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Exploring testate amoebae as taxonomic and functional bioindicators to inform peatland habitat status and blanket bog restoration

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ABSTRACT

Many UK blanket bog peatlands are degraded due to historical management including drainage, leading to reduced biodiversity, carbon sequestration and water storage. Currently, much restoration efforts including rewetting and revegetation strategies are being deployed aiming to restore habitats towards intact and ecohydrologically functioning ecosystems. However, it remains unclear how these efforts affect the key microbial consumers, testate amoebae (TA), especially their functional traits, and whether TA can be used as a generic tool to monitor the long-term hydrological restoration success. This study compared TA communities and their key functional traits at one intact and three near-intact sites versus three modified blanket bog sites with different habitat conditions (i.e., least modified, post-restoration, and degraded) to assess their environmental responses, bioindicator potential and explore their possible functional contribution to ecosystems in the process of recovery. The results showed: 1) TA community composition gradually changes from degraded to intact sites in both *Sphagnum* and surface peat, with distinct dominant TA species in each; 2) soil moisture, *Sphagnum* cover, and phosphorus content strongly relate to TA composition; 3) *Hyalosphenia subflava* and *Corythion dubium* indicate dry conditions, while *Archerella flavum* and *Amphitrema wrightianum* are indicators of wet conditions; 4) significantly higher community-weighted mean values of TA traits (biovolume, aperture width, siliceous tests) in surface peat at the least modified areas suggest TA's strong role in carbon and silica cycling following rewetting. Our findings support TA as bioindicators for tracking habitat hydrological conditions and restoration progress in blanket bogs, effectively linking community composition to ecosystem functions.

1. Introduction

Blanket bog peatlands are important, yet in good condition rare, UK habitats (Wilkie and Mayhew, 2003) which store large amounts of carbon (Evans and Lindsay, 2010) and are home to many plants and birds (Littlewood et al., 2010). Globally, approximately 13 % of blanket bogs are contained in the UK upland areas (Bain et al., 2011). To improve the production of livestock, a number of British blanket bogs were historically drained (mainly for grazing) including considerable government financial incentives during the past century (Evans, 1998; Sansom, 1999; Britton et al., 2017; Alday et al., 2022). However, together with large stocking densities, such management practices have put more than 70 % of upland areas in England and Wales at risk from overgrazing and almost half of the upland breeding bird species are threatened (Sansom, 1999). In some cases, extensive regions were modified, leading to degradation, with large areas of peat being severely exposed, dried and eroded, often forming large series of erosion gully networks (Tallis, 1998; Parry et al., 2014). So far, it is estimated that 70–80 % of UK blanket bogs are damaged to various degrees (Artz et al., 2014; Loisel and Gallego-Sala, 2022), with only around 10–20 % of them in protected sites to be classified as in ‘favorable’ condition (IUCN UK Peatland Programme, 2018; Jones, 2023). However, it is noted that a favorable condition mainly reflects a habitat assessment based on incomplete (arbitrary) pass/fail criteria such as focusing on vegetation composition, which often lacks evidence of measured functions (Ashby and Heinemeyer, 2021).

Besides the decline of biodiversity and erosion of surface peat, drainage and sheep trampling has significantly affected the vegetation composition (Shaw et al., 1996), likely shifting it from *Sphagnum* moss-dominated to more vascular plant-dominated communities (Lindsay, 2010). The resultant loss of *Sphagnum* cover, along with drier, exposed peat and potentially faster surface runoff can increase the downstream

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flood risk (Holden et al., 2007; Wilson et al., 2011; Shuttleworth et al., 2019). With rapid loss of water and water table drawdown, peat decomposition is accelerated by increased oxygen availability to soil microbes, which decompose the stored organic matter and its carbon into CO_2 and other products like dissolved organic carbon (DOC), thereby reducing the net carbon sequestration rate and potentially changing the ecosystem's C balance from C sink to C source (Armstrong et al., 2010; Evans and Lindsay, 2010). Water quality is then inevitably reduced by increased stream DOC concentration, and the deteriorated water quality increases the cost of processing for drinking water supplies (Holden et al., 2006). Consequently, to restore degraded upland blanket peatlands towards ecosystems that function hydrologically and ecologically, a range of restoration strategies have been increasingly developed and deployed over the past few decades, including reduced grazing levels and ditch/gully-blocking (Wilson et al., 2010; Rosenburgh, 2015; Shuttleworth et al., 2019; Watts, 2020; Chapman et al., 2022). It is therefore crucial to assess and monitor the peatland condition and restoration progress following these management interventions to evaluate the recovery success to functioning bog status or advise on further restoration needs. Using testate amoebae (TA) as hydrological bioindicators seems a promising way to achieve this aim as TA are known to show species-specific responses to environmental factors, especially wetness (Lousier, 1974; Marcisz et al., 2014), and TA have also been linked to C and silica cycle functions (Jassey et al., 2015; Puppe, 2020).

TA are the main component of peatland protists, which are particularly abundant in both *Sphagnum* moss and surface peat (Lamentowicz and Mitchell, 2005; Liu et al., 2019). Since TA are sensitive to a wide variety of environmental factors including hydrology (Koenig et al., 2017), air pollution (Nguyen-Viet et al., 2004), heavy metals (Nguyen-Viet et al., 2007) and have a rapid reproduction rate (Charman, 2001), they have been proposed as potential ideal ecological indicators to monitor environmental changes in peatlands (Payne, 2010; Meyer et al., 2013; Swindles et al., 2016; Creevy et al., 2018). During the past two decades, an increasing number of studies have been conducted to explore the use of TA as bioindicators in monitoring peatland restoration by virtue of assessing peat cores (Buttler et al., 1996; Jauhiainen, 2002; Davis and Wilkinson, 2004; Valentine et al., 2013) and modern moss samples (Vickery, 2006; Laggoun-Défarge et al., 2008; Swindles et al., 2016; Creevy et al., 2018; Evans et al., 2024). However, it is worth noting that most of these studies focused on raised bogs (Buttler et al., 1996; Davis and Wilkinson, 2004; Vickery, 2006; Laggoun-Défarge et al., 2008; Valentine et al., 2013), with only one study conducted in relation to short-term restoration in a modified semi-natural blanket bog (Swindles et al., 2016) and another one related to long-term restoration of afforested blanket bogs (Creevy et al., 2018). These studies showed a strong sensitivity of TA to hydrological change following restoration, with species indicative of wet environments such as *Archerella flavum* being relatively more abundant in post-restoration areas, while species associated with dry conditions, like *Nebela tincta*, were more prevalent in pre-restoration areas (Jauhiainen, 2002; Swindles et al., 2016). Moreover, species richness and diversity have been reported to increase, while density declines from the recent to advanced stages of recovery (Laggoun-Défarge et al., 2008). Undoubtedly, while this limited insight into TA communities has improved our understanding of their response to restoration strategies, a broader geographic range of site assessments remains lacking, especially for blanket bogs undergoing longer-term (~10 years) restoration periods and considering TA's functional traits.

As the primary microbial consumers, TA communities potentially play a crucial role in ecosystem functioning including carbon and nutrient cycling (Wilkinson, 2008; Jassey et al., 2015). Wilkinson et al. (2010) indicated that the heterotrophic TA (HTA) can influence the decomposition rate of organic matter (OM) by selectively preying on bacterial and fungal functional groups involved in carbon/nitrogen-cycling, thereby indirectly affecting carbon dioxide emissions and nutrient flow (Wilkinson and Mitchell, 2010). Their biovolume and aperture size were also reported to exhibit a strong positive correlation with trophic level, with larger specimens tending to have a broader range of food sources and being more likely to occupy higher trophic positions (Jassey et al., 2016). Furthermore, recent studies suggest that mixotrophic TA (MTA) can constitute over 70 % of the total peatland (in a raised bog) microbial biomass, and as such potentially significantly contribute to carbon fixation as they can acquire carbon sources through photosynthesis by their endosymbionts (e.g., green microalgae) (Jassey et al., 2015, 2016). Despite these reports, we still lack knowledge on how key functional traits like mixotrophic TA, body and aperture size vary across the blanket bog habitat spectrum. As a result, investigating changes in these traits of TA in response to restoration management will improve our understanding of their potential functional contribution to ecosystem processes and to better link habitat status to functions. Noticeably, previous studies investigated the TA communities either from peat (surface peat and/or peat core) samples or surface mosses samples (Davis and Wilkinson, 2004; Valentine et al., 2013; Swindles et al., 2016; Creevy et al., 2018); no study seems to have been carried out examining both aspects at the same time to explore their response to restoration management. Given the notable differences in environmental conditions (e.g., light, temperature, oxygen) and food sources between *Sphagnum* mosses and surface peat, the composition and recovery pattern of TA would likely vary to some extent between these two habitat niches.

Here, TA communities were investigated at three restoration blanket bog (which mostly experienced a combination of historical sheep grazing, and grouse moor heather management with high heather cover on drier peat, each consisting of degraded, post-restoration and least-modified areas) and compared to other sites representing near-intact to intact bog habitat condition counterparts (with hardly any or no recent management and low heather cover on very wet peat). This allowed an assessment of TA's potential as bioindicators for monitoring habitat hydrological status and ecosystem recovery trajectories, while also exploring their possible contributions to ecosystem functioning throughout the restoration process. The objectives of this study were to: 1) investigate shifts in TA community composition, taxonomic and functional traits (e.g., MTA, body/aperture size) in response to rewetting management across a gradient from degraded, post-restoration to the least modified, near-intact and very wet intact bog habitats; 2) explore the main drivers for TA development and distribution; 3) link their change of functional traits to ecosystem processes (e.g., C & N cycling); and 4) use TA from deep peat sections to allow a comparison of likely historic site hydrological conditions before management intensification. It was hypothesized that: 1) over time, restoration of degraded blanket bog peatlands by rewetting and regeneration shifts TA community composition, taxonomic and functional diversity towards that of undisturbed and wetter ‘near-intact to natural/intact’ conditions; 2) TA community composition is strongly related to soil moisture and *Sphagnum* moss cover and, to a lesser extent, to peat physical, chemical, and other environmental factors; 3) TA show species-specific sensitivity to rewetting strategies, and restoration management drives change in functional traits of TA towards assemblages resembling those in near-intact to natural/intact conditions but their recovery pattern is more pronounced in *Sphagnum* mosses than in surface peat; 4) TA communities in deep peat layers of blanket bog can serve as a valuable tool to infer and compare historical hydrological conditions before peatland management intensification and thus aid interpretation of comparability of generic site conditions.

2. Methods

2.1. Study sites

Samples were collected from six hill blanket bog sites and one intact bog (Butterburn), described as a topogenous border mire (BM) bog complex (including intermediate, ridge-raised and valley bogs) developed over valley/depression-shaped terrain (Eades et al., 2021), also referred to as watershed or basin peat mires (Wheeler et al., 2020, 2023). Such "soup bowl" peats are generally much wetter and have significantly deeper peat depth than typical "leaky slope" hill blanket bogs (Glatzel et al., 2023). Among the six hill blanket bogs, half of them are described as near-intact blanket bogs (Kielder Head, Whitelee English side and Whitelee Scottish side), and the other half as modified and/or degraded blanket bogs (Cray Moss, Fleet Moss and Stake Moss), each consisting of least modified, post-restoration and degraded areas. The very wet border mire and near-intact blanket bogs were chosen in this study as reference sites since they were considered not to or only to have been hardly disturbed by historic management. All of them are in the north of England, among which Whitelee, the northernmost, is situated right at the border between England and Scotland. The other three managed restoration blanket bog sites are all located in the Pennine uplands (Fig. 1, Fig. S1).

Detailed information about these seven sites, based on site surveys conducted during this study, is described below and in Table 1 (for related methods, such as peat moisture assessment, see the following sections).

The very wet intact border mire (conditions reflect sampling location and time - February 2021):

Butterburn border mire site (Butt): lies within the Kielder Forest Park, UK, at 55° 07′ 85"N; 2° 53′ 12"W (UK Grid Ref NY 661761) about 167 m above sea level (a.s.l.). The vegetation (i.e., sum of over and understory; at least 100 %) was dominated by *Sphagnum* moss ranging from 65 % to 85 % (mean: 73.3 %) in the sampling areas, followed by *Eriophorum* spp. (cotton-grass) sedge from 25 % to 40 % (mean: 36.7 %), with other mosses occupying less than 10 % (mean: 3.7 %). The soil was very wet with a peat moisture (by weight) content from 95.1 % to 96.2 %. The average peat depth was about 6.7 m and a mean slope of 1° across the sampling areas. However, this site most likely represents an intermediate/ridge-raised bog complex as has been recently highlighted for the border mire peatlands (Eades et al., 2021).

The three near-intact sites (conditions reflect sampling location and time – February 2021):

Kielder Head site (KH): lies within the Kielderhead National Nature Reserve, at 55° 27′ 85"N; 2° 45′ 38"W (UK Grid Ref NY 712983) about 408 m a.s.l. The vegetation was dominated by other mosses ranging from 33 % to 85 % (mean: 52.7 %) in the sampling areas, followed by heather from 28 % to 65 % (mean: 51.0 %), with *Sphagnum* moss and cotton-grass (henceforth referred to as sedge) occupying from 1 % to 45 % (mean: 27.3 %) and from 8 % to 25 % (mean: 15.0 %), respectively. The soil (at sampling time) was wet (from 89.8 % to 92.1 %), with an average peat depth of 1.8 m and a slope of 3° across the sampling areas.

Whitelee (English and **Scottish side) site (WE & WS)**: are located at the border between England and Scotland, which lies within the Whitelee Moor National Nature Reserve, at 55° 34′ 35"N; 2° 49 ′77"W

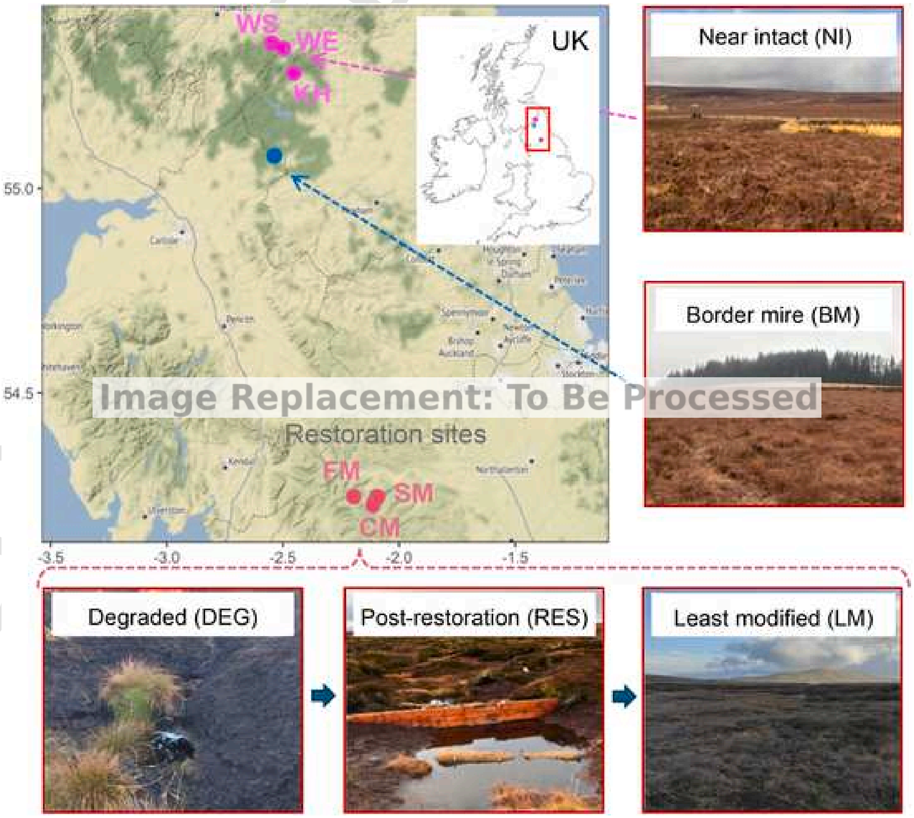

Fig. 1. Location of study sites. Restoration sites include Fleet Moss (FM), Stake Moss (SM) and Cray Moss (CM) each consisting of least modified (**LM**), post-restoration (**RES**), and degraded (**DEG**) areas; near-intact sites (**NI**) include Kielder Head (KH), Whitelee English side (WE) and Whitelee Scottish side (WS); intact border mire site (**BM**) includes the Butterburn (Butt) area.

Table 1

Location and sample number information for the sampling sites of the six-blanket bog and one border mire sites. Vegetation cover was assessed within replicated (3) 50 × 50 cm squares for Cray, Fleet and Stake Moss in December 2020 and for Butterburn, Kielder Head and Whitelee in February 2021 as total cover (at least 100 %) of: heather (H), cotton-grass sedge (S), *Sphagnum* moss (Sph), other mosses (OM), brash/litter/bare ground (BLB). Samples taken for testate amoebae extractions consisted of *Sphagnum* moss, surface peat (SP: 0–5 cm) and deep peat (DP: 50–55 cm).

Site	Peatland type	Altitude (m)	Peat depth (mean: m)	Vegetation cover (mean %)					Number of samples (Sph, SP, DP)
				H	S	Sph	OM	BLB	
Butterburn (Butt)	Border mire	167	6.7	4.3	36.7	73.3	3.7	7.7	3, 3, 3
Kielder Head (KH)	Blanket bog	408	1.8	51.0	15.0	27.3	52.7	8.7	3, 3, 3
Whitelee -English side (WE)	Blanket bog	480	2.9	28.7	19.7	77.7	13.3	3.7	3, 3, 3
Whitelee - Scottish side (WS)	Blanket bog	567	1.6	45.7	11.3	74.3	10.0	5.0	3, 3, 3
Cray Moss least modified/post-restoration/degraded	Blanket bog	581	3.6	4.0	45.7	61.7	4.3	13.3	3, 3, 3
		571	2.2	3.0	51.7	75.0	22.7	6.3	3, 3, 3
		570	1.3	2.0	44.7	1.3	83.3	8.0	3, 3, 3
Fleet Moss least modified/post-restoration/degraded	Blanket bog	564	1.4	6.0	10.3	58.3	15.7	12.3	3, 3, 3
		552	1.8	1.0	29.3	2.0	86.3	3.0	3, 3, 3
		566	3.1	4.7	10.7	0.0	67.7	15.0	3, 3, 3
Stake Moss least modified/post-restoration/degraded	Blanket bog	561	2.6	5.0	23.3	82.7	7.0	7.0	3, 3, 3
		571	2.7	4.3	19.0	8.3	29.3	30.3	3, 3, 3
		558	2.6	0.0	1.0	0.0	20.7	78.3	3, 3, 3

(UK Grid Ref NT 685056) about 524 m a.s.l. The vegetation was dominated by *Sphagnum* moss ranging from 30 % to 96 % (mean: 76.0 %) in the sampling areas, followed by heather from 16 % to 53 % (mean: 37.2 %), with other mosses and sedge occupying from 1 % to 33 % (mean: 11.7 %) and from 6 % to 24 % (mean: 15.5 %), respectively. The soil (at sampling time) was wet (from 89.0 % to 95.0 %), with an average peat depth of 2.3 m and a slope of 4° across the sampling areas. Noticeably, the English side was grazed whilst the Scottish side was not.

The three restoration management sites (conditions reflect sampling location and time – December 2020 and information was provided by the Yorkshire Peat Partnership's (YPP) peatland restoration plan (restoration activities took place at various times during 2012–2019))

Cray Moss: is situated within the parishes of Buckden and Bishopdale in Yorkshire Dales National Park, at 54° 23′ 06″N; 2° 10 '57″W (UK Grid Ref SD 932816) about 574 m a.s.l. Cray Moss was recently managed as a small upland farm with breeding ewes and suckler cattle, which likely caused large areas of moorland to further degrade. Restoration work on Cray Moss had been carried out in 2012 and 2013 by grip and gully blocking, reprofiling of hags, and revegetation of bare peat areas, forming a series of different habitat status consisting of least modified, post-restoration, and degraded areas. The vegetation in both the least modified and post-restoration areas were dominated by *Sphagnum* moss ranging from 25 % to 95 % (mean: 62 %) and from 35 % to 95 % (mean: 75 %), respectively. By contrast, other mosses were the main vegetation in degraded areas ranging from 80 % to 85 % (mean: 83 %). Likewise, the soil was relatively wet in the least modified and post-restoration areas, with an average peat moisture (at sampling time) of 92.1 % and 90.8 %, respectively. However, it was much drier in degraded areas with a mean peat moisture of only about 83.7 %. The average peat depth was about 2.5 m with a mean slope of 1° across the sampling areas.

Fleet Moss: is located in the parish of Bainbridge in the Yorkshire Dales National Park, at 54° 24′ 71″N; 2° 20 '92″W (UK Grid Ref SD 864835) about 560 m a.s.l. Fleet Moss was recently fenced to prevent sheep grazing and managed for limited grouse shooting. Restoration work on Fleet Moss had been carried out in 2014 and 2015 by grip blocking of actively eroding grips, forming a series of different habitat status consisting of least modified, post-restoration, and degraded areas. The vegetation in the least modified areas was dominated by *Sphagnum* moss ranging from 40 % to 70 % (mean: 58.3 %). However, for both post-restoration and degraded areas, the dominant vegetation were other mosses, ranging from 75 % to 99 % (mean: 86.3 %) and from 65 % to 95 % (mean: 78.3 %) respectively. Likewise, the soil was relatively wet in the least modified and post-restoration areas, with an average peat moisture (at sampling time) of 89.0 % and 88.7 %, respectively. By contrast, it was drier in degraded areas as the mean peat moisture was about 85.7 %, with an average peat depth of about 1.9 m and a mean slope of 2° across the sampling areas.

Stake Moss: is situated in the parish of Bainbridge in Yorkshire Dales National Park, at 54° 23′ 83″N; 2° 09 '83″W (UK Grid Ref SD 936825) about 564 m a.s.l. Stake Moss was until recently managed as a grouse moor with some sheep grazing. Restoration work on Stake Moss had been carried out in 2018 and 2019 by hydrological interventions (e.g., coir, timber, and stone dams) and revegetation with plug planting and heather brash, forming a series of different habitat status consisting of least modified, post-restoration, and degraded areas. The vegetation in the least modified areas was dominated by *Sphagnum* moss ranging from 60 % to 95 % (mean: 82.7 %). In post-restoration areas, the dominant vegetation were other mosses, ranging from 8 % to 60 % (mean: 29.3 %), followed by sedge, ranging from 12 % to 30 % (mean: 19 %). Interestingly, the brash and/or litter layer in the degraded areas covered the largest area, ranging from 65 % to 80 % (mean: 75 %). Correspondingly, the peat was relatively wet in the least modified areas, with an average peat moisture (at sampling time) of 93.3 %. By contrast, it was drier in both post-restoration and degraded areas as mean peat moisture was only about 87 % and 87.1 %, respectively. The average peat depth was about 2.7 m and a mean slope of 2° across the sampling areas.

2.2. *Sampling*

Samples (nine per type, each consisting of three replicates at each site) of the dominant *Sphagnum* mosses (Liu et al., 2024), surface peat (0–5 cm) and deep peat (50–55 cm) were collected from the very wet border mire (Butt) and three near-intact sites (KH, WE, WS) (Table 1). Peat depth was measured with an extendable drainage rod system and peat cores were taken with a 1 m box corer (see Heinemeyer et al., 2019). Another 27 samples per type (i.e., three replicates per site for each management/habitat area) were collected for the dominant *Sphagnum* mosses (or other mosses if *Sphagnum* moss was absent), surface peat (0–5 cm), and deep peat (50–55 cm) samples from the three managed restoration sites (i.e., Cray Moss, Fleet Moss, and Stake Moss), each of which included the least modified, post-restoration, and degraded habitat areas (Table 1). Vegetation type and cover (as sum of over and understory; at least 100 %) were recorded at the sampling locations in a 50 cm × 50 cm quadrat and corresponding photos were taken for further verification. Some other parameters including coordinates, elevation, slope (across a line of ~10 m), aspect, peat depth, height of *Sphagnum* layer, moss layer and litter depth were also recorded in the field.

2.3. Testate amoebae processing

Testate amoebae (TA) were prepared using a modified version of the method of Booth et al. (2010). For each sample, around 5 g of fresh *Sphagnum* moss or 2 cm^3 peat was used for preparation (Liu et al., 2024). The weighted sample was soaked and disaggregated in the 100 ml beaker with about 60–80 ml deionized water for at least 24 h and stirred occasionally. The sample liquid was first sieved through a 355 μm mesh to remove plant and coarse particulate matter and the filtrate was then sieved with a 10 μm mesh to filter some of the smaller particulates which tends to make analysis easier and more efficient. The material retained in the 10 μm mesh was carefully washed into 50 ml centrifuge tubes and centrifuged at 4000 rpm for 5 min. The settled material (after centrifugation) was preserved using distilled water with 10 % ethanol in a 15 ml tube. Rose Bengal was then added to stain the cytoplasm and thus differentiate living including encysted TA (stained with pink colour) from dead (empty) individuals. TA identification (Todorov and Bankov, 2019) and counting were performed under light microscopy at 200x and 400x magnification, with a target of tallying at least 150 individuals in each sample. Some of the functional traits of TA, such as mixotrophic TA (MTA), were recorded when counting. Species body size (length, width and height) and aperture size (length and width) of each species encountered were also measured with a digital camera (OLYMPUS SC-100) and specialised software (OLYMPUS cellSens Entry) (Table S1).

2.4. Peat physicochemical analysis

Around 2 cm^3 of peat subsample from the surface layer separated in the lab were weighed first and then dried at 105 °C in an aluminum weighing dish (70 mm) for a minimum of 72 h and reweighed to determine dry bulk density and peat moisture content (by weight loss). The subsamples used for determining the total soil C and N content were oven-dried at 105 °C and finely ground using a ball mill and finally determined by an elemental analyzer (Vario MACRO cube, Elementary, Germany). The soil C:N ratios were calculated as mass ratios. Additionally, 3 ± 0.1 g of fresh wet peat sample was added to 27 ml ultra-pure water and manually homogenized for 60 s, leaving it for 4 h; the pH was then measured in the water suspension with a calibrated digital pH meter. After that, the peat solutions were filtered and stored in the dark at 4 °C. Dissolved organic carbon (DOC) and total bound nitrogen (TbN) was measured in filtered (using 0.45 μm peat pore water [Rhizon; van Walt, Netherlands] samplers) water extracted from fresh peat samples (squashed inside a plastic bag for 1 min by hand) which were then measured using a VarioTOC (Elementar Analysensysteme GmbH, Hanau, Germany) instrument. For elemental analysis, around 0.5 g of dried peat for each sample was put into a 100 ml digestion tube. The digestion tubes were then transferred to a fume cupboard, and 10 ml of 70 % AnalaR Nitric acid was carefully pipetted into each tube, leaving the samples to stand overnight for full digestion. After that, the digested peat was filtered (using filter papers) and kept in labelled 50 ml centrifuge tubes, which were then diluted with ultra-pure water by a factor of at least 2 in 15 ml centrifuge tubes. Finally, a total of 14 elements (including key environmental and ecologically informative ones) Cu, Zn, Pb, Al, Ca, Fe, K, Mg, Na, Mn, Cd, P, As and Si were measured with Inductively Coupled Plasma (ICP) analysis using an ICP-7000 (ICP-OES) instrument.

2.5. Statistical analysis

Differences and recovery patterns of TA communities in relation to restoration (i.e., degraded, post-restoration, least modified management) and habitat status (i.e., Cray Moss, Fleet Moss, Stake Moss, near-natural and the very wet natural/'intact' border mire sites) were analyzed using both multivariate community data and a number of indices. Taxa occurring in less than 4 % of total number of samples and those whose relative abundance was overall less than 4 % were removed from further quantitative analyses to minimize the effects of rare species. The species abundance data was Hellinger transformed prior to all multivariate analyses (Legendre and Gallagher, 2001). Nonmetric Multidimensional Scaling (NMDS) ordination analysis was used to compare TA assemblages among the five different grouped sites and restoration management. To test the relationship between TA communities and environmental variables, redundancy analysis (RDA) was applied together with forward selection of the important variables. The constraining variables were selected based on all the 33 available environmental variables. Relative abundance of selected TA species showing significant change following restoration were calculated to display the detailed recovery pattern. Before doing so, a series of TA wet (including intermediate) indicators and TA dry indicators were determined in accordance with their reported hydrological preferences (Tolonen et al., 1992; Charman, 1997; Bobrov et al., 1999; Booth, 2001; Lamentowicz and Mitchell, 2005; Mieczan, 2009; Mieczan and Adamczuk, 2015; Liu et al., 2019) (Table S2). To investigate diversity patterns, Shannon Diversity Index (SDI) (Keylock, 2005) and Functional diversity (Rao quadratic entropy index) were calculated for each sample (Fournier et al., 2012). Functional traits including biovolume, body length, aperture width, test type (siliceous versus others) and mixotrophy were calculated as community weighted mean value (CWM), which is an index of functional composition expressed as the mean trait value of species present in the community weighted by their relative abundances (Laliberté et al., 2014; Marcisz et al., 2016) to explore the potentially functional contribution of TA to ecosystem processes. Among them, mixotrophy was determined as the proportion of mixotrophic species within a community (Fournier et al., 2015). The biovolume (μm^3) was calculated based on geometric shapes using dimensions measured under the microscope (length or diameter, width, and height)

Hemisphere: $\text{Biovolume} = Pi * r^3 * 2/3$
Saucer-shaped: $\text{Biovolume} = Pi/2 * r^2 * h$
Cylindrical-ovoid: $\text{Biovolume} = Pi/6 * d^2 * h$
Ovoid: $\text{Biovolume} = Pi/6 L * w * h$

where r is the radius, Pi (π) is approximately 3.14159, h the height, d the diameter, L the length, and w the width of the shell (Fournier et al., 2012). The aperture width of each species was measured under a microscope until the values exhibited a normal distribution (Table S1). The significant associations for the selected indices of interest including Shannon diversity, functional diversity, biovolume, aperture width, and test type among restored sites (i.e., Cray/Fleet/Stake Moss) and status (i.e., degraded/post-restoration/least modified) were tested using two-way analysis of variance (ANOVA). For those data which did not satisfy the assumptions of ANOVA, a Kruskal-Wallis test was applied. All analyses and graph plotting were performed with core packages of vegan, FD, car, ggpubr, ggplot2 in R (R Core Team, 2024).

3. Results

3.1. Testate amoeba communities in relation to habitat status and restoration in Sphagnum mosses

3.1.1. Taxonomic differences

A total of 45 testate amoebae (TA) taxa were identified from 122 *Sphagnum* samples collected within the seven peatland sites. NMDS ordination showed a distinct separation in TA community composition between the very wet border mire and the blanket bog sites, but no obvious difference was found among the modified/degraded restoration sites (Fig. 2a). However, interestingly, two points, which in fact came from the same sample (i.e., top 3 cm and bottom 3 cm of *Sphagnum* moss, respectively) at Stake Moss aligned closely to the area of near-intact and the very wet border mire samples (Fig. 2a). Further ordination analysis based on the combination of the three restoration sites revealed a clear gradient in TA community composition among habitat status, and a marked restoration trajectory for modified sites from degraded to post-restoration and to the least modified areas (Fig. 2b). Specifically, on average, the most abundant TA taxa within *Sphagnum* moss in degraded areas included *Euglypha tuberculata* (20.8 %), *Nebela tincta* (14.6 %), *Assulina muscorum* (13.6 %) and *Corythion dubium* (12.2 %); their relative abundance decreased dramatically in other habitats (Fig. 3a). Conversely, the species *Nebela collaris-bohemica* type and *Heleopera sylvatica* were particularly abundant in other habitats compared to degraded areas although *Nebela collaris-bohemica* type was lower in near-intact and the very wet border mire sites, which were replaced by the relative increase of *Archerella flavum*, *Amphitrema wrightianum* and *Euglypha strigosa* (Fig. 3a). As for TA species diversity (Shannon's index), it was only significantly higher in the very wet border mire site compared to samples from near-intact, least modified, and degraded areas (H (4) = 18.954, $p < 0.001$) (Fig. 4a). Interestingly, no statistically significant difference was found comparing the restoration sites' habitat conditions even though the median line in post-restoration areas seemed much higher than those in both the least modified and degraded areas (Fig. 4a).

3.1.2. Functional traits

TA functional diversity was significantly higher in the very wet border mire site (Butt) than in those of all other habitat types (H (4) = 28.427, $p < 0.001$). However, no statistically significant difference was found comparing restoration effects (Fig. 4c). For specific functional traits of TA, most of them did show separation for the very wet border mire site (Fig. 5a, c, e, g), but no significant difference was observed between restoration status except for body length (H (2) = 6.4773, $p < 0.05$) (Fig. S2a). It showed an increasing trend following rewetting management, with a significant difference between post-restoration and degraded areas (W = 248, $p < 0.05$) and between the least modified and degraded areas (W = 259, $p < 0.05$). However, no statistically significant difference was found between the least modified and post-restoration areas (Fig. S2a). It is noteworthy that MTA were significantly higher in abundance in the very wet border mire compared to the other habitat types (H (4) = 54, $p < 0.001$), although few points were abnormally high at the least modified areas (Fig. 5g) reflecting at Stake Moss (Fig. S3). In addition, at Stake Moss most TA functional traits (i.e., biovolume, test type) in post-restoration areas showed a similarity to degraded areas, while at Fleet and Cray Moss these traits in post-restoration areas were more similar to the least modified areas (Fig. S3).

3.2. Testate amoeba communities in relation to habitat status and restoration in surface and deep peat

3.2.1. Taxonomic differences

A total of 47 testate amoeba taxa were identified from 67 peat samples collected within the seven peatland sites. NMDS showed a substantially different TA community composition between surface peat (0–5 cm) and deep peat (50–55 cm) (Fig. 2c). For surface peat, a distinct separation was observed be-

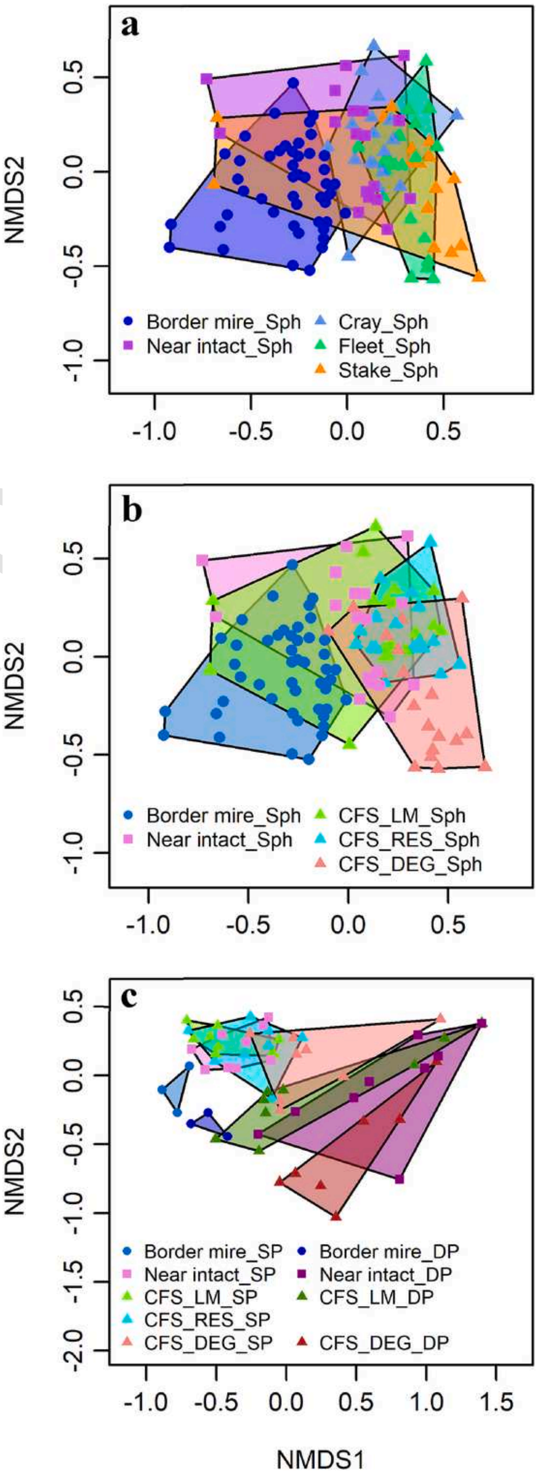

Fig. 2. NMDS analysis outputs for testate amoebae community composition in **(a)** *Sphagnum* moss (Sph) across the restoration sites (Cray Moss, Fleet Moss, and Stake Moss), **(b)** *Sphagnum* moss (**Sph**) and **(c)** surface and deep peat (**SP: 0–5 cm** and **DP: 50–55 cm**) across different habitat status consisting of least modified (**LM**), post-restoration (**RES**) and degraded (**DEG**) blanket bog areas from the restoration sites (**CFS**: combination of Cray, Fleet and Stake Moss) versus the three combined near intact sites (Kielder Head, Whitelee English/Scottish side) and the very wet border mire site Butterburn. Note: TA were not counted in CFS_RES_DP samples.

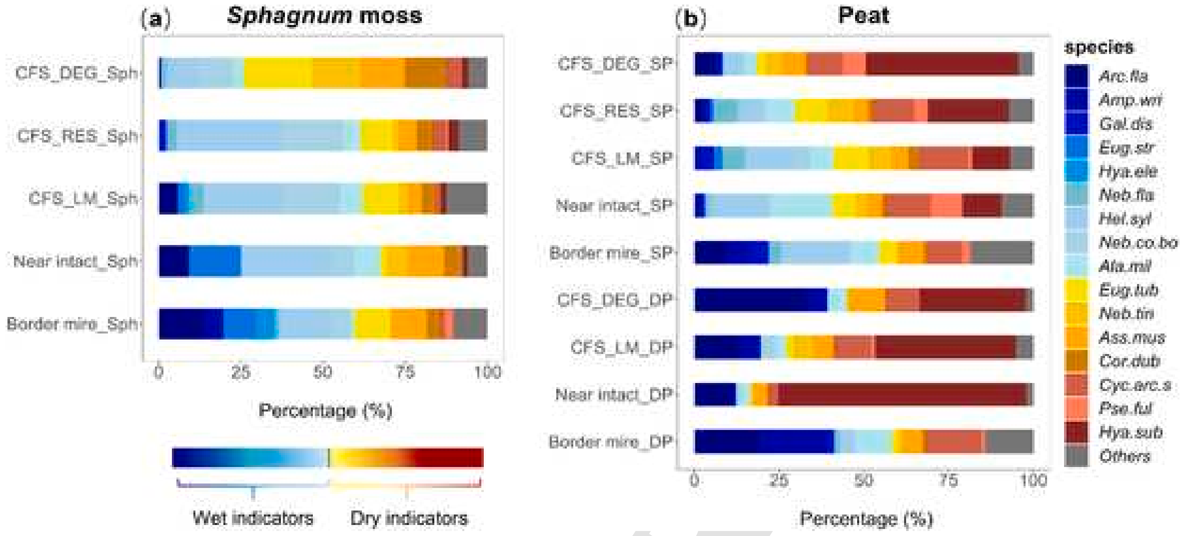

Fig. 3. Relative abundance of testate amoebae (TA) in **(a)** *Sphagnum* moss (**Sph**) and **(b)** surface and deep peat (**SP: 0–5 cm** and **DP: 50–55 cm**) at the restoration sites (**CFS**: combination of Cray, Fleet, and Stake Moss; **LM**: least modified; **RES**: post-restoration (e.g., ditch/gully-blocking); **DEG**: degraded) compared to the three combined near intact sites (Kielder Head, Whitelee English/Scottish side) and the very wet border mire site Butterburn. Species that showed significant change among habitat status and with the highest mean relative abundance of more than 5 % in either *Sphagnum* moss or peat were selected and displayed: *Arc.fla - Archerella flavum, Amp.wri - Amphitrema wrightianum, Ala.mil - Alabasta militaris, Ass.mus - Assulina muscorum, Cor.dub - Corythion dubium, Cyc.arc.s - Cyclopyxis arcelloides (small:* <50 *μm) type, Eug.stri - Euglypha strigosa, Eug.tub - Euglypha tuberculata, Gal.dis - Galeripora discoides, Hel.syl - Heleopera sylvatica, Hya.ele - Hyalosphenia elegans, Hya.sub - Hyalosphenia subflava, Neb.co.bo - Nebela collaris-bohemica type, Neb.fla - Nebela flabellulum, Neb.tin - Nebela tincta, Pse.ful - Pseudodifflugia fulva, Others* - all combined remaining species. The colour scheme indicates wet (blue) to dry (reddish brown) TA indicator species and the black dashed line between cyan and yellow represents the threshold between wet (including intermediate) and dry indicator species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

tween the very wet border mire and other habitat sites. Likewise, a clear recovery trajectory was found for modified sites from degraded to post-restoration and to the least modified areas (Fig. 2c). Among the 33 environmental variables, peat moisture (WC: water content), heather cover and phosphorus (P) concentration best explained the variation in TA community composition in peat (Fig. 6, Fig. S4). The primary and secondary compositional gradients, as revealed by axis 1 and axis 2 of the RDA ordination, represented 36.3 % and 15.6 % of the variability in the data set respectively (Fig. 6). In comparison, a near similarity was shown for the TA communities between the near-intact, least modified and degraded deep peat whereas they were considerably different from that in the very wet border mire (Fig. 2c and 3b). In detail, the dominant taxa of TA in degraded areas from surface peat were *Hyalosphenia subflava* (45.1 %), *Cyclopyxis arcelloides* (10.5 %), *Assulina muscorum* (6.9 %), *Pseudodifflugia fulva* (6.7 %), and *Heleopera sylvatica* (5.4 %); interestingly, *Archerella flavum* (6.2 %) commonly indicative of wet habitat was also found abundant in degraded areas (Fig. 3b). Among them, the *Hyalosphenia subflava* showed the most sensitive response to restoration management and habitat status. Their relative abundance declined progressively from degraded (45.1 %) to least modified areas (11.2 %) and even disappeared in the very wet border mire (Fig. 3b). Meanwhile, a clear decreasing trend was observed for *Pseudodifflugia fulva* in line with progressive restoration (Fig. 3b). By comparison, *Heleopera sylvatica* showed a consistent increasing trend from degraded (5.4 %) to least modified (14.7 %) and to the very wet border mire (19.8 %). While an apparent increasing trend over the course of restoration was also observed for few other species like *Galeripora discoides*, *Euglypha strigosa* and *Nebela collaris-bohemica* type, their relative abundance was much lower than that of *Heleopera sylvatica* (Fig. 3b).

As for the deep peat samples, the most abundant taxa included *Hyalosphenia subflava* and *Archerella flavum* in the near-intact, least modified, and degraded areas (Fig. 3b). Apart from *Archerella flavum*, *Amphitrema wrightianum* and *Alabasta militaris* dominated the very wet border mire but no *Hyalosphenia subflava* was found (Fig. 3b). Noticeably, the relative abundance of MTA including *Archerella flavum* and *Amphitrema wrightianum* in degraded areas (39.3 %) was very similar to the very wet border mire (39.1 %) which were both much higher than in the least modified (19.5 %) and near-intact (12.1 %) areas (Fig. 3b). TA species diversity showed a clear increasing trend from the driest to wettest habitat, within which it was particularly pronounced over the course of restoration, with TA diversity being significantly higher in the least modified areas than in degraded areas ($W = 78, p < 0.001$) (Fig. 4b).

3.2.2. *Functional traits*

No significant difference was found in TA functional diversity in surface peat over the course of restoration although there was an increasing trend from degraded to the least modified areas (Fig. 4d). However, TA functional diversity was shown to be significantly higher in the very wet border mire than in near-intact areas ($W = 27, p < 0.01$) and least modified areas ($W = 26, p < 0.05$) (Fig. 4d). Surprisingly, all the functional traits of TA indicated sensitivity to restoration in surface peat, but interestingly, most functional traits in near-intact and the very wet border mire areas were much lower than in the least modified areas (Fig. 5). Generally, a clear increasing trend was observed for biovolume, aperture width and siliceous shell over the course of restoration, all being significantly higher in the least modified areas compared to the degraded areas (Fig. 5b, d, f). By comparison, mixotrophic TA was particularly abundant in the very wet border mire although a few points were abnormally high in degraded areas (Fig. 5h). It is worth noting that an obvious similarity of most TA functional traits (i.e., biovolume,

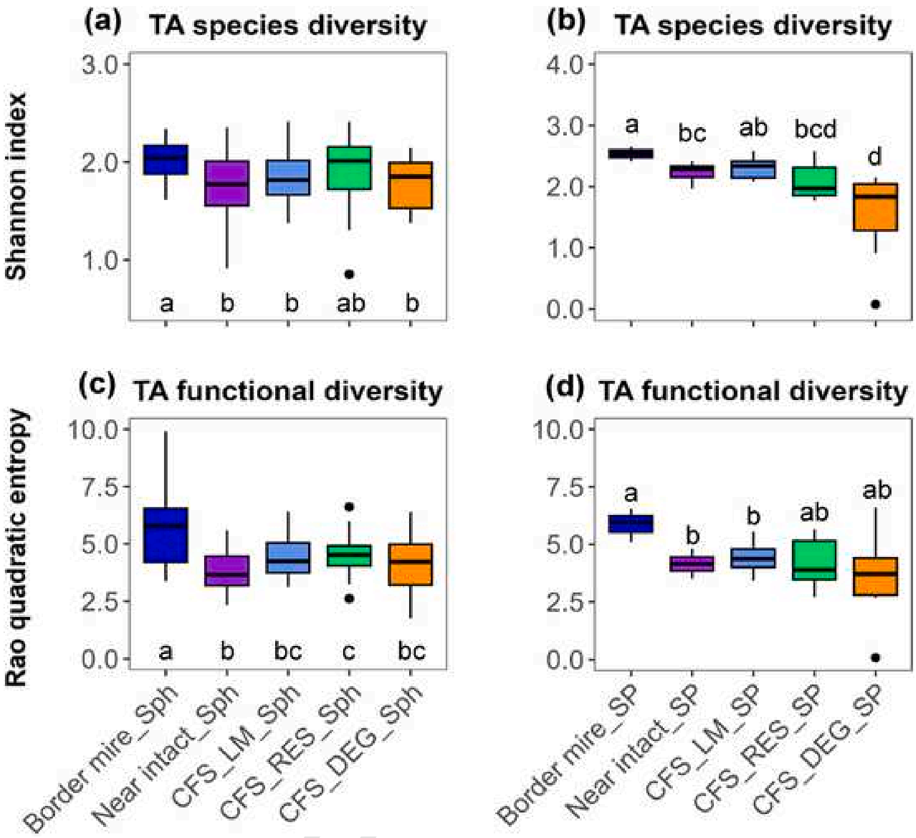

Fig. 4. Boxplots showing testate amoebae (TA) species diversity and functional diversity (as measured by the Rao quadratic entropy) at restoration sites (**CFS**: combination of Cray, Fleet, and Stake Moss; **LM**: least modified; **RES**: post-restoration (e.g., ditch/gully-blocking); **DEG**: degraded) compared to the three combined near intact sites (Kielder Head, Whitelee English/Scottish side) and the very wet border mire site Butterburn. (a) and (c) represent TA communities from *Sphagnum* mosses (**Sph**: combination of top 3 cm and bottom 3 cm segment), whereas (b) and (d) from corresponding surface peat (**SP**: 0–5 cm). Box shows the median (middle line), 25 % (bottom border) and 75 % (top border) ranges, with points representing outliers. Different letters indicate significant difference of the medians among habitat status (Kruskal-Wallis test, $p < 0.05$).

aperture width, test type) was found between post-restoration areas and degraded areas in Stake Moss, whereas these traits in post-restoration areas showed more resemblance to the least modified areas in both Fleet and Cray Moss (Fig. S5).

4. Discussion

Both *Sphagnum* moss and surface peat data suggest a clear recovery trajectory of TA community composition following restoration management (Fig. 2b and c). This is in line with the first aspect of the first hypothesis: *restoration of degraded blanket bog peatlands by rewetting and regeneration shifts TA community composition, taxonomic and functional diversity towards that found in undisturbed and wetter 'near-natural/intact' conditions over time*. It is not surprising as hydrology (either expressed as water table depth or soil moisture) and vegetation composition, especially the development of *Sphagnum* moss, have long been found to be the main determinants on TA's establishment and development (Mitchell et al., 1999; Creevy et al., 2018). This was also corroborated by the results from RDA analysis (Fig. 6), with both peat moisture and *Sphagnum* moss cover having increased considerably over the course of restoration. The resultant more diverse microhabitats were likely to be conducive to a broader colonization of certain species such as *Archerella flavum* whose relative abundance increased together with wetness. The second hypothesis was therefore also supported: *TA community composition is strongly affected by soil moisture and Sphagnum moss cover, and to a lesser extent by peat physical, chemical, and other environmental factors*. Undoubtedly, the distinct separation of TA community composition between the very wet border mire and other habitat status is to be expected due to the significant difference in the sites' ecohydrology (i.e., overall wetness) (e.g., Glatzel et al., 2023), which is also reflected in very high peat accumulation as seen in the vastly deeper mire site peat depth – unlike in a typical hill blanket bog. Previous studies have shown that a big difference of TA community may be observed between microhabitats even within small areas (20 × 20 cm quadrat), which suggests a high relevance of TA colonization and development to the ecological characteristics of the exact spot where they live (Mitchell et al., 2000). This likely explains why TA communities in the two wet samples (*Sphagnum* mosses with high moisture) from the near-intact (WE) and least modified areas (Stake Moss) closely resembled those of the very wet border mire site (Fig. 2a).

While both TA species diversity and functional diversity showed a similar recovery trajectory, their change pattern was to some degree different between *Sphagnum* and surface peat samples, particularly in post-restoration areas (Fig. 4). This may be related to the degree of degradation, stage of restoration and historical environmental characteristics. Studies have reported that species recovery following disturbance is mainly controlled by the creation of environmental conditions which is conducive to the establishment of new taxa (Creevy et al., 2018). In the managed restoration sites, the previous ditch establishment

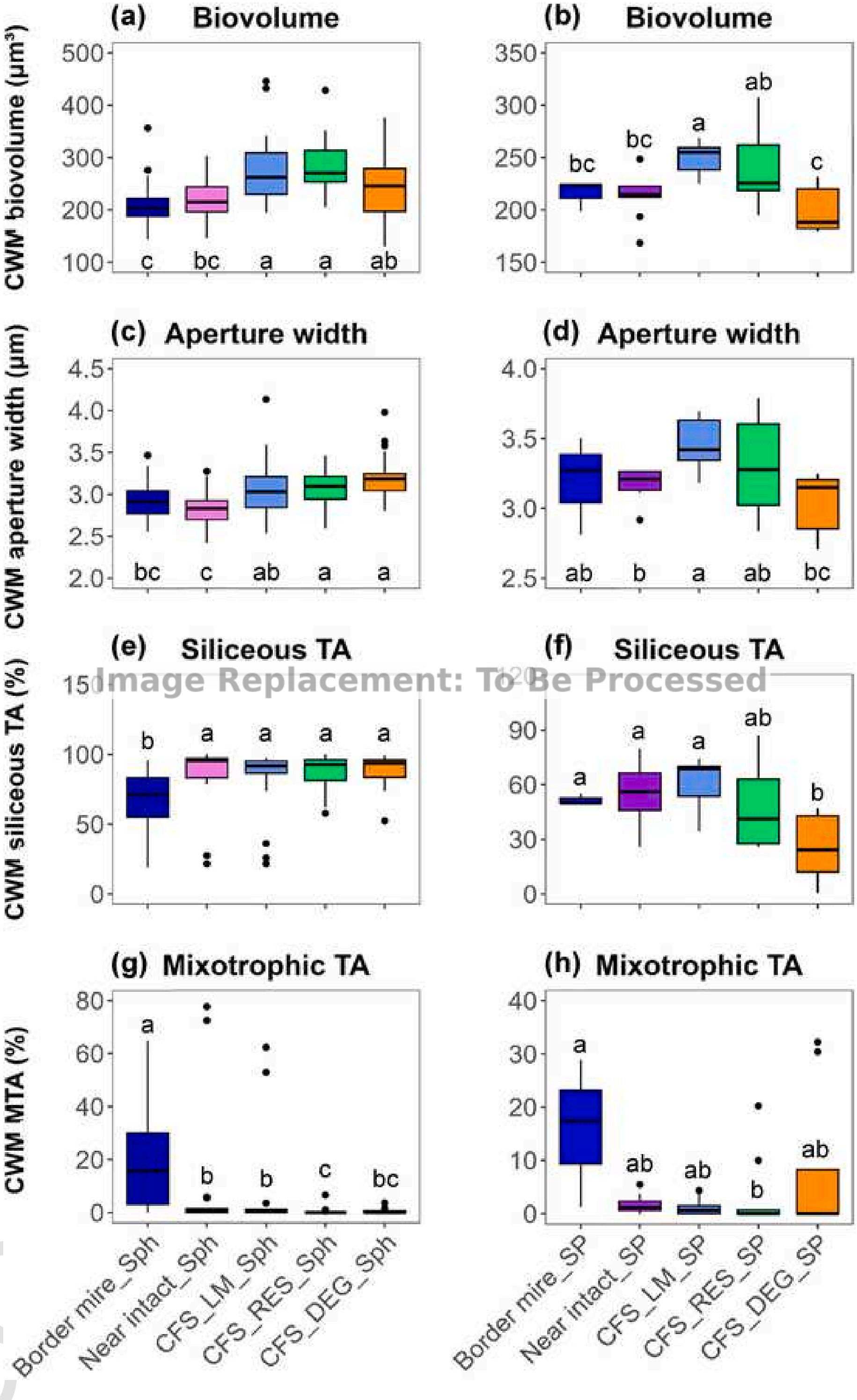

Fig. 5. Boxplots showing testate amoebae (TA) functional traits (**CWM**: community weighted mean value of traits) change pattern for samples taken from *Sphagnum* moss (**Sph**; **a**: biovolume, **c**: aperture width, **e**: siliceous TA, **g**: mixotrophic TA) and surface peat (**SP**: 0–5 cm; **b**: biovolume, **d**: aperture width, **f**: siliceous TA, **h**: mixotrophic TA) at restoration sites (**CFS**: combination of Cray, Fleet, and Stake Moss; **LM**: least modified; **RES**: post-restoration (e.g., ditch/gully-blocking); **DEG**: degraded) compared to the three combined near intact sites (Kielder Head, Whitelee English/Scottish side) and the very wet border mire site Butterburn. Boxes show the median (middle line), 25 % (bottom border) and 75 % (top border) ranges, with points representing outliers. Different letters indicate significant difference of the medians among habitat status (Kruskal-Wallis test, $p < 0.05$).

(mainly for increased sheep grazing) lowered water tables and very likely reduced biodiversity. However, it is probable that the ecosystem became more diverse compared to the least modified areas over the course of restoration (niche diversity), which likely led to slightly higher TA species and functional diversity in post-restoration areas for *Sphagnum* moss. Notably, this was not the case for surface peat as TA communities counted in this study included many empty (dead) shells which

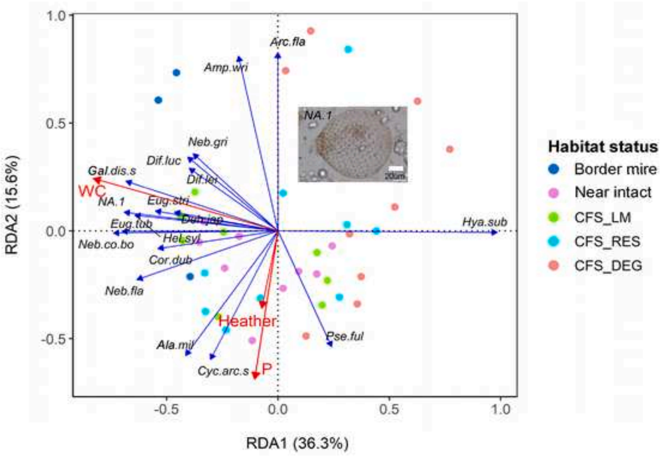

Fig. 6. Redundancy analysis (RDA) showing the relationship among habitat status, testate amoebae (TA) in surface peat and key selected (based on forward selection) environmental variables (i.e., WC: water content, Heather: *Heather* cover, P: phosphorus). **CFS**: combination of Cray, Fleet, and Stake Moss; **LM**: least modified; **RES**: post-restoration (e.g., ditch/gully-blocking); **DEG**: degraded. RDA axis 1 explains 36.3 % of total variation of TA community whereas axis 2 explains 15.6 % of the variance. Species that showed significant change ($p < 0.05$) were selected and displayed. Species codes: *Ala.mil - Alabasta militaris, Arc.fla - Archerella flavum, Amp.wri - Amphitrema wrightianum, Ass.mus - Assulina muscorum, Cor.dub - Corythion dubium, Cry.arc.s - Cryclopyxis arcelloides small type (*< 50 μm*), Dif.luc - Difflugia lucida, Eug.stri - Euglypha strigosa, Eug.tub - Euglypha tuberculata, Gal.dis.s - Galeripora discoides type, Hel.syl - Heleopera sylvatica, Hya.sub - Hyalosphenia subflava, Neb.co.bo - Nebela collaris-bohemica type, Neb.fla - Nebela flabellulum, Neb.tin - Nebela tincta, Pse.ful - Pseudodifflugia fulva type.* ***NA.1*** - it is likely to be *Assulina seminulum* but the aperture did not show any crenulated margins.

were probably more likely affected by historical environment/management conditions (before the onset of restoration), thereby compromising and decreasing the observable, direct effect from current restoration management. The remaining part of the first hypothesis in relation to taxonomic and functional diversity were therefore partially but well supported (i.e., *diversity shifts from degraded towards intact habitats*).

Previous studies have shown that TA could be used in monitoring the recovery success of degraded peatland following restoration by investigating either the core peat (Buttler et al., 1996; Jauhiainen, 2002; Davis and Wilkinson, 2004; Valentine et al., 2013) or surface mosses samples (Swindles et al., 2016; Creevy et al., 2018; Evans et al., 2024). This was also demonstrated by the present study, with taxa usually indicative of dry habitat such as *Hyalosphenia subflava*, *Nebela tincta*, *Assullina muscorum and Corythion dubium* being particularly abundant in degraded areas whereas species often served as wet indicators like *Archerella flavum*, *Amphitrema wrightianum* and *Heleopera sylvatica* were more plentiful in near-intact and the very wet border mire. But interestingly, the dominant dry indicator species were completely different between *Sphagnum* moss and surface peat. There are probably two underlying reasons. Firstly, it may be partly due to the preservation issues of TA stored in surface peat in blanket bog. Because of the wider range in water tables and/or soil moisture in hill blanket bogs compared to raised bogs (Howson et al., 2021; Glatzel et al., 2023), it may lead to a faster decomposition rate of easily decomposable TA shells in blanket bog peat. The decomposition rate was very likely further exacerbated by peatland management practices such as drainage. As most dry indicators (e.g., *Nebela tincta and Corythion dubium*) found in *Sphagnum* moss are idiosomic type whose tests formed by secreted siliceous plates, which have been reported to be fragile and easier to break down under adverse conditions such as desiccation compared to agglutinated and most of the secreted proteinaceous (including chitinous) shells, they were therefore rarely observed in surface peat. This was also probably the reason for the opposite change pattern of *Euglypha tuberculata* (siliceous plates) between *Sphagnum* moss and surface peat over the course of restoration, with its relative abundance extremely low in surface peat within degraded areas. By contrast, the corresponding dry indicators of *Pseudodifflugia fulva* (with agglutinated test) and *Hyalosphenia subflava* (with chitinous shell; Fig. S6) preserved relatively well in surface peat and were found abundantly in degraded areas. On the other hand, it may be highly related to the living environment (e.g., temperature, oxygen, living space, and suitable materials for test construction) and available food sources (e.g., more microbes and detritus in peat) as there is an apparent difference of them between *Sphagnum* moss and surface peat. As indicated above, dry indicators of TA with siliceous tests were mainly found in *Sphagnum* moss, whereas those with agglutinated and proteinaceous tests dominated the surface peat in degraded areas. It was not surprising as small particles crucial for agglutinated tests were mainly distributed in the peat. While the content of soluble silica, the raw material for siliceous tests in *Sphagnum* moss, was probably similar to that in surface peat, the higher temperature and greater oxygen availability around *Sphagnum* moss may promote the formation of siliceous plates. Besides, the spacious living space (water film) in *Sphagnum* moss was likely to be the reason for the colonization of larger dry indicators like *Nebela tincta*. Conversely, limited space (i.e., smaller pore size) in surface peat in degraded areas (with the shrinkage of peat) may be more suitable for smaller species.

Recent studies on TA functional traits suggest that TA potentially play an important role in carbon and nutrient cycling (Fournier et al., 2012; Jassey et al., 2015; Payne et al., 2016; Creevy et al., 2018). For example, Jassey et al. (2015) reported that over 70 % of total TA biomass was MTA in a raised bog and 13 % reduction of carbon dioxide fixation of the entire *Sphagnum* moss resulted from increased temperature was caused by the 50 % decrease of mixotrophic TA (i.e., *Archerella flavum*, *Heleopera sphagni*, and *Hyalosphenia papilio*). Nevertheless, no large proportion of MTA was found in the present study sites, even in the very wet border mire, where MTA (i.e., *Archerella flavum* and *Amphitrema wrightianum*) occupied only nearly 20 % of total TA abundance. Neither were any *Heleopera sphagni* observed, and only a few numbers of *Hyalosphenia papilio* were found in three samples. This is more likely due to the limited moisture content of *Sphagnum* moss rather than a lack of light, which has been observed as an indispensable condition for MTA survival (Schönborn, 1965) as samples were collected in relatively open areas without the shade of heather at the very wet border mire. However, interestingly, abundant MTA was found in one post-restoration plot and two degraded areas (where MTA were mostly dead) in surface peat (Fig. 5h). This was very likely to be explained by the removal/erosion of surface peat in these areas by external stressors causing degradation such as strong wind, flooding, and sheep trampling. TA counted in the surface peat could then, in fact, be derived from deeper peat that experienced wetter historical conditions rather than reflecting the current surface peat conditions.

The biovolume and aperture size of TA have been reported to be highly related to the decomposition of organic matter by feeding on microbes (e.g., bacteria, fungi) (Fournier et al., 2015; Jassey et al., 2016; Koenig et al., 2018b). Previous studies indicated that small species (e.g., *Assullina muscorum*, *Corythion dubium and Trinema lineare*) are easier to colonize a new habitat following disturbance resulting in drought than large species (Krashevska et al., 2016; Koenig et al., 2018a). This was corroborated by this study from surface peat, with small species, expressed by both small biovolume (including body size) and aperture size being particularly abundant in the driest degraded areas. Both values increased significantly over the course of restoration, reaching their peak in the least modified areas (Fig. 5b, and d). However, this change pattern was not found in

Sphagnum moss. No statistically significant increase was shown in the least modified areas although the biovolume was lower in degraded areas. More interestingly, the aperture size in degraded areas was a bit higher than that in post-restoration and the least modified areas (Fig. 5c). This was probably related to the sub-fossil taxa counted in surface peat (0–5 cm), the abnormally abundant small mixotrophic TA (*Archerella flavum*) in *Sphagnum* moss within one of the least modified plots, and the relatively higher amount of *Nebela tincta* (with large aperture size) in degraded areas from Stake Moss. As most TA can preserve well after death under favorable (waterlogged) conditions, the observed TA in surface peat reflected not only the effects of current management but also the historical conditions (maybe over the past fifty years) to some extent, which was more marked in post-restoration and the least modified areas than in degraded areas due to their better preservation conditions. Due to the replacement of TA with relatively larger aperture size by large numbers of smaller MTA (with small aperture size) in *Sphagnum* moss within one of the least modified plots from Stake Moss, the average aperture size declined dramatically in the least modified areas. This was likely also the reason for the relatively lower values of biovolume and aperture size of TA in near-intact and the very wet border mire sites for both *Sphagnum* moss and surface peat samples. Likewise, a higher proportion of *Nebela tincta* with large aperture size in degraded areas markedly increased the overall aperture size. Caution is therefore needed when linking TA biovolume/aperture size to habitat wetness, especially in the presence of large numbers of small MTA. Additionally, it is noted that the proportion of TA with siliceous shells was much less in surface peat than in *Sphagnum* moss. Despite a significant increase (from around 30 %–60 %) in surface peat during the restoration process, their relative abundance in *Sphagnum* moss was consistently higher than 90 %. This difference may indicate the substantial contribution of TA to silica cycling throughout the restoration. According to the discussion above in relation to TA functional traits, we could partly support the third hypothesis: *TA show species-specific sensitivity to rewetting strategies, and restoration management drives functional traits of TA change towards that similar as in near-natural/intact conditions, but their recover pattern is more pronounced in Sphagnum mosses than in surface peat.*

Although the combined data demonstrated the effectiveness of restoration strategies over a timescale of 2–9 years, one might also be interested in how fast the ecosystem could recover to a near-intact status. Swindles et al. (2016) showed a clear response of certain species of TA, especially those indicative of wet habitats (e.g., *Amphitrema stenostoma*, *A. flavum*, *Arcella discoides* type, *Diffugia bacillifera* and *Diffugia bacillarium*) to blanket bog restoration by investigating TA communities at 63, 234 and 771 days, respectively, after ditch blocking and reprofiling. The other study investigating the recovery of TA following peatland forest-to-bog restoration (Creevy et al., 2018) found that TA communities in post-restoration sites seventeen years after restoration management were still more similar to forested counterparts than to open bog areas. This was partly consistent with our results as TA communities showed a significant yet complex response during the first two years of restoration, while community composition and functional traits remained similar to those in degraded areas. However, six years post restoration made both community composition and functional traits of TA resemble that of least modified areas, and they showed greater similarity between post-restoration and the least modified areas for both *Sphagnum* moss and surface peat eight years after restoration (Figs. S3 and 5). This timescale aligns with findings from microbial studies on raised bog restoration, which reported that more than ten years is needed for a full recovery or to become similar to that in near-intact conditions (Andersen et al., 2013a, 2013b). Another microbial study suggested that more than twenty-five years are needed to recover a severely degraded blanket bog with bare peat to an ideal status (Elliott et al., 2015). These studies may indicate that a timescale of at least ten to twenty-five years of active restoration is needed for TA to fully recover both taxonomically and functionally in degraded blanket bog following drainage and peat erosion.

Despite the successful and widespread application of TA for palaeohydrological reconstruction in ombrotrophic peatland ecosystems (raised bog) due to their sensitivity to moisture change and assumed well-preserved traits after death (Woodland et al., 1998; Charman et al., 2006; Swindles et al., 2014; Liu et al., 2019; Bysouth and Finkelstein, 2021), their application in blanket bogs should be applied more cautiously as this study indicated an apparently dramatic decomposition rate (i.e., loss) of most TA taxa, particularly those with siliceous plates even in near-intact sites. It is noted that a large proportion of MTA (i.e., *Archerella flavum* and *Amphitrema wrightianum*) were found in deep peat layers at depths of 50–55 cm (which should reflect and reveal peat conditions prior to management intensification (i.e., at 50 cm depth estimated about 800 years before present (McCarroll et al., 2017)) and very likely outside the influence of recent water table reductions due to drainage). This does not mean they can survive in such a niche without light and enough oxygen, but they can be well preserved even in unfavorable conditions due to their chitinous shell materials. While the deep peat samples proved not good enough to serve as a pre-management intensification reference in relation to TA-derived wetness conditions due to the disproportional preservation of TA in different habitat status, the apparently high abundance of MTA in deep peat samples from managed sites, to a large extent, indicated relatively wet historical conditions (pre-management intensification). Thus, we have to overall reject the fourth hypothesis that: *TA communities present in blanket bog deep peat layers can serve as valuable bioindicators to infer and compare historical conditions and thus aid interpretation of comparability of generic site conditions*. In addition, the large amount of *Hyalosphenia subflava*, some of which were alive when sampling, in the deep peat and degraded surface peat may suggest that they are able to directly decompose organic matter at depth as peat detritus was observed within the body of many individuals, especially in deep peat samples (Fig. S6). However, it is also plausible that they consume associated microbes (e.g., bacteria) while indirectly feeding on humus particles.

The findings overall suggest that the application of TA as hydrological bioindicators near the peat surface is effective and promising in informing blanket bog habitat status and restoration progression. However, it is important to note that the inferred hydrological conditions can only provide limited information about the overall functional status of the habitat, especially when considering deeper peat samples. Further, little is known about the quantitative relationship between TA community composition (or specific species/groups) and habitat hydrological conditions (WTD or substrate moisture) and their linkages to ecosystem carbon functioning (carbon sink or source). While TA communities in post-restoration areas showed high similarity to those in the least modified areas, we still lack a clear and quantitative understanding of the extent of restoration success and the time required to fully restore bog functions. Moreover, it should be cautioned against directly comparing hill blanket bog recovery trajectories toward that of naturally much wetter topogenous bogs. As for the potential contribution of TA to ecosystem processes, there is currently a significant knowledge gap regarding TA feeding habits, which greatly hampers our understanding of their ecosystem functions, especially in terms of their direct impacts. Most studies are still based on inferring the relevance of key functional traits (body or aperture size) to carbon and nutrient cycling proxies (e.g., DOC, DON) from a microbial food web perspective. Finally, considering the substantial presence of dead TA within the top 5 cm peat layer, future studies could benefit from sampling the top 1 or 2 cm of peat for a more precise and accurate assessment of the impacts of peatland restoration work (ditch/gully blocking) on TA communities.

5. Conclusions

This study has shown a distinct separation of TA communities between the very wet border mire and modified blanket bogs, and that restoration management has shifted the TA community composition, taxonomic and functional diversity towards that similar to near-intact conditions. However, comparing peatland sites with large natural ecohydrological differences (i.e., topogenous bog versus hill blanket bog) should be used cautiously as a direct indication of expected habitat status or recovery. The significant response of some species, such as those indicative of dry habitats (e.g., *Hyalosphenia subflava*, *Assullina muscorum and Corythion dubium*) and those indicative of wet habitats (e.g., *Archerella flavum* and *Amphitrema wrightianum*) highlighted the potential of TA being used as hydrological bioindicators to monitor peatland restoration success. By contrast, the distinctive response pattern of functional traits of TA between *Sphagnum* moss and surface peat may suggest different functional roles they play, with TA in *Sphagnum* moss being more important in carbon uptake in waterlogged habitats, while TA in surface peat being highly related to peat decomposition and silica cycling in drier environments. In general, a timescale of ten to twenty-five years of active restoration may be needed for full recovery of TA communities and blanket bog functions, especially on sites following severe drainage and peat erosion. Additionally, the observed large proportion of *Hyalosphenia subflava* in surface and deep peat may indicate its strong relationship to carbon cycling. Apart from feeding on microbes, they may be actively involved in peat decomposition. However, uncertainties about differential preservation of TA taxa due to higher ranges in water tables in blanket bogs raise questions about using TA from deeper peat layers to infer and compare historic conditions. Overall, this study quantified the sensitivity of TA to restoration strategies of degraded peatlands and clearly demonstrates the link between community composition and ecosystem service provision (water/carbon), and the effectiveness of restoration work.

CRediT authorship contribution statement

Bing Liu: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andreas Heinemeyer:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Robert Marchant:** Writing – review & editing, Supervision. **Robert TE. Mills:** Writing – review & editing, Supervision.

Data availability

Data will be made available on reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

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Appendix A. Supplementary data

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