1 **How many is enough? Determining optimal count totals for**

2 **ecological and palaeoecological studies of testate amoebae.**

3

4 Richard J. Payne [1*] and Edward A.D. Mitchell [2,3]

5

6 [1] Geography, School of Environment and Development, The University of

7 Manchester, Oxford Road, Manchester, M13 9PL, United Kingdom.

8 [2] WSL Swiss Federal Research Institute, Ecosystem Boundaries Research Unit,

9 Wetlands Research Group, Station 2, CH–1015 Lausanne, Switzerland.

10 [3] Ecole Polytechnique Fédérale de Lausanne, Laboratory of Ecological Systems,

11 Station 2, CH–1015 Lausanne, Switzerland.

12

13 * Corresponding author: E-mail: r.j.payne@manchester.ac.uk

14

15 **Keywords:** Testate amoebae, Protists, Palaeoecology, Palaeohydrology,

16 Palaeoclimate, Peatlands.

17

18 **Running title:** Optimising testate amoebae counts in palaeoecology

1   **Abstract**

2   Testate amoebae are increasingly used in ecological and palaeoecological studies of

3   wetlands. To characterise the amoeba community. a certain number of individuals

4   need to be counted under the microscope. To date, most studies have aimed for 150

5   individuals, but that sample size is not based on adequate evidence. When testate

6   amoeba concentrations are low, it can be difficult or impossible to reach this total.

7   The impacts of lower count totals have never been seriously scrutinised. We

8   investigated the impact of count size on number of taxa identified, quantitative

9   inferences of environmental variables and the strength of the links between amoebae

10   and environmental data in the context of predicting depth to water table. Low counts

11   were simulated by random selection of individuals from four existing datasets.

12   Results show progressively diminishing returns by all criteria as count size increases

13   from low numbers to counts of 150. A higher count is required to identify all taxa

14   than to adequately characterise the community for transfer function inference. We

15   suggest that in most cases, it will be a more efficient use of time to count a greater

16   number of samples to a lower count. While a count of 50 individuals may be

17   sufficient for some samples from some sites we recommend that counts of 100

18   individuals should be sufficient for most samples. Counts need only be increased to

19   150 or more where the aim is to identify relatively minor, but still potentially

20   ecologically relevant community changes. This approach will help reduce lack of

21   replication and low resolution, which are common limitations in testate amoeba-based

22   palaeoecological and ecological studies.

23

1 Introduction

2 Testate amoebae are a group of unicellular eukaryotic micro-organisms characterised

3 by a decay-resistant shell (Meisterfeld 2000a, b). Testate amoeba analysis has been

4 used in a variety of ecological and palaeoecological applications (Foissner 1999;

5 Mitchell et al. 2008b) and has been intensively applied in palaeoecological studies

6 from peatlands with the aim to reconstruct mire palaeohydrology and thereby, at least

7 for ombrotrophic bogs, past climatic change (Charman 2001). By analysing testate

8 amoeba community changes down the length of a core and interpreting the results

9 with a transfer function model, it is possible to quantitatively reconstruct changing

10 mire surface wetness (Booth et al. 2004; Lamentowicz et al. 2008; Sillasoo et al.

11 2007; Woodland et al. 1998) as well as sea-levels (Gehrels et al. 2002; Roe et al.

12 2002) through the Holocene. Testate amoebae are also used over shorter time periods

13 to monitor the success of peatland restoration (Buttler et al. 1996; Davis and

14 Wilkinson 2004; Jauhiainen 2002; Laggoun-Défarge et al. 2008; Vickery and

15 Charman 2004) and the impact of pollution in aquatic or terrestrial ecosystems (Balik

16 1991; Kandeler et al. 1992; Kauppila et al. 2006; Kumar and Patterson 2000; Nguyen-

17 Viet et al. 2007; Nguyen-Viet et al. 2004; Patterson et al. 1996; Patterson and Kumar

18 2002; Reinhardt et al. 1998; Scott et al. 2001; Török 2001).

19   To adequately characterise the community composition of any microfossil

20 used in palaeoecology, a minimum number of individuals needs to be counted under

21 the microscope. For different biological proxies, the minimal count required ranges

22 between 50 and over 300, largely dictated by the number of taxa typically identified

23 in a sample. Lytie and Wahl (2005) explored the effect of pollen sample size on

24 vegetation reconstruction and observed that with counts as low as 150 grains, the

25 vegetation reconstruction was accurate within >90%. Other studies suggest that

counts of 200 (Barkeley 1934) to 250 grains (Hill 1996) are sufficient. However, the recommended sum is generally between 300 and 500 grains, and in order to obtain a precise estimate for rare taxa, counts of 500 to 1000 grains are preferred (Birks and Birks 1980). Heiri and Lotter (2001) investigated the impact of low counts on transfer function inference for Chironomids and concluded that counts as low as 50 specimens provided useful results. For charcoal, the minimum count (including exotic marker grains) was estimated at 200 (Finsinger and Tinner 2005). For diatoms using the random settling method, a total count of 300 has been shown to yield reasonable estimates for the most abundant taxa, and increasing the count to 400 did not greatly improve the accuracy (Boden 1991), although the reliability of this approach has been criticized (Meng 1994). Totals of 500-1000 valves are frequently used (Lowe and Walker 1997). In the case of foraminifera, the required minimum is 300-400 individuals (Lowe and Walker 1997).

For testate amoeba analysis, studies have usually aimed to count either 150 or, less commonly, 100, 200, or 300 individuals (Bobrov 2005; Charman et al. 2000; Tolonen 1986; Warner 1990). Whether such counts are actually sufficient has not been rigorously tested. Mitchell et al. (2000) looked at the relationship between testate amoeba count and number of taxa and found a plateau was reached after around 100 individuals. Warner (1990) recorded the number of taxa with increasing count for 8 samples and found a plateau was reached after around 60 individuals. Woodland et al. (1998) showed that cumulative species diversity reached a plateau after around 100 individuals.

Several studies have found it impossible, or at least impractical, to reach the usual total. Low test concentrations have been encountered in situations such as palaeoecological sequences with highly humified peats (Beyens and Chardez 1987;

1 Charman et al. 2001) and surface and sub-surface samples of fen peats (Jauhiainen

2 2002; Payne and Pates 2008). There are probably three factors contributing to these

3 problems: 1) low abundance of amoebae in the sediments on deposition, 2)

4 decomposition of tests over time and 3) a reduction in apparent test concentration due

5 to a high abundance of organic or inorganic material in the same size range as the

6 tests, which is not removed in sample preparation.

7   Low test concentrations present particular problems in palaeoecological

8 studies where an adequate count may not be achievable for parts of the sequence. This

9 leads to the question of whether some data is better than none: is it better to have

10 portions of a sequence with very low counts or to leave a gap in the palaeoecological

11 record? Some palaeoecological studies have presented records with very low counts

12 and have made palaeoenvironmental inferences based on these results, albeit with

13 caveats clearly stated (e.g. Beyens and Chardez 1987). However no study has been

14 undertaken to assess the relationship between number of testate amoeba shells

15 counted and data quality.

16   This study attempts to determine the minimum count levels required for

17 ecological and palaeoecological studies of testate amoebae by examining the impact

18 of count size on the number of taxa identified in three contrasting sites, by simulating

19 low counts using a random-selection approach with four previously established

20 modern training sets, and then applying the same approach to two palaeoecological

21 data sets.

22

23

Methods

General approach and aims

To determine the test count required to characterise a sample, we use four criteria: 1) The number of taxa. As count size increases, the number of taxa identified should increase and then stabilise as the total complement is reached. 2) Transfer function inferences of environmental variables. As count increases, transfer function inferred values and associated standard errors should stabilise. 3) The strength of the relationship between the amoeba community and environmental variables. As the amoeba community becomes better characterised with increasing count size, the strength of the relationship with key environmental controls should improve. 4) The inferred pattern of wet and dry periods in palaeoecological records: As count size increases, the pattern should become more and more similar to the full model.

Impact of count on number of taxa identified

Previous studies have looked at the impact of count size on the number of taxa identified (Warner 1990; Woodland et al 1998). However these studies only considered a limited number of samples from a limited range of sites, and it is useful to examine this with additional data. We present new data from the Ispani-2 peatland, a percolation bog in western Georgia (Connor et al. 2007; Joosten et al. 2003)(10samples), the Sterling peatland, a kettle-hole mire in southern Alaska (Payne et al. 2006)(10 samples) and Moidach More a blanket bog in eastern Scotland (150 samples). The sites represent a variety of peatland types and cover a large geographic range. Samples were extracted from the surface of the Ispani-2 and Moidach More

6

1   sites and just below the surface (10-20cm) in the Sterling site. The normal total of 150

2   tests was counted for each sample and the number of tests required to identify each

3   additional taxon recorded.

4

5   Impact of count on species-environment relationships and predicted water table depth

6

7   To assess the impact of count size on amoeba community recorded, low-counts were

8   simulated using four data sets from previous studies of peatland testate amoebae: 1)

9   the data of Payne & Mitchell (2007) from the Elatia mires in northern Greece, 2) the

10   data of Payne et al. (2008) from the Sürmene Ağaçbaşı Yaylası peatland in north-

11   eastern Turkey, 3) the data of Mitchell et al. (1999) from peatlands of the Jura

12   Mountains (France and Switzerland) and 4) the data of Payne et al. (2006) from

13   peatlands in southern Alaska (Table 1). Samples in these studies were counted to at

14   least the usual total of 150 and recorded as percentages. Species complement varied

15   from 4 to 27 taxa. Much of the count is represented by a few taxa, and a single taxon

16   typically accounts for at least 25% of the total. Counts of 10, 20, 30..140 were

17   simulated by randomly selecting individuals from the full data set, and each

18   individual was able to be selected more than once. The transfer function models

19   calculated in the original studies were used to infer depth to water table based on

20   these simulated data sets. Standard errors were calculated using bootstrapping (1000

21   cycles). The procedure was repeated nine additional times with random re-selection of

22   individuals on each occasion, and the bias from the predicted values of the original

23   model were calculated.

24   These experiments assume that a count of 150 tests is sufficient to characterise

25   the amoeba community, To test the impact of higher totals on transfer function

1 performance, a sub-set of samples was separately analysed. For some samples from

2 the Alaska and Jura studies, amoeba concentrations were high and over 250 tests were

3 counted. Counts of 150, 160,..., 240 were simulated for these samples using the same

4 repeated random selection approach as above. Transfer function inferences using the

5 simulated data sets were compared to those of the full model for these samples.

6   Ordination was used to test the impact of count on the strength of relationship

7 between species and environmental data. The percent variance explained by the depth

8 to water table (DWT) data was tested using redundancy analysis (RDA) with each of

9 the simulated low count data-sets in CANOCO ver.4.53 (ter Braak and Šmilauer

10 1997-2004). As these analyses are labour-intensive only one cycle of data-selection

11 was used for this experiment.

12

13 Impact of count on palaeoecological interpretation

14

15 Finally, the same random data-selection approach was used to simulate low counts for

16 two palaeoecological data sets from the Praz-Rodet peatland in Switzerland (Mitchell

17 et al. 2001) and a small sub-Arctic mire in eastern Alaska, USA (termed site DLB;

18 Payne & Blackford, unpublished data). Water table changes were inferred for each of

19 the simulated data sets using the transfer functions of Mitchell et al. (1999, 2001) and

20 Payne et al. (2006). Although low counts were simulated for the palaeoecological

21 data, the full data sets were used for the transfer functions. The impact of lower

22 palaeoecological counts was evaluated by comparing the pattern of inferred low and

23 high water tables, and the correlation between the inferred DWT values of the reduced

24 versus full data sets. Reconstructions were repeated with ten sets of random

1 reselection, and standard deviations were calculated to assess the repeatability of

2 predictions.

3

4

5 **Results**

6 Impact of count size on number of taxa identified

7

8 Fig. 1 shows average number of taxa encountered with various count sizes. With the

9 Ispani-2 and Sterling samples, plateaux in the number of taxa are reached with counts

10 of around 100 and 120 individuals, respectively. With the Moidach More samples

11 there is a continued but slowing increase in taxa complement through to 150

12 individuals. In 55 of these 150 samples, new taxa are still encountered with counts

13 over 130. It is probable that 150 individuals is insufficient to identify all taxa in these

14 samples. There is no simple relationship between the total number of taxa in a sample

15 and the count required to identify all taxa. The Sterling samples are more diverse than

16 the Moidach More samples, but a count of 130 individuals would identify all taxa in

17 the Sterling samples and miss taxa in more than a third of the Moidach More samples.

18 The count required to identify all taxa depends not just on the number of taxa but the

19 relative abundances of those taxa.

20

21 Impact of count size on species-environment relationships and predicted water table

22 depth

23

24 Fig. 2 shows box plots of transfer-function inferred water table depth with various

25 count totals for four representative samples from the Sürmene Ağaçbaşi Yaylasi site

1   using ten cycles of random selection of individuals. Increasing the count generally

2   reduces the scatter of predictions and brings them closer to the full-model predicted

3   result, but not necessarily the measured value. Clearer results are obtained when using

4   all samples and considering mean bias from the full-model predicted values (Fig. 3).

5   With all data sets, increasing count gives predictions that are closer to the full model.

6   This improvement is most rapid in the range of 10-50 individuals. Counts over 50 and

7   up to 100 produce a slight further improvement, this is most noticeable with the

8   outliers in the Turkey data. Increasing the count from 100 to 140 appears to make

9   little further difference. Fig 4 shows the results if this is extended to counts of up to

10   240 using just those samples from the Jura and Alaska data sets with counts $\geq$250. As

11   with the full data, the greatest difference is obtained by increasing the count to around

12   50 with less pronounced improvement beyond that. It is notable that increasing the

13   count beyond 150 seems to make a continued slight improvement in performance in

14   both data sets. It should, however, be noted that the data sets used to obtain this result

15   are small, with 12 and 18 samples for the Alaska and Jura data, respectively.

16   Increasing count reduces the standard error of predictions (Fig. 5). The count

17   required before values stabilise varies between data sets from 40 with the Alaska data

18   to perhaps 100 with the Turkey data. Increasing the count also increases the percent

19   variance explained in the redundancy analyses (Fig. 6). As these results are based on a

20   single cycle of random selection, there is more apparent 'noise' than with the results

21   discussed above. Values stabilise after counts of 100 in all sites. The increase is most

22   rapid with the Turkey data and least rapid with the Greece data.

23

24   Impact of count size on palaeoecological inferences

25

1   The palaeoecological reconstructions from Praz-Rodet and site DLB are shown in

2   Fig. 7a and Fig. 7b, respectively. With low counts, there is a great deal of high-

3   amplitude variability. Increasing the counts generally gives a record with less noise.

4   Some features of the record are only resolved with a higher count such as the double

5   peak at the base of the Praz-Rodet record, and the trough at 21-22 cm in the DLB

6   record. It is clearly the case that increasing the count size gives a less noisy TI-DWT

7   record, which agrees better with the results of the full model. However, it is perhaps

8   surprising that counting very few tests still shows many of the major trends. Even

9   with counts as low as 10 tests, the DLB record picks out the three distinct wet phases

10   at 26-29 cm, 53-56 cm and 61-71 cm. In the Praz-Rodet record, counts of ten tests

11   still show the distinctive peaks at 126, 166 and 312 cm, and troughs at 156 and 226

12   cm. Increasing count produces a slight decrease in boot-strapped errors, but this is

13   minor and makes little difference to the interpretation of the results. Inferences based

14   on a count of 10 individuals probably would not be very different from those based on

15   the full count. The correlation between the reduced and full data further shows that

16   counts as low as 10 already lead to a correlation coefficient of 0.6-0.75, and a

17   correlation of 0.9 is reached with counts of 50, with little further subsequent

18   improvement (Fig. 8). A plot of the mean standard deviation of the predictions shows

19   high standard deviation for lower counts, representing distinct differences between

20   individual cycles of data selection. As count size increases, the repeatability of

21   predictions improves. The improvement produced with higher counts declines after

22   around 40-50 individuals, but still continues to make a difference through to 140

23   individuals (Fig. 9).

24

25   **Discussion**

11

1   This is the first in-depth attempt to determine the minimum count sizes required for

2   ecological and palaeoecological studies of testate amoebae. We addressed the impact

3   of count size on species richness, community composition and the ecological and

4   palaeoecological information that can be obtained from the community structure data.

5   This study, therefore, has direct relevance for a range of ecological and

6   palaeoecological applications of testate amoebae (Charman 2001; Mitchell et al.

7   2008a).

8   Increasing count total increases the number of taxa identified, improves the

9   precision of transfer function prediction and reduces the standard error of those

10   predictions. As count size increased, there were progressively diminishing returns to

11   further increasing the number of tests counted. The point at which counting more tests

12   fails to produce any further improvement varies depending on what criterion is used.

13   For standard errors, there appears to be little gain from counting more than 60 tests.

14   To characterise a sample for redundancy analysis, 100 individuals seems to be

15   enough. A total of 100 tests also appears to be enough to achieve precision in transfer

16   function predictions, although slight further benefit may be obtained by counting

17   more individuals. To identify all the taxa in a sample, a count of at least 100 is

18   generally required and counts of 150 or more may be needed in samples that are

19   species-rich, or which include very rare taxa.

20   A frequent limitation of testate-amoeba based studies is low sampling

21   resolution and the lack of true replication. The sampling intensity of many ecological

22   studies may be insufficient to characterise the full range of testate amoeba

23   communities and environmental variables under consideration. In transfer function

24   studies this may lead to 'no-analogue' problems when the data are used for

25   environmental reconstruction. In most palaeoecological studies, only one core is

1   analysed, but it is possible that this core may not be truly representative of the site.

2   Studies that examined several cores show that differences in peat accumulation

3   patterns can be relatively important (Charman 2007). The sampling resolution of

4   cores is also frequently inadequate. It is common that some palaeoecological "events"

5   are documented by very few samples, and indeed often only a single sample.

6   Given these limitations we suggest that, when counting time is limited, it is

7   likely to be more useful to count a greater number of samples to a lower count total

8   than to count fewer samples to the usual total of 150. While a count of 50 tests may be

9   sufficient for some samples from some sites, we advocate a count of 100 tests based

10   on our analyses that examined depth to water table. This total should yield most of the

11   ecological information provided by counts of 150 tests, but for a significantly reduced

12   expenditure in counting time. The time taken to count a sample is only one

13   component of the total time invested in data generation. Additional time is required

14   for fieldwork, sub-sampling and preparation. However, in most cases the greatest

15   proportion of time is spent counting, and time savings in this would allow

16   considerably more samples to be analysed. Higher counts will still be required in

17   some situations where it is important to identify very fine environmental changes, or

18   if the focus of the study is on diversity. It is likely that even counts of 150 individuals

19   will fail to identify some taxa in some samples. Higher count totals may have more

20   value in transfer function studies that will be used for multiple inferences than for

21   individual palaeoecological studies. For most studies the small amount of information

22   lost in using a lower count will not be important, and the gain in number of samples

23   will be a worthwhile trade-off.

24   In some studies low test concentrations may make it impossible to reach a

25   count of 150, or even 50 tests in a reasonable time frame, particularly if the amount of

1   material is limited. Lower counts will inevitably reduce the accuracy and precision of

2   transfer function inferences. However ,our palaeoecological results suggest that a

3   meaningful palaeoecological signal may still predominate over random noise. Where

4   counts are very low, results must be treated with considerable caution, but it is still

5   possible for major changes to be shown.

6   Our findings are largely based on four modern training sets. Although these

7   training sets cover a large geographic range and considerable differences in amoebae

8   community structure, it is possible that our conclusions are not applicable to all such

9   samples. Our findings may also not be applicable to transfer functions that aim to

10   reconstruct different environmental variables, and particularly to testate amoebae

11   communities in other habitats that may have quite different diversity. The majority of

12   our experiments also assume that a count of 150 tests is in itself sufficient. If this is

13   not the case, then our conclusions may be erroneous.

14   Testate amoeba analysis and specifically its application for palaeohydrology,

15   is a relatively young technique compared to more established palaeoecological

16   methods such as palynology and diatom analysis. As the method becomes more

17   routinely applied it is necessary to address many of its underlying assumptions. Count

18   totals are one of these assumptions along with issues such as taphonomy, taxonomy

19   and the precise environmental controls on amoeba communities (Barber and Langdon

20   2007; Charman 2007; Charman et al. 2004; Mitchell et al. 2008a; Mitchell et al.

21   2008b; Payne 2007; Payne and Mitchell 2007; Swindles and Roe 2007). Testate

22   amoeba analysis is increasingly used in palaeoecology and ecology. Our results show

23   that most of the valuable data that lies in the structure of fossil testate amoeba

24   communities can be obtained at a reduced cost.

25

8

9 References

10 Balik V (1991) The effect of the road traffic pollution on the communities of testate

11 amoebae (Rhizopoda, Testacea) in Warsaw (Poland). Acta Protozool 30: 5-11

12 Barber KE, Langdon PG (2007) What drives the peat-based palaeoclimate record? A

13 critical test using multi-proxy climate records from northern Britain. Quat Sci Rev 26:

14 3318-3327

15 Barkeley F (1934) The statistical theory of pollen analysis. 15: 283-289

16 Beyens L and Chardez D (1987) Evidence from testate amoebae for changes in some

17 local hydrological conditions between c. 5000 BP and c. 3800 BP on Edgeøya

18 (Svalbard). Polar Res 5: 165-169

19 Birks HJB and Birks HH (1980) Quaternary Palaeoecology. Edward Arnold, London

20 Bobrov AA (2005) Testate amoebas and the regularities of their distribution in soil.

21 Eurasian Soil Sci 38: 1001

22 Boden P (1991) Reproducibility in the Random Settling Method for Quantitative

23 Diatom Analysis. Micropaleontology 37: 313-319

24 Booth RK, Jackson ST, Gray CED (2004) Paleoecology and high-resolution

25 paleohydrology of a kettle peatland in upper Michigan. Quat Res 61: 1–13

1   Buttler A, Warner BG, Grosvernier P, Matthey Y (1996) Vertical patterns of testate

2   amoebae (Protozoa: Rhizopoda) and peat forming vegetation on cutover bogs in the

3   Jura, Switzerland. New Phytol 134: 371-382

4   Charman DJ (2001) Biostratigraphic and palaeoenvironmental applications of testate

5   amoebae. Quat Sci Rev 20: 1753-1764

6   Charman DJ (2007) Summer water deficit variability controls on peatland water-table

7   changes: implications for Holocene palaeoclimate reconstructions. Holocene 17: 217-

8   227

9   Charman DJ, Brown AD, Hendon D, Karofeld E (2004) Testing the relationship

10 between Holocene peatland palaeoclimate reconstructions and instrumental data at

11 two European sites. Quaternary Sci Rev 23: 137–143

12 Charman DJ, Caseldine C, Baker A, Gearey B, Hatton J, Proctor C (2001)

13 Paleohydrological records from peat profiles and speleothems in Sutherland,

14 northwest Scotland. Quat Res 55: 223-234

15 Charman DJ, Hendon D, Woodland WA (2000) The identification of testate amoebae

16 (Protozoa: Rhizopoda) in peats. Quaternary Research Association, London, 147 pp

17 Connor SE, Thomas I, Kvavadze EV (2007) A 5600-yr history of changing

18 vegetation, sea levels and human impacts from the Black Sea coast of Georgia. 17:

19 25-36

20 Davis SR, Wilkinson DM (2004) The conservation management value of testate

21 amoebae as 'restoration' indicators: speculations based on two damaged raised mires

22 in northwest England. Holocene 14: 135–143

23 Finsinger W, Tinner W (2005) Minimum count sums for charcoal-concentration

24 estimates in pollen slides: accuracy and potential errors. Holocene 15: 293-297

1   Foissner W (1999) Soil protozoa as bioindicators: pros and cons, methods, diversity,

2   representative examples. Agric Ecosyst Environ 74: 95-112

3   Gehrels WR, Roe HM, Charman DJ (2002) Foraminifera, testate amoebae and

4   diatoms as sea-level indicators in UK saltmarshes: a quantitative multiproxy approach

5   (vol 16, pg 201, 2001). 17: 285-285

6   Heiri O, Lotter AF (2001) Effect of low count sums on quantitative environmental

7   reconstructions: an example using subfossil chironomids. J Paleolimn 26: 343-350

8   Hill TR (1996) Statistical determination of sample size and contemporary pollen

9   counts, Natal Drakensberg, South Africa. Grana 35: 119-124

10   Jauhiainen S (2002) Testacean amoebae in different types of mire following drainage

11   and subsequent restoration. Europ J Protistol 38: 59-72

12   Joosten H, Kaffke A, Matchutadze I (2003) The mires of the Kolkheti lowlands

13   (Georgia). 2003: 19-23

14   Kandeler E, Luftenegger G, Schwarz S (1992) Soil microbial processes and testacea

15   (Protozoa) as indicators of heavy-metal pollution. Z Pflanzen Bodenk 155: 319-322

16   Kauppila T, Kihlman S, Makinen J (2006) Distribution of arcellaceans (testate

17   amoebae) in the sediments of a mine water impacted bay of Lake Retunen, Finland.

18   Water Air Soil Pollut 172: 337

19   Kumar A, Patterson RT (2000) Arcellaceans (thecamoebians): new tools for

20   monitoring long- and short-term changes in lake bottom acidity. Environ Geol 39:

21   689-697

22   Laggoun-Défarge F, Mitchell EAD, Gilbert D, Disnar J.-R, Comont L, Warner B,

23   Buttler A (2008) Cutover peatland regeneration assessment using organic matter and

24   microbial indicators (bacteria and testate amoebae). J Appl Ecol 45: 716-727

1 Lamentowicz M, Obremska M, Mitchell EAD (2008) Autogenic succession, land-use

2 change, and climatic influences on the Holocene development of a kettle hole mire in

3 Northern Poland. Rev Palaeobot Palynology: in press

4 Lowe JJ, Walker MJC (1997) Reconstructing Quaternary Environments. Prentice

5 Hall, 446 pp

6 Lytle DE, Wahl ER (2005) Palaeoenvironmental reconstructions using the modern

7 analogue technique: the effects of sample size and decision rules. Holocene 15: 554-

8 566

9 Meisterfeld R (2000a) Order Arcellinida Kent, 1880. In: JJ Lee, GF Leedale, P

10 Bradbury (eds.), The illustrated guide to the protozoa. Society of protozoologists,

11 Lawrence, Kansas, USA, pp. 827-860

12 Meisterfeld R (2000b) Testate amoebae with filopodia. In: JJ Lee, GF Leedale, P

13 Bradbury (eds.), The illustrated guide to the protozoa. Society of protozoologists,

14 Lawrence, Kansas, USA, pp. 1054-1084

15 Meng L (1994) How Accurate Is the Random Settling Method for Quantitative

16 Diatom Analysis - a Test Using Lycopodium Spore Tablets. Micropaleontology 40:

17 261-266

18 Mitchell EAD, Borcard D, Buttler AJ, Grosvernier P, Gilbert D, Gobat JM (2000)

19 Horizontal distribution patterns of testate amoebae (Protozoa) in a *Sphagnum*

20 *magellanicum* carpet. Microb Ecol 39: 290-300

21 Mitchell EAD, Buttler AJ, Warner BG, Gobat JM (1999) Ecology of testate amoebae

22 (Protozoa : Rhizopoda) in *Sphagnum* peatlands in the Jura mountains, Switzerland

23 and France. Ecoscience 6: 565-576

1   Mitchell EAD, Charman DJ, Warner BG (2008a) Testate amoebae analysis in

2   ecological and paleoecological studies of wetlands: past, present and future.

3   Biodiversity and Conservation. 17: 2115-2137

4   Mitchell EAD, Payne RJ, Lamentowicz M (2008b) Potential implications of

5   differential preservation of testate amoebae shells for paleoenvironmental

6   reconstruction in peatlands. J Paleolimnol 40: 603-618

7   Mitchell EAD, van der Knaap WO, van Leeuwen JFN, Buttler A, Warner BG, Gobat

8   JM (2001) The palaeoecological history of the Praz-Rodet bog (Swiss Jura) based on

9   pollen, plant macrofossils and testate amoebae (Protozoa). Holocene 11: 65-80

10   Nguyen-Viet H, Bernard N, Mitchell EAD, Cortet J, Badot PM, Gilbert D (2007)

11   Relationship Between Testate Amoeba (Protist) Communities and Atmospheric

12   Heavy Metals Accumulated in Barbula indica (Bryophyta) in Vietnam. Microb Ecol

13   53: 53-65

14   Nguyen-Viet H, Gilbert D, Bernard N, Mitchell EAD, Badot P-M (2004) Relationship

15   between atmospheric pollution characterized by NO2 concentrations and testate

16   amoebae abundance and diversity. Acta Protozool 43: 233-239

17   Patterson RT, Barker T, Burbidge SM (1996) Arcellaceans (thecamoebians) as

18   proxies of arsenic and mercury contamination in northeastern Ontario lakes. J

19   Foraminifer Res 26: 172-183

20   Patterson RT, Kumar A (2002) A review of current testate rhizopod (thecamoebian)

21   research in Canada. Paleogeogr Paleoclimatol Paleoecol 180: 225-251

22   Payne R (2007) Laboratory experiments on testate amoebae preservation in peats:

23   implications for palaeoecology and future studies. Acta Protozool 46: 325-332

Payne R, Mitchell EAD (2007) Testate amoebae-environment relationships and a hydrological transfer function from mires in the Central Rhodope Mountains, Greece. Protist 158: 159-171

Payne R, Pates J (2008) Vertical stratification of testate amoebae in the Elatia Mires, northern Greece: Palaeoecological evidence for a wetland response to recent climatic change, or autogenic processes? Wetlands Ecol Manage in press DOI 10.1007/s11273-008-9112-8

Payne RJ, Charman DJ, Eastwood W (2008) Testate amoebae as palaeoclimatic proxies in Sürmene Ağaçbaşi Yaylasi peatland (Northeast Turkey). Wetlands 28: 311-323

Payne RJ, Kishaba K, Blackford JJ, Mitchell EAD (2006) Ecology of testate amoebae (Protista) in south-central Alaska peatlands: building transfer-function models for palaeoenvironmental studies. Holocene 16: 403-414

Reinhardt EG, Dalby AP, Kumar A, Patterson RT (1998) Arcellaceans as pollution indicators in mine tailing contaminated lakes near Cobalt, Ontario, Canada. Micropaleontology 44: 131-148

Roe HM, Charman DJ, Gehrels WR (2002) Fossil testate amoebae in coastal deposits in the UK: implications for studies of sea-level change. J Quaternary Sci 17: 411-429

Scott DB, Medioli FS, Schafer CT (2001) Monitoring in Coastial Environments Using Foraminifera and Thecoamoebian Indicators. Cambridge Unversity Press, Cambridge

Sillasoo U, Mauquoy D, Blundell A, Charman D, Blaauw M, Daniell JRG, Toms P, Newberry J, Chambers FM, Karofeld E (2007) Peat multi-proxy data from Mannikjarve bog as indicators of late Holocene climate changes in Estonia. Boreas 36: 20-37

1   Swindles GT, Roe HM (2007) Examining the dissolution characteristics of testate

2   amoebae (Protozoa: Rhizopoda) in low pH conditions: Implications for peatland

3   palaeoclimate studies. Paleogeogr Paleoclimatol Paleoecol 252: 486-496

4   ter Braak CJF, Šmilauer P (1997-2004) CANOCO ver.4.53. . Biometris- Plant

5   Research International, Wageningen, The Netherlands

6   Tolonen K (1986) Rhizopod analysis. In: BE Berglund (ed.), Handbook of Holocene

7   Palaeoecology and Palaeohydrology. John Wiley and Sons, Chichester, pp. 645-666

8   Török JK 2001. Diversity of testacean Protozoa in River Tsiza after a cyanide and

9   metal pollution at the beginning of year 2000: Preliminary study. Opusc Zool

10   Budapest XXXIII: 91-98

11   Vickery E, Charman DJ (2004) Biomonitoring of peatland restoration using testate

12   amoebae. In: JTA Verhoeven, E Dorland and M Coemans (eds.), 7th INTECOL

13   International Wetlands Conference, Utrecht, NL, 25-30 July 2004, p. 342

14   Warner BG (1990) Testate Amoebae (Protozoa). In: BG Warner (ed.), Methods in

15   Quaternary Ecology. Geoscience Canada, St. John's, Newfoundland, pp. 65-74

16   Woodland WA, Charman DJ, Sims PC (1998) Quantitative estimates of water tables

17   and soil moisture in Holocene peatlands from testate amoebae. Holocene 8: 261-273

18

19

1   **Tables & Figures**

2

3   Figure 1. Impact of increasing test count on number of taxa recorded in samples from

4   three contrasting peatlands, shown as mean and standard deviations.
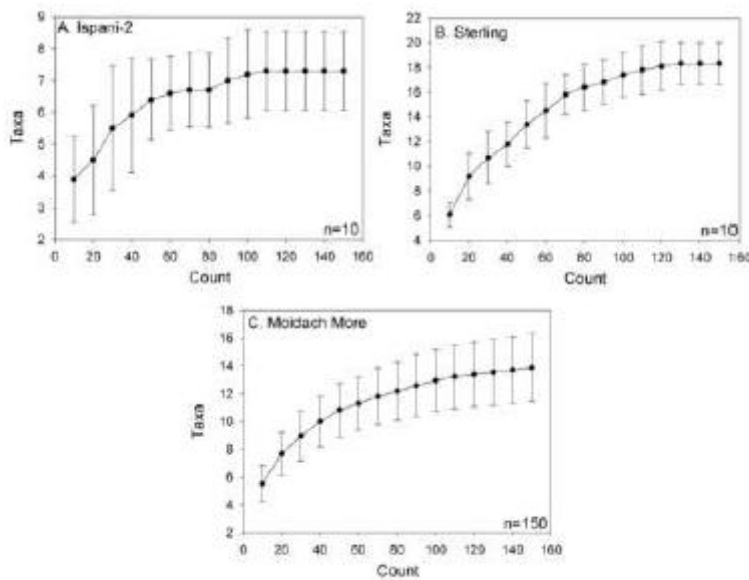
5

6   Figure 2. Box plots showing impact of increasing simulated test count on transfer

7   function predicted depth to water table (DWT) for four samples spanning the

8   hydrological gradient in the Sürmene Ağaçbaşi Yaylasi site. Results based on ten

9   cycles of random re-selection of individuals. Solid horizontal line shows full model

10   predicted value, dashed line shows measured value. Box plots show median (central

11   line), first and third quartiles (grey box), tenth and ninetieth percentiles ('whiskers')

12   and fifth and ninety-fifth percentiles (dots).
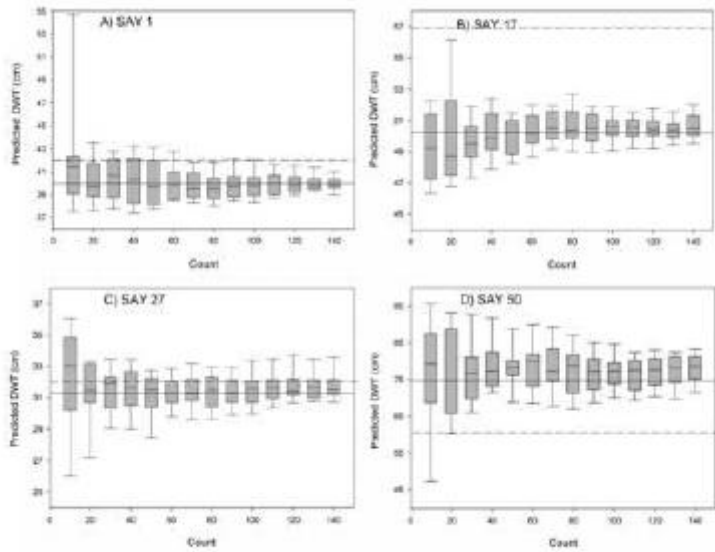
1

2   Figure 3. Mean bias from full model predicted DWT for simulated low-counts with

3   all samples from four data-sets, based on ten cycles of random re-selection of

4   individuals. Box plots as for Fig. 2.
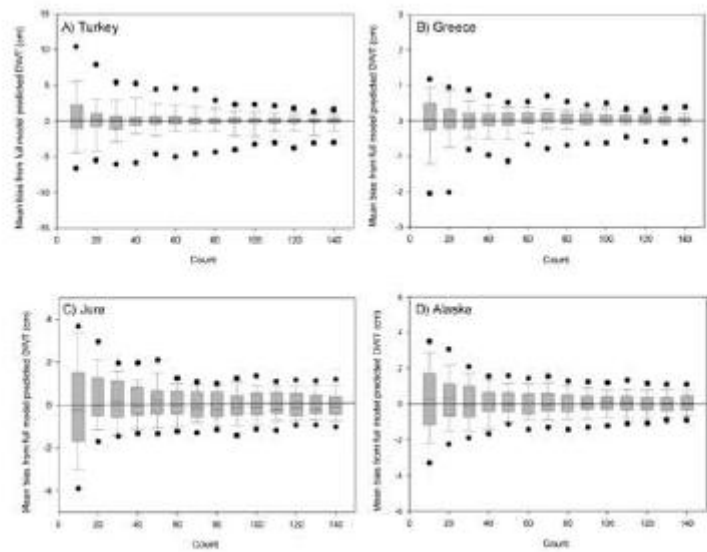

5

6   Figure 4. Mean bias from full model predicted DWT with simulated low-counts

7   extended to 240 tests using 12 samples from the Alaska training set and 18 samples

8   from the Jura training set in which at least 250 tests were counted. Box plots as for

9   Fig. 2.
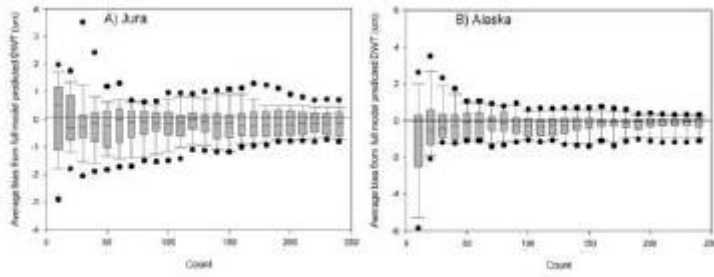
1

2   Figure 5. Boot-strapped error estimates (mean of ten cycles of random selection) for

3   all samples from four data sets. Box plots as for Fig. 2.
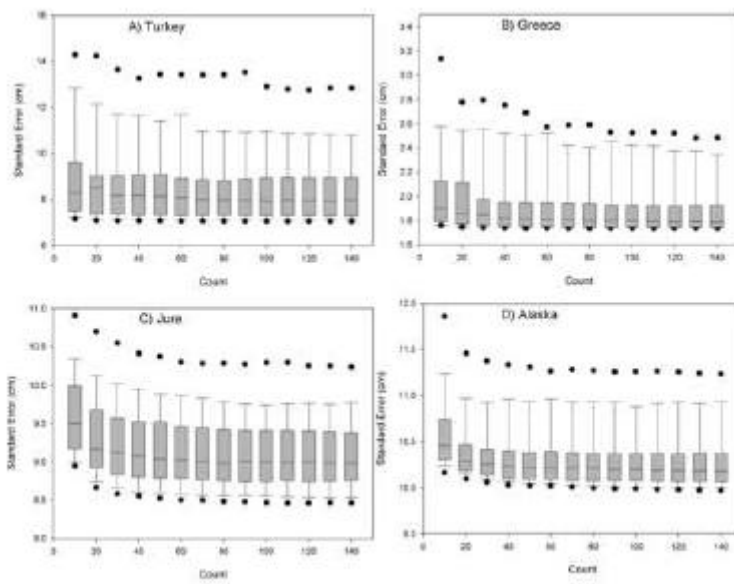
4

5

6   Figure 6. Impact of increasing simulated count on % variance in amoebae species data

7   explained by DWT. All relationships are significant at $P<0.001$.
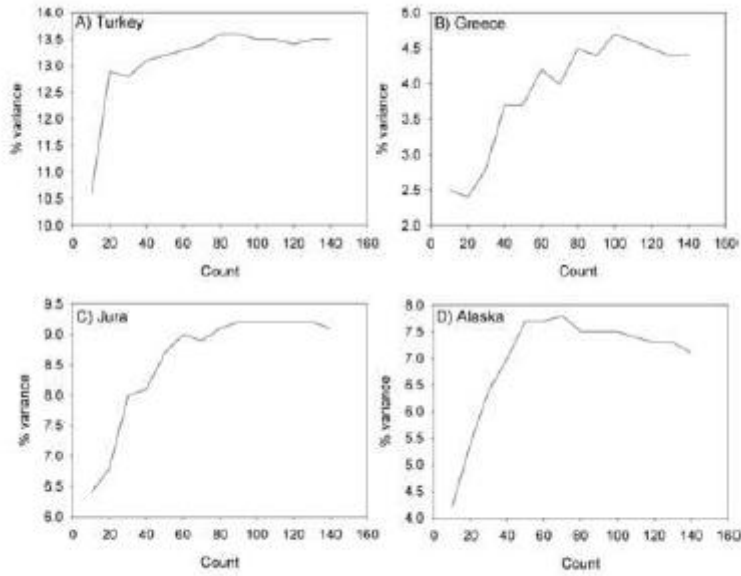
1

2   Figures 7a & b. Testate amoebae inferred depth to water table (TI-DWT) with

3   simulated counts of 10, 20 and 50 tests for palaeoecological sequences from the Praz-

4   Rodet peatland, Switzerland (7a) and site DLB, Alaska (7b).
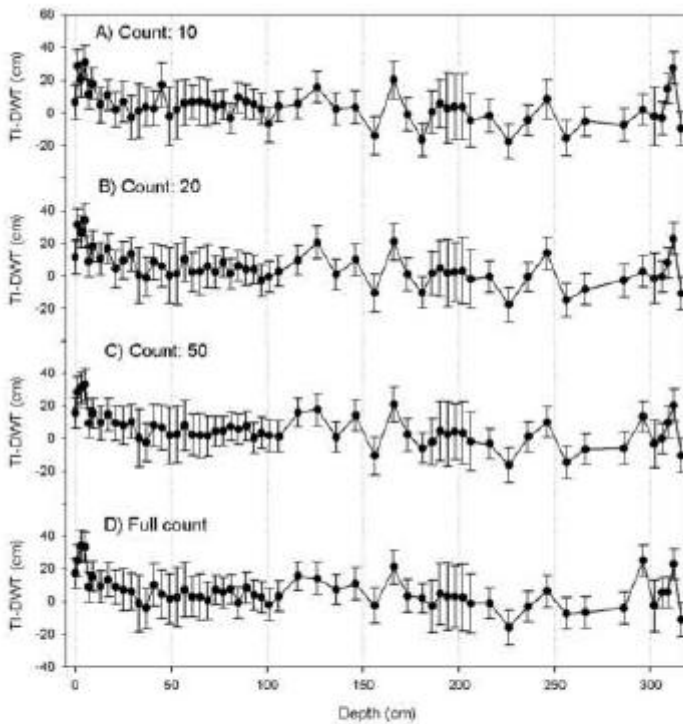
5

1 Figure 8. Relationship between count and r^2 of the regression between the inferred

2 DWT from the full and the reduced data sets from the Praz-Rodet peatland,

3 Switzerland (8a) and site DLB, Alaska (8b).
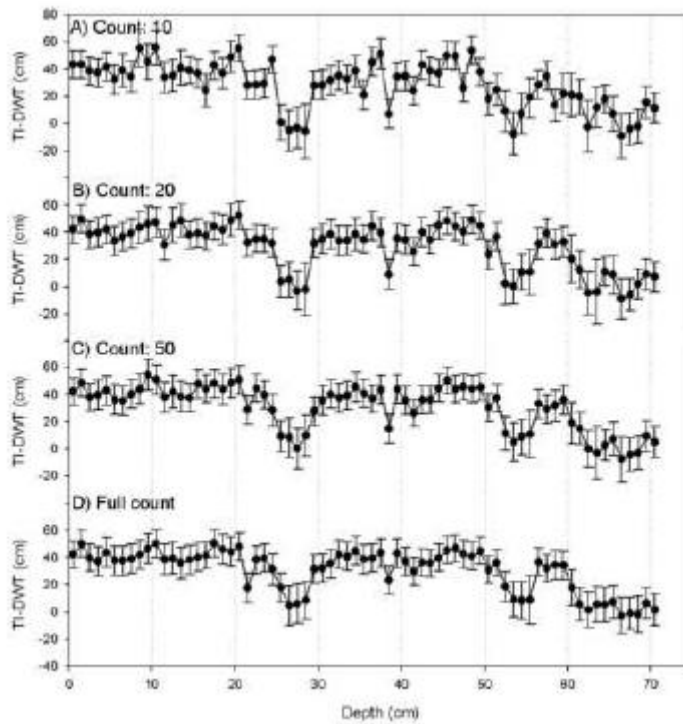
4

5 Figure 9. Mean standard deviation of predictions based on ten cycles of random data

6 selection for the Praz-Rodet peatland, Switzerland (9a) and site DLB, Alaska (9b).
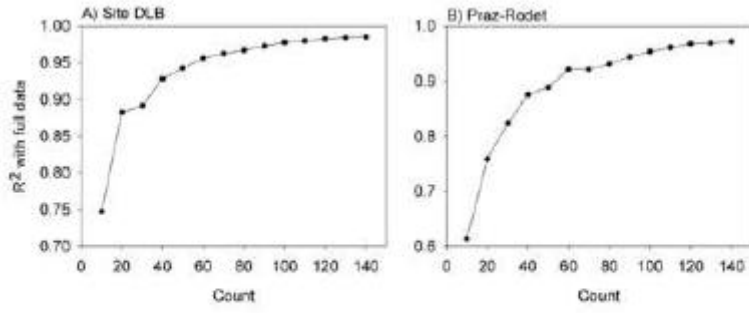
1
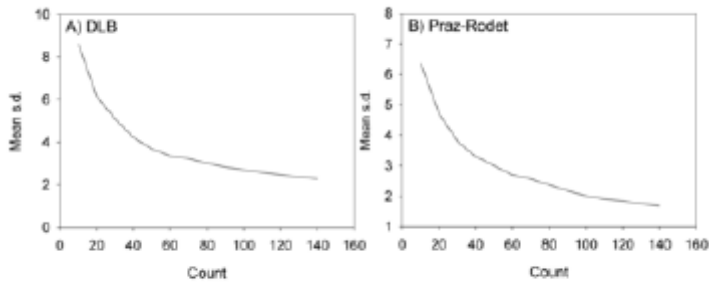
2

1

2

3

4

Table 1. Attributes of the training sets employed in this study showing number of samples, structure of transfer function model and jack-knifed root mean square error of prediction (RMSEP), Maximum Bias and R

Location	n	Model structure	$RMSEP_{jack}$ [cm]	Max $Bias_{jack}$ [cm]	R^2_{jack}	Reference
Alaska	91	WA-PLS (2 component)	9.7	14	0.55	Payne et al. (2006)
Greece	57	ML	1.8	2	0.82	Payne & Mitchell (2007)
Jura	48	WA-PLS (2 component)	8.0	21	0.62	Mitchell et al. (1999)
Turkey	42	ML	7.1	21	0.81	Payne et al. (2008)

5

6

7