

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Ecology of testate amoebae in an Amazonian peatland and development of a transfer function for palaeohydrological reconstruction

Manuscript for MICROBIAL ECOLOGY

- (1) Graeme T. Swindles, School of Geography, University of Leeds, UK
- (2) Monika Reczuga, Department of Biogeography and Palaeoecology, Adam Mickiewicz University, Poland & Faculty of Biology, Adam Mickiewicz University, Poland
- (3) Mariusz Lamentowicz, Department of Biogeography and Palaeoecology, Adam Mickiewicz University, Poland
- (4) Cassandra Raby, Institute of Integrative Biology & Institute of Zoology, University of Liverpool, UK
- (5) T. Edward Turner, School of Geography, University of Leeds, UK
- (6) Dan Charman, Geography, College of Life and Environmental Sciences, University of Exeter, UK
- (7) Angela Gallego-Sala, Geography, College of Life and Environmental Sciences, University of Exeter, UK
- (8) Elvis Valderrama, Putumayo Cdra. 24, Calle Garcia Calderon 246, Iquitos, Peru
- (9) Christopher Williams, School of Geography, University of Leeds, UK
- (10) Frederick Draper, School of Geography, University of Leeds, UK
- (11) Euridice N. Honorio Coronado, School of Geography, University of Leeds, UK
- (12) Katy Roucoux, School of Geography, University of Leeds, UK
- (13) Tim Baker, School of Geography, University of Leeds, UK
- (14) Donal J. Mullan, School of Natural Sciences and Psychology, Liverpool John Moores University, UK

31 Tropical peatlands represent globally important carbon sinks with a unique biodiversity and are cur-
32 rently threatened by climate change and human activities. It is now imperative that proxy methods are
33 developed to understand the natural ecohydrological dynamics of these systems and for testing peat-
34 land development models. Testate amoebae have been used as environmental indicators in ecological
35 and palaeoecological studies of peatlands, primarily in ombrotrophic *Sphagnum*-dominated peatlands
36 in the mid- and high latitudes. We present the first ecological analysis of testate amoebae in a tropical
37 peatland, a nutrient-poor domed bog in western (Peruvian) Amazonia. Litter samples were collected
38 from different hydrological microforms (hummock to pool) along a transect from the edge to the interior
39 of the peatland. We recorded 47 taxa from 21 genera. The most common taxa are *Cryptodiffugia ovi-*
40 *formis*, *Euglypha rotunda* type, *Phryganella acropodia*, *Pseudodiffugia fulva* type and *Trinema lineare*.
41 One species found only in the southern hemisphere, *Argygnia spicata*, is present. *Arcella* spp., *Cen-*
42 *tropyxis aculeata* and *Lesqueresia spiralis* are indicators of pools containing standing water. Canonical
43 Correspondence Analysis and Non-Metric Multidimensional Scaling illustrate that water table depth
44 is a significant control on the distribution of testate amoebae, similar to the results from mid- and
45 high latitude peatlands. A transfer function model for water table based on weighted averaging partial
46 least-squares (WAPLS) regression is presented and performs well under cross-validation ($r_{\text{apparent}}^2 =$
47 0.76 , $\text{RMSE} = 4.29$; $r_{\text{jack}}^2 = 0.68$, $\text{RMSEP} = 5.18$). The transfer function was applied to a 1-m peat
48 core and sample-specific reconstruction errors were generated using bootstrapping. The reconstruction
49 generally suggests near-surface water tables over the last 3,000 years, with a shift to drier conditions
50 at c. cal. AD 1218-1273.

51 1 Introduction

52 Tropical peatlands are thought to contain approximately 88.6 Gt of carbon, equivalent to 15-19 %
53 of the global peatland carbon pool [1, 2]. They support important ecosystems and are found in both
54 lowland and upland areas in SE Asia, Africa and Central and South America [3, 4, 5, 6]. A wide variety
55 of peatlands have recently been discovered in the subsiding Pastaza-Marañón basin in Peruvian Ama-
56 zonia including minerotrophic palm swamps and ombrotrophic domed bogs, classified by differences in
57 surface nutrient status, topography and vegetation communities [5, 7]. Peat thickness is also variable
58 (from <1 to 7.5 m) [5, 7]. These peatlands are different to those in SE Asia as they have not been
59 heavily disturbed by human activity and the domed bogs may therefore be the best remaining exam-
60 ples in the world [7, 8]. Peatlands have also been recently reported from Central Amazonia (Brazil),
61 although peat thicknesses are not as great as in the west [9].

62
63 Peatlands in the tropics are vulnerable to destabilisation through climate-induced changes and human
64 activities including deforestation, drainage and burning [2, 10, 11]. To fully understand how tropical
65 peatlands may respond to such drivers of change, knowledge of their developmental history and past
66 ecohydrological dynamics is needed. The use of testate amoebae for palaeohydrological reconstruction
67 is well established for mid-latitude peatlands [12, 13, 14, 15, 16, 17] and subarctic/boreal peatlands
68 to a lesser extent [18]. However, their potential as hydrological indicators in tropical peatlands has
69 not yet been assessed despite several ecological studies of testate amoebae in the tropics [19, 20, 21].
70 Hydrological reconstructions from tropical peatlands may prove particularly useful as relatively little
71 hydrological monitoring data exists, especially from sites in Africa and S. America.

72
73 This paper has three aims:

- 74 (1) To describe the testate amoebae communities in an Amazonian peatland;
- 75 (2) To determine the most important environmental parameters that influence the testate amoeba
76 communities;
- 77 (3) To elucidate if testate amoeba transfer functions for the reconstruction of hydrological change can
78 be developed in these environments.

79 We test the hypothesis that water table depth is the strongest environmental control on the distribution
80 of testate amoebae in an Amazonian peatland.

81 **2 Study site**

82 Aucayacu is a nutrient-poor domed peatland in Peruvian Amazonia which now operates as an om-
83 brotrophic 'raised bog' system [7]. It is situated on alluvial fan sediments between a stream of the
84 Pastaza fan and the Tigre River (Figure 1). The peatland was initially a nutrient rich minerotrophic
85 system that gradually became an ombrotrophic raised bog [8]. Aucayacu represents the deepest peat-
86 land that has been discovered in the Amazon basin (up to 7.5 m thick) and peat initiation at the site
87 has been dated to c. 8870 cal. BP [8]. The vegetation of Aucayacu is characterised by 'pole' and
88 'dwarf' forest communities.

89
90 At the nearby city of Iquitos (Figure 1), average annual rainfall of up to 3000 mm is typical, with
91 the wet season spanning the months November to March when the ITCZ has migrated just south of
92 the equator and is positioned over northern Peru [22]. Even the dry season from June to September
93 can experience monthly rainfall totals of up to 170 mm [23]. Owing to its equatorial position, the
94 altitude of the midday sun is always close to vertical, leading to nearly constant monthly temperatures
95 throughout the year. The average annual temperature at Iquitos is 26°C, with a diurnal range of
96 approximately 10°C (30-32°C daytime temperatures and 21-22°C at night) [23]. The climate of this
97 region is classed as equatorial under the Köppen climate classification (*Af*).

98 **3 Methods**

99 Linear transects from the edge to the interior of the Aucayacu peatland were established and 100 sur-
100 face sampling points were selected to cover the entire microtopographical/hydrological gradient. The
101 transect was surveyed using a Leica level and staff and the locations of the sample points recorded
102 using a hand-held GPS. Litter samples of approximately 5 cm³ were sampled from each point and
103 placed into ziplock bags. The size and shape of each microform along with the vegetation composition
104 (within an area of 5 m²), % litter and vegetation cover was recorded at each location (Supplementary
105 file 1, 2). A hole was augered at each sampling point and the water table depth measured at regular
106 intervals until it equalised before being measured with a metal ruler (Supplementary file 3). The water
107 table measurements were carried out over a three day period to ensure they were internally consistent.
108 pH and conductivity measurements were carried out on water samples extracted from the boreholes

109 using calibrated field meters. A 1-m core was extracted from the interior of the Aucayacu peatland
110 using a Russian corer following the parallel hole method [24, 25].

111
112 Following courier transport, all samples were returned to the laboratory and stored in refrigeration at
113 4°C prior to further analysis. Approximately one half of each litter sample was weighed, oven dried
114 and re-weighed to determine moisture content. The samples were subsequently burnt in a muffle fur-
115 nace at 450°C for 8 hours to determine loss-on-ignition [26]. Testate amoebae were prepared using a
116 modified version of the standard method [27]. Samples of known volume were sieved through a 300 µm
117 sieve and no fine-sieving was carried out following [28]. The samples were stored in deionised water.
118 Testate amoebae were counted under transmitted light at 200-400× and identified using morphology,
119 composition, size and colour to distinguish taxa. At least 100 specimens were counted per sample
120 [29]. The taxonomy uses a morphospecies approach in certain circumstances, where a designation that
121 includes other species has been classed as a "type". Testate amoebae were identified using several
122 standard keys [30, 31, 32, 33, 34]. Scanning electron microscope images were taken using a Hitachi
123 S-3700N scanning electron microscope (<http://www.sem-eds.amu.edu.pl/>). The core was sub-sampled
124 in the laboratory and samples were prepared for testate amoebae analysis as outlined above.

125
126 Above ground plant material (e.g. leaf fragments, wood and seeds) were extracted from the peat
127 samples and AMS ¹⁴C dates at ¹⁴Chrono (Queen's University Belfast) and the SUERC Accelerator
128 Mass Spectrometer Laboratory (East Kilbride, Scotland). All samples were acid-alkali-acid washed
129 prior to analysis. We looked for Spheroidal Carbonaceous Particles (SCPs) in the top 50 cm of the
130 peat core in an attempt to date the recent century [35]; however, none were present.

131
132 The gradient length of the contemporary data was determined using Detrended Correspondence Analy-
133 sis (DCA). As the data are characterised by a long gradient, Canonical Correspondence Analysis (CCA)
134 was used to explore the relationships between testate amoebae taxa and environmental variables. The
135 relative contributions of the environmental variables were investigated using a series of partial CCAs
136 [36], enabling an estimation of how the total variance is partitioned and the intercorrelations between
137 variables. Monte-Carlo permutation tests were used to determine the statistical significance of these
138 analyses (e.g. Dale and Dale 2002). Our use of CCA enables direct comparisons with previous studies

139 of peatland testate amoebae that have relied on this technique. A number of environmental variables
140 (plant functional types, distances and heights, peat thickness, % litter and vegetation cover, number
141 of plant taxa, microform area) were considered as indirect factors and were included as passive (sup-
142 plementary) variables in the analysis. As there have been some criticisms of the use of the χ^2 distance
143 in CCA [38, 39], Nonmetric Multidimensional Scaling (NMDS), [40, 41] was also used to examine the
144 relationship between testate amoebae and environmental variables. In contrast to CCA, NMDS does
145 not make assumptions about species distributions over environmental gradients. Species data were
146 square root transformed prior to NMDS ordination and Sorensen distance measure was used. The
147 optimum solution was identified through comparison of final stress values. The analysis was carried
148 out using the Vegan package in R version 2.15.1 [42, 43]. The Shannon Diversity Index (SDI) was used
149 to examine the community diversity [44]. The SDI is defined as:

150

151

152

$$SDI = \sum_{i=1}^s \left(\frac{X_i}{N_i} \right) \ln \left(\frac{X_i}{N_i} \right)$$

153

154

155 where X_i is the abundance of each taxon in a sample, N_i is the total abundance of the sample,
156 and s is equal to the species richness of the sample. Environments are considered to be healthy if
157 the SDI falls between 2.5 and 3.5, in transition between 1.5 and 2.5, and stressed between 0.1 and 1.5
158 [45, 46].

159

160 Detrended Canonical Correspondence Analysis (DCCA) was performed on the dataset to determine
161 the gradient length and therefore to deduce whether linear or unimodal-based regression methods
162 would best represent the taxon-environment relationships [47]. Transfer functions were constructed
163 using several regression models - Weighted averaging (WA), tolerance-downweighted weighted averag-
164 ing (WA-Tol), locally-weighted weighted averaging (LWWA), weighted averaging partial least-squares
165 (WA-PLS) and maximum likelihood (ML). Models were also developed using the modern analogue
166 technique (MAT) and weighted modern analogue technique (WMAT). The models were built using C2
167 [48]. The performance of the models was assessed using r^2 and the root mean square error of prediction
168 (RMSEP) with leave-one-out cross validation (jack-knifing) and bootstrapping. The transfer function

169 models were improved through removal of 19 samples with high residual values (>10 cm). A further 11
170 samples were screened out based on influence of other (non-hydrological) factors. The best performing
171 model was the one based on WA-PLS (component 2). The water table transfer function was applied to
172 subfossil data and sample-specific errors of prediction were generated by 999 bootstrap cycles [49, 50].

173 4 Results

174 4.1 Site characteristics

175
176 The topographic survey illustrates that Aucayacu is a domed peatland with a steep rand (Figure
177 2). Environmental parameters vary within the peatland and there is clear evidence of river influence
178 at the edge of the site causing higher pH and reduced loss-on-ignition (through delivery of minerogenic
179 material). A silty clay deposit underlies the peatland and there is a small natural levee at the peatland
180 edge (Figure 2). The vegetation survey suggests the presence of at least 87 plant taxa (Figure 3;
181 Supplementary file 1), the most commonly encountered trees and 'small trees' include *Alibertia* sp. 1,
182 *Iryanthera ulei*, *Virola pavonis*, *Zygia* sp. 1 and *Oxandra euneura*. The most commonly occurring palm
183 trees are *Mauritia flexuosa* and *Oenocarpus mapora*. Understory herbs include *Trichomanes pinnatum*
184 and *Pariana* sp. 1. There are variations in the distribution of plant functional types (PFTs) and the
185 relative contribution of different plant families across the site (Figure 3). For example, individuals from
186 the families Myristicaceae and Arecaceae become more abundant with distance from the river. Plants
187 from the Euphorbiaceae and Annonaceae families are well represented in both the peatland margins
188 and interior, whereas there is a zone of plants from the Rubiaceae family approximately 500-800 m
189 from the river.

190
191 A series of microforms were encountered in the Aucayacu peatland including hollows and pools (the
192 latter characterised by standing water), flat areas ('litter flats'), raised ridges and mounds of accu-
193 mulated litter ('litter hummocks' and 'ridges') and raised areas caused by litter accumulation around
194 the roots of large trees ('tree hummocks'). The pools and hollows become larger in the interior of the
195 peatland and more aligned to the contours, similar to Northern peatlands (Figure 2, Supplementary
196 file 2). The microforms are characterised by contrasting water table depths (Figure 4, Supplementary
197 file 3).

199 *4.2 Ecology of testate amoebae*

200

201 A total of 47 testate amoebae taxa from 21 genera were identified at Aucayacu (Figures 5, 6ab,
 202 Table 1). The most common taxa are *Cryptodiffugia oviformis*, *Euglypha rotunda* type, *Phryganella*
 203 *acropodia*, *Pseudodiffugia fulva* type and *Trinema lineare*. One species found only in the southern
 204 hemisphere, *Argygnia spicata*, is present [51]. *Arcella* spp., *Centropyxis aculeata* and *Lesqueresia spi-*
 205 *ralis* are indicators of pools with standing water. We recorded one potentially new species of *Arcella* -
 206 details of which will be published elsewhere. The Shannon Diversity Index values of the samples range
 207 between 0.8-2.8.

208

209 CCA axes one (eigenvalue = 0.149) and two (eigenvalue = 0.063) explain 11.7% of the variance in
 210 the testate amoebae data (Figure 7). The hydrological variables (moisture content and water table)
 211 and loss-on-ignition are strongly correlated to axis one. The associated Monte Carlo permutation test
 212 shows that CCA axis one is highly significant ($p < 0.001$, 999 random permutations), pH and conductiv-
 213 ity are correlated with axis two. A series of partial CCAs show that water table depth explains 15.3%
 214 of the variance in the data ($p < 0.002$). pH explains 12.9% ($p < 0.002$), Moisture content explains 8.9%
 215 ($p < 0.025$), conductivity explains 11.8% ($p < 0.030$) and loss-on-ignition explains 10.0% ($p < 0.0470$).
 216 The strong influence of hydrological variables is also illustrated by the NMDS ordination as water
 217 table and moisture content are correlated with NMDS coordinate 1 (Figure 7).

218

219 *4.3 Transfer function and application to core*

220

221 The performance statistics for the transfer function models are shown in Table 2. The best perform-
 222 ing transfer function model (Figure 8) is based on weighted averaging partial least-squares (WAPLS)
 223 component 2 ($r_{\text{apparent}}^2 = 0.53$, RMSE = 7.70, $r_{\text{jack}}^2 = 0.40$, RMSEP = 9.13). After the screening
 224 of samples (Supplementary file 5) the model performance greatly improved ($r_{\text{apparent}}^2 = 0.76$, RMSE
 225 = 4.29; $r_{\text{jack}}^2 = 0.68$, RMSEP = 5.18). The most common subfossil testate amoebae present in the
 226 core from Aucayacu include *Hyalosphenia subflava* "major" ($> 60\mu\text{m}$), *Hyalosphenia subflava* "minor"
 227 ($< 60\mu\text{m}$), *Phryganella acropodia*, *Trigonopyxis arcula* "polygon aperture", *Centropyxis aculeata* and

228 *Cryptodiffugia oviformis*. The transfer function was applied to the subfossil data and there were no
229 missing modern analogues. The directional changes in the water table reconstruction are mirrored by
230 principal components analysis (PCA) axis one scores, suggesting that the transfer function is correctly
231 representing the structure in the subfossil data (Supplementary file 6). The reconstruction suggests
232 near-surface water tables over the last 3,000 years (Figure 9; Supplementary file 7) with a marked
233 shift to drier conditions at c. 50 cm (c. cal. AD 1218-1273).

234 5 Discussion

235 To our knowledge this is the first study examining the ecology of testate amoebae in a tropical peat-
236 land. We have demonstrated that testate amoebae are sensitive hydrological indicators in Amazo-
237 nian peatlands, suggesting they have the potential to be used more widely in tropical peatland re-
238 search. The multivariate statistical analysis illustrates the strong hydrological controls on the dis-
239 tribution of testate amoebae, similar to the research findings from mid- and high latitude peatlands
240 [12, 13, 14, 15, 16, 17, 18]. pCCA also shows that pH is an important control on testate amoebae in the
241 Aucayacu peatland - two species (*Trinema grandis*, *Pyxidicula operculata*) are indicators of higher pH
242 conditions. However, the statistical analysis also demonstrates that a large proportion of variance in
243 the testate amoeba data remains unexplained. This may be due to a combination of inter-correlations
244 between environmental variables and unmeasured environmental (edaphic/abiotic) factors. Such fac-
245 tors may include the characteristics of the canopy (determining the amount of moisture reaching the
246 peatland surface), litter quality, diversity and decomposition [21], variations in nutrient status, and
247 other unmeasured geochemical factors. It has also been suggested that short-term environmental vari-
248 ability may be an important factor in the community dynamics of testate amoebae [52]. There is evidence
249 that the Aucayacu stream has an influence on the SW margin of the peatland (increased pH and
250 loss-on-ignition - Figure 2) which may affect the testate amoebae communities there. There is also a
251 possibility that occasional high-magnitude river flooding events affects the peatland interior, although
252 there is no evidence for this.

253
254 There appears to be differences in the ecology of certain species compared to the findings from mid- and
255 high latitude peatlands. For example, *Hyalosphenia subflava*, *Diffugia pulex* and *Trigonopyxis arcuata*
256 are not unambiguous dry indicators as reported from mid-latitude *Sphagnum* peatlands [15, 17, 53].

257 However, the ecology of these taxa may be complex as they have been observed in wet fen environ-
258 ments as well as dry bog hummocks in subarctic and boreal peatlands [18]. The large abundance of
259 *Diffugia pulex* and *Hyalosphenia subflava* in this tropical peatland is particularly interesting as there
260 have been some problems finding modern analogues for these taxa in temperate peatlands [12, 14, 54]
261 and it has also been suggested that these two taxa are characteristic of highly variable conditions [52].
262 However, *Centropyxis aculeata* is consistently a wet indicator in both tropical and temperate peatlands
263 [55]. There is morphological variability of certain taxa reported here such as the marked differences in
264 the aperture of *Trigonopyxis arcuata*. Ogden and Hedley (1980) describe the highly variable aperture
265 shape of *T. arcuata* [31], which was also noted by Bobrov et al. (1995) in populations from Russia and
266 Canada including the occurrence of 3-point, 4-point and almost polygonal/circular apertures [56].

267
268 Palaeohydrological reconstructions based on testate amoebae may prove particularly useful for exam-
269 ining the developmental history of tropical peatlands. Previous pollen and stratigraphic data suggest
270 that Amazonian peatlands undergo major vegetation transitions in their developmental history [57].
271 Testate amoebae may provide important information about the role of changing hydrology across such
272 ecological transitions. However, poor preservation of tests may hinder this for older sections of the
273 subfossil record [58, 59, 60].

274
275 Relatively little is currently known about the ecohydrological dynamics of tropical peatlands. Peatland
276 development models [61, 62] modified for tropical ecosystem PFTs, productivity and decomposition
277 (e.g. [63]) may shed light on the long-term ecohydrological and C dynamics of these systems. Of
278 particular interest is how peatlands respond to climatic shifts [64]. Testate amoebae-based reconstruc-
279 tions may therefore prove useful for testing the hydrological outputs of such models and understanding
280 peatland responses to changing climate. In the case of Amazonian peatlands, changing flooding regime
281 through time and river channel migration may also affect the peatland development trajectories.

282
283 Previous palaeoenvironmental studies of lakes and swamps in Amazonia have suggested distinct phases
284 of climate changes during the Holocene. For example, there is compelling evidence for a period of in-
285 creased precipitation from several areas of Amazonia at c. 700-1300 cal. BP [65, 66, 67, 68, 69].
286 Further work is needed to examine the strength of the climatic signal preserved in Amazonian peat-

287 lands through i) the generation of modern and palaeoecological data from other suitable sites; ii) the
288 development of robust core chronologies; iii) high-resolution sampling; iv) multiproxy approaches; iv)
289 inter and intra-site comparison studies and v) the comparison of peat-based reconstructions to in-
290 dependent palaeoclimatic data. Furthermore, hydrological monitoring data will help understand the
291 sub-annual and inter-annual hydrological dynamics of Amazonian peatlands.

292
293 There are major concerns about the effects of climate change and human activity on tropical peatlands
294 as they are globally important carbon sinks [1, 2]. Future climate change scenarios for north-east Peru,
295 derived from an average of 21 climate models and expressed as relative changes from the 1961-1990
296 baseline climate to the year 2100, project an average annual increase in temperatures of 3.0 – 3.5 °C
297 and a 5 – 10% increase in precipitation across the region [23]. Agreement between models is generally
298 low (particularly for precipitation), but nonetheless a consistent pattern of warming and increasing
299 precipitation is projected [23]. As the Amazonian rainforest is also of special interest as a biodiversity
300 hotspot [70], further research is needed to examine the sensitivity of Amazonian peatlands to climate
301 change and loss of biodiversity from human impacts. Testate amoebae may prove to be a particularly
302 useful tool in this endeavour.

303 6 Conclusions

304 (1) We present the first analysis of testate amoebae from a tropical peatland (Aucayacu, Peruvian
305 Amazonia). We recorded 47 testate amoebae taxa from 21 genera in surface litter samples. The most
306 common taxa are *Cryptodiffugia oviformis*, *Euglypha rotunda* type, *Phryganella acropodia*, *Pseudodif-*
307 *flugia fulva* type and *Trinema lineare*. *Arcella* spp., *Centropyxis aculeata* and *Lesqueresia spiralis* are
308 indicators of pools with standing water.

309
310 (2) Multivariate statistical analysis illustrates that water table depth is the most important control
311 on the distribution of testate amoebae in the peatland explaining 15.3% of the variance in the data
312 ($p < 0.002$). pH is the next most important variable explaining 12.9% ($p < 0.002$). A transfer function
313 model for water table based on weighted averaging partial least-squares (WAPLS) regression is pre-
314 sented and performs well under cross validation ($r_{\text{apparent}}^2 = 0.76$, RMSE = 4.29; $r_{\text{jack}}^2 = 0.68$, RMSEP
315 = 5.18).

317 (3) The transfer function was applied to a 1-m peat core and sample-specific reconstruction errors
318 were generated using bootstrapping. The reconstruction generally suggests near-surface water tables
319 over the last 3,000 years, with a marked shift to drier conditions at c. cal. AD 1218-1273. Testate
320 amoebae may prove very useful for reconstructing the hydrological dynamics of tropical peatlands in
321 Amazonia and elsewhere.

322 **7 Acknowledgements**

323 This work was funded by a Royal Society research grant to GTS (grant no. 481831). We thank Outi
324 Lähteenoja for advice on accessing the Aucayacu peatland and Ricardo Farroñay Peramas and Denis
325 del Castillo Torres of the Instituto de Investigaciones de la Amazonía Peruana in Iquitos for assisting
326 with fieldwork planning. Aristidis Vasques is acknowledged for piloting the boats and helping us run
327 the field campaign. Many thanks to the villagers of Bellavista and Malvinas for assistance in the field
328 (especially Lucho Freyre and David Huayaban). Scanning electron micrographs (SEM) were taken
329 in The Scanning Microscopy and Microanalysis Laboratory, Faculty of Geographical and Geological
330 Sciences, Adam Mickiewicz University. We kindly thank Monika Lutynska for technical support.

331 **8 Figure captions**

332 Figure 1. Map showing the location of the Aucayacu peatland, Loreto region, Peruvian Amazonia.
333 Location of the study site is shown on a Landsat TM RGB false color image (NASA Landsat Program,
334 Orthorectified, WRS-2, Path 007, Row 063, downloaded from <http://earthexplorer.usgs.gov/>). Band
335 4 was assigned to red, band 5 was assigned to green and band 7 was assigned to blue.

336

337 Figure 2. Topographic and stratigraphic profile of the Aucayacu peatland with environmental variables
338 measured along the transects.

339

340 Figure 3. Plant families and plant functional types in the Aucayacu peatland (abundance plotted
341 against distance from the river).

342

343 Figure 4. Boxplot of water table depths measured for each microform type.

344

345 Figure 5. Contemporary percentage testate amoebae data from Aucayacu peatland, ranked in or-
346 der of water table depth. The total count and Shannon Diversity Index are also shown.

347

348 Figure 6a. Light microscope images of testate amoebae from Aucayacu: A. *Sphenoderia fissirostris*;
349 B. *Centropyxis aerophila*; C. *Nebela penardiana*; D. *Lesqueresia spiralis*; E. *Cryptodiffugia oviformis*;
350 F. *Diffugia pulex*; G. *Tracheleuglypha dentata*; H. *Centropyxis aculeata*; I. *Physochila griseola*; J.
351 *Quadrullella symmetrica*; K. *Trigonopyxis arcuata* "3-point aperture"; L. *Centropyxis ecornis*.

352

353 Figure 6b. SEM images of testate amoebae from Aucayacu: A. *Argygnia spicata*; B. *Hyalosphenia*
354 *subflava* "major" ($> 60\mu m$); C. *Euglypha rotunda* type; D. Aperture of *Euglypha rotunda* type; E.
355 *Nebela barbata*; F. *Trinema lineare*; G. *Tracheleuglypha dentata*; H. *Physochila griseola*.

356

357 Figure 7. (a) CCA of testate amoebae from Aucayacu and environmental variables (water table
358 depth, moisture content, pH, conductivity and loss-on-ignition. Abbreviated species codes and sample
359 numbers are shown (see Table 1). (b) CCA showing environmental variables and other factors plotted
360 as supplementary variables (plant functional types, number of plant taxa - S, % litter and vegetation,
361 depth of peatland, distance from river, height above water level, microform area). (c) NMDS ordina-
362 tion of the species and environmental data.

363

364 Figure 8. Graph of observed versus model estimated water table depth for (a) complete dataset;
365 (b) screened dataset.

366

367 Figure 9. Percentage subfossil testate amoebae diagram from Aucayacu and water table reconstruc-
368 tion. Radiocarbon dates are shown.

369

370 Table 1. Taxon codes.

371

372 Table 2. Transfer function model performance statistics.

373

374 Supplementary file 1. Vegetation survey data from Aucayacu.

375

376 Supplementary file 2. Photographs of peatland microforms at Aucayacu.

377

378 Supplementary file 3. Water table equalisation graphs.

379

380 Supplementary file 4. CCA results.

381

382 Supplementary file 5. Samples removed from the refined transfer function.

383

384 Supplementary file 6. Aucayacu water table reconstruction compared to PCA axis 1 scores.

385

386 Supplementary file 7. ^{14}C dates.

387 **References**

- 388 [1] Page SE, Rieley JO, Banks BG (2008) Global and regional importance of the tropical peatland
389 carbon pool. *Global Change Biology* 17: 798-818.
- 390 [2] Moore S, Evans CD, Page SE, Garnett MH, Jones T, Freeman C, Hooijer A, Wiltshire AJ, Limin
391 SH, Gauci V (2013) Deep instability of deforested tropical peatlands revealed by fluvial organic
392 carbon fluxes. *Nature* 493: 660-663.
- 393 [3] Anderson JAR (1964) The structure and development of the peat swamps of Sarawak and Brunei.
394 *The Journal of Tropical Geography* 18: 716.
- 395 [4] Joosten H (2009) *The Global Peatland CO₂ Picture*. Wetlands International, Ede: 33 pp.
- 396 [5] Lahteenoja O, Ruokolainen K, Schulman L, Alvarez J (2009a) Amazonian floodplains harbour
397 minerotrophic and ombrotrophic peatlands. *Catena* 79: 140145.
- 398 [6] Lahteenoja O, Ruokolainen K, Schulman L, Oinonen M (2009b) Amazonian peatlands: an ignored
399 C sink and potential source, *Global Change Biology* 15: 2311-2320.
- 400 [7] Lahteenoja O, Page SE (2011) High diversity of tropical peatland ecosystem types in the Pastaza-
401 Maraon basin, Peruvian Amazonia. *Journal of Geophysical Research* 116: G02025.
- 402 [8] Lahteenoja O, Reategui Y, Rasanen M, del Castillo D, Oinonen M, Page SE (2012) The large
403 Amazonian peatland carbon sink in the subsiding astaza-Maraon foreland basin, Peru. *Global*
404 *Change Biology* 18: 164178.
- 405 [9] Lahteenoja O, Flores B, Nelson B (2013) Tropical peat accumulation in Central Amazonia. *Wet-*
406 *lands* 33: 495-503.
- 407 [10] Page SE, Siegert F, Rieley JO, Boehm HDV, Jaya A, Limin S (2002) The amount of carbon
408 released from peat and forest fires in Indonesia in 1997. *Nature* 420: 61-65.
- 409 [11] Miettinen J, Shi C, Liew SC (2012) Two decades of destruction in Southeast Asia's peat swamp
410 forests. *Frontiers in Ecology and the Environment* 10: 124128.
- 411 [12] Woodland WA, Charman DJ, Sims PC (1998) Quantitative estimates of water tables and soil
412 moisture in Holocene peatlands from testate amoebae. *The Holocene* 8: 261273.

- 413 [13] Lamentowicz M, Mitchell EAD (2005) The ecology of testate amoebae (Protists) in sphagnum in
414 North-western Poland in relation to peatland ecology. *Microbial Ecology* 50, 1: 48-63.
- 415 [14] Charman DJ, Blundell A, ACCROTELM Members (2007) A new European testate amoebae
416 transfer function for palaeohydrological reconstruction on ombrotrophic peatlands. *Journal of Qua-*
417 *ternary Science* 22: 209221.
- 418 [15] Swindles GT, Charman DJ, Roe HM, Sansum PA (2009) Environmental controls on peatland
419 testate amoebae (Protozoa: Rhizopoda) in the North of Ireland: Implications for Holocene palaeo-
420 climate studies. *Journal of Paleolimnology* 42: 123-140.
- 421 [16] Turner TE., Swindles GT, Charman DJ, Blundell A (2013) Comparing regional and supra-regional
422 transfer functions for palaeohydrological reconstruction from Holocene peatlands. *Palaeogeography,*
423 *Palaeoclimatology, Palaeoecology* 369: 395408.
- 424 [17] Amesbury MJ, Mallon G, Charman DJ, Hughes PD, Booth RK, Daley TJ, Garneau M (2013)
425 Statistical testing of a new testate amoebae transfer function for water-table depth reconstruction
426 on ombrotrophic peatlands in Atlantic Canada and far north-eastern United States. *Journal of*
427 *Quaternary Science* 28: 27-39.
- 428 [18] Lamarre A, Magnan G, Garneau M, Boucher E (In Press) A testate amoeba-based transfer
429 function for paleohydrological reconstruction from boreal and subarctic peatlands in northeast-
430 ern Canada. *Quaternary International*.
- 431 [19] Bobrov AA (2001) Findings of the Tropical Group Testate Amoebae (Protozoa: Testacea) at the
432 Far East (Sikhote Alin Reserve). *Biology Bulletin of the Russian Academy of Sciences* 28: 401-407.
- 433 [20] Krashevskaya V, Bonkowski M, Maraun M, Scheu S (2007) Testate amoebae (protista) of an eleva-
434 tional gradient in the tropical mountain rain forest of Ecuador. *Pedobiologia* 51: 319331.
- 435 [21] Krashevskaya V, Maraun M, Scheu S (2012) How does litter quality affect the community of soil pro-
436 toists (testate amoebae) of tropical montane rainforests? *FEMS Microbiology Ecology* 80: 603607.
- 437 [22] Martinez R, Ruiz D, Andrade M, Blacutt L, Pabon D, Jaimes E, Leon G, Villacis M, Quintana J,
438 Montealegre E, Euscategui CH (2011) Synthesis of the climate of the Tropical Andes. Pages 97-109
439 in Herzog SK, Martinez R, Jorgensen, PM, Tiessen H (eds.). *Climate change and biodiversity in*

- 440 the Tropical Andes. MacArthur Foundation, Inter-American Institute of Global Change Research
441 (IAI) and Scientific Committee on Problems of the Environment (SCOPE), Sao Jose dos Campos
442 and Paris, 348 pp., ISBN: 978-85-99875-05-6.
- 443 [23] Met Office (2011) *Climate: Observations, Projections and Impacts*. Peru. Met Office: Exeter.
- 444 [24] Jowsey PC (1966) An improved peat sampler. *New Phytologist* 65: 245-248.
- 445 [25] De Vleeschouwer F, Chambers FM, Swindles GT (2010) Coring and sub-sampling of peatlands
446 for palaeoenvironmental research. *Mires and Peat* 7: 1-10.
- 447 [26] Schulte EE, Hopkins BG (1996) Estimation of soil organic matter by weight-loss-on-ignition. In
448 FR Magdoff et al. (eds) *Soil organic matter: analysis and interpretation*. SSSA Spec. Publ. 46,
449 Madison, WI.
- 450 [27] Hendon D, Charman DJ (1997) The preparation of testate amoebae (Protozoa: Rhizopoda) sam-
451 ples from peat. *The Holocene* 7: 199-205.
- 452 [28] Payne R (2009) The standard preparation method for testate amoebae leads to selective loss of
453 the smallest shells. *Quaternary Newsletter* 119: 16-20.
- 454 [29] Payne R, Mitchell E (2009) How many is enough? Determining optimal count totals for ecological
455 and palaeoecological studies of testate amoebae. *Journal of Paleolimnology* 42: 483-495.
- 456 [30] Charman DJ, Hendon D, Woodland W (2000) The identification of peatland testate amoebae.
457 *Quaternary Research Association Technical Guide* 9: 147pp.
- 458 [31] Ogden CG, Hedley RH eds. (1980) *An Atlas to Freshwater Testate Amoebae*, London: Oxford
459 University Press.
- 460 [32] Mazei Y, Tsyganov AN (2006) *Freshwater Testate Amoebae*. Moscow, KMK.
- 461 [33] Meisterfeld R (2000) Arcellinida. *The Illustrated Guide to the Protozoa, Second Edition*: 827-859.
- 462 [34] Meisterfeld R (2000) Testate Amoebae with Filopodia. *The illustrated Guide to the Protozoa,*
463 *Second Edition*: 1054-1083.
- 464 [35] Swindles GT (2010) Dating recent peat profiles using spheroidal carbonaceous particles (SCPs).
465 *Mires and Peat* 7: 1-10.

- 466 [36] Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological
467 variation. *Ecology* 73: 1045-1055.
- 468 [37] Dale B, Dale AL (2002) Application of ecologically based statistical treatments to micropalaeon-
469 tology. In: Haslett SK (ed) *Quaternary environmental micropalaeontology*. Arnold, London.
- 470 [38] Rao CR (1995) A review of canonical coordinates and an alternative to correspondence analysis
471 using Hellinger distance. *Qüestiió* 19: 2363.
- 472 [39] Legendre P, Gallagher E (2001) Ecologically meaningful transformations for ordination of species
473 data. *Oecologia* 129: 271-280.
- 474 [40] Kruskal JB (1964) Nonmetric multidimensional scaling: a numerical method. *Psychometrika* 29:
475 115-129.
- 476 [41] McCune B, Grace JB (2002) *Analysis of Ecological Communities*. MJM Press.
- 477 [42] Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos
478 P, Henry M, Stevens H, Wagner H (2012) *vegan: Community Ecology Package*. [http://CRAN.R-](http://CRAN.R-project.org/package=vegan)
479 [project.org/package=vegan](http://CRAN.R-project.org/package=vegan)
- 480 [43] R Core Team (2012). *R: A language and environment for statistical computing*. R Foundation for
481 Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- 482 [44] Shannon CE (1948) A mathematical theory of communication. *The Bell System Technical Journal*
483 27: 379-423 and 623-656.
- 484 [45] Magurran AE (1988) *Ecological Diversity and Its Measurement*. Princeton: Princeton University
485 Press.
- 486 [46] Patterson RT, Kumar A (2000) Assessment of arcellacea (thecamoebian) assemblages, species and
487 strains as contaminant indicators in variably contaminated James Lake, north Eastern Ontario.
488 *Journal of Foraminiferal Research* 30: 310-320.
- 489 [47] Birks HJB (1995) Quantitative palaeoenvironmental reconstructions. In: Maddy D, Brew JS
490 (eds) *Statistical Modelling of Quaternary Science Data*. Technical guide 5. Quaternary Research
491 Association, Cambridge.

- 492 [48] Juggins S (2003) C2 user guide. Software for ecological and palaeoecological data analysis and
493 visualisation. University of Newcastle, Newcastle Upon Tyne.
- 494 [49] Birks HJB, Line JM, Juggins S, Stevenson AC, ter Braak CJF (1990) Diatoms and pH recon-
495 struction. *Philosophical Transactions of the Royal Society B* 27: 263278.
- 496 [50] Line JM, ter Braak CJF, Birks HJB (1994) WACALIB version 3.3: a computer program to
497 reconstruct environmental variables from fossil assemblages by weighted-averaging and to derive
498 sample-specific errors of prediction. *Journal of Paleolimnology* 10: 147152.
- 499 [51] Deflandre G (1936) Etude monographique sur le genre *Nebela* Leidy. *Annales de Protistologie* 5:
500 201-286.
- 501 [52] Sullivan ME, Booth RK (2011) The potential influence of short-term environmental variability
502 on the composition of testate amoeba communities in *Sphagnum* peatlands. *Microbial Ecology* 62:
503 80-93.
- 504 [53] Turner TE, Swindles GT (2012) Ecology of testate amoebae in moorland with a complex fire
505 history: implications for ecosystem monitoring and sustainable land management. *Protist* 163:
506 844-855.
- 507 [54] Mitchell EAD, Charman DJ, Warner BG (2008) Testate amoebae analysis in ecological and pale-
508 oecological studies of wetlands: past, present and future. *Biodiversity and Conservation* 17: 2115-
509 2137.
- 510 [55] Booth RK, Zygmunt JR (2005) Biogeography and comparative ecology of testate amoebae inhab-
511 iting *Sphagnum*-dominated peatlands in the Great Lakes and Rocky Mountain regions of North
512 America. *Diversity and Distributions* 11: 577-590.
- 513 [56] Bobrov AA, Yazvenko SB, Warner BG (1995) Taxonomic and ecological implications of shell
514 morphology of three testaceans (Protozoa: Rhizopoda) in Russia and Canada. *Archiv für Protis-
515 tenkunde* 145: 119-126.
- 516 [57] Roucoux KH, Lawson IT, Jones TD, Baker TR, Coronado ENH, Gosling WD, Lahteenoja O
517 (2013) Vegetation development in an Amazonian peatland, *Palaeogeography, Palaeoclimatology,
518 Palaeoecology* 374: 242-255.

- 519 [58] Wilmshurst JM, Wisser SK, Charman DJ (2003) Reconstructing Holocene water tables in New
520 Zealand using testate amoebae: Differential preservation of tests and implications for the use of
521 transfer functions. *The Holocene* 13: 61-72.
- 522 [59] Swindles GT, Roe HM (2007) Examining the dissolution characteristics of testate amoebae (Pro-
523 tozoa: Rhizopoda) in low pH conditions: Implications for peatland palaeoclimate studies. *Palaeo-*
524 *geography, Palaeoclimatology, Palaeoecology* 252: 486496.
- 525 [60] Mitchell E, Payne R, Lamentowicz M (2008) Potential implications of differential preservation of
526 testate amoeba shells for paleoenvironmental reconstruction in peatlands. *Journal of Paleolimnology*
527 40: 603-618.
- 528 [61] Frohking S, Roulet NT, Tuittila E, Bubier JL, Quillet A, Talbot J, Richard PJH (2010) A new
529 model of Holocene peatland net primary production, decomposition, water balance, and peat ac-
530 cumulation. *Earth System Dynamics* 1: 121.
- 531 [62] Morris PJ, Belyea LR, Baird AJ (2011) Ecohydrological feedbacks in peatland development: A
532 theoretical modelling study. *Journal of Ecology* 99: 11901201.
- 533 [63] Kurnianto, S (2013) Modeling carbon accumulation dynamics in tropical peat swamp forests
534 (abstract), *New Frontiers in Tropical Biology: The Next 50 Years (A Joint Meeting of ATBC and*
535 *OTS)*.
- 536 [64] Swindles GT, Morris PJ, Baird AJ, Blaauw M, Plunkett G (2012) Ecohydrological feedbacks
537 confound peat-based climate reconstructions. *Geophysical Research Letters* 39: L11401.
- 538 [65] Bush MB, Colinvaux PA (1988) A 7000-year pollen record from the Amazon lowlands, Ecuador.
539 *Vegetatio* 76: 141154.
- 540 [66] Frost I (1988) A Holocene sedimentary record from Anañgucocha in the Ecuadorian Amazon.
541 *Ecology* 69: 6673.
- 542 [67] Liu KB, Colinvaux PA (1988) A 5200-year history of Amazon rain forest. *Journal of Biogeography*
543 15: 231248.

- 544 [68] Behling H, Berrio J, Hooghiemstra H (1999) Late Quaternary pollen records from the middle
545 Caquetá river basin in central Columbian Amazon. *Palaeogeography, Palaeoclimatology, Palaeoecology* 145: 193213.
546
- 547 [69] Correa-Metrio A, Cabrera KR, Bush MB (2010) Quantifying ecological change through discrimi-
548 nant analysis: a palaeoecological example from the Peruvian Amazon. *Journal of Vegetation Science*
549 21: 695704.
- 550 [70] Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartin I, Sanchez-
551 Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lund-
552 berg J, Stadler T, Sarkinen T, Antonelli A (2010) Amazonia through time: Andean uplift, climate
553 change, landscape evolution, and biodiversity. *Science* 330: 927-931.