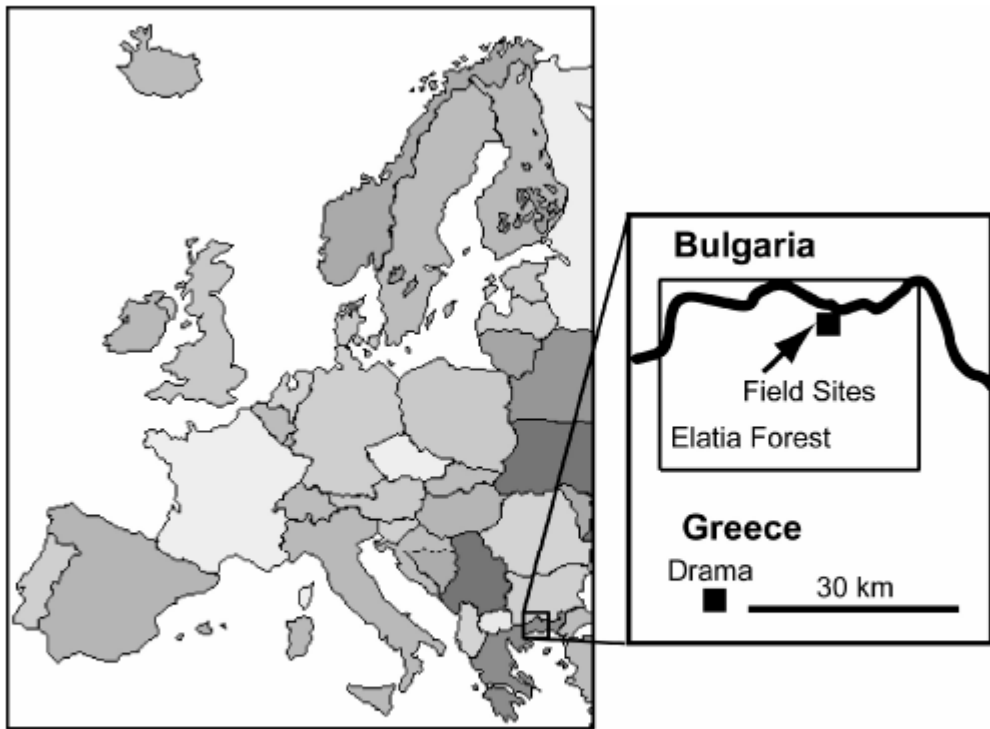
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1

2 FIGURES and TABLE

3

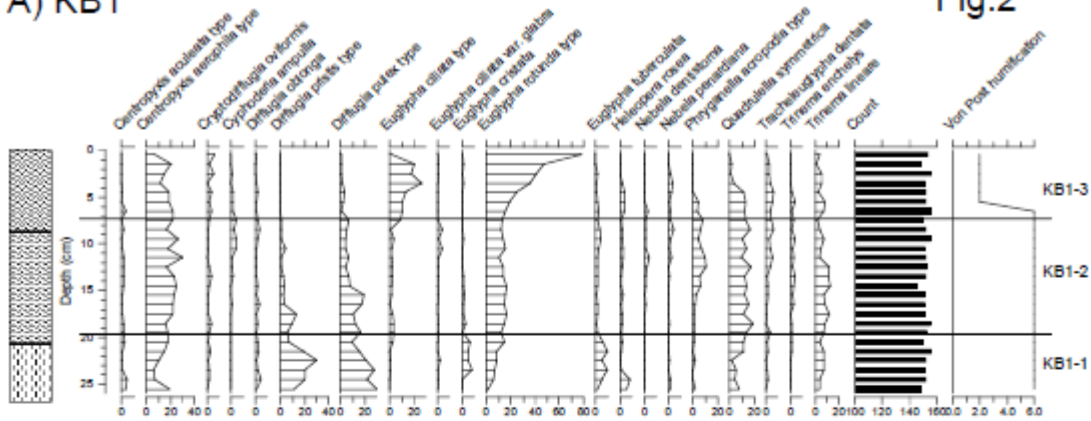
4 Figure 1. Location of the Elatia Mires.



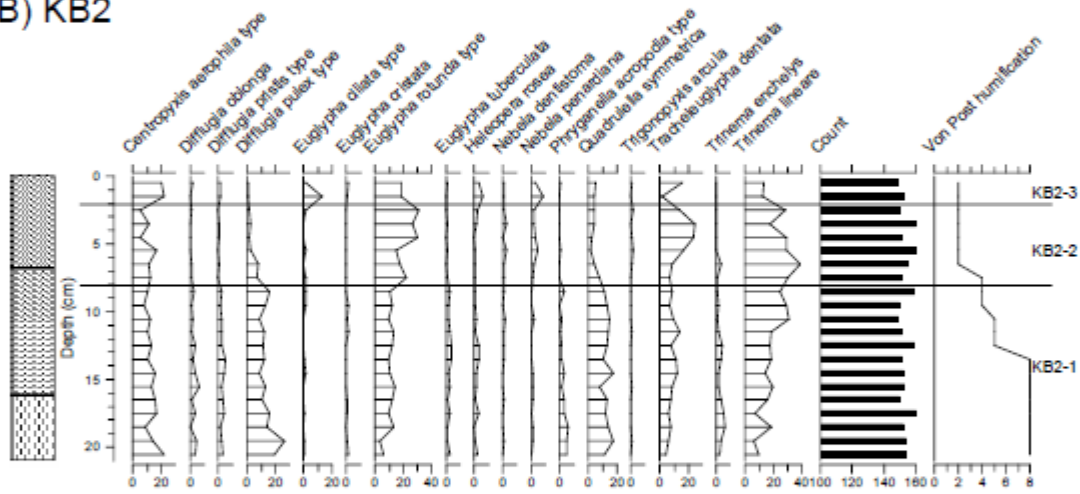
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6 Figure 2. Testate amoebae profiles from three monoliths. Showing peat stratigraphy,
7 major testate amoebae species (% of total), testate amoebae total count, humification
8 expressed on the Von Post scale, and testate amoebae zones. Stratigraphic columns show
9 moss-dominated peat (solid wavy lines), peat of mixed composition with mosses most
10 abundant (interrupted wavy lines), peat of mixed composition with macrofossils most
11 abundant (interrupted vertical lines) and macrofossil-dominated peat (solid vertical lines).

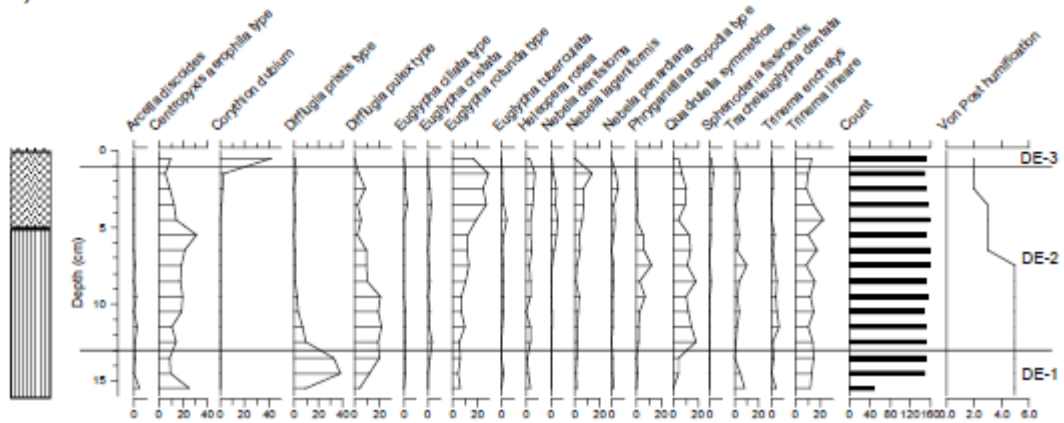
A) KB1



B) KB2



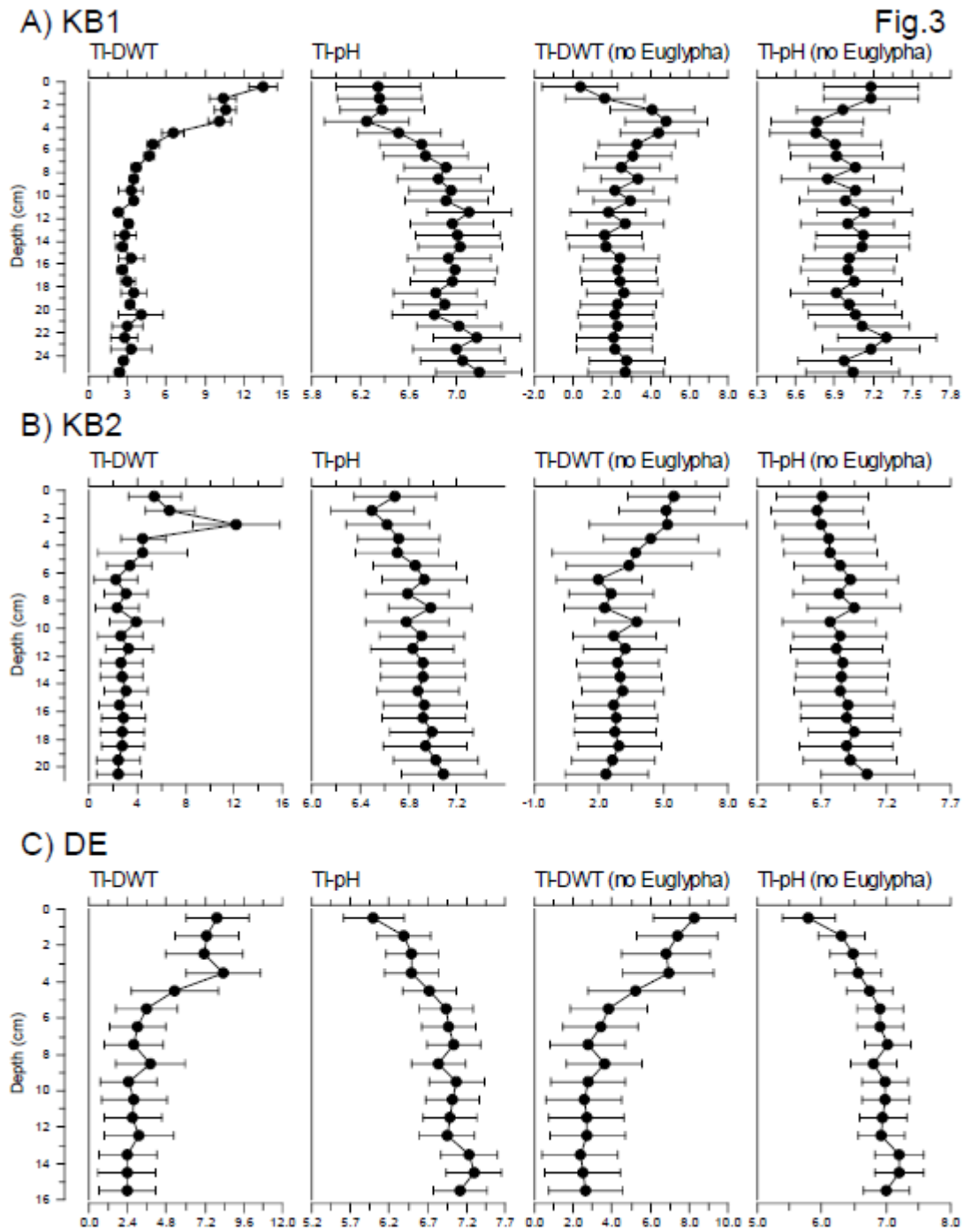
C) DE



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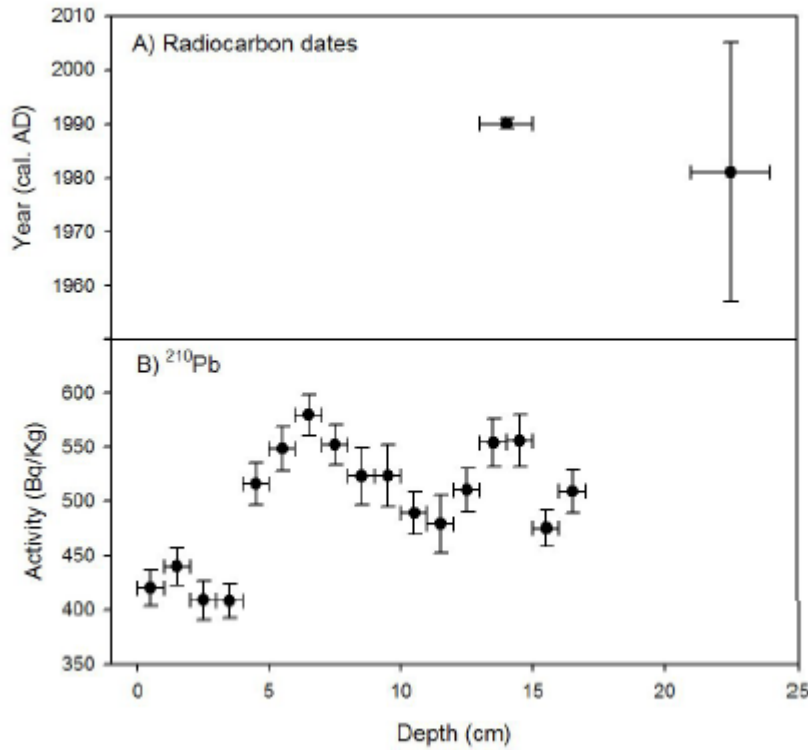
2

1 Figure 3. Testate amoebae inferred depth to water table (TI-DWT) and testate amoebae
2 inferred pH (TI-pH) from the three peat monoliths with boot-strapped error estimates.
3 The transfer function model was based on one-off DWT and pH measurements so the
4 units of reconstruction are depth to water table (cm) and pH based on an October 2005
5 datum. Also showing TI-DWT and TI-pH reconstructions excluding *Euglypha* species,
6 which might be lost from the palaeoecological record.



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2 Figure 4. Radiocarbon dates and ^{210}Pb profile for monolith KB2.



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Table 1. Radiocarbon dates from the peat monoliths

Monolith	Depth (cm)	Date code	Percent modern carbon (pMc)	Calibrated age range (95% probability) (cal. years AD)
DE	15-16	GdA-1177	120.55 ± 0.37	1958-1961 (9.3%) 1985-1988 (90.5%)
KB1	21-24	GdA-1015	121.45 ± 0.36	1958-1961 (52.7%) 1984-1986 (42.3%)

KB2	13-15	GdA-1016	116.17 ± 0.33	1956-1958 (0.2%) 1989-1991 (94.8%)
KB2	21-24	GdA-1178	110.88 ± 0.53	1957-1958 (10.3%) 1995- (85.1%)

1