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

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Abstract

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

1 Introduction

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

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

This paper has three aims:

(1) To describe the testate amoebae communities in an Amazonian peatland;

(2) To determine the most important environmental parameters that influence the testate amoeba communities;

(3) To elucidate if testate amoeba transfer functions for the reconstruction of hydrological change can be developed in these environments.

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

2 Study site

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

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

3 Methods

Linear transects from the edge to the interior of the Aucayacu peatland were established and 100 surface sampling points were selected to cover the entire microtopographical/hydrological gradient. The transect was surveyed using a Leica level and staff and the locations of the sample points recorded using a hand-held GPS. Litter samples of approximately 5 cm^3 were sampled from each point and placed into ziplock bags. The size and shape of each microform along with the vegetation composition (within an area of 5 m^2), % litter and vegetation cover was recorded at each location (Supplementary file 1, 2). A hole was augered at each sampling point and the water table depth measured at regular intervals until it equalised before being measured with a metal ruler (Supplementary file 3). The water table measurements were carried out over a three day period to ensure they were internally consistent. pH and conductivity measurements were carried out on water samples extracted from the boreholes using calibrated field meters. A 1-m core was extracted from the interior of the Aucayacu peatland using a Russian corer following the parallel hole method [24, 25].

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

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

The gradient length of the contemporary data was determined using Detrended Correspondence Analysis (DCA). As the data are characterised by a long gradient, Canonical Correspondence Analysis (CCA) was used to explore the relationships between testate amoebae taxa and environmental variables. The relative contributions of the environmental variables were investigated using a series of partial CCAs [36], enabling an estimation of how the total variance is partitioned and the intercorrelations between variables. Monte-Carlo permutation tests were used to determine the statistical significance of these analyses (e.g. Dale and Dale 2002). Our use of CCA enables direct comparisons with previous studies of peatland testate amoebae that have relied on this technique. A number of environmental variables (plant functional types, distances and heights, peat thickness, % litter and vegetation cover, number of plant taxa, microform area) were considered as indirect factors and were included as passive (supplementary) variables in the analysis. As there have been some criticisms of the use of the χ^2 distance in CCA [38, 39], Nonmetric Multidimensional Scaling (NMDS), [40, 41] was also used to examine the relationship between testate amoebae and environmental variables. In contrast to CCA, NMDS does not make assumptions about species distributions over environmental gradients. Species data were square root transformed prior to NMDS ordination and Sorensen distance measure was used. The optimum solution was identified through comparison of final stress values. The analysis was carried out using the Vegan package in R version 2.15.1 [42, 43]. The Shannon Diversity Index (SDI) was used to examine the community diversity [44]. The SDI is defined as:

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

where Xi is the abundance of each taxon in a sample, Ni is the total abundance of the sample, and s is equal to the species richness of the sample. Environments are considered to be healthy if 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 [45, 46].

Detrended Canonical Correspondence Analysis (DCCA) was performed on the dataset to determine the gradient length and therefore to deduce whether linear or unimodal-based regression methods would best represent the taxon-environment relationships [47]. Transfer functions were constructed using several regression models - Weighted averaging (WA), tolerance-downweighted weighted averaging (WA-Tol), locally-weighted weighted averaging (LWWA), weighted averaging partial least-squares (WA-PLS) and maximum likelihood (ML). Models were also developed using the modern analogue technique (MAT) and weighted modern analogue technique (WMAT). The models were built using C2 [48]. The performance of the models was assessed using r^2 and the root mean square error of prediction (RMSEP) with leave-one-out cross validation (jack-knifing) and bootstrapping. The transfer function models were improved through removal of 19 samples with high residual values (>10 cm). A further 11 samples were screened out based on influence of other (non-hydrological) factors. The best performing model was the one based on WA-PLS (component 2). The water table transfer function was applied to subfossil data and sample-specific errors of prediction were generated by 999 bootstrap cycles [49, 50].

4 Results

4.1 Site characteristics

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

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

4.2 Ecology of testate amoebae

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

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

4.3 Transfer function and application to core

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

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

5 Discussion

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

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

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

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

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

Previous palaeoenvironmental studies of lakes and swamps in Amazonia have suggested distinct phases of climate changes during the Holocene. For example, there is compelling evidence for a period of increased precipitation from several areas of Amazonia at c. 700-1300 cal. BP [65, 66, 67, 68, 69]. Further work is needed to examine the strength of the climatic signal preserved in Amazonian peatlands through i) the generation of modern and palaeoecological data from other suitable sites; ii) the development of robust core chronologies; iii) high-resolution sampling; iv) multiproxy approaches; iv) inter and intra-site comparison studies and v) the comparison of peat-based reconstructions to independent palaeoclimatic data. Furthermore, hydrological monitoring data will help understand the sub-annual and inter-annual hydrological dynamics of Amazonian peatlands.

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

6 Conclusions

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

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

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

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8 Figure captions

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

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

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

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

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

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

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

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

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

Figure 9. Percentage subfossil testate amoebae diagram from Aucayacu and water table reconstruction. Radiocarbon dates are shown.

Table 1. Taxon codes.

Table 2. Transfer function model performance statistics.

Supplementary file 1. Vegetation survey data from Aucayacu.

Supplementary file 2. Photographs of peatland microforms at Aucayacu.

Supplementary file 3. Water table equalisation graphs.

Supplementary file 4. CCA results.

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

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

Supplementary file 7. ^{14}C dates.

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