DAVIS EXPEDITION FUND
REPORT ON EXPEDITION / PROJECT

Expedition/Project Title:	Testate amoebae as a bioindicator for tropical peatland health
Travel Dates:	Out: 30/06/22 In: 3/08/22 (Elizabeth) and 18/08/2022 (Tasnim)
Location:	Sarawak, Malaysia.
Group Members:	Elizabeth Stroud, Tasnim Hanafiah
Aims:	To conduct a preliminary study on testate amoeba diversity as an environmental indicator for SE-Asian tropical peatlands To see whether testate amoeba diversity can be assessed in a fast, cheap, non-destructive manner

Photography consent form attached: (*please refer to your award letter)*

☒ Yes
☐ No

Testate amoebae as a bioindicator of tropical peatland health

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INTRODUCTIONS

Southeast Asian tropical peatlands, or peat swamp forests (PSFs), are a key habitat for unique biodiversity and make up a significant portion of the global carbon reservoir (Page et al., 2011; Kurnianto et al., 2015). In the Bornean Malaysian state of Sarawak, PSFs are an extensive feature, totalling a land area of 0.33 million hectares (Forest Department Sarawak, 2018). These forests are situated on low-lying coastal plains and along lower river systems inland, where water tables are near or at the soil surface. Their waterlogged condition slows down the decay of organic matter, resulting in a large capacity for carbon storage which is ideal for mitigating climate change (Wosten et al., 2008). In addition, the PSFs are also valued as a natural resource for local communities due to their economic and ecological benefits (Tawan et al., 2008). They provide ecosystem services such as water cycling, and supply commodities including agricultural land, medicine, food and shelter.

However, if PSFs are overexploited and mismanaged, degradation will occur and ultimately lead to the depletion of resources and major losses in biodiversity. Severe droughts and anthropogenic draining and burning of PSFs have already been shown to release large amounts of greenhouse gases into the atmosphere, further exacerbating global warming (Inubushi et al., 2003; Arai et al., 2014). Low water tables may also lead to increased mineralisation of peat physiochemistry and consequently the colonisation of non-bog plant species, diminishing the once specialist and unique ecosystem (Koenig et al., 2018). Tracking the health of tropical peatlands is therefore imperative in administrating social, economic and environmental sustainability.

Effective ecosystem monitoring requires constant, widespread and efficient data collection. This ideally entails the development of cheap reproducible methods that are not destructive of the system being monitored and are accessible for use in citizen science projects to build the large datasets required to map and track changes of ecosystems on a global scale. Peatland monitoring often involves destructive methods which are unsuitable for repetitive monitoring of sensitive and protected sites (De Vleeschouwer et al., 2010).

The unicellular protist group testate amoeba (TA) is an ideal indicator for biomonitoring peatland health. They have been shown to be a sensitive hydrological indicator in temperate peatlands (Turner & Swindles, 2012; Swindles et al., 2015; Swindles et al., 2016a) and are widely used in boreal peat studies (Lamentowicz & Mitchell, 2005). Tracking their community composition may be a cheaper and quicker alternative to biomonitoring methods such as metagenomics which involve the use of expensive technology and laborious processes (Kitson & Bell, 2020; Dom et al., 2021). However,

the potential value of TA in tropical peatland research has only recently been uncovered, though this is generally limited to studies in South America (Marcisz et al., 2020). As a result, there is a knowledge gap in whether TA can be used to indicate tropical peatland health on a wider scale. This study aims to reduce this gap by investigating if TA can be used as a bioindicator in SE Asian PSFs.

Our preliminary study will determine if TA morphotype occurrence, composition and diversity varies between PSFs of differing condition using cheap, reproducible and non-destructive methods. By i) identifying TA morphotypes whose occurrence play a strong role in differentiating tropical peatland sites and ii) establishing if the community composition and diversity of these morphotypes vary with PSF condition, the use of TA as a proxy for tropical peatland health will be explored. Surface water and ground material samples will be collected, and TA will be identified to morphotype level to increase the accessibility of our methods. Sarawak Tropical Peatland Research Institute (TROPI) provided us with six accessible tropical peatland sites in Sarawak in varying condition due to diverse management practices, and associated peat and water table depth. Previous studies identified specific TA species to be associated with drier peatlands (Charman et al., 2000). We therefore expected drained tropical peatland plantations to have significantly different TA morphotype association, composition and diversity compared to undrained, near natural peatlands.

METHODS

Study site

Ground material was sampled in July 2022 from six peatland sites, including three undrained near natural forests, one undrained forest fragment and two drained peatlands that were converted into oil palm plantations. Table 1 sets out each site's sampling details and characteristics observed on their respective dates. Tree identification and canopy height measurement for general site descriptions was done by a local tree expert from TROPI at the time of sampling.

The first three sites sampled were near natural forests located at a tropical peat dome at Maludam National Park, Betong: Mixed Peat Swamp Forest (MPS), Alan Batu (ABt), and Alan Bunga (ABg) (Fig. 1; Table 1). There is a very gradual undulating slope from the centre of the park (Fig. 1). The sites were categorised according to their phasic zonation and vegetation associations (see Dom et al., 2021). Prior to being gazetted in 2000, the national park underwent selective logging until the 1990s (Monda et al., 2018). According to TROPI staff, ABg was the least disturbed forest out of the three sites prior to the national park's gazettement. Previous studies showed mean water table depth (WTD) pattern for the different forests was ABg > ABt > MPS in 2011-2014 (Sangok et al., 2017) and ABg > MPS > ABt in 2017-2018 (Dom et al., 2021), which differ with the MPS > ABg > ABt pattern from our single WTD measurements in 2020. However, this is acceptable as they are still higher WTD than plantations and thus valid for the comparisons within the scope of this study.

The first drained site sampled was a young (four-year-old) oil palm plantation (YP) in Betong (Fig. 1; Table 1). YP was converted from an ABt-type peat swamp forest into an oil palm (*Elaeis guineensis*) plantation in 2015. Oil palm trees were arranged in regular 8 m grids and underwent periodical harvesting as well as clearing of lopped fronds into stacking rows. Understory vegetation was sparse, made up of damiana (*Turnera* spp.) hedges to encourage pollinators, at least three fern species and 'kelait' seedlings.

Our last sites were a mature plantation (MP) and forest fragment within MP known as Alan Swamp Forest (ASF) in Sibu. MP was converted from an ombotrophic peat dome in 2004, where the land underwent soil compaction to increase soil bulk density to reduce tree leaning and toppling (Melling et al., 2008). Oil palm trees here were planted in 8 m grids 13 years prior to sampling and harvesting has not occurred for several years. Oil palm plantations are usually replanted every 25-30 years (Ishikura et al., 2018), meaning both MP and YP were in their first cultivation cycle. There was

noticeably more understory vegetation and leaf litter in MP compared to YP. The understory vegetation included moss, ferns (*Stenochlaena palustris*) and 'otan belalang'. Finally, we chose to sample ASF due to the availability of an artificial pool at the site. It was an undrained peatland which we suspected was of intermediate condition due disturbance from bordering with a plantation (such as fertiliser or pesticide runoff) which may affect TA assemblage. Detailed site history for ASF and YP are unavailable to us for the time being.

Table 1: Study site characteristics based on measurements and observations on specified dates.

Sampling Site (GPS coordinate)	**Type of site**	**Sampling condition (Date, Weather, Temperature, Humidity)**	**Prevalent tree species**	**WTD (cm)**	**Canopy height (m)**
MPS (1°25'N 111°7'E)	Near natural forest	12 July 2022 Cloudy with light rain 27.5°C 85.7 % humidity	Dominant: Serait (*Nephelium maingayi*) Others: • Medang (*Litsea* spp.) • Alan (*Shorea albida*) • Ara *(Ficus* spp.*)* • Merbulan (*Blumeodendron* spp.)	-23.3	12-13
ABt (1°27'N 111°9'E)	Near natural forest	12 July 2022 Sunny with partial cloud cover 30°C 84.9 % humidity	Dominant: Keruntum (*Combretocarpus rotundatu*) Others: • Jongkong (*Dactylocladus stenostachys*) • Ubah (*Eugenia* spp.) • Medang • Alan • Serait	-43.2	13-14
ABg (1°26'N 111°8'E)	Near natural forest	12 July 2022 Mainly cloudy with some sun 28.4°C 92.2 % humidity	Dominant: Alan Others: • Jongkong • Ubah	-25.2	9-10
YP (1°23'N 111°23'E)	Oil palm plantation	14 July 2022 Sunny with thin cloud cover 28.8°C	Oil palm	-75.6	4

		79 % humidity			
MP (2°11'N 111°51'E)	Oil palm plantation	16 July 2022 Sunny with partial cloud cover N/A N/A	Oil palm	-70.0	10
ASF (2°11'N 111°51'E)	Forest fragment	16 July 2022 Cloudy and cool N/A N/A	Dominant: Alan Others: • Serait • Makaranga (*Macaranga* spp.) • Mangkoyong	-85.5	10-12

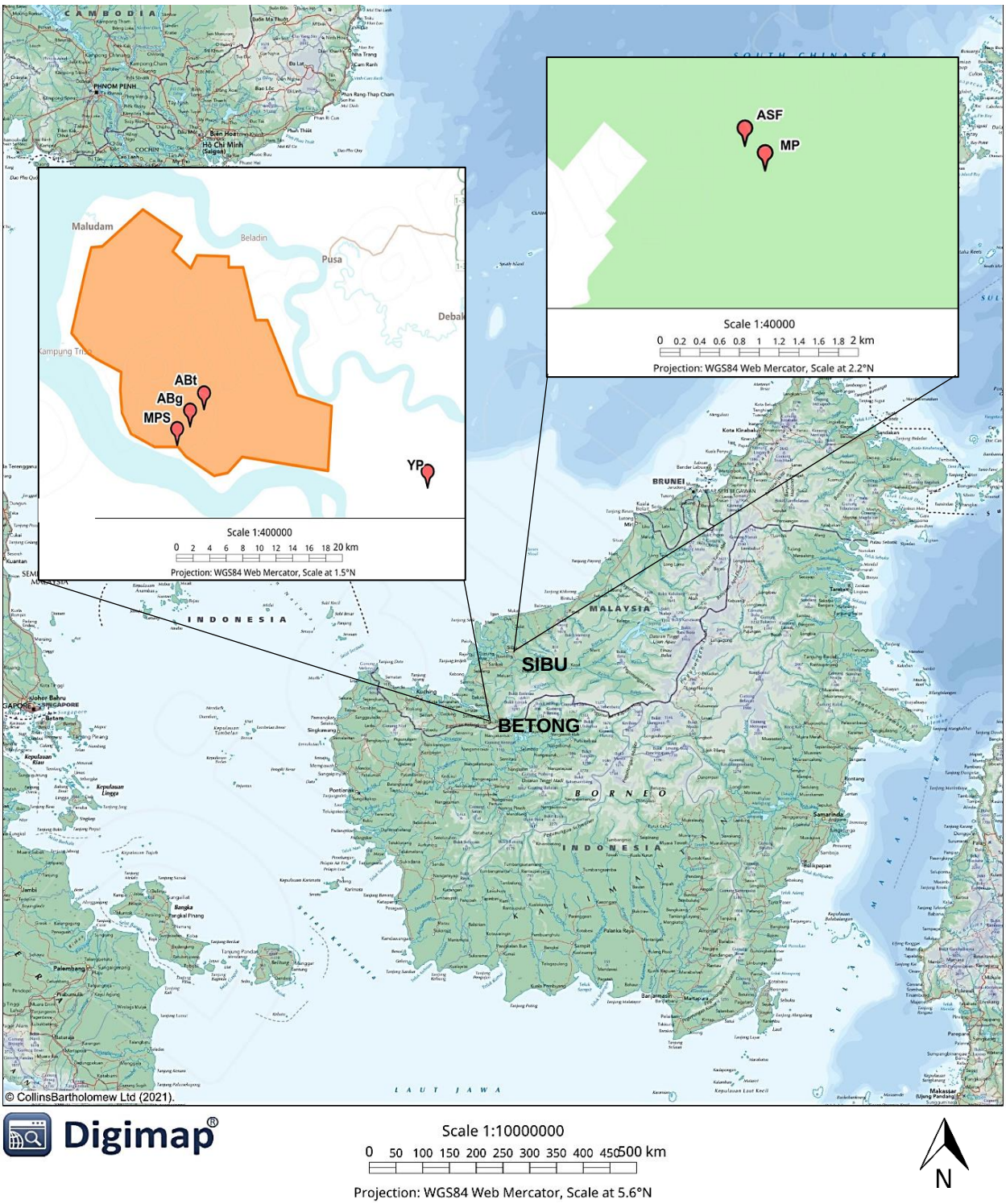

Figure 1. **Study site locations where samples were collected**. Undrained sites included Alan Batu (ABt), Alan Bunga (ABg) and Mixed Peat Swamp (MPS) in Maludam National Park (represented by an orange polygon) in Betong, and Alan Swamp Forest (ASF) in Sibu. Drained sites included a young plantation (YP) in Betong and a mature plantation (MP) in Sibu. In previous papers, ABg was the furthest site from the coast, followed by ABt and then MPS (see Dom et al., 2021; Sangok et al., 2017; Melling et al., 2016). However, the coordinates in our study showed that ABt was the furthest from the coast, followed by ABg and then MPS. We are currently unable to determine the cause of this discrepancy. Digital Map Data © Collins Bartholomew (2022).

Study specimen

Testate amoebae (TA) are a diverse polyphyletic group of unicellular amoeboid protists characterised by morphologically distinct partially enclosed tests (Swindles et al., 2014; Mitchell et al., 2008). Tests are typically between 7-500 µm diameter and rigid. They can be smooth and calcareous, composed of siliceous plates, or fragments of other organism remains, bound by proteinaceous material (Clarke et al., 2003). The amoebae eukaryotic cells can be up to 100 µm-long, filling part of the test interior and extending pseudopodia through the pseudostome (the mouth or aperture) for locomotion and food capture. In extreme conditions, TA can undergo encystment, which withdraws the cell to the edge of the test and forms a plug or seal over the pseudostome to reduce dehydration or temperature exposure (Clarke et al., 2003). When amoebae die, the tests remain preserved for millennia (Charman, 2001).

TA can occur in aquatic or moist habitats, including in stagnant pools, leaf litter, soils, wetlands and moss (Mitchell et al., 2008; Swindles et al., 2014). They play an important role in nutrient cycling, having highest biomass in upper soil depths rich in humus and litter (Schonborn, 1992). Small TA are limited to feeding on unicellular algae, bacteria, yeast, microfungi and detritus, whilst large TA can ingest protozoa including small amoebae, rotifers and larger detritus (Clarke, 2003). TA population densities can be greatly reduced by fungal infections and predation by ciliated protozoa and verrucosid amoebae (Bovee, 1960; Finley et al., 2001).

TA morphologies reflect environmental conditions. Polymorphism is common in many TA species, with habitat conditions such as water film thickness, light and food availability, and temperature influencing the range of test shape, colour and transparency (Clarke et al., 2003). Using morphotypes to classify individual TA avoids the complexities of separating morphologically overlapping species (Finlay et al., 2001).

Fieldwork

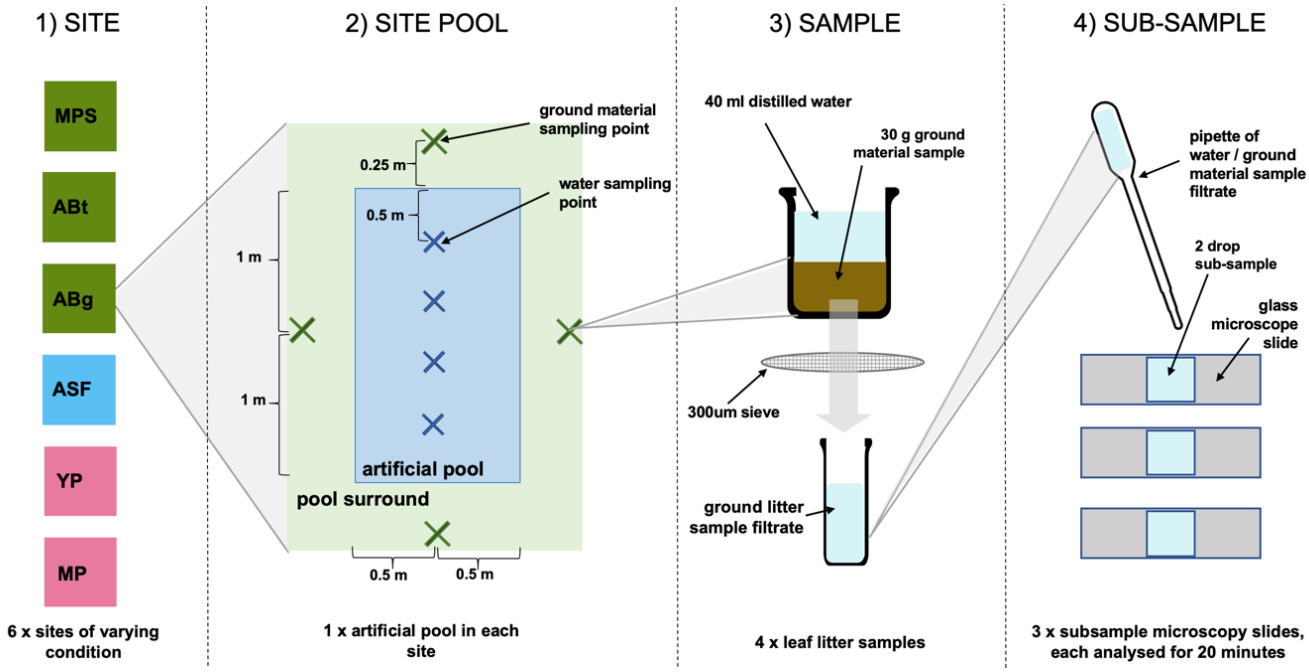

Figure 2. Sampling design and analysis process for water (n = 24) and ground material samples (n = 24); 1) six tropical peatland sites of varying condition, i) near natural (green) Mixed Peat Swamp Forest (MPS), Alan Batu (ABt) and Alan Bunga (ABg), ii) fragment (blue) Alan Swamp Forest (ASF), and iii) drained (pink) Young Plantation (YP) and Mature Plantation (MP); 2) water and ground material sampling locations around artificial pools located in each site; 3) preparation of ground material samples for microscopy; 4) preparation of subsample microscopy slides from each sample.

Due to low water tables caused by the dry season, the sampling design was organised around artificial sampling pools. Each study site had a single 2 m long x 1 m wide x 2 m deep pools dug between 2015 – 2017 by TROPI for regular monitoring. Water samples were taken at four locations at regular distance intervals across the pool surface using labelled 50 ml falcon tubes (Fig. 2; *Appendix B*). We then sampled a handful of ground material (abbrev. to GM henceforth) from each side of the pool (Fig. 2) and placed them into a labelled Ziplock bag (Swindles et al., 2014; *Appendix C*). The GM samples included a mixture of loose soil, twigs and leaf litter in varying proportions. Long rubber gloves were worn throughout sampling. Pool water and GM samples were stored and transported in a polystyrene cool box for up to six days before being stored in a laboratory cool room (4°C) over the duration of analysis (Swindles et al., 2014). This procedure was repeated for the artificial pool at each site.

Laboratory Analyses

I. Sample preparation

We filtered each pool water sample through a 300 μm sieve into a glass beaker, pouring the filtered contents into a clean falcon tube for storage. We then added 10 g (±0.5 g) of each GM sample in 40 g (±1 g) distilled water in a glass beaker. We swirled the beaker clockwise for one minute, ensuring all materials were fully submerged, then swirled it anticlockwise for another minute. Contents of the beaker were then filtered through a 300 μm sieve into a glass beaker and squeezed using a metal spatula to get as much water out into the beaker. We repeated filtering for a second time with a clean 300 μm sieve, this time without squeezing the contents to ensure no debris went through the sieve. We then transferred the GM sample filtrate into a clean falcon tube for storage. All samples were kept in a cool box that was stored in the laboratory cool room (4^{o}C) when not used for microscopy.

II. Microscopy

Before extracting a sample filtrate from its falcon tube, we inverted the tube three times to ensure microbes were well distributed. Using 3 ml Pasteur pipettes, we placed two drops of the sample filtrate on a flat microscope glass slide and carefully placed a cover slip to prevent formation of air bubbles. We analysed the slide through a compound microscope (400x magnification) for 20 minutes, photographing any microbe that was a possible TA candidate using a smartphone attached to a microscope lens (see Fig. 3; Swindles et al., 2014). The microscopy period was set to 20 minutes as through trial-and-error, we found this was the optimal duration that minimised sample desiccation and maximised the number of TA encounters. Graticules were unavailable in the laboratory, so we were unable to record the microbe size. Three slides were analysed for each water sample.

Figure 3. Setup for microscopy and testate amoebae imaging.

III. Identification and enumeration

We identified a specimen in our images as a TA if it (i) had a test, (ii) had at least one apparent mouth or aperture and (iii) was within the range of common test shapes and colours (based on several identification guides, see Ogden & Hedley, 1980; Hingley, 1993; Charman et al., 2000; Clarke, 2003). We included dead TAs and empty tests in our enumeration (Swindles et al., 2014). Morphotypes were categorised and enumerated based on their shape, colours and textures (see Table 2; Fig. 4). To minimise inter-observer error, morphotyping and enumeration was conducted by both researchers together.

Table 2. Testate amoebae morphotype categories.

Morphotype	Shape	Colour and Texture
A	Disk/Subspherical	brown, smooth
B		brown, textured
C		colourless, textured
D		pink-yellow, smooth
E		pink-yellow, embellished
F		pink-yellow, textured
G		brown, embellished
H		pink-yellow, smooth, spiny
I	Flask	colourless, textured
J		colourless, embellished
K		colourless, smooth
L		brown, smooth
M		brown, textured
N		grey, textured

O		pink-yellow, textured
P		pink-yellow, smooth
Q		pink-yellow, embellished
R	Slipper / Ovoid	pink-yellow, smooth
S		brown, textured, spiney
T		brown, textured
U		colourless, smooth
V		pink-yellow, embellished
W		pink-yellow, textured
X		brown, embellished
Y		brown, embellished, spiny

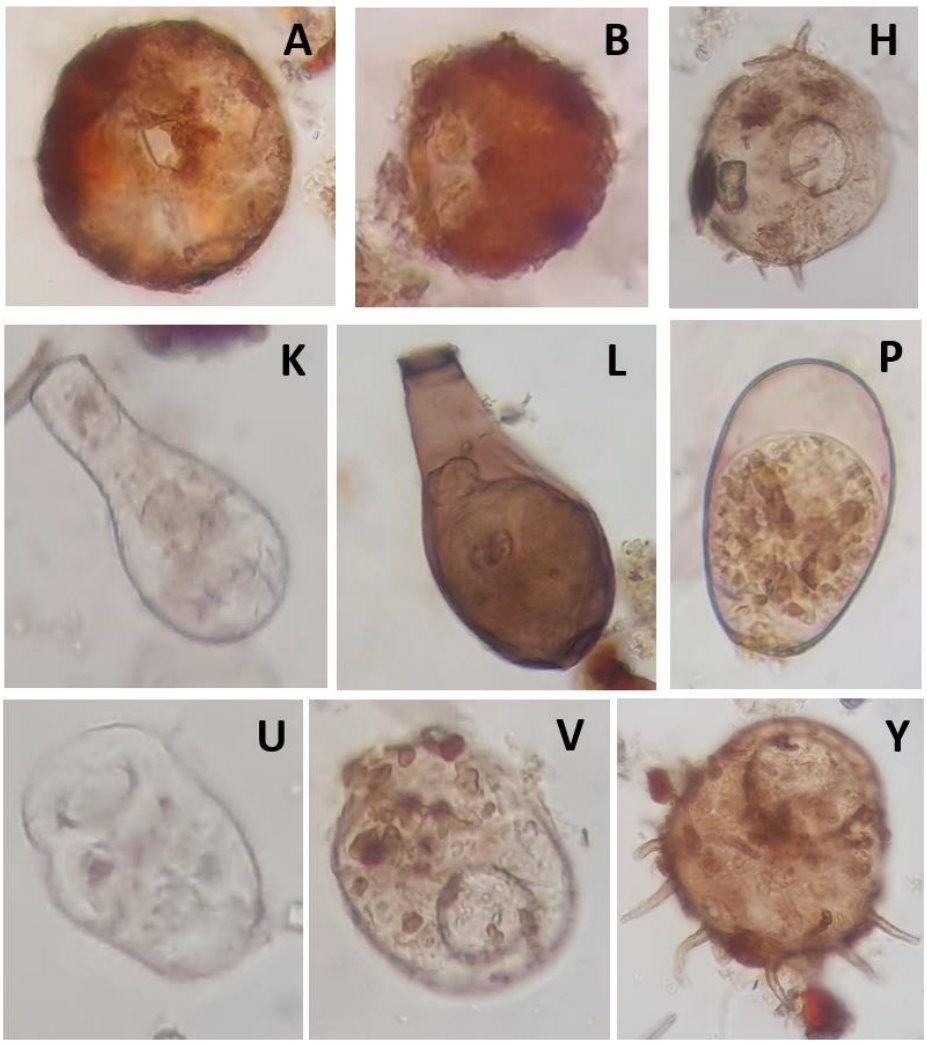

Figure 4. Light microscope images of several testate amoebae from our samples labelled under their morphotype categories. A - Brown, smooth subspherical, **B** - Brown, textured subspherical, **H** - Pink-yellow, smooth, subspherical, **K** - Colourless, smooth flask, **L** - Brown, smooth flask, **P** - Pink-yellow, smooth flask, **U** - Colourless, smooth slipper, **V** - Pink-yellow, embellished slipper, **Y** - Brown, embellished, spiny slipper. Images are not to scale.

Data Analyses

Statistical analyses and generation of graphics were carried out in R version 4.2.1. To test whether testate amoebae morphotype and abundance predicted tropical peat swamp condition, the Bray-Curtis distances were calculated using R and principal coordinate decomposition computed using the Principal Coordinate Analysis (PCoA; pcoa function in the ape package). The first two principal coordinates were plotted against each other to show any clustering between sites. Original variables were projected onto the ordination plot and influential TA morphotypes were identified (biplot.pcoa function in the ape function). A plot showing the clustering between different site types and projections of the most influential TA morphotypes was generated (ggplot function in the ggplot2 package).

The effect of tropical peatland site type on TA morphotype occurrences and abundances were determined using permutational multivariate analysis of variance on the Bray-Curtis distance matrix (PERMANOVA; adonis2 function in the vegan package) using 999 permutations and $p < 0.05$ as the significance threshold. An ANOVA-test (aov function in the stats package) was used to determine if there was a significant difference in the average abundance of each influential TA morphotypes per slide, between different site types. A Tukey's post hoc test (TukeyHSD function in the stats package) was used to determine statistical significance between specific sites. Finally, the alpha diversity of each site was measured using Shannon's diversity index (Shannon, 1948). ANOVA-tests were conducted to determine if there was a significant difference in alpha diversity among individual sites, types of sites and drainage status of sites.

RESULTS

We found no microbes in the pool water samples during microscopy and focused our analyses on the GM samples only. The PCoA revealed TA diversity and abundance to be significantly different between individual tropical peatland sites (PERMANOVA; $F_{23,5}$ = 2.734, p = 0.001), types of sites (PERMANOVA; $F_{23,2}$ = 2.747, p = 0.02), and site drainage status (PERMANOVA; $F_{23,1}$ = 4.700, p = 0.002), with some evident clustering of drained and non-drained sites. The two-dimensional PCoA plot (Fig. 5) shows that the first principal coordinate accounted for 30.22 % of total variation and largely separated the YP from the other tropical peatland sites, but there was some overlap with ABt. MPS and ABt appeared to be the only sites not limited by the second principal coordinate (20.92 % of total variation), but the axis failed to separate any sites. The projections of variables in the PCoA biplot revealed testate amoebae morphotypes P and K to be most influential in differentiating sites (Fig. 5).

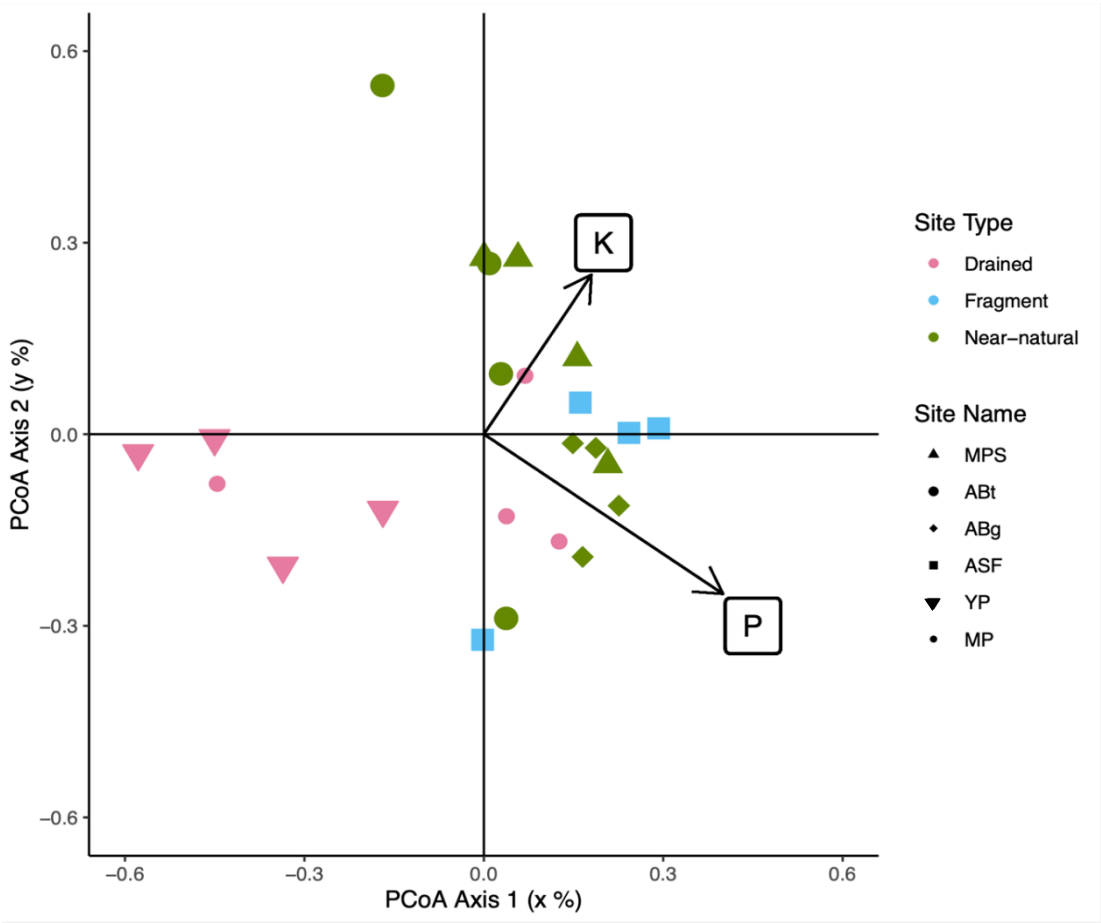

Figure 5. A two-dimensional plot of the Principal Coordinate Analysis (PCoA) of testate amoebae abundance data showing the clustering of TA morphotypes in different site types. The first and second principal coordinates account for 30.22 % and 20.92 % of total variation respectively. The arrows indicate the direction and power of the most influential TA morphotypes, P and K, extracted from the PCoA biplot. The fragment and near natural site types are taken to be forest sites, and the drained site type plantation.

There was no significant difference between the number of K morphotypes found in different types of peat swamp sites (ANOVA, $F_{21,2} = 2.317$, $p = 0.123$; Fig. 6).

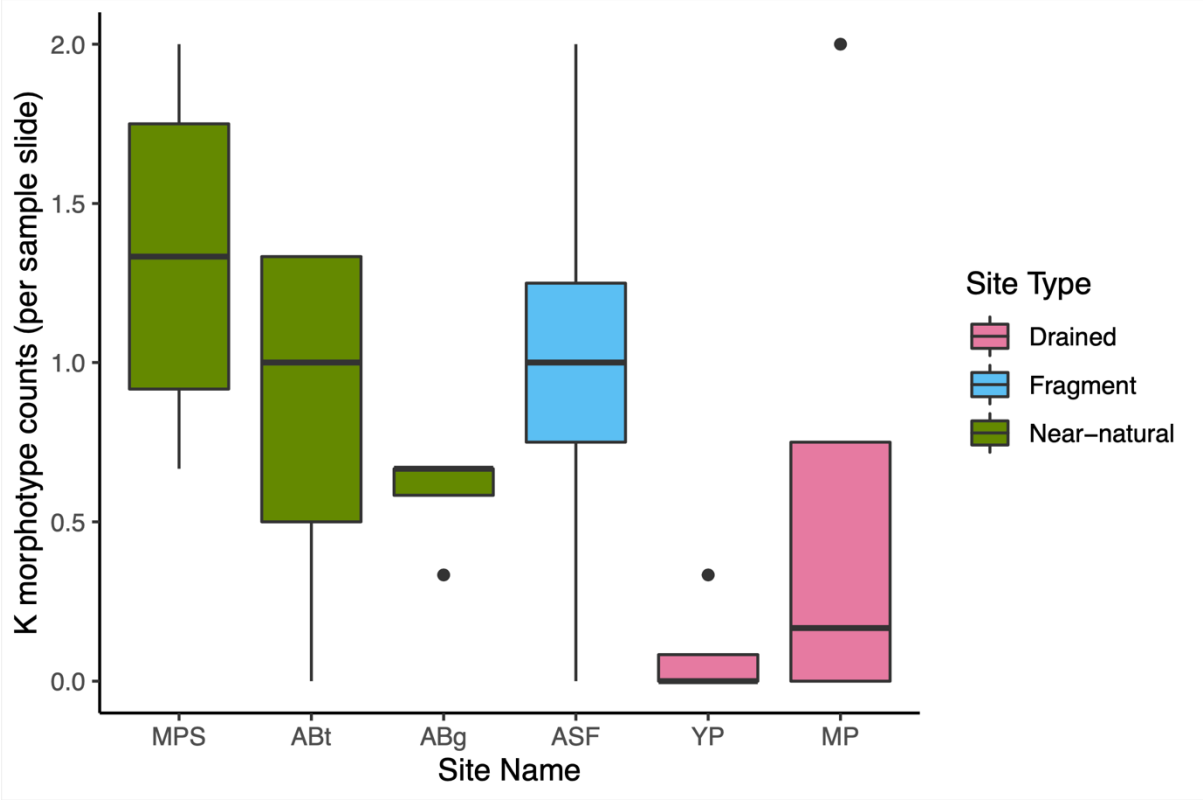

Figure 6: The difference in K morphotype counts in tropical peatland sites of different condition. Morphotype counts are taken as an average of counts from 3 slides which were used for the analysis of each sample. The fragment and near natural site types are taken to be forest sites, and the drained site type plantation.

Abundance of the P morphotype significantly varied between different types of peat swamp forest site (ANOVA, $F_{21,2}$= 4.275, p = 0.028; Fig 6). Drained sites had significantly fewer P morphotypes than the fragmented site (Tukey-test, Δμ = 1.833, p = 0.041), but not the near natural sites (Tukey-test, Δμ = 1.222, p = 0.072).

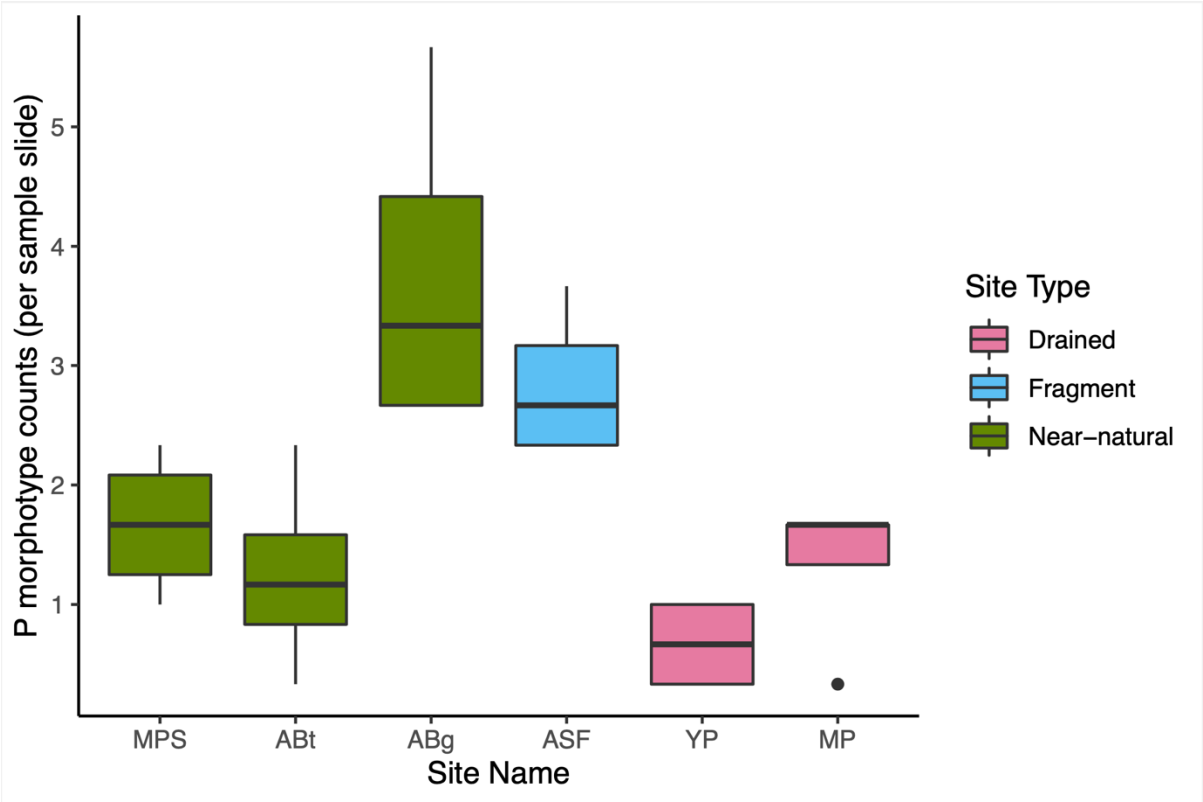

Figure 7: The difference in P morphotype counts in tropical peatland sites of different condition. Morphotype counts are taken as an average of counts from 3 slides which were used for the analysis of each sample. The fragment and near natural site types are taken to be forest sites, and the drained site type plantation.

The testate amoebae alpha diversity is not significantly different between the individual tropical peatland sites (ANOVA, $F_{18,5} = 1.707$, $p = 0.184$), site types (ANOVA, $F_{21,2} = 3.177$, $p = 0.062$), and site drainage status (ANOVA, $F_{22,1} = 0$, $p = 0.987$).

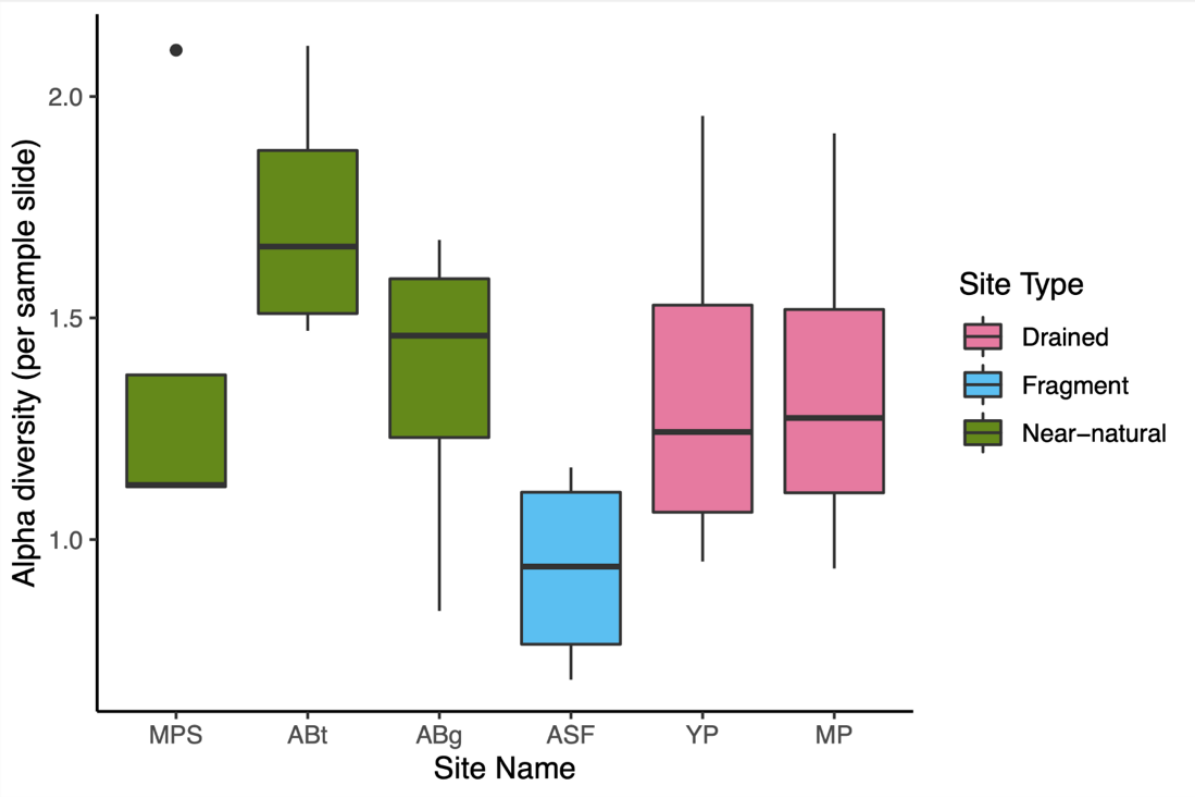

Figure 8: The difference in testate amoebae alpha diversity in tropical peatland sites of different condition. Morphology counts are taken as an average from three slides which were used for the analysis of each sample. The fragment and near natural site types are taken to be forest sites, and the drained site type plantation.

DISCUSSION

Our preliminary study found that the ground material in SE Asian tropical peatland sites of varying condition have significantly different compositions of TA morphotypes. However, TA alpha diversity did not differ among these sites. Two morphotypes, K and P, were found to be influential in differentiating sites, though only morphotype P was found to significantly differ in abundance between sites.

TA morphotype community composition

We found that TA community composition in the drained plantation sites significantly differed to those in undrained forests. The TA morphotype assemblage of YP overlapped the least with other sites, indicating a most disparate TA community composition. YP was the most intensely managed site with regular clearing of ground litter and the lowest vegetation diversity, reducing leaf litter availability and variety. YP was also a drier plantation compared to MP as the former was more intensely drained and had less canopy cover, leading to greater sun exposure. This may have limited hummus heterogeneity and creation, and water film thickness on ground material at the site. These are extreme conditions which only few specific TA species can tolerate (Krashevska et al., 2007). The TA reproductive rate may also have been constrained by low food and test construction material availability (Clarke, 2003). Furthermore, overlap of MP with undrained sites (Fig. 5) was possibly due to the lack of management MP experienced in recent years, allowing conditions to return closer to that of undrained forest levels. Confidence in these findings would be increased by collecting data on specific TA microhabitat conditions and having more plantation sites of YP and MP condition to compare.

Alpha diversity of TA did not differ among sites, site types or drainage status of sites. This may have been because TA respond less to environmental change when in soil than in leaf litter (Krashevska et al., 2016). Our undrained ground material samples mainly consisted of leaf litter, whereas drained GM samples were mostly soil, possibly explaining their similarities in diversity. By sampling both leaf litter and surface soil as separate ground material samples, the influence of site-specific environmental factors on ground material TA diversity could be better understood.

The majority of TA morphotype enumerations were either of dead TA or empty tests. Though this is expected for ground surface samples (Wilkinson, 2010; Lamentowicz et al., 2013; Wanner et al., 2015) and most field TA studies include empty tests in enumerations (Jassey et al., 2011; Swindles et

al., 2016b), their exclusion has been found to provide more accurate data on species distribution and their association with moisture level (Lizoňová et al., 2019). By distinguishing between occupied and empty tests, more precise environmental-species associations could be drawn. In addition, it would allow studies to include an estimate of a site's 'shell quotient' (i.e. ratio of live TA and empty tests), which has been suggested as an indicator of overall microbial activity of a habitat (Schonborn, 1992; Wanner, 1991; Meisterfeld, 1980; Volz, 1951). The low number of live TA in our study may have been exacerbated by the time samples were kept in storage and under microscopy light. This could be avoided by analysing samples in situ or improving preservation of TA during transport and microscopy. For example, mounting slides with glycerol prevents specimen desiccation during microscopy and provides clearer images (Hendon & Charman, 1997).

Influential indicator morphotypes K and P

TA morphotypes P and K were the most influential in differentiating tropical peatland sites (Fig. 5). The abundance of these morphotypes alone failed to separate sites of differing condition, suggesting consideration of total TA composition analysis is necessary to build an effective environmental proxy. By directly measuring the microclimate of collection sites and comparing to the abundance of our TA morphotypes, their specific environmental associations could be highlighted (Swindles et al., 2014).

I. K morphotype

Our PCoA analysis suggests that K morphotype was influential in separating drained sites from the undrained sites, which indicates that it is more likely to occur in wetter peatlands. However, the various species that would have been categorised under this morphotype provide us with conflicting inferences. The K morphotype encompassed *Euglypha rotunda* and *Euglypha tuberculata,* which are typical of intermediate to high hydrological conditions (Charman et al., 2000; Hedley and Ogden, 1973). In contrast, the morphotype category also include *Nebela collaris* and *Nebela militaris*, which are associated with drier conditions (de Graaf, 1956; Corbet, 1973; Heal, 1961), as well as *Cryptodifflugia oviformis* which is reported as a cosmopolitan (Charman et al., 2000). This indicates that our sampling may not have been representative, and that our morphological categorisations failed to distinguish between important species with diverse hydrological associations. This could possibly be improved by adding another morphospecies category which distinguishes between plated

and smooth tests. Mounting with glycerol may enable this distinction in texture by providing images with better definition (Hendon & Charman, 1997).

II. P morphotype

High abundance of the P morphotype was found in peatland sites ABg and ASF. The only known variable uniquely shared by these sites is domination by Alan trees, suggesting the P morphotype may be linked to Alan leaf litter, or an associated environmental driver we did not measure. Building a comprehensive characterisation of the TA macro-and microclimate in each sampling site is required to determine the drivers of the P morphotype abundance.

Species with differing environmental preferences are included in our P morphotype category. *Hyalosphenia subflava,* a morphospecies found to dominate TA compositions (Krashevska et al., 2020) and included in the P category, has been identified as an indicator of variable hydrological conditions (Sullivan and Booth, 2011; Swindles et al., 2014; Krashevska et al., 2020). In contrast *Nebela tincta,* another species accounted for in the morphotype, is often regarded as xerophilous (Tolonen, 1986; Tolonen et al, 1992), although Warner (1987) reported its occurrence in 'very wet' conditions. Again, morphological categorisations failed to distinguish between important species with diverse hydrological associations.

Sampling methods

Our simple, inexpensive ground surface material sampling approach and light microscopy morphology analyses effectively differentiated peatland sites of varying condition. Our results demonstrate the value of ground material over surface water for yielding TA. Sampling water at multiple depths and from natural pools would confirm the worth of water samples in tropical peatland TA studies. Ground material samples have the potential to reflect a wider range of environmental characteristics including the feeding preference of TA.

Incorporating TA functional traits into analyses can reveal more about species-environment relationships, nutrient cycling and energy flow. Testate length to aperture size ratio has been used to separate TA into low trophic level species that feed on bacteria and algae, and high trophic level species that feed on protists, and micro-metazoans (Jassey et al., 2013). TA size has also been found to relate to food, temperature, water film thickness (Clarke et al., 2003), and site drainage. For example, the size of *H. subflava*, represented in our P morphology, decreases with increasing

moisture. Finding a community of P morphotypes with a variety in test size could therefore be an indicator of highly variable hydrological regime (Krashevska et al., 2020). Having access to a graticule and taking these measurements would allow insight into TA morphotype ecological functions and response to macro- and micro-environmental processes (Lamentowicz et al., 2013; Song et al., 2013).

The use of ground material samples alone is unrepresentative of the sites. This is because the sampling approach was organised around artificial pools, with the assumption that water would form the centre of the analysis as the easiest and least destructive sampling method. Despite this bias, the ground material still revealed differences in TA composition between sites, showing the potential for the method to be incorporated into existent survey routes. A further study sampling a range of ground material across a random transect instead has the potential to strengthen and expand the conclusions of this study (Swindles et al., 2014).

The problem of site replication in this preliminary study is another reason to treat our findings with caution. We had a disproportionate number of samples from undrained sites compared to drained sites, introducing bias in our comparative analyses. This was mainly caused by issues in accessing plantations and time constraints. Further studies with greater site replication would be required to make more generalised conclusions on TA ecology difference between peatlands of drained and undrained conditions in Sarawak.

CONCLUSIONS

Ground material in SE Asian tropical peatland sites of varying condition contain significantly different compositions of testate amoebae morphotypes. This preliminary study shows the promise of this cheap, accessible monitoring method, but the underlying environmental drivers of TA community differentiation are uncertain. We recommend a revision of morphological categorisations to reflect functional types and microhabitat preferences, expand sampling representation, and sampling of specific environmental variables. Following this, a citizen science project trial would establish the utility and replicability of the methods.

REFERENCES

Arai, H. et al. (2014). Land use change affects microbial biomass and fluxes of carbon dioxide and nitrous oxide in tropical peatlands. *Soil Science Plant Nutrition* 60:423–34.

Bovee, E. C. (1960). Studies of feeding behavior of amebas. I. Ingestion of thecate rhizopods and flagellates by verrucosid amebas, particularly *Thecamoeba sphaeronicleolus*. *Journal of Protozoolologie* 7, 55-60.

Charman, D. J. (2001). Biostratigraphic and palaeoenvironmental applications of testate amoebae. *Quaternary Science Reviews* 20(16-17) 1753-1764.

Charman, D. J., Hendon, D. & Woodland, W. A. (2000). The Identification of Testate Amoebae (Protozoa: Rhizopoda) in Peats. *Quartenary Research Association.*

Clarke, K. J. (2003). Guide to the Identification of Soil Protozoa – Testate Amoebae. *The Freshwater Biological Association.*

Corbet, S. A. (1973). An illustrated introduction to the testate Rhizopods in *Sphagnum* with special reference to the area around Malham Tarn, Yorkshire. *Field Studies* 3, 801-838.

De Graaf, F. (1956). Studies on Rotaria and Rhizopoda from the Netherlands. *Biologisch Jaarboek Dodonea* 23, 145-217.

De Vleeschouwer, F., Chambers, F.M. and Swindles, G.T., 2010. Coring and sub-sampling of peatlands for palaeoenvironmental research. *Mires and peat*, 7.

Dom, S. P., et al. (2021). Linking prokaryotic community composition to carbon biogeochemical cycling across a tropical peat dome in Sarawak, Malaysia. *Scientific Reports* 11(1).

Finlay, B. J. et al. (2001). Biodiversity of terrestrial protozoa appears homogenous across local and global spatial scales. *Protist* 152, 355-366.

Forest Department Sarawak. (2018). Types and Categories of Sarawak's Forests. https://forestry.sarawak.gov.my/page-0-160-593-Types-and-Categories-of-Sarawak-s-Forests.html

Heal, O. W. (1961). The distribution of testate amoebae (Rhizopoda: testacea) in some fens and bogs in northern England. *Journal of the Linnean Society, Zoology* 44, 369-382.

Hendon, D. and Charman, D. J. (1997). The preparation of testate amoebae (Protozoa:Rhizopoda) samples from peat. *The Holocene* 7, 199-205.

Hingley, M. (1993). Microscopic Life in Sphagnum. *The Richmond Publishing Co. Ltd.*

Inubushi, K. et al. (2003). Seasonal changes of CO2, CH4 and N2O fluxes in relation to land-use change in tropical peatlands located in coastal area of South Kalimantan. *Chemosphere* 52:603–8

Ishikura, K., et al. (2018). Soil carbon dioxide emissions due to oxidative peat decomposition in an oil palm plantation on tropical peat. *Agriculture Ecosystems & Environment* 254 202-212.

Jassey, V. E. J., et al. (2013). Above- and belowground linkages in Sphagnum peatland: climate warming affects plant-microbial interactions. *Global Change Biology* 19(3) 811-823.

Jassey, V. E. J., et al. (2011). Fine-Scale Horizontal and Vertical Micro-distribution Patterns of Testate Amoebae Along a Narrow Fen/Bog Gradient. *Microbial Ecology* 61(2) 374-385.

Kitson, E. and Bell, N. G. A. (2020). The Response of Microbial Communities to Peatland Drainage and Rewetting. A Review. *Frontiers in Microbiology*, 11: 582812.

Koenig et al. (2018). Impact of two hot and dry summers on the community structure and functional diversity of testate amoebae in an artificial bog, illustrating their use as bioindicators of peatland health. *Mires and peat* 21 (8), 1–24.

Krashevska, V., et al. (2007). Testate amoebae (protista) of an elevational gradient in the tropical mountain rain forest of Ecuador. *Pedobiologia* 51(4) 319-331.

Krashevska, V., et al. (2016). Changes in structure and functioning of protist (testate amoebae) communities due to conversion of lowland rainforest into rubber and oil palm plantations. *PLoS One*, 11(7).

Krashevska, V., et al. (2020). Testate Amoeba Species- and Trait-Based Transfer Functions for Reconstruction of Hydrological Regime in Tropical Peatland of Central Sumatra, Indonesia. *Frontiers in Ecology and Evolution* 8.

Kurnianto, S. et al. (2015). Carbon accumulation of tropical peatlands over millennia: a modeling approach. *Global Change Biology*, 21:431-444

Lamentowicz, M. and Mitchell, E.A.D. (2005). The Ecology of Testate Amoebae (Protists) in Sphagnum in North-western Poland in Relation to Peatland Ecology. *Microbiology Ecology* 50, 48–63

Lamentowicz, M., et al. (2013). Seasonal patterns of testate amoeba diversity, community structure and species-environment relationships in four Sphagnum-dominated peatlands along a 1300 m altitudinal gradient in Switzerland. *Soil Biology & Biochemistry* 67 1-11.

Lizonova, Z., et al. (2019). Small-scale Variation of Testate Amoeba Assemblages: the Effect of Site Heterogeneity and Empty Shell Inclusion. *Microbial Ecology* 77(4) 1014-1024.

Marcisz, K., et al. (2020). Testate Amoeba Functional Traits and Their Use in Paleoecology. *Frontiers in Ecology and Evolution* 8.

Meisterfeld, R. (1980). The structure of Testaceans (Rhizopoda, Testacea) in the soils of Solling. *Verhandlungen der Gesellschaft für Ökologie* 8, 435-447.

Melling, L. et al. (2016). Chapter 4: Peatland in Malaysia. In: Osaki, M., Tsuji, N. (Eds.), *Tropical Peatland Ecosystems,* pp. 59-73.

Melling, L., Chua, K.H., Lim, K.H., (2008). Managing peat soils under oil palm. In: Agronomic Principles & Practices of Oil Palm Cultivation. 3. pp. 1-33.

Mitchell, E. A. D., D. J. Charman and B. G. Warner (2008). Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodiversity and Conservation* 17(9) 2115-2137.

Monda, Y., et al. (2018). Association of growth and hollow stem development in Shorea albida trees in a tropical peat swamp forest in Sarawak, Malaysia. *Trees-Structure and Function* 32(5) 1357-1364.

Ogden, C. G. & Hedley, R. H. (1980). An Atlas of Freshwater Testate Amoebae. British Museum (Nat. His.) *Oxford University Press.*

Page, S. E., Rieley, J. O. and Banks, C. J. (2011). Global and regional importance of the tropical peatland carbon pool. *Global Change Biology* 17, 798-818.

Schonborn, W. (1992). The role of protozoan communities in freshwater and soil ecosystems. *Acta Protozoologie* 31, 11-18.

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Techincal Journal* 27, 379–423 and 623–656.

Song, L. H., et al. (2018). Seasonal dynamics in the community structure and trophic structure of testate amoebae inhabiting the Sanjiang peatlands, Northeast China. *European Journal of Protistology* 63 51-61.

Sullivan, M. E. and R. K. Booth (2011). The Potential Influence of Short-term Environmental Variability on the Composition of Testate Amoeba Communities in Sphagnum Peatlands. *Microbial Ecology* 62(1) 80-93.

Swindles, G. T., et al. (2016a). Evaluating the use of dominant microbial consumers (testate amoebae) as indicators of blanket peatland restoration. *Ecological Indicators* 69 318-330.

Swindles, G. T. et al. (2016b). Palaeoecology of testate amoebae in a tropical peatland. European *Journal of Protistology* 5, 181–189.

Swindles, G. T. et al. (2015). Evaluating the use of testate amoebae for palaeohydrological reconstruction in permafrost peatlands. *Palaeogeography, Palaeoclimatology and Palaeoecology* 424, 111–122.

Swindles, G. T., et al. (2014). Ecology of Testate Amoebae in an Amazonian Peatland and Development of a Transfer Function for Palaeohydrological Reconstruction. *Microbial Ecology* 68(2) 284-298.

Tawan, C. S., Ipor I.B., Wan Sulaiman W.H. (2008). Floral diversity of the peat swamp forests of Sarawak. In: After Wise Use – The Future of Peatlands, Proceedings of the 13th International Peat Congress: Tropical Peatlands.

Tolonen, K., Warner, B. G., Vasander, H. (1992). Ecology of testaceans (Protozoa: Rhizopoda) in mires in Southern Finland: I. Autecology. *Archiv fur Protistenkunde* 142, 119-138.

Tolonen, K. (1986). Rhizopod analysis. In: Berglund, B. E. (Ed.), *Handbook of Holocene palaeoecology and palaeohydrology*. John Wiley, Chichester, pp. 645-666.

Turner, T. E. and Swindles, G. T. (2012). Ecology of testate amoebae in moorland with a complex fire history: implications for ecosystem monitoring and sustainable land management. *Protist* 163(6), 844-855.

Volz, P. (1951). Studies on the microfauna of the forest floor. *Zool. Jb. Syst.* 79, 514-566.

Wanner, M. (1991). Studies on the ecology of testate amoebae (Protozoa: Rhizopoda) in forests of Southern Germany. *Archiv für Protistenkunde* 140(2-3), 237-288

Wanner, M., et al. (2015). Testate amoebae colonizing a newly exposed land surface are of airborne origin. *Ecological Indicators* 48 55-62.

Warner, B. G. (1987). Abundance and diversity of testate amoebae (Rhizopoda, Testacea) in *Sphagnum* peatlands in southwestern Ontario, Canada. *Archiv fur Protistenkunde* 133, 173-189.

Wilkinson, D. M. (2010). Have we underestimated the importance of humans in the biogeography of free-living terrestrial microorganisms? *Journal of Biogeography* 37(3) 393-397.

Wosten, J. H. et al. (2008). Peat–water interrelationships in a tropical peatland ecosystem in Southeast Asia. *Catena (Giessen)*. 73 (2), 212–224.

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APPENDIX

Appendix A: Table of Expenditure

Item (2 pax)	**Cost (£)**
Return flight Edinburgh to Kuala Lumpur	1636.28
Return flight Kuala Lumpur to Kuching	140.16
Accommodation in Sarawak	414.07
Transportation in Sarawak	93.12
Food in Sarawak	615.17
Vaccinations	820.14
Equipment	557.94
TOTAL	4276.88

Appendix B: Pool water sampling

Figure 8: Elizabeth taking pool water samples at one of the near natural sites under TROPI staff supervision

Appendix C: Ground material

Figure 9: Tasnim and Elizabeth sorting out ground material samples