

**Exploring testate amoebae as bioindicators  
in UK upland peatlands: habitat status,  
vegetation management, peatland  
restoration and ecosystem functioning**

**Bing Liu**

**PhD**

**University of York**

**Environment and Geography**

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## Abstract

Many UK blanket bogs are modified and often classified as being in a degraded state due to various management regimes including drainage and potentially also heather burning. While many restoration efforts (e.g., ditch-blocking, alternative heather cutting) are tried to restore these peatlands, we still lack monitoring tools to assess if, when and over what time such interventions achieve restoration goals towards improved ecology of the bog and associated functions. Testate amoebae (TA), as known bioindicators, could provide such a monitoring tool. TA communities were investigated at a series of modified blanket bog peatland sites under different heather management regimes, across blanket bog restoration sites and compared to near intact peatland communities to 1) investigate the optimal sampling strategies of TA for indicating site/habitat wetness condition, 2) assess the potential of TA as hydrological bioindicators to monitor the restoration trajectory and success, 3) explore their possible functional contribution to ecosystem processes.

TA communities from *Sphagnum* mosses were shown to best reflect the wetness gradient among sites. TA species richness and density reached their highest value in either late autumn or early winter. Clear vertical separation of TA communities (e.g., living status, specific species) was observed along *Sphagnum* moss sections.

TA communities showed species-specific sensitivity to hydrological changes, with species indicative of dry habitat (e.g., *Hyalosphenia subflava*, *Corythion dubium*) being particularly abundant in the degraded and uncut peatland areas, while those representing wet habitat (e.g., *Amphitrema flavum*, *A. wrightianum*) being more common in the near intact sites.

TA key functional traits (e.g., body and aperture size) showed significant responses to management interventions. The distinctive change in TA community between living *Sphagnum* and peat may suggest different functional roles they play, with mixotrophic TA in *Sphagnum* being more important in carbon fixation in very wet habitats, while heterotrophic TA communities in peat are crucial in decomposition in drier habitats.

Overall, this study highlights the potential of TA as hydrological bioindicators to monitor peatland recovery, and also clearly demonstrates the link between biodiversity, management, and likely inferred ecosystem functioning of peatland ecosystems.

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# List of Abbreviations

ANOVA: Analysis of Variance

BD: bulk density

BM: border mire

Butt: Butterburn

CFS: combination of Cray Moss, Fleet Moss and Stake Moss peatland sites

CWM: community weighted mean value

DEG: degraded

DOC: dissolved organic carbon

DP: deep peat (50-55 cm) sample section

ETs: effect traits

FI: prescribed burning (fire)

FTs: functional traits

HTA: heterotrophic testate amoebae

Ind.: individuals

KH: Kielder Head peatland site

LB: mowing with brash left

LM: least modified

Moss: Mossdale

MTA: mixotrophic testate amoebae

NMDS: Nonmetric Multidimensional Scaling



NI: near intact

Nidd: Nidderdale

NMW: combination of Nidderdale, Mossdale and Whitendale peatland sites

OM: organic matter

RES: restored

RDA: Redundancy Analysis

RTs: response traits

SDI: Shannon Diversity Index

SOC: Soil organic carbon

SP: surface peat (0-5 cm) sample section

Sph: *Sphagnum* moss

SSU rDNA: Small Subunit ribosomal DNA

TA: testate amoebae

Uncut: no heather management

WC: water content

WE: Whitelee (English side) peatland site

WS: Whitelee (Scottish side) peatland site

WTD: water table depth

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## **Declaration**

I declare that this thesis is a presentation of original work, and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as references.

Specifically, I and my supervisor (Andreas Heinemeyer) conceived the project. Andreas found and contacted the sites, and both of us did the fieldwork with the help of Jenny Sharman (from the Yorkshire Peat Partnership) and my wife (Mengya Wang). I did the lab work, data analyses and wrote the chapters contained in this thesis. All my supervisors helped me with the improvement of language and contents, which mainly came from Andreas.

# Chapter 1: Introduction and literature review

## 1.1 Introduction

### 1.1.1 Research context

Peatlands represent a significant terrestrial carbon pool, storing about 30% of global soil organic carbon (SOC) even though covering only 3% of the world's land surface (Yu et al. 2011). Peatlands therefore have the potential to affect global climate change through their role as either sink or source of atmospheric carbon dioxide and as source of atmospheric methane (Frolking et al. 2006, Turner and Swindles 2012). In the UK, blanket bogs are one of the most important peatland types, which store an estimated 2,300 Mt of SOC (Billett et al. 2010), offer important drinking water recourses, whilst also providing diverse wetland habitats for many upland plant and bird species (Carroll et al. 2015, Heinemeyer et al. 2019b). However, many of them (about 87% in England) are classified as being in a degraded status (Pike 2021) due to a series of historical and current management practices (Jones 2023). Among them, prescribed burning, a practice of producing a diverse vegetation structure and young heather (*Calluna vulgaris*) shoots to promote the population of red grouse, has been increasingly claimed to degrade peatlands with potential detrimental impacts on many of the peatland ecosystem services such as water storage, drinking water quality, flood prevention and carbon storage (Holden et al. 2012, Clay et al. 2015, Holden et al. 2015, Pereira et al. 2021). However, the evidence base on the claimed impacts of prescribed fire are not clear (Davies et al. 2016, Harper et al. 2018). Nonetheless, some alternative heather management strategies have been promoted by policy makers and scientists in recent years, with cutting (heather mowing) being one of the alternative managements to facilitate restoration on heather-dominated peatlands (Heinemeyer et al. 2019b, Morton and Heinemeyer 2019). Noticeably, impacts from cutting, often done with heavy and large machinery, are even less known than for burning (Harper et al. 2018, Ashby and Heinemeyer 2021), yet negative impacts and trade-offs are also likely (Heinemeyer et al. 2023). It is therefore of paramount importance to assess and monitor the peatland conditions following these different management strategies to provide evidence toward informed and unbiased peatland management and restoration recommendations for policy makers. Apart from prescribed burning, drainage ditches have been dug in a

number of British upland blanket bogs to improve the production of livestock (mainly for grazing and with considerable government financial incentives) during the past century (Evans 1998, Sansom 1999, Britton et al. 2017, Alday et al. 2022). Together with large stocking densities, such management practices have put more than 70% of the heather moorland in England and Wales at risk from overgrazing and almost half of the upland breeding bird species were under threat (Sansom 1999). Notably, extensive regions were modified, 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), which pose a great threat to carbon fluxes and water quality (Holden et al. 2006, Armstrong et al. 2010). Consequently, to restore the degraded upland blanket bog peatlands towards hydrological and ecological functioning ecosystems, in addition to alternative heather management, a range of restoration strategies including reduced levels of grazing and ditch/gully-blocking have been developed and deployed over the past decades, in an attempt to improve biodiversity, crucial ecosystem services and their benefits to society (Wilson et al. 2010, Rosenburgh 2015, Shuttleworth et al. 2019, Watts 2020, Chapman et al. 2022). Surprisingly, little is known on how rewetting strategies affect ecosystem hydrological and ecological functions (Ashby and Heinemeyer 2021). It is therefore crucial to assess and monitor peatland condition and determine the extent of restoration success towards intact and functioning bogs following alternative vegetation management and peatland restoration.

### **1.1.2 What are testate amoebae?**

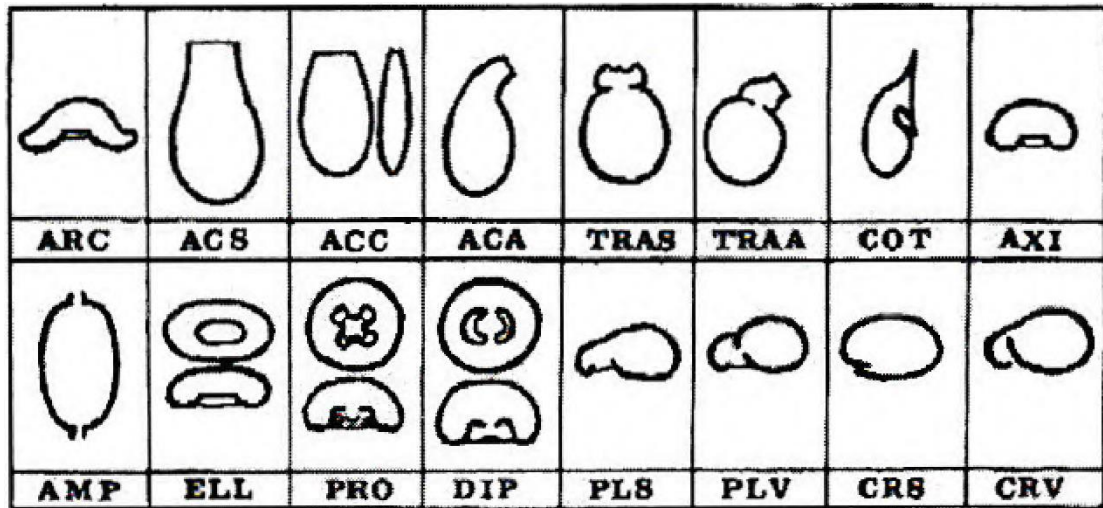
Testate amoeboid organisms, otherwise called testate amoebae (TA), are unicellular protists, which are commonly found in many moist or aquatic habitats such as mosses, soils (particularly peat), lakes and rivers but also occur in estuarine environments (Ogden and Hedley 1980, Meisterfeld 2002b). They are small (mostly between 20 to 200  $\mu\text{m}$  – approximately the size of pollen grain), abundant (1,000 -10,000 individuals  $\text{g}^{-1}$  dry weight peat, occupying about 30% micro-biomass of peatland), and diverse (over 2,500 species described to date, and usually between 10 and 30 species in any given sample) (Mitchell et al. 2008a, Qin et al. 2013). They are characterized by decay-resistant tests or shells covering the entire body, forming either from proteinaceous, calcareous, or siliceous material and some of them also build agglutinated shells by gluing together organic or mineral particles from their surrounding environment

(Tolonen et al. 1992, Mitchell et al. 2008a). Thus, many of them could be well-preserved in fossil peat deposits or other geological sediments after death. Due to their relationship with hydrological conditions, TA have been widely and successfully applied for palaeohydrological reconstruction (Charman 1997, Booth 2002, Charman et al. 2007, Qin et al. 2013, Marcisz et al. 2016). They are also fast in reproduction, have a short life cycle, show a narrow ecological niche and sensitivity to various environmental factors such as atmospheric pollution, dryness, and heavy metals. These characteristics thus make them valuable bioindicators in monitoring current environmental conditions (Foisnner 1987, Nguyen-Viet et al. 2007b, Payne 2010, Payne et al. 2016). In addition, TA, especially heterotrophic (HTA) organisms are usually considered to be the top predators of bacteria and fungi (Wilkinson 2008). They therefore play a crucial role in the process of decomposition of dead organic matter in soils (Wilkinson and Mitchell 2010). Some TA species are mixotrophic (MTA), which have the ability to absorb carbon dioxide (Jassey et al. 2015). Consequently, TA are likely to play an essential role in ecosystem processes, mainly carbon cycling (Tolonen et al. 1992, Aoki et al. 2007, Jassey et al. 2015).

### **1.1.3 Structural characteristics**

#### **1.1.3.1 Shell morphology**

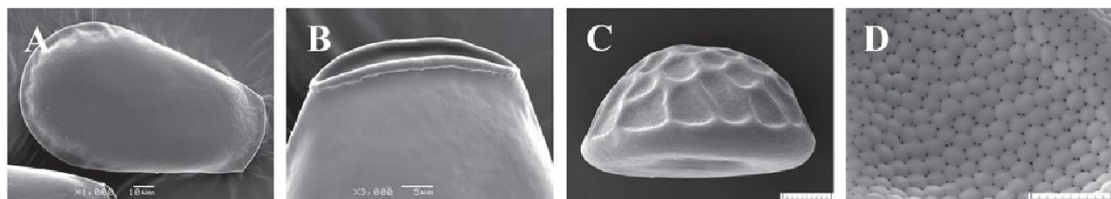
Testate amoebae shells exhibit a great variety of shapes, sizes, and compositions that reflect the diversity of this group of organisms. They were therefore classified into sixteen morphological types in accordance with their shape, symmetry of shells and structure of the aperture (Fig. 1.1) (Bonnet 1975).



**Figure 1.1** Morphological types of testate amoebae (TA) shells: ARC - arcella; ACS – simple acrostomy; ACC – compressed acrostomy; ACA – arched acrostomy; TRAS – simple trachelostomy; TRAA – arched trachelostomy; COT – cotylostomy; AXI – axial; AMP – amphistomy; ELL – ellipsostomy; PRO – propylostomy; DIP – diplostomy; PLS – simple plagiostomy; PLV – plagiostomy with visor; CRS – simple cryptostomy; CRV – cryptostomy with visor (taken from Todorov and Bankov (2019), fig. 1).

Based on the shell materials and composition, TA were further divided into four main groups with different types of shells, that is, proteinaceous, siliceous, agglutinate and calcareous (Ogden and Hedley 1980, Meisterfeld 2002a).

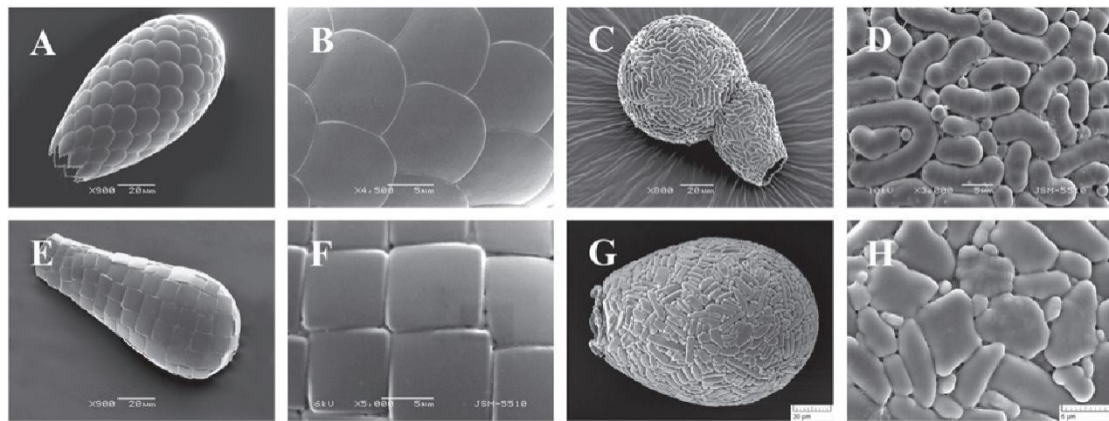
**Proteinaceous shells:** are composed of organic materials such as chitin, cellulose, or proteins. They are typically transparent or lightly pigmented and can exhibit various shapes and sizes depending on the species. For example, *Hyalosphenia spp* usually have the shells with a smooth and rigid homogeneous coat composed of mucoprotein while *Arcellidae spp* have shells constructed of numerous regularly arranged, hollow building units or alveoli (Fig. 1.2).



**Figure 1.2** Testate amoebae with proteinaceous shells: (A, B) *Hyalosphenia papilio* – shell composed of mucoprotein; (C, D) *Arcella gibbose* – shell composed of numerous hollow building units (alveoli) (taken from Todorov and Bankov (2019), fig. 2).

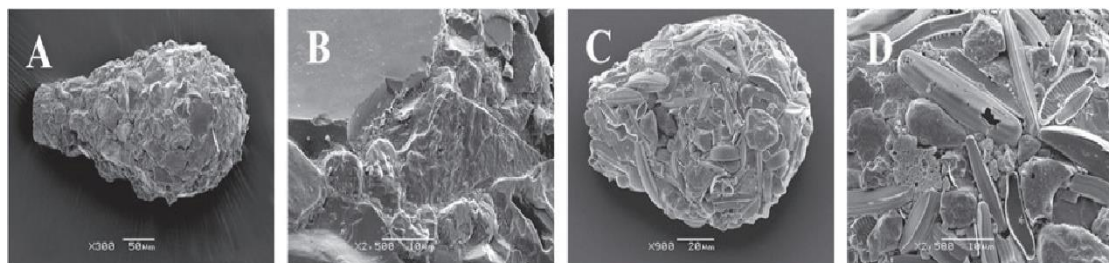


**Siliceous shells:** are composed of either self-produced endogenous siliceous shell-plates (idiosomes) or combined with external siliceous materials such as diatom frustules. They are mostly transparent though species like *Assullina muscorum* type have a light-brown color. The shells can exhibit various structures and patterns, with shape, size and arrangement of plates being species-specific but having a valuable taxonomic significance. For example, the shape of idiosomes can vary from oval or circular in the *euglyphids* (Euglyphida), through rods in *Lesquereusia* and nail-like in *Netzelia* to quadrangular in *Quadrulella* (Fig. 1.3).



**Figure 1.3** Testate amoebae with siliceous shells composed of self-produced siliceous shell-plates (idiosomes): (A, B) *Euglypha tuberculata*; (C, D) *Lesquereusia epistomium*; (E, F) *Quadrulella longicollis*; (G, H) *Netzelia oviformis* (taken from Todorov and Bankov (2019), fig. 4).

**Agglutinate shells:** are composed of particles that are glued together with organic or inorganic cement. The particles can include mineral grains, organic debris and other biological materials such as diatom frustules from the environment (xenosomes). They are typically opaque and can exhibit various shapes and sizes. For example, some agglutinate-shelled TA, like *Diffugia* spp have shells covered with mineral particles, while others such as *Thecamoebida* spp have shells made of organic debris (Fig. 1.4).



**Figure 1.4** Testate amoebae with agglutinate shells: (A-D) shells composed of foreign material from the environment (xenosomes); (A, B) *Diffugia oblonga*; (C, D) *Pontigulasia rhumbleri* (taken from Todorov and Bankov (2019), fig. 3).

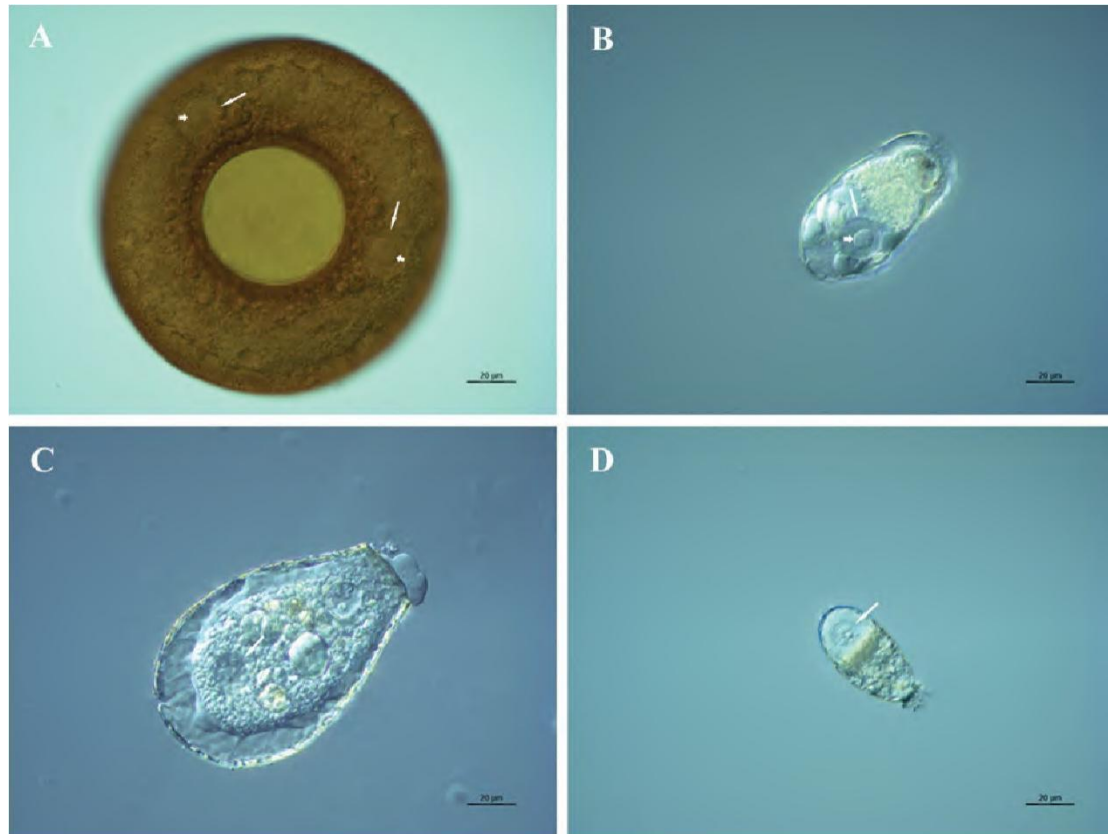
**Calcareous shells:** are made of calcium carbonate or other calcium-containing minerals. This type of shell is the least presented and is characteristic to only two genera *Paraquadrula* and *Cryptodiffugia* (not found in any of the study sites). The first one has quadrangular calcite shell-plates embedded in a sheet-like organic cement matrix, while the second has a smooth shell surface, with the wall being made of two layers, a thin organic outer layer and a thick inner layer of amorphous calcium phosphate (Hedley et al. 1977).

### 1.1.3.2 Cytoplasm

The cytoplasm of TA is a complex, three-dimensional network of interconnected membranes, cytoskeletal filaments, and various organelles; it is a semi-fluid, gel-like substance that fills the space inside the test or shell that surrounds the cell body. The cytoplasmic matrix provides structural support and serves as a scaffold for the organelles within the cell (Ogden and Meisterfeld 1991). Nevertheless, cell morphology of most taxa with agglutinate shells (xenosomes) have been scarcely studied due to the opaqueness of the robust and thick test walls, which prevent the observation of the cytoplasm by light and transmission in electron microscopy. Thus, much of the current knowledge on the cell structure of TA is mainly based on the studies of species with proteinaceous, siliceous or calcareous shells (Charret 1964, Hedley and CG 1974, Harrison et al. 1976, Golemansky et al. 1987, Ogden 1991). In general, the cytoplasm is often divided into two zones. The anterior zone is a granular cytoplasm which contains numerous mitochondria, many food and digestive vacuoles and peripherally located vesicles. The posterior zone is a dense cytoplasm and includes nucleus, surrounded by a compact mass of granular endoplasmic reticulum, numerous ribosomes, one or more Golgi complexes, as well as several contractile vacuoles located laterally of the nucleus, close to the plasmalemma (Ogden and Hedley 1980, Meisterfeld 2002a, Todorov and Bankov 2019).

One of the most prominent organelles in the cytoplasm is the **nucleus**, which contains the cell's genetic material in the form of DNA. The nucleus is surrounded by a double membrane called the nuclear envelope, which separates it from the rest of the cytoplasm. The DNA in the nucleus is organized into chromosomes, which carry the genetic information that is necessary for cell growth, reproduction, and other essential functions (Wanner et al. 1997). Most species have one nucleus and rarely the nuclei are

two (many *Arcella* species), about ten to twenty (*Arcella polypora*), and up to several tens or hundreds (e.g., *Arcella megastoma*, *Diffugia urceolata*, *Phryganella nidulus*). According to the classification of Raikov (1982), the nuclei can be divided into two main types, with the first (vesicular nuclei) having one large, central nucleolus while the other (ovular nuclei) having several to many small nucleoli (Fig. 1. 5).

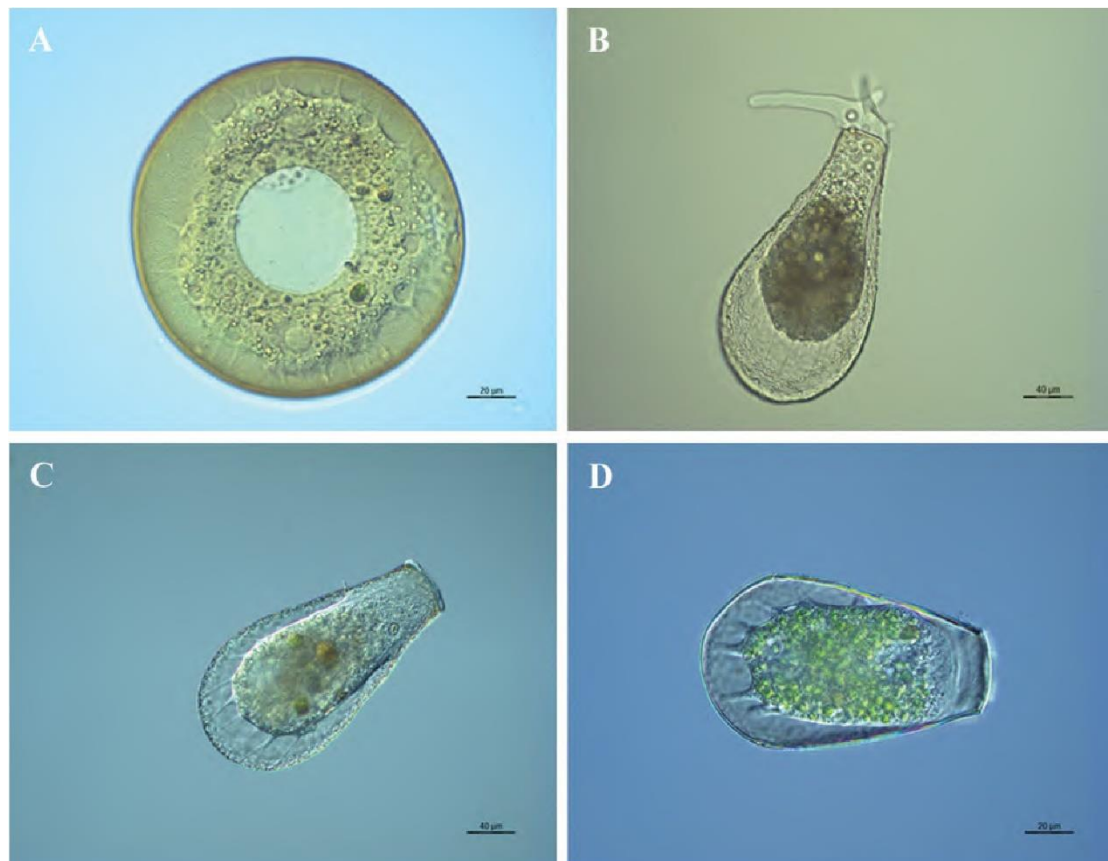


**Figure 1.5** Nuclei of testate amoebae: (A, B) vesicular nuclei of *Arcella discoidea* (A) and *Trinema galeata* (B); (C, D) ovular nuclei of *Nebila collaris* (C) and *Tracheleuglypha dentata* (D) (large arrows – nucleus, small arrows - nucleolus) (taken from Todorov and Bankov (2019), fig. 5).

Another important organelle in the cytoplasm is **mitochondria**, which are responsible for producing energy through cellular respiration. Mitochondria have an inner and outer membrane and are often found distributed throughout the cytoplasm. Mitochondria generate ATP, the energy currency of the cell, by converting the energy stored in nutrients such as glucose into a form that the cell can use (González-Miguéns et al. 2022).

Finally, the cytoplasm of TA contains various types of **vesicles and vacuoles**, which are involved in storage, transport, and digestion (Anderson 1994). Vacuoles are especially important for regulating water balance within the cell, and many testate

amoebae have specialized contractile vacuoles that can expel excess water and maintain cellular hydration (Anderson 2017). It is also worth noting that the cytoplasm usually fills the entire shell in the smaller euglyphids (e.g., *Corthion*, *Euglypha*, *Sphenoderia*, *Trinema*), while it fills only half or two-third of the shell in larger species, where the cell is attached to the posterior part of the shell wall with numerous thin cytoplasmic strands (epipodes) (Fig. 1.6) (Todorov and Bankov 2019).



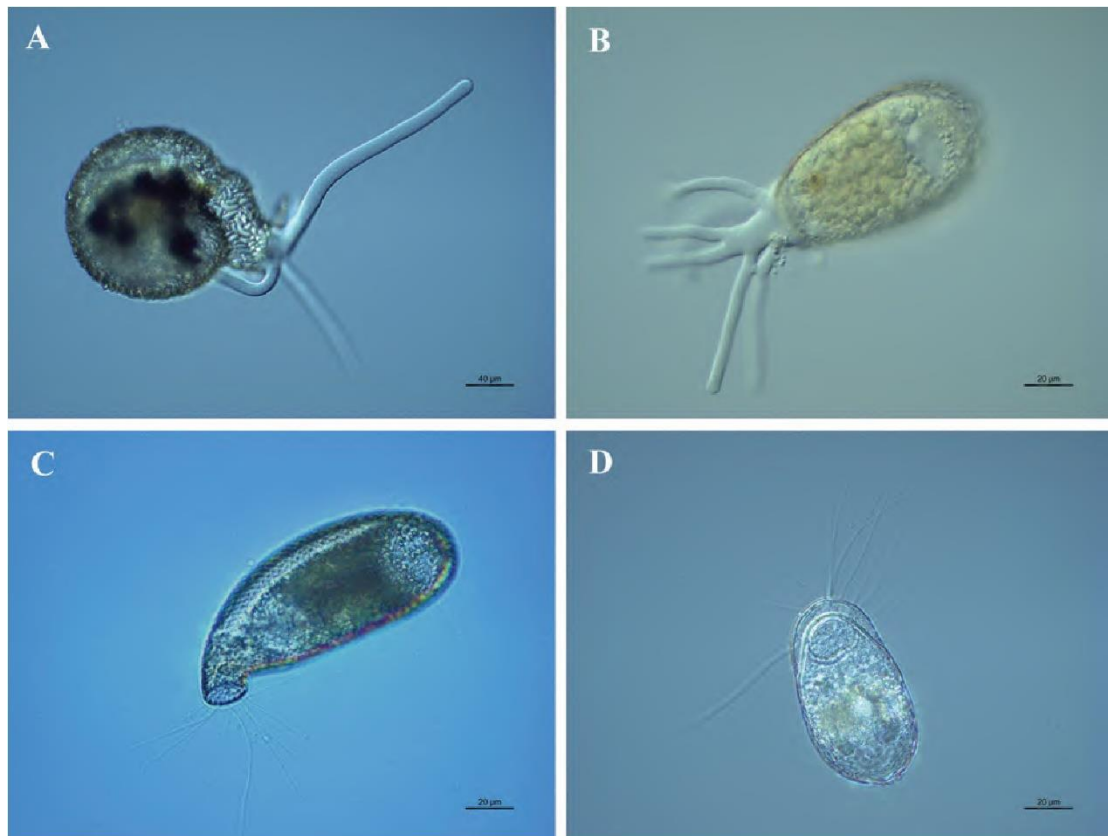
**Figure 1.6** Thin cytoplasmic strands (epipodes) attaching the cell to the shell wall: (A) *Arcella discoides*; (B) *Gibbocarina galeata*; (C) *Longginebela tubulosa*; (D) *Hyalosphenia papilio* (taken from Todorov and Bankov (2019), fig. 6).

### 1.1.3.3 Pseudopodia

Pseudopodia are extensions of the cell membrane that are used by amoebae for various functions, including movement, feeding, and sensory perception (Mast 1926). In testate amoebae, these extensions are more rigid and enclosed within the shell or test, which protects them and provides additional support. They are composed of actin filaments, which are thin, flexible protein fibers that can rapidly polymerize and depolymerize. These actin filaments form a complex network throughout the cytoplasm and are anchored to the cell membrane (Ogden and Meisterfeld 1991). When the amoeba wants

to extend a pseudopodium, it sends a signal to the actin filaments, causing them to polymerize and push against the cell membrane. As the actin filaments continue to polymerize, the pseudopodium extends further outward (Taylor et al. 1976).

The shape and size of the pseudopodia can vary greatly depending on the species of TA and the environmental conditions (Mitchell et al. 2008a). Some species have long, thin pseudopodia (**filopodia**) that are used for locomotion and feeding, while others have short, stout pseudopodia (**endolobopodia**) that are used for gripping and anchoring the cell to the substrate (Smith et al. 2009). Some TA have branched or bifurcated pseudopodia (**ectolobopodia**) that can help to trap prey or create a more complex network for exploring the environment (Fig. 1.7) (Wylezich et al. 2002).



**Figure 1.7** Pseudopodia of testate amoebae: (A, B) endolobopodia of *Lesquereusia gibbosa* (A) and *Heleopera sylvatica* (B); (C, D) filopodia of *Cyphoderia ampulla* (C) and *Trinema galeata* (D) (taken from Todorov and Bankov (2019), fig. 7).

In addition to movement and feeding, pseudopodia also play a role in sensory perception. TA can use their pseudopodia to detect changes in their environment, such as changes in light, temperature, or chemical gradients. By extending their pseudopodia

towards a stimulus, TA can gather information about their surroundings and respond accordingly (Han et al. 2008).

#### **1.1.3.4 Reproduction**

Testate amoebae reproduce both asexually and sexually, depending on the species and environmental conditions (Bobrov and Mazei 2004, Lahr et al. 2011).

**Asexual reproduction:** is the most common method of reproduction in TA. In this process, the amoeba replicates its genetic material (DNA) and divides into two identical daughter cells through a process called binary fission. Binary fission begins with the replication of the chromosomes in the parent cell. Once the chromosomes have been replicated, they attach to the cell membrane and the cell starts to elongate. Eventually, the cell constricts in the middle, forming a furrow that separates the cell into two identical daughter cells (Fig. 1.8) (Ogden 1979, Ogden 1987, Raikov and Mignot 1991). The doubling time of daughter cells is between two and twelve days depending on the species and environmental conditions, usually shorter in a laboratory than in natural conditions (Heal 1964, Hedley and CG 1974, Ogden 1981, Ogden 1989). Each daughter cell receives an equal share of the parent cell's genetic material and cytoplasm, and they both grow to their full size over time. This process of binary fission can continue, resulting in exponential growth in the population of TA.

It was believed for a long time that TA can only reproduce asexually (Penard 1890, Dangeard 1923), until in the late 20<sup>th</sup> century Valkanov (1962, 1966) studied the reproduction of TA in more detail and revealed four types of copulation. Currently, it is suggested that TA and many other microbial eukaryotes, can also reproduce sexually (Lahr et al. 2011, Tekle et al. 2017, Hofstatter et al. 2018).

**Sexual reproduction** in TA involves the fusion of two haploid cells (gametes) to form a diploid zygote. The zygote undergoes meiosis to produce haploid cells that can grow and divide by binary fission to form new individuals (Raikov 1995). The production of gametes occurs through a process called gametogenesis. In this process, the parent cell undergoes meiosis, a type of cell division that results in the formation of four haploid cells (Goodenough and Heitman 2014). Two of these haploid cells fuse together during fertilization, forming a diploid zygote. The zygote develops into a cyst, a protective structure that allows it to survive harsh environmental conditions. Inside the cyst, the



zygote undergoes meiosis, producing four haploid cells that can grow and divide by binary fission to form new individuals (Lüftenegger and Foissner 1991, Mignot and Raikov 1992, Iudina and Sukhanova 2000).



**Figure 1.8** Reproduction of testate amoebae by binary fission: (A) *Sphenoderia lenta*; (B) *Heleopera sylvatica*; (C) *Arcella dentata* (the shell of parent is dark-brown because the alveoli are enriched with iron, used to strengthen the shell wall; this inorganic material is not usually present in the alveoli of young animals and their shells are light yellowish) (taken from Todorov and Bankov (2019), fig. 8).

### 1.1.3.5 Cysts

Apart from the reproductive cysts, TA can form resistant cysts by secreting a protective layer around themselves in response to adverse environmental conditions such as desiccation, lack of nutrients, extreme temperature and other environmental stresses (Samba-Louaka 2023). The cysts are typically spherical or ovoid in shape and can range in size from a few micrometers to several hundred micrometers in diameter, depending on the species (Fig. 1.9). They are often composed of organic or inorganic materials, such as chitin, silica, or calcium carbonate, which can be opaque but mostly translucent in appearance. The protective layer surrounding the cysts helps to prevent desiccation and damage from environmental stressors. This layer can also contain specialized structures, such as pores or spines that help to regulate gas exchange and protect the cyst from physical damage (Page 1981). In such an encysted state, TA can remain viable for extended periods of time, sometimes for many years. This allows the organism to survive unfavorable environmental conditions until suitable conditions for growth and reproduction return. In the period of drought, many peat- and moss-dwelling species are able to generate short-time cysts (precysts), which differ from the resistant cysts by their relatively thin membrane enclosing the cell. When conditions improve, this type of cysts can often quickly germinate and grow into active amoebae

once again. Usually, TA forming cysts also seal the shell aperture with a plug of either siliceous or extraneous particles (Fig. 1.9) (Todorov and Bankov 2019).



**Figure 1.9** Cysts of testate amoebae: (A, B) short-time cysts of *Heleopera rosea* (A) and *Hyalophenia papilio* (B); (C) resistant cyst of *Nebela collaris* and additional sealing of the shell aperture with a plug of siliceous and extraneous particles (taken from Todorov and Bankov (2019), fig. 9).

#### **1.1.4 Feeding preferences - a key TA characteristic for their contribution to ecosystem processes**

In answering what functional roles TA are likely to play in ecosystem processes (e.g., carbon and nutrient cycling), an essential question may be raised, that is, “from where does it get its supply of energy and nutrients”? Although we still do not have a clear and comprehensive understanding of the food sources of TA, it is clear that they can utilize a range of foods, including bacteria, fungi, algae and other smaller protozoa (Ogden and Hedley 1980, Coûteaux and Dévaux 1983, Schröter 2001). And some of them are also reported to be able to feed on quite large organisms, for example, by puncturing the cells of filamentous algae (Stump 1935), or even catching nematodes (Yeates and Foissner 1995) and planktonic rotifers (Han et al. 2008) in aquatic systems. Likewise, in terrestrial systems, the predation of TA (e.g., *Nebela tinctoria*, *Hyalosphenia papilio*) on large organisms (e.g., rotifers) was also observed in a few studies (Gilbert et al. 2000, Gilbert et al. 2003). Through the observation of TA’s feeding preferences under a microscope, it is commonly supposed that body and/or aperture size are likely to be the main factor to determine its predation habits, with smaller taxa primarily feeding on bacteria or other smaller microbes, while larger species having a greater range of food from bacteria to fungi, other protists (e.g., flagellates, ciliates and small TA) and even to mesofauna (e.g. nematodes, rotifers) (Ogden and Hedley 1980, Gilbert et al. 2000). However, this generalization may be a simplification and is seemingly not suitable for all species, for example, the large taxon *Trigonopyxis arcuata* that is usually



abundant in forest litter, appears to be a fungal specialist (Sutton and Wilkinson 2007). However, it is still not clear if the TA that have been described as fungal specialists do actually feed directly on hyphae, or if they only consume exudates from the hyphae, or bacteria feeding on such exudates (Wilkinson and Mitchell 2010).

Apart from the feeding preferences of HTA (heterotrophy) mentioned above, there are some MTA (mixotrophy) which contain endosymbiotic algae (zoochlorellae), which have the potential to acquire their source of energy by endosymbiont's photosynthesis (Jassey et al. 2015). While this symbiotic relationship between some TA and zoochlorellae has long been known (for example, Leidy (1879) recorded and remarked on the abundance of chlorophyll corpuscles in TA such as *Hyalosphenia papilio*), very limited studies have been conducted to quantify the energetic benefits of such endosymbionts to any TA taxon. However, it is worth noting that Schönborn (1965) observed the death of some MTA when they are deprived of light, indicating, to some extent, the importance of endosymbionts to some MTA (Schönborn 1965).

With the development of molecular tracer techniques in the past decades, stable isotope methods (i.e., ratios of carbon and nitrogen) have been increasingly and successfully used to provide time-integrated information about feeding relationships and food web structure in aquatic food webs (Anderson and Cabana 2009, Carscallen et al. 2012, Mao et al. 2012). Thus, a combination of traditional (i.e., observation and counting the digestive vacuole contents of TA) and molecular (i.e.,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) methods to investigate the feeding behavior of TA seems promising. This was clearly confirmed by subsequent studies conducted by Jassey et al. (2012, 2013). The results showed that the feeding type of TA (e.g., bacterivory, algivory or both) is highly related to their trophic level and which position they occupy in the food chain depends on their feeding behavior, the availability and density of their food resources, and their metabolism (i.e., mixotrophy versus heterotrophy). In addition, these studies also further demonstrate the energetic benefits of mixotrophy (e.g., *Hyalosphenia papilio*) when the density of its preferential prey is low, highlighting the dynamics in predator-prey abundance could modify the functional role of TA, especially mixotrophic TA in the microbial food web (Jassey et al. 2012, Jassey et al. 2013).

### **1.1.5 Taxonomy**

Testate amoebae are a polyphyletic group of protozoa that produce (to likely varying degree) decay-resistant and morphologically distinct tests, which could usually be identified to species level (Beyens and Meisterfeld 2002, Meisterfeld 2002a). According to their pseudopod morphology, TA have been traditionally divided into two main groups. The first group has lobose pseudopodia (order Arcellinida) that are relatively broad and blunt extensions of the cell membrane, such as *Arcella* type, *Centropyxis* type, *Nebela* type and *Diffflugia* type. The second group has filose pseudopodia (order Euglyphida) that are slender, thread-like extensions of the cell membrane, mainly represented by *Euglypha* type, *Assulina* type, *Corythion* type and *Trinema* type. The phylogenetic position of these groups (i.e., Arcellinida and Euglyphida) among Amoebozoa and Cercozoa, respectively, has been established on the basis of SSU rDNA sequence data (Bhattacharya et al. 1995, Cavalier-Smith and Chad 1997, Wylezich et al. 2002, Nikolaev et al. 2005). The rest of TA possessing anastomosing networks of reticulopodia and two symmetrical pseudostomes (e.g., *Amphitrema* sp. and *Archerella* sp.) were previously, based on their morphology, classified in the phylum Granuloreticulosea (Bovee 1985) and then in Rhizaria (Meisterfeld 2002b). More recently, this group has been demonstrated to be included in the Stramenopila by phylogenetic analyses of Small Subunit ribosomal DNA (SSU rDNA) sequence data (Gomaa et al. 2013). Despite the fact that using molecular data have helped to clarify the relationships between different groups of TA and have provided insights into their evolutionary history, DNA sequences are only available for a limited number of taxa and the higher-level classification of TA still relies primarily on morphological characters (Mitchell et al. 2008a).

### **1.1.6 What is peat and peatland?**

Peat is the remains of plant and animal constituents accumulating in the place where it has been formed under more or less waterlogged (anoxic) and acidic conditions resulting from incomplete decomposition (Rydin et al. 2013). It is distinguished from other soil types by its relatively high content of organic matter which can range from 30% to virtually 100% depending on the definitions and conventions used (Lindsay 2010). A wide range of plant materials may be involved in the process of peat formation such as woody parts, stems, leaves, rhizomes, roots, and bryophytes (primarily *Sphagnum* moss). Over time, the partially decomposed plant material accumulates and

undergoes chemical changes, resulting in the formation of peat, which is typically dark brown to black in color. It has a spongy texture (low bulk density) and thus contains a high amount of water, usually between 90% and 95%.

Peatland is a terminology used to describe the peat-covered areas/terrain. It usually requires a minimum depth of peat for an area to be classified as peatland (Rydin et al. 2013). However, the precise values in the specific definitions regarding the minimum depth of peat often vary depending on the countries and organizations. In Canada, the minimum depth is 40 cm (National Wetlands Working Group 1997), but in many other countries and in the peatland area statistics of the International Mire Conservation Group, the minimum is 30 cm (Joosten and Clarke 2002). Overall, peatlands are home to a unique assemblage of plant and animal species adapted to these conditions and play a crucial role in carbon storage, water regulation, and biodiversity conservation. They can include various types such as bogs, fens, marshes, and swamps, each with distinct hydrological and ecological characteristics (Rydin et al. 2013). As for bogs, they are often subdivided into raised bogs and blanket bogs. However, there are some special mires (e.g., valley bogs) between them characterized by an irregular terrain consisting of a series of hills, ridges, basins, troughs, and small valleys, for example, those spanning the border between Cumbria and Northumberland, just to the south of the Scottish-English border. While typical valley bogs are highly different from blanket bogs in both hydrology (e.g., water resources) and topography (V-shape versus relatively flat slope), there are other special bogs in this area with often very deep peat over bedrock depressions, which are classified as ridge-raised mire or intermediate bog, collectively referred to as Border Mires (Eades et al. 2021). In fact, Eades et al. (2021) highlights the difference in Border Mire peatlands versus more typical blanket bogs and states that “this is not a ‘problem’ restricted to the Border Mires, but is relevant to many upland peatland areas, including the Flow Country (to which the Border Mires could be considered an English analogue)”. Throughout the thesis, this type of bog will be referred to as border mire (BM) bog.

### **1.1.7 What is a blanket bog?**

Blanket bog is a type of peatland ecosystem that is characterized by its extensive coverage of large areas, often covering entire hill or plateau landscapes (Tipping 2008). These ecosystems are found primarily in cool, wet climates, such as in Ireland, the UK,

Scandinavia, Canada, and parts of Russia (Chico 2020). The often-thick layer of peat that accumulates in blanket bogs is primarily composed of partially decomposed plant materials, such as *Sphagnum* moss, heather, and cotton grass, with typical peat depth varying between 50 cm and 3 m on average, although some can exceed 5 m (Williamson et al. 2017). Blanket bog is “ombrotrophic”, that is, the water and mineral supply comes entirely from atmospheric sources (rainwater, mist, cloud-cover). Water chemistry is therefore usually nutrient-poor and acidic, which is beneficial to acid-loving plant communities. Typical plant species found in blanket bogs include heather, bilberry, crowberry, cotton grass, and various species of mosses (e.g., *Sphagnum* moss). These plant species play an important role in maintaining the structure and function of the ecosystem, by contributing to the accumulation of peat, water holding capacity and regulating water flow (Cuckston 2017). Blanket bogs are also important habitats for a range of animal species, many of which are adapted to the unique conditions of the ecosystem. These include species such as the golden plover, dunlin, hen harrier and various species of insects and other invertebrates (Conaghan et al. 2000, Gallego-Sala and Colin Prentice 2013).



**Figure 1.10** Mossdale (northern England) is the location of one sample site in this study. The site is a typical UK blanket bog mostly covered by heather-dominated vegetation with periodical burning (foreground) and cutting (patch in the centre) with considerable sedge and *Sphagnum* moss presence in places. Peat is mostly about 1.5 m deep. Photo: A. Heinemeyer

### **1.1.8 What is a border mire (valley, ridge raised and intermediate) bog?**

Border Mire (BM) is a type of peatland characterized by large depressions or basins that are filled with peat and other waterlogged organic material. These bogs are typically found in valleys or low-lying areas where water accumulates and slowly drains away, creating a unique and specialized ecosystem (Newbould 1960). They are similar to other types of bogs, such as raised bogs and blanket bogs, in that they are fed by rainfall and are typically located in areas with high levels of precipitation. However, it also differs from above mentioned bogs in many aspects. For example, despite the water source from rainwater, BM bogs are often also fed by groundwater (which includes upwelling water originating from the valley sides) (Wassen et al. 1990). One of the defining features of BM bogs is the very waterlogged conditions and the presence of a very deep layer of peat. The peat layer is often much greater than 3 m, and it is composed primarily of *Sphagnum* mosses and other ‘water-loving’ plants that thrive in the near permanently waterlogged, acidic, nutrient-poor conditions found in valley bogs (Walker et al. 2001). As such the Border Mires are similar to the Flow Country bogs, both of which are unlike typical blanket bogs in England (Eades et al. 2021). Border mire bogs are important ecosystems because they provide near constantly water logged peatland habitat for a variety of rare and endangered species, including bog turtles, dragonflies, and carnivorous plants such as pitcher plants, sundews and bladderworts (McDonald 2015).



**Figure 1.11** Butterburn (northern England) is the location of one sample site in this study. The site is a UK border mire (likely an intermediate or ridge raised) bog mostly covered by *Sphagnum*-dominated vegetation with considerable sedge presence in places. Peat is mostly about 6.7 m deep. Photo: Bing Liu

## **1.1.9 Management practices on British blanket bogs**

### **1.1.9.1 Prescribed burning of heather**

The use of fire has been a common vegetation management practice in the uplands of the UK for hundreds, even thousands of years (Worrall et al. 2010, Davies et al. 2016). While the earliest evidence of human managed burning can date back to the late Mesolithic or early Neolithic time (about 4000 years ago) for clearing land and as hunting strategy (Fyfe et al. 2003, Davies et al. 2008), it was not until the late medieval period when burning was recorded to become a common land management practice, particularly in southern England and Scotland (1300s) (Rackham 2020). In the mid-19<sup>th</sup> century, with the development of intensification of grouse shooting in the UK, the use of prescribed burning for heather-dominated habitat management to promote the production of red grouse (*Lagopus lagopus scotica*) started to spread rapidly (Harper et al. 2018). Specifically, the practice originated in Scotland in the 18<sup>th</sup> century, where it was primarily a sport for the wealthy landowners and aristocracy. At this time, heather moorland was seen as a valuable resource for grouse shooting, as it provided ideal



habitat for the birds and offered a challenging and exciting sport for the shooters (Dodgshon and Olsson 2006). More recently, the potential role of prescribed fire as a management tool to potentially lower the increasing wildfire risk on UK peatlands (Belcher et al. 2021) has been recognized (Davies et al. 2016, Harper et al. 2018, Heinemeyer 2023).

Prescribed burning of heather involves setting controlled fires in heather moorland (including grouse moors), typically during the winter months when the risk of fire impacts on the soil is lower. The fires are started by (often trained and experienced) personnel who use a variety of techniques, including the use of hand-held tools and drip torches, to ignite and control the flames (Fig. 1.12A) (Heinemeyer et al. 2019a). The purpose of this practice is to remove the old, woody vegetation (e.g., heather) and create a mosaic of burnt patches of different ages to provide red grouse with both food from new young shoots and protection from predators in the taller, older patches (Heinemeyer et al. 2019b).

In the UK, it was estimated that about 18% of peatlands (Worrall et al. 2010) and 30% of blanket bog (Heinemeyer et al. 2019a) are under such a burn management regime. Despite the fact that some burning occurs within a given area each year (Davies et al. 2008), it is usually carried out within a cycle of between eight and twenty-five years (depending on site climate and heather growth rates, but often on a 10–15-year rotation) in patches of about 0.5-1.0 ha in size (Harper et al. 2018). Such rotational burning management promotes the regrowth of ling heather (*Calluna vulgaris*) and generates different age structures of heather patches to support high grouse populations, but this practice has been controversial in recent decades due to concerns about the impacts on biodiversity and key ecosystem services. For example, peat soils could be damaged or eroded by burning if it is not performed correctly or if there is a loss of control of the fires, releasing additional carbon (in addition to that from vegetation combustion) into the atmosphere, thereby contributing to climate change. Burning can also damage or kill bog mosses which play an essential role in maintaining peat hydrological function by their high-water holding capacity, and therefore could threaten the continued formation of peat (Lindsay 2010). However, the claims around fire impacts and the concept of *Sphagnum* as a key peat-forming species are often unevidenced and ill-defined (Davies et al. 2016, Harper et al. 2018, Ashby and Heinemeyer 2021,

Heinemeyer and Ashby 2023). Additionally, prescribed burning has also been linked to affecting water quality by increasing the amount of dissolved organic carbon in streams within burnt catchments (Yallop and Clutterbuck 2009), although the overall impacts are not clear (Harper et al. 2018).

As a result, regulations and guidelines have been introduced to govern the use of prescribed burning in the UK (although they vary between the devolved UK regions). These regulations aim to ensure that the practice is carried out in a sustainable and environmentally responsible way (e.g., guidance as part of the Heather and Grass Burning Code; Defra 2014). For example, prescribed burning may only be conducted in areas where it is deemed necessary for conservation of land management purposes, and it must be carefully planned and managed to minimize the risk of damage to peat soils and other sensitive ecosystem services (Allen et al. 2013, Davies et al. 2022).

#### **1.1.9.2 Alternative cutting of heather**

As the use of prescribed burning mentioned above (sometimes in conjunction with drainage) has been considered to have potentially contributed to blanket bog degradation (Lindsay 2010), alternative cutting (often also referred to as mowing) management of heather is of particular interest to policy makers and land managers (Heinemeyer et al. 2019b).

Heather cutting is usually carried out using flail mowers due to their ability to effectively cut through dense vegetation. These machines are designed with a rotating drum that contains multiple blades or flails. They can be attached to tractors or other vehicles or mounted to their own wheeled or tracked chassis for more maneuverability in rough terrain (MacDonald 1996). The flails (normally set between 12.5 cm and 15 cm above the ground as cutting too low can damage the roots of the plants, while cutting too high can leave patches of uncut heather) rotate at a high speed, cutting through vegetation as they pass over it. The shredded material is then normally left on the ground as mulch, which can help to protect the soil from desiccation, but sometimes is also collected (e.g., for peatland restoration projects elsewhere). Noticeably, where brash is left on site as a thick mulch, it can also affect plant growth by smothering regrowth of heather and other vegetation (Fig. 1.12B) (Heinemeyer et al. 2019b). In fact, on balance, the issues/benefits of either management are far from clear, especially



lacking monitoring impacts on key ecosystem processes over an entire management cycle (Heinemeyer et al. 2023).

Compared to burning, a clear advantage of cutting is that this practice is much less constrained by weather conditions (Tucker 2003), even though access for cutting machinery is relatively easier when habitat condition is drier, and the ground is firmer (MacDonald 1996). However, it is not always accessible or suitable for all areas to be cut by larger machinery due to a range of reasons such as the slope of the moor (uneven and steep), and ground conditions (very boggy or rocky) (Heinemeyer et al. 2019a). Another potential advantage of cutting with brash left on site is the reduced water loss via evapotranspiration from the brash covered ground compared to exposed burn areas, thereby limiting any negative rain and erosion effects, whilst enhancing infiltration and also returning organic matter (and thus some of its carbon) to the soil (Heinemeyer et al. 2019b). Furthermore, brash could be particularly effective in spreading *Sphagnum* propagules across larger areas, which is therefore beneficial to restoring many areas of bare peat towards active bog vegetation. Nevertheless, cutting could have some drawbacks. For example, heavy cutting machinery can change the physical properties of surface (compaction). However, the most obvious impact of cutting by machinery is the possible reduction of surface micro-topography by cutting off the tops of tussocks and/or hummocks as shown by Heinemeyer et al. (2019a). It could, thus, possibly affect surface water runoff and also impact nesting habitat conditions for ground nesting birds such as dunlins (Heinemeyer et al. 2019a).

### **1.1.9.3 Drainage ditches**

The earliest drainage in blanket bogs in the UK can be dated back to the medieval period, when small-scale ditching was carried out to improve drainage on a local level (Rippon et al. 2006). This was often done by farmers or local communities, who would dig simple channels to allow water to drain from the bog into nearby streams or rivers (Ramchunder et al. 2009). One example of early drainage is found in the Peak District, where some of the earliest recorded instances of ditching on blanket bogs have been discovered. Archaeological evidence suggests that these ditches were dug by hand using simple tools such as shovels and were likely used to improve the drainage of the surrounding land for agricultural purposes (Rothwell et al. 2005).

However, it was not until the 20th century (around 1950s) that more extensive drainage of blanket bogs began to take place in the UK (Fig. 1.12C) (Bonn et al. 2016). This was driven mainly by the increasing demand for agricultural land (e.g., improvement of livestock and red grouse production) and the development of new drainage technologies such as steam-powered pumps, use of drainage pipes and deep ploughing. These techniques allowed farmers to drain larger areas of blanket bog more efficiently. By 1970, approximately 100,000 ha of land were being drained annually (Robinson and Armstrong 1988). Meanwhile, drainage was also conducted as part of afforestation programs (Cannell et al. 1993). In some areas of intense peat erosion (e.g., English Peak District), large networks of gullies also served as drainage routes while these gullies even act as the primary drainage system in some of the most degraded regions (Evans and Lindsay 2010a).

Drainage had (and often still has) significant impacts on the peatland environment. For example, drainage of the bogs reduces the amount of water stored in the peat, which makes it more vulnerable to erosion and degradation (Holden et al. 2007a). It also disrupts the natural water cycle and modifies the hydrological flow pathways, which may affect downstream water quality (because of the increased sediment and nutrients) and flood risk (Holden et al. 2006). Moreover, the loss of bogs can lead to a decline in biodiversity as many species of plants (e.g., sundew) and animals (e.g., golden plover) that depend on the wetland habitat are lost. As the water table drops, the drying out of the peat layer also leads to an increase in greenhouse gas emissions from decomposition and other products such as dissolved organic carbon (DOC) (Lindsay 2010). In fact, most of the negative impacts attributed to burning are likely (entirely or at least partly) due to drainage (as a confounding factor), which often coincides with burning (Ashby and Heinemeyer 2021).

Today, there is growing awareness of the importance of protecting blanket bogs, and efforts are being made to restore damaged areas (Caporn et al. 2018). The commonly used restoration techniques in relation to drainage include drainage ditch blocking, gully reprofiling, bare peat revegetation and *Sphagnum* moss reintroduction (Bonn et al. 2016). These efforts are aimed at reversing the damage that has been done to the bogs and restoring their natural functions as habitats for wildlife, carbon sinks, and sources of clean water (Dixon et al. 2014, Rosenburgh 2015, Howson et al. 2021b).

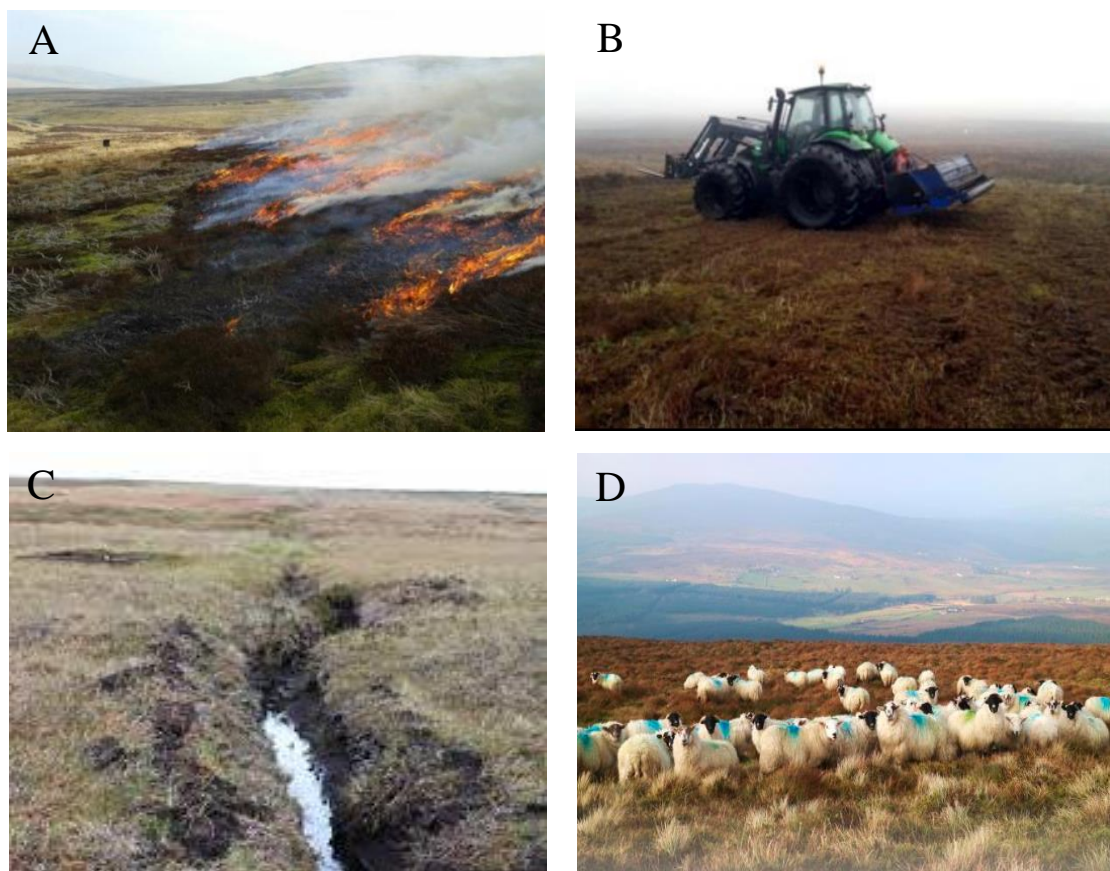
However, prescribed fire and cutting are also still considered as a potential tool to restore heather-dominated peatlands to a more ‘functioning’ bog, with a higher proportion of other bog species, especially *Sphagnum* moss (Heinemeyer 2023).

#### **1.1.9.4 Sheep grazing**

Sheep grazing has a long and significant history in the UK, which can be traced back to prehistoric times (Boyd et al. 1964). Evidence suggests that sheep were domesticated in the Middle East around 10,000 years ago and they were brought to the UK by Neolithic farmers around 6,000 years ago (Balasse et al. 2019). Despite the long history of sheep grazing, it only became widespread in the UK during the medieval period, particularly in areas with poor soil that was unsuitable for arable farming (Ratcliffe 1984). Sheep were then gradually grazed on upland areas such as blanket bogs, which provided natural grazing land. Sheepwalks were also established in these areas, and shepherds were employed to manage the flocks (Gosset 2017). The wool trade became a major industry during this time, with the finest wool coming from areas such as Cotswolds and other areas of central regions of England (Rose 2017).

In the 19<sup>th</sup> and 20<sup>th</sup> centuries, the demand for wool increased significantly, and sheep farming became a more intensive industry. Meanwhile, sheep grazing in upland areas including blanket bogs also became more intensive, with larger flocks and more widespread grazing practices in the following century (Fig. 1.12D). By 1986, around 71% of peatland had been stocked at a rate greater than its maximum bearing capacity (two sheep per hectare) (Holden et al. 2007b). However, such overgrazing can have a considerable impact on the biodiversity and ecosystem services supplied by blanket bogs through vegetation variation from *Sphagnum*-dominated towards more vascular (e.g., heather) species (Ward et al. 2007). It can also be impacted directly by sheep trampling which may initiate and enhance peat erosion (Evans and Warburton 2011). For example, heavy grazing on blanket bogs can not only reduce *Sphagnum* species (and their cover) (Milligan et al. 2018), but also increase the abundance of *Molinia caerulea* and *Eriophorum vaginatum* (Shaw et al. 1996). Sheep trampling and associated tracks can significantly increase overland flow production (Holden et al. 2007a) and the subsequently eroded peat can therefore increase sediment loads (e.g., particulate organic carbon) into watercourses, reducing water quality (Bonn et al. 2016).

As a result, the UK government introduced a series of policies during the past century to promote the conservation and restoration of degraded blanket bogs (Caporn et al. 2018). These policies included reducing grazing pressure on the bogs, particularly during the breeding season, and restoring damaged areas through strategies such as blocking drainage ditches and planting vegetation (e.g., *Sphagnum* mosses) (Rosenburgh 2015, Marrs and McAllister 2020). Today, grazing on blanket bogs is often managed through agreements between farmers, landowners, and conservation organizations, to ensure that the bogs are protected while also maintaining the viability of sheep farming in upland areas (Riesch et al. 2019).



**Figure 1.12** Management practices in blanket bogs in the UK: (A) prescribed burning of heather (B) alternative cutting of heather with brush left on site; (C) drainage (<https://www.fas.scot/downloads/practical-guide-managing-peatlands-and-upland-habitats/>) and (D) sheep grazing (<https://meatpromotion.wales/en/news-industry-info/welsh-hill-sheep-breeders-take-part-in-a-pioneering-environmental-project>) (Photos: A and B from A. Heinemeyer, while C and D from online).

### 1.1.10 Blanket bog conditions

In the UK, the definitions of blanket bog condition seem arbitrary and ill-defined (Ashby and Heinemeyer 2021), and it is a complex picture due to various (and often historic) land use and management impacts (e.g., afforestation, drainage, grazing) (Evans et al. 2017). According to the degree of damage or the extent of impacts from human activities, blanket bogs in this study were generally classified into three categories (based on some ecological yet often uncertain or arbitrary criteria): intact blanket bog, modified blanket bog and degraded blanket bog.

**Intact blanket bog:** refers to blanket bogs that have remained largely untouched by human activity and are in their (near) natural state (often and in this study therefore referred to ‘near natural’). These peatlands usually have thick layers of peat that have accumulated over thousands of years, often in cool and wet climates with high rainfall, where *Sphagnum* mosses are often the dominant plant species. Intact bogs play a crucial role in carbon sequestration, acting as significant long-term carbon sinks, thereby helping to mitigate climate change (Lindsay 2010). Furthermore, they are supporting crucial hydrological functions, acting as natural water reservoirs and releasing it gradually. This capacity helps regulate water flow, potentially alleviating flooding downstream (although the evidence is unclear; see Allott et al. 2019) and maintaining drinking water supply and good water quality (Bonn et al. 2016). Intact blanket bogs are also essential habitats that support specialized plant and animal communities (Howson et al. 2021a).

**Modified blanket bog:** refers to blanket bogs that have been evidently altered or disturbed, to some degree but not seriously, by human activities (e.g., drainage, peat extraction, afforestation, grazing) or natural processes (e.g., wildfire, climate change). Unlike intact blanket bogs, which retain their characteristic features, modified blanket bogs have undergone changes that may have impacted their structure, hydrology and ecological functions. Nonetheless, they may still play a positive role in carbon sequestration and water regulation, but likely to a lesser extent compared to intact blanket bogs (notably data to support this are often lacking as pointed out by Ashby and Heinemeyer 2021; Heinemeyer et al 2023).

**Degraded blanket bog:** refers to blanket bogs that have undergone significant deterioration or decline in their ecological condition. Degradation often occurs when a blanket bog experiences severe and persistent impacts that disrupt its natural processes,

diminish its biodiversity, and impair its ecological functions (Bonn et al. 2016). It can be caused by various factors, which include above mentioned reasons (e.g. drainage). The most common characteristics of degraded blanket bogs are loss of peat and/or carbon storage, reduced water retention ability and quality, loss of specialized plant species such as *Sphagnum* moss. In general, they are most likely playing a negative role in carbon sequestration and water regulation and to a much larger extent than in modified blanket bogs (Lindsay 2010).

### **1.1.11 Restoration of degraded blanket bogs**

As mentioned above, degradation of blanket bogs in the UK has been a common phenomenon and caused by a range of factors, including drainage, peat extraction, overgrazing, and burning (McCarroll et al. 2016). These practices can have significant negative impacts on the ecosystem, such as the loss of carbon storage capacity, loss of biodiversity, and changes in hydrology (e.g., reduction of water quality) (Bain et al. 2011). To restore degraded blanket bogs, a series of strategies have been developed mainly during the past century in a bid to address the specific causes of degradation and promote the restoration of ecosystem function and biodiversity (Parry et al. 2014, Cuckston 2017, Caporn et al. 2018). Some of the strategies commonly applied are described as follows:

**Blocking drainage ditches:** it is an effective and commonly used technique for restoring degraded peatlands. This strategy involves the construction of dams or other structures to prevent water from flowing out of the bog. By blocking the ditches, the water table is allowed to rise, which promotes peat accumulation and the re-establishment of vegetation. The use of heather bales, peat turves (Fig. 1.13A), or plastic piles is a common method for blocking drainage ditches (Armstrong et al. 2009).

**Gully blocking/reprofiling:** gully blocking is similar to drainage ditches blocking, aiming to rewet the adjacent peat mass by raising water tables, stabilizing peat erosion and reducing carbon loss from the gully system. The use of heather bales (Fig. 1.13B), wooden dams (Fig. 1.13C), low stone walls (Fig. 1.13D), and plastic piling has been the common gully-blocking method. Sediment deposition behind the blocks create locations for the colonization of common blanket bog vegetation such as cotton grass. By contrast, gully reprofiling is a process that involves modifying the shape and

structure of an existing gully in order to improve its stability and reduce erosion. It is usually carried out where the sides of gullies are too steep to stabilize the peat and vegetation. The commonly used technique is to fill in parts of the gully with peat turves from the surrounding area to create a more gradual slope. Wherever needed, bare peat is often revegetated by seeding with a nurse crop and also with heather seed from a mulch of cut heather (Artz et al. 2018).

**Revegetation:** it is another important strategy for the restoration of degraded peatlands. This strategy involves the promotion of the re-establishment of vegetation cover, which can help to stabilize the peat (particularly the bare area) and provide habitat for a range of species. Reseeding with native vegetation is a common technique for restoring vegetation cover. This involves the use of seed mixed containing a variety of plant species, including heather, cotton grass, and bog myrtle. Transplanting of *Sphagnum* mosses and allowing natural regeneration to occur are other techniques used for restoring vegetation cover (Bonn et al. 2016).

**Reducing grazing pressure:** overgrazing by livestock can damage the vegetation cover and lead to soil erosion. Rotational grazing is a common technique used to reduce grazing pressure (Bonn et al. 2016). This involves moving (or excluding) grazing animals around the landscape to prevent overgrazing in any one area. Rotational grazing can help to maintain the ecological health of the bogs while still allowing livestock to graze.

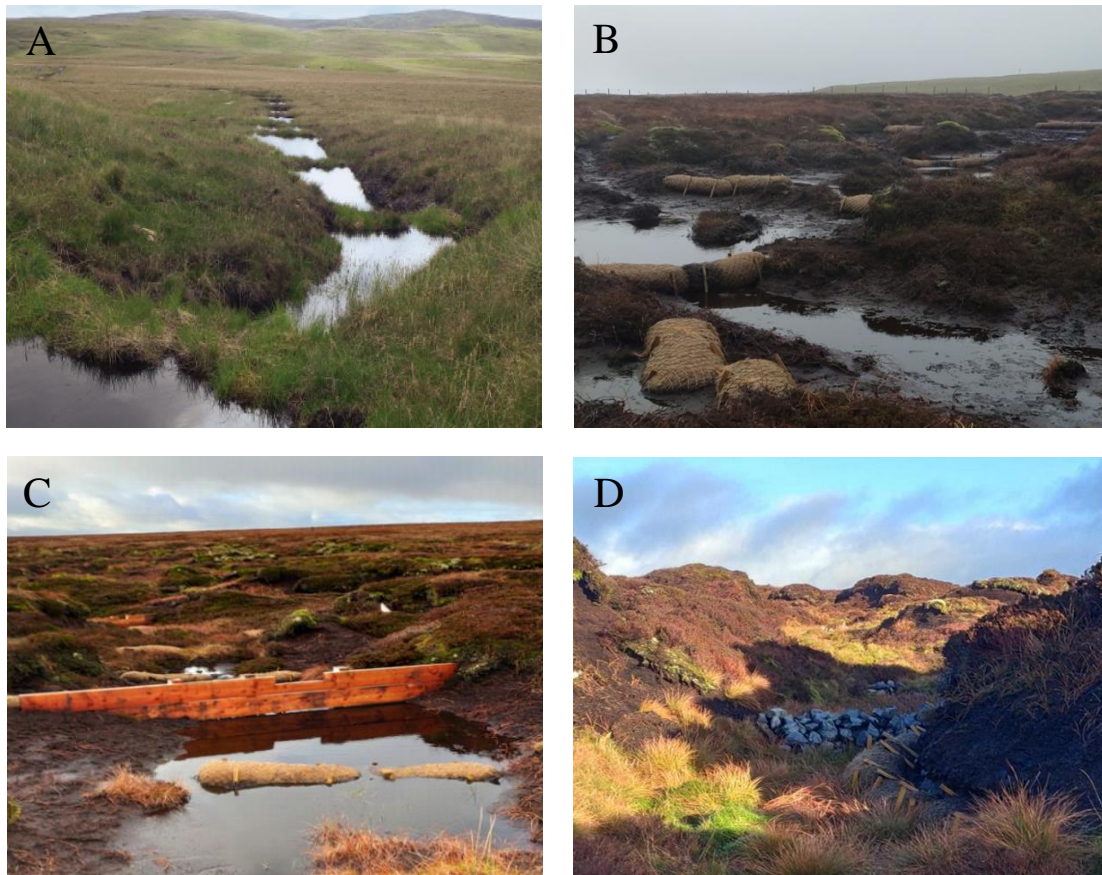
**Control of invasive species:** invasive species can threaten the biodiversity of peatlands by outcompeting native vegetation. Mechanical removal and the use of herbicides are common techniques for controlling invasive species, although this technique must be carefully managed to prevent further degradation of the peat.

**Sustainable land and vegetation management practices:** this strategy includes reduced fertilization and the use of organic fertilizers, as well as the use of rotational grazing to prevent overgrazing in any one area. Sustainable land management practices can help to maintain the biodiversity and ecological integrity of blanket bogs while still allowing for economic uses of the land. In addition, the use of this strategy can help to prevent further degradation of the bogs. Although it is still unclear what type of heather management (i.e., burning or cutting) is a better tool to support restoration of heather-

dominated peatlands, there are clear indications that either management will lead to some benefits, but that impacts/benefits are site dependent and need to consider long-term impacts and wildfire aspects (Heinemeyer et al. 2023).

Noticeably, restoration of degraded blanket bogs in the UK has made significant progress in recent years. For example, the Pennine PeatLIFE project, which aims to restore 5,000 hectares of degraded blanket bog in northern England, has already restored over 2,000 hectares of blanket bog since it began in 2017 (Jones 2023). The UK government (England Peat Action Plan) has also set a target to restore 35,000 hectares of degraded peatland (including blanket bogs) by 2025 (Pike 2021). However, despite the progress made in restoration during the past decades, there are still several challenges that need to be resolved. For example, many blanket bogs in the UK are located in upland areas that are difficult to access and work on (Andersen et al. 2017). In addition, the complex nature of peatland ecosystems means that restoration efforts can be unpredictable, and there is a need for ongoing monitoring and evaluation to assess the effectiveness of different techniques (Parry et al. 2014).





**Figure 1.13** Restoration of degraded blanket bogs in the UK: (A) ditch-blocking with peat turves (<https://www.iucn-uk-peatlandprogramme.org/projects/dungonnell-blanket-bog-catchment-management-plan-0>) (B) gully-blocking with heather bales; (C) gully-blocking with wooden dams (D) gully-blocking with stone walls (Photos: A from online, while B, C, and D were taken by Bing Liu when sampling peat cores).

## 1.2 Literature review

### 1.2.1 Modern ecology of TA

The earliest research on testate amoebae (TA) across the world can date back to the early 19<sup>th</sup> century (1815) when Leclerc described the genus of *Diffflugia*. From then on, more and more researchers started to notice and focus on the study of this microorganism (Greenwood 1886, Calkins 1926). In the beginning, researchers mainly concentrated on the work of morphological description and species identification/classification (Leidy 1879, Penard 1890, Cash and Hopkinson 1909). With the improvement of micro-techniques such as scanning electron microscopes, it greatly boosted the species discovery and description of TA (Burdman 1919). It was

not until the late 20th century that Ogden et al. (1980) published the first atlas of freshwater TA. Not long after that, modern molecular biology technology has also been applied in the study of TA taxonomy and biodiversity (Wanner et al. 1997, Lara et al. 2007, Lara et al. 2011, Kosakyan et al. 2013). Study of TA ecology and biogeography were then broadly carried out all over the world in all kinds of ecosystems, particularly in peatlands (Beyens et al. 1992, Wilkinson 1994, Charman and Warner 1997, Mitchell et al. 2000).

The first study of TA in peatlands was conducted by Harnisch in 1927, in which the author described the distribution characteristics of TA under four different water conditions (Harnisch et al. 1927). In 1936, Jung qualitatively investigated the relationships between TA and eight categories of peats by ranking the peat with different moisture content, the F-scale (Jung 1936). In the late 20<sup>th</sup> century, with the improvement of experimental methods and statistical techniques, a growing number of studies started to quantitatively explore the relationship between TA and environmental factors (Warner 1987, Tolonen et al. 1992, Charman and Warner 1997). Among the factors, water table depth or substrate moisture has been consistently reported to be the main control in determining TA establishment and distribution in *Sphagnum*-dominated ombrotrophic (i.e. raised bog) peatlands (Charman and Warner 1992, Mitchell et al. 1999, Booth 2001, Payne et al. 2006). By contrast, in minerotrophic peatlands (fens), despite the importance of hydrology, other factors like plant functional type composition, hydrochemistry (e.g., pH, nutrient content) play a more important role on TA community structure (Opravilová and Hajek 2006, Jassey et al. 2014). In recent years, *Sphagnum* metabolites, such as phenolic compounds, were also reported to be highly related to TA communities (Jassey et al. 2011a, Jassey et al. 2016).

In addition, during the past three decades, TA have also been largely investigated in response to a variety of external environmental stresses such as sulphur (Payne et al. 2010), ozone (Payne et al. 2012), nitrogen (Mitchell and Gilbert 2004), CO<sub>2</sub> (Mitchell et al. 2003), heavy metals (Nguyen-Viet et al. 2008), particulate pollutants (Meyer et al. 2010) and fire (Qin et al. 2013). All of them showed sensitivity of TA to these environmental changes and this will be summarized in more detail in the below section (1.2.3). It is noteworthy that studies investigating the ecology of TA in peatlands mainly focused on analyzing TA communities dwelling in *Sphagnum* mosses (Mitchell et al.

1999, Booth and Zygmunt 2005, Qin et al. 2013, Mazei et al. 2017, Liu et al. 2019), with only few investigated TA from other vegetation types, for example, mainly other mosses (Booth 2001, Mitchell and Gilbert 2004, Mieczan 2009, Lizoňová and Horsák 2017).

## **1.2.2 Seasonal and vertical variation of TA in *Sphagnum* moss**

### **1.2.2.1 Seasonal changes**

Seasonal studies of TA communities have considerably increased since the late twentieth century (Schonborn 1982, Lousier and Parkinson 1984, Schönborn 1986, Jax 1992, Jax 1996). However, only a few of them investigated TA by analyzing *Sphagnum* mosses from the peatland ecosystem (Heal 1964, Gilbert et al. 2003, Warner et al. 2007, Song et al. 2018).

Heal (1964) conducted the first study to assess the population dynamics of TA species (*Nebela tinctoria* type, *Hyalosphenia papilio*, and *Amphitrema flavum*) on *Sphagnum* moss in a British peatland (a valley bog) across a whole year. The result showed that all three species reached their greatest numbers during the spring-summer season (Heal 1964). Similar seasonal patterns of TA abundance were reported in the following studies carried out in Europe (Gilbert et al. 2003, Mieczan 2007, Marcisz et al. 2014b) and Asia (Song et al. 2018). All these studies attributed seasonal patterns to the favorable hydrological conditions during this period. Interestingly, Lamentowicz et al. (2013) revealed a bimodal distribution of TA density among seasons along an altitudinal gradient in Switzerland, with the first peak in the spring and the second in the summer or autumn which depends on the sites included in the analysis. However, in a study carried out in North America, no clear seasonal change of TA abundance was found among the peatlands (Warner et al. 2007).

As for species richness or diversity, studies also showed distinctive change patterns among seasons (Mazei and Tsyganov 2007, Lamentowicz et al. 2013, Marcisz et al. 2014b, Song et al. 2018). For example, Mazei et al. (2007) reported an increase pattern of TA species richness over the growing season while Lamentowicz et al. (2013) showed an inconsistent change pattern among sites over the same time period. Moreover, species diversity was found not substantially changed by Mazei et al. (2007)

but significantly dropped from spring to summer by both Marcisz et al (2014) and Song et al. (2018). TA community composition was also reported either significantly changed among seasons in open bog/fen which experienced considerable hydrological changes or not clearly changed in swamp where both soil moisture and water table appeared constant over the course of sampling period (Warner et al. 2007, Marcisz et al. 2014b).

While TA encystment status is potentially to be important in the functioning of their role in ecosystem processes, only few studies have investigated their seasonal change patterns to date (Heal 1964, Gilbert et al. 2003). As mentioned above in the study of Heal (1964), encystment status of TA was also recorded for two of the studied species (i.e., *Nebela tincta* type and *Hyalosphenia papilio*). The change pattern of them was shown to be largely different. Specifically, the numbers of encysted *Nebela tincta* type only slightly increased from late autumn to early of winter and dropped towards January, while encysted *Hyalosphenia papilio* dramatically decreased from middle of autumn to early of winter but slightly increased towards January. Moreover, a large proportion of encysted *Hyalosphenia papilio* also occurred in February and March (Heal 1964). Interestingly, Gilbert et al. (2003) found that the total encysted individuals of TA reached their highest numbers in April and lowest in the summer period, however, noticeably, no data was available during the winter period in their study.

#### **1.2.2.2 Vertical changes**

Studies of TA vertical distribution in *Sphagnum* moss can date back to 1945 when Heinis described the TA assemblages in the top 10 cm of *Sphagnum* segment (Heinis 1945). Although various vertical sampling intervals have been employed in the following studies, for example, Booth (2002) studied the vertical distribution of TA by qualitatively separating green as upper and brown as lower portion of *Sphagnum* stem, while Mitchell et al. (2004) quantitatively divided the *Sphagnum* segment into intervals of 0-1 cm, 1-3 cm, and 3-5 cm, a clear vertical change pattern of TA has been found across all these investigations (Heal 1962, Booth 2002, Mitchell and Gilbert 2004, Mazei and Tsyganov 2007, Jassey et al. 2011a).

Overall, except in extremely dry microhabitats, studies have found at least three general variation patterns of TA along the segment of *Sphagnum* moss. Specifically, in the

upper green segment, mostly refer to the top 3 cm, mixotrophic TA that contain symbiotic zoochlorellae, such as *Amphitrema flavum*, *Hyalosphenia papilio* and *Heleopera sphagni*, are usually more abundant mainly due to the requirement of light by their symbionts for photosynthesis (Heal 1962, Mazei and Tsyganov 2007). However, the mixotrophic taxon, *Amphitrema wrightianum*, is more often observed in the middle segment (approximately from 4 to 10 cm), which have been considered as a compromise between light requirement and suitable materials (e.g., peat or mineral particles) for test construction (Chacharonis 1954, Mitchell and Gilbert 2004). By contrast, agglutinate TA that generate shells by incorporating foreign materials such as sand grains, and diatom frustules are generally more common in the lower segment of the *Sphagnum* moss as the quantity of these foreign matters increases with depth along the segment, with only little being present in the upper portions (Heal 1962, Mieczan 2009, Roe et al. 2017).

Additionally, some other species like *Assulina muscorum* which has xerophile tendencies are usually found abundant in the upper parts of *Sphagnum* moss, whereas species that have hygrophile tendencies such as *Nebela militaris* and *Heleopera sylvatica* type are often restricted to the lower segment (Heal 1962, Booth 2002, Mitchell and Gilbert 2004). It seems likely that materials for the construction of siliceous shells are possibly also more abundant in the lower portions (Chacharonis 1954, Booth 2002). Moreover, another study found that spined taxa of *Euglypha* such as *Euglypha strigosa*, are generally more common in lower wetter portions of *Sphagnum* moss while spineless species are usually preferential to drier habitats (Bobrov et al. 1999). Interestingly, despite the fact that most *Nebela* species were found more frequent in the lower segment, *Nebela tinctoria* (more likely refer to the *Nebela-collaris-bohemica* group) was more often observed in upper portions of *Sphagnum* moss, which may be highly dependent on the availability of their food sources as they commonly prey on other smaller TA (primarily *Euglypha* and *Assulina* spp) and diatoms (Booth 2002).

As for species richness and/or diversity and abundance, studies consistently showed that all of them increased with depth along the *Sphagnum* stem (Booth 2002, Mitchell and Gilbert 2004, Mieczan 2009). This has been mostly explained by the vertical transport of TA after death and/or growth of the moss, which eventually incorporates

some of the taxa occurring predominantly on upper parts of *Sphagnum* moss into the lower communities (Booth 2002, Roe et al. 2017). Conversely, the proportion of living TA was found to be substantially higher in the upper regions (around 75%) than in the lower sections (about 25%), which suggested that upper communities are likely to be more representative of the environmental conditions at the time of sampling (Booth 2002, Mazei and Tsyganov 2007).

### **1.2.3 TA as bioindicator in peatlands**

During the past few decades, TA have been increasingly and successfully used as bioindicators in a wide range of contexts from monitoring ecosystem restoration to fire history, climate change and particularly environmental pollution (Gilbert et al. 1998, Wanner and Xylander 2003, Marcisz et al. 2014a, Swindles et al. 2016, Creevy et al. 2018). Before making a list of what is known about their application as bioindicators, it is worth knowing firstly what is meant by bioindication? According to O'Brien et al (1993), bioindication is a term used frequently, yet is rarely defined, and it is usually used in different contexts in different ways to mean quite different things. However, in a broad sense, the aim of bioindication is simply the use of biota as indicators of a particular 'state' and they can roughly be divided into three categories, which are, environmental, ecological, and biodiversity indicators, though they were considered somewhat overlapping (McGeoch and Chown 1998, Payne 2013).

The investigation of TA as bioindicators in peatlands has a history probably as long as the study of these organisms themselves (Harnisch et al. 1927). As mentioned above, in the early stage, the interests were mainly concentrated on the exploration of relationships between TA (species richness, abundance, community composition) and environmental factors, with water table depth (WTD) and/or soil moisture and pH being consistently reported to be the primary controls (Warner 1987, 1991, Tolonen et al. 1992). Due to their sensitivity to WTD, TA-based transfer functions were also developed and applied successfully for the reconstruction of past hydrology change (Warner and Charman 1994, Charman 1997). After that, interest in exploring the potential of TA as peatland bioindicators increased drastically (Mitchell et al. 2003, Payne 2010, Koenig et al. 2018a). In general, the bioindicators aspects can be mainly summarized into four categories in relation to the response of TA to: 1) atmospheric and soil pollution (e.g., NO<sub>2</sub>, CO<sub>2</sub>, heavy metals, acid); 2) prescribed fire/wildfire (short

and long term); 3) climate change (temperature & water tables); 4) restoration/regeneration (e.g., grip blocking, tree felling, peat extraction).

### **1.2.3.1 Response of TA to atmospheric and soil elemental/nutrient pollution**

Gilbert et al. (1998) assessed the structure of the microbial loop in *Sphagnum fallax* and the effects of short-term nitrogen enrichment on the microbial communities. The results showed that TA were well represented in *Sphagnum* peatland (14% of total microbial biomass) and the high nitrogen input (50 kg ha<sup>-1</sup>) decreased the relative importance of TA (Gilbert et al. 1998). These results were also supported by his subsequent study through evaluating the impact of fertilization (PKCa and NPKCa) on the different microbial communities, with TA occupied 48% of the total microbial biomass and the supply of nutrients resulted in a decrease in the relative proportion of TA (Gilbert et al. 1998). Mitchell et al. (2003) then investigated the potential effects of the increasing atmospheric CO<sub>2</sub> concentration on the microbial communities in *Sphagnum* peatlands. A similar result was recorded that CO<sub>2</sub> concentration modified the structure of microbial communities and the biomass of TA decreased by 13% after the increase of atmospheric CO<sub>2</sub> concentration (almost double the amount than the control plot), which corresponds to the projected concentration for year 2120 (Mitchell et al. 2003).

Likewise, TA was also shown to be highly sensitive to heavy metals, especially for Pb and Cu. However, their response mechanisms seem different to some degree. In 2007, Nguyen-Viet et al. conducted a controlled Pb addition (0 (control), 625, and 2500 ug L<sup>-1</sup> of Pb<sup>2+</sup>) experiment to explore the impacts of lead pollution on the microbial community. The biomass of TA experienced a dramatic and significant decrease in both Pb addition treatments after 6, 12, and 20 weeks in comparison with the control (Nguyen-Viet et al. 2007). Not surprisingly, species richness and total density of TA were also recorded declined and changed over time in response to the Pb treatment in his subsequent paper. Furthermore, the species-specific response of TA to Pb contamination was also identified, with *Nebela carinata*, *Euglypha strigosa* and *Hyalosphenia sphagni* being the most sensitive ones (Nguyen-Viet et al. 2008). By contrast, the effects of copper concentration on the TA community showed an opposite change pattern in terms of abundance in the study of Asada et al. (2009). The abundance of TA was observed especially high in the area with high copper concentration (as high

as 16,000  $\mu\text{g/g}$ , with 4,550  $\mu\text{g/g}$  being the mean) even though species diversity was low (Asada and Warner 2009).

In addition, sulfate and sulphuric acid deposition were also reported to have a significant impact on TA community even though the samples extracted for TA analysis were seven and ten years after the treatment respectively (sulphuric acid and sodium sulfate) (Payne et al. 2010, Payne 2010). The result showed a significant decrease in the abundance of *Trinema lineare*, *Corythion dubium* and *Euglypha rotunda*, which were all regarded as bacterivores, with the input of sodium sulfate. This indicated a potential community shift via a microbial loop through feeding pathways due to functional group changes in the TA community, further influencing the ecosystem processes such as carbon and nutrient flow (Payne et al. 2010).

### **1.2.3.2 Response of TA to prescribed fire and wildfire**

The interests in exploring the potential of TA as bioindicators in relation to fire seems to only have emerged during the past two decades, with the first research conducted by Wanner et al. (2003) to investigate the impacts of small, short fires (low intensity) caused by routine shooting exercises in military training areas on TA community. However, no significant effects on abundance or biomass of TA had been revealed from the short-term (a few days post fire) and long-term (nine-12 months post fire) samples (Wanner and Xylander 2003). Moreover, the subsequent study carried out again by Wanner (2012) to track the immediate effects (one hour before and after) of short-term (within minutes) prescribed burning (controlled small scale burning over one hectare with air temperatures above the soil surface between 500°C and 800°C) on TA community in a continental *Calluna* heathland corroborated the author's previous study and highlighted the effectiveness of prescribed burning (transient fires) as a useful tool for habitat management without affecting soil TA communities (Wanner 2012). However, the investigation by Qin (2017) in a nutrient poor fen experiencing intense wildfire, which destroyed much of the surface vegetation, revealed a contrasting result. TA community composition was observed to have re-organized or changed greatly three days after the end of fire, primarily by a shift from taxa with tests constructed of idiosomes to those built of xenosomes, with the most likely explanation by the direct destruction of many idiosome tests due to extreme heat (Qin et al. 2017).



Meanwhile, in a more comprehensive study, Turner et al. (2012) clearly recorded marked differences in TA communities among the samples collected from three zones (moorland) with different fire intensity, that is, zone A (strong intensity - large uncontrolled wildfire), zone B (low intensity - traditional grouse shooting heather moorland management practice), and zone C (unmanaged wet moorland). The taxon *Hyalosphenia subflava* was also observed as a clear indicator of severe disturbance, which was found abundant in the peat surface following a severe wildfire event (Turner and Swindles 2012). Thus, the type of the fire (size, intensity, duration and severity) has consistently been considered to be one of the most important factors controlling the effects of fire on the TA community (Turner and Swindles 2012, Wanner 2012, Qin et al. 2017).

With the functional traits of TA such as metabolism type (mixotrophy and heterotrophy), aperture and body size increasingly being applied in TA research in the past few years, so far only two studies seem to have been conducted using a trait-based approach to investigate the potential of TA (taxonomy and functional traits) as bioindicators in response to fire by virtue of peat core evidence (Marcisz et al. 2016, Marcisz et al. 2019). Both of them suggested that TA functional traits may serve as a good proxy of past environmental changes as traits like mixotrophy, small hidden apertures (plagiostomic apertures) and test types (idiosomes and xenosomes) were shown to be strongly correlated with fire history, for example, some of TA which are common before fire, mainly mixotrophs and those with proteinaceous tests, can temporarily re-appear after fire (Marcisz et al. 2016, Marcisz et al. 2019).

### **1.2.3.3 Response of TA to climate related factors**

Although many studies have shown that TA are sensitive to climate-related factors like temperature (seasonal research) and precipitation (WTD or moisture), hardly any experimental studies have been carried out to test the effects of temperature and hydrological changes on TA communities until recent years (Lamentowicz and Mitchell 2005, Marcisz et al. 2014b, Andrews et al. 2022).

Jassey et al. (2011) conducted the first controlled microcosm experiment (10, 20, and 25°C) using sixteen *Sphagnum (fallax)* peat cores (25 cm x 15 cm) for eight growing weeks' time to investigate the effects of a temperature gradient on TA communities to

assess their potential as bioindicators in response to climate change. The results showed that the community composition of TA changed in the warmed treatments, and an increased trend of their density and biomass was observed in *Sphagnum* upper segments (0-3 cm of the green capitulum). Moreover, the biomass of TA was also shown to be highly related to the biomass of bacteria and to the total biomass of other groups (Jassey et al. 2011b).

After that, Koenig et al. (2018) conducted a similar study to explore the impacts of two hot and dry summers on the community structure and functional diversity of TA. In addition to significant changes in community composition, the species-specific response of TA to relatively high temperature were also observed, with smaller taxa (*Corythion dubium*, small *Euglypha* sp.) that are indicative of dry conditions increased and mixotrophy (i.e., with carbon fixation potential) decreased in the two hot and dry summers (Koenig et al. 2018a). In the same year, Koenig et al. (2018) carried out the first hydrology-controlled study to investigate the effect of manipulated water table depth (wet: -4 cm, intermediate: -15 cm, and dry: -25 cm) on TA assemblages over time using a mesocosm approach. The results confirmed the importance of water level as a strong environmental factor in controlling TA community change. Specifically, after one year, the typical mixotrophic TA (*Hyalosphenia papilio*) decreased in the dry treatment and the community composition shifted towards a dominance of dry indicators (*Nebela tinctoria* complex, *Corythion dubium*, *Euglypha compressa*) (Koenig et al. 2018b).

Likewise, in the subsequent study conducted by Anna et al. (2020) assessing the response of microbial community biomass to warming and reduced precipitation in a peatland ecosystem, the biomass of *Hyalosphenia papilio* (Mixotrophy) was observed to decrease in either heated plots or the plots with reduced precipitation and the strongest reduction was further observed in plots where warming was combined with a reduction in precipitation (Basińska et al. 2020).

#### **1.2.3.4 Response of TA to restoration**

Two approaches (i.e., peat core versus modern surface sample) have mainly been applied in exploring the potential of TA as bioindicators for peatland restoration and regeneration, that is, 1) using TA from peat cores to investigate water table changes in

the recent past (few decades) or long time-scale (hundreds and even thousands of years); 2) using modern surface samples to monitor current conditions following management (e.g. ditch blocking, tree-felling). In general, all these studies showed a positive outcome of using TA as a sensitive new way for monitoring habitat changes in peatlands with respect to restoration or regeneration using either method (Buttler et al. 1996, Davis and Wilkinson 2004, Valentine et al. 2013, Swindles et al. 2016, Creevy et al. 2018).

In order to understand the dynamics of TA in the process of regeneration following peat extraction, Buttler et al. (1996) and Laggoun et al. (2008) analyzed the TA communities from the peat cores (monolith of secondary peat that was cut to the depth of the old cutover surface) and surface samples (time series - 0, 29, (51-58) years), respectively. Both suggested that TA communities changed significantly over the succession but experienced a similar convergence pattern regardless of the initial conditions. Specifically, (i) the species richness and diversity increased but density declined from the recent to the advanced regeneration stages; (ii) the biomass and average size of species declined but were higher at the reference site (unexploited site) (Buttler et al. 1996, Laggoun-Défarage et al. 2008). Meanwhile, the sensitivity of TA, especially some indicator species in response to environmental change (e.g., dry or wet event) was further revealed by another two peat core studies relating to the restoration of damaged bogs. The results showed that the species indicative of dry conditions disappeared, or their numbers decreased after the restoration, while cooler and/or wetter conditions promoted the resurgence of some hygrophilous taxa like *Amphitrema wrightianum* (Jauhiainen 2002, Davis and Wilkinson 2004).

As restoration work has been implemented increasingly over recent years across the damaged British peatlands (drained and/or harvested bogs), this creates a good opportunity to monitor the recovery status of the degraded upland blanket bog peatland and further assess the effectiveness or successfulness of the restoration strategies (Wilson et al. 2011a, Parry et al. 2014, Andersen et al. 2017). In 2016, Swindles et al. (2016) conducted the first study to evaluate the use of TA as indicator of blanket peatland restoration by comparing the TA communities in three treatments (i) control (unblocked), (ii) re-profiled (dammed and re-profiled) and dammed (with pools of open water behind each dam). The results highlight the potential of TA as bioindicators of

peatland restoration success as a considerable change of TA communities in response to ditch blocking were observed, and this was especially significant in samples from the dammed and reprofiled treatments. Moreover, the same study showed an increasing trend in the diversity and the wet-indicator species (including *Amphitrema stenostoma*, *Archerella flavum*, *Arcella discoides* type, *Diffflugia bacillifera* and *Diffflugia bacillarum*) also appeared unambiguously in the recovery sites, reflecting a shift towards wetter conditions. Interestingly, WTD was not recorded as a significant impact factor on the TA community in this sloping blanket bog, which suggested a poor relationship between surface moisture and WTD. However, this poor relationship is likely to be correlated with the low water table across the sites before blocking and saturated conditions usually lead to frequent overland flow due to its steep slope in blanket bogs. Finally, the study suggested that an indicator species approach is probably more appropriate for monitoring the early stages of peatland restoration than examining change at the community level (Swindles et al. 2016).

At the same time, another influential study carried out by Payne et al. (2016) investigated the impacts of tree encroachment on peatland TA communities using ten transects of 30-45 m length across the forested to open bog ecotone, with the results probably indicated a functionally significant change in TA community, that is, the tree encroachment reduced and even lost the mixotrophic TA (e.g. *Hyalosphenia papilio*, *Archerella flavum*) while increased the heterotrophic, especially smaller bacterivorous TA (e.g. *Trinema lineare*, *Corythion dubium*). Furthermore, TA from the open bog end of the transects generally had larger apertures, indicating larger prey and higher trophic position, which is likely to have a considerable impact on ecosystem processes (e.g., carbon and nutrient cycling) (Payne et al. 2016). This result was then confirmed by Creevy et al. (2018) in investigating the potential of TA as a functional significant bioindicator in forest-to-bog restoration. As for why they changed in this pattern, both suggested that light conditions, moisture content and availability of food resources are likely to be the main controls. It has been known for some time that MTA are highly sensitive to light conditions. With the growth of trees, the ground-level light level will be reduced due to the dense plant cover. Thus, not surprisingly, MTA such as *H. papilio* and *A. flavum* were almost only found in undisturbed open bog as they cannot survive without photosymbionts and require light to survive. Moisture content has been reported to be a significant control on TA in forested peatlands, with larger taxa tending

to live in wetter conditions, vice versa (Charman and Warner 1992). Therefore, it is perhaps not surprising that large taxa were the most dominant ones in the open bog sites as the open bog was recorded to be wetter and less acidic compared to the forested sites (with lower water tables due to drainage and high evapotranspiration losses), and the available food sources with larger food items such as microalgae and ciliates are likely to be more abundant in the open peatland (Payne et al. 2016, Creevy et al. 2018).

## **1.2.4 Functional roles of TA in ecosystem processes**

### **1.2.4.1 TA's potential contribution to carbon and nitrogen cycling**

While TA have been increasingly inferred to be an essential component in the process of carbon and nitrogen cycling, mostly in forest systems and much less so in peatlands, we still only have limited understanding of their functional roles and the mechanism that they interact with other microbial groups such as microalgae, bacteria, fungi, nematodes, and other protists (e.g. flagellates, ciliate) (Wilkinson and Mitchell 2010, Lamentowicz et al. 2013, Jassey et al. 2015). In terms of exploring the role of TA in ecosystem processes, the key requirements are probably the quantitative data on their occurrence in different habitats and their interactions with other microbial groups. In general, all studies can be mainly divided into four stages, which are, 1) early work up to the 1960s; 2) key studies from 1960s to the 1980s; 3) post 2000 and 4) recent work on C and N cycles.

#### **Early work up to the 1960s**

The first quantitative study of soil TA was conducted by Volz et al. (1934) who investigated the communities of TA in two forests (i.e., *hornbeam-ash* vs *pine-beech*). The results showed a higher density of TA ( $20.7 \times 10^3$  individuals g dry soil<sup>-1</sup>) in the mixed hornbeam-ash forest than in the pine-beach mixed forest ( $3.6 \times 10^3$  individuals per g dry soil) in the top centimeter of the soil. Moreover, a sharp decline in density with depth was also observed between 3 and 4 cm depth in the hornbeam-ash forest ( $3.4 \times 10^3$  individuals per g dry soil). Over the next 20 years, quantitative studies relating to TA seem to have disappeared until 1963, Heal et al. compared the TA communities in three deciduous woodlands in the UK. They recorded a much higher density of TA in the forest with high trees than in the two coppices from the top

centimeter samples and the density decreased sharply with depth as well. This study also suggested that humus with a higher organic matter content contain higher density of TA (Heal 1963). Following that, Heal et al. (1964) investigated the TA communities in a valley bog in northern England during the summer and found  $50-100 \times 10^6/m^2$  testates (Heal 1964).

### **Key studies from the late 1960s to the 1980s**

A big improvement in relation to TA's productivity (i.e., seasonal change in density), relationship with organic matter decomposition (litter versus humus layer), breakdown of tests (idiosome vs agglutinated tests) has been made during this period, with the main contribution from Chardez, Couteaux, and Lousier et al.

Chardez et al. (1970) investigated the density and biomass of TA in an Oak-Hornbeam Forest in spring, summer, and winter of two consecutive years. During the first year (1967), the samples were collected in February, April, and August, with the highest density ( $1.1 \times 10^3 \text{ ind. g}^{-1}$ ) being observed in August and comparable between February ( $0.8 \times 10^3 \text{ ind. g}^{-1}$ ) and April ( $0.9 \times 10^3 \text{ ind. g}^{-1}$ ). The samples were then taken in February, May, and December in the second year (1968). They found that the density was highest ( $2.3 \times 10^3 \text{ ind. g}^{-1}$ ) in May, lower in December ( $1.4 \times 10^3 \text{ ind. g}^{-1}$ ) and least in February ( $1.1 \times 10^3 \text{ ind. g}^{-1}$ ). Thus, both results indicated that densities could change quite strongly between and within the year and numbers are lowest at the end of winter but are likely to be high in the beginning of winter when the soil is covered by freshly fallen litter (Chardez and Krizelj 1970). At the same time, a similar seasonal investigation of TA's community was done by Couteaux et al. (1967) using a new method by direct counts on a weight basis. She counted the density of TA in the humus layer of an oak forest, with the total densities ranging from ca.  $5 \times 10^3 \text{ ind. g dry soil}^{-1}$  to ca.  $40 \times 10^3 \text{ ind. g dry soil}^{-1}$ . The lowest numbers were recorded in January and February and high values in April to December, with the highest density in November and December. She attributed the highest numbers in early winter to an input of organic matter from leaf fall, while the low values in winter are likely to be caused by the lowering of available moisture due to frost (Couteaux 1967).

Subsequently, Couteaux et al. (1972) compared the TA communities of the same oak forest done earlier with that of a spruce forest. In this study, she analyzed the

communities from the litter (L) and humus (H) layer separately. Interestingly and opposite to the hypothesis, the communities in the H layer from the two forests were very similar and the same was observed for the L layer. However, the communities differed between the L and H layer in terms of relative abundance of different taxa in both forests. Specifically, the density and Shannon diversity were both lower in the humus than the litter, which suggested that the micro-environmental conditions in the litter are likely to be more variable. Cousteaux also observed that species with compressed tests and those with an eccentric ventral aperture, which are assumed better adapted to the environment with a thin or variable water film, were more abundant in the litter layer whereas taxa with a terminal aperture or hemispheric ones were more abundant in the humus. Furthermore, the small taxa (size range 25-45  $\mu\text{m}$ ) were also recorded as the dominant species in both forests (Cousteaux 1972).

In terms of nutrient cycling, apart from the population size, the rate at which material from testates is made available to other organisms is clearly important. This probably can be achieved by predation on TA. Unfortunately, no quantitative data have been reported on this point though Chardez observed a few examples of predation on small TA by other large TA, ciliates, naked TA (Chardez 1985) and earthworms (Chardez 1992). However, Lousier (1981) quantified the rate at which empty TA was broken down in culture. The results showed a high rate of loss, with 74-94% of 'platelet' shells (self-secreting tests) lost after 1 week and 42-68% of shells constructed from particles (agglutinated tests) lost during the same period. Obviously, the decay rates of the two types of tests were significantly different, with the platelet shells experiencing an exponential decline, while the agglutinated shells had a linear rate of loss from the cultures (Lousier and Parkinson 1981). This difference between the two test types was also revealed apparent in the preservation of subfossil shells in peat bogs where more recent work has shown that there was a clear pattern of decreasing preservation of idiosome tests (platelet tests) with depth, but not for agglutinated tests (Mitchell et al. 2008b).

Based on these studies, Lousier et al. (1984) proposed a few assumptions with respect to the roles of TA may play in carbon and nutrient cycling, with the detailed suppositions as follows:

1. TA feed on other soil microbial groups and are therefore likely to modify the

structure/composition of the soil micro-food-web.

2. They speed up the turnover of both microbial biomass and soil organic matter.
3. TA may be involved in the decomposition of soil litter (i.e., plant remains) (the importance of this is still not clear due to our limited knowledge of their feeding habits).
4. They are likely to be important as prey for other organisms. (This point was later expanded on by Bamforth et al. (1995) who suggested that the key point is that TA may form an important link in the transfer of energy and nutrients from bacteria to animals-such as various types of “worms”).

### **Post 2000 and recent work on C and N Cycles**

Following the work in the 1970s and 1980s, studies in relation to the role of TA in carbon and nutrient cycling were rare during the 1990s until the PhD work of Schroter (2001, 2003). Schroter investigated the decomposer systems of four coniferous forests (mainly *Picea abies*) on a latitudinal gradient from NE France to northern Sweden. His results showed that the biomass of TA occupied on average 68.5% of the total biomass of ‘animals’ (no other group of protozoa was identified, and TA was treated as animals) and that the contribution of TA to carbon and nitrogen cycling was estimated to represent 79.9% and 96.5% respectively of that of all animals (Schröter et al. 2003). By contrast, a similar result was also reported by Mitchell et al. (2003) who analyzed the TA communities in five European *Sphagnum*-dominated peatlands, that is, TA occupied on average 59% of the biomass of animals (including heterotrophic protists) and 17% of the total microbial biomass (Mitchell et al. 2003). In addition, Schroder et al. (2003) suggested that the species richness of TA ranged from 34 to 40, apparently showing a decreasing trend with increasing latitude. Such a pattern is common in macro-organisms but has only been convincingly described in a small number of microbial groups (Sherratt and Wilkinson 2009). Notably, the nitrogen deposition from atmospheric pollution also declined with increasing latitude in the study area, which may modify the species richness and community composition of microbial groups including TA. Further results showed that mineralization rate was low at the more northern sites (low nitrogen) and the decomposer community was dominated by fungi. In contrast, the mineralization rate was higher at further south sites (higher nitrogen)



and bacteria played a prominent role. Thus, the importance of TA was thought to be in releasing nitrogen immobilised by bacteria (Schröter et al. 2003).

However, there may be a closer relationship between TA, fungi, and nutrient cycling than was presumed by Schroter et al. (2003). This was supported by the study of Vohnik et al. (2008) who has suggested a potential new role for TA in nutrient cycling in nutrient poor soils via mycorrhizal fungi, including ericoid mycorrhizae, where plants form a symbiosis in which the fungal partner aids the plant in the uptake of nutrients, especially nitrogen compounds (Aerts 2003), in exchange for carbon. Vohnik et al. (2008) analyzed the soils under three species of *Rhododendron* (shrubs in the Ericaceae) in a series of central European sites, with the results showing that an intimate connection existed between TA tests and the hyphae of the ericoid mycorrhizal fungi (Vohník et al. 2008). Specifically, they found that a large proportion of the shells (over 40%) of the genus *Trigonopyxis* was colonized by fungal mycelium. Notably, heather is also a strongly ericoid mycorrhizal plant on heathlands/peatlands (Leake and Read 1989; Heinemeyer et al 2019b). As *Trigonopyxis* are usually recorded as large species, they are presumably a good source of nutrients for the plants to access through their mutualistic fungal associates. However, it was not clear if the TA were dead before fungal colonization.

In recent years, more and more functional traits (e.g., mixotrophic TA, body/aperture size, test type) of TA have been revealed and applied in the exploration of TA's ecology and palaeoecology, many of which have also highlighted the potential and importance of TA in the energy and nutrient cycling (Jassey et al. 2013a, Jassey et al. 2015, Payne et al. 2016, Creevy et al. 2018). Traditionally, functional traits (FTs) are defined as traits that affect fitness (Violle et al. 2007) and they can be further divided into: (1) response traits (RTs) (e.g., shell biovolume, shell compression and aperture position) that reflect species response to environmental change and (2) effect traits (ETs) (e.g., mixotrophy and aperture size) that reveal species impact on ecosystem processes. In other words, shifts in RTs at the community level are inferred as being driven by changes in environmental conditions, while shifts in ETs may indicate changes in the process at the ecosystem scale. However, some traits can possibly also act as both response and effect traits, such as mixotrophy and shell biovolume. Some of the functional traits of TA and their potential ecological preferences and/or functional roles

were summarized as follows (Fournier et al. 2016, Marcisz et al. 2016, Koenig et al. 2018a):

- 1) Mixotrophy (RTs & ETs): the presence of photosymbionts is interpreted as an adaptation to wet oligotrophic conditions such as those existing in bog pools. Mixotrophy is both a response trait (MTA being indicative of wet conditions (Mitchell et al. 1999) and an effect trait (mixotrophs have been shown to enhance carbon fixation) (Stoecker et al. 2009, Jassey et al. 2015).
- 2) Test length (RTs & ETs): used as a proxy for biovolume. This is a response trait - larger taxa have longer generation times than small taxa and are thus expected both to be more sensitive to perturbation and to recover more slowly from perturbations, and thus can be viewed as K-strategists following Grime (1977). As larger taxa generally have a large pseudostome, this is also, to some extent, thought of as an effect trait.
- 3) Pseudopod morphology (RTs & ETs): filose or lobose. These equal two of the three phylogenetic groups distinguished in TA (Meisterfeld 2002a, Nikolaev et al. 2005, Gomaa et al. 2013): *Euglyphida* that have filose pseudopods and *Arcellinida* that have lobose pseudopods. *Euglyphida* include mainly smaller, bacteria-feeding taxa and are generally considered as r-strategists while *Arcellinida* include both smaller and larger taxa and are generally considered as K-strategists. The ratio lobose/filose has been shown to increase from early to late soil developmental stages and is thus used to define the maturity of ecosystems (Bonnet 1964).
- 4) Test material (RTs & ETs): agglutinate or autogenous. TA either agglutinate material from the surrounding environment (xenosomes, either prey skeleton re-used in test construction or inorganic particles) or autogenously secrete siliceous (idiosomes, which is related to Si-cycling) or proteinaceous tests (Meisterfeld 2002b). The availability of building material and/or the higher cost of self-secretion can constrain species distribution (Gilbert and Mitchell 2006, Schwind et al. 2016).
- 5) Aperture size (ETs): the pseudostome size is an effect trait as it determines the range of prey – taxa with a large pseudostome occupy higher trophic positions in microbial food webs (i.e., they are predators of other protists and micro-eukaryotes) while taxa with a small pseudostome are mainly bacterivorous (Jassey et al. 2013a).

- 6) Test compression (RTs): compressed or non-compressed. Compressed tests are believed to be better suited to live in low moisture conditions with a thin water film, but at a higher cost (more shell material compared to cell biomass), while non-compressed tests (spherical or round in cross-section) may be adapted to very wet conditions (Bonnet 1964).
- 7) Aperture position (RTs): central, sub-terminal or terminal. The position of the aperture is believed to represent an adaptation to moisture, where an exposed, terminal aperture is better adapted to wetter conditions, whereas a central, hidden aperture is better adapted to drier conditions (Bonnet 1964).

Among the studies using TA functional traits, the most attractive one is the work conducted by Jassey et al. (2015) who quantified the contribution of mixotrophic protists to carbon fixation in response to climate warming with a combination of field and microcosm experiments. The results showed that mixotrophs in the *Sphagnum* bryosphere play an important role in modulating peatland C cycle responses to experimental warming as the biomass of the dominant MTA reduced 50% in the five years of consecutive summer warming with peaks of +2 to +8°C. And in their microcosm experiment, they found the reported 50% reduction of MTA biomass in the field was linked to a significant reduction of C uptake (-13%) of the entire *Sphagnum* bryosphere. Thus, their findings indicated that decreased abundance of MTA with climate warming could lead to decreased peatland C fixation.

#### **1.2.4.2 TA's potential contribution to Si cycling**

Silicon (Si) is the second most abundant element in the Earth's crust; it is therefore not surprising that numerous organisms have been evolutionarily adapted to use Si for the synthesis of siliceous structures (called biosilicification), with idiosomic TA being one of them. In general, TA can be assigned to two subgroups according to their shell structure: (i) *Arcellinida* and (ii) *Euglyphida*. In terms of *Euglyphida*, they have filose pseudopodia and almost all of them are characterized by siliceous shells made up of self-synthesized silica platelets (idiosomes). Specifically, the idiosomes of TA are synthesized in so-called silica deposition vesicles in the cell cytoplasm and deposited on the cell surface by exocytosis, where they are bound together by organic cement (Anderson 1994, Anderson 1995).

While the silica cycle does not get the same prominence as the carbon or nitrogen cycle in most studies, it is crucial to the working of the global system. On geological time scales, silicate weathering is the main sink for atmospheric carbon dioxide (so linking to the C cycle) and on shorter time scales, leakage of silica from soils to aquatic systems is important for diatom primary production, which are able to fix large quantities of carbon dioxide via photosynthesis because up to 54% of the biomass in the oceans is represented by diatoms (Tréguer and De La Rocha 2013). Although research on phytogenic BSi (i.e., BSi synthesized by plants) have been established and developed in the past decades, studies investigating protozoic Bsi (i.e., BSi synthesized by TA) and their potential contribution to Si-cycling have only been mentioned at the beginning of 21<sup>st</sup> century (Clarke 2003, Cary et al. 2005, Conley et al. 2006).

After that, Aoki et al. (2007) conducted the first quantitative work on protozoic Si pools and calculated the BSi in the shells of different euglyphid TA taxa. Based on these results, they further calculated the annual biosilicification of idiosomic TA using data of annual mean population densities from literature, with the results showing that annual biosilicification of idiosomic TA was comparable to silica released by trees through litter (Aoki et al. 2007). Following this, Sommer et al. (2013) carried out a similar quantitative analysis, with a special focus on biogenic silica synthesized by trees and TA. They found that protozoic Si pools were small ( $1.9 \text{ kg Si ha}^{-1}$ ) compared to the average plant uptake of Si ( $35 \text{ kg Si ha}^{-1} \text{ y}^{-1}$ ) but biosilicification of idiosomic TA ( $17 \text{ kg Si ha}^{-1} \text{ y}^{-1}$ ) was comparable to the Si uptake by trees, which clearly corroborated the findings of Aoki et al. (2007) (Sommer et al. 2013).

Since then, more studies have been conducted, especially in recent years, to explore the contribution of idiosomic TA to Si cycling by analyzing and quantifying the Si pools and annual biosilicification rates in various environments, for example, in initial ecosystems (e.g. uncovered soil vs soil covered with initial vegetation, post sand-mined catchment) (Puppe et al. 2014, Creevy et al. 2016, Puppe et al. 2016, Puppe et al. 2017) and in disturbed soils (e.g. plantation, wildfire, heavy metals) (Krashevskaya et al. 2016, Haynes 2017, Qin et al. 2017, Wanner et al. 2020). In general, all the results highlight the importance and potential of idiosomic TA for Si cycles in terrestrial ecosystems, but we still lack clear and comprehensive understanding about their contribution and particularly the question on how long does it take for idiosomes to dissolve?

## **1.3 Summary of key knowledge gaps**

Based on the literature review four key knowledge gaps were identified:

- 1) Most TA ecological studies focused on analyzing *Sphagnum* moss samples, with only few investigated other vegetation types, for example, mainly other mosses. Little is still known about what type of vegetation samples are best for the study of TA to be used as hydrological indicators to appropriately indicate the site/habitat wetness.
- 2) While studies of TA seasonal change and vertical distribution in *Sphagnum* moss have been conducted in raised bogs and fens, no studies have been carried out in blanket bogs where vegetation cover is highly different from the abovementioned habitats.
- 3) Many UK upland blanket bogs have been or are under prescribed burning and restoration management, but little is known about how these interventions affect TA communities, and if they can be used as hydrological bioindicators to assess and monitor the long-term recovery trajectories and success.
- 4) While TA have been reported to play a crucial role in carbon cycling, nothing is known about their potential functional contributions to ecosystem processes over the course of recovery following prescribed burning and active rewetting strategies.

## **1.4 Thesis aims, objectives and structure**

### **1.4.1 Thesis aims**

The overarching aim of this study on British upland blanket bogs is to:

- 1) Assess the potential of TA to be used as hydrological bioindicators to assess the peatland habitat status and monitor recovery trajectories and/or success of restoring degraded blanket bog peatlands (comparing heather management and following restoration management).

The other two aims of this study in British upland blanket bogs are to:

- 2) Investigate the optimal sampling strategies of testate amoebae (TA) in indicating habitat/site wetness conditions in blanket bogs.

- 3) Explore the potential functional contributions of TA over the course of recovery following blanket bog vegetation management and restoration measures.

### **1.4.2 Thesis objectives**

The main objectives of this study on British upland blanket bogs are to:

- 1) Investigate TA communities among different plant types (*Sphagnum* moss, other mosses, litter of heather and sedge) on two heather-dominated blanket bogs and one intact peatland (Border Mire).
- 2) Develop an understanding of TA seasonal and vertical variability on *Sphagnum* mosses in a blanket bog.
- 3) Examine the response of TA (taxonomy and functional traits) to heather management strategies (prescribed burning versus alternative cutting compared to unmanaged and aging heather) within blanket bog.
- 4) Examine the response of TA (taxonomy and functional traits) to restoration strategies (ditch/gully-blocking) within blanket bog.

### **1.4.3 Thesis structure**

The thesis is composed of six chapters. Chapter 1 gives a general introduction of TA, blanket bogs, related upland peatlands, and associated management practices in the UK and a detailed literature review of TA as bioindicators and their potential roles in ecosystem processes. Chapter 2 and 3 are the results of objective 1 and 2, which aim to investigate the most effective sampling approaches for TA as hydrological bioindicators in British blanket bogs. Chapter 4 and 5 are the results of objective 3 and 4, which aim to evaluate the viability of using TA as hydrological bioindicators for monitoring the progress and effectiveness of peatland management and restoration efforts, whilst exploring their potential functional contribution throughout the recovery process. From chapter 2 to 5, each comprises sections of abstract, introduction, methods, results, discussion and conclusions (and where applicable a supplementary graph section). Chapter 6 provides a general discussion focusing on the linkages between the main chapters, summarizing the core findings and their implications, leading into the sections discussing limitations and future work followed by overall conclusions.

# **Chapter 2: Investigation of testate amoeba communities among different plant functional types and their potential in indicating the restoration wetness trajectory of peatland habitats**

## **2.1 Abstract**

Many degraded blanket bog peatlands in the UK have undergone restoration management by a series of rewetting strategies in recent years. Whilst water tables are assumed to rise after restoration work, there remain questions as to how effective the measures are and how quick a recovery is to support a trajectory towards an intact bog status. As well-known wetness indicators, testate amoebae (TA) have been applied to assess their potential as bioindicators as part of monitoring peatland restoration success. However, little is still known about what kind of TA samples are best to be used in such an application. In this study, TA communities were taken from lawn (flat) areas for different plant functional types (*Sphagnum* moss, other mosses and surface litter of heather and sedge) at two blanket bogs and one intact border mire across a strong hydrological gradient to explore the optimal sampling strategy. The results showed that: 1) the wetness gradient among sites was best indicated by TA communities (i.e., by the proportion of relative abundance of wet and dry indicators) found in *Sphagnum* moss; 2) similar TA communities were found on different combinations of *Sphagnum* mosses (i.e., all *Sphagnum* moss, monospecific *S. capillifolium* and all *Sphagnum* moss without rare species); 3) both dominant and second abundant *Sphagnum* moss species showed a similar performance in indicating wetness difference among sites as all combined *Sphagnum* moss species did. Overall, considering the cost and practicality, this study recommends that sampling the dominant *Sphagnum* moss species from lawn areas is a simplified but effective method to assess the potential of TA as hydrological bioindicators and to monitor the restoration (rewetting) success of degraded blanket bog.



## 2.2 Introduction

Peatlands represent a significant terrestrial carbon pool, storing about 30% of global soil organic carbon even though covering only 3% of the world's land surface (Yu et al. 2011). They therefore have the potential to affect global climate change through their role as both sink and source of atmospheric carbon dioxide and as source of atmospheric methane (Frolking et al. 2006, Turner and Swindles 2012). In the UK, blanket bogs are one of the most important peatland types, which store an estimated 2,300 Mt of soil organic carbon (SOC) (Billett et al. 2010), whilst also providing diverse wetland habitats for many upland plant and bird species (Carroll et al. 2015, Heinemeyer et al. 2019a). However, many of them are classified as being in a degraded state, with low biodiversity and reduced carbon and water storage due to historical management practices such as drainage, grazing and potentially also rotational burning of vegetation (Wilson et al. 2011c, Heinemeyer et al. 2018, Alday et al. 2022). Currently, much restoration work has been deployed in degraded peatland areas to improve biodiversity and restore peat hydrological functioning and associated key ecosystem services (related to carbon and water storage) by ditch/gully-blocking, alternative cutting (as compared to burning) and other rewetting and revegetation strategies (Wilson et al. 2011a, Heinemeyer et al. 2019a, Heinemeyer et al. 2019b, Heinemeyer and Ashby 2023). It is therefore important to assess and monitor the peatland's condition and recovery success (towards intact and functioning bog status) following these restoration strategies.

Undoubtedly, the most direct way to monitor restoration success is to record vegetation regrowth patterns and measure ecosystem services related parameters such as carbon fluxes and water table depth regularly and frequently (Heinemeyer et al. 2019b). However, this usually requires a long-term commitment and intensive measurement plan, which is not only time-consuming but also expensive (see the Peatland-ES-UK long-term study by Heinemeyer et al. 2023). Alternatively, an indicator approach could be a relatively cheaper and faster way of environmental monitoring where an organism (or part of it or a community) is used to indicate environmental changes, offering qualitative or semi-quantitative information about the quality of the ecosystem (Markert et al. 1999, Sumudumali and Jayawardana 2021, Freitas et al. 2022). Historically, small mammals and birds with rapid metabolisms have been proven especially useful in

perceiving the presence or absence of toxic gases (Burrell 1914). For example, canaries were successfully used by miners to detect the presence of carbon monoxide (CO), as they show signs of distress or inertia within a few minutes of exposure to hazardous environments. This early indication therefore served as a life-saving measure, preventing disasters from occurring (Payne 2013). In recent decades, despite the interests in other organisms (e.g., fish, bat, lichen) (Asif et al. 2018), protists have received increasing attention in their potential as bioindicators (Láng and Kóhidai 2012, Meyer et al. 2012, Desrosiers et al. 2013). Among them, testate amoebae (TA) have mostly been explored and applied in peatland research.

TA are the main component of protists, which are ubiquitous in moist or aquatic habitats such as lakes and rivers but also in mosses and soils (especially in peat) (Charman 2001, Booth 2008). They are small (mostly 20-200  $\mu\text{m}$ ), abundant (e.g., 1,000-10,000 individuals  $\text{g}^{-1}$  dry weight peat) and diverse (over 2,500 described species) (Qin et al. 2013). In most cases, they can produce a test or shell either from proteinaceous, calcareous, chitinous or siliceous material (Mitchell et al. 2008a). Some of them can also form an agglutinated test by gluing together organic or mineral particles from their surrounding settings, which is fairly decay-resistant, making them preserve well after death even in extreme environment, such as low pH conditions (Beyens and Meisterfeld 2002, Smith et al. 2009). Due to their key characteristics such as short life cycle, great sensitivity (e.g., to hydrology, heavy metals), abundant and diverse presence in different environments and worldwide distribution (Foissner 1999, Mitchell et al. 2008a, Payne 2013), TA were proposed as ideal bioindicators and have been used to monitor a range of environmental changes such as water quality, fire history, air and soil pollution (Nguyen-Viet et al. 2007a, Payne 2010, Meyer et al. 2013, Marcisz et al. 2016). During the past two decades, an increasing number of studies have also 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). Despite the positive reporting of their application in this area, little is known about what kind of TA samples can be most sensitive and effective to indicate the wetness change in response to peatland rewetting strategies from surface vegetation samples, especially in blanket bogs where vegetation composition is highly different from raised bogs and fens.

Most studies which investigated the ecology of TA (especially their relationship with environmental variables) and their potential as bioindicators were based on the collection of various moss samples representing the highest diversity of the microhabitats (e.g., hummock, lawn, hollow) in *Sphagnum*-dominated peatlands, mostly in raised bogs and fens (Lamentowicz and Mitchell 2005, Mieczan 2009, Jassey et al. 2014, Lizoňová and Horsák 2017). While this method has been shown to be effective in linking TA communities to environmental variables (Booth 2001, Lamentowicz et al. 2010), it might not be as effective and likely could underestimate the potential of TA as hydrological indicators in response to restoration strategies if most of the samples are collected from hummocks, as *Sphagnum* moss species growing in hummocks were often reported to be less sensitive to water table change than those in lawns and hollows (Rydin 1985, Robroek et al. 2007, Bengtsson et al. 2021). Furthermore, few studies focused on sampling the representative lawn communities (e.g., Mitchell et al. 2000) and monospecific genus (e.g., *S. fallax*) (Jassey et al. 2011a). Both aspects look promising to be applied in UK modified blanket bogs where heather often dominates the habitat, followed by sedges, and mosses, with often only few and often low cover of *Sphagnum* moss species mostly forming lawns. However, there are some potential problems as, for example, different types and numbers of *Sphagnum* moss species are likely to be collected when sampling the representative lawn communities. Firstly, there is little understanding on the effects of unbalanced (different replicates of *Sphagnum* moss species) samples on statistical robustness (Ateş et al. 2019), which is often the case in species limited systems. Secondly, it is often difficult or even impossible to find the same species among sites as *Sphagnum* mosses can be rare and often just occupy a low proportion of total vegetation cover in many blanket bogs and the same species do not necessarily best represent the hydrological conditions of each habitat.

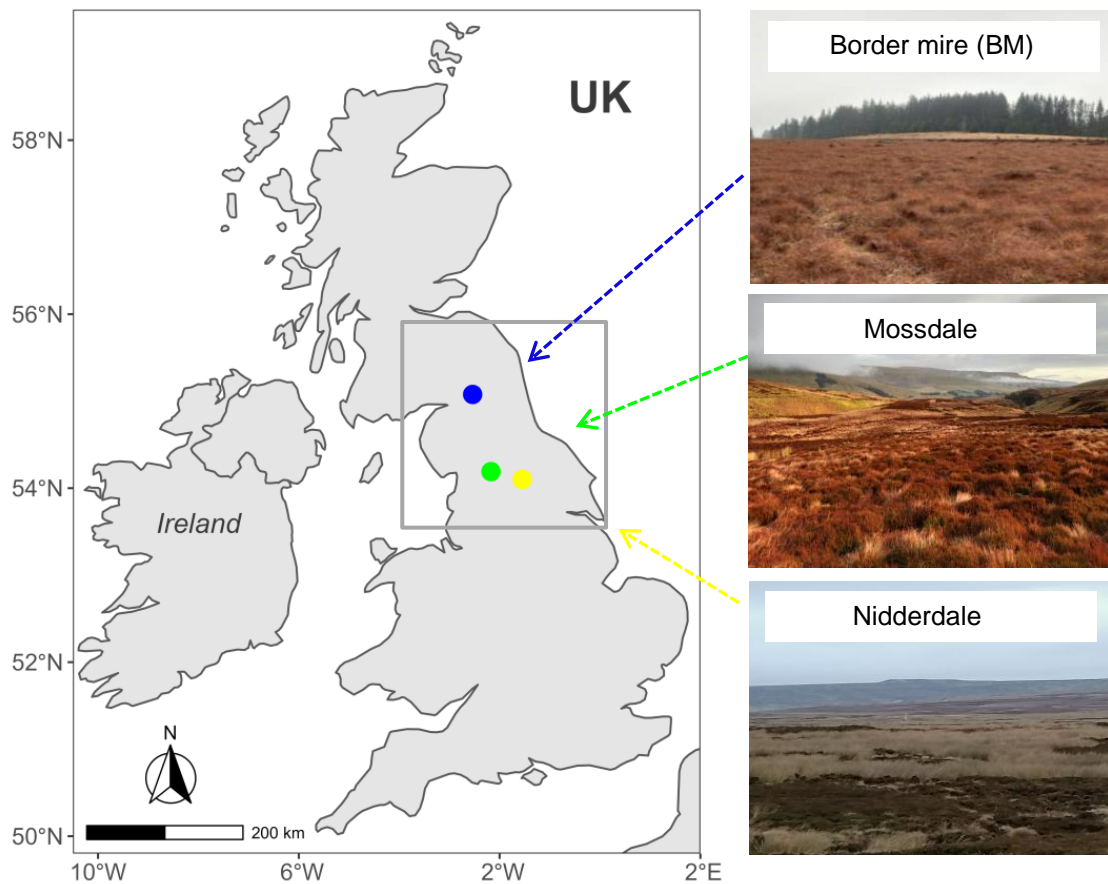
Here, TA communities were investigated from different plant functional types (i.e., *Sphagnum* moss, other mosses, surface litter of heather and sedge) at two blanket bog and one intact border mire sites across a strong hydrological (wetness) gradient to answer three questions: 1) Do TA in *Sphagnum* moss perform better than other plant functional types to reflect/indicate the wetness difference of habitats? 2) Do different numbers of replicates for individual *Sphagnum* moss species samples matter? 3) Do TA in the dominant *Sphagnum* moss species perform better than other *Sphagnum* samples to reflect the plot/site wetness condition?

Due to the heterogeneity of tissue structure, the way and capacity for different plant/litter types to absorb and hold water are distinctive, especially when considering hyaline cells in *Sphagnum* moss, which make their response to water table and capturing moisture changes significantly different. It is therefore hypothesized that: 1) TA communities from *Sphagnum* moss are better indicators of habitat wetness change than other moss types or plant litter; 2) different number of *Sphagnum* moss replicates do not affect TA's indicator value for habitat wetness; 3) TA from the dominant *Sphagnum* moss performs best as wetness indicator.

## 2.3 Methods

### 2.3.1 Study sites

Samples were collected from two modified blanket bog sites (Nidderdale: 54°10'07"N; 1°55'02"W and Mosssdale: 54°19'01"N; 2°17'18"W) and one intact border mire (BM; intermediate/ridge-raised bog) (Butterburn: 55° 07'69"N; 2° 53'73"W, also considered as a valley mire complex developed over numerous small valley/depression-shaped terrain) (Eades et al., 2021). The two modified blanket bog sites are located in Northern England, while the intact border mire, the northernmost, is situated right at the border between England and Scotland (Fig. 2.1; Table 2.1). Both Nidderdale and Mosssdale are heather-dominated and under grouse moor management, offering fairly flat areas, with an average slope of  $4 \pm 3^\circ$  and  $6 \pm 3^\circ$  respectively (see Heinemeyer et al. 2023). Typically, they were managed with a ~20-year burn rotation and had a long history of burning (more than 100 years; based on estate information and confirmed by charcoal records as outlined in Heinemeyer et al. 2018, indicating an average 16- and 22-year burn rotation over the past 65 years (i.e., from 1950 to 2015) at Nidderdale and Mosssdale, respectively). Both grouse moor sites had more than 50% *Calluna vulgaris* (ling heather) cover, with some other bog vegetation in the form of *Eriophorum* (cotton-grass), *Sphagnum* and other moss species, and both sites had a low sheep stocking density of  $< 0.5$  ewes  $\text{ha}^{-1}$ . By contrast, Butterburn BM has not been reported to have been disturbed by historic management, with only a low heather cover (~4.3%) and an average slope of  $1 \pm 1^\circ$  across the sampling plots. The environmental condition of the three sites (based on either long-term weather station data or field investigation) differed. The sites' wetness increases from Nidderdale to Mosssdale (based on average



**Figure 2.1** Location of study sites in the UK. Nidderdale and Mossdale are both ‘modified’ heather-dominated blanket bog sites under grouse moor management, whilst Butterburn (Butt) is an ‘intact’ border mire with low heather and high *Sphagnum* moss cover.

surface peat water content, see Table 2.1) to the intact border mire (Butterburn), which is topographically wetter and thus has a deeper peat depth than other near intact blanket bogs. Based on Heinemeyer et al. (2019b), Nidderdale, the driest site, showed low *Sphagnum* cover (~6.4%), with mostly *Hypnum jutlandicum* as the other moss cover (~82.7%); the wetter site (Mossdale) had larger *Sphagnum* moss cover (~48.9%) and also mostly *Hypnum jutlandicum* as other moss (~24%). The assessment for Butterburn BM, the wettest site, showed the largest *Sphagnum* moss cover (~73.3%), but with low cover of other moss species (~3.7%). More details on basic site conditions and mean vegetation cover are presented in Table 2.1.

**Table 2.1** Location information for the three peatland sampling sites in the UK. Note: number of samples share the same sequence as type of samples. Vegetation cover was assessed within 5 x 5 m squares for Nidderdale and Mossdale in December 2020 but within 50 x 50 cm squares for Butterburn in February 2021 as total cover (at least 100%) of: heather (H), sedge (S), *Sphagnum* moss (Sph), other mosses (OM), brash/litter/bare ground (BLB). Samples taken for testate amoebae extractions consisted of *Sphagnum* moss, other mosses and surface litter of heather and sedge. Surface water content was measured in the top 5 cm of peat.

Site	Peatland type	Altitude (m)	Surface peat water content (%)	Peat depth (mean: m)	Vegetation cover (mean %)					Number of samples (H, S, Sph, OM)
					H	S	Sph	OM	BLB	
Butterburn (Butt)	Border mire	167	95.6	6.7	4.3	36.7	73.3	3.7	7.7	5, 6, 24, 12
Mossdale (Moss)	Blanket bog	390	90.7	1.5	81.1	3.3	48.9	24	10	5, 6, 24, 12
Nidderdale (Nidd)	Blanket bog	450	87.3	1.7	69.8	14.5	6.4	82.7	18.2	6, 7, 25, 10

### 2.3.2 Sampling

Different plant functional types (*Sphagnum* moss, other mosses, surface litter of heather and sedge) were sampled from the three sites (Nidderdale, Mossdale and Butterburn) in December 2020 and February 2021. Sampling plots were chosen at relatively flat (lawn) positions. At each plot, up to five different *Sphagnum* species (although some *Sphagnum* species were sampled repeatedly if the total number of *Sphagnum* species was less than five in a given location) and four to five other plant samples (other mosses, litter of heather and sedge) were collected randomly from five locations, respectively (Supplementary Fig. S2.1). Each location was defined as a 5x5 m plot area. In the case of Nidderdale and Mossdale, these plots were replicated, uncut heather areas, with a separation of approximately 100-200 m, forming part of a larger-scale experimental block experiment (see Heinemeyer et al. 2019b; 2023). Meanwhile, in Butterburn, the plot areas were randomly selected, ensuring a minimum distance of 50 m between them. A total of 142 samples were collected, among which 73 were *Sphagnum* moss species (including *S.capillifolium*, *S.fallax*, *S.fimbriatum*, *S.medium*, *S.palustre*, *S.papillosum*), 34 were other mosses (mainly including *Aulacomnium palustre*, *Hypnum jutlandicum*, *Pleurozium schreberi*, *Polytrichum commune*), 16 were litter of heather and 19 were litter of sedge (Table 2.1). In addition, vegetation type and total cover (i.e., sum of over and understorey; at least 100%) were assessed in 50 x 50 cm squares around the

sampling areas for Butterburn, Nidderdale and Mossdale. However, vegetation was also investigated inside the fixed 5 x 5 m long-term experimental plots for Nidderdale and Mossdale, where samples in this study were collected from. Additionally, surface peat (0-5 cm) samples in three of the five plots in each site were collected for the measurement of site wetness (peat water content). Peat depth was measured by using extendable drainage rods (see Heinemeyer et al., 2019b).

### **2.3.3 TA processing**

Testate amoebae (TA) were prepared using a modified version of the method of Booth et al. (2010). For each sample, around 5 g fresh *Sphagnum* moss or other plant samples were used for preparation. To extract TA the sample was soaked and disaggregated in the 100 ml beaker with 60-80 ml deionized water for at least 24 hours and stirred occasionally. At the same time, One tablet of *Lycopodium clavatum* was added for density calculation. The sample liquid was washed 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 and tends to make analysis easier and more efficient. The material retained in the 10 mesh µm was carefully washed into 50 ml centrifuge tubes and centrifuged at 4000 rpm for 5 minutes. 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 color) from dead (empty) individuals. TA identification and counting were performed under light microscopy at 200x and 400x magnification, with a target of tallying at least 150 individuals in each sample.

### **2.3.4 Peat moisture measurement**

Around 2 cm<sup>3</sup> peat subsamples from the surface layer separated in the lab were weighed first and then dried at 105°C in 10-30 ml crucibles for a minimum of 72 hours and reweighed to determine peat moisture (water content).

### **2.3.5 Statistical analyses**

The number of TA species in each sample was counted for each type of vegetation (*Sphagnum* moss, other mosses, litter of heather and sedge) to compare their species

richness. Relative abundance of wet and dry TA indicators was mainly calculated to indicate the wetness condition of habitats. Before doing so, a series of TA wet and intermediate indicators (*Amp.fla*: *Amphitrema flavum*, *Amp.wri*: *Amphitrema wrightianum*, *Hya.ele*: *Hyalosphenia elegans*, *Hel.syl*: *Heleopera sylvatica*, *Eug.stri*: *Euglypha strigosa*, *Neb.fla*: *Nebela flabellulum*, *Neb.co.bo*: *Nebela collaris-bohemica* type, *Neb.mil*: *Nebela militaris*, *Cyc.arc*: *Cryclopixis arcelloides* type) and TA dry indicators (*Eug.tub*: *Euglypha tuberculata*, *Neb.tin*: *Nebela tinctoria*, *Eug.rot*: *Euglypha rotunda*, *Pse.ful*: *Pseudodiffugia fulva*, *Tri.arc*: *Trigonopyxis arcula*, *Ass.mus*: *Assulina muscorum*, *Cor.dub*: *Corythion dubium*) were determined in accordance with their reported indication of hydrological preferences (Tolonen et al. 1992, Charman 1997, Bobrov et al. 1999, Booth 2001, Lamentowicz and Mitchell 2005, Mieczan 2009) (Table 2.2). Moreover, taxa present in less than 4% of the total number of samples and those whose relative abundance of overall less than 4% were eliminated from the TA community dataset to reduce the influence of rare taxa. Species that either showed significant change (and a mean relative abundance of more than 5%) or were particularly abundant (with a relative abundance of more than 10%) were selected for the calculation of relative TA abundance. The significant associations for each TA species among sites were tested using Kruskal-Wallis's tests. Finally, regression analysis was applied to compare the performance of different plant types and different combinations of *Sphagnum* moss species by calculating the linear regression line equation, significance level (*p*) and correlation coefficient (*R*) between the total relative abundance of wet TA indicators and surface peat moisture measured at sampling time (at the sampling locations). All the analyses and plotting of graphs were performed using R (R Core Team 2013).



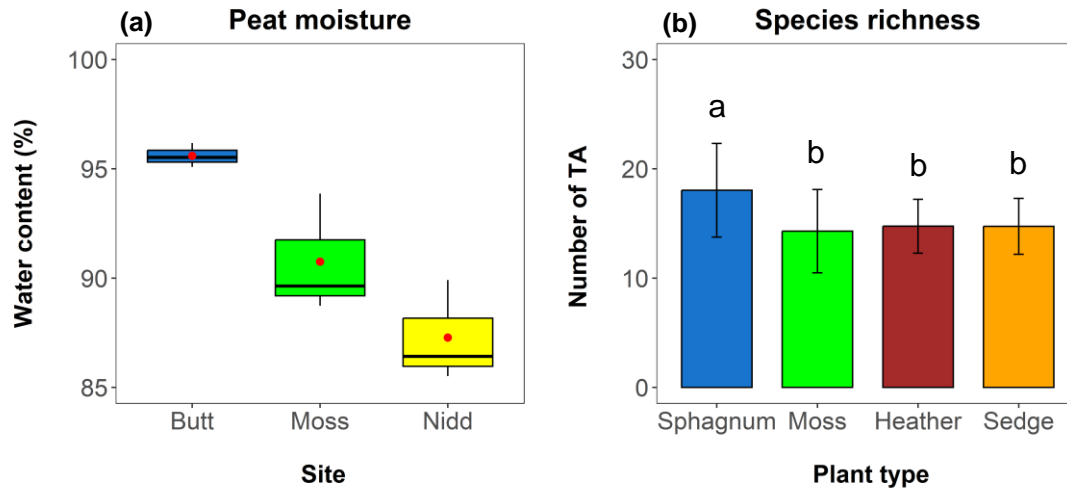
**Table 2.2** Classification of testate amoebae species as wet, intermediate and dry indicators in accordance with their commonly reported hydrological preferences based on combined literature information (i.e., references).

Wet indicators	Intermediate indicators	Dry indicators	References
<i>Amphitrema flavum</i> ,	<i>Euglypha strigosa</i> ,	<i>Euglypha tuberculata</i> ,	Tolonen et al. 1992,
<i>Amphitrema wrightianum</i> ,	<i>Nebela flabellulum</i> ,	<i>Nebela tinctoria</i> ,	Charman 1997,
<i>Hyalosphenia elegans</i> ,	<i>Nebela collaris-bohemica</i> type,	<i>Euglypha rotunda</i> ,	Bobrov et al. 1999,
<i>Heleopera sylvatica</i>	<i>Nebela militaris</i> ,	<i>Pseudodifflugia fulva</i> ,	Booth 2001,
	<i>Cryclopixys arcelloides</i> type	<i>Trigonopyxis arcula</i> ,	Lamentowicz and Mitchell 2005,
		<i>Assulina muscorum</i> ,	Mieczan 2009
		<i>Corythion dubium</i>	

## 2.4 Results

### 2.4.1 Hydrological conditions among the three sites

Overall surface peat moisture in the intact border mire (Butterburn) was higher (at sampling time) than at both blanket bogs (Mosssdale and Nidderdale), of which Mosssdale was higher than Nidderdale, but this was not statistically significant ( $H(2) = 5.9556$ ,  $p = 0.05091$ ) although there seems to be a large separation (Fig. 2.2a). Specifically, the median and mean surface peat moisture in the intact border mire was 95.5% and 95.6%, whereas they were 89.6% and 90.7% in Mosssdale and 86.4% and 87.3% in Nidderdale, respectively (Fig. 2.2a).

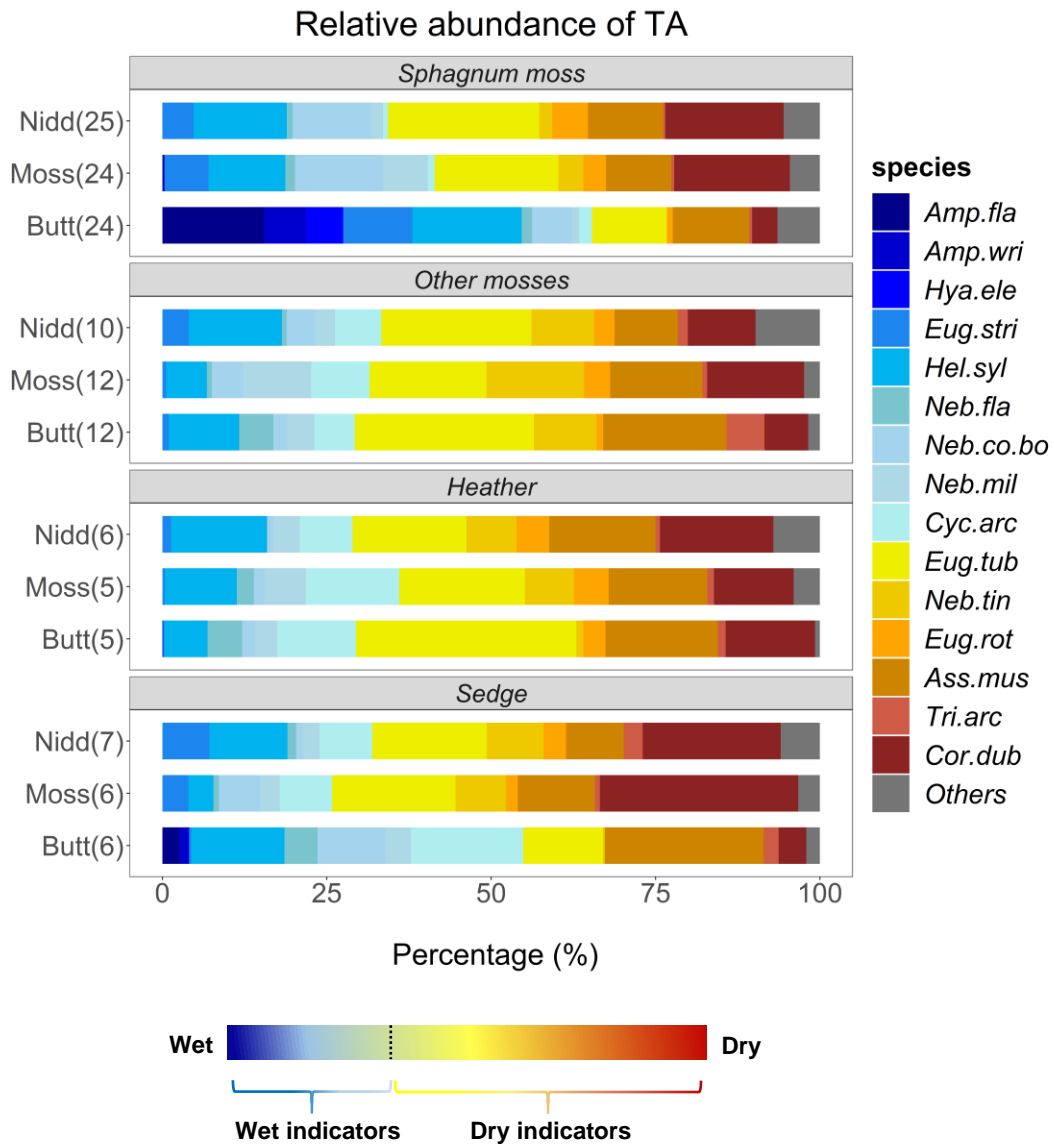


**Figure 2.2** Differences in (a) species richness (number of species) of testate amoebae (TA) in *Sphagnum* moss ( $n = 73$ ), other mosses ( $n = 34$ ), surface litter of heather ( $n = 16$ ) and sedge ( $n = 19$ ) at the combined Nidderdale (Nidd), Mossdale (Moss), and Butterburn (Butt) sites; (b) surface peat (0-5 cm) moisture of the three study sites (note the truncated y-axis). The box shows the median (middle line), 25% (bottom border), 75% (top border) ranges and red points inside the box represent mean values. Different letters indicate a significant difference of the species richness means (Tukey's HSD tests,  $P < 0.05$ ). Standard deviations are presented by the error bars.

## 2.4.2 TA communities from different plant functional types

A total of 51 TA taxa were identified from 144 samples within the three peatland sites. Noticeably, species richness of TA from *Sphagnum* moss ( $18 \pm 4$ ) was significantly higher ( $F(3) = 10.24, p < 0.001$ ) than from other mosses ( $14 \pm 4$ ), heather ( $15 \pm 2$ ) or sedge ( $15 \pm 3$ ) litter (Fig. 2.2b). However, no difference was found between the latter three vegetation types (Fig. 2.2b). According to the relative abundance of selected wet (including intermediate) versus dry indicators, TA on *Sphagnum* moss was shown to best indicate the wetness difference among the three sites (Fig. 2.3). In detail, the total relative abundance of wet TA indicators occupied 34.3% of total species in Nidderdale (Nidd), which increased to 41.4% in Mossdale (Moss) and 65.4% in Butterburn (Butt). Among the species, the well-defined TA wet indicators (i.e., *Amphitrema flavum*, *A. wrightianum*) and TA dry indicators (i.e., *Euglypha tuberculata*, *Corythion dubium*) showed the clearest wetness pattern, with relative abundance of the wet *Amphitrema flavum* and *A. wrightianum* types being significantly lower in Nidderdale (0%, 0.1% respectively) and Mossdale (0%, 0.1%) than in Butterburn (15.5%, 6.4%) (Fig. 2.3, Table 2.3). Conversely, the relative abundance of the dry *Euglypha tuberculata* and *Corythion dubium* types were significantly higher in Nidderdale (23%, 18%) and Mossdale (18.8%, 17.6%) than in Butterburn samples (11.3%, 3.8%) (Fig 2.3, Table

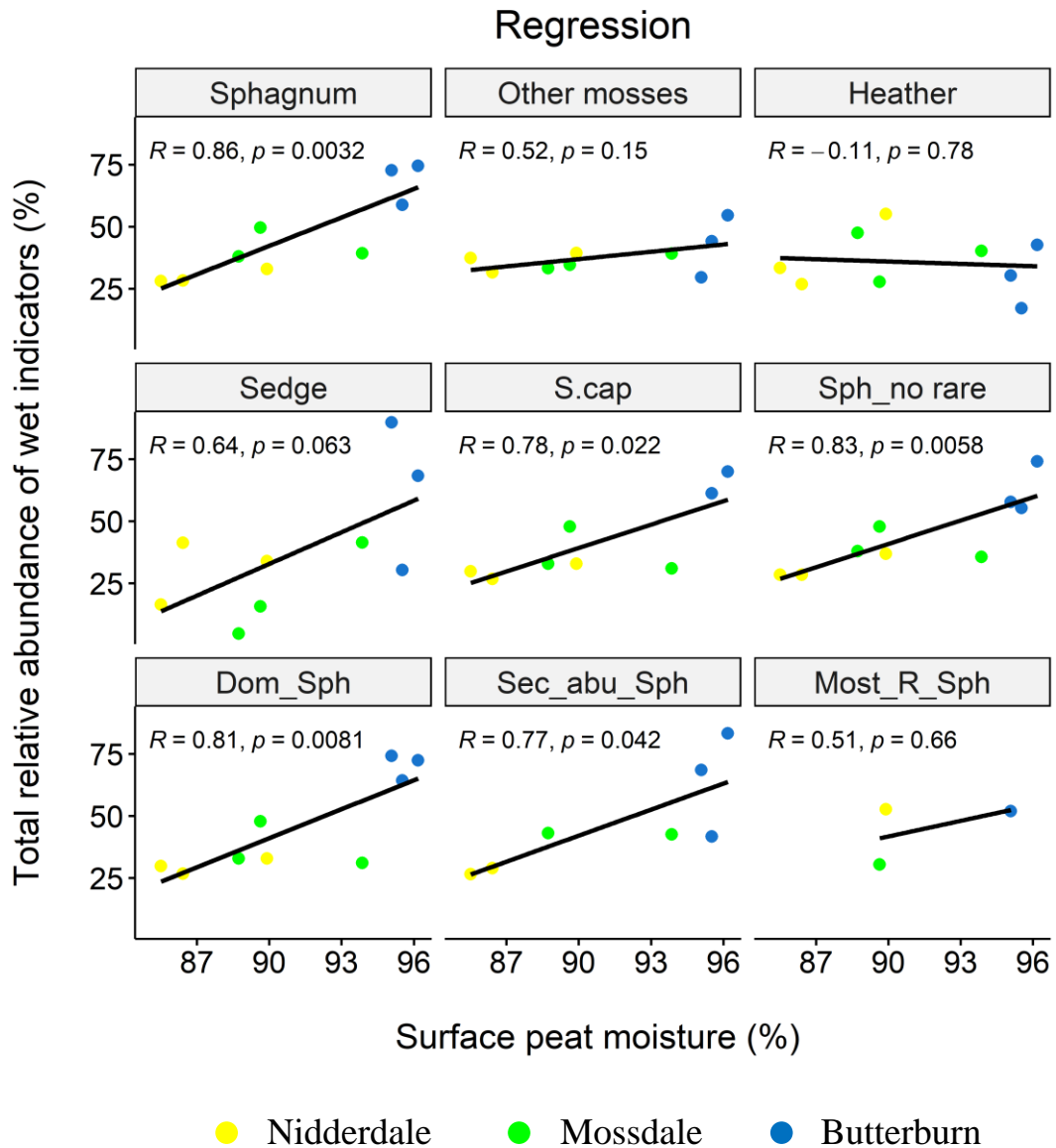
2.3). By contrast, the general pattern of wetness gradient was not clearly reflected by TA communities from other plant types even though TA on sedge litter showed some similarity but to a lesser extent (Fig. 2.3). The total relative abundance of wet TA indicators was much lower in each site from other mosses (Nidd: 33.3%, Moss: 31.5%, Butt: 29.3%), heather (Nidd: 28.9 %, Moss: 36.0%, Butt: 29.4%) and sedge (Nidd: 31.9%, Moss: 25.8%, Butt: 54.9%). Both well-defined wet and dry indicators mentioned above did not change dramatically among sites for other mosses and heather while they changed to a larger, still not significant extent, on sedge (Fig. 2.3, Table 2.3). The correlation coefficients expressed as R between the total relative abundance of wet TA indicators and surface peat moisture were 0.86 (*Sphagnum*), 0.52 (other moss), -0.11 (heather) and 0.64 (sedge), with only R for *Sphagnum* moss ( $p = 0.0032$ ) shown to be statistically significant (Fig. 2.4).



**Figure 2.3** Relative abundance of testate amoebae (TA) species in *Sphagnum* moss, other mosses, surface litter of heather and sedge at Nidderdale (Nidd), Mossdale (Moss), and Butterburn (Butt) sites respectively. The number of samples analyzed at each site is indicated within the bracket on the y-axis. Species that either showed significant change between sites (and their mean relative abundance of more than 5%) or were particularly abundant (with relative abundance more than 10%) were selected and displayed: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Cyc.arc* - *Cryclopixys arcelloides* type, *Eug.rot* - *Euglypha rotunda*, *Eug.stri* - *Euglypha strigosa*, *Eug.tub* - *Euglypha tuberculata*, *Hya.ele* - *Hyalosphenia elegans*, *Hel.syl* - *Heleopera sylvatica*, *Hya.sub* - *Hyalosphenia subflava*, *Neb.co.bo* - *Nebela collaris-bohemica* type, *Neb.fla* - *Nebela flabellulum*, *Neb.mil* - *Nebela militaris*, *Neb.tin* - *Nebela tinctoria*, *Pse.ful* - *Pseudodiffugia fulva*, *Tri.arc* - *Trigonopyxis arcuata*, 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.

**Table 2.3.** Results of Kruskal Wallis tests (KW\_test,  $P < 0.05$ ) for testate amoebae (TA) in *Sphagnum* moss (Sph), other mosses (OM), surface litter of heather (H) and sedge (S) among sites (B: Butterburn, M: Mossdale, N: Nidderdale). The highest mean values of individual TA (H\_Mean) (%) among sites is presented. Note: Taxa in bold indicate significant differences (and their mean relative abundance of more than 5%) between sites and vegetation samples or its highest mean value of more than 10% (when not significant).

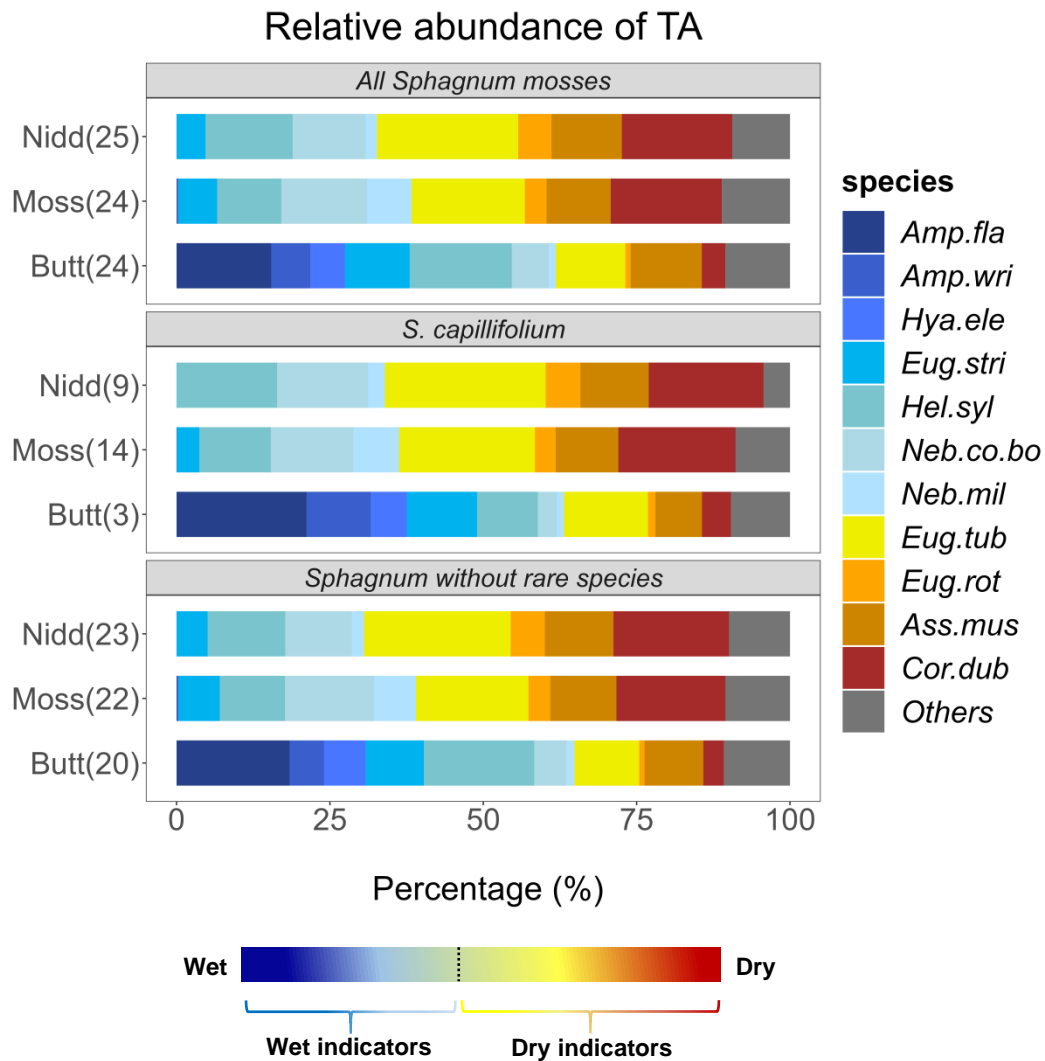
Taxa	B_M_N_Sph_TA		B_M_N_OM_TA		B_M_N_H_TA		B_M_N_S_TA	
	KW_test	H_Mean	KW_test	H_Mean	KW_test	H_Mean	KW_test	H_Mean
<b>Amp.fla</b>	<b>&lt;0.001</b>	18.2	1	0	1	0	0.052	2.6
<b>Amp.wri</b>	<b>&lt;0.001</b>	5.6	1	0	0.333	0.1	0.052	1.4
Arc.d.s	0.008	2.4	0.058	1.7	0.198	1.3	0.16	1.5
<b>Ass.mus</b>	0.532	<b>10.8</b>	0.266	<b>18.7</b>	0.529	<b>17.1</b>	0.079	<b>24.2</b>
Ass.sem	<0.001	4.2	0.178	0.3	0.33	0.6	0.01	0.9
Cor.con	<0.001	1.4	-	-	-	-	-	-
Cen.cas	-	-	<0.001	2	0.037	3.3	0.158	1.0
<b>Cor.dub</b>	<b>&lt;0.001</b>	18	<b>0.043</b>	14.7	0.673	<b>13.6</b>	0.088	<b>30.2</b>
Cyc.arc.l	-	-	0.884	4.6	0.338	9.0	0.484	4.9
<b>Cyc.arc.s</b>	-	-	0.534	4.7	0.203	7.8	0.225	<b>15.9</b>
Cry.ovl	<0.001	4.3	-	-	-	-	-	-
<b>Eug.rot</b>	<b>&lt;0.001</b>	5.4	0.013	4	0.365	5.3	0.012	3.4
<b>Eug.stri</b>	<b>0.001</b>	9.5	0.414	4.0	0.571	1.3	0.428	7.2
<b>Eug.tub</b>	<b>&lt;0.001</b>	23.3	0.361	<b>27.2</b>	0.338	<b>33.5</b>	0.286	<b>18.8</b>
<b>Hel.syl</b>	<b>0.049</b>	18.2	0.131	<b>14.2</b>	0.358	<b>14.6</b>	0.329	<b>14.2</b>
<b>Hya.ele</b>	<b>&lt;0.001</b>	6.8	1	0	1	0	1	0
Hya.sub	<0.001	1.6	0.013	3.4	0.169	0.7	0.081	1.3
<b>Neb.co.bo</b>	<b>&lt;0.001</b>	14.5	0.1	4.8	0.115	2.0	<b>0.027</b>	10.3
<b>Neb.fla</b>	0.315	1.4	0.202	5.2	<b>0.043</b>	5.2	0.072	5.0
Neb.gri	<0.001	1.7	-	-	-	-	-	-
<b>Neb.mil</b>	<b>&lt;0.001</b>	6.8	<b>0.024</b>	10.3	0.682	6.4	0.105	3.9
<b>Neb.tin</b>	<0.001	3.3	<b>0.01</b>	14.9	<b>0.028</b>	7.6	<b>0.013</b>	8.6
Pse.ful	<0.001	3.1	0.006	2.1	0.344	0.2	0.820	1.1
<b>Tri.arc</b>	-	-	<b>0.009</b>	5.8	0.705	1.2	0.619	2.9
Tri.lin	<0.001	1.5	0.017	1.3	0.029	1.6	0.023	1.8



**Figure 2.4** Regression analyses between total relative abundance of wet TA indicators (sampled in December 2020 and February 2021) and surface peat moisture in vegetation samples of different plant functional types across Nidderdale (Nidd: yellow), Mossdale (Moss: green), and Butterburn (Butt: blue) sites. Sphagnum: all combined *Sphagnum* mosses, S.cap: *Sphagnum capillifolium*, Sph\_no rare: *Sphagnum* without rare species, Dom\_Sph: dominant *Sphagnum* moss, Sec\_abu\_Sph: second abundant *Sphagnum* moss, Most\_R\_Sph: most rare *Sphagnum* moss. The significance level (*p*) and correlation coefficient *R* values are displayed.

### 2.4.3 TA communities from all combined *Sphagnum* mosses, monospecific *S. capillifolium* and *Sphagnum* mosses without rare species

The total relative abundance of wet and dry TA indicators showed a similar change pattern among sites for different combinations of *Sphagnum* mosses. All indicated an increasing wetness gradient from the drier Nidderdale to wetter Mossdale modified blanket bog to the intact border mire at Butterburn. Specifically, for the selected species from all combined *Sphagnum* mosses (which is slightly different from those selected above when compared to other plant functional types), the total relative abundance of TA wet indicators occupied 32.7% of all species in Nidderdale, while it increased to 38.3% in Mossdale and 61.8% in Butterburn (Fig. 2.5). In comparison, TA communities were not observed to be macroscopically different from both *S. capillifolium* and *Sphagnum* mosses without rare species, with total relative abundance of wet indicators being 34% and 30.6% respectively in Nidderdale, 36.3% and 39.0% in Mossdale, and 63.2% and 64.9% in Butterburn (Fig. 2.5). Noticeably, the well-defined wet (i.e., *Amphitrema flavum*, *A. wrightianum*) and dry (i.e., *Euglypha tuberculata*, *Corythion dubium*) indicators shared a similar proportion in corresponding sites for all three groups but showed most difference between the intact border mire and modified blanket bogs (Fig. 2.5). The mean relative abundance of *Amphitrema flavum* and *A. wrightianum* types were significantly higher in Butterburn (18.4%, 7.5%) than in Mossdale (0.1%, 0.1%) and Nidderdale (0%, 0%), while *Euglypha tuberculata* and *Corythion dubium* types were considerably lower in Butterburn (11.9%, 4.0%) than in Mossdale (19.7%, 18.3%) and Nidderdale (24.4%, 18.5%) (Fig. 2.4, Table 2.4). The correlation coefficients (R) between the total relative abundance of wet TA indicators and surface peat moisture were 0.86 (all combined *Sphagnum* moss), 0.78 (monospecific *S. capillifolium*) and 0.83 (*Sphagnum* mosses without rare species) (Fig. 2.4).



**Figure 2.5** Relative abundance of testate amoebae (TA) species in all *Sphagnum* moss, *Sphagnum capillifolium*, and *Sphagnum* without rare species samples at Nidderdale (Butt), Mossdale (Moss), and Butterburn (Butt) sites respectively. The number of samples analyzed at each site is indicated within the bracket on the y-axis. Species that either showed significant change between sites (and a mean relative abundance of more than 5%) or which were particularly abundant (with a relative abundance of more than 10%) were selected and displayed: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Eug.rot* - *Euglypha rotunda*, *Eug.stri* - *Euglypha strigosa*, *Eug.tub* - *Euglypha tuberculata*, *Hya.ele* - *Hyalosphenia elegans*, *Hel.syl* - *Heleopera sylvatica*, *Neb.co.bo* - *Nebela collaris-bohemica* type, *Neb.mil* - *Nebela militaris*, 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 indicators species.

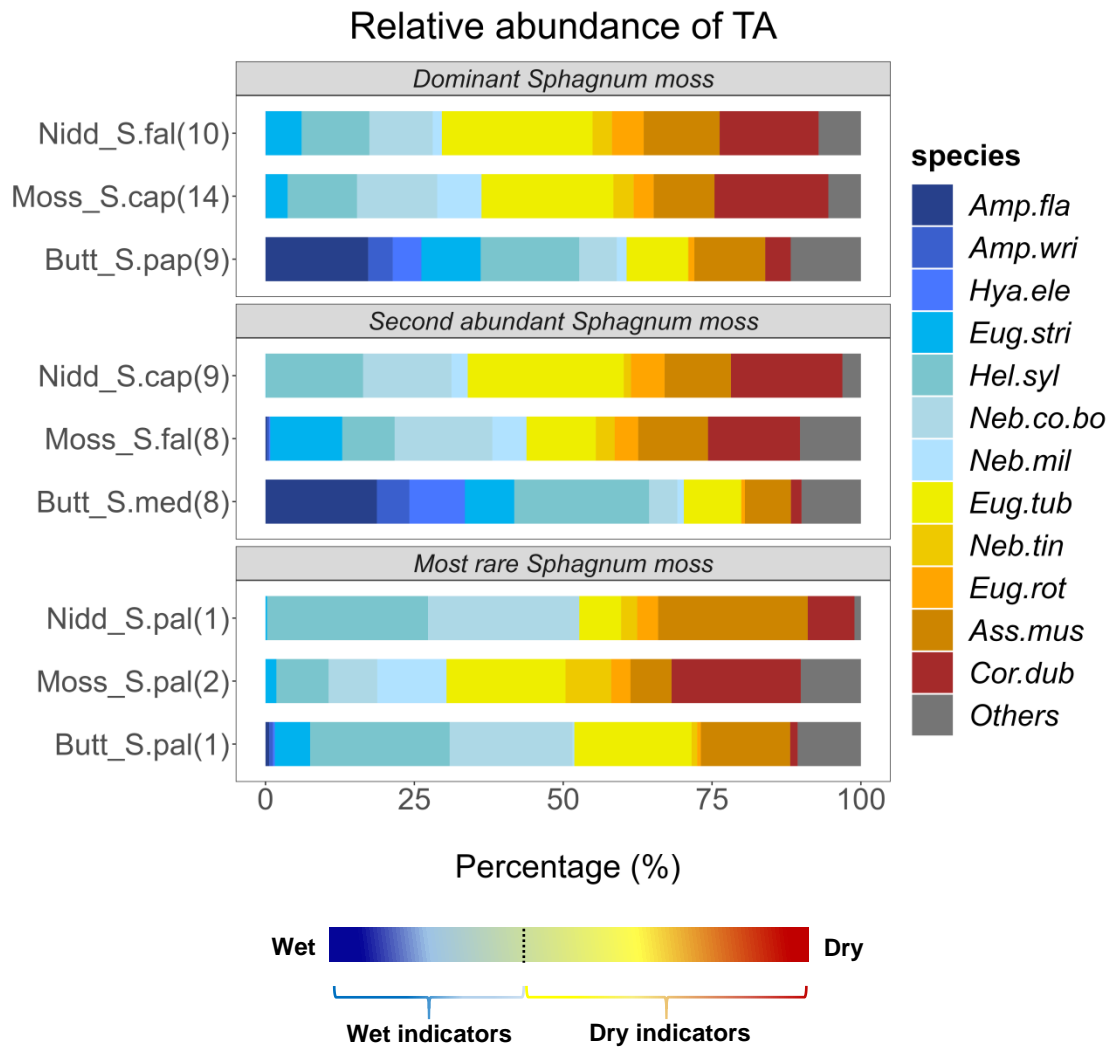


**Table 2.4** Results of Kruskal Wallis tests (KW\_test,  $P < 0.05$ ) for testate amoebae (TA) in all *Sphagnum* moss (all\_Sph), *Sphagnum capillifolium* (S.cap), and *Sphagnum* without rare species (no\_rare\_Sph) among sites (B: Butterburn, M: Mossdale, N: Nidderdale). The highest mean values of individual TA (H\_Mean\_%) among sites are presented. Note: Taxa in bold indicate significant differences (and their mean relative abundance of more than 5%) between sites and vegetation samples or its highest mean value of more than 10% (when not significant).

Taxa	B_M_N_all_Sph_TA		B_M_N_S.cap_TA		B_M_N_no_rare_Sph_TA	
	KW_test	H_Mean_%	KW_test	H_Mean_%	KW_test	H_Mean_%
<b>Amp.fla</b>	<b>&lt;0.001</b>	18.2	<b>&lt;0.001</b>	21.2	<b>&lt;0.001</b>	18.2
<b>Amp.wri</b>	<b>&lt;0.001</b>	5.6	<b>&lt;0.001</b>	10.5	<b>&lt;0.001</b>	5.6
Arc.d.s	0.008	2.4	0.559	0.3	0.008	2.4
<b>Ass.mus</b>	0.532	<b>10.8</b>	0.687	<b>11.1</b>	0.532	<b>10.8</b>
Ass.sem	<0.001	4.2	<0.001	4.6	<0.001	4.2
Cor.con	<0.001	1.4	0.399	-	<0.001	1.4
<b>Cor.dub</b>	<b>&lt;0.001</b>	18.0	0.088	<b>19.1</b>	<b>&lt;0.001</b>	18.0
Cyc.arc.l	-	-	0.337	0.3	-	-
Cyc.arc.s	-	-	0.108	0.7	-	-
Cry.ovi	<0.001	4.3	-	-	<0.001	4.3
<b>Eug.rot</b>	<b>&lt;0.001</b>	5.4	<b>0.039</b>	5.7	<b>&lt;0.001</b>	5.4
<b>Eug.stri</b>	<b>0.001</b>	9.5	<b>&lt;0.001</b>	11.4	<b>0.001</b>	9.5
<b>Eug.tub</b>	<b>&lt;0.001</b>	23.3	0.094	<b>26.2</b>	<b>&lt;0.001</b>	23.3
<b>Hel.syl</b>	<b>0.049</b>	18.2	0.64	<b>16.4</b>	<b>0.049</b>	18.2
<b>Hya.ele</b>	<b>&lt;0.001</b>	6.8	<b>&lt;0.001</b>	5.9	<b>&lt;0.001</b>	6.8
Hya.sub	<0.001	1.6	0.1	0.9	<0.001	1.6
<b>Neb.co.bo</b>	<b>&lt;0.001</b>	14.5	<b>0.035</b>	14.8	<b>&lt;0.001</b>	14.5
Neb.fla	0.315	1.4	0.091	1.3	0.315	1.4
Neb.gri	<0.001	1.7	-	-	<0.001	1.7
<b>Neb.mil</b>	<b>&lt;0.001</b>	6.8	<b>0.034</b>	7.4	<b>&lt;0.001</b>	6.8
Neb.tin	<0.001	3.3	0.094	4.1	<0.001	3.3
Pse.ful	<0.001	3.1	0.096	4.1	<0.001	3.1
Tri.arc	-	-	0.085	0.6	-	-
Tri.lin	<0.001	1.5	0.066	1.3	<0.001	1.5

#### 2.4.4 TA communities from the dominant, second abundant and rarest *Sphagnum* mosses

TA community composition from the dominant *Sphagnum* moss was shown to be similar to the second abundant *Sphagnum* moss species. However, both were substantially different from that on the rarest *Sphagnum* mosses (Fig. 2.6). In detail, the total relative abundance of TA wet indicators on the dominant *Sphagnum* mosses occupied 34.0% of total species in Nidderdale, while it reached 36.3% in Mossdale and 60.6% in Butterburn, respectively. By contrast, on the second abundant *Sphagnum* mosses, the total TA wet indicators showed a slightly higher proportion at Mossdale (43.8%) and Butterburn (70.3%) but a slightly lower proportion in Nidderdale (29.6%). Interestingly, for the rarest *Sphagnum* moss, the total relative abundance of TA wet indicators in Nidderdale was particularly high (52.7%), which was even slightly higher than in Butterburn (51.9%) whereas it was much lower in Mossdale (30.4%) (Fig. 2.6). Furthermore, the well-defined TA wet (i.e., *Amphitrema flavum*, *A. wrightianum*) and dry (i.e., *Euglypha tuberculata*, *Corythion dubium*) indicators showed a similar wetness pattern for both the dominant and the second abundant *Sphagnum* mosses and all of them (i.e., well-defined wet and dry TA indicators) were significantly different among sites (Fig. 2.6, Table 2.5). The correlation coefficients (R) between the total relative abundance of TA wet indicators and surface peat moisture were 0.81, 0.77, 0.51 for the dominant, second abundant and rarest *Sphagnum* species, respectively (Fig. 2.4)



**Figure 2.6** Relative abundance of testate amoebae (TA) in dominant *Sphagnum* moss, second abundant *Sphagnum* moss, and most rare *Sphagnum* moss species across Nidderdale (Nidd), Mossdale (Moss), and Butterburn (Butt) sites respectively. S.fal: *Sphagnum fallax*, S.cap: *Sphagnum capillifolium*, S.pap: *Sphagnum papillosum*, Sph.pal: *Sphagnum palustre*, S.med: *Sphagnum medium*. The number of samples analyzed at each site is indicated within the bracket on the y-axis. Species that either showed significant change between sites (and their mean relative abundance of more than 5%) or which were particularly abundant (with a relative abundance of more than 10%) were selected and displayed: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Eug.rot* - *Euglypha rotunda*, *Eug.stri* - *Euglypha strigosa*, *Eug.tub* - *Euglypha tuberculata*, *Hya.ele* - *Hyalosphenia elegans*, *Hel.syl* - *Heleopera sylvatica*, *Hya.sub* - *Hyalosphenia subflava*, *Neb.co.bo* - *Nebela collaris-bohemica* type, *Neb.mil* - *Nebela militaris*, *Neb.tin* - *Nebela tinctoria*, 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.

**Table 2.5** Results of Kruskal Wallis tests (KW\_test,  $P < 0.05$ ) for testate amoebae (TA) in the dominant *Sphagnum* moss (D\_Sph), second abundant *Sphagnum* moss (SA\_Sph), and most rare *Sphagnum* moss species (MR\_Sph) among sites (B: Butterburn, M: Mossdale, N: Nidderdale). The highest mean values of individual TA (H\_Mean\_%) among sites is presented. Note: Taxa in bold indicates significant differences (and their mean relative abundance of more than 5%) between sites and vegetation samples or its highest mean value of more than 10% (when not significant).

Taxa	B_M_N_D_Sph_TA		B_M_N_SA_Sph_TA		B_M_N_MR_Sph_TA	
	KW_test	H_Mean_%	KW_test	H_Mean_%	KW_test	H_Mean_%
<b>Amp fla</b>	<b>&lt;0.001</b>	17.2	<b>&lt;0.001</b>	18.7	0.223	0.6
<b>Amp wri</b>	<0.001	4.1	<b>0.019</b>	5.5	0.223	0.6
Arc.d.s	0.964	0.3	0.039	4.4	0.223	0.3
<b>Ass.mus</b>	0.447	<b>11.9</b>	0.939	<b>12.7</b>	0.259	<b>25.1</b>
Ass.sem	<0.001	4.3	<0.001	3.8	0.223	2.2
Cen.cas	0.131	0.2	0.59	0	0.607	0.5
<b>Cor.dub</b>	<b>&lt;0.001</b>	19.1	<b>&lt;0.001</b>	16.6	0.259	<b>21.7</b>
Cyc.arc.l	0.956	0.4	0.655	1.0	0.264	1.2
Cyc.arc.s	0.034	1.7	<0.001	0.8	0.259	2.0
<b>Eug.rot</b>	<b>&lt;0.001</b>	5.7	<b>0.002</b>	5.3	0.407	3.5
<b>Eug.stri</b>	<b>&lt;0.001</b>	10.0	0.108	<b>12.1</b>	0.259	5.9
<b>Eug.tub</b>	<b>0.004</b>	26.2	<b>0.022</b>	25.3	0.259	<b>20.0</b>
<b>Hel.syl</b>	0.249	<b>16.5</b>	0.11	<b>22.6</b>	0.259	<b>27.0</b>
<b>Hya.ele</b>	<0.001	4.8	<b>0.07</b>	9.3	0.223	0.3
Hya.sub	0.005	0.9	0.01	1.3	0.223	0
<b>Neb.co.bo</b>	<b>0.007</b>	14.8	<b>0.029</b>	16.4	0.259	<b>25.4</b>
Neb fla	0.057	2.2	0.463	1.6	0.325	5.0
<b>Neb.mil</b>	<b>0.01</b>	7.4	0.052	5.7	0.259	<b>11.6</b>
Neb.tin	0.005	3.4	0.003	3.3	0.259	7.7
Pse.ful	0.008	2.5	0.037	3.3	0.223	0.3
Tri.arc	0.044	0.6	0.038	0.2	0.259	1.6
Tri.lin	0.002	1.3	0.01	1.9	0.259	4.9

## 2.5 Discussion

TA communities extracted from different plant functional types suggest a superior performance of *Sphagnum* moss compared to other plant types (i.e., other mosses, litter of heather and sedge) to indicate the wetness gradient among the studied peatlands. The first hypothesis is therefore supported: *TA communities on Sphagnum moss are able to better indicate the wetness change of peatland habitats than other plant types*. This is probably highly related to the mechanism of different vegetation to absorb and hold water (and thus amplify moisture differences) as TA's establishment and distribution have long been reported to be primarily controlled by water table depth or substrate moisture. Compared to other plants, *Sphagnum* mosses have large, dead and hollow hyaline cells within which they can hold a large amount of water, often reaching 15 to 20 times as much water as their dry weight (Keddy 2010, McCarter and Price 2014). Unlike *Sphagnum* moss, vascular plants, such as heather and sedge, have roots and conducting tissues (called xylem) by which water and nutrients are drawn up and transported throughout the plant mainly via indirect water transport due to evapotranspiration from leaf stomata to control water loss (Rydin et al. 2006). Therefore, whereas *Sphagnum* moss dries out in response to water table changes or other desiccation conditions, the water content of living vascular plant tissues is relatively constant or just slightly affected by and buffered against normal hydrological changes, such as decline of water table depth. However, while *Sphagnum* moss can store large amounts of water in its hyaline cells, the moisture content of dead plant litter (e.g., heather and sedge samples) is mostly impacted by surface rainfall and air humidity. As this litter is not connected to living xylem (and lacks the hyaline cell buffering capacity of *Sphagnum* moss), it tends to dry out quickly. Consequently, quickly drying plant litter is disconnected from the overall site wetness and overall water table depths and thus does not relate well to changes in the TA community composition, particularly for the well-defined TA wet (i.e., *Amphitrema flavum*, *A. wrightianum*) and dry (i.e., *Euglypha tuberculata*, *Corythion dubium*) indicators. These are probably the reasons for the similar TA community composition among sites (with a clear wetness gradient) from both heather and sedge litter. Nonetheless, the relative abundance of stronger wet indicators (i.e., *Amphitrema flavum*, *Euglypha strigosa*) in sedge litter was still overall slightly higher than in heather litter. This is likely due to their respective water content as sedge litter is usually found in dense and soft litter

layers, keeping moisture within it, and in overall wetter areas compared to heather litter with looser and harder leaf layers (i.e., needle-like leaves and woody branches).

Moreover, the mosses (*Sphagnum* moss and other mosses) not only lack roots and leaf stomata but also do not have specialized conducting tissues. They acquire water either from rain or water bodies below through an external capillary network formed by spaces between the leaves, and between the stem and branches (Rydin et al. 2013). Mosses are therefore more prone to be impacted by hydrological changes. Interestingly, despite the similar way to absorb rainwater, TA communities from other mosses were dramatically different from that on *Sphagnum* moss. This may again be explained by their highly differential water-holding capacity in relation to the *Sphagnum* hyaline cells (i.e., providing storage and thus buffering capacity against drying out). However, other mosses can only store limited quantities of water inside their tissues, mainly within the small living chlorophyllous cells. *Sphagnum* mosses therefore offer a niche for a broader range of TA species to colonize and dwell, especially for those with high moisture preferences. This was also corroborated by the significantly higher richness of TA on *Sphagnum* moss than on all other plant types in this study (Fig. 2.2b).

While TA from *Sphagnum* mosses were shown to be better to indicate the wetness gradient among sites, different types and number of *Sphagnum* moss species have the potential to cause an indication bias, either overestimating or underestimating, as different species show distinctive responses to hydrological changes (Li et al. 1992, Robroek et al. 2009, Bu et al. 2013, Bengtsson et al. 2021). For example, hummock forming species (e.g., *S. fuscum*, *S. capillifolium*) have repeatedly been suggested to be less sensitive than lawn (e.g., *S. magellanicum*) and hollow habitat (e.g., *S. fallax*) species to changes in water level (e.g., long periods of drought or desiccation) due to their higher water-holding capacity and superior efficiency in transporting water from water table to capitula (Robroek et al. 2007, Bu et al. 2013). However, this study did not find any significant difference between TA communities from different combinations of *Sphagnum* moss species (i.e., comparing all *Sphagnum* moss to monospecific species: *S. capillifolium* or *Sphagnum* mosses without rare species), which was, to a large extent, consistent with the second hypothesis: *different number of Sphagnum moss replicates does not affect TA's indicator value for habitat wetness*. This may be partly related to the topography of the sample areas, as our samples were

collected in relatively flat and homogeneous plots. Despite the discrepancy in keeping capitula moist, water content of capitula among species was likely to be similar as they grow in the same layer and/or overlap with each other due to the lateral transport of water through contact between leaves and branches (Li et al. 1992, Rydin et al. 2006). Meanwhile, a random combination of *Sphagnum* moss species with different topographic position preferences (i.e., hummock, lawn, hollow) would theoretically be able to compromise the over- or under-effects from hollow and hummock preferred species (whereas samples in this study were all taken from near flat areas). TA communities were therefore not considerably different between combined all *Sphagnum* mosses and the monospecific species (*S. capillifolium*) although the correlation coefficient was a bit lower for *S. capillifolium* (Fig. 2.4). But noticeably, only *S. capillifolium* was sampled with a relatively abundant occurrence (more than three in each site) at all study sites, being particularly abundant at Nidderdale and Mossdale. This may suggest that *S. capillifolium* has a wider ecological niche and greater desiccation tolerance and resilience than other studied *Sphagnum* moss species.

Rare *Sphagnum* species seemed to have no substantial effects on TA community composition when combined with or removed from other *Sphagnum* moss species. This is likely the result of a large number of samples being analyzed (more than 20 in each site), which likely reduced the impact of rare species to a negligible degree. However, the rarest *Sphagnum* species (*S. palustre*) itself was inferior in indicating the habitat wetness (Fig. 2.6). The relatively much lower abundance of well-defined TA wet indicators (i.e., *Amphitrema flavum*, *A. wrightianum*) from *S. palustre* in the very wet intact border mire (Butterburn) may suggest that other factors such as nutrients could be more important than water table depth or substrate moisture in determining the growth of this species. It is also possible that *S. palustre* was by chance sampled from a relatively higher position (similar to a hummock) in the intact border mire (but sample height above peat surface was not recorded). By contrast, both the dominant and second abundant *Sphagnum* moss species at each site performed as well as the combined all *Sphagnum* mosses did. Expectedly, the dominant *Sphagnum* species was slightly superior to the second abundant species. This is consistent with the third hypothesis: *TA on the dominant Sphagnum moss performs better than on the less dominant Sphagnum moss species*. In general, good performance for both dominant and second

most abundant *Sphagnum* moss species may indicate that the water content of both species was similar.

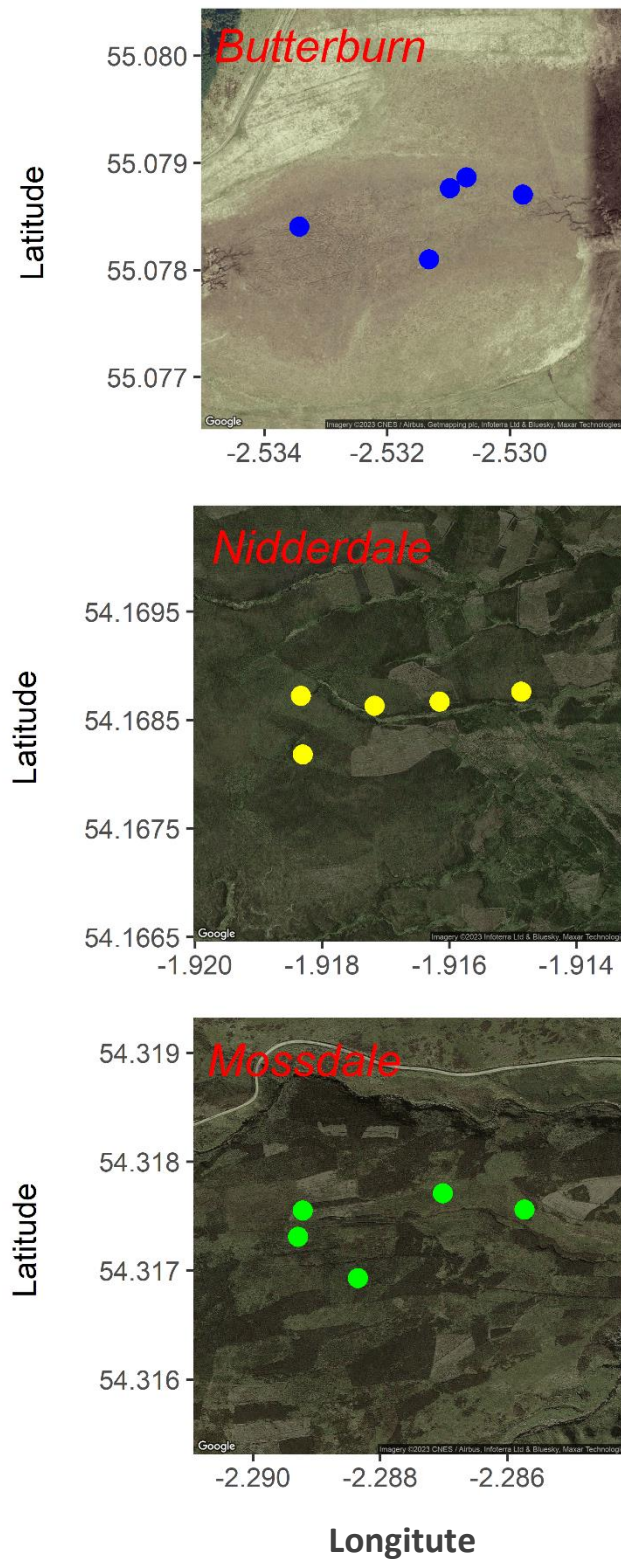
The findings overall suggest that sampling TA communities from different *Sphagnum* moss species in a relatively flat (lawn) position provides a fairly consistent and representative indication of habitat wetness condition. However, it is often time-consuming and expensive to process the concomitant large number of samples as both extraction and identification of TA are slow. In addition, it is often difficult and even impossible to find the same *Sphagnum* moss species existing across several different and spatially dispersed study sites, especially for drier blanket bogs with often low cover of *Sphagnum* moss. However, despite the similar performance of the monospecific species (i.e., *S. capillifolium*) versus the combined all *Sphagnum* mosses, sampling just the same species may not be the optimal option. Given the limitations mentioned above, this study recommends that sampling the dominant *Sphagnum* moss species from a lawn position is a more practical, simplified, and informative method when using TA as hydrological bioindicators in peatlands. However, all these assertions are mainly based on the deduction from previous studies and the limited observation from the current study. Future work should also measure fundamental hydrological and physicochemical parameters from different plant functional types to further confirm and support the inferences and recommendations. More work is also needed to focus on the functional differences (e.g., carbon fixation, water transport and holding capacity) between *Sphagnum* moss species, especially when they grow in the same microsite.



## 2.6 Conclusions

This study shows a superior performance of TA sampling from *Sphagnum* moss compared to other plant types (i.e., other mosses, heather and sedge litter) to indicate the hydrological condition of peatland habitats by investigating their associated testate amoebae (TA) communities. Despite the problems of rare *Sphagnum* species not assisting with predicting the wetness gradient among sites, a combination with or removal from other *Sphagnum* species and an unbalanced number of samples had no considerable impacts on the general performance of TA as wetness indicators. Moreover, the similar TA communities on *S. capillifolium* compared to those on combined all *Sphagnum* moss species may suggest a small effect of different *Sphagnum* species when they are sampled from relatively flat (lawn) position. Overall, considering the cost and practicality, the present study recommends sampling the dominant *Sphagnum* species from a lawn position to be a more simplified yet effective method if the aim is to assess the potential of TA as hydrological bioindicators or when using them to monitor the restoration (rewetting) success on blanket bogs.

## 2.7 Supplementary material



**Supplementary Fig S2.1** Detailed plot location of study sites in the UK. Nidderdale and Mossdale are both ‘modified’ heather-dominated blanket bog sites under grouse moor management, whilst Butterburn is an ‘intact’ border mire with low heather and high *Sphagnum* moss cover.

## **Chapter 3: Seasonal change and vertical distribution of testate amoeba communities on *Sphagnum* moss in a blanket bog**

### **3.1 Abstract**

Many modified or degraded blanket bogs in the UK have been undergoing restoration by a series of rewetting strategies in recent years. While testate amoebae (TA), as well-known hydrological indicators, have been increasingly applied to assess their potential in monitoring peatland restoration success, we still have little understanding on the most appropriate sampling strategy, particularly in which season and which position of TA samples (taken from *Sphagnum* moss) are most optimal to be collected in modified heather-dominated blanket bogs. In this study, TA communities were taken from three different *Sphagnum* moss species (i.e., entire section of *S.capillifolium*, *S.fallax*, and *S.palustre*) at nine fixed locations in a heather-dominated blanket bog to investigate their seasonal (March, June, September, January) and vertical (top 3 cm, middle and bottom 3 cm) variation. The results showed that: 1) TA species richness and density consistently increased from March to September, with density reaching the highest value in the following January; 2) despite the similarity of TA community composition among seasons, average proportion of encysted TA was much higher in both June and January than in March and September; 3) there was a clear and marked vertical separation of TA in living status and density, with living TA constantly decreasing (but dead TA and density increasing) from top to bottom *Sphagnum* segments; 4) idiosomic TA (with siliceous shell) were particularly abundant (more than 90% of total abundance) in all *Sphagnum* segments, which may indicate a substantial contribution and/or role of TA to silicon cycling in blanket bogs. Overall, based on the seasonal and vertical variation of TA mentioned above, along with the field observation of the living status of *Sphagnum* mosses, the present study recommends sampling the entire *Sphagnum* section in late autumn or early winter in British blanket bogs by counting living individuals as an appropriate sampling strategy to harness the potential of TA as hydrological bioindicators and to monitor the restoration (rewetting) success of degraded peatlands.

## 3.2 Introduction

Peatlands play an important role in the global carbon cycle as they store approximately 30% of the world's soil carbon (Blodau 2002), which is equivalent to twice the amount of carbon stored in all the forests combined (Dunn and Freeman 2011). However, peatlands cover only 3% of the Earth's land area (Joosten et al. 2016). They therefore possess the capacity to significantly influence global climate change by being either carbon sinks or sources (Frolking et al. 2006). In the UK, blanket bogs are one of the most important peatland types, which are estimated to occupy about 15% of the global blanket bog areas, and store around 2,300 Mt of soil organic carbon (Billett et al. 2010). They are also home to numerous specialized and rare upland plants and animals (Bain et al. 2011). However, many of them are classified as being in a degraded state, exhibiting low biodiversity and reduced carbon storage and water quality, likely as a result of a combination of various past management practices such as drainage, peat extraction, grazing and potentially rotational burning of vegetation (Cooper et al. 2001, Wilson et al. 2011c, Heinemeyer et al. 2018, Alday et al. 2022). However, the separation of cause and effect of individual aspects remains uncertain, although drainage seems the main cause of observed negative impacts (Ashby and Heinemeyer 2021). Presently, extensive restoration efforts are underway in degraded peatland regions, focusing on revitalizing peat hydrological functioning, enhancing biodiversity, and improving crucial ecosystem services such as carbon sequestration (Parry et al. 2014, Pilkington et al. 2015, Heinemeyer et al. 2023). Consequently, it is crucial to evaluate and closely monitor the peatland conditions and their progress in recovering towards intact and functional bogs after undertaking these restoration endeavors.

Among possible approaches, using testate amoebae (TA) as bioindicators seems a promising way to achieve this purpose as TA are known to show sensitive and species-specific responses to environmental changes, especially water table depth or substrate moisture (Lousier 1974, Marcisz et al. 2014a). Due to their assumed well-preserved shells after death, they have been widely and successfully applied for palaeohydrological changes (Charman 1997, Booth 2002, 2008, Swindles et al. 2014, Liu et al. 2019). Many studies have also reported that TA are particularly abundant in *Sphagnum*-dominated peatlands, within which living TA mainly dwells in *Sphagnum* mosses (Booth 2002, Mieczan 2009, Jassey et al. 2011b). In Chapter 2, it was found

that sampling the dominant *Sphagnum* species from lawn positions is likely to be a simple yet effective method if the aim is to use TA to monitor the restoration success in blanket bogs. Nevertheless, another key aspect that needs to be further investigated is when and from which segment to sample the *Sphagnum* moss associated TA communities to best represent the ecological site conditions.

Seasonal monitoring of TA has been increasingly applied in peatlands during the past few decades (Heal 1964, Gilbert et al. 2003, Warner et al. 2007, Lamentowicz et al. 2013, Song et al. 2018), but the results regarding changes in pattern of community structure, species richness/diversity and density/biomass are not completely consistent (Mieczan 2007, Warner et al. 2007, Marcisz et al. 2014b). For example, most studies reported that TA reach their highest abundance in either spring or summer (Heal 1964, Gilbert et al. 2003, Mieczan 2007, Song et al. 2018), while other studies showed no clear and consistent change tendency of species abundance during growing season (from May to September) (Mazei and Tsyganov 2007) or different peak time of density among sites (Lamentowicz et al. 2013). Furthermore, community structure was reported to be significantly different between spring and summer in open bog and fen (Warner et al. 2007, Marcisz et al. 2014b) but not clearly distinct in a swamp (Warner et al. 2007). However, what these studies have in common is that they primarily attribute these seasonal discrepancies to hydrological fluctuations although other factors like pH, nutrient, and available food sources are probably more important when substrate moisture or water tables are relatively constant over the course of investigation (Lamentowicz et al. 2013, Marcisz et al. 2014b, Song et al. 2018). Noticeably, these studies were mainly carried out during the vegetation growing season (mostly from April to October) in either *Sphagnum*-dominated bog/fen or tree-dominated swamp. In fact, little is known about TA communities (including encystment status) in the winter, especially in heather dominated (*Calluna vulgaris*) blanket bogs where vegetation cover is highly different from the above-mentioned studies and habitats.

Apart from seasonality, vertical variation of TA (living and dead) in *Sphagnum* mosses is another key factor worth considering to determining which position/segment is best for sampling to indicate the plot conditions as vertical micro-distribution of TA has previously been reported (Heal 1962, Booth 2002, Mitchell et al. 2004, Jassey et al.

2011a, Song et al. 2016). On the whole, three general patterns of TA along the *Sphagnum* segments have been commonly recognized. Specifically, in the upper green segment (mostly from the top 3 cm), mixotrophic species that contain symbiotic zoochlorellae (i.e., those that photosynthesise), like *Amphitrema flavum*, *Heleopera sphagni*, and *Hyalosphenia papilio*, are relatively more abundant mainly due to their requirement of light for photosynthesis (Heal 1962, Booth 2002). Interestingly, another mixotrophic TA species, *Amphitrema wrightiamum*, is more often found in the middle segment (mostly from 4 to 10 cm), which can be considered as a compromise/balance of light requirement of the algal symbiont and their own requirement of suitable materials for test construction (Mitchell and Gilbert 2004, Jassey et al. 2011). By contrast, agglutinate taxa that incorporate foreign materials like sand grains or fungal hyphae into their shells (e.g., *Trigonopyxis arcula*, *Cyclopyxis arcelloides*, *Diffflugia leigyi*) are more common in the lower segment (Heal 1962). This was primarily explained by the increased availability of test materials and food sources with depth (Chacharonis 1954, Song et al. 2016, Roe et al. 2017). Additionally, vertical variation along the *Sphagnum* section has also been linked to a gradient from mostly alive TA in the aerobic, upper parts to mostly dead specimens in the more anaerobic, lower parts (Booth 2002). As a result, these spatial variations are largely associated with the gradient of light, temperature, oxygen and available test materials and food sources (Jassey et al. 2011). Despite this information on vertical TA distribution, we still lack more specific knowledge on the vertical pattern of TA in heather-dominated blanket bogs (in which *Sphagnum* mosses are often less abundant and mostly shaded by taller heather).

Here, TA communities were investigated seasonally (March, June, September, January) in a UK heather-dominated blanket bog for top, middle and bottom segments of three different (at this site dominant) *Sphagnum* mosses (i.e., entire section of *S.capillifolium*, *S.fallax*, and *S.palustre*) at nine fixed locations to explore the optimal season and vertical position for TA community sampling. The objectives of this study were to investigate firstly, the seasonal changes of TA in community composition, species richness, density and encystment status, and secondly, the vertical variation of TA in community composition (including specific species), living status and density. Due to the considerable seasonal differences in temperature, precipitation and peat moisture in heather-dominated UK blanket bogs (e.g., Heinemeyer et al. 2023), it was hypothesized

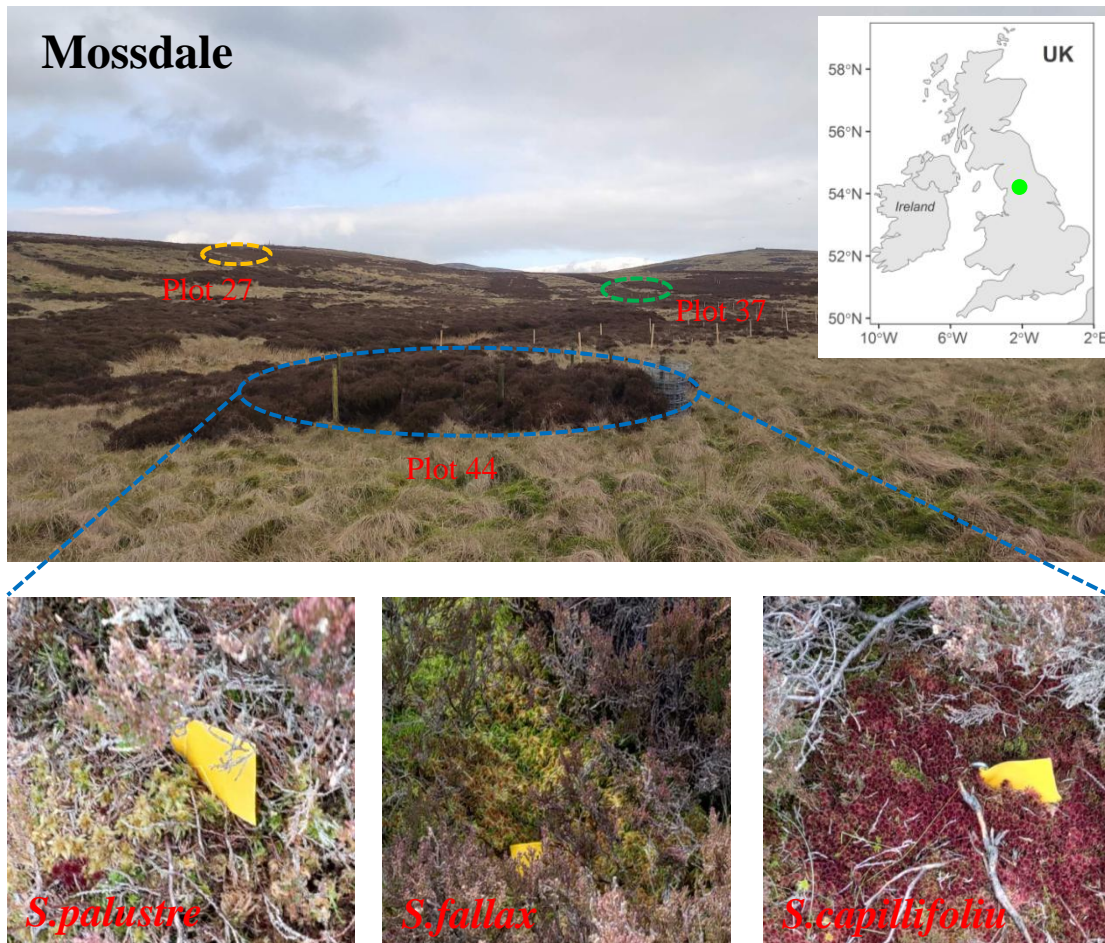
that: 1) TA community composition, species richness, density and encysted TA are significantly different among seasons; 2) there are distinctive vertical variations along *Sphagnum* segments in TA community composition, living status and density, with mixotrophic testate amoebae (MTA) being more abundant in the upper and intermediate regions while agglutinate taxa being more common in the lower region.

### 3.3 Methods

#### 3.3.1 Study site

Samples were collected from one modified blanket bog site, Mossdale (54°19'01"N; 2°17'18"W), which is located in upper Wensleydale within the Yorkshire Dales National Park at about 390 m above sea level (a.s.l) (Fig. 3.1). This site has a low sheep stocking density of < 0.5 ewes ha<sup>-1</sup> and is under grouse moor management with a ~20-year burn rotation as part of a long history of heather burning (more than 100 years; based on estate information and confirmed by charcoal records) (Heinemeyer et al. 2018). However, sample plots were located within the 'mown' catchment (burning stopped in 2012 and was replaced with cutting heather). Moreover, Mossdale offers fairly flat areas with mature *Calluna vulgaris* (ling heather) cover of ~81.1 % and a considerable proportion of other bog vegetation (see Heinemeyer et al. 2019; 2023), mainly in the form of ~3.3 % *Eriophorum* (cotton-grass), ~24 % other mosses (*Hypnum jutlandicum*) and ~48.9 % *Sphagnum* moss species. During the study period (February 2021 - January 2022), the mean annual air temperature was 7.2°C and annual total precipitation was 1453 mm. The site lies on limestone which is overlain by thin sandstone covered with mudstone (Hall 1979). The soil is a poorly draining organic peat (Winter Hill series) with an average slope of  $6 \pm 3^\circ$  and an average peat depth of  $1.2 \pm 0.4$  m at the experimental plots and peat depth across the catchments ranging from 0.3 m to 2.1 m (manual peat rod measurements made in 2012; see Heinemeyer et al. 2019). Most of the drainage grips within the study areas, which were dug about 50 years ago, were found to be naturally infilled by 2012 (Heinemeyer et al. 2019). There are no natural erosion gullies at this site.





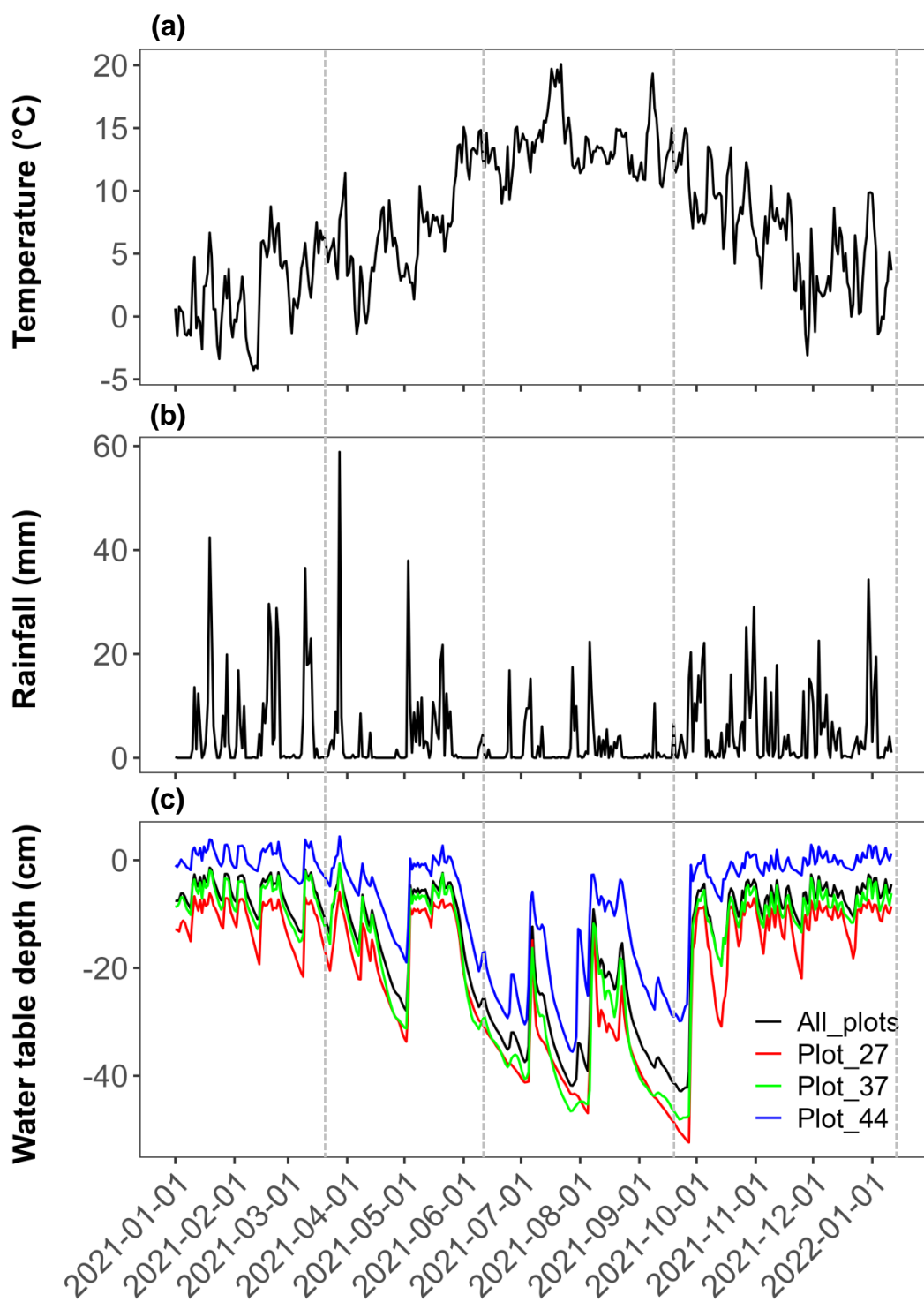
**Figure 3.1** Location of the modified heather-dominated blanket bog study site (Mossdale) in the UK (green dot). Three 5 x 5 m areas (plots 44, 37, 27 of an existing experimental design; see Heinemeyer et al. 2019; 2023) were selected for seasonal monitoring (reflecting a gradient of wetness from wet to dry, respectively). In each plot, patches of three *Sphagnum* moss species (*S. capillifolium*, *S. fallax*, *S. palustre*) were marked using a yellow peg as fixed locations.

### 3.3.2 Sampling

Three different types of *Sphagnum* mosses (*S. capillifolium*, *S. fallax*, and *S. palustre*) were sampled seasonally (2021: March, June, September, and 2022: January) at three fixed locations within each 5 x 5 m plot (three plots in total labeled as plot numbers 27, 37, 44) in the uncut heather-dominated plots (heather age of approximately 35 years) located within the ‘mown’ catchment (Fig. 3.1) (this and other additional site data in the following sections were part of the Peatland-ES-UK project; Heinemeyer et al. 2019; 2023). The sampling areas were all in the shade of/under cover of dense heather (mean height of about 50 cm and cover of around 75%). A total of 36 samples were collected



across the year, consisting of 12 samples each for *S. capillifolium*, *S. fallax*, and *S. palustre*. In addition, hourly climate (air temperature at 1 m above ground level and precipitation at ground level) data over the course of investigation was recorded (see Fig. 3.2a, b) and collected from the automated weather station (see Heinemeyer et al. 2019; 2023) at the center of the site (~200 m northwards of plot 27). Also, continuous monitoring data (every six hours; Heinemeyer et al. 2019; 2023) of water table depth was logged at each sampling plot (Fig. 3.1, Fig. 3.2c).



**Figure 3.2** Seasonal change of daily (a) air temperature (b) rainfall, and (c) water table depth (zero indicates the peat surface and negative values drying peat) measured at the Mossdale (heather-dominated blanket bog) site from January 2021 to January 2022 (dashed line represent sampling times) at the uncut (i.e., no recent heather management) plots (labeled as plot 27, 37, 44) where *Sphagnum* moss was sampled. Data from Heinemeyer et al. 2023.

### 3.3.3 TA processing

Each *Sphagnum* moss was cut into three segments to represent the upper (top 3 cm: living area), middle (between upper and bottom: combination of potential living part and litter), and bottom (lowest 3 cm: litter) regions of *Sphagnum* moss, respectively, for vertical investigation (all segments were combined for seasonal investigation) (Supplementary Fig. S3.1). Testate amoebae (TA) were extracted using a modified version of the method of Booth et al. (2010). Around 5 g fresh *Sphagnum* moss for each sample was used for preparation. The weighted sample was soaked and disaggregated in the 100 ml beaker with about 60-80 ml deionized water for at least 24 hours and stirred occasionally. At the same time, one tablet of *Lycopodium clavatum* was added for density calculation. The sample liquid was first sieved through a 355  $\mu\text{m}$  mesh to remove plant and coarse particulate matter and the filtrate was then sieved with a 10  $\mu\text{m}$  mesh to filter some of the smaller particulates which tends to make analysis easier and more efficient. The material retained in the 10 mesh  $\mu\text{m}$  was carefully washed into 50 ml centrifuge tubes and centrifuged at 4,000 rpm for five minutes. 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 color) from dead (empty) individuals. TA identification and counting were performed under light microscopy at 200x and 400x magnification, with a target of tallying at least 150 individuals in each sample.

### 3.3.4 Statistical analyses

The number of TA species in each sample was counted for each type of *Sphagnum* moss to compare their species richness among seasons. TA density in each sample was also calculated for both seasonal and vertical comparisons. For all other analyses, taxa present in less than 4% of the total number of samples and those whose relative abundance of overall less than 4% were eliminated from the TA community dataset to reduce the influence of rare taxa. Nonmetric Multidimensional Scaling (NMDS) ordination analysis was used to compare TA assemblages among seasons and along the different segments of *Sphagnum* moss (upper, middle and bottom). Prior to NMDS, the species abundance data was Hellinger transformed (Legendre and Gallagher 2001). Relative abundance of wet and dry TA indicators was mainly calculated to indicate the wetness condition of habitats among seasons. Before doing this, a series of TA wet and

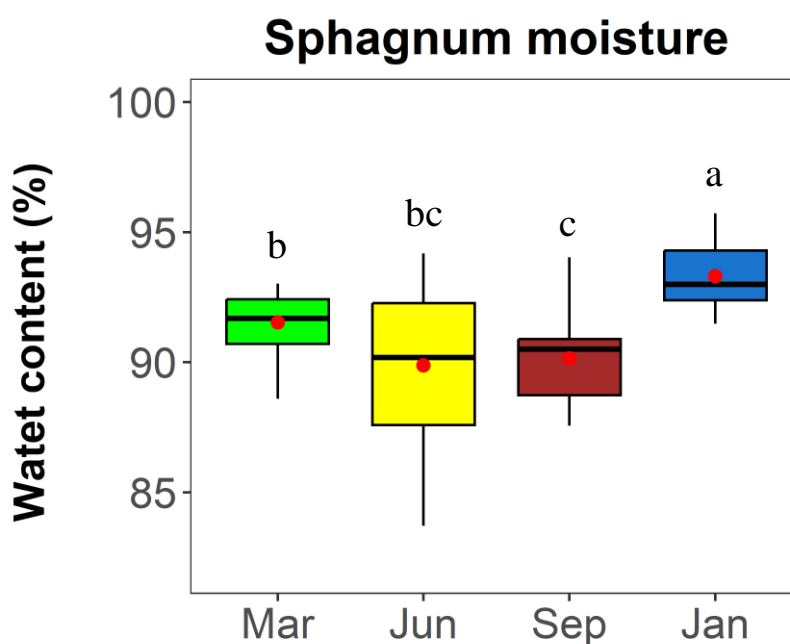
intermediate indicator species (*Arc.art* - *Arcella artocrea*, *Eug.stri*: *Euglypha strigosa*, *Hel.syl*: *Heleopera sylvatica*, *Neb.co.bo*: *Nebela collaris-bohemica* type, *Neb.fla* - *Nebela flabellulum*, *Neb.mil*: *Nebela militaris*, *Cyc.arc*: *Cryclopixis arcelloides* type) and dry indicator species (*Eug.tub*: *Euglypha tuberculata*, *Neb.tin*: *Nebela tincta*, *Tri.arc*: *Trigonopyxis arcula*, *Ass.mus*: *Assulina muscorum*, *Cor.dub*: *Corythion dubium*) were determined and classified in accordance with their previously reported hydrological preferences (Tolonen et al. 1992, Charman 1997, Bobrov et al. 1999, Booth 2001, Lamentowicz and Mitchell 2005, Mieczan 2009). The proportion of encysted TA was also calculated (as the ratio of total encysted TA to total TA) to compare the encystment status among seasons. The significant associations for species richness, density, encysted TA and each TA species among seasons were tested using Kruskal-Wallis's tests. All the analyses and plotting of graphs were performed using R (R Core Team 2013).

## 3.4 Results

### 3.4.1 Climate and hydrological conditions

Mean air temperature and total precipitation (based on hourly data obtained from a weather station; see Heinemeyer et al. 2019; 2023) showed an approximately opposite seasonal change pattern throughout the year (Fig. 3.2a, b). Specifically, temperature displayed an arched pattern from January 2021 to January 2022, peaking in the summer, with the highest value reaching 20.1°C in July and lowest value being -4.3°C in February (Fig. 3.2a). By contrast, precipitation showed more dynamic monthly changes, but the total seasonal rainfall reached its maximum (426.1 mm) in winter (sum from December to February) and its minimum (189.8 mm) in summer (sum from June to August) (Fig. 3.2b). Seasonal change of water table depth (WTD) was largely explained by air temperature ( $R^2 = 0.54$ ) (Supplementary Fig. S3.4a) but much less so to rainfall ( $R^2 = 0.13$ ) (Supplementary Fig. S3.5a). However, rainfall correlated slightly better with water tables within one day ( $R^2 = 0.17$ ) after the rain (Supplementary Fig. S3.5e). Noticeably, this correlation was not improved by removing the low amount of rainfall (rainfall >1mm:  $\text{Rad}j^2 = 0.08$ , rainfall >2mm:  $\text{Rad}j^2 = 0.12$ , rainfall >5mm:  $\text{Rad}j^2 = 0.04$ ) for those with WTD more than -30 cm (Supplementary Fig. S3.5b,c,d) but dramatically improved for those with WTD less than -30 cm when rainfall was more than 2 mm

( $\text{Radj}^2 = 0.35$ ) (Supplementary Fig. S3.5c). The highest mean WTD (based on 6-hourly data obtained from a logger in each plot; see Heinemeyer et al. 2019; 2023) of the studied plots (-5.6 cm) occurred in the winter, while it reached the lowest level (-28.5 cm) in the summer (Fig. 3.2c). Although all three studied plots (i.e., plot 27, 37, 44) showed similar patterns, the WTD in plot 44 was the highest (wettest) and plot 27 was consistently the lowest (driest) (Fig. 3.2c). Noticeably, *Sphagnum* moisture content showed significant seasonal changes, but the range of change was relatively small, with the highest water content of combined *Sphagnum* mosses (mean: 93.3%, median: 93.0%) in January, and lowest value (mean: 89.9%, median: 90.2%) in June (Fig. 3.3).

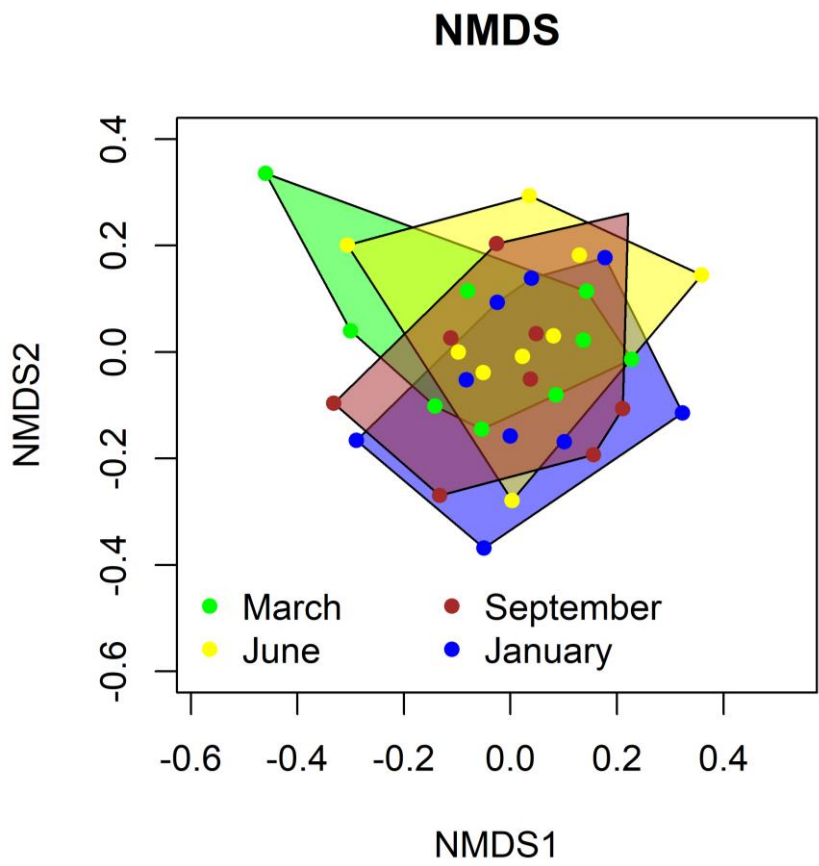


**Figure 3.3** Seasonal change pattern of *Sphagnum* moss moisture across 2021-Mar: March, Jun: June, Sep: September; and 2022-Jan: January. Box shows the median (middle line), 25% (bottom border) and 75% (top border) ranges, with asterisks representing outliers. Red points represent the mean value. Different letters indicate significant difference of the medians among seasons (Wilcoxon test,  $P < 0.05$ ).

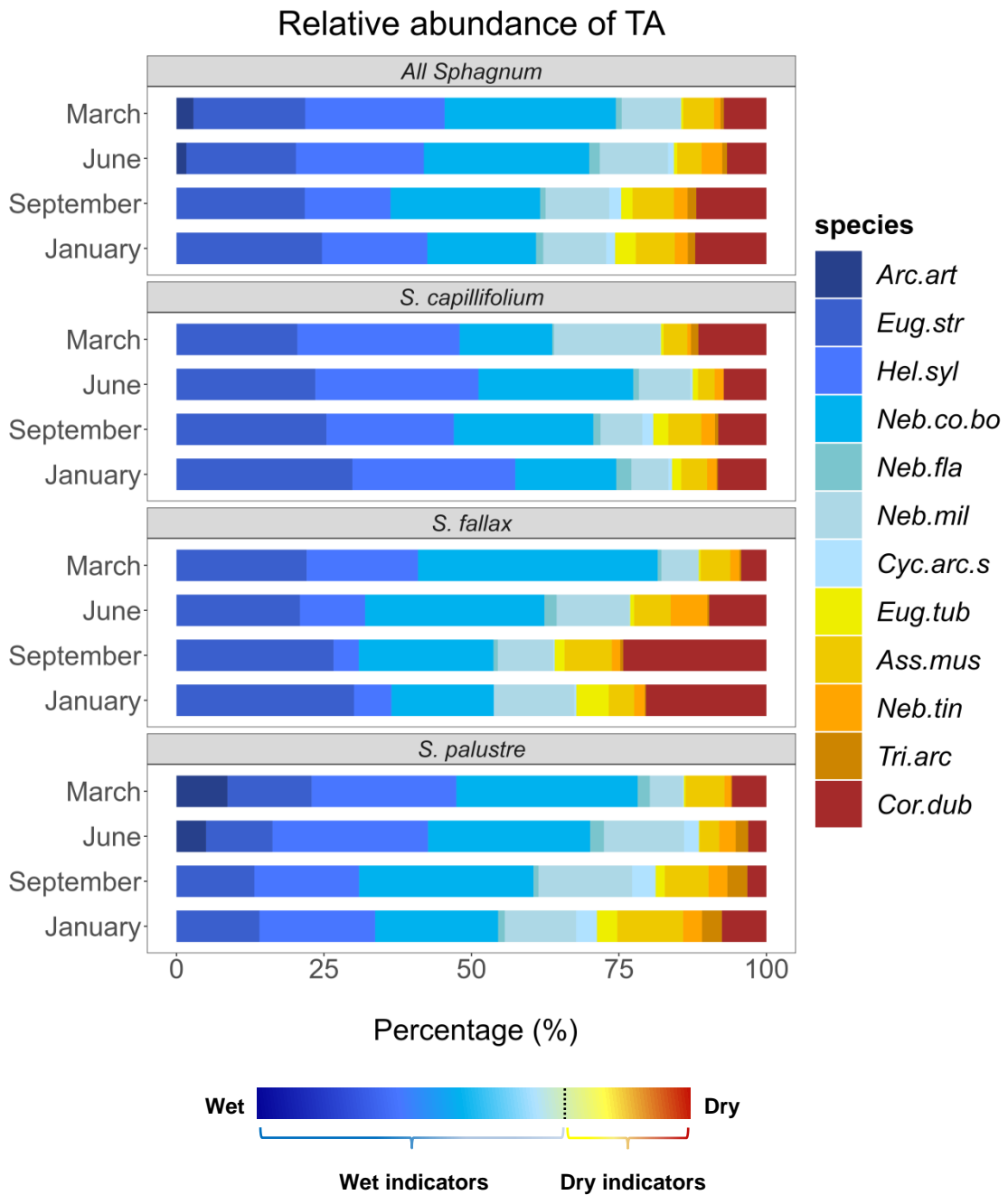
### 3.4.2 Seasonal change of TA in *Sphagnum* mosses

A total of 38 TA taxa were identified from 36 samples collected throughout the year. NMDS ordination showed no considerable difference for the living TA community composition among seasons (Fig. 3.4). Nevertheless, the total relative abundance of living dry indicators from the combined *Sphagnum* mosses in September (24.6%) and January (25.6%) was higher than in March (14.4%) and June (15.7%) (Fig. 3.5). Among the species, only *Euglypha tuberculata* ( $H(3) = 18.493$ ,  $p < 0.001$ ) and *Cyclopyxis arcelloides* ( $H(3) = 11.818$ ,  $p = 0.008$ ) showed statistically significant seasonal

differences across the year, but at a very low proportion, with the highest mean value being 3.5% in January and 2.0% in September respectively (Fig. 3.5, Table 3.1). Noticeably, while there was no statistical significance, the relative abundance of *Corythion dubium* type was obviously higher in September (11.9%) and January (12.1%) than in March (7.2%) and June (6.7%) (Fig 3.5). Interestingly, individual *Sphagnum* moss species showed different degrees of response to seasonal change, with TA communities in *S. fallax* and *S. palustre* being more distinctive than in *S. capillifolium* (Fig. 3.5, Supplementary Fig. S3.2).



**Figure 3.4** Non-metric multidimensional scaling (NMDS) analysis of living testate amoebae (TA) community composition across 2021 (March: green, June: yellow, September: brown) and 2022 (January: blue) in *Sphagnum* moss samples (combination of *S. capillifolium*, *S. fallax*, and *S. palustre*) collected at Mossdale (heather-dominated blanket bog).



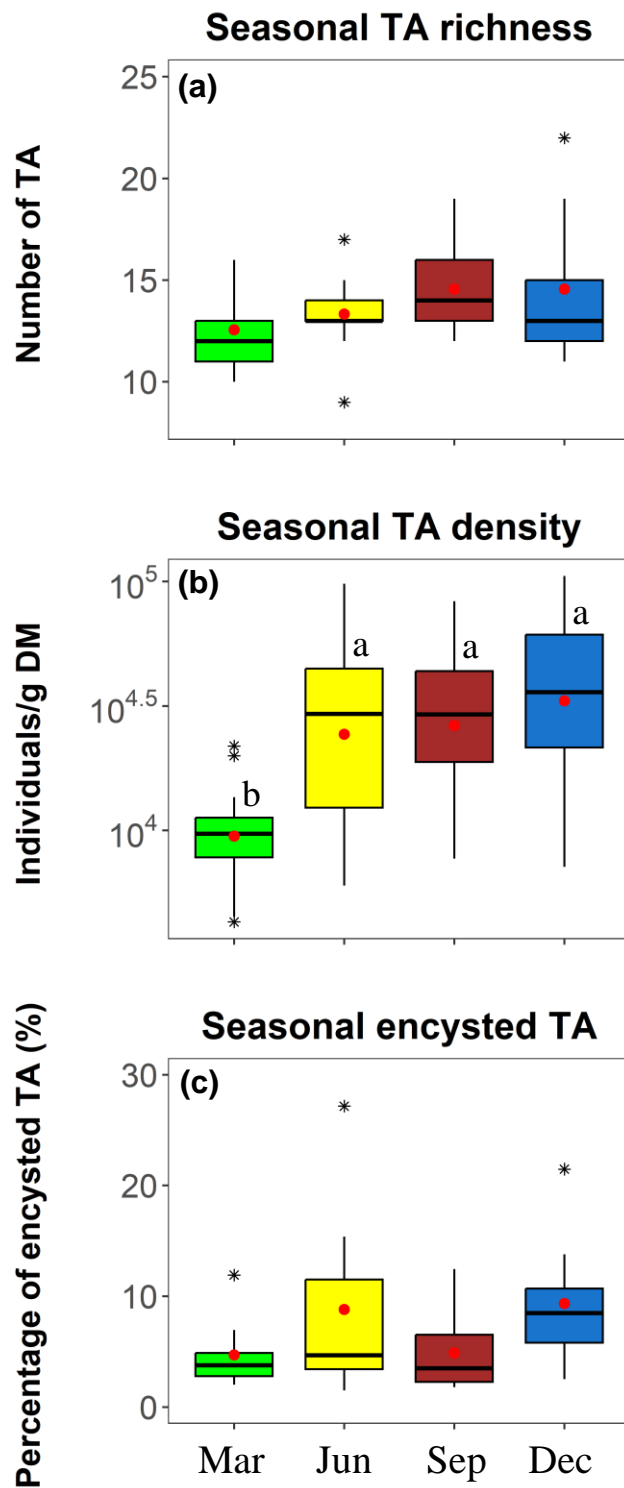
**Figure 3.5** Relative abundance of living testate amoebae (TA) among seasons on combined all *Sphagnum* moss, *S. capillifolium*, *S. fallax*, *S. palustre*, respectively at Mossdale. Species that occurred more than 4% of total number of samples and those whose relative abundance all more than 4% were selected and displayed: *Arc.art* - *Arcella artocrea*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Cyc.arc.s* - *Cyclopyxis arcelloides* small type, *Eug.stri* - *Euglypha strigosa*, *Eug.tub* - *Euglypha tuberculata*, *Hel.syl* - *Heleopera sylvatica*, *Neb.co.bo* - *Nebela collaris-bohemica* type, *Neb.flu* - *Nebela flabellulum*, *Neb.mil* - *Nebela militaris*, *Neb.tin* - *Nebela tinca*, *Tri.arc* - *Trigonopyxis arcula*. The colour scheme indicates wet (blue) to dry (reddish brown) TA indicator species and black dashed line starting from yellow color represents the threshold between wet (including intermediate) and dry indicators.

**Table 3.1.** Results of Kruskal Wallis test (KW\_test,  $P < 0.05$ ) for living testate amoebae (TA) in seasonal samples for either all combined *Sphagnum* moss or the key species (*S. capillifolium*, *S. fallax*, *S. palustre*) collected at Mossdale (heather-dominated blanket bog). The highest mean value of individual TA (H\_Mean\_%) among seasons was presented with numbers in bold indicating species with significant seasonal change.

Taxa	All_Sph_TA		<i>S. capillifolium</i> _TA		<i>S. fallax</i> _TA		<i>S. palustre</i> _TA	
	KW_test	H_Mean_%	KW_test	H_Mean_%	KW_test	H_Mean_%	KW_test	H_Mean_%
Arc.art	0.560	2.9	1	0	1	0	0.530	8.6
Ass.mus	0.671	7.0	0.578	5.6	0.578	8.0	0.972	11.2
Cor.dub	0.298	12.1	0.468	11.5	<b>0.029</b>	24.3	0.270	7.5
Cyc.arc.s	<b>0.008</b>	2.0	0.063	1.9	0.243	0.4	0.116	4.0
Eug.str	0.424	24.7	0.516	29.8	0.478	30.1	0.740	14.3
Eug.tub	<b>0.001</b>	3.5	0.350	2.5	0.089	5.5	<b>0.028</b>	3.4
Hel.syl	0.312	23.6	0.459	27.7	0.319	18.9	0.789	26.4
Neb.co.bo	0.059	29.0	0.172	26.2	0.079	40.6	0.442	30.7
Neb.fla	0.676	1.8	0.697	2.6	0.452	2.1	0.872	2.3
Neb.mil	0.915	11.6	0.715	18.0	0.828	13.6	0.953	15.9
Neb.tin	0.556	3.5	0.633	2.3	0.735	6.2	0.785	3.2
Tri.arc	0.525	1.5	0.066	1.3	0.821	0.5	0.082	3.4

Species richness of TA showed an increasing, but not statistically significant trend from March (median: 12, mean: 13) to September (median: 14, mean: 15) (Fig. 3.6a). While the average number of TA in January 2022 (15) was the same as that in September 2021, the median was slightly lower in January (13) (Fig. 3.6a). TA density (individuals/g dry matter of *Sphagnum* moss (DM)) increased consistently from March 2021 to January 2022, and it was significantly higher ( $H(3) = 38.89$ ,  $p < 0.001$ ) in June (median: 29433, mean: 32220), September (median: 29315, mean: 31045), and January (median: 35945, mean: 42839) than in March (median: 9697, mean: 10155) (Fig. 3.6b). As for encysted TA, the median percentage was much higher in January (8.5%) than in other months (March: 3.8%, June: 4.7%, September: 3.5%) (Fig. 3.6c). However, the average proportion of encysted TA in June (8.8%) was only slightly lower than in January (9.3%) (Fig. 3.6c).

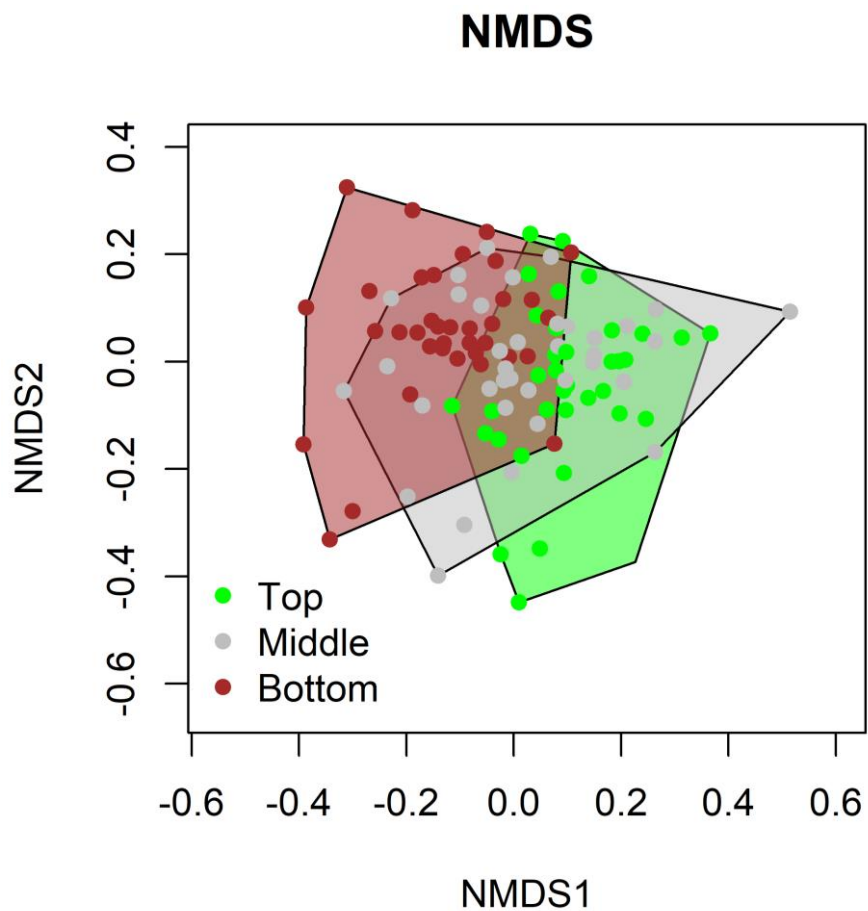




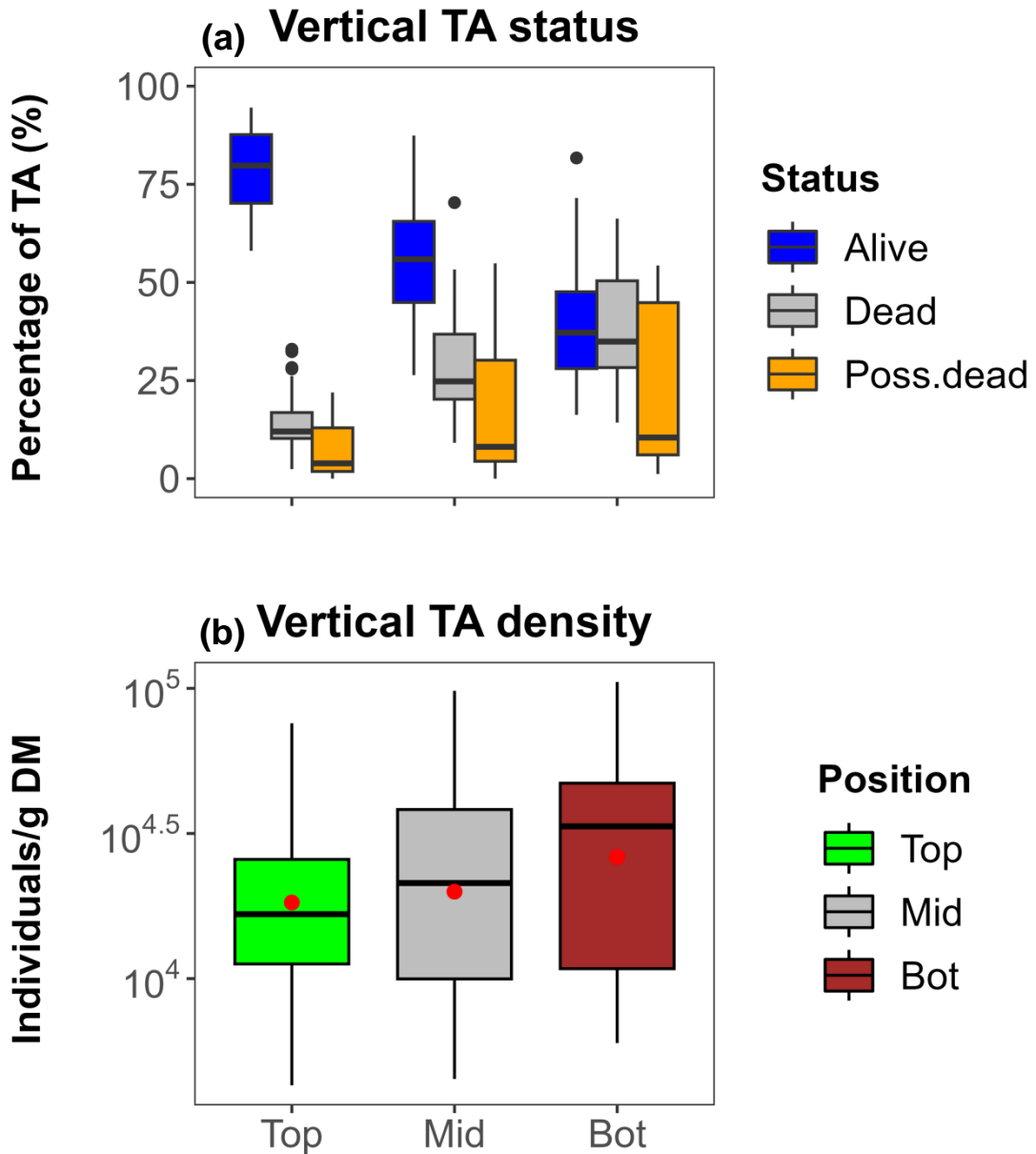
**Figure 3.6** Seasonal change pattern of (a) species richness: number of living testate amoebae (TA), (b) living TA density, (c) encysted TA in *Sphagnum* moss (combination of *S. capillifolium*, *S. fallax*, and *S. palustre*) across 2021-Mar: March, Jun: June, Sep: September; and 2022-Jan: January. Box shows the median (middle line), 25% (bottom border) and 75% (top border) ranges, with asterisks representing outliers. Red points represent the mean value. Different letters indicate significant difference of the medians among seasons (Wilcoxon test,  $P < 0.05$ ).

### 3.4.3 Vertical distribution of TA along *Sphagnum* sections

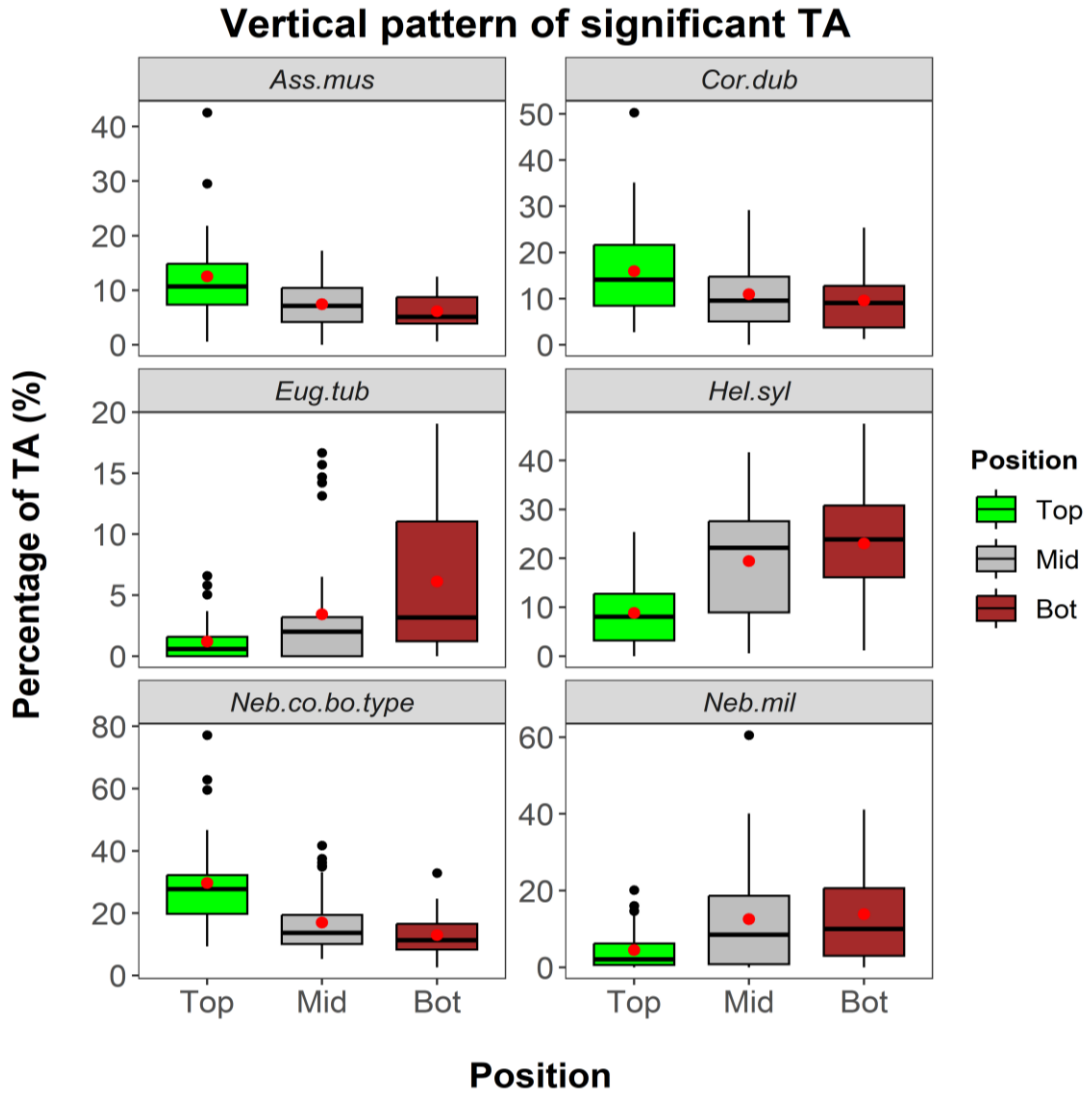
NMDS ordination showed clear and marked separation of TA community composition from top through middle to bottom parts of the *Sphagnum* sections (Fig. 3.7). Specifically, living TA decreased consistently in their relative abundance from top (median: 79.8%) to bottom (median: 40.2%) segments while both dead and possible dead TA increased progressively from top (median: 12.0%, 3.9% respectively) to bottom sections (median: 34.9%, 10.5%) (Fig. 3.8a). TA density (individuals/g DM) also showed an increasing trend for the *Sphagnum* from top (median: 16699) to bottom (median: 33466) segments (Fig. 3.8b). As for individual species, taxa *Euglypha tuberculata* ( $H(2) = 14.954, p < 0.001$ ), *Heleopera sylvatica* ( $H(2) = 29.658, p < 0.001$ ), and *Nebela militaris* ( $H(2) = 13.105, p = 0.001$ ) significantly increased from top to bottom segment whereas taxa *Assulina muscorum* ( $H(2) = 20.703, p < 0.001$ ), *Corythion dubium* ( $H(2) = 9.922, p = 0.007$ ) and *Nebela collaris-bohemica* type ( $H(2) = 36.842, p < 0.001$ ) significantly decreased accordingly (Fig. 3.9). Proteinaceous TA and agglutinated TA showed a clear, gradual but opposite change pattern along the *Sphagnum* section. However, both of them occupied only a low proportion, with proteinaceous TA being 3.0% in the upper, 1.0% in the middle, and 0.7% in the bottom regions while agglutinated TA representing 2.3%, 3.0% and 5.7% accordingly. By contrast, idiosomic TA (with siliceous shell) dominated all segments with 94.7%, 96%, and 93.6% respectively (Supplementary Fig. S3.3).



**Figure 3.7** Non-metric multidimensional scaling (NMDS) analysis output for testate amoebae (TA) community composition on different sections (Top: 0-3 cm, Middle: ~3 cm between top and bottom, Bottom: lowest 3 cm) of *Sphagnum* moss (combination of *S. capillifolium*, *S. fallax*, and *S. palustre*) at Mossdale (heather-dominated blanket bog).



**Figure 3.8** Vertical change pattern of testate amoebae (TA) for (a) status (alive: stained with pink color, dead: empty inside the body, and possible dead (poss. dead: likely dead as not stained with pink color but showed some visible food and/or cytoplasm inside the body)) and (b) density on different sections (Top: 0-3 cm, Mid-Middle: ~3 cm between top and bottom, Bot-Bottom: lowest 3 cm) of *Sphagnum* moss samples (combination of *S. capillifolium*, *S. fallax*, and *S. palustre*) collected at Mossdale (heather-dominated blanket bog). Box shows the median (middle line), 25% (bottom border) and 75% (top border) ranges, with black points representing outliers. Red points represent the mean value.



**Figure 3.9** Vertical pattern of all selected testate amoebae (TA) which showed significant changes between different sections (Top: 0-3 cm, Mid-Middle: ~ 3 cm between top and bottom, Bot-Bottom: lowest 3 cm) of *Sphagnum* moss samples (combination of *S. capillifolium*, *S. fallax*, and *S. palustre*) collected at Mossdale (heather-dominated blanket bog). *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Eug.tub* - *Euglypha tuberculata*, *Hel.syl* - *Heleopera sylvatica*, *Neb.co.bo.type* - *Nebela collaris-bohemica* type, *Neb.mil* - *Nebela militaris*. Box shows the median (middle line), 25% (bottom border) and 75% (top border) ranges, with black points representing outliers. Red points represent the mean value.

**Table 3.2** Results of Kruskal Wallis tests (KW\_test,  $P < 0.05$ ) for seasonal encysted testate amoebae (TA) and vertical TA on *Sphagnum* moss samples (combination of *S. capillifolium*, *S. fallax*, and *S. palustre*) collected at Mossdale (heather-dominated blanket bog). Highest mean values of the individual TA (H\_Mean\_%) species are presented with the number in bold indicating species with significant seasonal or vertical change.

Taxa	Seasonal_ensysted_TA		Vertical_TA	
	KW_test	H_Mean_%	KW_test	H_Mean_%
Arc.are	0.785	1.4	-	-
Arc.art	-	-	0.864	1.9
Ass.mus	0.445	1.5	<b>&lt;0.001</b>	<b>12.6</b>
Bul.ind	0.109	2.6	-	-
Cen.arc	-	-	0.739	0.7
Cor.dub	0.218	7.3	<b>0.007</b>	<b>16.0</b>
Cyc.arc.s	-	-	<0.001	2.4
Deh.jap	0.536	3.0	-	-
Eug.rot	0.099	5.9	0.129	2.4
Eug.str	0.302	39.0	0.366	22.1
Eug.tub	0.089	3.2	<b>&lt;0.001</b>	<b>6.1</b>
Hel.pet	-	-	0.111	0.9
Hel.syl	0.161	12.2	<b>&lt;0.001</b>	<b>23</b>
Hya.ele	0.292	2.0	-	-
Neb.co.bo	<b>0.035</b>	41.6	<b>&lt;0.001</b>	<b>29.7</b>
Neb.flu	0.225	2.0	0.021	1.8
Neb.mil	0.993	17.8	<b>0.001</b>	<b>13.9</b>
Neb.tin	0.958	2.2	0.49	2.3

## 3.5 Discussion

### 3.5.1 Seasonal change of TA in *Sphagnum* mosses

Seasonal monitoring of three different types of *Sphagnum* mosses suggests an overall similar testate amoebae (TA) community composition (largely unaffected by season), although the relative abundance of wet and dry TA indicator species in individual *Sphagnum* moss was somewhat different in relation to seasonal environmental changes (Figs. 3.4, 3.5). This is largely inconsistent with the first hypothesis: *TA community composition is significantly different among seasons*. It probably can be explained by the captured relatively small seasonal range in average *Sphagnum* sample moisture (only ranging from 89.9% to 93.3%) as TA's establishment and distribution have long been reported to be mainly controlled by water table depth or substrate moisture. While water table depth changed considerably throughout seasons, *Sphagnum* moisture seemed not largely affected. This is most likely related to the limited moisture samples versus daily water tables and also the high and dense coverage of heather in the study plots, which offered shading and protection for *Sphagnum* mosses, especially reducing transpiration and wind desiccation in the summer, and using water even in winter with ongoing transpiration from its evergreen leaves. Previous studies carried out by Warner et al. (2007) and Marcisz et al. (2014) also demonstrated similar protective effects of vascular vegetation on seasonal substrate/*Sphagnum* moisture, thereby resulting in low dissimilarity of TA assemblages throughout the year. However, another important aspect was rainfall before sampling took place, which was confirmed by the continuous climate (temperature and precipitation) monitoring that was conducted every six hours in the study site (Fig. 3.2), reducing any moisture differences at sampling time.

Although *Sphagnum* moisture in September was similar as that in June, the higher total relative abundance of dry TA indicators, especially the common bacterivorous taxa *Corythion dubium* and *Euglypha tuberculata* in September is likely the result of more abundant fresh, labile plant litter, which stimulates the activity of microbes, hence increasing the availability of food sources (Lamentowicz et al. 2013). The similar relative abundance of wet and dry TA indicators in January as that in September seemed not affected by the statistically significantly increased *Sphagnum* moisture, which may suggest that the actual available water is key for TA colonization and development as

the majority of water in January was frozen. The alternating freeze-thawing in winter is therefore more likely to be beneficial to small individuals. It is also possible that TA communities or at least part of them in *Sphagnum* moss in January were in fact those living in December and/or November when the continuous freeze-thawing begins. Moreover, the visible differential response of TA communities among *Sphagnum* mosses to seasonal environmental change (mainly hydrology) is most likely due to differences in *Sphagnum* species' capacity of absorbing, transporting and holding water. For example, common hummock-forming species (e.g., *S. capillifolium*, and *S. palustre*) have repeatedly been suggested to be less sensitive than hollow-forming (e.g., *S. fallax*) species to changes in water level (e.g., long periods of drought or wetness) due to their higher water-holding capacity and superior efficiency in transporting water from water table to capitula (Li et al. 1992, Robroek et al. 2007, Bu et al. 2013). It is noteworthy that while individual *Sphagnum* species have their own niche preference of microsites (e.g., hummock, lawn, hollow), all of them have the ability to grow in the same microsite such as the relatively flat area (lawn) where samples in the present study were collected from.

Studies have shown that species richness and density of TA are highly positively related to substrate moisture (expressed as either water table depth or soil/*Sphagnum* moisture), with both of them being higher in wetter conditions, and vice versa (Mieczan 2007, Marcisz et al. 2014b, Song et al. 2018). Interestingly, species richness and density were found to increase in June and September compared to in March even though both water table depth and *Sphagnum* moisture were lower in the summer and early autumn than in the early spring (Fig. 3, Fig. 3.6a, b). However, this is partly consistent with the study by Mazei et al (2007) who reported increased species richness from the end of spring to the beginning of autumn, but unfortunately seasonal change of WTD was not clearly reported in their research. Nonetheless, it may suggest that other factors like temperature, length of daylight, food sources, and interspecific interactions, or combination of them are more important in TA's colonization and development than substrate moisture under relatively constant sampling moisture during this study. The continuously higher density of TA in January may further indicate that environmental conditions during winter are likely to be more favorable to a variety of species, especially those with small size (e.g., *Corythion dubium* and *Euglypha tuberculata* type) for which higher relative abundance in January have been reported (Mazei and



Tsyganov 2007, Lamentowicz et al. 2013). Noticeably, TA density dropped sharply in March in this study (Fig. 3.6b). It is not surprising as low temperature and frequent freezing (likely from late autumn to the end of winter) may cause high mortality of TA (Lamentowicz et al. 2013). The dead TA are then most likely carried away along the *Sphagnum* stem by ice water when thawing and further washed away by the high winter/spring rains. This is also probably the reason for the reduced TA species richness in January and March.

Formation of cysts has been widely considered to be a response mechanism of TA to adverse environmental conditions (Heal 1962, 1964, Gilbert et al. 2003, Roe et al. 2017) such as drought (Booth and Zygmunt 2005), frost (Mitchell et al. 2000), and lack of food (Laminger and Sturn 1984). The higher average percentage of encysted TA in January and June in this study supports this idea to a large degree, thus indicating that both high (around 20°C) and low temperatures (below zero) and freezing greatly increased the tendency of TA to encyst to cope with the unfavorable weather. However, the relatively higher average proportion of encysted TA in June than in March and September is in fact primarily due to the extremely high value (27.2%) from one *Sphagnum* sample (i.e., *S. fallax*) in plot 27 where the sampling area was mostly exposed (least shaded by heather) to sunlight and WTD was consistently the lowest (Figs. 3.1, 3.2c). Moreover, this sample was also shown to have the highest percentage of encysted TA (21.5%) in January. This may further suggest that extreme hot or cold weather are very likely to increase the number of encysted TA, especially in the habitats of open bogs and fens, thereby affecting the structure and functioning of TA communities and associated microorganisms (e.g., bacteria, fungi). Despite the functional importance of TA as carbon fixer (mixotrophic TA; Jassey et al. 2015) and microbial consumers (heterotrophic TA), little is known about how the periodic encystment of TA affects the biogeochemical processes. More research is needed in this field, particularly given the increasing levels of global warming and extreme weather.

### **3.5.2 Vertical distribution of TA along *Sphagnum* stem**

Both combined and individual *Sphagnum* moss data suggest a clear transition and separation of TA community composition along the *Sphagnum* sections from top to bottom (Fig. 3.7). This is consistent with the first half of the second hypothesis: *there*

is distinctive vertical variation along the *Sphagnum* plants in their TA community composition. Such vertical variation of TA communities along the *Sphagnum* section has been consistently observed by a variety of previous studies (Heal 1962, Mitchell and Gilbert 2004, Jassey et al. 2011, Roe et al. 2017). But noticeably, TA community composition in each corresponding segment in this study was dramatically different from many of other studies (Booth 2002, Song et al. 2016). For example, mixotrophic species like *Amphitrema flavum*, and *Hyalosphenia papilio* that are usually found abundant in raised bogs or fens in the upper regions of *Sphagnum* section were only rarely and occasionally observed from the wettest plot (44) in this blanket bog (Fig 3.2c). None of these species was found in the other two drier plots (27, 37) (Fig. 3.2c), which may suggest that the limiting factor for the colonization of these two taxa is more likely to be moisture rather than the shortage of sunlight as mixotrophic TA most often occur in habitats with soil water contents of more than 95% (Warner et al. 2007, Jassey et al. 2011). The other two mixotrophic TA species (i.e., *Heleopera sphagni* and *Amphitrema wrightiamum*) that often dwell in the middle segment of *Sphagnum* moss, considered as a compromise between light requirement and availability of materials (e.g., mineral particles, diatom frustules) for test construction, were not found in any plot. This could probably be explained by the lack of available mineral particles, or a shortage of diatom frustules for test construction, which is supported by the relatively low abundance of agglutinate taxa in both middle and bottom segments of *Sphagnum* moss (Supplementary Fig. S3.3). The remaining part of the second hypothesis in relation to test constituents was therefore largely not supported: *mixotrophic testate amoebae (MTA) are more abundant in the upper and intermediate regions while agglutinate taxa are more common in the lower region*. By contrast, the extremely high proportion of TA with siliceous shell in all segments may imply a sufficient supply of available materials for the construction of siliceous tests on *Sphagnum* moss in blanket bogs and also possibly further indicate a considerable contribution of TA to silicon cycling in this habitat.

Patterns of TA living status (alive versus dead) observed in the present study along the *Sphagnum* sections is highly consistent with many other studies conducted in other habitats such as raised bogs and fens (Heal 1962, Booth 2002, Roe et al. 2017). These studies have primarily attributed the higher proportion of dead individuals in the lower portion of *Sphagnum* moss to the vertical transport of TA after death from top to bottom

and/or to the growth/senescence of *Sphagnum* moss. However, it is also possible that the relatively anaerobic condition in the lower regions itself is not suitable for most TA species to live and, even if they survive, this condition is likely to shorten their generation turnover, thereby increasing the proportion of dead individuals accordingly. These are also likely the reason for the increased TA density from top to bottom of *Sphagnum* moss. Noticeably, the low proportion of unidentified living status of TA (i.e., possibly dead) showed a similar pattern as dead TA along the *Sphagnum* sections (Fig. 3.8a). While visible food and cytoplasm containing organs (e.g., vacuole) were detected inside their body, the uncolored protoplast may indicate that most of them had died, although there is a possibility for inadequate staining. However, it remains unclear whether dead TA with well-preserved organs/cytoplasm are not easily stained or if there are other reasons (e.g., low concentration of stains and short length of dying time) causing inefficient staining in living TA. Therefore, further quantitative examination of the efficiency of stains (e.g., Rose Bengal) in staining TA should be tested by controlling both the concentration of staining agent and the length of dying time.

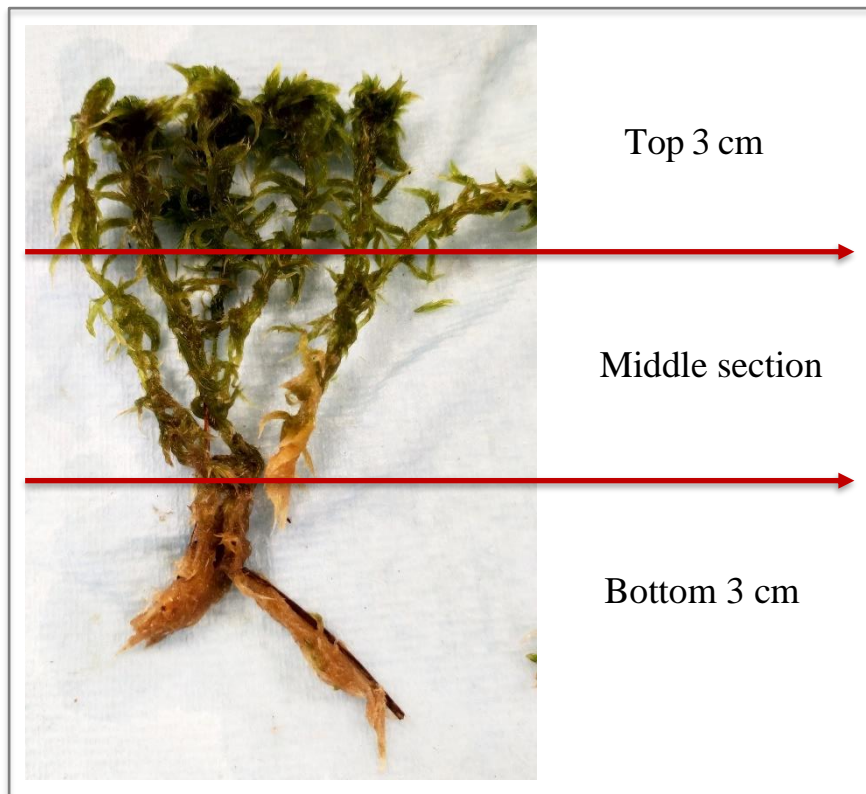
While total relative abundance of idiosomic TA (with siliceous shell) was similar in different segments, significant species-specific vertical separation has been shown for most taxa in the present study (Fig. 3.9). This spatial variation has also been reported in other studies. For example, *Assulina muscorum*, and *Corythin dubium* were mostly observed abundant in the upper segment, whereas *Heleopera sylvatica*, *Nebela militaris* and *Euglypha tuberculata* were more frequent in the lower segment (Chacharonis 1954, Heal 1962, Mitchell and Gilbert 2004, Roe et al. 2017). As for these change patterns, there are at least two possible reasons. On the one hand, it may be partly due to the hydrological gradient across *Sphagnum* moss as the upper segment is usually drier and less tolerant to drought than the lower and more saturated segments. The well-defined xerophiles (e.g., *Assulina muscorum*, *Corythin dubium*) that are usually of smaller size are therefore expected to mainly dwell in the upper regions, while the well-defined hydrophiles (e.g., *Heleopera sylvatica* and *Nebela militaris*) with often larger size are more likely to inhabit the wetter lower parts. On the other hand, it is probably also related to the availability of materials for test construction as some species such as *H. sylvatica* whose test is partly composed of quartz particles were primarily restricted to lower regions of the *Sphagnum* section. This was partly corroborated by the relatively higher abundance of *H. sylvatica* in the lower segment in this study. However, it was

not the case for *Nebela collaris-bohemica* type, which is often considered as an intermediate moisture indicator. Despite its large size, their relative abundance was found to be much higher in the upper regions (Fig. 3.9). As a result, other factors such as available food sources may be more important than hydrology in determining the establishment and distribution of this species in *Sphagnum* moss. For example, Gilbert et al. (2003) reported that *N. collaris-bohemica* type has a wide range of food sources, with 45% of the identified prey being micro-algae, within which 33% were diatoms. As these algae are usually more abundant at the top of *Sphagnum* moss, because of their need of light for photosynthesis, the upper segment thus offers more favorable environmental conditions and sufficient food supplies for *N. collaris-bohemica* type. It therefore may indicate the complexity of vertical micro-distribution for intermediate TA indicators, which more likely depends on their special requirements such as food, or materials for test construction.

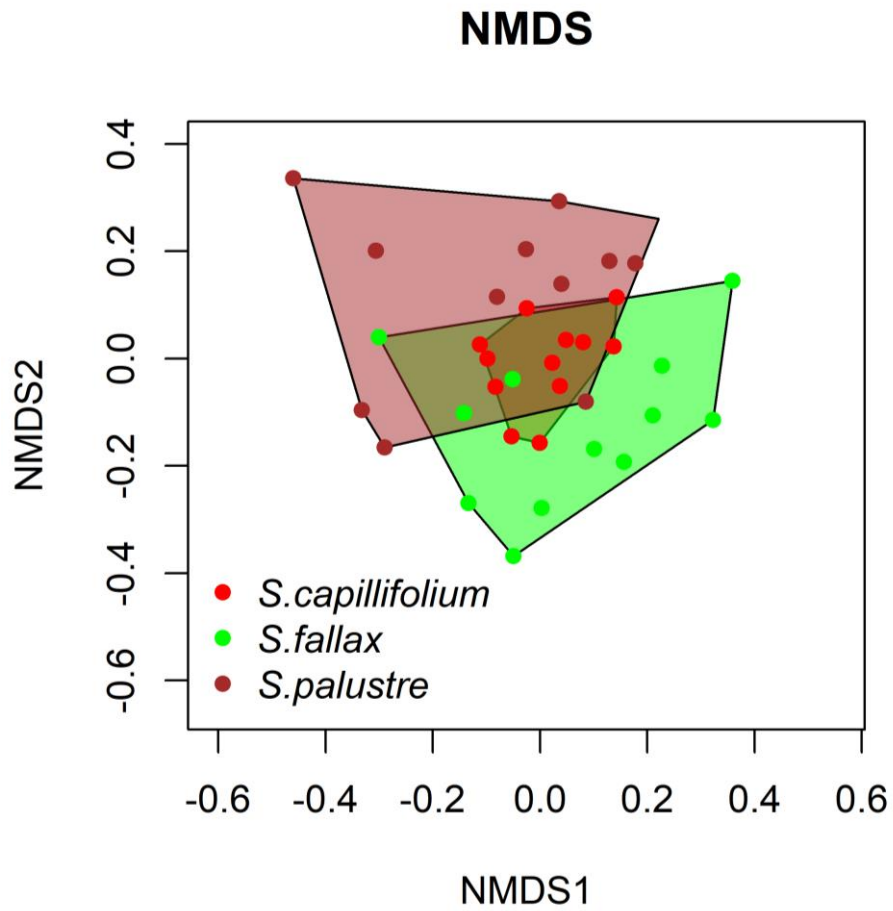
### 3.6 Conclusions

This study shows a considerable seasonal change of TA in species richness and density and also a clear and marked vertical separation of TA in living status and niche preference. Despite the similarity of TA community composition among seasons, a relatively higher average abundance of encysted TA and one particularly high value in both June and January highlight the capability of TA to form cysts in response to extreme hot/dry and cold weather. This change may also suggest a non-negligible influence on ecosystem processes (e.g., C-cycling). Moreover, the extremely high proportion of idiosomic TA (with siliceous shell) in all segments of *Sphagnum* moss may imply a sufficient supply of available materials for the construction of siliceous tests in blanket bogs and also possibly further indicate a considerable contribution of TA to silica cycling in this peatland habitat. Overall, based on the seasonal and vertical variation of TA mentioned above, along with the field observation of the living status of *Sphagnum* mosses, the present study recommends sampling the entire *Sphagnum* section in late autumn or early winter in British blanket bogs as an appropriate sampling strategy. If the aim is to reflect the environmental conditions at the sampling time, this study recommends counting only the living individuals. However, if the aim is to reflect the full range of environmental conditions during the past or recent years, this study recommends counting both living and dead TA for analysis. Nevertheless, it has to be admitted that all these assertions are mainly based on the deduction from previous studies and the observation from the present study focusing only on a single and very wet UK blanket bog site. Further fundamental and comprehensive biotic (e.g., primary producers, other microbial consumers, decomposers) and abiotic (e.g., hydrological and physicochemical parameters) factors from *Sphagnum* moss sampled across a wider range of wetness conditions needs to be measured to confirm and support the inferences and recommendations.

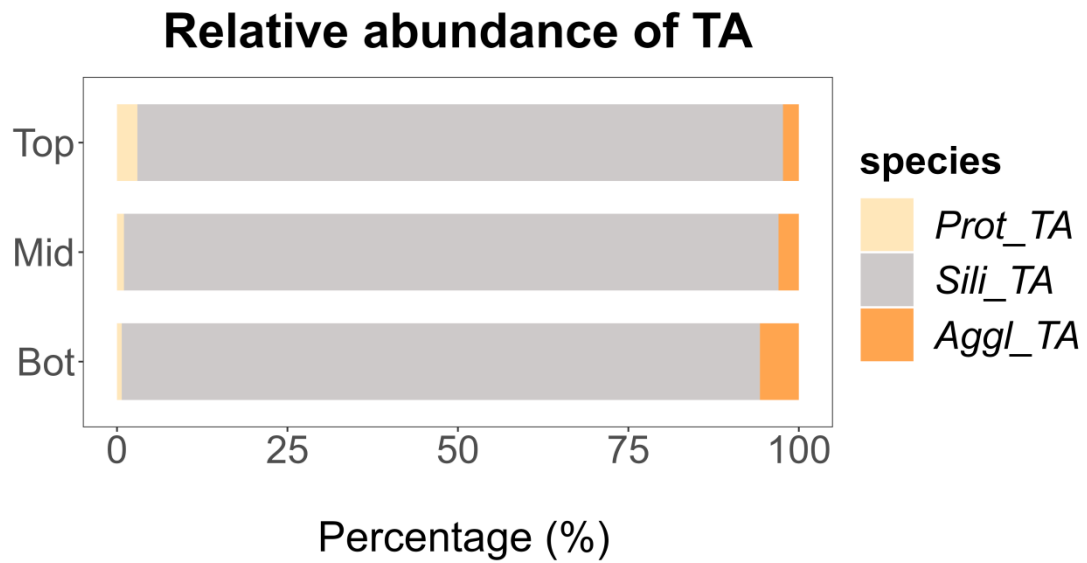
### 3.7 Supplementary materials



**Supplementary Figure S3.1** Separation of *Sphagnum* moss into three segment (top 3 cm, ~3 cm middle section and bottom 3 cm).

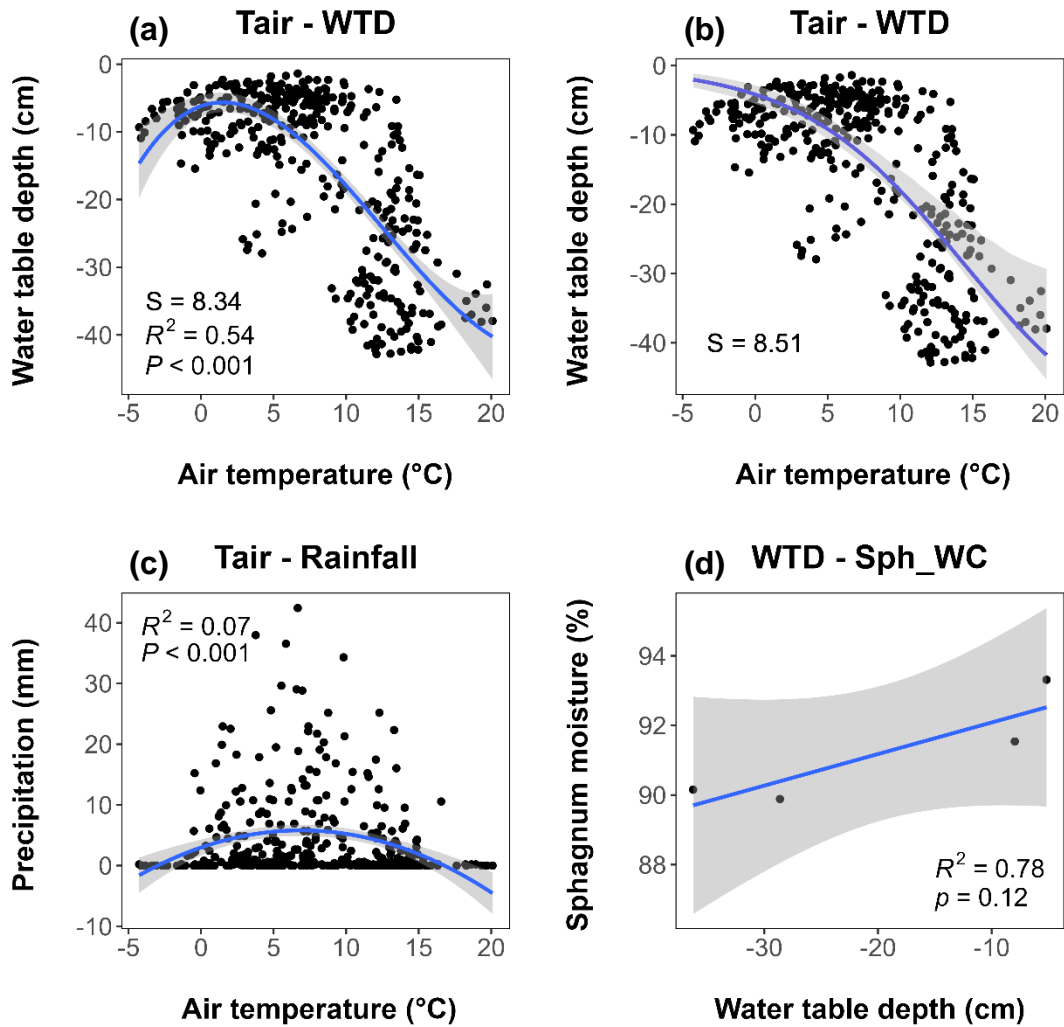


**Supplementary Figure S3.2** Non-metric multidimensional scaling (NMDS) analysis of living testate amoebae (TA) community composition on *S. capillifolium*, *S. fallax*, and *S. palustre* sampled at Mossdale (heather-dominated blanket bog).



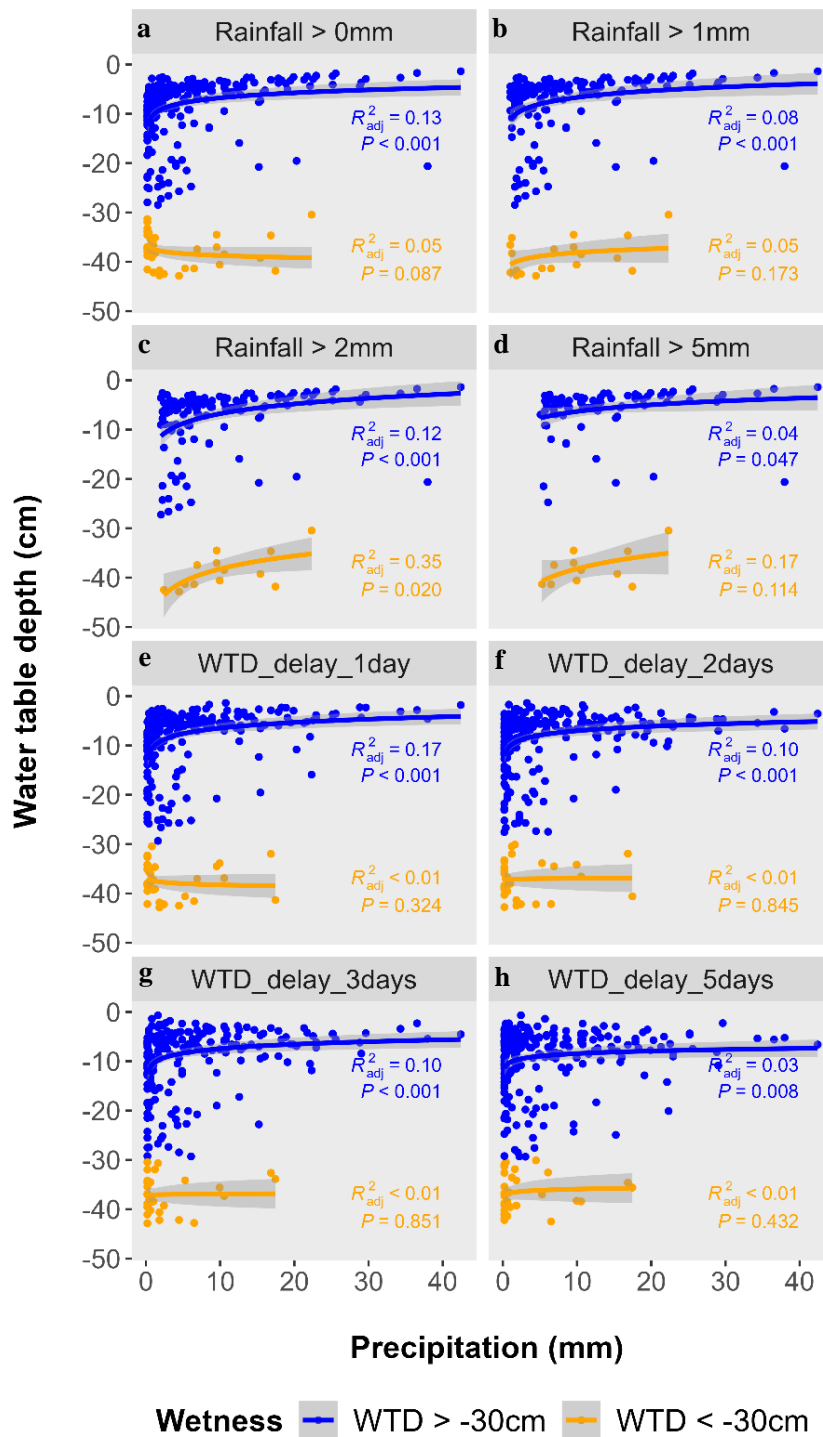
**Supplementary Figure S3.3** Relative abundance of living testate amoebae (TA) with different types of test materials (TA with proteinaceous shell: Prot\_TA (light orange), TA with siliceous shell: Sili\_TA (grey), TA with agglutinated particles: Aggl\_TA (orange) on different sections (Top: 0-3 cm, Mid-Middle: ~ 3 cm between top and bottom, Bot-Bottom: lowest 3 cm) of *Sphagnum* moss (combination of *S. capillifolium*, *S. fallax*, and *S. palustre*) sampled at Mosssdale (heather dominated blanket bog).





**Supplementary Figure S3.4** Regression analysis for Mossdale site from January 2021 to January 2022 of daily (a) air temperature versus water table depth (WTD) (Polynomial regression with degree of three); (b) air temperature versus water table depth (Inverted sigmoidal regression); (c) air temperature versus rainfall (Polynomial regression with degree of two) and (d) *Sphagnum* moisture versus water table depth (linear regression). The gray shading areas represent the 95% confidence intervals. Regression coefficient square (R squared) and significance level ( $p$  value) are shown in a, c and d. S (standard error of regression) are shown in a and b; WTD is for uncut plots.

## Rainfall - WTD



**Supplementary Figure S3.5** Logarithmic regression analysis for Mossdale site from January 2021 to January 2022 of daily (a) rainfall (> 0mm) versus water table depth (WTD); (b) rainfall (> 1mm) versus WTD; (c) rainfall (> 2 mm) versus WTD, (d) rainfall (> 5 mm) versus WTD; and (e) rainfall versus WTD one day after rain; (f) rainfall versus WTD two days after rain; (g) rainfall versus WTD three days after rain and (h) rainfall versus WTD five days after rain. The gray shading areas represent the 95% confidence intervals. Regression coefficient square (R-squared) and significance level ( $p$  value) are shown in each plot; WTD is for uncut plots.

# Chapter 4: Heather management impacts on testate amoeba communities within upland blanket bog peatlands

## 4.1 Abstract

Most UK blanket bog peatlands are classified as being degraded in their habitat state and ecological functions due to management interventions that include historic drainage and likely also heather management such as burning, although the impacts of the latter (including alternatives to burning) are unclear and lack robust experimental evidence (Heinemeyer et al., 2023). Currently, restoration efforts, such as ditch-blocking and alternative heather cutting, are being applied to restore these peatlands. However, we still lack the monitoring tools to assess if such interventions achieve restoration towards ‘intact’ bog status (especially in relation to its hydrology) and associated functions. As known bioindicators, testate amoebae (TA) probably can provide a potentially additional tool to characterize the status and impact of management interventions, particularly in relation to peat hydrology. Here, TA communities, and their key functional traits (e.g., mixotrophic TA, biovolume/aperture width, test type), were investigated in *Sphagnum* and peat samples across managed (burnt versus cut heather treatments) and unmanaged (uncut control) heather-dominated areas and were also compared to near intact blanket bog and an intact intermediate/ridge raised bog in the border mires to assess their potential as habitat condition bioindicators and explore their possible linkages to ecosystem processes. The results showed that: 1) TA community composition, species and functional diversity were distinctly separated between the very wet intact border mire and other peatland sites, but were not significantly different between heather management treatments (with only a small difference between managed and unmanaged plots); 2) species of *Hyalosphenia subflava* and *Corythion dubium* may represent good dry indicators while *Amphitrema flavum* and *A.wrightianum* may serve as good wet indicators; 3) TA communities found in *Sphagnum* moss and surface peat likely have different functional roles - being more important in C fixation in waterlogged habitats, while more crucial in peat decomposition and/or silica cycling in relatively dry habitats, respectively; 4) apart

from feeding on microbes, the *Hyalosphenia subflava* may be actively involved in peat decomposition; 5) application of TA from deeper peat layers to infer and compare historic conditions seems constrained due to highly disproportional degradation of some shell types in blanket bogs, where water tables can severely drop periodically. Overall, this study highlights the potential and some limitations of TA as bioindicators to monitor hydrological changes and also clearly demonstrates the link between biodiversity, management, and likely inferred ecosystem functioning (water management and carbon/silica cycling).

## 4.2 Introduction

Peatlands represent a significant terrestrial carbon pool, storing about 30% of global soil organic carbon (Yu et al. 2011). They, therefore, have the potential to affect global climate change by functioning as both a sink and a source of atmospheric carbon dioxide, as well as being a source of atmospheric methane (Frolking et al. 2006, Turner and Swindles 2012). With the growing anthropogenic disturbances (mainly via drainage and ensuing agriculture) on peatlands over millennia and especially during the last two hundred years since the onset of the industrial revolution across the world, large areas of global peatlands have been severely damaged, thereby emitting a large amount of greenhouse gasses (i.e., mainly as CO<sub>2</sub>) (Miettinen and Liew 2010, Law et al. 2015, Dohong et al. 2017, Lilleskov et al. 2019, Regensburg et al. 2022). Blanket bogs are an extensive type of peatland in the UK uplands, but they are largely classified as being in a degraded state, suffering from a range of drivers of change, especially over the past few hundred years, such as drainage and atmospheric pollution and likely also from rotational burning of vegetation (Heinemeyer et al. 2018).

Since around 1850, with the onset of driven grouse (*Lagopus lagopus scotica*) shooting, rotational prescribed vegetation burning has been intensified on grouse moors to create a mosaic of patches of different ages of ling heather (*Calluna vulgaris*; henceforth heather). This practice produces a diverse vegetation structure and allows the regeneration of younger, more palatable and nutritious heather shoots (as food for red grouse), whilst also provides shelter amongst older heather (as location for nesting), to promote large populations of red grouse for shooting (Heinemeyer et al. 2019b). Meanwhile, burning has been increasingly claimed to degrade peatlands with potential

detrimental impacts on many of the peatland ecosystem services such as water storage, drinking water quality, flood prevention and carbon storage (Holden et al. 2012, Clay et al. 2015, Holden et al. 2015, Pereira et al. 2021). However, the evidence-based impacts of prescribed fire are not clear (Davies et al. 2016, Harper et al. 2018), especially as drainage often co-occurred with burning and is more likely the factor responsible for peatland degradation (Heinemeyer and Ashby 2021), which has also been shown for drainage related DOC export (Williamson et al. 2021). Nonetheless, some alternative heather management strategies have been promoted by policy makers and scientists in recent years, with cutting (heather mowing) being one of the most typical options to facilitate restoration on heather peatlands (Heinemeyer et al. 2019b, Morton and Heinemeyer 2019). Noticeably, impacts from cutting, often done with heavy and large machinery (Fig 4.1), are even less known than for burning (Harper et al. 2018, Heinemeyer and Ashby 2021), yet negative impacts and trade-offs are also likely (Heinemeyer et al. 2019b). As both burning and cutting remove the aboveground vegetation, along with the impacts of mowing machinery on the peat surface microtopography (Heinemeyer et al. 2019a), it likely increases surface runoff, especially for burnt areas due to the increased water repellency of organic materials (hydrophobicity) (Heinemeyer et al. 2018). This water repellency also reduces water infiltration (Holden et al. 2012) and may further lower the water table and stimulate erosion, drying and warming of the surface peat and thus enhancing microbial decomposition rates (Brown et al. 2013). However, previous studies were primarily short-term and lacked a robust experimental design (Ashby and Heinemeyer 2021) and as such overlooked the fact that long-term impacts can be very different (Heinemeyer et al. 2023). In contrast, cutting with leaving brash might ensure a wetter peat by reducing evapotranspiration and enhancing infiltration (Heinemeyer et al. 2019), although this benefit seems to be short-term (Heinemeyer et al. 2023) and long-term brash decomposition likely results in greater carbon losses (Heinemeyer et al. 2023), as predicted in modelling scenarios (Worrall et al. 2013). Moreover, leaving heather unmanaged is another management option, although the impacts are also understudied, yet indications are for a less diverse and drier peat habitat (Heinemeyer et al. 2023).

With large amounts of resources and funding spent on peatland restoration and to evidence restoration success, it is therefore of paramount importance to assess and monitor the peatland conditions following these different management and restoration

strategies. Among the approaches to monitor the restoration trajectory and success using testate amoebae (TA), which are well-known biological indicators, seems a promising way to support this purpose as TA are known to show species specific responses to environmental factors, especially water tables and peat wetness (Lousier 1974, Marcisz et al. 2014a).

TA represent the main component of protists, which are particularly abundant in both *Sphagnum* moss and surface peat (Charman 2001, Booth 2008). Since they are sensitive to a wide variety of environmental factors such as hydrology, air pollution, heavy metals and have a rapid reproduction rate, TA were proposed as ideal biological indicators and have been applied to monitor a range of environmental changes (Nguyen-Viet et al. 2007a, Payne et al. 2010, Meyer et al. 2013, Swindles et al. 2016, Creevy et al. 2018). During the past two decades, a few studies have been conducted to explore the effects of fire on TA, but the results were not entirely consistent (Wanner and Xylander 2003, Turner and Swindles 2012, Wanner 2012, Marcisz et al. 2016, Qin et al. 2017). Wanner et al. (2003, 2012) showed that transient burning with low intensity following active military training and landscape management had little effects on soil TA communities (Wanner and Xylander 2003, Wanner 2012) whereas Qin et al. (2017) reported a strong impact of severe wildfire on TA tests, with idiosome tests being likely to be damaged directly over the course of combustion (Qin et al. 2017). By investigating the impacts of different intensities of fire on TA communities dwelling on moss, Turner et al. (2012) suggested that TA community composition was indeed influenced by strong fire, with taxon *Hyalosphenia subflava* likely being a good indicator of burning as it was found particularly abundant following a severe wildfire event (Turner and Swindles 2012). Additionally, Marcisz et al. (2016, 2019) reported strong sensitivity of some key functional traits of TA such as mixotrophs and species body size, to fire activities by virtue of the evidence from core peat, even though the intensity, severity and lasting time of the fire was not clearly indicated. Undoubtedly, these insights have improved our understanding of TA at both a taxonomic and functional level in response to fire, but we still lack a broader range of site assessments, especially on blanket bogs, including knowledge on how key functional traits of TA, like mixotrophic TA, biovolume and aperture size change in the process of natural recovery following common British heather management strategies (i.e., prescribed burning and alternative mowing).

As the main microbial consumers in peatland (Mitchell et al. 2000), TA communities potentially play a pivotal role in ecosystem functioning such as carbon and nutrient cycling (Wilkinson 2008, Jassey et al. 2015). Wilkinson et al. (2010) suggested that the heterotrophic TA (HTA) can affect the decomposition rate of organic matter (OM) through preferentially preying on carbon/nitrogen-cycling related functional groups of bacteria and fungi, thereby indirectly influencing carbon dioxide emission and nutrient flow (Wilkinson and Mitchell 2010). The biovolume and aperture size of TA were also reported to be strongly positively related to trophic level, with larger specimens usually having a wider range of food sources and being likely to occupy a higher trophic position (Jassey et al. 2016). Furthermore, recent studies also highlight the non-negligible contribution of mixotrophic TA (MTA) to carbon fixation as they can also acquire carbon sources through photosynthesis by their endosymbionts (e.g., green microalgae) (Jassey et al. 2015). As a result, investigating the alteration of key functional traits of TA in response to heather management will also facilitate our understanding of their potential linkage to key ecosystem processes such as carbon cycling. Noticeably, previous studies investigated the response of TA communities to fire either from peat samples (surface peat and/or peat core) or surface moss/litter layer samples. No study has been carried out to examine both habitat aspects at the same time. Given the significant discrepancy in the living environment (e.g., light, temperature, oxygen) and food sources between surface peat and *Sphagnum* moss layers, the composition and recovery pattern of TA communities are likely to be somewhat different between these two habitats.

Here, TA communities were investigated in *Sphagnum* and peat samples at three heather-dominated peatlands (two of which are managed as grouse moors) in northern England, within which heather was managed by either burning or cutting (different old heather areas were managed in 2013, 2015 and 2018) and those areas were compared to uncut control areas. These managed sites and their time point samples were further compared to other nearby sites representing near intact blanket bog (likely to be wetter) and intact border mire (very wet) peatland habitat conditions to assess the potential of TA as bioindicators to monitor the habitat hydrological status and recovery trajectories of the ecosystem and explore their possible linkages to ecosystem functioning. The objectives of this study were to: 1) investigate the TA community and changes over time in their composition, taxonomic and functional groups/traits (e.g., MTA,

body/aperture size) in response to prescribed heather burning and cutting treatments versus no management (uncut) as a control; 2) compare TA communities in the managed and unmanaged heather areas to corresponding near intact and intact (very wet) habitats; 3) explore the main drivers for TA colonization and development; 4) link changes of TA functional traits over the course of recovery to ecosystem processes (e.g., carbon, nitrogen and silica cycling). It was therefore hypothesized that on blanket bog peatlands: 1) cutting is superior to burning for the hydrological restoration of heather-dominated peatlands although subsequent vegetation recovery following cutting shifts TA communities (including functional traits) towards that of near-natural conditions, whereas burnt areas will develop towards unmanaged heather-dominated areas; 2) site/plot wetness is overall more important than management and other factors (e.g., peat physicochemical property) in determining TA establishment and development; 3) TA communities in *Sphagnum* moss play different functional roles from those in surface peat and their recovery pattern is more pronounced in *Sphagnum* moss than in peat samples; 4) TA communities in blanket bog deep peat layers can be used to infer and compare historic environmental conditions (i.e., before the onset of intensification of management).

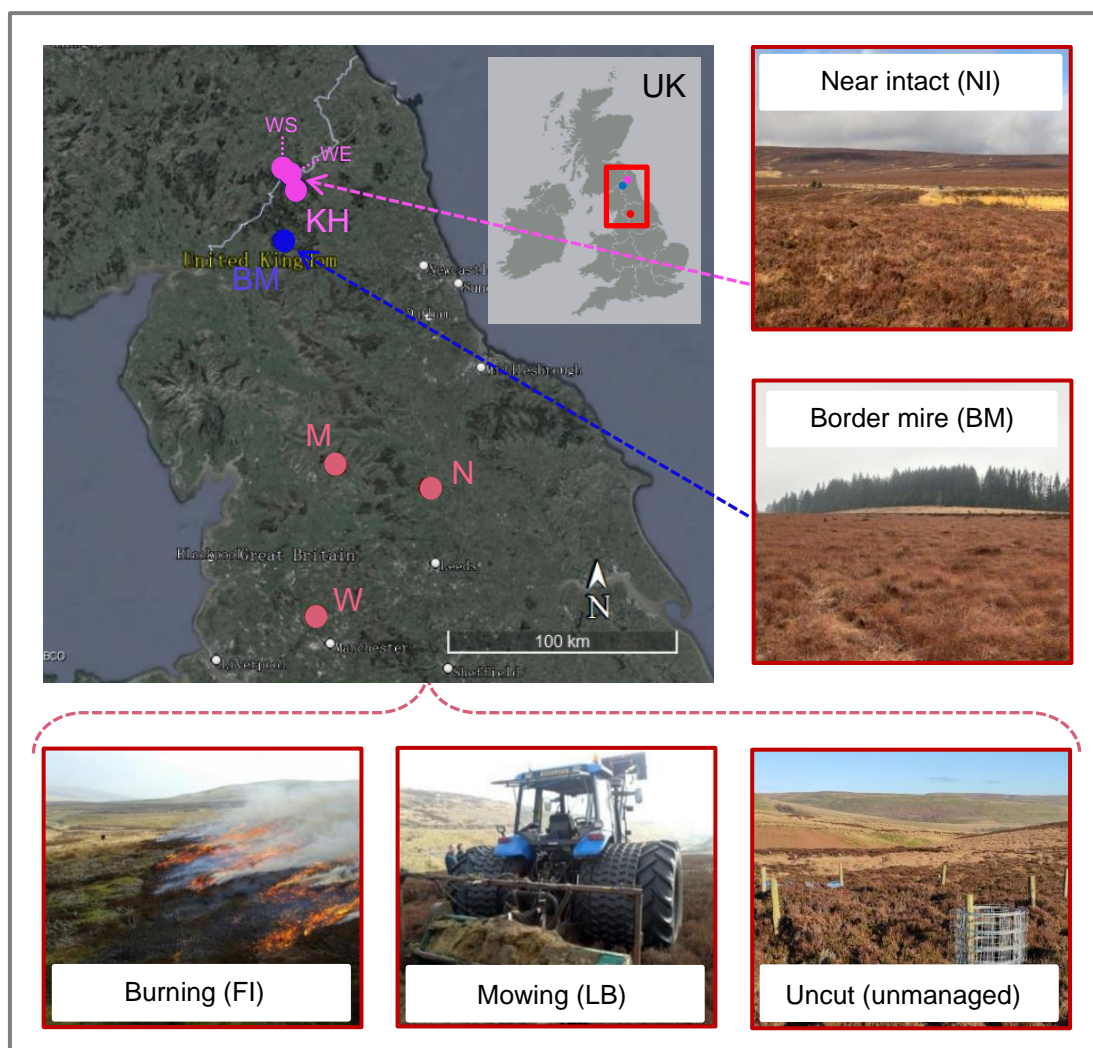
## 4.3 Methods

### 4.3.1 Study sites

Samples were collected from six blanket bog sites and one from a very wet intact border mire (Butterburn, considered as an intermediate/ridge-raised bog mire complex developed over valley-shape terrain (Eades et al. 2021), which is topographically wetter and thus has a much deeper peat depth than typical blanket bog). Among the six blanket bogs, half of them is classified as near intact blanket bog (Kielder Head, Whitelee English side and Whitelee Scottish side) and the other half is managed (past and/or present drainage, grazing, burning) heather dominated (Nidderdale, Mossdale and Whitendale) with different management areas (burning & cutting versus uncut control areas). The very wet intact border mire and near intact blanket bogs were chosen in this study as reference sites since they were considered not or only to have been slightly disturbed by historic management (e.g., grazing). 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 sites are all located furthest South in northern England (Fig. 4.1, Supplementary Fig. S4.1).



**Figure 4.1.** Location of border mire (Butt), near intact sites (KH and WE&WS) and managed experimental sites (burning & mowing with brash left versus uncut control) in the UK. B-Butt: Butterburn, K-KH: Kielder Head, W-WE: Whitelee English side, W-WS: Whitelee Scottish side, N-Nidd: Nidderdale, M-Moss: Mossdale, W-Whit: Whitendale.

The managed sites have low sheep stocking densities of  $< 0.5$  ewes  $\text{ha}^{-1}$ . The sites were chosen based on a set of key criteria: all were classed as modified/degraded (i.e., heather-dominated, and recent burn rotation and/or drainage) blanket bog (Evans et al. 2017) with a mean peat depth of over 1 m (Histosol). Typically, the grouse moor sites were managed on average with a 22-year burn rotation (based on peat core charcoal peak information; Heinemeyer et al. 2018) and two (Nidderdale and Mossdale) had a long history of rotational vegetation burning (more than 100 years; based on estate information). Moreover, in mature areas all sites show more than 50% *Calluna vulgaris*

(ling heather) cover, with at least some existing bog vegetation in the form of *Eriophorum* (cotton-grass; forming some hummocks) and *Sphagnum* moss species. More importantly, these three sites each consisted of two adjacent catchments, which were managed as part of a patch-level rotation either by burning or cutting with leaving brash (with unmanaged/uncut control plots located within the cut catchment) at three time points (2013, 2015 and 2018) in different areas (i.e., new tall heather areas were either burnt or cut to cover the entire catchment area over time as part of a long-term management plan) to compare the effects of alternative management strategies on biodiversity, carbon dynamics and water quality. This study was set up to screen for the most suitable management techniques to address the dominance of heather and facilitate the support of active blanket bog vegetation (peat-forming enhancing species, e.g., *Sphagnum* moss). Detailed information about these six sites is described below and is also shown in Table 4.1 (for measurement methods see below sections 4.3.2-4).

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

**Butterburn border mire site (BM):** 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 cover; at least 100%) is dominated by *Sphagnum* moss ranging from 65% to 85% (mean: 73.3%) across the sampling plots, followed by *Eriophorum* spp. sedge from 25% to 40% (mean: 36.7%), with other mosses occupying less than 10% (mean: 3.7%). The peat was very wet with a peat moisture content at sampling time of (from 95.1% to 96.2 %). The average peat depth was about 6.7 m and a mean slope of 1° across the sampling plots. However, this is not a typical blanket bog but an intermediate/ridge-raised mire complex as has been recently highlighted for the border mires (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 (i.e., sum of over and understory cover; at least 100%) is dominated by other mosses ranging from 33% to 85% (mean: 52.7%) in the sampling plots, 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 peat was wet at sampling (peat moisture content from 89.8% to 92.1 %), with an average peat depth of 1.8 m and a slope of 3° across the sampling plots.

**Whitelee (English versus 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 (UK Grid Ref NT 685056) about 524 m a.s.l. The vegetation (i.e., sum of over and understorey; at least 100%) is dominated by *Sphagnum* moss ranging from 30% to 96% (mean: 76.0%) in the sampling plots, 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 was wet at sampling (peat moisture content from 89.0% to 95.0 %), with an average peat depth of 2.3 m and a slope of 4° across the sampling plots. Noticeably, the English side is grazed, and the Scottish side is not.

The three heather-dominated sites (conditions reflect sampling location and information obtained from the DEFRA report by Heinemeyer et al. (2019) and the subsequent Peatland-ES-UK report update (Heinemeyer et al. 2023)) and cover a range from dry (Nidderdale) to wet (Mosssdale) with Whitendale being intermediate (based on mean water table depth data in Heinemeyer et al. 2019):

**Nidderdale (Nidd)** is located on the Middlesmoor estate in upper Nidderdale, which lies within the Yorkshire Dales National Park, UK, at 54° 10' 07"N; 1° 55' 02"W (UK Grid Ref SE 055747) about 450 m a.s.l. The site showed a mean ( $\pm$  standard deviation during 2012-2021) annual air temperature of  $7.4 \pm 0.4^\circ\text{C}$  and annual total precipitation of  $1426 \pm 277$  mm during the ten years study period, and a mean annual water table depth was  $-12.5 \pm 6.4$  cm. The soil is poorly drained organic peat (Winter Hill series), with an average peat depth of  $1.6 \pm 0.3$  m across the experimental plots and an average slope of  $4 \pm 3^\circ$ . Most of the grips within the study area, which were dug about 50 years ago (~1970), were mostly naturally infilled by 2010 and no further grip blocking took place during the study period. There are a few gullies (similar to grips but naturally formed) at this site. The detailed mean vegetation cover (%) in each type of treatment (burnt/mown/uncut) is displayed in Table 4.1.

**Whitendale (Whit)** is located within the Forest of Bowland (an Area of Outstanding Natural Beauty; AONB), Lancashire, at 53° 59' 04"N; 2° 30' 03"W (UK Grid Ref SD 672543) about 410 m a.s.l. The mean ( $\pm$  standard deviation during 2012-2021) annual air temperature was  $7.8 \pm 0.4^{\circ}\text{C}$  and annual total precipitation was  $1795 \pm 272$  mm during the ten years study period. The mean annual water table depth was  $-9.0 \pm 6.9$  cm. The soil is a poorly drained organic peat in the Winter Hill series with an average peat depth of  $1.7 \pm 0.4$  m at the experimental plots and an average slope of  $8 \pm 3^{\circ}$ . The peat depth across the entire catchment area ranged from 0.2 m to 4.5 m (i.e., with shallower area on steep slopes). This study area had no grips, although several gullies (possibly related to historic overgrazing) are still present in both catchments. The detailed mean vegetation cover (%) in each type of treatment (burnt/mown/uncut) is displayed in Table 4.1.

**Mossdale (Moss)** is located in Upper Wensleydale within the Yorkshire Dales National Park at 54° 19' 01"N; 2° 17' 18"W (UK Grid Ref SD 813913) about 390 m a.s.l. The site showed a mean ( $\pm$  standard deviation during 2012-2021) annual air temperature of  $7.4 \pm 0.3^{\circ}\text{C}$  and annual total precipitation of  $1912 \pm 325$  mm during the ten years study period, with mean annual water table depth around  $-7.7 \pm 5.7$  cm. The soil is a poorly drained organic peat (Winter Hill series), with an average peat depth of  $1.2 \pm 0.4$  m at the experimental plots and an average slope of  $6 \pm 3^{\circ}$ . The peat depth across the two catchments ranged from 0.3 m to 2.1 m. Most of the grips/ditches within the study area, which were dug about 50 years ago, were naturally infilled by 2010. There are no gullies at this site. The detailed mean vegetation cover (%) in each type of treatment (burnt/mown/uncut) is displayed in Table 4.1.

**Table 4.1** Location data of sampling sites in the UK. Vegetation cover was assessed in December 2020 within 50 x 50 cm squares for Nidderdale, Mossdale and Whitendale but in February 2021 (due to Covid travel restriction) for Butterburn, Kielder Head and Whitelee as total cover (at least 100%) of: heather (H), 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/ Management	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	15	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	5.0	3, 3, 3
Whitendale (Whit)									
burnt/ cut/ uncut	Blanket bog	384	1.4	12.7	27.9	56.1	24.3	4.7	9, 9, 9
		390	1.4	21.9	17.0	14.9	53.8	9.7	9, 9, 9
		370	1.9	23.3	16.3	27.3	50.7	7.7	3, 3, 3
Mossdale (Moss)									
burnt/ cut/ uncut	Blanket bog	353	1.0	7.1	7.1	50.3	28.1	17.8	9, 9, 9
		334	1.2	12.3	16.9	40.9	39.1	12.9	9, 9, 9
		324	1.5	34.0	9.3	50.0	28.3	10.7	3, 3, 3
Nidderdale (Nidd)									
burnt/ cut/ uncut	Blanket bog	461	1.6	40.7	14.0	17.9	53.2	11.3	9, 9, 9
		457	1.6	23.9	19.4	23.8	45.9	27.3	9, 9, 9
		478	1.7	57.7	16.0	28.3	42.7	4.7	3, 3, 3

### 4.3.2 Sampling

Samples (nine each; three replicates at each site) of the dominant *Sphagnum* mosses, surface peat (0-5 cm) and deep peat (50-55 cm) were collected from the very wet border mire and the other three near intact sites in February 2021 (Table. 4.1). Another 63 each (three replicates per management/habitat area at each site) were collected for the dominant *Sphagnum* moss (replaced by other mosses if there was no *Sphagnum* moss), surface peat (0-5 cm), and deep peat (50-55 cm) samples from the three heather-dominated managed sites (i.e., Nidderdale, Mossdale and Whitendale) which each

included uncut, burnt, and cut with leaving brush areas in December 2020 (Table. 4.1). Vegetation type and cover (as sum of over and understory; at least 100%) were recorded at the sampling location in a 50 cm x 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, other moss layer and litter depth were also recorded in the field (Supplementary Fig. S4.2).

### 4.3.3 TA processing

Testate amoebae (TA) were prepared using a modified version of the method of Booth et al. (2010). For each sample, around 5 g fresh *Sphagnum* or 2 cm<sup>3</sup> peat was used for preparation. The weighted sample was soaked and disaggregated in the 100 ml beaker with about 60-80 ml deionized water for at least 24 hours and stirred occasionally. At the same time, one tablet of *Lycopodium clavatum* was added for density calculation. 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 mesh µm was carefully washed into 50 ml centrifuge tubes and centrifuged at 4,000 rpm for five minutes. 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 color) from dead (empty) individuals. TA identification 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, 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 specialized software (OLYMPUS cellSens Entry). The biovolume (µm<sup>3</sup>) was calculated based on geometric shapes using dimensions measured under the microscope (length or diameter; width, and height):

$$\text{Hemisphere: Biovolume} = \text{Pi} \cdot r^3 \cdot 2/3$$

$$\text{Saucer-shaped: Biovolume} = \text{Pi}/2 \cdot r^2 \cdot h$$

$$\text{Cylindrical-ovoid: Biovolume} = \text{Pi}/6 \cdot d^2 \cdot h$$

Ovoid: Biovolume =  $\frac{\pi}{6}L*w*h$

Where r is the radius, Pi the Archimedes's constant, h the height, d the diameter, L the length, and w the width of the shell (Fournier et al. 2012).

#### **4.3.4 Peat physicochemical analysis**

Around 2 cm<sup>3</sup> peat subsamples from the surface layer separated in the lab were weighed first and then dried at 105°C in 10-30 ml crucibles for a minimum of 72 hours and reweighted to determine dry bulk density and peat moisture content. 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. 3 ± 0.1 g of fresh wet peat sample was added to 27 ml ultrapure water and manually homogenized for 60 seconds, leaving it for 4 hours. The pH was then measured in the water suspension with a digital pH meter. After that, the peat solutions were filtered and stored in the dark at 4°C until further analysis. Dissolved organic carbon (DOC) and total bound nitrogen (TbN) was measured in filtered (using 0.45 µm Pore Rhizon samplers) water extracted from squashed, fresh peat samples (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 labeled 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 nutrients of ecological/environmental value) Cu, Zn, Pb, Al, Ca, Fe, K, Mg, Na, Mn, Cd, P, As and Si were measured by Inductively Coupled Plasma (ICP) analysis with an ICP-7000 (ICP-OES) instrument.

#### **4.3.5 Statistical analyses**

Differences in TA communities between managed (burnt & mown treatments versus uncut control) sites, near intact sites and the very wet border mire were analyzed using both multivariate community data and a number of indices. Taxa present in less than 4%

of total number of samples and those whose relative abundance overall less than 4% were eliminated from the TA community dataset to reduce the influence of rare taxa. 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 heather management. In order 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 management were calculated to display the detailed recovery pattern. To investigate diversity patterns, Shannon Diversity Index (SDI) (Shannon 2001) and Functional diversity (Rao quadratic entropy index) were calculated for each sample (Fournier et al. 2012). Functional traits including biovolume, aperture width, test type and mixotrophic TA 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 in ecosystem processes. Mixotrophy was determined as the proportion of mixotrophic species within a community (Fournier et al. 2015). Biovolume (body size) was calculated based on geometric shapes and specific formulae (see above in TA processing). The aperture width of each species was measured under a microscope until their value reached a normal distribution. The significant associations for the selected indices of interest including Shannon diversity, functional diversity, biovolume, aperture width, test type among managed sites (i.e., Nidderdale, Mossdale, Whitendale) and treatments (i.e., burning & cutting versus uncut control) were tested using two-way analysis of variance (ANOVA). For those data which did not satisfy the assumptions of ANOVA, a Kruskal-Wallis's test was applied. All these analyses and plotting of graphs were performed using R (R Core Team 2019).



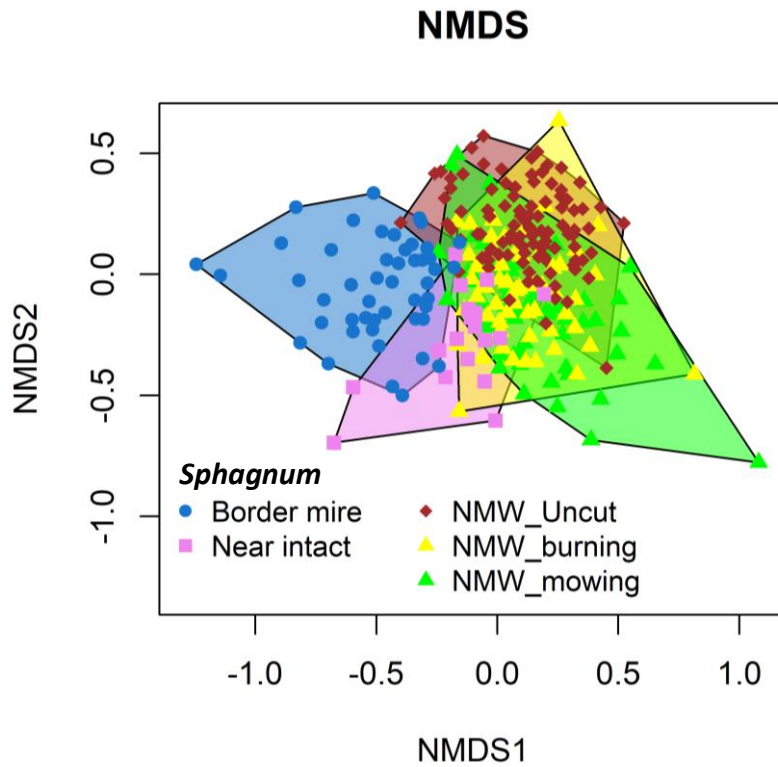
## 4.4 Results

### 4.4.1 TA communities versus habitat status and management (burning versus cutting versus uncut) in *Sphagnum* moss samples

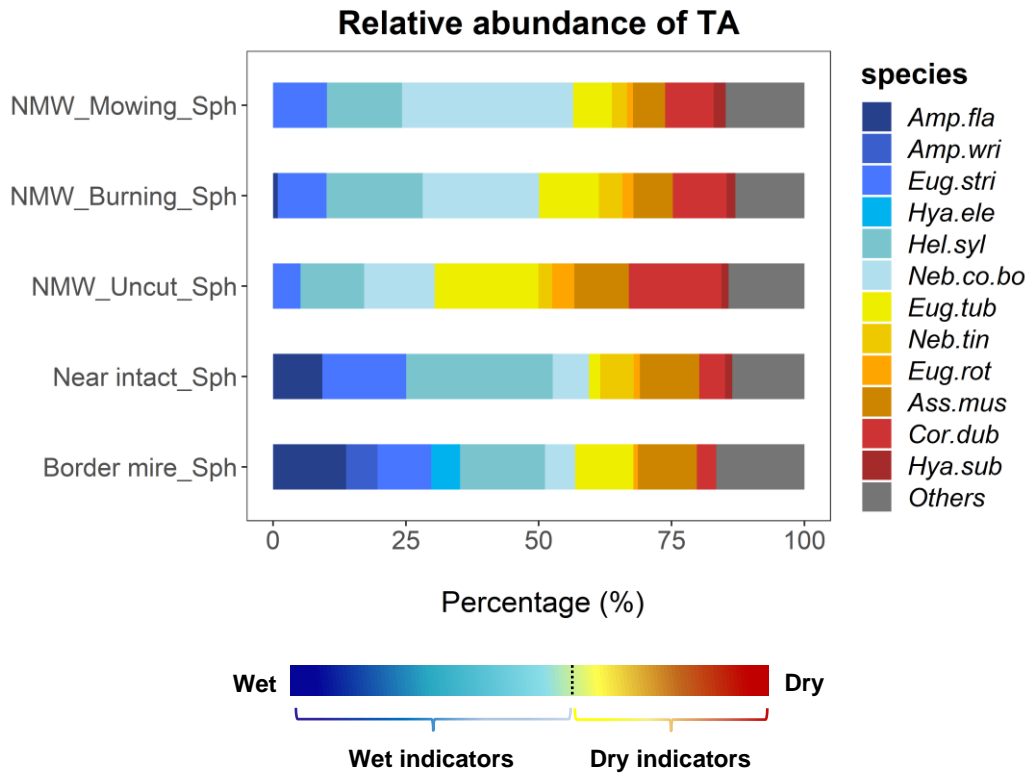
#### 4.4.1.1 Taxonomic differences

A total of 55 testate amoebae (TA) were identified from 142 samples within the seven peatland sites. NMDS ordination showed no significant separation in TA community composition between years of (i.e., time since) management for both burning and cutting with leaving brash (henceforth referred to as 'cut or mown') treatment (Supplementary Fig. S4.3a) and no strong differences were observed among sites (i.e., Nidderdale, Mossdale, and Whitendale) although one sample from Whitendale was placed slightly further away from others (Supplementary Fig. S4.4a). Considering the little effects of time and sites, samples were combined accordingly. The further ordination analysis still revealed no significant difference in TA community composition between burnt and mown treatments, but a small difference was shown between managed (i.e., combined burning & cutting) and unmanaged (uncut control) areas (Fig. 4.2), the latter being grouped much closer together. Noticeably, TA communities were considerably distinct between the very wet border mire (i.e., an intermediate/ridge-raised mire complex and not a typical blanket bog) and other habitat status, and also between the near intact sites and the managed sites (including uncut areas) (Fig. 4.2). Specifically, on average, the most abundant TA taxa in the plots of mown treatment included *Nebela collaris-bohemica* type (32.2%), *Heleopera sylvatica* (14.1%), *Euglypha strigosa* (10.1%), *Corythion dubium* (9.2%), *Euglypha tuberculata* (7.3%), and *Assulina muscorum* (6.0%) (Fig. 4.3). The plots of the burnt treatment shared similar dominant species and relative abundance as that in cut plots, that is, *Nebela collaris-bohemica* type (21.9%), *Heleopera sylvatica* (18.1%), *Euglypha tuberculata* (11.3%), *Corythion dubium* (10.1%), *Euglypha strigosa* (9.2%), and *Assulina muscorum* (6.0%) (Fig. 4.3). Compared to the managed plots, some of the dominant species in unmanaged (uncut control) plots showed higher relative abundance (e.g., *Euglypha tuberculata*: 19.5%, *Corythion dubium*:17.4%, and *Assulina muscorum*:10.2%), whereas some were lower, such as *Nebela collaris-bohemica* type (13.3%), *Heleopera sylvatica* (12.0%), and *Euglypha strigosa* (5.0%) (Fig. 4.3).

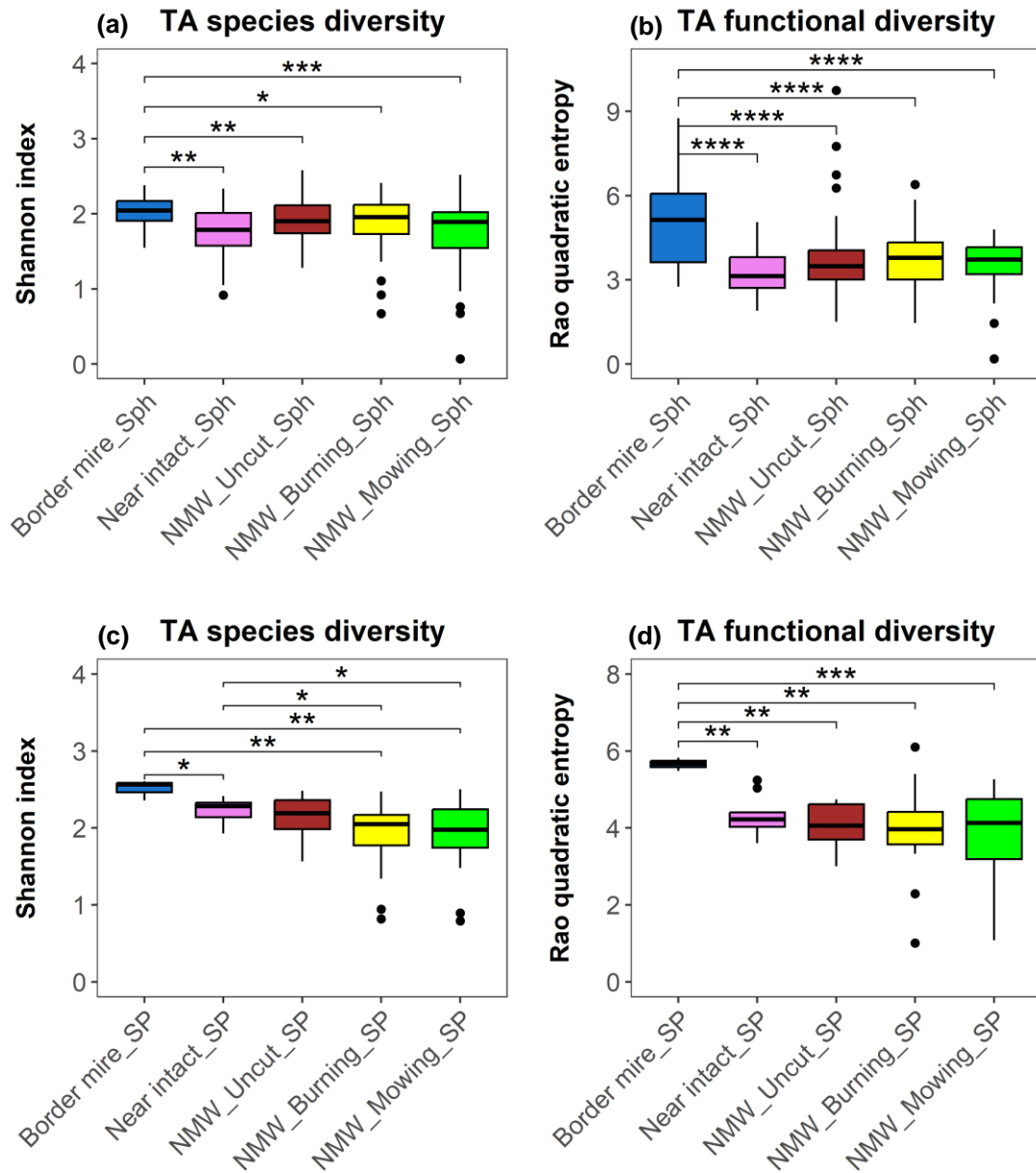
However, the abundant *Nebela collaris-bohemica* type in managed and unmanaged areas was significantly lower in both near intact sites (6.8%) and the very wet border mire (5.7%). Moreover, *Amphitrema flavum* only appeared with a very low amount in burnt (0.9%) and uncut (0.1%) areas and was relatively more abundant in near intact sites (9.3%) and the very wet border mire (13.8%) (Fig. 4.3). *Amphitrema wrightianum* type (5.9%) and *Hyalosphenia elegans* type (5.4%) were also only dominant in the very wet border mire (Fig. 4.3). Overall, TA species diversity (Shannon's index) was statistically significantly higher in the very wet border mire compared to near intact, managed (i.e., burnt & cut) and unmanaged (uncut control) plots ( $H(4) = 16.963$ ,  $p < 0.01$ ) (Fig. 4.4a). Interestingly, no significant difference in TA diversity was found between the managed and unmanaged plots although the median line in burnt plots seemed higher than that in both uncut and cut plots (Fig. 4.4a).



**Figure 4.2** Non-metric multidimensional scaling (NMDS) analysis of testate amoebae (TA) community composition in *Sphagnum* moss (combination of top 3 cm and bottom 3 cm segments) across the managed sites (NMW: combination of Nidderdale, Mossdale and Whitendale) versus the combined near intact (purple square) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn (blue circle). Burning (yellow triangle): represents the combination of burning treatments (2013, 2015, and 2018), whereas mowing (green triangle) stands for the combination of mowing (cut with brash left) treatments (2013, 2015, and 2018); Uncut (brown rhombus): stands for the unmanaged control areas.



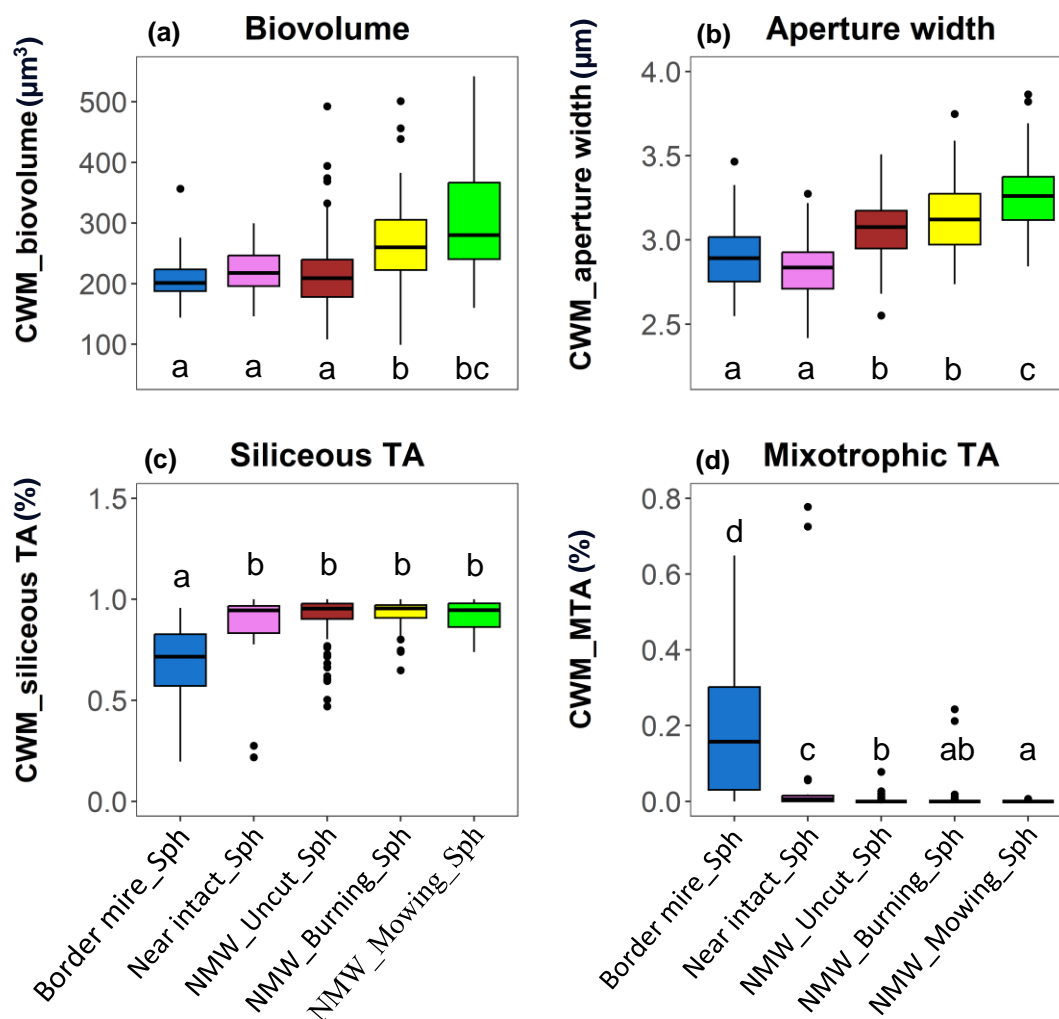
**Figure 4.3** Relative abundance of testate amoebae (TA) in *Sphagnum* moss (Sph) samples at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed (**Burning**: burnt treatment versus **Mowing**: cut with brash left treatment) and unmanaged (**Uncut** control) areas compared to the combined near intact (**NI**) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire () site Butterburn (Butt). Species that showed significant change and whose mean relative abundance more than 5% were selected and displayed: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Cyc.arc.s* - *Cryclopoxis arcelloides small type* (< 50  $\mu$ m), *Eug.tub* - *Euglypha tuberculata*, *Hel.syl* - *Heleopera sylvatica*, *Hya.sub* - *Hyalosphenia subflava*, *Neb.mil* - *Nebela militaris*, *Neb.tin* - *Nebela tinctoria*, *Pse.ful* - *Pseudodiffugia fulva type*, 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.



**Figure 4.4** Boxplots showing TA species diversity and functional diversity at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed (Burning: combination of burnt treatment (2013, 2015 and 2018: yellow); Mowing: combination of mown treatment (2013, 2015 and 2018: green) and unmanaged (Uncut control: brown) areas compared to the combined near intact (purple) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn (blue); (a) and (b) represent TA communities from *Sphagnum* mosses (Sph: combination of top 3 cm and bottom 3 cm segments), whereas (c) and (d) are from corresponding surface peat (SP: 0-5 cm). Asterisks indicate the significance level (Wilcoxon test), \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

#### 4.4.1.2 Functional differences

TA functional diversity was significantly higher in the very wet border mire than in those of all other habitat types ( $H(4) = 43.421, p < 0.001$ ) (Fig. 4.4b). However, no statistically significant difference was found between the managed and unmanaged plots and also between the burnt and mown treatments (Fig. 4.4b). For specific functional traits, both biovolume and aperture width displayed marked differences between managed treatment and other habitat types (Fig. 4.5a, b). In detail, biovolume was significantly higher in both burnt ( $W = 1484, p < 0.01$ ) and cut ( $W = 1056, p < 0.01$ ) plots than in uncut plots but did not markedly differ between burnt and cut plots even though the median line seemed much higher in cut plots (Fig. 4.5a). Interestingly, the aperture width of TA in cut plots was significantly higher than in burnt plots ( $W = 1052, p < 0.01$ ) but did not differ between burnt and uncut plots (Fig. 4.5b). It is worth noting that TA with siliceous shells and MTA showed an opposite change pattern, with siliceous TA significantly lower ( $H(4) = 77.887, p < 0.001$ ) in the very wet border mire than other habitat types (Fig. 4.5c, d). While no statistically significant difference was found neither between the managed and unmanaged plots nor between the burnt and mown treatment for siliceous TA, their total mean relative abundance was extremely high and even more than 90% in the managed and unmanaged areas (Fig. 4.5c, Supplementary Fig. S4.4). However, MTAs were particularly abundant and significantly higher in the very wet border mire compared to other habitat types ( $H(4) = 158.68, p < 0.001$ ) although a few points were abnormally high in the near intact sites and burnt plots from Nidderdale (Fig. 4.5d, Supplementary Fig. S4.6).



**Figure 4.5** Boxplots showing the change pattern of TA key functional traits (based on community weighted mean value (CWM)) from *Sphagnum* moss samples (**Sph**: combination of top 3 cm and bottom 3 cm segments) at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed **Burning**: combination of burnt treatment (2013, 2015 and 2018: yellow); **Mowing**: combination of mown treatment (2013, 2015 and 2018: green) and unmanaged (**Uncut** control: brown) areas compared to the combined near intact (purple) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site (Butterburn (blue)). Different letters indicate the significance level (Kruskal-Wallis test,  $p < 0.05$ ).

## 4.4.2 TA communities versus habitat status and management (burning versus cutting versus uncut) in surface and deep peat samples

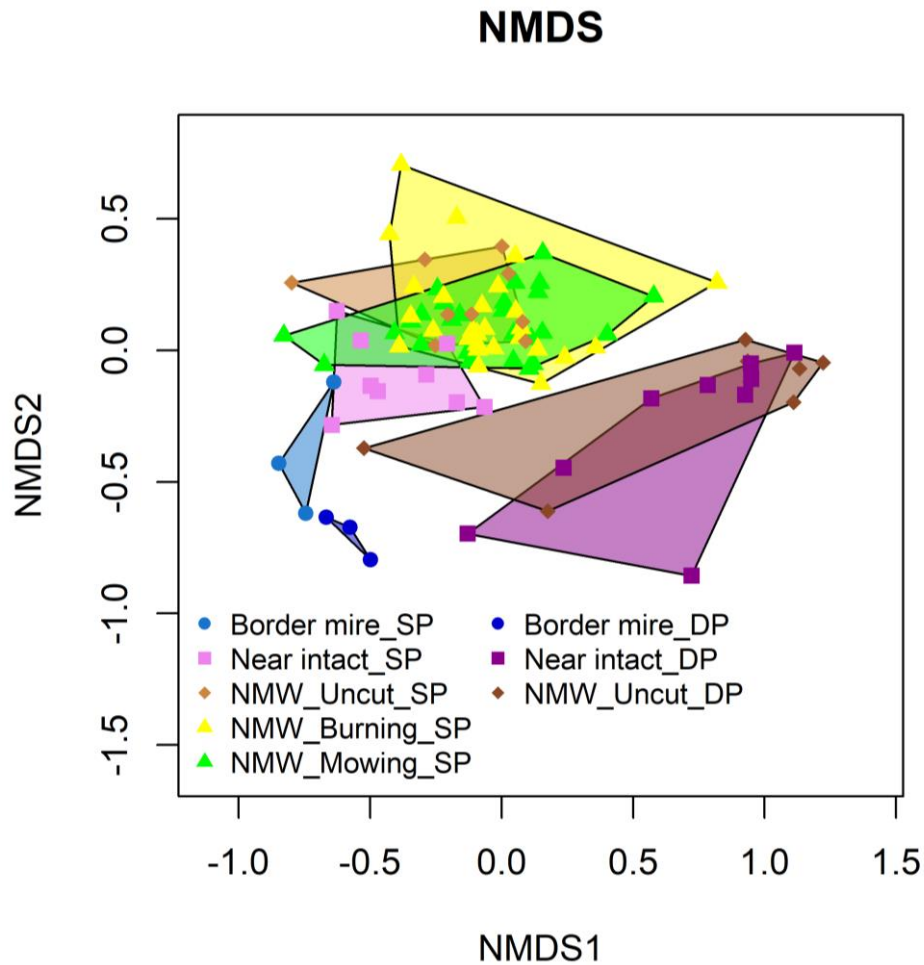
### 4.4.2.1 Taxonomic differences

A total of 51 TA taxa were identified from 95 samples collected within the seven peatland sites. NMDS ordination analysis showed a considerably different TA community composition between surface peat (0 to 5 cm) and deep peat (50 to 55 cm)

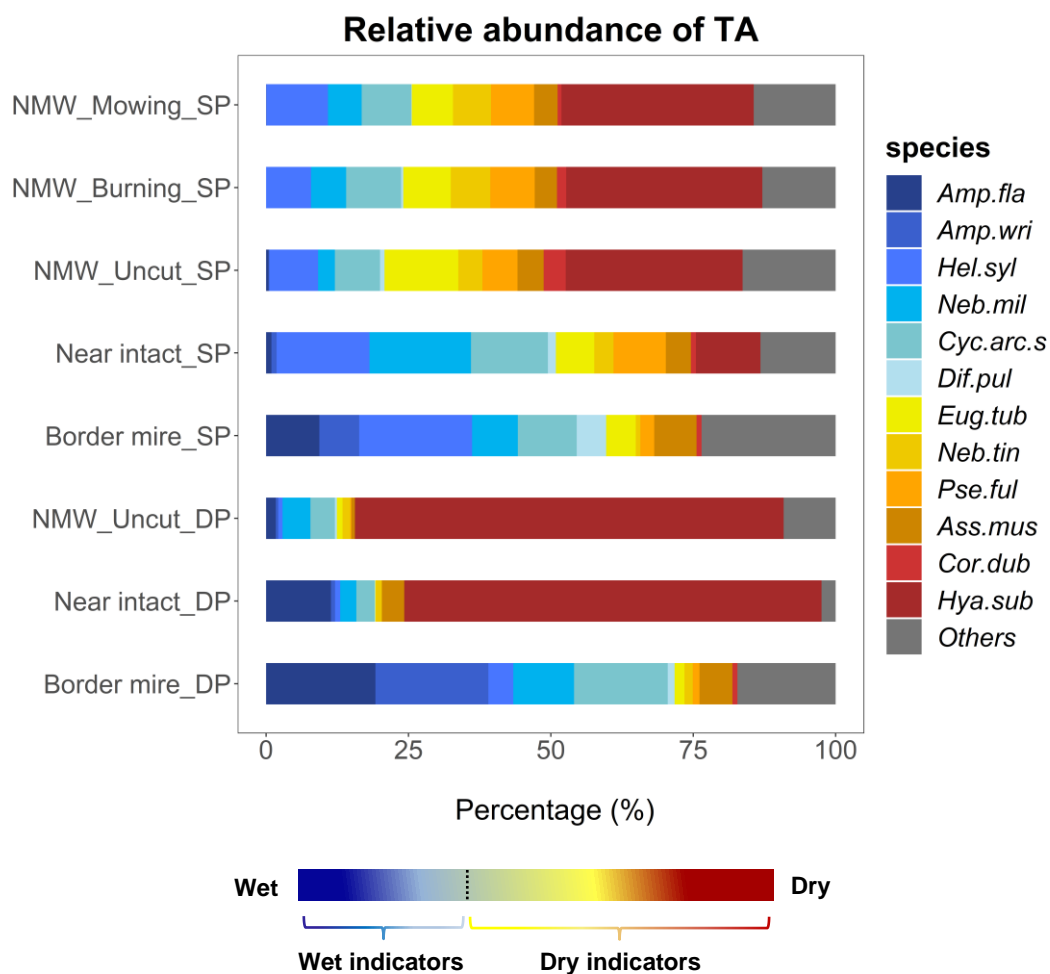
(Fig. 4.6). For surface peat, a distinct separation was observed between the very wet border mire and other habitat sites (Figs. 4.6, 4.7). TA communities were also shown to be highly different between the near intact sites and managed sites (including uncut control plots), but no obvious difference was found between the managed and uncut plots (Figs. 4.6, 4.7). Among the 33 environmental variables, peat moisture (WC: water content) was only selected to best explain the variation in TA community composition by RDA and forward selection analyses due to the strong correlations between some variables (Fig. 4.8, Supplementary Fig. S4.7). Nevertheless, other factors including *Sphagnum* moss cover, heather cover, Si and Zn concentration also play an important role, but to a lesser extent in determining the establishment and development of TA (Fig. 4.8). The primary and secondary compositional gradients, as revealed by axis 1 and axis 2 of the RDA ordination, represented 14.4% and 2.6% of the variability in the data set respectively (Fig. 4.8). In terms of the detailed TA communities, the dominant taxa in uncut plots included *Hyalosphenia subflava* (31.0%), *Euglypha tuberculata* (13.0%), *Heleopera sylvatica* (8.6%), *Cyclopyxis arcelloides* (7.9%), and *Pseudodiffugia fulva* (6.2%) (Fig. 4.7). Both burnt and cut plots shared very similar TA communities and relative abundance of dominant species compared to uncut plots, with only *Euglypha tuberculata* occupying a slightly lower proportion in the managed (burnt: 8.3%, cut: 7.2%) plots. While both *Nebela militaris* (2.9%) and *Corythion dubium* (3.9%) were not dominant species in the uncut plots, they indeed showed non-negligible difference compared to managed plots, with *Nebela militaris* higher and *Corythion dubium* lower in both burnt and cut plots. By contrast, the relative abundance of the most abundant species (i.e., *Hyalosphenia subflava*) in managed and unmanaged plots decreased dramatically in the near intact sites (11.3%) and even disappeared in the very wet border mire. Conversely, *Heleopera sylvatica*, relatively abundant in the managed and unmanaged plots, was even more abundant in the near intact sites (16.3%) and the very wet border mire (19.8%). Noticeably, *Amphitrema flavum* (9.3%) and *A. wrightianum* (7.0%) which are often indicative of wet habitat were particularly abundant in the very wet border mire (Figs. 4.7, 4.9d). Overall, TA species diversity showed a clear increasing trend from the mown to uncut and then all the way to the very wet border mire plots, but no significant difference was found between the managed (i.e., burnt & mown treatments) and uncut plots (Fig. 4.4c). As for deep peat samples, a near similarity was shown for TA communities between the uncut and near intact plots whereas they were markedly different from that in the very wet border mire



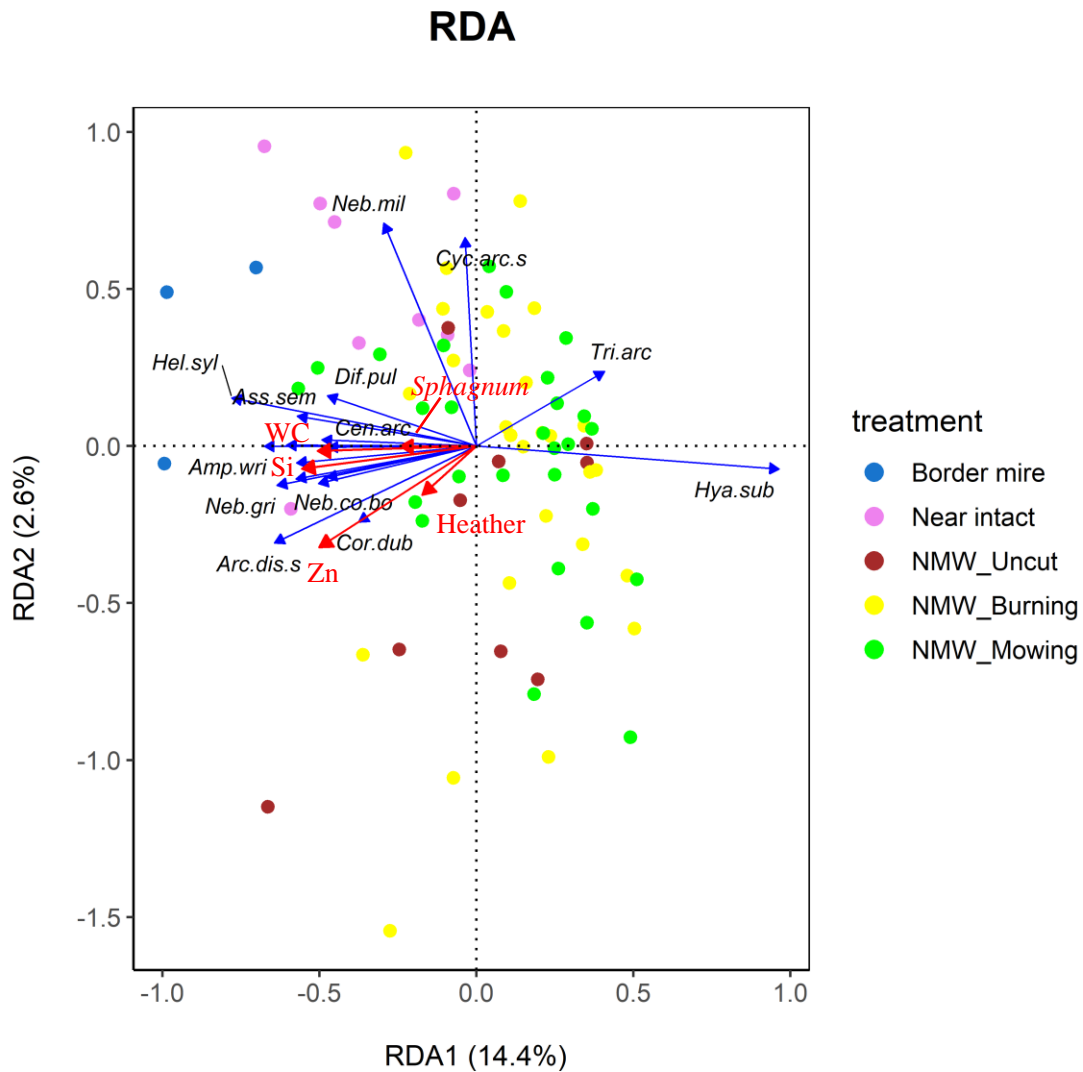
(Figs. 4.6, 4.7). Specifically, the dominant species in the very wet border mire were *Amphitrema wrightianum* (19.8%), *A. flavum* (19.2%), *Cyclopyxis arcelloides* (16.4%), and *Nebela militaris* (10.7%) and all of them were relatively much lower in both near intact and uncut plots (Fig. 4.7). Interestingly, no *Hyalosphenia subflava* was found in the very wet border mire whereas it was extremely abundant in both the near intact (75.3%) and uncut (73.3%) plots (Fig. 4.7).



**Figure 4.6** Non-metric multidimensional scaling (NMDS) analysis of testate amoebae (TA) community composition in surface peat (SP: 0-5 cm) and deep peat (DP: 50-55 cm) across the managed sites (NMW: combination of Nidderdale, Mossdale and Whitendale) versus the combined near intact (purple square) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn (blue circle). **Burning** (yellow triangle): represents the burning treatment whereas **Mowing** (green triangle) stands for the mowing (with brush left) treatment; **Uncut** (brown rhombus): stands for the unmanaged control areas. Note that symbol color was shown in the caption only for surface peat, the colours for deep peat are in darker shades but there was no assessment for managed NMW in DP.



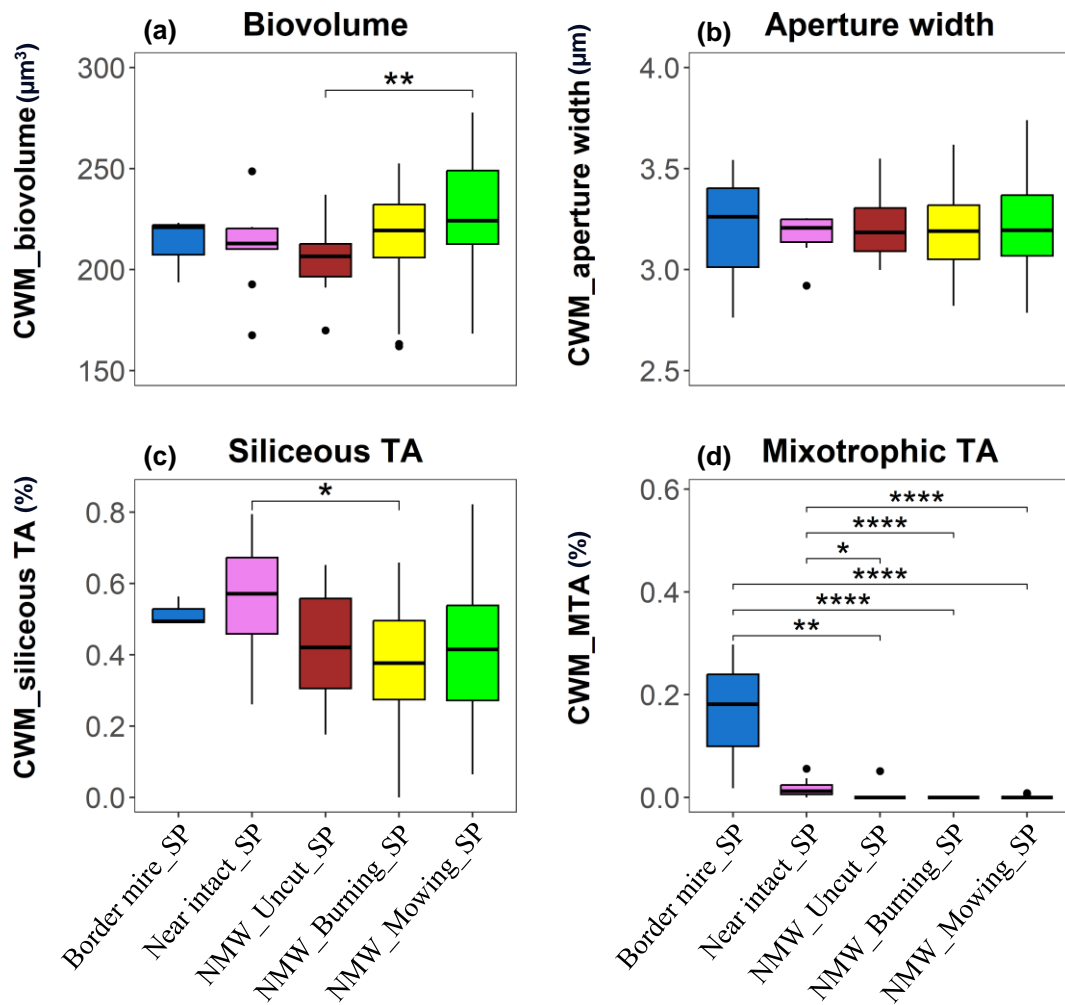
**Figure 4.7** Relative abundance of testate amoebae (TA) in surface peat (SP: 0-5 cm) and deep peat (DP: 50-55 cm) samples at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed (Burning: burnt treatment versus Mowing: mown with brash left treatment) and unmanaged (Uncut control) areas compared to the combined near intact sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn. Species that showed significant change and whose mean relative abundance more than 5% were selected and displayed: *Amp.flu* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Cyc.arc.s* - *Cryclopixis arcelloides small type* (< 50  $\mu$ m), *Eug.tub* - *Euglypha tuberculata*, *Hel.syl* - *Heleopera sylvatica*, *Hya.sub* - *Hyalosphenia subflava*, *Neb.mil* - *Nebela militaris*, *Neb.tin* - *Nebela tincta*, *Pse.ful* - *Pseudodiffugia fulva type*, 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. Note that only Uncut samples were assessed for DP at NMW sites (reflecting the same historical management across the NMW sites).



**Figure 4.8** Redundancy analysis showing the relationship among sites, habitat status, testate amoebae species, selected (based on forward selection) environmental variables (i.e., WC: water content, Si: silicon, Zn: zinc) and key vegetation cover (i.e., *Sphagnum*, *Heather*). Managed sites (NMW: combination of Nidderdale, Mossdale and Whitendale) and habitats (**Uncut** (brown): unmanaged control, **Burning** (yellow): burnt management, **Mowing** (green): mown with brash left management) were compared to the combined near intact (purple) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn (blue). Axis 1 explains 14.4 % of total variation of TA community whereas Axis 2 explains 2.6 % of the variance. Species that showed significant change ( $p < 0.001$ ) were: *Amp fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Arc.dis.s* - *Arcella discoides* type, *Ass.sem* - *Assulina seminulum*, *Cen. are* - *Centropyxis areaona*, *Cen.cas* - *Centropyxis cassis*, *Cor.dub* - *Corythion dubium*, *Cry.ovi* - *Cryptodiffugia oviformis*, *Cry.arc.s* - *Crycopyxis arcelloides small type* ( $< 50 \mu m$ ), *Dif.lei* - *Diffugia leidy*, *Dif.pul* - *Diffugia pulex*, *Hel.syl* - *Heleopera sylvatica*, *Hya.sub* - *Hyalosphenia subflava*, *NA.1* - *Assulina* type, *Neb.co.bo* - *Nebela collaris-bohemica* type, *Neb.gri* - *Nebela griseola*, *Neb fla* - *Nebela flabellulum*, *Neb.mil* - *Nebela militaris*.

#### 4.4.2.2 Functional differences

TA functional diversity was significantly higher in the very wet border mire than in other habitat types ( $H(4) = 9.0506$ ,  $p < 0.05$ ). However, no statistically significant difference was found between the managed and unmanaged (uncut) plots even though the median line in cut plots seemed higher than in both burnt and uncut plots (Fig. 4.4d). For specific functional traits, most of them did not show any significant difference between the uncut and any treatment of the managed plots except biovolume, which was significantly higher in cut than uncut plots ( $W = 52$ ,  $p < 0.01$ ). Interestingly, no significant difference was found in aperture width of TA for any paired habitat type (Fig. 4.9b). Furthermore, TA with siliceous shell was only significantly higher in the near intact sites than in the burnt plots ( $W = 132$ ,  $p < 0.05$ ) although the median line in burnt plots seemed much lower than in both cut and uncut plots (Fig. 4.9c). It is worth noting that the proportion of siliceous TA in managed and uncut plots was relatively low, only occupying about 40% of all types of TA (Supplementary Fig. S4.4b). Similarly, mixotrophic TA in the very wet border mire was relatively abundant and significantly higher than those in all other habitat types (Fig. 4.9d). While a significant difference of MTA was revealed between the near intact plots and both managed and uncut plots, the proportion of them was still relatively low in the near intact plots (Figs. 4.7, 4.9d).



**Figure 4.9** Boxplots showing the change pattern of TA key functional traits (based on community weighted mean value (CWM)) from surface peat (SP: 0-5 cm) samples at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed (Burning: combination of burnt treatment (2013, 2015 and 2018: yellow); Mowing: combination of mown treatments (2013, 2015 and 2018: green) and unmanaged (Uncut control: brown) areas compared to the combined near intact (purple) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn (blue). Asterisks indicate the significance level (Kruskal-Wallis test), \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

## 4.5 Discussion

TA extracted from both *Sphagnum* moss and surface peat samples worked well to allow the identification of the differences of TA communities and functional types. Both datasets suggest a distinct separation in TA community composition, species and functional diversity between the very wet border mire and other habitat type, but no clear and consistent recovery trajectory was revealed by TA communities over the course of eight years of natural recovery following vegetation management (i.e., burnt and mown treatments) (Supplementary Figs. S4.8,4.9). This is to a large extent inconsistent with the first hypothesis: *cutting is better than burning for the hydrological restoration of heather-dominated peatlands although subsequent vegetation recovery following cutting shifts TA communities (including functional traits) towards that of near-natural conditions, whereas burnt areas will develop towards unmanaged heather-dominated areas*. This may be highly related to the wetness of sites as hydrology (either expressed as water table depth (WTD) or soil moisture), which has long been reported to be the main controls on TA communities (Charman 1997, Mitchell et al. 1999), has been shown to be significantly higher in the very wet border mire but not strikingly changed during the period of recovery. It was also corroborated by the RDA analysis, with peat moisture being the most important environmental factor in explaining TA variability (Fig 4.8). We can therefore partly support the second hypothesis *that site/plot wetness is overall more important than management and other factors (e.g., peat physicochemical property) in determining TA establishment and development*. It is noteworthy that hydrology can be influenced not only by management practices, but also by vegetation cover/composition and annual climatic conditions (Cerdà and Doerr 2005, Whittington and Price 2006). The confounding factors including drainage, sheep grazing, and site/plot conditions (e.g., climate/slope) are also likely to complicate the hydrological condition in managed plots. As a result, the relatively small change in peat moisture resulting from management practices was highly susceptible to variations in the aforementioned factors, thereby leading to significant and dynamic inter-annual hydrological fluctuations. This may be the reason for the inconspicuous and irregular change pattern of TA communities during the course of recovery, especially for surface peat samples, which indicates a slow natural recovery rate of the ecosystem following these kinds of heather management strategies.

Interestingly, neither *Sphagnum* moss nor surface peat data revealed a statistically significant difference in TA community composition, species and functional diversity between burnt and mown treatments (Figs. 4.2, 4.4, 4.6). This is also inconsistent with the first hypothesis. However, it is not surprising as both burnt and mown treatments did not result in distinct differences in peat physical properties, a key characteristic in determining hydrological conditions (Heinemeyer et al., 2023). Despite the assumed compaction potentially caused by heavy machinery for vegetation-cutting, the peat showed resilience and no lasting plot-level impact on either peat depth or bulk density (Heinemeyer et al. 2019). The peat moisture was therefore not dramatically different between burnt and cut plots following management practices, although mean moisture and water table depth in the first five years was reported approximately 2 cm higher (wetter) in cut plots (Heinemeyer et al., 2019). However, the burnt plots gradually became wetter over time (Heinemeyer et al., 2023). This was probably the main reason for the slight difference in TA communities between the management practices.

While TA community composition was similar between burnt and cut plots, it was substantially different between the near intact sites and managed (including uncut control areas) sites (Figs. 4.2, 4.6). This was probably highly related to the historical management of the managed sites. For example, previous management by digging a series of ditches in the 1960 s/70s to mainly improve sheep grazing on two of these studied grouse moors lowered the water table depth. Although the other site (Whitendale) did not undergo historical drainage, the presence of large natural gullies may have also greatly affected the hydrology (Heinemeyer et al. 2023). The resulting drier surface peat then created favorable conditions for the colonization of taxa tolerating or preferring dryer habitat like *Hyalosphenia subflava*. This was most likely the reason for the remarkable abundance of this species in uncut plots (with dense, old heather likely causing strong evapotranspiration drying). Conversely, this drying has negative impacts on species with relatively wetter hydrological preferences such as *Amphitrema flavum*, and *A. wrightianum*. Their relative abundance was therefore dramatically reduced and even disappeared in most of the uncut plots. It is noteworthy that both the burnt and mown treatment resulted in a clear indication of increased wetness of the peatland habitat compared to the uncut heather plots, as shown by their higher relative abundance of total wet TA indicator species. This indication was highly consistent with the results of measured peat moisture (supplementary Fig. S4.10) and

WTD data (Heinemeyer et al. 2023), which clearly demonstrate the effectiveness of TA to be used as bioindicators to monitor hydrological changes in the habitats. But interestingly, the dominant indicator species were distinctly different between *Sphagnum* moss and surface peat. This may be explained by the difference of suitable materials for test construction and available food sources (e.g., more microbes and detritus in peat). For example, the main dry indicators (e.g., *Corythion dubium*, and *Euglypha tuberculata*) found in *Sphagnum* moss are idiosome type whose tests formed by secreted siliceous plates which was reported to be relatively more abundant in *Sphagnum* mosses (Gilbert et al. 2003). By contrast, the corresponding dry indicator species, *Hyalosphenia subflava* (with chitinous shell), was notably abundant in the surface peat, possibly due to the substantial presence of microbes in this niche. These may therefore suggest the potential different functional roles of TA communities between *Sphagnum* moss and surface peat.

Recent studies linking functional traits of TA to ecosystem processes suggest that TA play an essential 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 MTA occupied more than 70% of total TA biomass in a raised bog and 13% reduction of CO<sub>2</sub> uptake of the entire *Sphagnum* moss caused by increased temperature was related to the 50% decrease of MTA biomass (i.e., *Amphitrema flavum*, *Heleopera sphagni*, and *Hyalosphenia papilio*). Nonetheless, much less proportion of MTA was counted in the present study, even in the wettest border mire, where relative abundance of MTA including *Amphitrema flavum* and *A. wrightianum* from *Sphagnum* mosses only accounted for 19.6% of total TA abundance. However, no *Heleopera sphagni* was observed across all samples. *Hyalosphenia papilio* was also found scarce, with relative abundance of more than 4% being only counted in one sample. This was more likely to be attributed to the limited moisture content of *Sphagnum* mosses than a shortage of light, which has been reported as an essential requirement for MTA survival (Schönborn 1965) as light was assumed adequate for MTA colonization in any of the sampling sites. It was, to a large extent, evidenced by the extremely abundant MTA from two very wet *Sphagnum* samples obtained from a near-intact and a burnt plot. Although it is typically challenging to determine the relative significance of moisture and light in governing MTA colonization and development, this study suggests that peat moisture may hold greater importance than light for MTA establishment,



especially in cases where light availability is sufficient, as MTA was found relatively abundant in the very wet border mire. This may also indicate a potential contribution of TA within *Sphagnum* mosses in C fixation in relatively wet habitats.

Biovolume and/or aperture size (width) of TA have been recently reported to be highly linked to the decomposition of organic matter (Fournier et al. 2015, Jassey et al. 2016, Payne et al. 2016, Koenig et al. 2018b). Some studies have related the biovolume/aperture size to the wetness of habitat, with large species (> 60 µm) more abundant in wetter habitat while small (< 60 µm) species more abundant in drier habitat (Jassey et al. 2016, Payne et al. 2016). This was just partly corroborated by this study as both biovolume and aperture width of TA from *Sphagnum* mosses in the near intact sites and the very wet border mire were smaller than that in the burnt and cut plots (drier). The most probable reason for this was the replacement of relatively large species (*Nabela collaris-bohemica* type) in the burnt and cut plots by a large amount of smaller MTA, that is, *Amphitrema flavum* and *A. wrightianum* (with small aperture size) in the reference sites due to the significant increase of peat moisture, which greatly reduced the community weighted mean value of biovolume and aperture size. Therefore, a cautious approach should be taken to infer a correlation between biovolume/aperture size and ecosystem functioning (peat decomposition) on the basis of hydrological conditions if a considerable quantity of small MTA occurs. However, because of the extremely low abundance of MTA in the burnt, cut and uncut plots, it is plausible that peat decomposition is increased in the drier uncut plots compared to the burnt and cut plots as small species (e.g., *Assullina muscorum*, *Corythion dubium*, *Pseudodiffugia fulva*), which have been reported to predominantly feed on microbes, were found more abundant in uncut plots.

Previous studies showed that type of fire (size, intensity, duration and severity) is likely to be the most important factor in controlling TA community composition, with idiosomic TA (with siliceous shell) being likely easier to be broken down by intense fire (Turner and Swindles 2012, Qin et al. 2017), whereas low intensity and short-term prescribed burning might have no significant effects on TA communities (Wanner and Xylander 2003, Wanner 2012). This is supported by our results as the proportion of TA with siliceous shell was remarkably similar between burnt and cut plots in *Sphagnum* mosses, even though it was visibly, but not statistically significantly, lower in the burnt

than cut plots in surface peat. This may indicate the limited effects of prescribed burning of heather-dominated peatlands on TA communities, especially for TA with siliceous shell. But noticeably, more than 90% of total TA was TA with siliceous shell from *Sphagnum* mosses in the managed and uncut plots, whereas it only occupied about 40% from surface peat accordingly. Moreover, the majority of siliceous TA observed in surface peat were dead while most of them in *Sphagnum* mosses were alive. These may suggest that siliceous TA mainly dwell in *Sphagnum* mosses and most of them observed in the surface peat actually originated from *Sphagnum* mosses by vertical transport after death. Given its abundance and the characteristic of being relatively more susceptible to breakdown under adverse conditions such as desiccation, TA with siliceous shells within *Sphagnum* mosses may play a pivotal role in silica cycling in relatively dry habitats. According to the discussion above in relation to TA functional traits, the third hypothesis can be only partly supported: *TA communities in Sphagnum moss fulfill distinct functional roles compared to those in the surface peat and their recovery pattern is more pronounced in Sphagnum moss than in peat samples.*

Despite the fact that TA have been broadly and successfully applied for palaeohydrological reconstruction in ombrotrophic peatland ecosystems (raised bog) due to their characteristics of sensitivity to moisture change and assumed well-preserved shells after death (Woodland et al. 1998, Charman et al. 2006, Swindles et al. 2014, Liu et al. 2019, Bysouth and Finkelstein 2021), its application should be cautiously considered in blanket bogs (with often lower water tables, especially in summer, see Heinemeyer et al. 2023) as this study indicated a potentially dramatic degradation/decomposition rate (i.e., loss) of most TA taxa, particularly those with siliceous shells even in near intact sites. However, some MTA were still counted in the deep peat at a depth of 50 to 55 cm although it was only slightly higher than that in the corresponding surface peat samples (Fig. 4.7). This does not mean they can survive in such a niche with a big shortage of light and oxygen, instead it indicates better preservation after death, even in unfavorable conditions, due to their chitinous shell, which is hard to break down. While the deep peat samples proved not good enough to serve as a pre-management comparison reference, to investigate the effects of current management on TA communities due to the disproportional preservation of TA in different habitat status, the similar amount of MTA in deep peat to surface peat for uncut plots may reflect a similar hydrological condition for about five hundred years

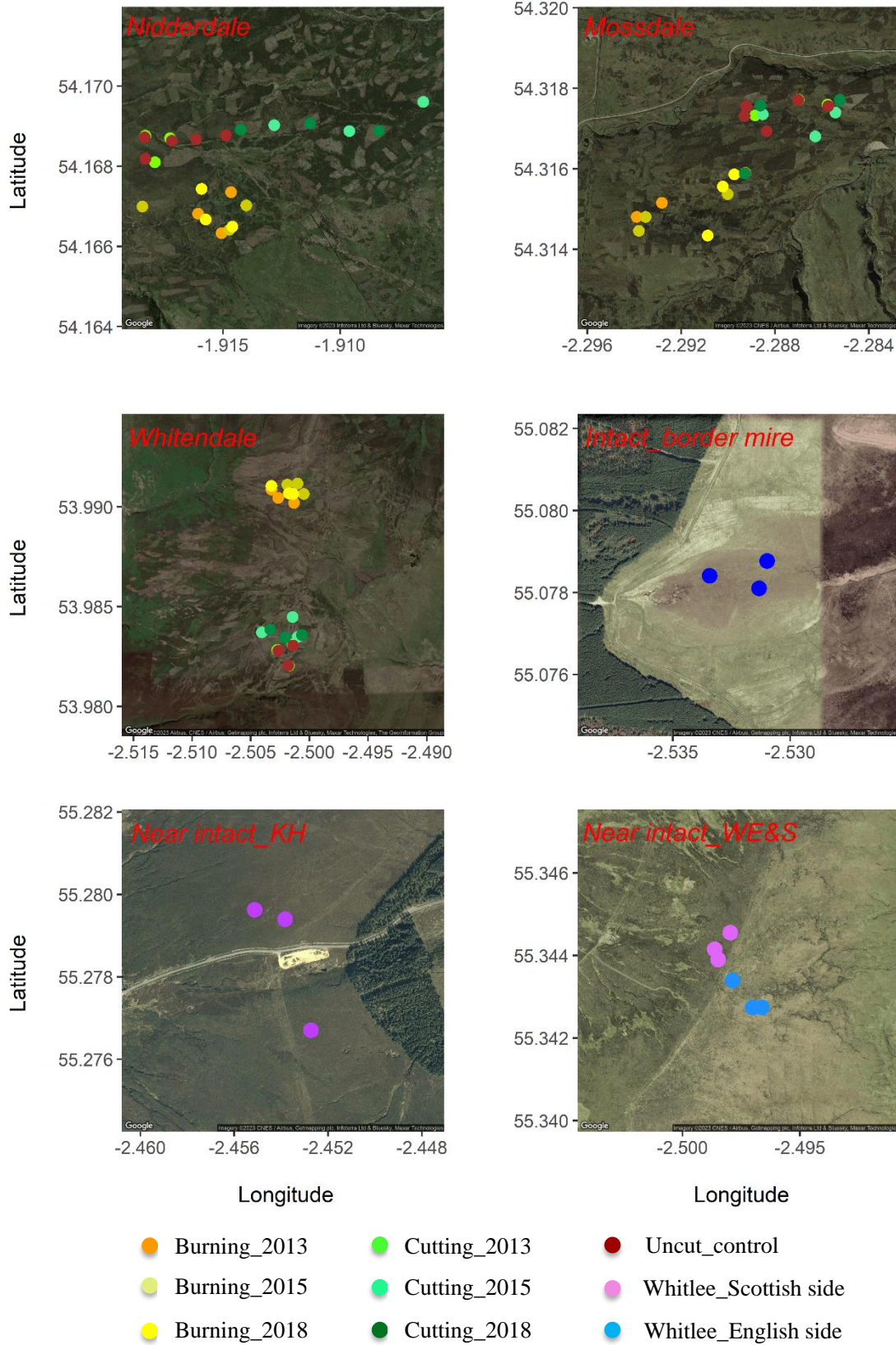
ago. This may further suggest a long history of prescribed burning practices taking place in the currently managed sites, which is confirmed by charcoal records revealing frequent fires over many hundreds of years at the three managed sites (Heinemeyer et al. 2018) and thousands of years for a study from a nearby location at Mossdale (Webb et al. 2022). Thus, we have to reject the fourth hypothesis that: *TA communities in deep peat layers in blanket bogs can be used to infer and compare historic conditions*. Additionally, the large proportion of *Hyalosphenia subflava*, some of which were likely alive when sampling, in surface and deep peat from uncut and managed plots may indicate that they are directly linked to decomposition process of organic matter as peat detritus/humus particles were observed abundantly in many individuals, especially from the deep peat samples (Supplementary Fig. S4.11). However, it is also possible that they feed on peat particles for consuming associated microbes (e.g, bacteria).

Undoubtedly, the application of TA as hydrological indicators to inform habitat restoration has been shown to be efficient and promising in this study, but the inferred hydrological conditions can only provide limited information on the habitat status. The small changes in habitat hydrology revealed by TA dry and wet indicators over the eight years of natural recovery after vegetation management, whilst confirming observations based on WTD records, could hardly predict if the habitat can recover to the near intact status and how long it might take (if this is actually possible and not limited by site climatic conditions). As for their potential functional roles, most of them are still based on the inference of functional traits from a microbial food web perspective. However, we currently still have a big knowledge gap on their feeding habits, which greatly hinders our understanding of their ecosystem functions. Finally, due to the large proportion of dead TA counted in the top five-centimeter peat, future studies should better sample the top one- or two-centimeters of peat to more precisely and accurately reflect the effects of peatland management (e.g., burnt and/or mown treatment) on TA communities.

## 4.6 Conclusion

This study shows a distinct separation of TA community composition, species and functional diversity between the very wet border mire and other habitat status, but no statistically significant difference was found between burning and mowing treatments. The significant response of species indicative of dry habitats (e.g., *Hyalosphenia subflava*, *Corythion dubium*) and those indicative of wet habitats (e.g., *Amphitrema flavum* and *A. wrightianum*) highlighted the potential of TA as bioindicators to monitor hydrological changes. The indicator value was especially sensitive to larger differences in overall habitat wetness (very wet border mire) and did only reveal small changes in respect to heather management, which confirmed small measured WTD differences (uncut drier than both management and mown slightly wetter than burnt). By contrast, the notable differences in dominant indicators and functional traits (e.g., MTA, biovolume/aperture size, test type) between *Sphagnum* mosses and surface peat may imply different functional roles they play, with TA in *Sphagnum* mosses appearing to have a greater significance in C fixation in relatively wet habitats, while TA in surface peat being more important in peat decomposition and silica cycling in relatively dry habitats. Furthermore, the observed large amount of *Hyalosphenia subflava* in surface and deep peat from managed sites may indicate its strong linkage to carbon cycling. Apart from feeding on microbes, they may actively take part in the peat decomposition process. 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 a large sensitivity of TA to habitat status and less so to heather management practices and clearly demonstrates the link between biodiversity, management, and likely inferred ecosystem functioning (water management / carbon and silica cycling).

## 4.7 Supplementary materials

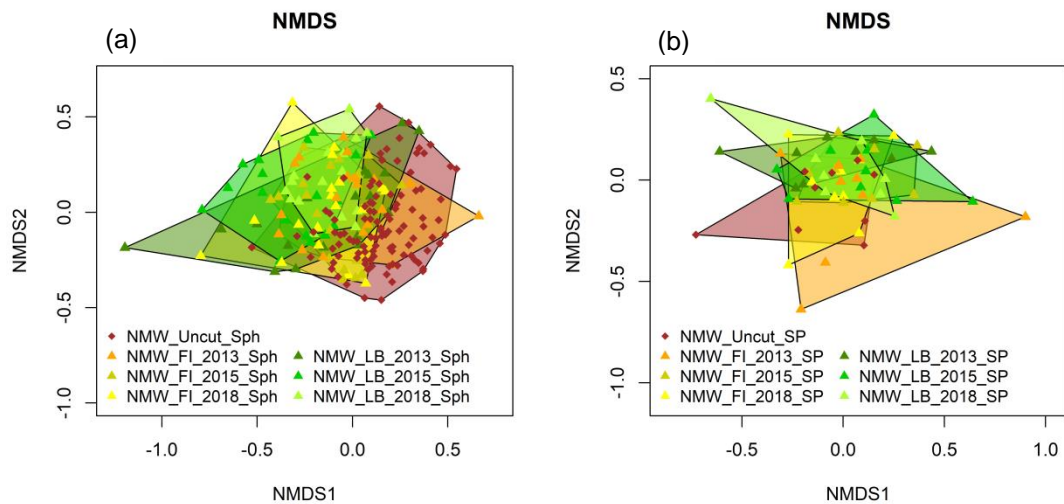


**Supplementary Fig. S4.1** Sampling location of each plot/area among sites. Reference sites: Intact border mire (Butterburn), and near intact sites (KH: Kielder Head and WE&S: Whitelee English/Scottish side). Managed experimental sites: Nidderdale, Mossdale and Whitendale (burning & cutting with brush left versus uncut control) in the UK.

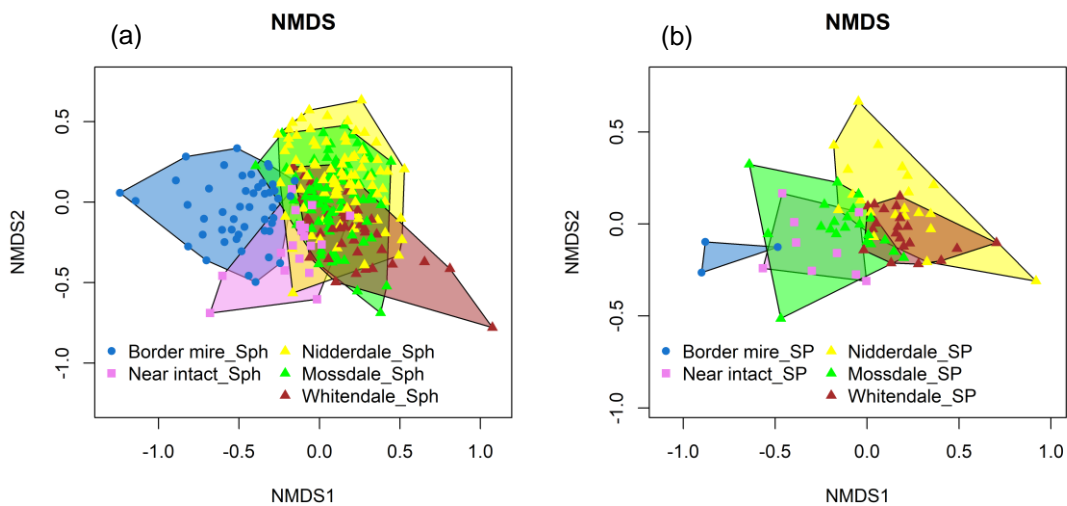




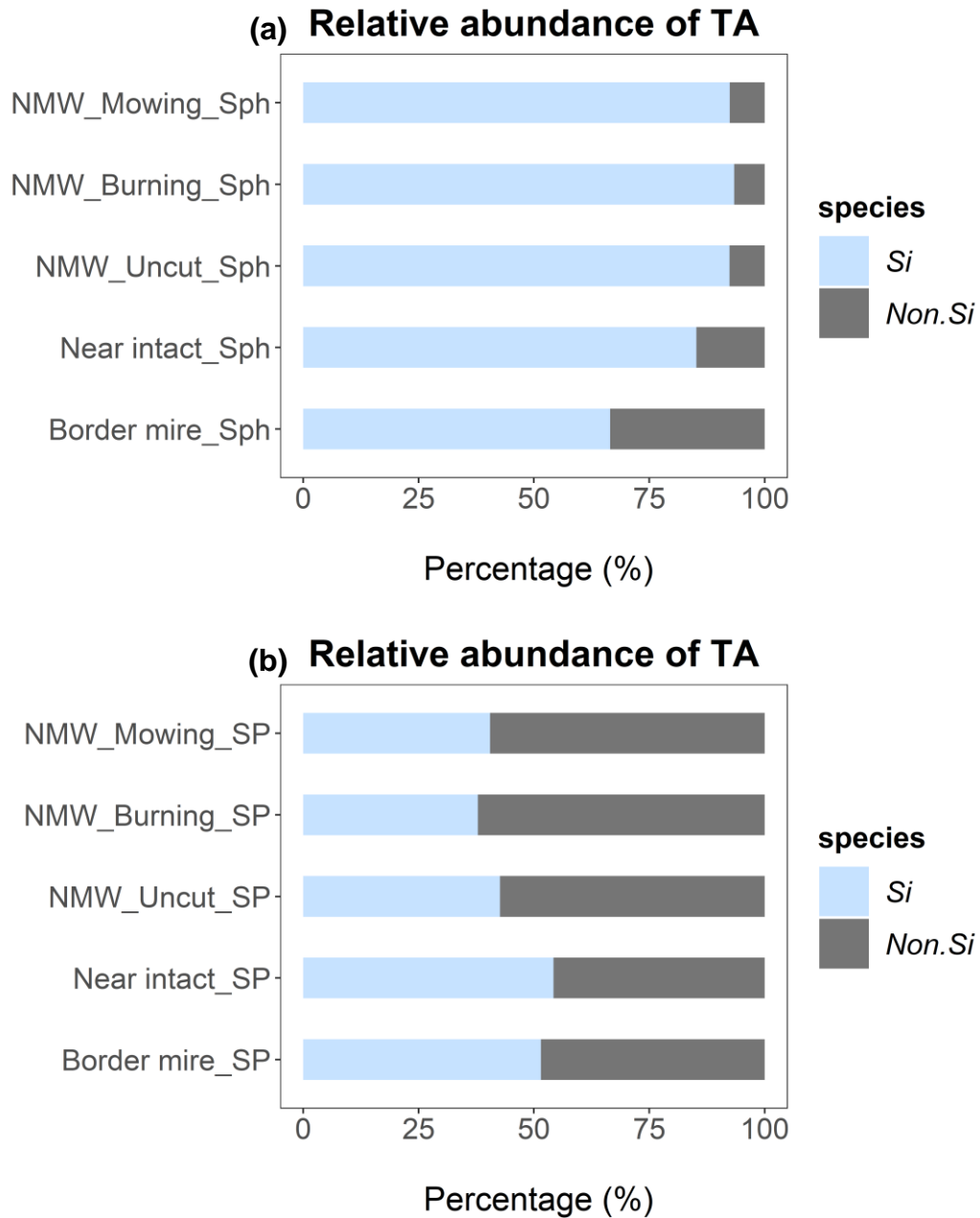
**Supplementary Fig. S4.2** Photos showing measurement of site parameters (i.e., peat depth, slope, aspect, temperature, slope, height of *Sphagnum* moss, other moss, litter) and vegetation investigation during the fieldwork (pictures are from Mossdale).



**Supplementary Fig. S4.3** Non-metric multidimensional scaling (NMDS) analysis of testate amoebae (TA) community composition across different management interventions. (a) compares TA communities between the managed (**FI**: burnt treatment in 2013 (orange triangle), 2015 (pale yellow triangle), and 2018 (yellow triangle)) versus **LB**: mown with brush left treatment in 2013: dark green triangle, 2015: green triangle, and 2018: light green triangle) and unmanaged (Uncut control: brown rhombus) areas for combined sites (**NMW**: combination of Nidderdale, Mossdale, and Whitendale) for *Sphagnum* moss (**Sph**: combination of top 3 cm and bottom 3 cm segments) whereas (b) for surface peat (**SP**: 0-5 cm).

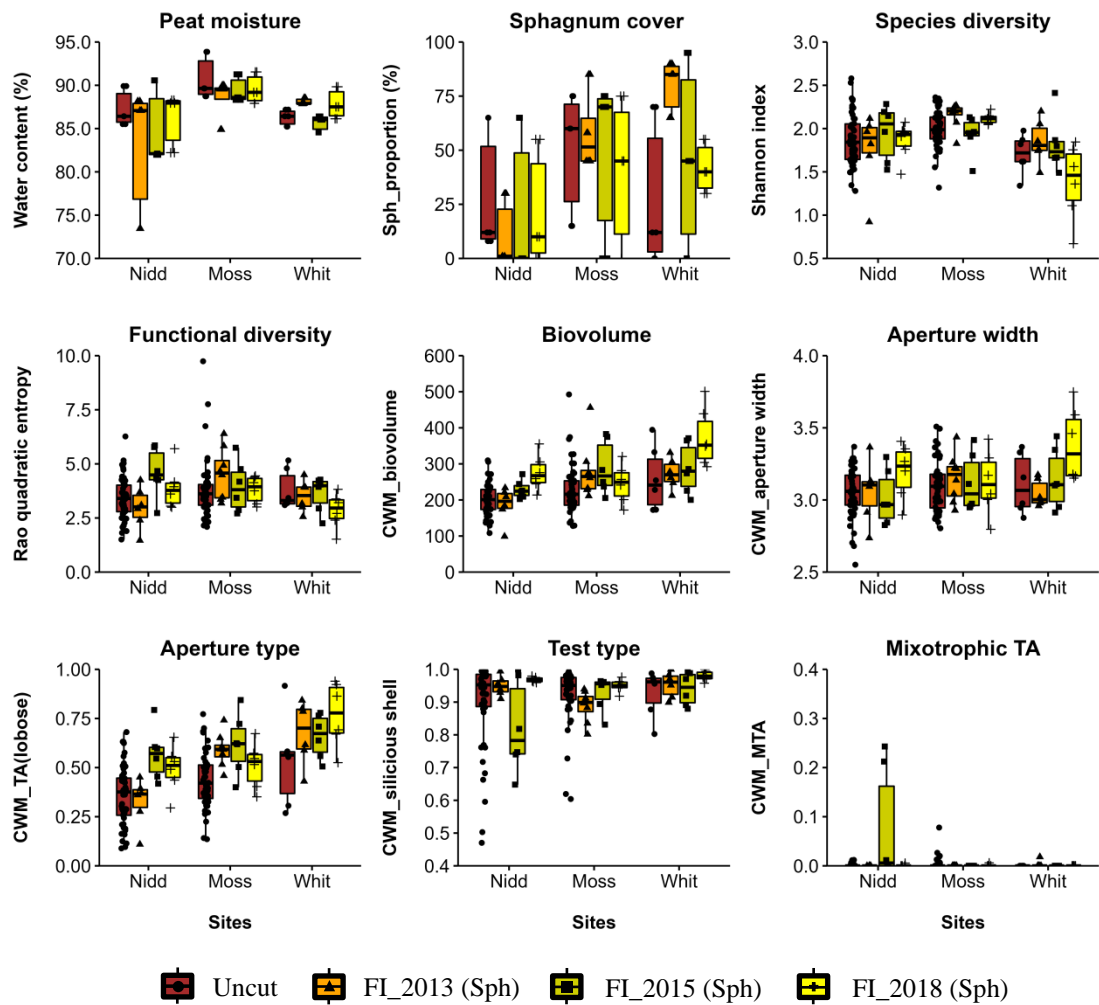


**Supplementary Fig. S4.4** Non-metric multidimensional scaling (NMDS) analysis of testate amoebae (TA) community composition in (a) *Sphagnum* moss (**Sph**: combination of top 3 cm and bottom 3 cm segments) and (b) surface peat (**SP**) across the managed sites (i.e., Nidderdale: yellow triangle, Mossdale: green triangle, and Whitendale: brown triangle) versus the combined near intact (purple square) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn (blue circle).



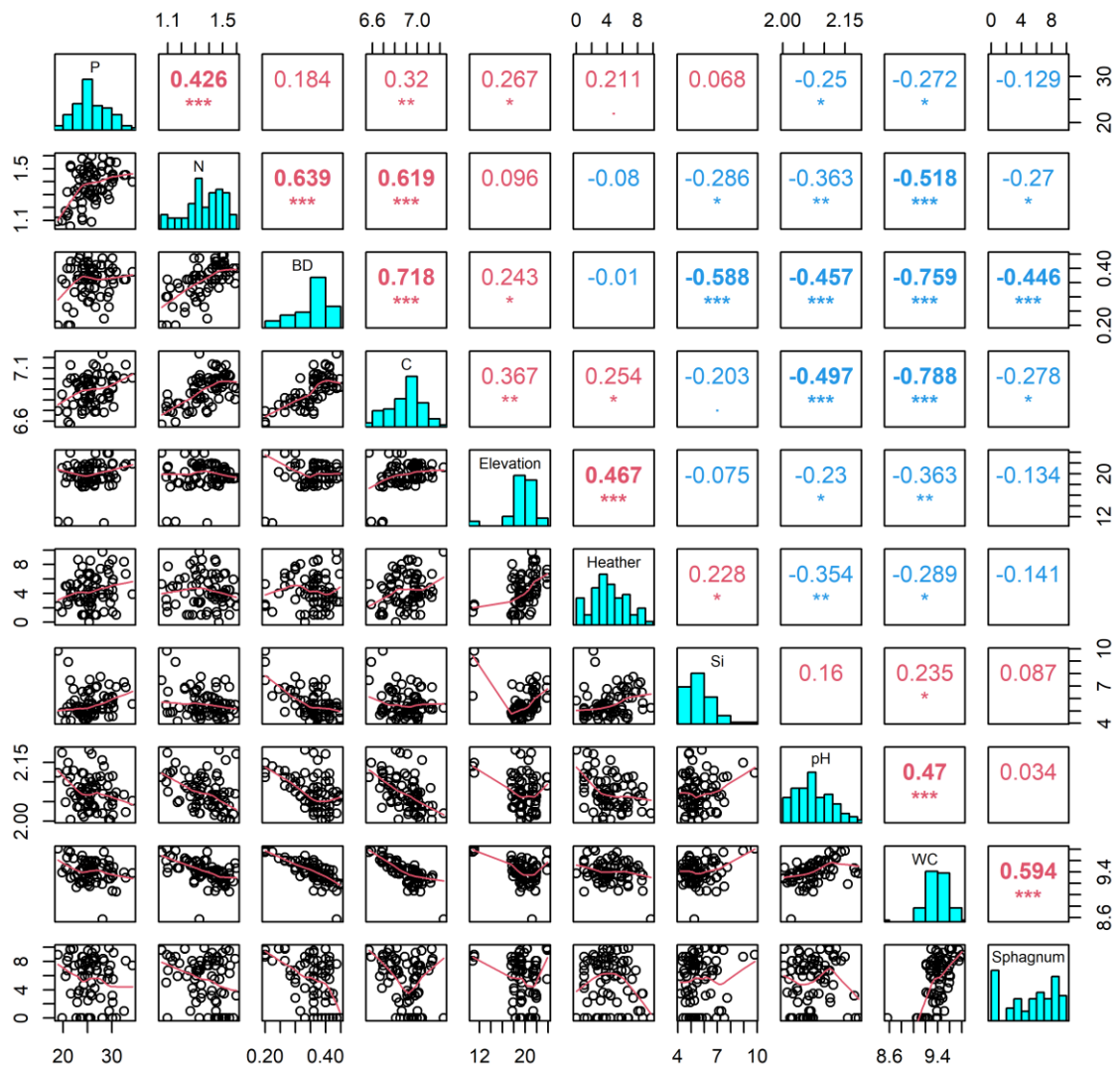
**Supplementary Fig. S4.5** Relative abundance of testate amoebae (TA) in *Sphagnum* moss (Sph: combination of the top 3 cm and bottom 3 cm) and surface peat (SP: 0-5 cm) at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed (Burning: burnt treatment versus Mowing: cut with brush left treatment) and unmanaged (Uncut control) areas compared to the combined near intact sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire (VB) site Butterburn. Species are grouped as: Si - idiosomic TA (with siliceous shell) and Non.Si - other TA.



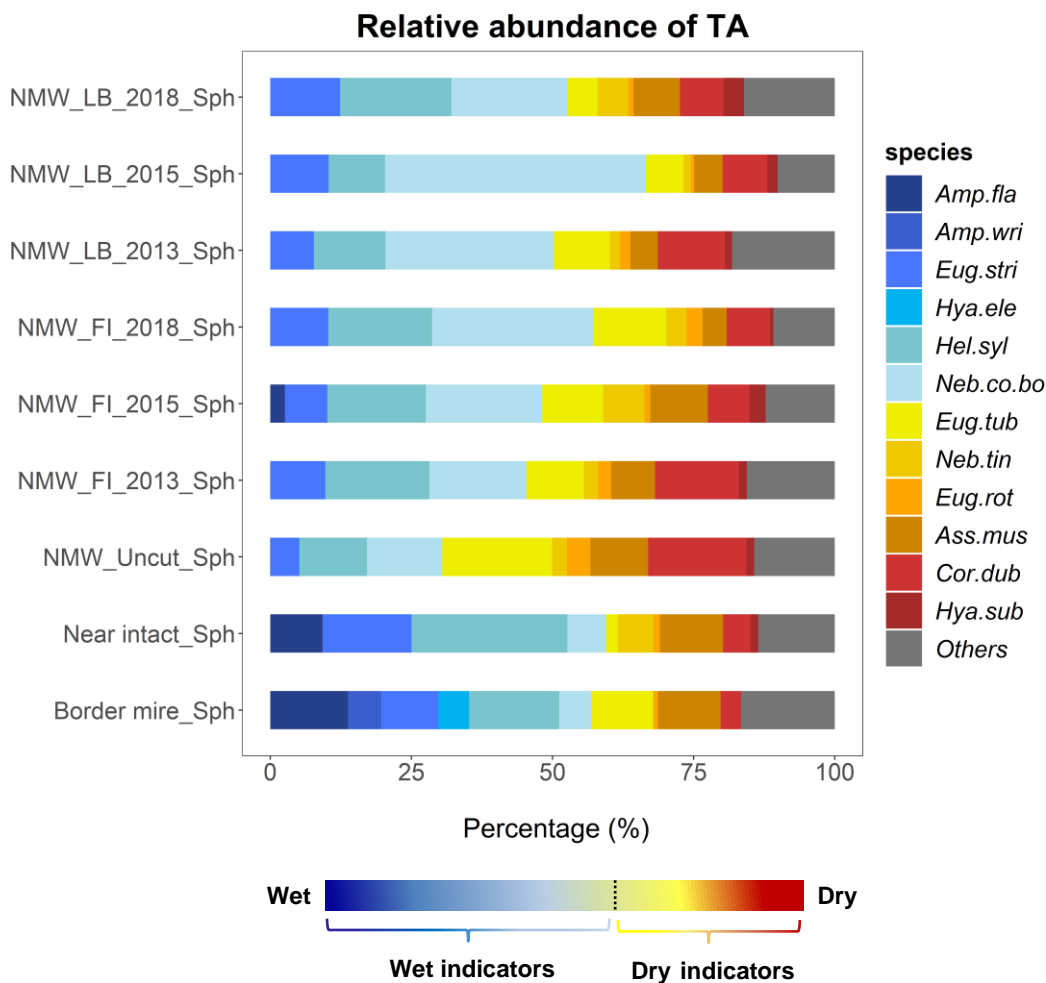


**Supplementary Fig. S4.6** Boxplots showing key environmental factors (peat moisture and *Sphagnum* cover), TA diversity (species and functional diversity) and functional traits (based on community weighted mean value (CWM)) in *Sphagnum* mosses (**Sph**: combination of top 3 cm and bottom 3 cm segments) at the managed (**FI**: burnt treatment in 2013, 2015, and 2018) and unmanaged (Uncut control) areas from the three sites (i.e., Nidd: Nidderdale, Moss: Mossdale, Whit: Whitendale).

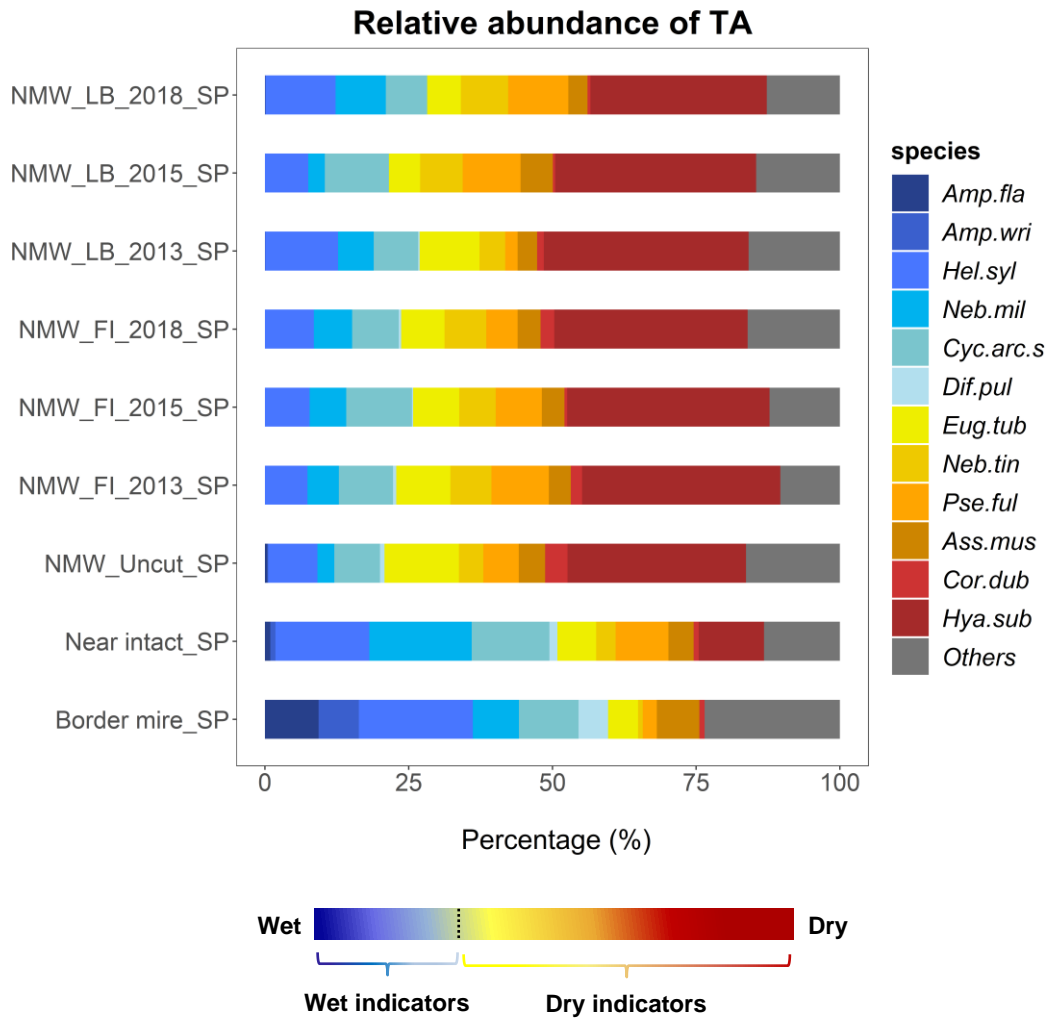
## Pearson Correlation Matrix



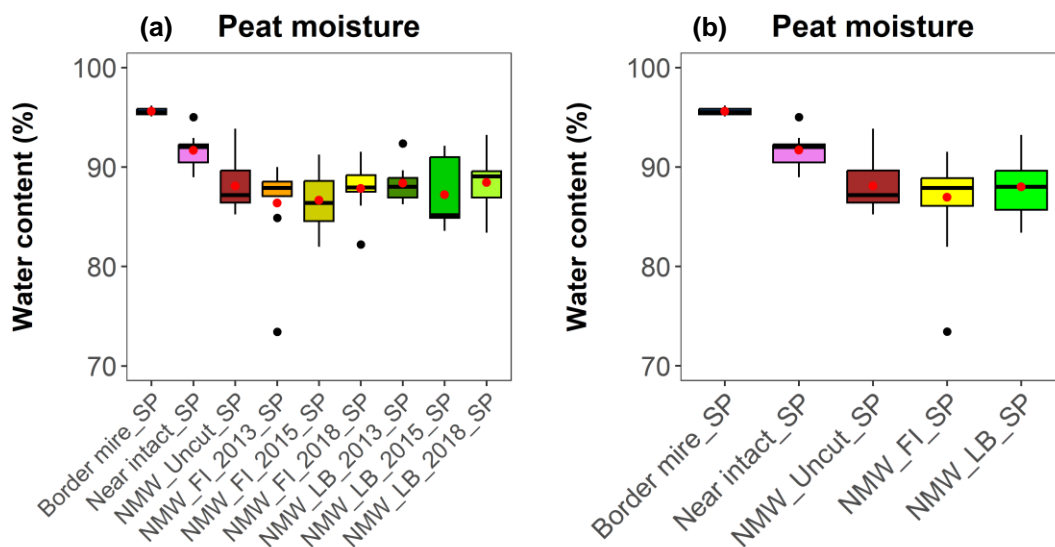
**Supplementary Fig. S4.7** Multipanel display of pairwise correlations between key environmental variables with Pearson  $r$  correlations for P: phosphorus, N: nitrogen, BD: bulk density, C: carbon, Elevation: height above sea level, Heather: percentage heather cover, Si: silicon, pH: acidity, WC: water content (peat moisture), Sphagnum: percentage of *Sphagnum* cover. Asterisks indicate the significance level (ANOVA test), \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Supplementary Fig. S4.8** Relative abundance of testate amoebae (TA) in *Sphagnum* moss (Sph: combination of the top 3 cm and bottom 3 cm) at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed (FI: burnt treatment in 2013, 2015 and 2018; LB: cut with leaving brash treatment in 2013, 2015 and 2018) and unmanaged (Uncut control) areas compared to the combined near intact sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn. Species that showed significant change and whose mean relative abundance more than 5% were selected and displayed: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Eug.rot* - *Euglypha rotunda*, *Eug.stri* - *Euglypha strigosa*, *Eug.tub* - *Euglypha tuberculata*, *Hel.syl* - *Heleopera sylvatica*, *Hya.ele* - *Hyalosphenia elegans*, *Hya.sub* - *Hyalosphenia subflava*, *Neb.co.bo* - *Nebela collaris-bohemica* type, *Neb.tin* - *Nebela tinctoria*, Others - all combined remaining TA 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.



**Supplementary Fig. S4.9** Relative abundance of testate amoebae (TA) in surface peat (SP: 0-5 cm) at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed (FI: burnt treatment versus LB: cut with brash left treatment) and unmanaged (Uncut control) areas compared to the combined near intact sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn. Species that showed significant change and whose mean relative abundance more than 5% were selected and displayed: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Cyc.arc.s* - *Cryclopixis arcelloides small type* (< 50  $\mu$ m), *Eug.tub* - *Euglypha tuberculata*, *Hel.syl* - *Heleopera sylvatica*, *Hya.sub* - *Hyalosphenia subflava*, *Neb.mil* - *Nebela militaris*, *Neb.tin* - *Nebela tincta*, *Pse.ful* - *Pseudodifflugia fulva type*, 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.



**Supplementary Fig. S4.10** Boxplots showing peat moisture at surface peat (SP: 0-5 cm) at the combined Nidderdale, Mossdale, and Whitendale sites (NMW) for managed (FI: burnt treatment in 2013: pale yellow, 2015: yellow and 2018: orange; LB: cut with brush left treatment in 2013: dark green, 2015: green and 2018: light green) and unmanaged (Uncut control: brown) areas compared to the combined near intact (purple) sites (i.e., Kielder Head, Whitelee (English & Scottish side)) and the very wet border mire site Butterburn (blue); (a) and (b) represent separated and combined management of years, respectively.

## *Sphagnum* Moss



## Surface Peat



## Deep Peat



**Supplementary Fig. S4.11.** Photos of typical *Hyalosphenia subflava* from *Sphagnum* moss (A, B, C) with clean body inclusions, surface peat: 0-5 cm (D, E, F) with some small black materials (like bacteria and/or peat detritus) inside the body and deep peat: 50-55 cm (G, H, I) with loads of black materials (like bacteria and/or peat detritus) inside the body respectively.

# Chapter 5: Impacts of peatland restoration on testate amoeba communities within upland blanket bogs

## 5.1 Abstract

Many blanket bog peatlands in the UK are classified as being in a degraded state, characterized by low biodiversity and reduced carbon sequestration and water storage, mostly due to habitat modification by historical management practices including drainage. Currently, much restoration work by ditch-blocking, other rewetting and revegetation strategies have been deployed in degraded peatland areas to improve biodiversity and key ecosystem services (related to carbon cycling and water storage). However, we still lack the fundamental understanding of how blanket bog management and restoration influences the main microbial consumers, testate amoebae (TA), and especially their functional traits. Further, we also lack knowledge if TA species, as known bioindicators, can be used as a tool to assess and monitor the long-term restoration trajectory and success. In this study, TA communities were investigated at three modified blanket bog sites, each with three habitat conditions (i.e., least modified, post-restoration, and degraded) to assess the potential of TA as hydrological bioindicators within restoration projects and explore likely key environmental factors impacting on TA communities. TA functional traits were further explored by comparing TA species composition of post-restoration (two to nine-years post restoration) and degraded locations against those of adjacent least modified areas, near intact blanket bog sites and a very wet intact border mire site in Northern England. The results showed that: 1) there is a clear change in TA community composition from degraded through post-restoration to near intact and intact peatland sites in both surface peat and living *Sphagnum* mosses, but the dominant species were considerably different between peat and moss samples; 2) soil moisture, *Sphagnum* cover, and P-content were shown to be highly related to TA community composition; 3) the taxa *Hyalosphenia subflava* and *Corythion dubium* may serve as a good dry indicators, whereas the mixotrophic taxa (i.e., *Amphitrema flavum* and *A. wrightianum*) are likely to be useful as reliable wet indicators; 4) the community-weighted mean (CWM) value of TA functional traits in surface peat including biovolume, aperture width, and test type (siliceous) were significantly higher in least modified than in degraded areas, whereas;

5) most TA functional traits in *Sphagnum* mosses did not markedly change following restoration management except for aperture type, which showed a completely opposite pattern to that in surface peat. Overall, the data support the potential of TA to be used as bioindicators to assess habitat hydrological condition and track long-term restoration progression in blanket bogs. The study also clearly demonstrates the link between biodiversity, restoration, and likely inferred ecosystem functioning (water management and carbon/silica cycling) and confirms the long-term effectiveness of rewetting strategies (e.g., ditch/gully-blocking) as tools for restoring peatland ecohydrological and related ecosystem functions.

## 5.2 Introduction

Blanket peatlands (bogs) are important, yet in good condition rare UK habitats (Wilkie and Mayhew 2003), and store large amounts of carbon (Garnett et al. 2000) and are home to many specific plants and birds (Littlewood et al. 2010). Globally, approximately 13% of blanket bogs contained in the UK upland areas (Bain et al. 2011). In order to improve the production of livestock, a number of British blanket bogs were historically drained (mainly for grazing and with 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 the heather moorland in England and Wales at risk from overgrazing and almost half of the upland breeding bird species were under threat (Sansom 1999). Sometimes, extensive regions were modified and often caused 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 around 80% of UK blanket bogs are damaged to various degrees (Cris et al. 2011), with only 58% of them in the protected sites to be classified as in ‘favourable’ condition (Bonn et al. 2016). However, while a favourable condition mainly reflects a habitat assessment based on arbitrary pass/fail criteria such as focusing on vegetation composition, it does not relate to 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),



which thus likely changed from *Sphagnum* moss-dominated towards more vascular-dominated communities (Lindsay 2010). The resultant loss of *Sphagnum* cover, drier and exposed peat with potential faster surface runoff can therefore likely increase the downstream flood risk (Holden et al. 2007a, Wilson et al. 2011b, 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<sub>2</sub> 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 2010b). Water quality is then inevitably reduced by increased stream DOC concentration, and deteriorated water quality increases the cost for processing in drinking water supplies (Holden et al. 2006). Nevertheless, drainage impacts in blanket bogs are fairly limited, especially on flat areas, mostly only around 5 cm in between ditches and only increasing very near the ditches (Holden et al. 2004, Wilson et al. 2010, Luscombe et al. 2016). Consequently, to restore the degraded upland blanket peatlands towards hydrological and ecological functioning ecosystems, a range of restoration strategies including reduced levels of grazing and ditch/gully-blocking have been increasingly developed over the past decades, in an attempt to improve biodiversity, crucial ecosystem services and their benefits to society (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 success towards intact and functioning bogs following these management strategies to validate the success or advise on further restoration needs. Among the approaches, using testate amoebae (TA) as biological indicators 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. 2014a), and have also been linked to C-cycle functions (Jassey et al. 2015, Jassey et al. 2016).

TA represent the main component of protists, which are particularly abundant in both *Sphagnum* moss and surface peat (Lamentowicz and Mitchell 2005, Liu et al. 2019). Since they 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. 2007a) and have a rapid reproduction rate (Charman 2001), TA have been proposed as ideal ecological indicators and applied 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). 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 short-term study conducted in relation to modified semi-natural blanket bogs (Swindles et al. 2016) and another long-term study related to restoration of afforested peatland (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 *Amphitrema flavum* being relatively more abundant in post-restoration sites, *vice versa* (Jauhiainen 2002, Swindles et al. 2016). Moreover, species richness and diversity have been reported to be increased but density declined over the course of recovery (Laggoun-Défarge et al. 2008). Undoubtedly, whilst this limited insight on TA composition has improved our understanding of TA in response to restoration strategies, we still lack a broader geographic range of site assessments, especially on blanket bogs, including knowledge on how key functional traits of TA, like mixotrophic TA, biovolume and aperture size change in the process of a longer timescale of restoration such as ten years.

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 impacting carbon dioxide emission 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 have indicated that mixotrophic TA (MTA) can account for 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, Jassey et al. 2016). As a result, investigating the alteration of key functional traits of TA in response to restoration management will facilitate our understanding of their potential functional contribution to ecosystem processes. 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 surface peat and *Sphagnum* mosses, the composition and recovery pattern of TA would likely vary to some extent between these two niches.

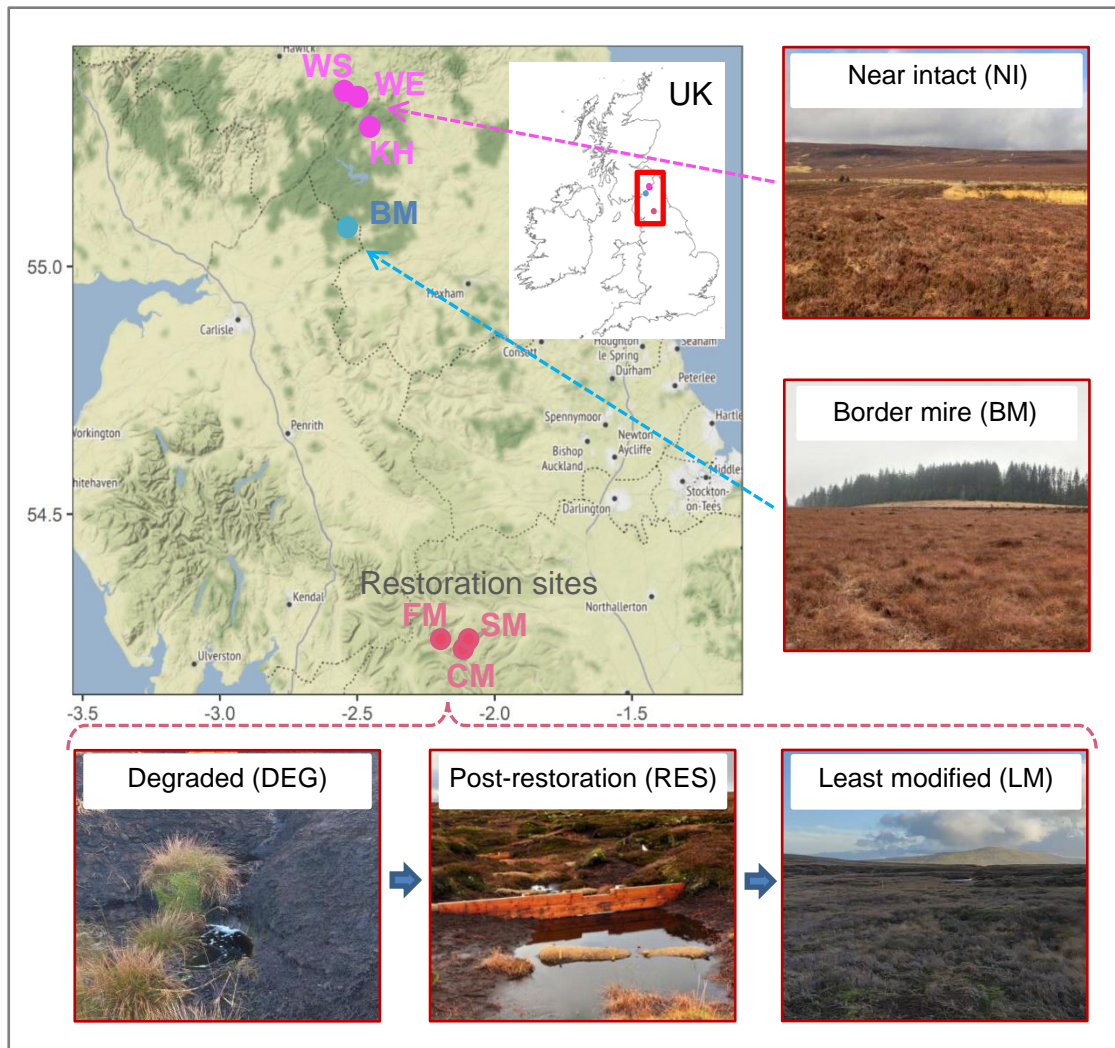
Here, TA communities were investigated at three historically managed blanket bogs (mostly a combination of sheep grazing and recent grouse moor management with high heather cover on drier peat) and compared to other sites representing near intact to intact habitat condition counterparts (with hardly any or no recent management and low heather cover on wet peat), to assess the potential of TA to be used as bioindicators to monitor the habitat status and potential recovery trajectories of the ecosystem and also explore their possible contribution to ecosystem functioning over the course of restoration. 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 least modified, near intact and very wet bog habitat status; 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 historic site hydrological conditions before management intensification. It was therefore 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 natural/intact' conditions; 2) 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; 3) TA show species-specific sensitivity to rewetting strategies, and restoration management causes functional traits of TA (e.g., mixotrophic

TA, biovolume and aperture size) to change towards assemblages resembling those in near natural/intact conditions but their recover pattern is more pronounced in *Sphagnum* mosses than in surface peat; 4) TA communities present in deep peat layers of blanket bog can serve as valuable tool to infer and compare historical hydrological conditions before intensification of peatland management.

## **5.3 Methods**

### **5.3.1 Study sites**

Samples were collected from six blanket bog sites and one very wet border mire (Butterburn, considered as an intermediate/ridge-raised bog mire complex developed over depression/valley-shape terrain (Eades et al. 2021), which is generally wetter and often has much deeper peat depth (~6 m) than typical blanket bogs. Among the six blanket bogs, half of them is near intact blanket bogs (Kielder Head, Whitelee English side and Whitelee Scottish side), and the other half are 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 or only to have been slightly 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 northern England (Fig. 5.1, Supplementary Fig. S5.1).



**Figure 5.1** Location of study sites. Restoration sites including least modified (**LM**), postrestoration (**RES**), and degraded (**DEG**) areas; Near intact sites (**NI**) including Kielder Head (**KH**), Whitelee English side (**WE**) and Whitelee Scottish side (**WS**); Intact border mire site (**BM**) consisting of Butterburn (Butt) and restoration sites included Fleet Moss (**FM**), Stake Moss (**SM**) and Cray Moss (**CM**).

Detailed information about these seven sites (based on site surveys conducted during this study - for related methods such as for peat moisture see the following sections) is described below and in Table 5.1.

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

**Butterburn border mire site (BM):** 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%) is 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 of (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 is most likely an intermediate/ridge-raised mire 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 is 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 versus 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 (UK Grid Ref NT 685056) about 524 m a.s.l. The vegetation is 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 is grazed, and the Scottish side is not.

The three restoration management sites (conditions reflect sampling location and time and information 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 the 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 caused large areas of moorland to 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 least modified and post-restoration areas are dominated by *Sphagnum* moss ranging from 25% to 95% (mean: 62%) and from 35% to 95% (mean: 75%) respectively. By contrast, other mosses are 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 and 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 is currently 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 least modified areas is dominated by *Sphagnum* moss ranging from 40% to 70% (mean: 58.3%). However, for both post-restoration and degraded areas, the dominant vegetation are 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 average peat moisture (at sampling time) being 89.0% and 88.7% respectively. By contrast, it was drier in degraded areas as mean peat moisture was about 85.7%, with 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 the 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 least modified areas is dominated by *Sphagnum* moss ranging from 60% to 95% (mean: 82.7%). In post-restoration areas, the dominant vegetation are 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 occupied the largest area, ranging from 65% to 80% (mean: 75%). Correspondingly, the peat was relatively wet in the least modified areas, with average peat moisture (at sampling time) being 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 is about 2.7 m and a mean slope of 2° across the sampling areas.

### **5.3.2 Sampling**

Samples (nine for each consisting of three replicates at each site) of the dominant *Sphagnum* mosses, 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 5.1). 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 each (i.e., three replicates at each site per management/habitat area) were collected for the dominant *Sphagnum* mosses (replaced by other mosses if there was no *Sphagnum* moss), 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) which each included the least modified, post-restoration, and degraded habitat areas (Table 5.1). Vegetation type and cover (as sum of over and understory; at least 100%) were recorded at the sampling locations in a 50 cm x 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.



**Table 5.1** Location and sample number information for the sampling sites of the six-blanket bog and one border mire sites. Vegetation cover was assessed in December 2020 within replicated (3) 50 x 50 cm squares for Cray, Fleet and Stake Moss and in February 2021 for Butterburn, Kielder Head and Whitelee as total cover (at least 100%) of: heather (H), 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	
<b>Butterburn (Butt)</b>	Border mire	167	6.7	4.3	36.7	73.3	3.7	7.7	3, 3, 3
<b>Kielder Head (KH)</b>	Blanket bog	408	1.8	51	15	27.3	52.7	8.7	3, 3, 3
<b>Whitelee - English side (WE)</b>	Blanket bog	480	2.9	28.7	19.7	77.7	13.3	3.7	3, 3, 3
<b>Whitelee - Scottish side (WS)</b>	Blanket bog	567	1.6	45.7	11.3	74.3	10	5.0	3, 3, 3
<b>Cray Moss</b>									
least modified/	Blanket bog	581	3.6	4.0	45.7	61.7	4.3	13.3	3, 3, 3
post-restoration/		571	2.2	3.0	51.7	75.0	22.7	6.3	3, 3, 3
degraded		570	1.3	2.0	44.7	1.3	83.3	8.0	3, 3, 3
<b>Fleet Moss</b>									
least modified/	Blanket bog	564	1.4	6.0	10.3	58.3	15.7	12.3	3, 3, 3
post-restoration/		552	1.8	1.0	29.3	2.0	86.3	3.0	3, 3, 3
degraded		566	3.1	4.7	10.7	0.0	67.7	15.0	3, 3, 3
<b>Stake Moss</b>									
least modified/	Blanket bog	561	2.6	5.0	23.3	82.7	7.0	7.0	3, 3, 3
post-restoration/		571	2.7	4.3	19.0	8.3	29.3	30.3	3, 3, 3
degraded		558	2.6	0.0	1.0	0.0	20.7	78.3	3, 3, 3

### 5.3.3 TA 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* or 2 cm<sup>3</sup> peat was used for preparation. The weighted sample was soaked and disaggregated in the 100 ml beaker with about 60-80 ml deionized water for at least 24 hours and stirred occasionally. At the same time, one tablet of *Lycopodium clavatum* was added for density calculation. 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 mesh  $\mu\text{m}$  was carefully washed into 50 ml centrifuge tubes and centrifuged at 4,000 rpm for five minutes. 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 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). The biovolume ( $\mu\text{m}^3$ ) was calculated based on geometric shapes using dimensions measured under the microscope (length or diameter, width, and height)

Hemisphere: Biovolume =  $\text{Pi} \cdot r^3 \cdot 2/3$

Saucer-shaped: Biovolume =  $\text{Pi}/2 \cdot r^2 \cdot h$

Cylindrical-ovoid: Biovolume =  $\text{Pi}/6 \cdot d^2 \cdot h$

Ovoid: Biovolume =  $\text{Pi}/6L \cdot w \cdot h$

Where r is the radius, Pi ( $\pi$ ) is approximately 3.14159, h the height, d the diameter, L the length, and w the width of the shell (Fournier et al. 2012).

### 5.3.4 Peat physicochemical analysis

Around 2  $\text{cm}^3$  of peat subsample from the surface layer separated in the lab were weighed first and then dried at 105°C in 10-30 ml crucibles for a minimum of 72 hours 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 ultrapure water and manually homogenized for 60 s, leaving it for 4 hours; the pH was then measured in the water suspension with a 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] 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.

### **5.3.5 Statistical analyses**

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 intact and the very wet border mire sites) were analysed 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 minimise 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. In order 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. To investigate diversity patterns, Shannon Diversity Index (SDI) (Shannon 2001) and Functional diversity (Rao quadratic entropy index) were calculated for each sample (Fournier et al. 2012). Functional traits including biovolume, body

length, aperture width, aperture type, test type 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 in ecosystem processes. Among them, mixotrophy was determined as the proportion of mixotrophic species within a community (Fournier et al. 2015). Biovolume (body size) was calculated based on geometric shapes and specific formulae (see above in TA processing). The aperture width of each species was measured under a microscope until their value reached a normal distribution. The significant associations for the selected indices of interest including Shannon diversity, functional diversity, biovolume, aperture width, aperture type, 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 these analyses and plotting of graphs were performed using R (R Core Team 2019).

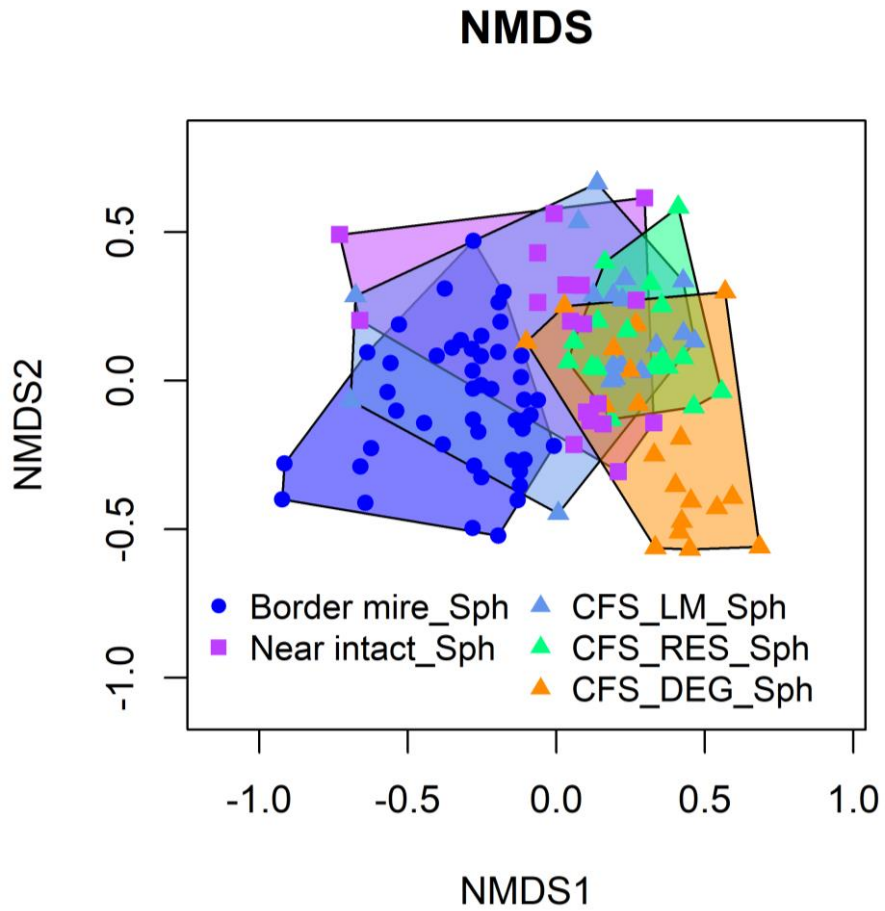
## **5.4 Results**

### **5.4.1 Testate amoeba communities in relation to habitat status and restoration in *Sphagnum* mosses**

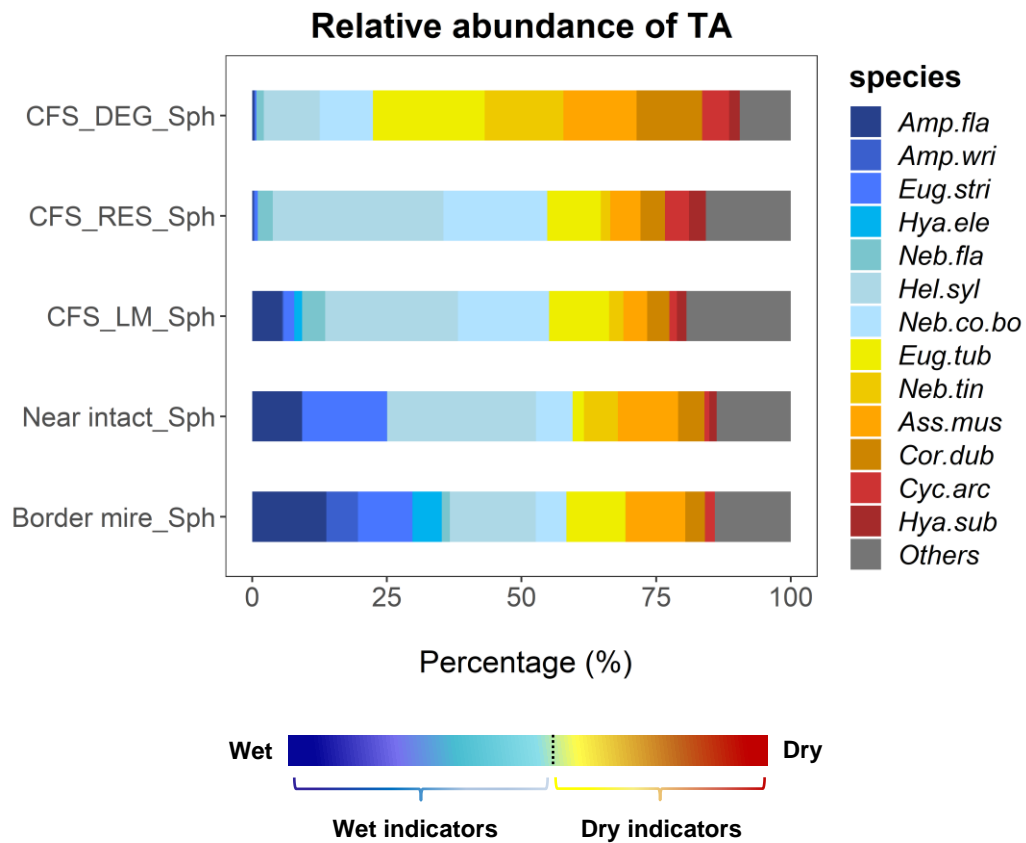
#### **5.4.1.1 Taxonomic differences**

A total of 45 testate amoeba (TA) taxa were identified from 122 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 compared to the near intact sites except for two points, which in fact came from the same sample (i.e., top and bottom 3 cm of *Sphagnum* moss at least modified areas in Stake Moss), aligning closer to the area of the near intact and the very wet border mire samples (Supplementary Fig. S5.2). The 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

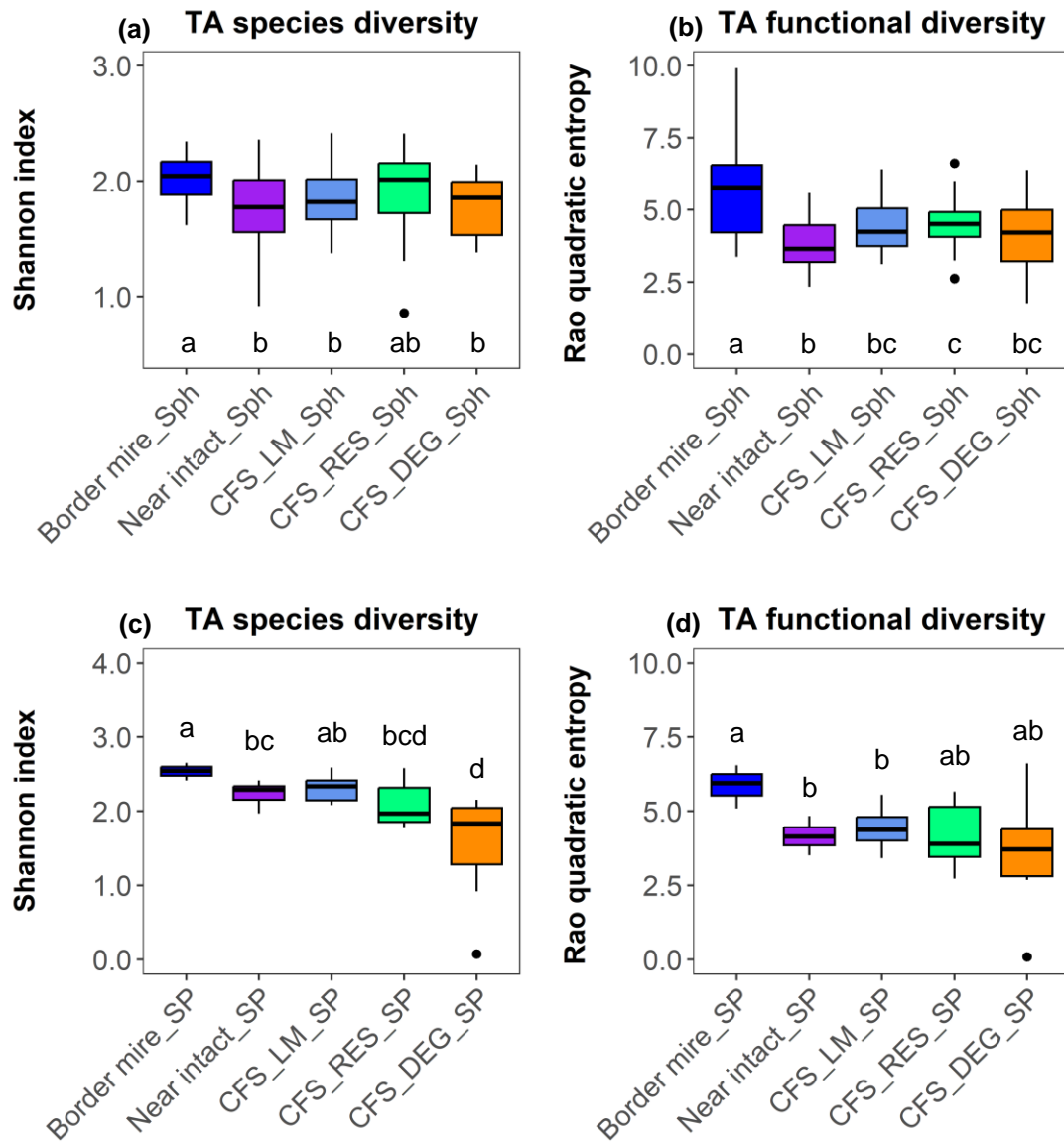
from degraded to post-restoration and to least modified areas (Fig. 5.2). Specifically, on average, the most abundant TA taxa within *Sphagnum* moss in degraded areas included *Euglypha tuberculata* (20.8%), *Nebela tincta* (14.6%), *Assulina muscorum* (13.6%) and *Corythion dubium* (12.2%); their relative abundance decreased dramatically in other habitats (Fig. 5.3). 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 areas, which were replaced by the relative increase of *Amphitrema flavum*, *A. wrightianum* and *Euglypha strigosa* (Fig. 5.3). As for TA species diversity (Shannon's index), it was only significantly higher in the very wet border mire site (i.e., likely a valley mire and not a true blanket bog) compared to samples from near intact, least modified, and degraded areas ( $H(4) = 18.954, p < 0.001$ ) (Fig. 5.4). Interestingly, no statistically significant difference was shown comparing the restoration sites' habitat conditions even though the median line in post-restoration areas seemed much higher than those in both least modified and degraded areas (Fig. 5.4).



**Figure 5.2.** NMDS analysis outputs for testate amoebae community composition in *Sphagnum* moss (**Sph**) 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.



**Figure 5.3** Relative abundance of testate amoebae (TA) in *Sphagnum* moss 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 and whose mean relative abundance more than 5% were selected and displayed: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Cry.arc* - *Cryclopixis arcelloides* type, *Eug.stri* - *Euglypha strigosa*, *Eug.tub* - *Euglypha tuberculata*, *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*, *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.

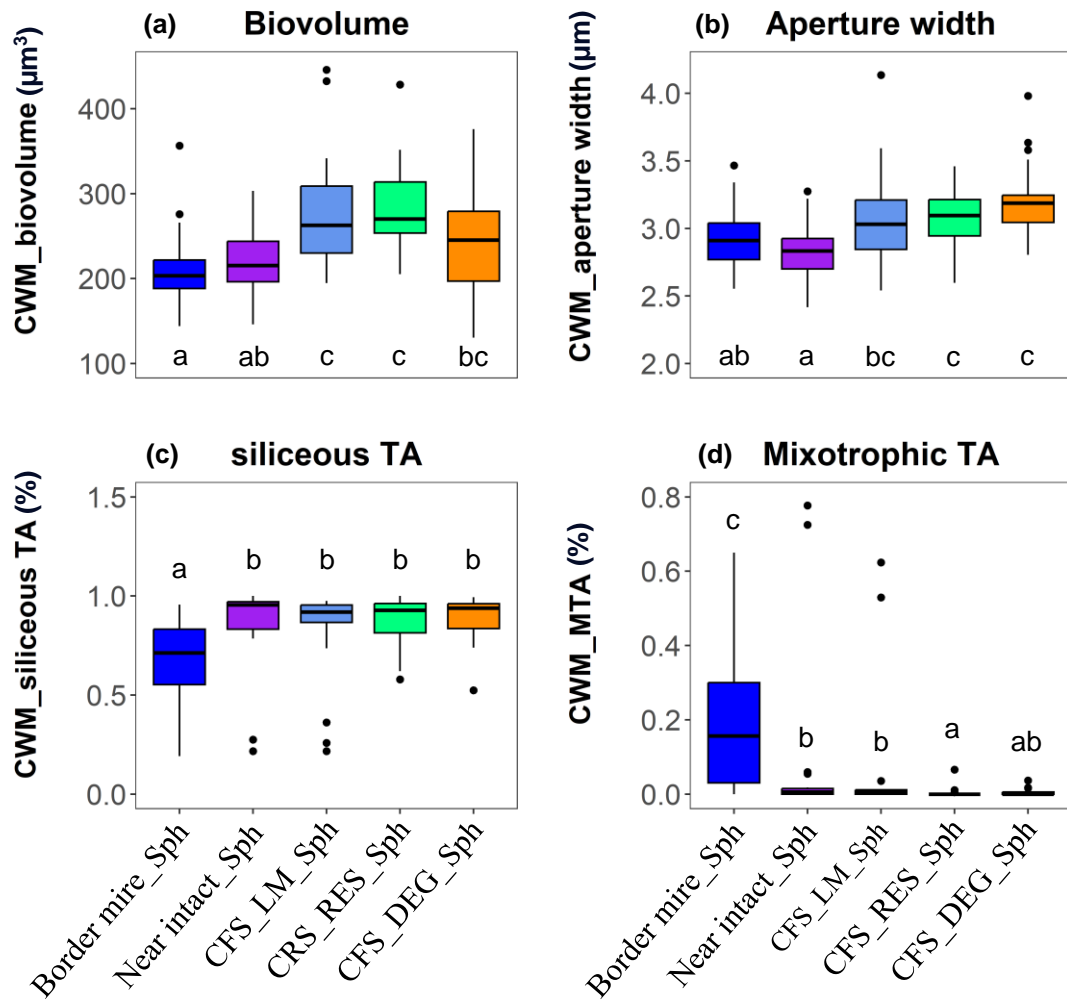


**Figure 5.4** Boxareas for testate amoebae (TA) species diversity and functional diversity at restoration sites (**CFS**: combination of Cray, Fleet, and Stake Moss; **LM**: least modified; **RES**: post-restoration (e.g., ditch/gully-blocking); **DEG**: degraded) compared to the three combined near intact sites Kielder Head, Whitelee English/Scottish side and the very wet border mire site Butterburn. (a) and (b) represent TA communities from *Sphagnum* mosses (**Sph**: combination of top 3 cm and bottom 3 cm segment), whereas (c) 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 circle points representing outliers. Different letters indicate significant difference of the medians among habitat status (Wilcoxon test,  $p < 0.05$ ).



#### 5.4.1.2 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. 5.4). For specific functional traits of TA, most of them did show separation for the very wet border mire (Butt) site but no significant difference was observed between restoration status except for body length ( $H(2) = 6.4773$ ,  $p < 0.05$ ) and pseudopod type ( $H(2) = 11.58$ ,  $p < 0.01$ ) (Fig. 5.5, Supplementary Fig. S5.3a, c). Both analyses showed an increasing trend following rewetting management, with a significant difference between post-restoration and degraded areas (body length:  $W = 248$ ,  $p < 0.05$ ; pseudopod type:  $W = 280$ ,  $p < 0.01$ ) and between least modified and degraded areas (body length:  $W = 259$ ,  $p < 0.05$ ; pseudopod type:  $W = 279$ ,  $p < 0.01$ ). However, no statistically significant difference was found between the least modified and post-restoration areas (Supplementary Fig. S5.3a, c). 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 least modified areas in Stake Moss (Fig. 5.5, Supplementary Fig. S5.4). In addition, at Stake Moss most TA functional traits (i.e., biovolume, aperture type, 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 least modified areas (Supplementary Fig. S5.4).



**Figure 5.5** Boxplots to show testate amoebae functional traits (CWM: community weighted mean value of traits) change pattern from *Sphagnum* moss 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. Box shows the median (middle line), 25% (bottom border) and 75% (top border) ranges, with circle points representing outliers. Different letters indicate significant difference of the medians among habitat status (Wilcoxon test,  $p < 0.05$ ).

## 5.4.2 Testate amoeba communities in relation to habitat status and restoration in surface and deep peat

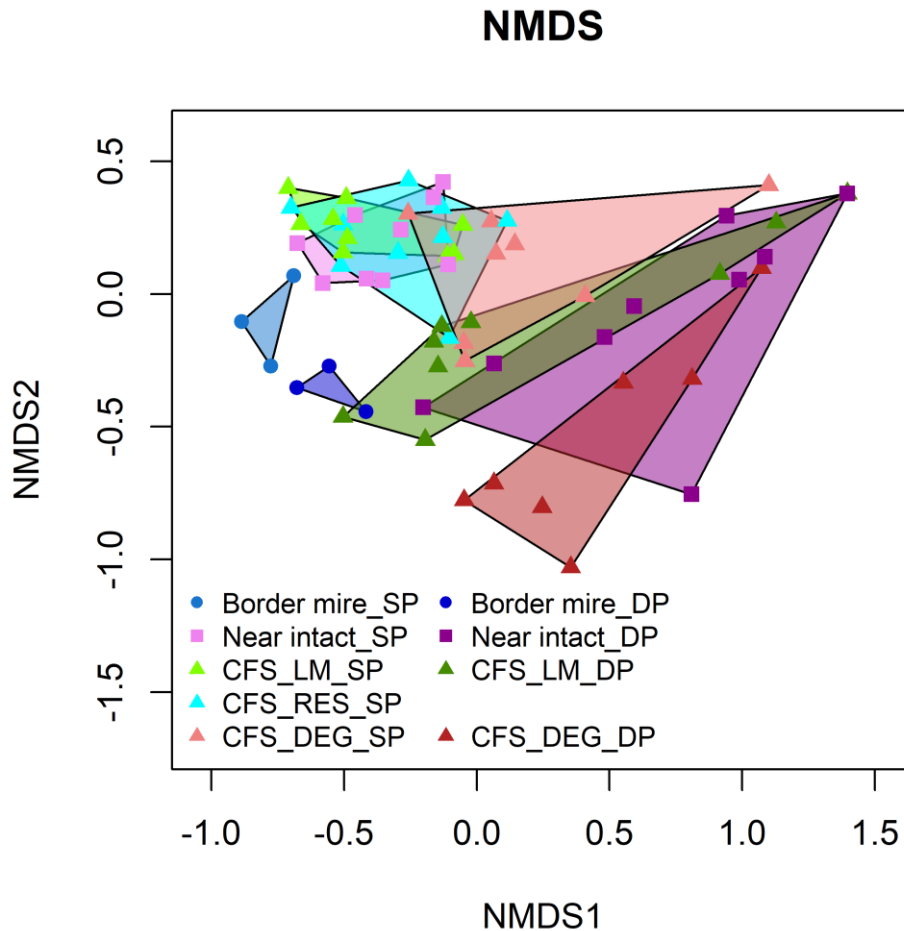
### 5.4.2.1 Taxonomic differences

A total of 47 testate amoeba taxa were identified from 67 samples collected within the seven peatland sites. NMDS showed a substantially different TA community

composition between surface peat (0 to 5 cm) and deep peat (50 to 55 cm) (Fig. 5.6). For surface peat, a distinct separation was observed between 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 least modified areas (Fig. 5.6). Among the 33 environmental variables, peat moisture (WC: water content), heather cover and Phosphorus concentration were selected to best explain the variation in TA community composition by RDA and forward selection analyses due to the strong correlations between some variables (Fig. 5.7, Supplementary Fig. S5.5). 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. 5.7). 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. 5.7, Fig. 5.8). 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, *Amphitrema flavum* (6.2%) commonly indicative of wet habitat was also found abundant there (Fig. 5.8). 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. 5.8). Meanwhile, a clear decreasing trend was observed for *Pseudodifflugia fulva* in line with progressive restoration (Fig. 5.8). By comparison, *Heleopera sylvatica* showed a consistent increasing trend from degraded (5.4%) through least modified (14.7%) and then to the very wet border mire (19.8%). While an apparent increasing trend over the course of restoration was also observed for few of other species like *Arcella discoidea*, *Euglypha strigosa* and *Nebela collaris-bohemica* type, their relative abundance was much lower than that of *Heleopera sylvatica* (Fig. 5.8).

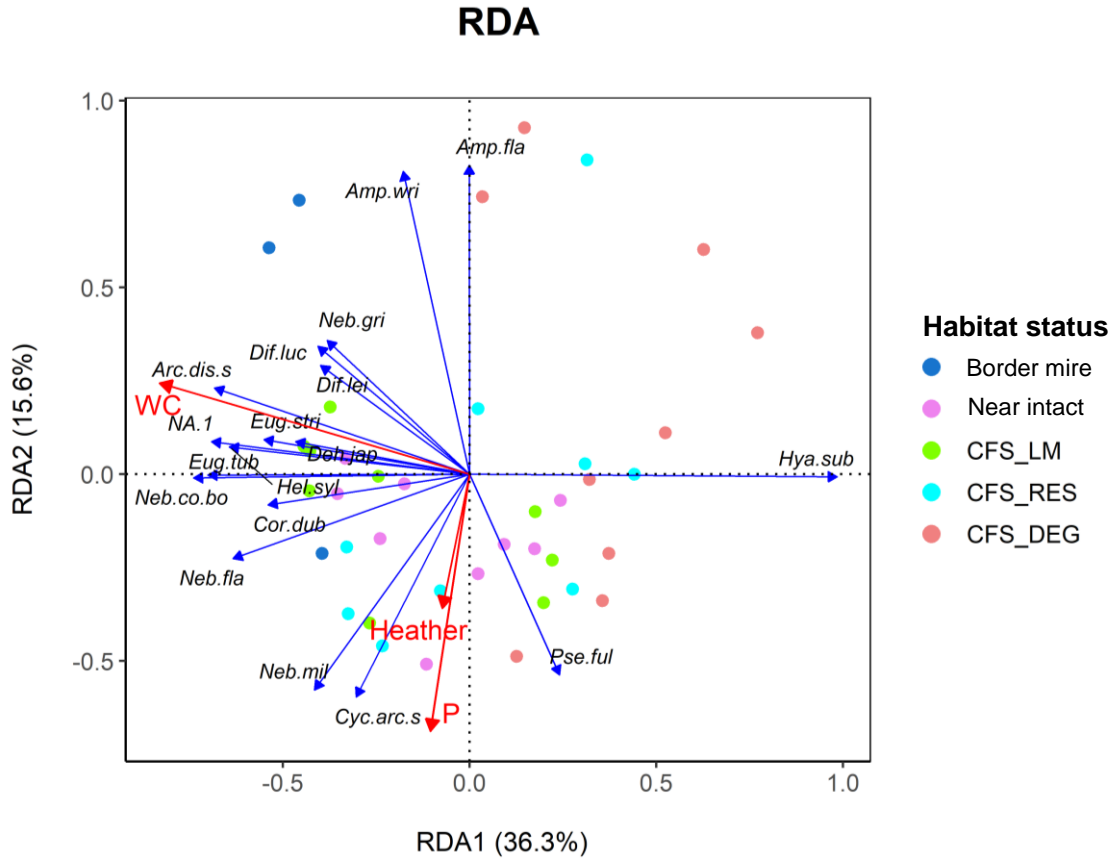
As for the deep peat samples, which should reflect and reveal peat conditions from before intensification in management (i.e., at 50 cm depth estimated about 800 years before present (McCarroll et al. 2017) and very likely outside the influence of recent water table reductions due to drainage), the most abundant taxa included *Hyalosphenia subflava* and *Amphitrema flavum* in near intact, least modified, and degraded areas (Fig.

5.8). Apart from *Amphitrema flavum*, *A. wrightianum* and *Nebela militaris* dominated the very wet border mire but no *Hyalosphenia subflava* was found (Fig. 5.8). Noticeably, the relative abundance of MTA including *Amphitrema flavum* and *A. 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

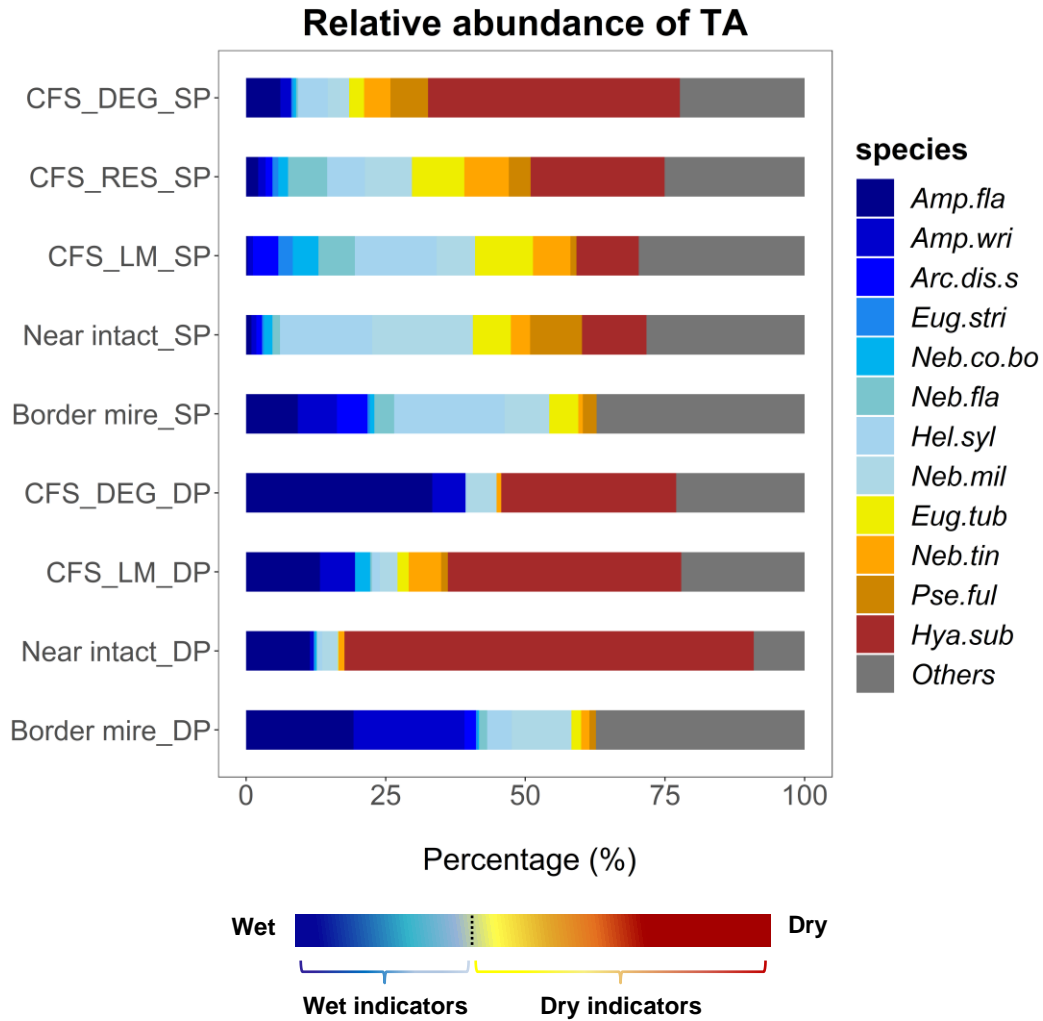


**Figure 5.6** NMDS analysis for testate amoebae (TA) community composition in the surface (SP: 0-5 cm) and deep (DP: 50-55 cm) peat across sites and habitat spectrum versus the three combined near intact sites Kielder Head, Whitelee English/Scottish side and the very wet border mire site Butterburn. CFS: combination of Cray, Fleet, and Stake Moss; LM: least modified; RES: post-restoration; DEG: degraded. Note: TA were not counted in CFS\_RES\_SD samples as they represent the situation between least modified and degraded management.

(Fig. 5.8). 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 least modified areas than in degraded areas ( $W = 78, p < 0.001$ ) (Fig. 5.4c).



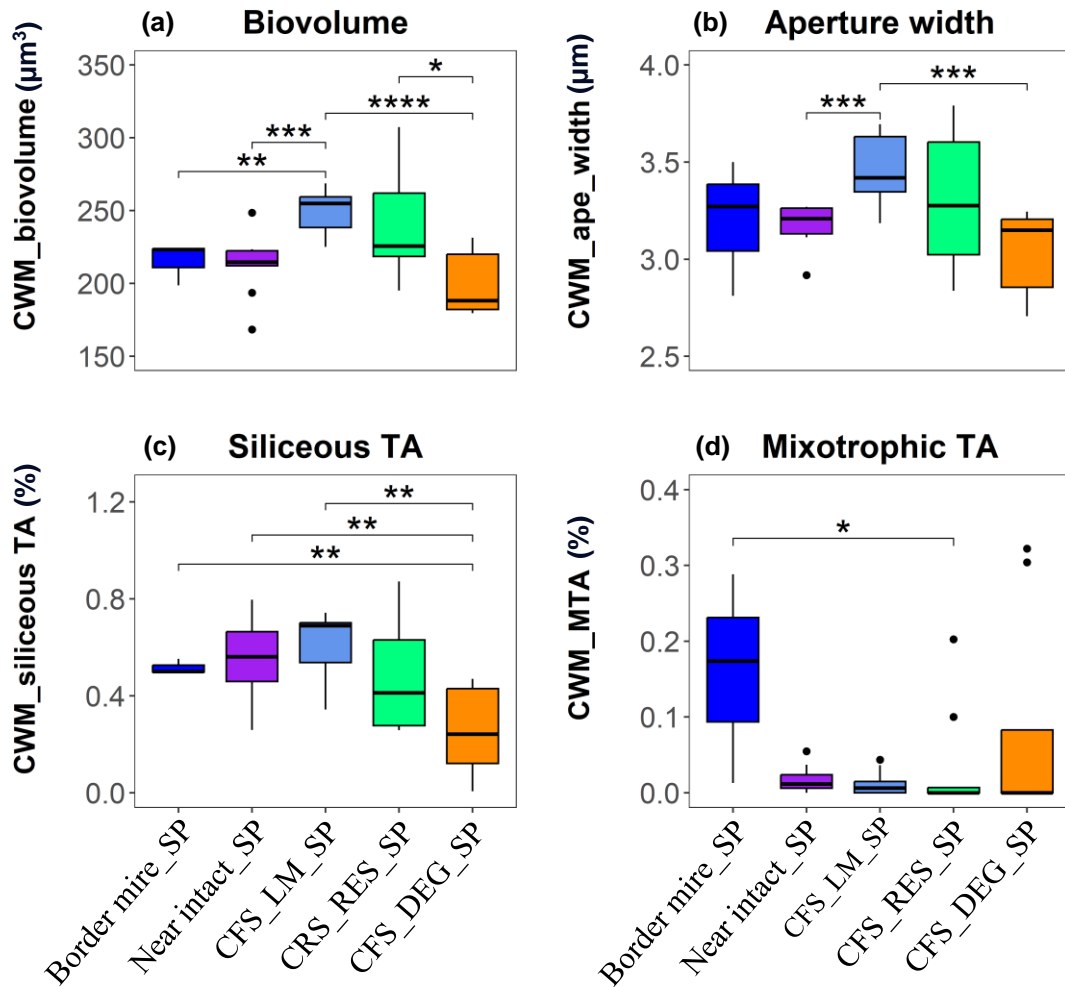
**Figure 5.7** Redundancy analysis (RDA) showing the relationship among habitat status, testate amoebae (TA) and key selected (based on forward selection) environmental variables (i.e., WC: water content, Heather: *Heather* cover, P: phosphorus). CFS: combination of Cray, Fleet, and Stake Moss; LM: least 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 to display here. Species codes: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Arc.dis.s* - *Arcella discooides* type, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Cry.arc.s* - *Cryclopoxis arcelloides* small type ( $< 50 \mu\text{m}$ ), *Dif.luc* - *Diffugia lucida*, *Eug.stri* - *Euglypha strigosa*, *Eug.tub* - *Euglypha tuberculata*, *Hel.syl* - *Heleopera sylvatica*, *Hya.sub* - *Hyalosphenia subflava*, *Neb.co.bo* - *Nebela collaris-bohemica* type, *Neb.fla* - *Nebela flabellulum*, *Neb.mil* - *Nebela militaris*, *Neb.tin* - *Nebela tinctoria*, *Pse.ful* - *Pseudodiffugia fulva* type.



**Figure 5.8** Relative abundance of testate amoebae (TA) from peat samples [**SP**: surface peat (0-5 cm), **DP**: deep peat (50-55 cm)] 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. Species that showed significant change and whose mean relative abundance more than 5% were selected and displayed: *Amp.fla* - *Amphitrema flavum*, *Amp.wri* - *Amphitrema wrightianum*, *Arc.dis.s* - *Arcella discoidea* type, *Ass.mus* - *Assulina muscorum*, *Cor.dub* - *Corythion dubium*, *Cry.arc* - *Cryclopixys arcelloides* type, *Eug.stri* - *Euglypha strigosa*, *Eug.tub* - *Euglypha tuberculata*, *Hel.syl* - *Heleopera sylvatica*, *Hya.ele* - *Hyalosphenia elegans*, *Hya.sub* - *Hyalosphenia subflava*, *Neb.co.bo* - *Nebela collaris-bohemica* type, *Neb.fla* - *Nebela flabellulum*, *Neb.mil* - *Nebela militaris*, *Neb.tin* - *Nebela tinctoria*, *Pse.ful* - *Pseudodiffugia fulva* type, 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.

#### 5.4.2.2 Functional traits

No significant difference was found in TA functional diversity over the course of restoration although there was an increasing trend from degraded to least modified areas (Fig. 5.4d). However, TA functional trait diversity was shown to be significantly higher in the very wet border mire than that in near intact areas ( $W = 27, p < 0.01$ ) and least modified areas ( $W = 26, p < 0.05$ ) (Fig. 5.4d). 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. 5.9). 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. 5.9a, b, c). For pseudopod type, the proportion of TA with lobose pseudopods showed a decreasing trend over the course of restoration and its value was significantly lower in least modified areas than in the degraded areas ( $W = 13, p < 0.05$ ) (Supplementary Fig. S5.2d). Similarly, mixotrophic TA was particularly abundant in the very wet border mire although a few points were abnormally high in degraded areas (Fig. 5.9d). It is worth noting that an obvious similarity of most TA functional traits (i.e., biovolume, aperture width, aperture type, 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 least modified areas in both Fleet and Cray Moss (Supplementary Fig. S5.6).



**Figure 5.9** Boxplots to show testate amoebae (TA) functional traits (CWM: community weighted mean value of traits) change pattern from surface peat (SP: 0-5 cm) 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 with the three combined near intact sites Kielder Head, Whitelee English/Scottish side and the very wet border mire site Butterburn. Box shows the median (middle line), 25% (bottom border) and 75% (top border) ranges, with circle points representing outliers. Asterisks indicate the significance level (Kruskal-Wallis test), \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

## 5.5 Discussion

Both *Sphagnum* moss and surface peat data suggest a clear recovery trajectory of TA community composition following restoration management based on NMDS ordination analysis (Fig. 5.2, Fig. 5.6). This is in line with the first hypothesis that: *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, with both peat moisture and *Sphagnum* moss cover having improved considerably over the course of restoration. The resultant more diverse microhabitats were likely to be conducive to a broader colonisation of certain species such as *Amphitrema flavum* whose relative abundance increased together with wetness. The second hypothesis was therefore 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 wetness (which is also reflected in very high peat accumulation as seen in the vastly deeper mire site peat depth – unlike in a typical blanket bog). Previous studies have shown that a big difference of TA community may be observed between microhabitats even within small areas (20 x 20 cm quadrat), which suggests a high relevance of TA colonisation and development to the ecological characteristics of the exact spot where they live (Mitchell et al. 2000). This is probably the reason why TA communities in two wet samples (*Sphagnum* mosses with high moisture) from the near intact (WE) and least modified areas (Stake Moss) were particularly similar to the very wet border mire site (Supplementary Fig. S5.2).

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. 5.4). 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 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 the TA communities counted in this study included large number of empty (dead) shells which 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.

Previous studies have shown that TA can 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). This was also demonstrated by the present study, with taxa usually indicative of dry habitat such as *Hyalosphenia subflava*, *Nebela tinctoria*, *Assullina muscorum*, *Corythion dubium* being particularly abundant in degraded areas whereas species often served as wet indicators like *Amphitrema flavum*, *A. 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. On the one hand, 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 blanket bogs compared to raised bogs (Howson et al. 2021b), 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 tinctoria*, *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 in degraded areas. By contrast, the corresponding dry indicators of *Pseudodiffugia fulva* (with agglutinated test) and *Hyalosphenia subflava* (with chitinous shell) 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 the siliceous tests in *Sphagnum* moss was probably similar to that in surface peat, the higher temperature and more sufficient oxygen content around *Sphagnum* moss may promote the metabolism of the formation of siliceous plates. Besides, the spacious living space (water film) in *Sphagnum* moss was likely to be the reason for the colonisation of larger dry indicators like *Nebela tinctoria*. 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 in relation to TA functional traits revealed that TA 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., *Amphitrema 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 relative abundance of MTA (i.e., *Amphitrema flavum* and *A. wrightianum*) just occupied near 20% of total TA abundance. Neither was any *Heleopera sphagni* observed, and only a few numbers of *Hyalosphenia papilio* were found in three samples. This was more likely to be caused by the limited moisture content of *Sphagnum* moss rather than the lack of light, which has been observed as an indispensable condition for MTA survival (Schönborn 1965) as light was assumed sufficient for MTA colonisation in any of the sampling sites. While it is often difficult to distinguish the relative importance between moisture and light in controlling MTA establishment and development, this study suggests that water content is likely to be more important than light for MTA colonisation when light is enough as an increasing trend of MTA was shown over the course of restoration compared to near intact and the very wet border mire sites in *Sphagnum* moss. However,

interestingly, abundant MTA was found from one post-restoration plot and two degraded areas in surface peat (Fig. 5.9). 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, which experienced more wet historical conditions rather than reflecting the current surface peat conditions.

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, Jasey et al. 2016, Koenig et al. 2018b). Previous studies indicated that small species (e.g., *Assullina muscorum*, *Corythion dubium*, *Trinema lineare*) are easier to colonise a new habitat following disturbance resulting in drought than large species (Krashevskaya 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, especially in least modified areas (Fig. 5.9). However, this change pattern was not found in *Sphagnum* moss. No statistically significant increase was shown in least modified areas although the biovolume was lower in degraded areas. More interestingly, the aperture size in degraded areas was even a bit higher than that in post-restoration and least modified areas (Fig. 5.5). This was probably related to the sub-fossil taxa counted in surface peat (0-5 cm), abnormally abundant small mixotrophic TA (*Amphitrema flavum*) on *Sphagnum* moss in one least modified plot and relatively higher amount of *Nebela tinctoria* (with large aperture size) in degraded areas from Stake Moss. As most of TA can preserve well after death under favourable (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 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 amounts of smaller MTA (with small aperture size) of *Sphagnum* moss in one least modified plot from Stake Moss, the average aperture size declined dramatically in the least modified areas. This was probably also the reason for the relatively lower value 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 tinctoria* with large aperture size in degraded areas markedly increased the overall aperture size.

Previous studies have suggested that the ratio of TA with lobose to filose pseudopods can be a good index to assess the maturity of an ecosystem, with higher ratio values usually indicating a more mature and stable environment, while lower values often representing a more dynamic and disturbed status (Bonnet 1964, Arrieira et al. 2015). This ratio index application was only supported by *Sphagnum* samples, where the proportion of TA with lobose pseudopods increased progressively over the course of restoration (Supplementary Fig. S5.3c). However, the corresponding change pattern was completely opposite in surface peat in each managed restoration site (Supplementary Figs. S5.3d, 5.6), thus, it was unlikely to be an anomaly. This may indicate a different response mechanism of TA in pseudopods following restoration strategies between *Sphagnum* moss and surface peat. It was also possible the result of disproportional decomposition rate of Euglyphida (have filose pseudopods) and Arcellinida (have lobose pseudopods) as Euglyphida were primarily constructed by siliceous plates while Arcellinida were mainly built by foreign agglutinated or secreted proteinaceous and chitinous materials. The relatively easier decomposition characteristics of siliceous plates under unfavourable conditions like drought in degraded areas, followed by post-restoration areas may be the reason why the proportion of TA with siliceous tests in surface peat showed an increasing trend over the course of restoration whereas there was no observable difference in *Sphagnum* moss. 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 causes functional traits of TA such as mixotrophic TA, biovolume and aperture size 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 findings with combined data demonstrated the effectiveness of restoration strategies over fairly short time scales (between 2-9 years), one might also be interested in how fast the ecosystem could recover to the 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 bacillarum*) to blanket bog restoration by investigating TA communities 63, 234 and 771 days respectively after ditch blocking and reprofiling (Swindles et al. 2016). The other study investigating the recovery of TA following peatland forest-to-bog restoration (Creevy et al. 2018) suggested that TA communities in post-restoration sites seventeen years after restoration management are still more similar to forested counterparts than to the open bog areas. This was partly similar to our results as TA communities responded markedly, yet in a complex way, over two years post restoration, but community composition and functional traits were still similar to that 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 were more similar between post-restoration and least modified areas for *Sphagnum* moss in many aspects eight years after restoration (Supplementary Figs. S5.4, 5.6). It was similar to the results from microbial investigation following restoration in raised bog, which reported that a timescale of more than ten years is needed for a full recovery or to become similar to that in near intact conditions (Andersen et al. 2013a, Andersen et al. 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 of degraded blanket bog following drainage and peat erosion is needed for TA to fully recover taxonomically and functionally.

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. Noticeably, a large proportion of MTA (i.e., *Amphitrema flavum* and *A. wrightianum*) was found in deep peat with a depth of 50 to 55 cm. This does not mean they can survive in such a niche without light and enough oxygen, but they can be well preserved even in unfavourable conditions due to their chitinous shell materials. While the deep peat samples proved not good enough to serve as a pre-management intensification reference, to investigate

the effects of current management on TA communities due to the disproportional preservation of TA in different habitat status, the apparently highly selective abundance of MTA in deep peat samples for managed sites at least reflected the relatively wet historical conditions. Thus, we have to 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*. In addition, the large amount of *Hyalosphenia subflava*, some of which were alive when sampling, in the deep peat and degraded surface peat may indicate that they are able to directly involve the decomposition process of organic matter as peat detritus were observed in the body of many individuals, especially in the deep peat samples. However, it is also plausible that they consume associated microbes (e.g., bacteria) while feeding on humus particles.

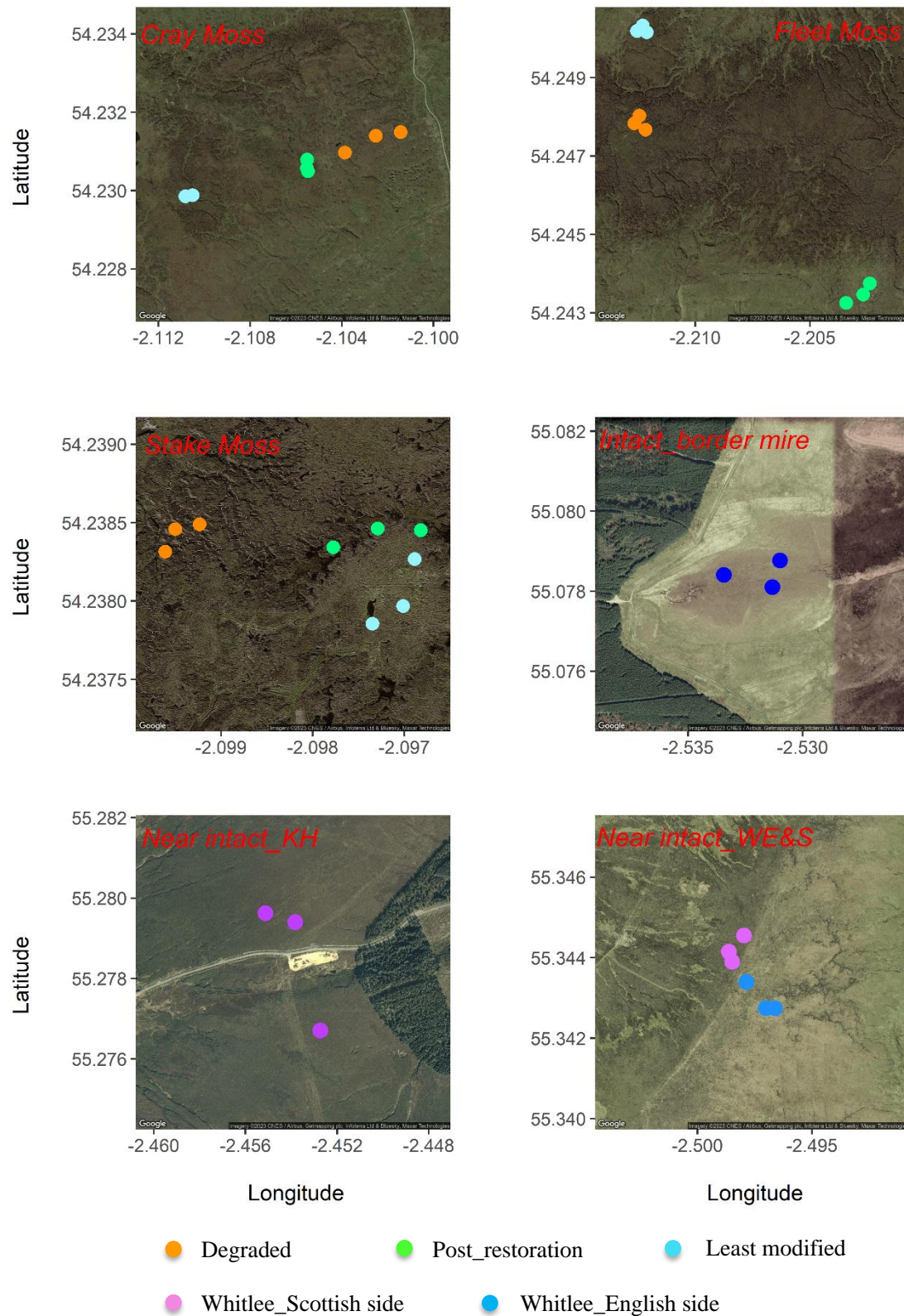
The findings overall suggest that application of TA as hydrological bioindicators is effective and promising in informing habitat restoration progression. However, it is important to note that the inferred hydrological conditions can only provide limited information about the overall health status of 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 least modified areas, we still lack a clear and quantitative understanding on how much the restoration work has achieved and how long it needs for achieving fully recovered bog functions. Regarding 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, particularly in their direct way. Most of the 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 five-centimeter peat layer, future studies should aim to sample the top one or two centimeters of peat for a more precise and accurate assessment of the impacts of peatland restoration work (ditch/gully blocking) on TA communities.

## 5.6 Conclusions

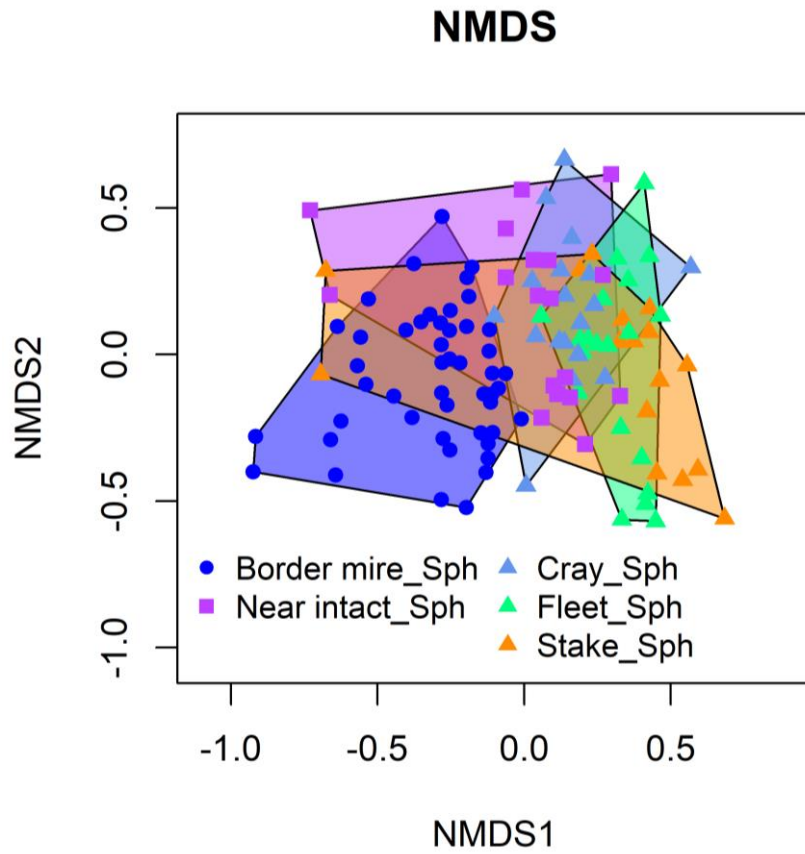
This study has shown a distinct separation of TA communities between the very wet border mire and modified blanket bogs and that restoration management has shifted the TA community composition, taxonomic and functional diversity towards that similar to near intact conditions. However, a timescale of 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. The significant response of some species, such as those indicative of dry habitats (e.g., *Hyalosphenia subflava*, *Assullina muscorum*, *Corythion dubium*) and those indicative of wet habitats (e.g., *Amphitrema flavum* and *A. 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 in drier environment. 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 biodiversity, restoration, and ecosystem service provision (water/carbon) and the effectiveness of restoration work.



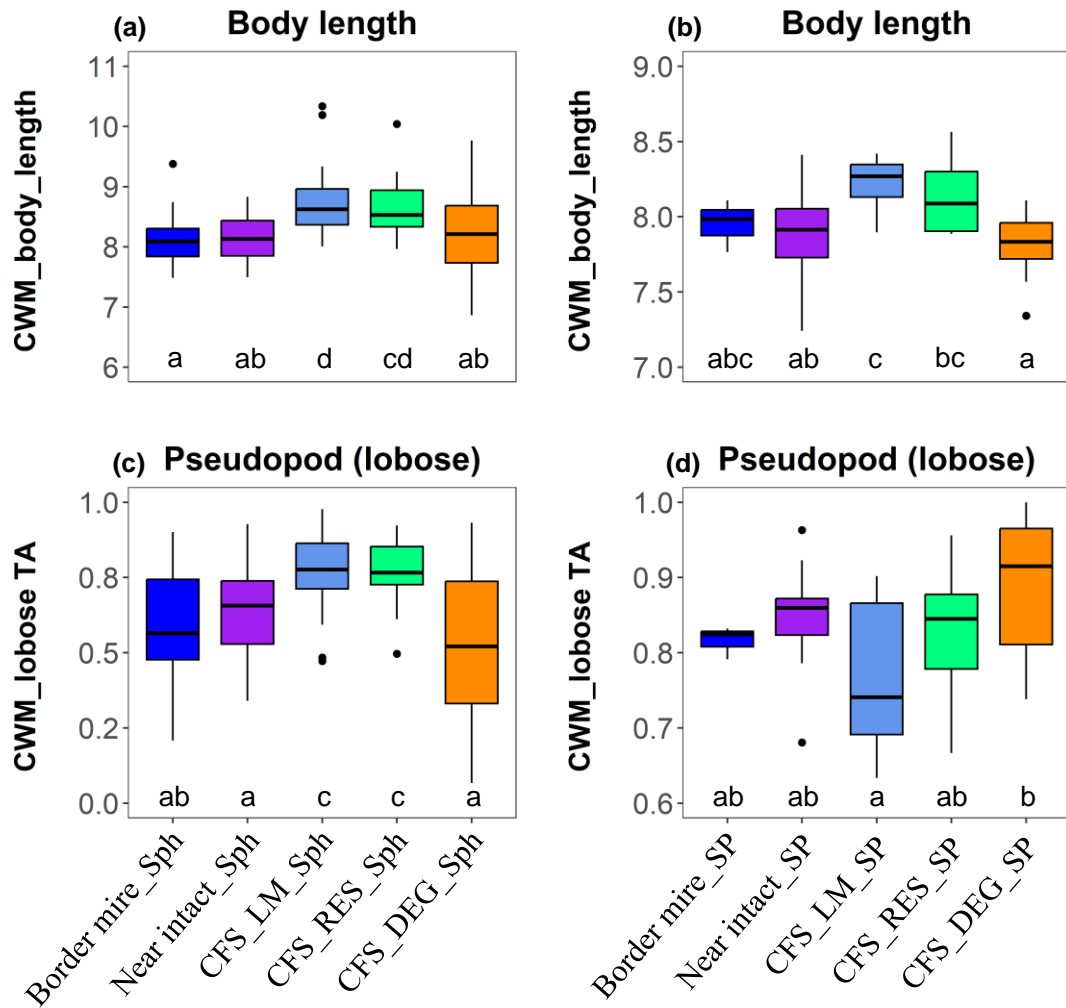
## 5.7 Supplementary materials



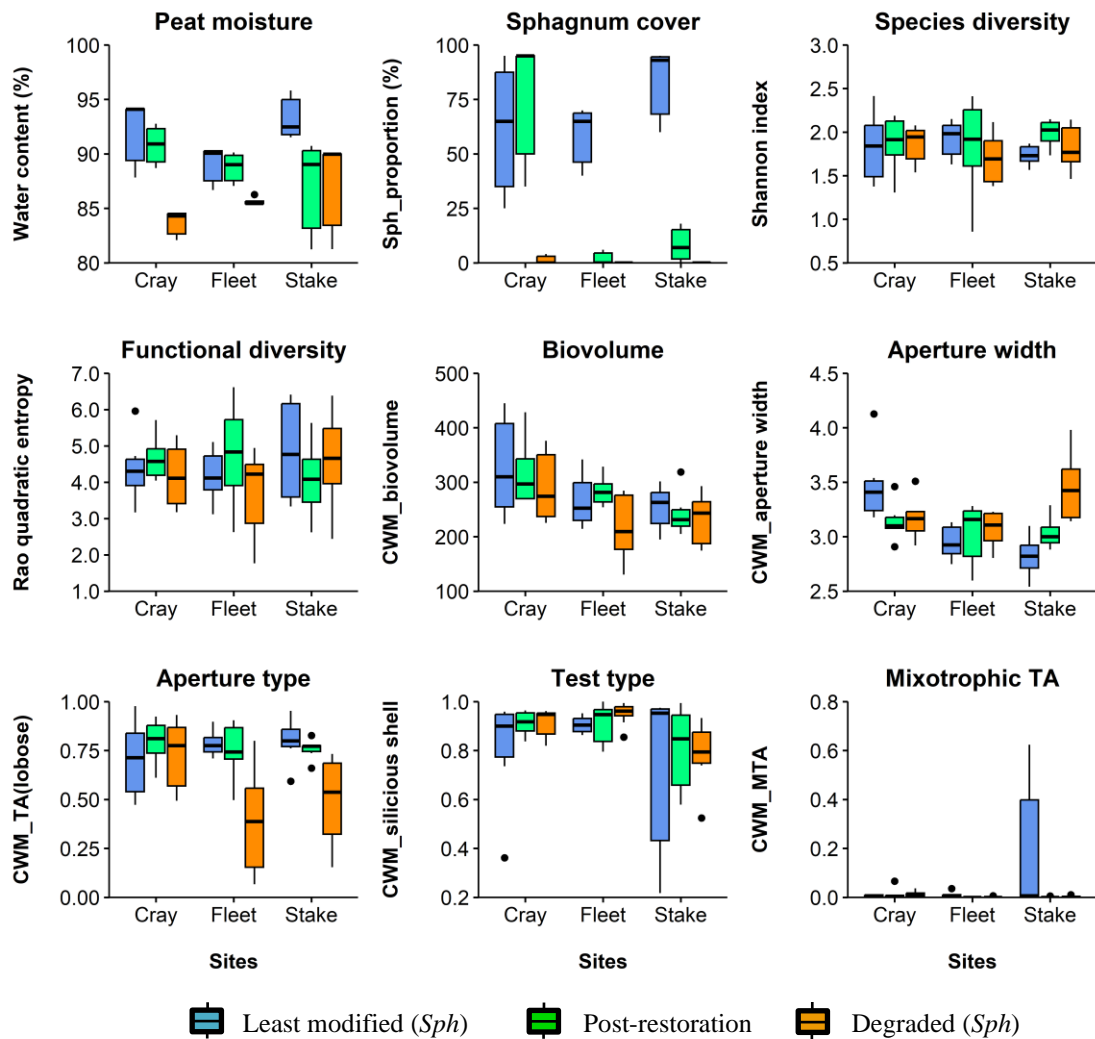
**Supplementary Fig. S5.1** Sampling location of each plot/area among sites. Reference sites: Intact border mire (Butterburn), and near intact sites (KH: Kielder Head and WE&WS: Whitelee English/Scottish side). Restoration sites: Cray Moss, Fleet Moss and Stake Moss (composed of degraded, post\_restoration and least modified areas) in the UK.



**Supplementary Fig. S5.2** NMDS analysis outputs for testate amoebae community composition in *Sphagnum* moss (Sph) across the restoration sites (Cray Moss, Fleet Moss, and Stake Moss) versus the three combined near intact sites Kielder Head, Whitelee English/Scottish side and the very wet border mire site Butterburn.

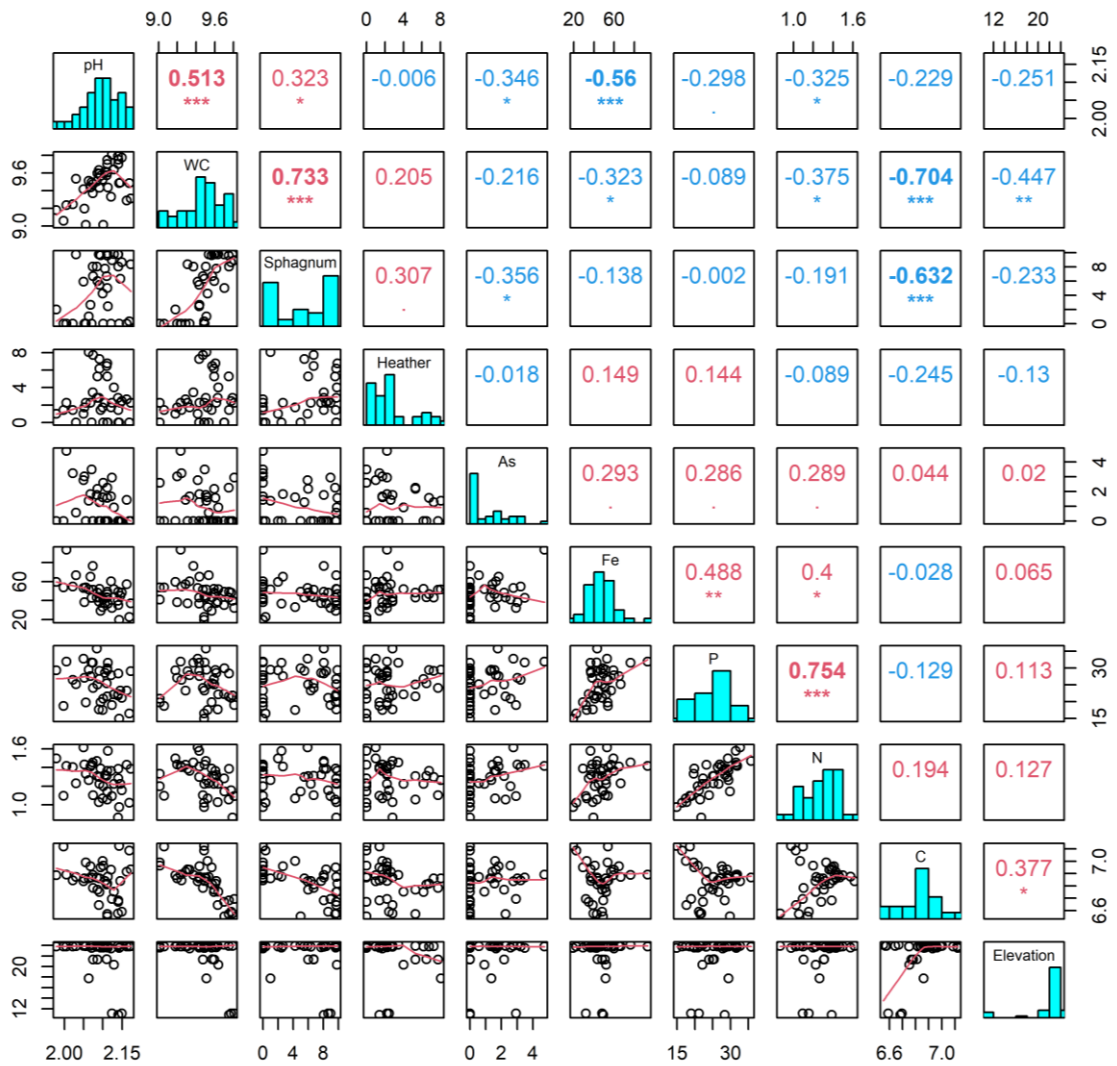


**Supplementary Fig S5.3** Boxplots to show testate amoebae functional traits (body length and pseudopod type; CWM: community weighted mean value of traits) change pattern from *Sphagnum* moss (**Sph**: a and c) and surface peat (**SP**: 0-5 cm; b and d) at restoration sites (**CFS**: combination of 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. Box shows the median (middle line), 25% (bottom border) and 75% (top border) ranges, with circle points representing outliers. Different letters indicate significant difference of the medians among habitat status (Wilcoxon test,  $P < 0.05$ ).

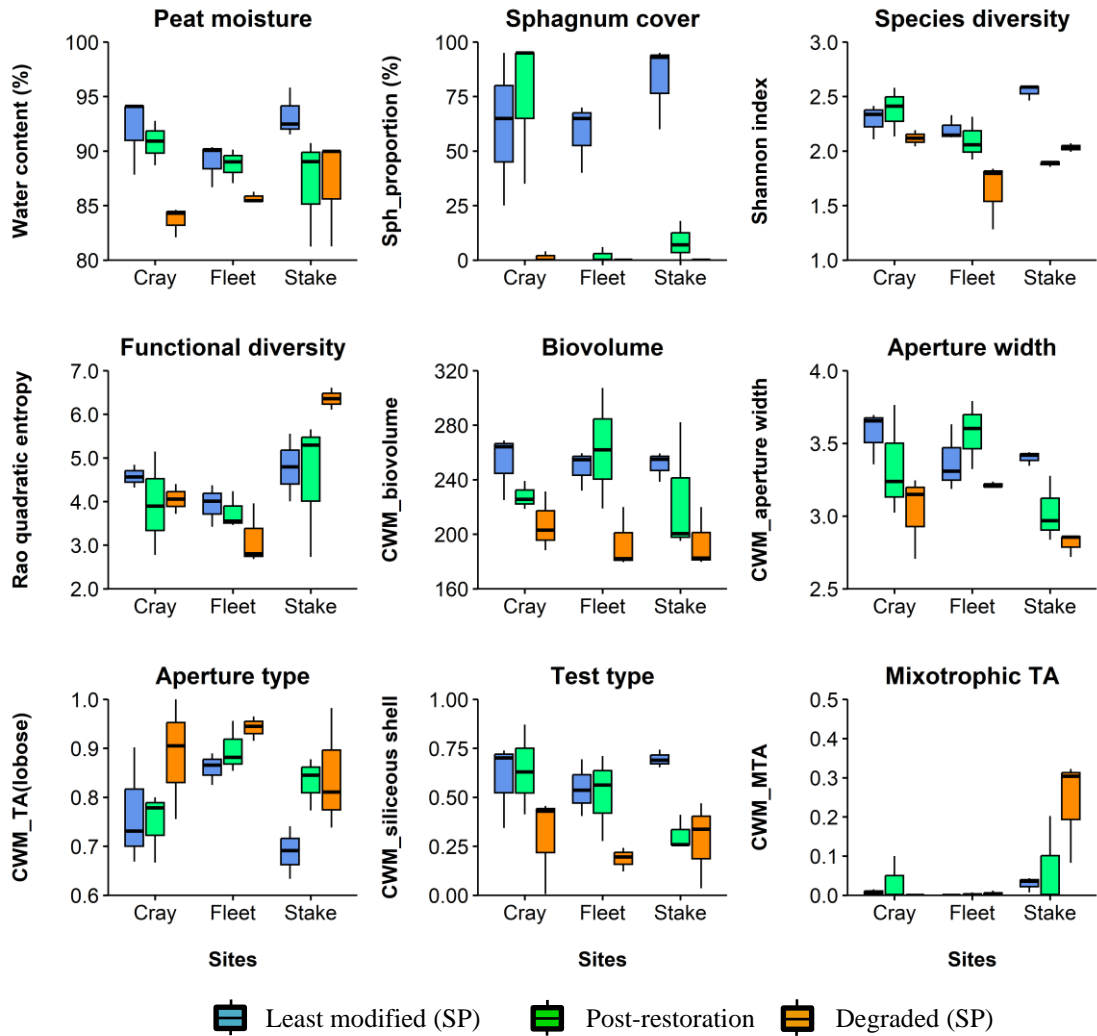


**Supplementary Fig. S5.4** Boxplots to show sample area key environmental factors (peat moisture and *Sphagnum* cover), testate amoebae diversity and functional traits (CWM: community weighted mean value of traits) in *Sphagnum* mosses (Sph: combination of top 3 cm and bottom 3 cm segment) from the three restoration (e.g., ditch/gully-blocking) sites with year of restoration management: **Cray**: Cray Moss (2012/2013); **Fleet**: Fleet Moss (2014/2015); **Stake**: Stake Moss (2018/2019) and per habitat status (least modified; restored; degraded).

## Pearson Correlation Matrix



**Supplementary Fig. S5.5** Multipanel display of pairwise relationships between key environmental variables with Pearson  $r$  correlations. pH: pondus hydrogenii, WC: water content (peat moisture), Sphagnum: *Sphagnum* cover, Heather: *Heather* cover, As: arsenic, Fe: iron, P: phosphorus, N: nitrogen, C: carbon; Elevation: height above sea level.



**Supplementary Fig. S5.6** Boxplots to show sample area key environmental factors (peat moisture and *Sphagnum* cover), TA diversity and functional traits (CWM: community weighted mean value of traits) in surface peat (SP: 0-5 cm) from the three restoration (e.g., ditch/gully-blocking) sites with year of restoration management: **Cray**: Cray Moss (2012/2013); **Fleet**: Fleet Moss (2014/2015); **Stake**: Stake Moss (2018/2019) and per habitat status (least modified; post-restoration; degraded).

## **Chapter 6: General discussion and conclusions**

### **6.1 Linkages between main chapters**

The main chapters of this study are from chapters 2 to 5. All these chapters closely work together in response to the principal aim of this thesis, i.e., assess the potential of testate amoebae to be used as hydrological bioindicators to monitor the recovery trajectories and/or success of restoring degraded peatlands following heather management and hydrological interventions. To achieve this aim, the first and also most critical questions that need to be answered are: what testate amoebae (TA) samples are best to be collected in indicating the site conditions (mainly hydrology)? and when is it optimal for the sampling? Definitely, the quality of the sample may also directly determine the validity and accuracy of the research results. Based on this, chapters 2 and 3 were designed to mainly address these two key questions respectively. Following these two chapters, chapters 4 and 5 separately focused on investigating testate amoeba communities in response to heather management (prescribed burning versus alternative mowing compared to an unmanaged heather control) and hydrological restoration interventions (ditch/gully blocking) in a bid to achieve the principal aim.

### **6.2 Core findings and their implications**

#### **6.2.1 Optimal sampling strategies of testate amoebae as hydrological bioindicators in blanket bogs**

Chapter 2 investigated TA communities among different plant functional types (*Sphagnum* moss, other mosses versus litter of heather and sedge) at two blanket bogs and one very wet valley bog across a strong hydrological gradient. TA communities from *Sphagnum* mosses were shown to be superior to other moss or plant litter to indicate the wetness gradient among the studied sites. This is probably highly related to the tissue structure of different vegetation/litter and their mechanism to absorb and hold water. Specifically, vascular plant litter from heather and sedge was structurally not intact (dead) and thus could not control water uptake or loss. In contrast, moss samples (including *Sphagnum* moss and other mosses) were obtained from living/intact plant parts. Although they lack roots and leaf stomata for water control, they possessed



intact leaf structures and some water storage mechanisms (Rydin et al. 2013). Importantly, compared to other mosses, *Sphagnum* mosses have large hyaline cells within which they can hold 15 to 20 times as much water as their dry weight, whereas other mosses can only store limited quantities of water, mainly within the small *chlorophyllous* cells (McCarter and Price 2014). *Sphagnum* mosses are thus more sensitive to changes in hydrology, which are, in turn, reflected in their TA community composition.

While different types and replicates of *Sphagnum* moss species have the potential to show distinctive responses to hydrological changes (Bengtsson et al. 2021), no significant differences were found between TA communities from different combinations of *Sphagnum* moss species (i.e., comparing all *Sphagnum* mosses to monospecific species: *S. capillifolium* or *Sphagnum* mosses without rare species). This could likely be attributed, in part, to the geographic features of the sampled regions, where samples were collected in comparatively flat and homogeneous plots (as compared to more diverse hummock and hollow positions). The relatively large number of samples being analyzed (more than 20 in each site) is likely another reason for a reduced impact of rare species. However, the rarest *Sphagnum* species (*S. palustre*) itself was shown to be inferior in indicating habitat wetness. Despite the good performance of the monospecific *Sphagnum* species (*S. capillifolium*), finding the same species of *Sphagnum* moss across multiple study sites, which are often spatially dispersed, is frequently challenging and sometimes even unattainable. Noticeably, both the dominant and second most abundant *Sphagnum* moss species at each site demonstrated comparable performance to the collective presence of all *Sphagnum* mosses.

Another key aspect that needs to be further considered for optimal sampling strategy is when and from which segment to sample the *Sphagnum* moss associated TA communities can best represent the site hydrological conditions? In order to answer this question, chapter 3 investigated TA communities seasonally (March, June, September, January) and vertically (top 3 cm, middle region and bottom 3 cm) from three different *Sphagnum* mosses (entire segments of *S. capillifolium*, *S. fallax*, and *S. palustre*) at nine fixed locations in a blanket bog. TA species richness, and density were shown to consistently increase from March to September, with density increasing up until the



following January. Although Mazei et al. (2007) also reported a similar increasing trend of species richness from the end of spring to the beginning of autumn, it is largely different from many other studies that showed highly positive relationships for both of them with substrate moisture (Mieczan 2007, Marcisz et al. 2014). This may suggest that factors such as temperature, daylight duration, availability of food sources, interspecific interactions, or a combination of them, play a more important role in the colonization and development of TA than the moisture of the substrate, which was relatively constant or similar when taking the samples in this study. This was likely explained by precipitation events before some samples were taken. Ideally a wider range of seasonal moisture conditions should be sampled. Moreover, the higher average proportion of encysted TA in both June (most hot period) and January (most cold period) than in March and September further supported previous ideas that TA have the ability to encyst to cope with unfavorable conditions such as drought (Booth and Zygmunt 2005), and frost (Mitchell et al. 2000).

Apart from seasonal variation, vertical micro-distribution of TA communities along *Sphagnum* segments has been reported by a few previous studies (Heal 1962, Mitchell and Gilbert 2004, Jassey et al. 2011). While mixotrophic TA (e.g., *Amphitrema flavum*, and *Hyalosphenia papilio*) are usually found abundant in the upper regions of *Sphagnum* segments in raised bogs and agglutinate TA (e.g., *Centropyxis aerophila*, and *Diffugia bacillifera*) are often more frequently observed in the corresponding lower parts in fens, both of them were only rarely and occasionally counted in this blanket bog. Instead, idiosomic TA (with siliceous shell) were particularly abundant (more than 90% of total abundance) in all segments of *Sphagnum* moss. Nevertheless, a distinct and evident vertical change of TA was revealed in terms of both living status and density. The living TA exhibited a consistent decrease, while the presence of dead TA and density increased noticeably from the upper to the lower regions. This has been primarily attributed to the vertical transport of TA after death along the *Sphagnum* segments and/or to the growth/senescence of *Sphagnum* moss. Further, significant species-specific vertical separation has also been shown for most taxa in the present study, which is highly consistent with other studies (Chacharonis 1954, Booth 2002, Roe et al. 2017). For example, *Assulina muscorum*, and *Corythin dubium* were abundant in the upper regions, while *Heleopera sylvatica*, *Nebela militaris* were more common in the lower regions (Mitchell and Gilbert 2004). These spatial variances are

considered to be predominantly linked to the gradient of light, temperature, oxygen levels, and the availability of test materials and food sources.

Overall, the key findings indicate that investigation of TA communities among different plant functional types and their seasonal and vertical variation provides us with valuable and empirically supported guidance for sampling purposes. Given the simplicity and practicality, along with the results mentioned above, it is recommended that sampling the dominant *Sphagnum* moss species (entire segments including capitula) from a lawn position in late autumn or early winter is a more practical, simplified, and informative approach when utilizing TA as hydrological bioindicators to monitor restoration (rewetting) success of degraded peatland in blanket bogs. If the objective is to accurately represent the environmental conditions at the time of sampling, this study suggests counting only the living individuals. However, if the goal is to encompass the complete spectrum of environmental conditions over the past or recent years, this study recommends counting both living and dead TA for analysis.

### **6.2.2 Potential of testate amoebae as hydrological bioindicators to monitor peatland management and restoration**

Chapter 4 and 5 investigated TA communities from both *Sphagnum* moss and surface peat (0-5 cm) samples at a series of peatlands in relation to heather management (prescribed burning versus alternative cutting compared to an unmanaged/uncut heather control) and hydrological restoration (ditch/gully blocking and revegetation – including degraded, restored, least modified areas), respectively. TA community composition from both types of samples was shown to be distinctly separated between the very wet intact valley bog and managed sites but was not considerably different between the burnt and mown treatments (with only a small difference between uncut and managed plots). By contrast, a noticeable shift in TA community composition was observed from degraded through restored and to near intact/intact sites even though the dominant species varied significantly between moss and peat samples. This is probably highly related to the moisture levels of the sites as the wetness in the intact valley bog was significantly higher than in other peatland sites, but it did not strikingly change between the management and during the period of recovery. However, the hydrology in restored sites has dramatically improved over the course of restoration, as also shown by its

much higher total relative abundance of TA wet indicators compared to the degraded plots. The RDA analysis further supported this explanation, indicating that the moisture content of peat was the primary environmental factor that accounted for the variability in TA community.

Although TA community composition showed similarities between burnt and cut plots, there were significant differences observed between the near intact sites and the managed sites. Previous management by digging ditches to promote sheep grazing is likely to be the main reason, which lowered the water table depth, drying out the surface peat, thereby increasing the relative abundance of dry indicator species such as *Hyalosphenia subflava*, and *Corythion dubium* but decreasing the wet indicator species (e.g., *Amphitrema flavum*, *Heleopera sylvatica*). Noticeably, both the burnt and mown treatments resulted in increased moisture levels in the habitats, as evidenced by their greater total relative abundance of TA wet indicators compared to the unmanaged (uncut control) plots. The indications of wetness in the cut plots appeared to be even slightly higher than in the burnt plots, which strongly aligns with the findings of measured peat moisture and water table depth (WTD), within which mean WTD was reported approximately 2 cm higher in the mown plots in the first five years following management than in the burnt plots, while the uncut pots were shown to be consistently and increasingly becoming drier (Heinemeyer 2023).

As indicated above, a clear recovery trajectory in TA community composition has been revealed following active restoration efforts by ditch and gully blocking (and some revegetation). This was further corroborated by the proportion of wet and dry TA indicators, with total relative abundance of wet TA indicators progressively increasing from degraded towards least modified areas, vice versa. It is noteworthy that some species were shown to be especially sensitive to changes in water level. For example, taxa typically associated with dry habitats, such as the *Hyalosphenia subflava*, *Nebela tinca*, *Assullina muscorum* and *Corythion dubium* type were notably abundant in degraded plots. Conversely, species commonly regarded as indicators of wet conditions, such as the *Amphitrema flavum*, *A. wrightianum* and *Heleopera sylvatica* were more plentiful in the near intact sites and the highly saturated valley bog. This is highly consistent with previous studies that monitored the recovery success of degraded peatland by investigating TA communities either from the core peat (Buttler et al. 1996,

Davis and Wilkinson 2004, Valentine et al. 2013) or surface mosses samples (Swindles et al. 2016, Creevy et al. 2018).

While TA communities exhibited significant and intricate responses during the two-year period following restoration, the community composition remained similar to that observed in degraded plots. However, after six years of restoration, both the community composition and indicator species of the restored plots closely resembled those found in the least modified plots. Furthermore, there were notable similarities found between the restored plots and the least modified plots, particularly regarding *Sphagnum* moss, eight years after the restoration, in various aspects. In general, based on the core findings summarized above, testate amoebae have been demonstrated to be species-specific sensitivity to hydrological changes. These results also clearly highlight the effectiveness of TA as hydrological bioindicators for monitoring peatland management and restoration.

### **6.2.3 Potential functional contribution of testate amoebae in the process of peatland recovery**

In chapter 4 and 5, key functional traits of TA (i.e., mixotrophic TA (MTA), biovolume, aperture size, and test type) were also investigated and analyzed in response to heather management and peatland restoration. Noticeably, a recent study that attempted to quantify the contribution of MTA to carbon fixation has demonstrated this function of them to a non-negligible degree (Jassey et al. 2015). In their study, MTA were found to occupy over 70% of the total TA biomass in the studied raised bog. The 13% reduction in carbon dioxide uptake by the entire *Sphagnum* moss, resulting from an increased temperature treatment, was associated with a 50% decrease in MTA biomass (i.e., *Amphitrema flavum*, *Heleopera sphagni*, and *Hyalosphenia papilio*). While in this study very low abundance of MTA (even near zero in some sites) was recorded on managed and restored sites, the larger proportion of MTA (19.6%) in the very wet valley bog may indicate an increasing contribution of MTA for C uptake over the course of recovery in a long-term scale (decades to hundreds of years). Despite the overall low density, MTA were shown to be particularly abundant in few sporadic locations (from near intact sites, least modified and burnt areas, respectively) where peat moisture was relatively high. This may suggest that substrate moisture, rather than light, is more

likely to be the limiting factor in determining the establishment and development of MTA following heather management or restoration efforts.

Biovolume and/or aperture size of TA have been recently reported to be highly related to the decomposition of organic matter (Fournier et al. 2015, Jassey et al. 2016, Payne et al. 2016). Several studies have also established a connection between the body length of TA and the wetness of their habitat (Jassey et al. 2016, Payne et al. 2016). Specifically, larger species ( $> 60 \mu\text{m}$ ) have been found to be more prevalent in wetter habitats, whereas smaller species ( $< 60 \mu\text{m}$ ) are more abundant in drier habitats. The findings obtained from both *Sphagnum* and surface peat samples in the present study provided further support to this observation. Small species, characterized by small biovolume (including body size) and aperture size, that were often considered to mainly feed on microbes, were found notably abundant in the driest uncut and degraded plots. Both of these values (biovolume and aperture size) exhibited a significant increase throughout the restoration process, particularly in surface peat, and they also displayed an upward trend from the uncut plots to the burnt plots, and further to the mown plots. As a result, these may imply a considerable contribution of TA in carbon and nutrient cycling over the course of peatland recovery.

Contribution of TA with siliceous shells to silica cycling has been rarely studied in peatlands (Puppe 2020, Qin et al. 2021). Despite their characteristics of being easily broken down under unfavorable conditions (e.g., desiccation), siliceous TA occupied more than 90% of total TA population from *Sphagnum* mosses in both managed and restored sites. By contrast, they only comprised approximately 40%, on average, of the TA derived from surface peat accordingly. Noticeably, in surface peat, the proportion of TA with siliceous shells in burnt plots was just slightly lower than in both uncut and mown plots. This may suggest that prescribed burning of heather-dominated blanket bogs has minimal effects on siliceous TA, which aligns with previous studies that have demonstrated the limited impact of low intensity and short-term fires on TA communities (Wanner and Xylander 2003, Wanner 2012). Moreover, the proportion of siliceous TA was found to be lowest in the degraded plots in surface peat, with its relative abundance gradually increasing towards the least modified plots. This suggests that the decomposition rate of dead TA with siliceous shells would decrease during the restoration process. Consequently, despite the successful and widespread application of

TA for palaeohydrological reconstruction in ombrotrophic peatland ecosystems (raised bog), (Woodland et al. 1998, Charman et al. 2006, Swindles et al. 2014, Liu et al. 2019, Bysouth and Finkelstein 2021), its application in blanket bogs should be more cautious considering the dramatic decomposition rate (i.e., loss) of most TA taxa, particularly those with siliceous plates even in near intact sites.

It is noteworthy that species of *Hyalosphenia subflava*, including some potentially living specimens at the time of sampling, were shown to be particularly abundant in both surface and deep peat samples obtained from uncut and degraded plots. Large amounts of peat detritus/humus particles were also observed in the body of many individuals, especially in the deep peat samples. This observation may suggest that these species are capable of actively participating in the decomposition process of organic matter, beyond their role as microbial consumers, by directly contributing to nutrient cycling regulation. However, it is also possible that they feed on humus particles as a means to consume associated microbes, such as bacteria.

To sum up, the notable differences in dominant indicators and functional traits (MTA, biovolume/aperture size, and test type) observed between *Sphagnum* mosses and surface peat over the course of recovery following heather management and water intervention may suggest distinct functional roles they play. TA associated with *Sphagnum* mosses appears to be more important in carbon fixation within relatively wet habitats, while TA in surface peat plays a crucial role in peat decomposition and silica cycling within relatively dry habitats. Moreover, some key species (e.g., *Hyalosphenia subflava*) seem to be able to live in deeper peat layers, likely contributing to decomposition processes.

### **6.3 Limitations and future work (recommendations)**

While TA have been successfully assessed as hydrological bioindicators in monitoring peatland management and restoration, it has to be admitted that there are still some limitations which preclude our clear understanding on their ecology and particularly their possible functional roles in ecosystem processes. The potential limitations identified in this study and recommendations for future work are listed as follows:

- 1) In chapter 2 and 3, the assertions and explanations of the main results primarily rely on deductions from previous studies and observations from the present study. It is therefore essential for future work to measure a wider range of fundamental and comprehensive biotic factors (e.g., primary producers, other microbial consumers, decomposers) and abiotic factors (e.g., hydrological and physicochemical parameters) to further validate and reinforce the inferences and recommendations.
- 2) While chapter 2 also attempted to investigate the effects of different types and numbers of *Sphagnum* moss on the performance of using TA to indicate the habitat wetness, limited and uneven replicates of samples made it difficult to achieve this goal. A more systematic and comparative experimental design needs to be considered in future studies. Further investigations should also prioritize examining the functional disparities among *Sphagnum* moss species, particularly when they coexist within the same microsite.
- 3) The reasons behind the inefficient staining of living TA, such as determining whether dead TA with well-preserved organs/cytoplasm is not easily stained or if there are other contributing factors (e.g., low stain concentration and short dyeing time), remains unclear. It is essential to conduct further quantitative investigations into the staining efficiency of stains (e.g., Rose Bengal) by controlling the concentration of the staining agent and the duration of dyeing.
- 4) The key aspect of achieving the aims of this study is to accurately define and classify the wet and dry TA indicators. However, there is not one well-defined concept, and the classification varies from study to study. It is therefore necessary and urgent to compile the published literature to generate a relatively clear and consistent reference in the future, even though it may be challenging due to a series of limitations (e.g., different sampling strategy, different approaches to measure water-related parameters).
- 5) Although TA play a significant functional role as both carbon fixers (mixotrophic TA) and microbial consumers (heterotrophic TA), there is limited understanding on how the periodic encystment of TA impacts biogeochemical processes. More research is needed in this field, especially considering the escalating levels of global warming and the occurrence of extreme weather events.

- 6) Siliceous TA have been shown to be particularly abundant in *Sphagnum* mosses and have relatively higher decomposition rate in drier habitats in blanket bogs, but little is known on how they contribute to the silica cycling in this ecosystem. More work is necessary to quantify their abundance/biomass, biosilicification rate and their actual contribution to silica cycling in peatlands, particularly in blanket bogs.
- 7) TA show species-specific sensitivity to changes in hydrology, which highlights their potential as a tool to assess and monitor the hydrological restoration trajectory and success of degraded peatland. However, little is known about the quantitative relationship between TA community composition and habitat 'health' status (i.e., in relation to ecosystem functions). More work is needed, for example, to quantify the relationship between TA community composition and habitat wetness condition (WTD or substrate moisture) and the linkages between habitat hydrological condition and ecosystem carbon functioning (e.g., carbon sink or source). Additionally, other monitoring tools (e.g., long-term measurement of carbon fluxes, water quality, and vegetation composition) need to be applied as to obtain informative and robust evidence to inform recommendations for peatland management and restoration.
- 8) While functional traits of TA (e.g., MTA, biovolume/aperture size, and test type) can potentially be applied to infer their potential roles in ecosystem processes, we still lack understanding about their contribution in carbon and nutrient cycling. More work is needed to explore their feeding habitats, a key characteristic for their contribution to ecosystem processes, and quantify their contribution and role by combining information with other microorganisms (e.g., primary producers, other microbial consumers, and decomposers) and actual carbon fluxes of research subjects (e.g., *Sphagnum* moss).



## 6.4 Concluding remarks

This study confirms the value of TA as hydrological bioindicators within blanket bogs but also highlights key considerations and caveats around sampling methods and location. The main conclusions are as follows:

- 1) Findings suggest that sampling the dominant *Sphagnum* species from lawn positions in late autumn or early winter is a simple but effective method if the aim is to use TA to monitor the restoration (rewetting) success in UK blanket bogs.
- 2) The distinct hydrological response of some species, such as those indicative of dry habitats (e.g., *Hyalosphenia subflava*, *Corythion dubium*) and those indicative of wet habitats (e.g., *Amphitrema flavum* and *A. wrightianum*) confirms the potential and ecological value of TA being used as hydrological indicators to monitor the restoration success.
- 3) By contrast, the distinctive response pattern of functional traits of TA (MTA, body/aperture size, Siliceous TA) between *Sphagnum* moss and surface peat may suggest different functional roles, 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 relatively dry habitats.
- 4) Additionally, the observed large amount of *Hyalosphenia subflava* in the surface and deep peat may indicate its strong relationship to C-cycling; apart from feeding on microbes, they may actively take part in peat decomposition processes.
- 5) However, differences between heather-management were limited but agreed with observations of water table depth at the heather-management sites, suggesting some vegetation management impact on peat wetness, with both cutting and burning increasing wetness depending on time since management, whilst unmanaged heather showed the driest habitat. This may further indicate that site climatic wetness (rainfall) seemed to be the most important factor explaining TA community composition.
- 6) Moreover, uncertainties remain about differential preservation of key TA indicator taxa due to higher ranges in water tables in blanket bogs (versus more stable water

levels in most often assessed raised bogs), which raise questions about using TA from deeper peat layers to infer and compare historic hydrological conditions.

Overall, this study highlights the potential of TA as a tool to assess and monitor the hydrological recovery trajectory and success of current peatland management and restoration practices. It also clearly demonstrates the link between biodiversity, management, restoration, and likely inferred ecosystem functioning (water management / carbon and silica cycling) and the effectiveness of active restoration work but raises questions about inferring past information from blanket bog deeper peat layers.

# Appendices

## Summary:

- **Appendix 1:** Raw data table of testate amoebae from each sampling plot on different litter of plant functional types (**Chapter 2**)
- **Appendix 2:** Raw data table of testate amoebae in relation to their seasonal change and vertical distribution on *Sphagnum* mosses (**Chapter 3**)
- **Appendix 3 (a, b, c, d):** Raw data table of testate amoebae in relation to heather management, the corresponding environmental variables and functional trait values of selected species (**Chapter 4**)
- **Appendix 4 (a, b, c):** Raw data table of testate amoebae in relation to peatland restoration and the corresponding environmental variables (**Chapter 5**)

**Appendix 1 (Chapter 2).** Raw data table of testate amoebae from each sampling plot on different litter of plant functional types (*Sphagnum* moss, other mosses, heather, sedge). Butt: Butterburn, N: Nidderdale, M: Mossdale, B: bottom 3 cm (treated as litter), UC: uncut.

Plot	Plant_type	Amp fla	Amp.wri	Arc.d.s	Ass.mus	Ass.sem	Cen.cas	Cor.con	Cor.dub	Cyc.arc.l	Cyc.arc.s	Eug.rot	Eug.stri	Eug.tub	Hel.syl	Hya.ele	Hya.sub	Neb.co.bo	Neb fla	Neb.gri	Neb.mil	Neb.tin	Pse.ful	Tri.arc	Tri.lin
Butt-plot1-sph1-B	S.medium	0	0	0	18	8	0	0	1	1	5	2	6	32	40	62	0	3	0	0	1	0	12	0	0
Butt-plot1-sph2-B	S.papillosum	82	7	1	12	3	0	0	1	3	4	0	10	5	11	0	0	4	0	17	0	0	0	0	0
Butt-plot1-sph3-B	S.capillifolium	31	3	0	9	12	0	0	0	0	0	0	9	10	30	23	0	4	0	6	3	0	25	0	0
Butt-plot1-sph4-B	S.medium	64	18	0	3	2	0	0	6	11	0	0	5	1	3	1	0	0	0	9	0	0	4	0	0
Butt-plot1-sph5-B	S.medium	3	0	0	10	6	0	0	1	9	2	2	0	21	65	25	0	4	1	1	0	0	4	1	0
Butt-plot2-sph1-B	S.capillifolium	0	43	0	12	1	0	0	1	0	0	2	14	56	27	0	0	5	0	0	0	0	0	1	0
Butt-plot2-sph2-B	S.medium	3	12	0	19	2	0	0	0	0	7	0	1	54	45	0	0	0	1	1	1	0	25	1	0
Butt-plot2-sph3-B	S.papillosum	3	0	0	17	16	0	0	1	3	13	0	0	9	50	0	0	6	5	2	0	1	34	0	0
Butt-plot2-sph4-B	S.medium	40	43	1	4	1	0	0	5	0	1	2	8	6	7	0	0	0	0	13	1	0	0	0	0
Butt-plot2-sph5-B	S.fallax	2	27	0	49	1	0	0	10	15	0	0	19	25	2	0	0	4	1	3	0	1	5	0	0
Butt-plot3-sph2-B	S.palustre	0	1	1	24	3	0	0	4	0	1	2	4	48	53	1	0	4	10	7	1	0	1	4	0
Butt-plot3-sph3-B	S.medium	15	1	0	11	9	0	0	0	0	0	0	9	23	55	22	0	11	3	0	0	0	9	1	0
Butt-plot3-sph4-B	S.papillosum	7	3	0	32	1	0	0	20	0	3	5	35	15	24	0	0	2	3	5	4	0	1	1	0
Butt-plot3-sph5-B	S.fallax	0	1	1	52	0	0	0	16	1	0	2	17	47	16	0	0	11	3	2	1	0	3	1	0
Butt-plot4-sph1-B	S.papillosum	39	0	0	7	10	0	0	0	1	1	0	14	0	30	45	0	3	5	3	3	1	1	1	0
Butt-plot4-sph2-B	S.papillosum	0	40	0	41	4	0	0	1	7	0	3	0	21	11	17	0	6	10	0	0	0	0	1	0
Butt-plot4-sph3-B	S.papillosum	0	2	0	9	4	0	0	0	0	2	0	2	25	48	36	0	3	4	0	24	0	7	2	0
Butt-plot4-sph4-B	S.papillosum	9	0	0	26	1	0	0	12	0	2	4	26	35	38	2	0	3	4	0	1	0	11	1	0
Butt-plot4-sph5-B	S.medium	0	0	0	15	3	0	0	6	0	1	0	25	18	41	25	0	9	6	0	13	0	13	0	0
Butt-plot5-sph1-B	S.fimbriatum	1	6	0	25	0	0	1	19	0	3	2	68	15	8	0	0	3	5	5	0	0	0	2	1
Butt-plot5-sph2-B	S.capillifolium	33	0	0	9	3	0	0	10	0	1	6	12	47	7	9	0	4	1	4	6	0	1	1	1
Butt-plot5-sph3-B	S.papillosum	15	1	0	9	5	0	0	5	0	2	2	1	89	26	0	0	2	3	1	0	0	3	0	0
Butt-plot5-sph4-B	S.medium	31	2	0	12	3	0	0	3	0	1	4	10	25	31	13	0	8	1	11	1	0	0	0	0
Butt-plot5-sph5-B	S.papillosum	9	6	0	15	2	0	0	11	1	6	3	17	36	33	0	0	5	9	11	0	1	0	1	0
N-UC1-sph1-B	S.capillifolium	0	0	0	23	0	0	1	38	0	0	12	0	61	41	0	1	10	0	0	2	0	0	1	0
N-UC1-sph2-B	S.palustre	0	0	0	47	0	0	0	21	0	0	6	0	14	58	0	0	20	0	0	0	6	0	0	2
N-UC1-sph3-B	S.capillifolium	0	0	0	8	0	1	0	16	0	0	11	0	70	30	0	0	21	0	0	12	0	0	0	3
N-UC1-sph4-B	S.capillifolium	0	0	0	26	0	4	13	17	4	1	19	0	42	14	0	0	12	0	0	2	6	0	0	2
N-UC1-sph5-B	S.capillifolium	0	0	0	9	0	0	0	12	1	0	7	0	72	55	0	1	12	0	0	5	0	0	0	0
N-UC2-sph1-B	S.fallax	0	0	3	11	0	1	5	12	1	1	3	2	38	54	0	7	8	2	0	3	1	1	0	0
N-UC2-sph2-B	S.capillifolium	0	0	2	1	0	0	1	56	0	0	12	0	38	29	0	2	11	0	0	1	0	2	2	0
N-UC2-sph3-B	S.fallax	0	0	15	14	0	0	0	18	5	1	7	5	50	41	0	8	5	2	0	1	0	14	1	0
N-UC2-sph4-B	S.fallax	0	0	4	6	0	0	0	23	1	0	11	0	51	49	0	1	17	0	0	1	1	0	1	0
N-UC2-sph5-B	S.fallax	0	0	1	27	0	0	0	13	0	0	18	0	82	28	0	13	6	0	0	2	1	0	0	0
N-UC3-sph1-B	S.capillifolium	0	0	0	4	0	0	0	6	0	0	7	0	46	83	0	0	24	1	0	0	0	0	0	0
N-UC3-sph2-B	S.capillifolium	0	0	0	12	0	0	0	39	0	0	3	0	83	14	0	0	4	0	0	3	0	1	0	11
N-UC3-sph3-B	S.capillifolium	0	0	0	9	0	0	0	71	0	0	6	0	40	4	0	0	6	0	0	35	0	0	0	10
N-UC3-sph4-B	S.fallax	0	0	0	15	0	0	0	31	0	0	21	0	83	5	0	0	11	0	0	12	13	0	0	0
N-UC3-sph5-B	S.fallax	0	0	8	12	0	0	0	10	0	0	3	60	12	22	0	0	10	15	0	1	2	1	0	6
N-UC4-sph1-B	S.fimbriatum	0	0	7	2	0	0	0	30	0	0	21	2	72	20	0	0	6	5	0	1	2	2	0	1
N-UC4-sph2-B	S.fallax	0	0	1	11	0	0	0	26	0	0	5	9	52	11	0	0	17	0	0	5	28	2	0	0
N-UC4-sph3-B	S.fallax	0	0	0	0	0	0	0	9	0	0	3	0	80	15	0	0	25	8	0	7	6	0	0	0
N-UC4-sph4-B	S.fimbriatum	0	0	13	4	0	1	1	20	1	3	3	38	7	22	0	14	2	3	0	1	0	0	2	1
N-UC4-sph5-B	S.fimbriatum	0	0	7	12	0	0	0	54	0	1	2	20	5	9	0	4	3	1	0	2	0	0	5	1
N-UC5-sph1-B	S.fallax	0	0	16	20	0	0	0	22	1	0	6	8	40	20	0	0	29	4	0	2	0	0	0	0
N-UC5-sph2-B	S.fallax	0	0	4	16	0	0	0	43	0	0	13	0	15	36	0	0	20	5	0	2	0	0	0	0
N-UC5-sph3-B	S.fimbriatum	0	0	31	6	0	0	0	21	1	8	5	13	17	13	0	13	2	3	0	4	2	3	2	0
N-UC5-sph4-B	S.papillosum	0	0	0	9	0	0	0	14	1	4	6	0	42	53	0	0	27	0	0	4	1	0	1	0
N-UC5-sph5-B	S.capillifolium	0	0	0	7	0	0	0	13	0	0	4	0	46	70	0	0	17	0	0	4	2	0	0	0
M-UC1-sph1-B	S.capillifolium	0	0	0	3	0	0	0	9	0	1	5	0	61	27	0	0	9	14	0	21	2	0	0	1
M-UC1-sph2-B	S.fallax	0	0	36	3	1	0	2	33	0	1	9	37	14	7	0	0	4	3	1	0	0	0	1	1
M-UC1-sph3-B	S.capillifolium	0	0	0	8	0	2	4	47	0	0	3	2	35	8	0	0	0	1	0	47	13	0	1	4

M-UC1-sph4-B	S.capillifolium	0	0	3	8	1	0	0	19	0	0	14	14	54	20	0	0	9	1	2	1	1	1	0	1
M-UC1-sph5-B	S.fallax	1	1	15	1	0	0	3	22	0	1	0	49	5	28	3	6	2	10	2	2	1	0	0	0
M-UC2-sph1-B	S.fallax	0	0	0	6	0	0	0	62	0	0	13	25	4	12	0	0	23	0	0	0	0	0	0	14
M-UC2-sph2-B	S.fallax	0	0	0	9	0	0	7	27	0	0	5	60	2	5	0	1	15	1	0	4	8	0	0	19
M-UC2-sph3-B	S.capillifolium	0	0	0	10	0	1	3	40	0	0	7	5	27	9	0	1	28	4	0	24	4	0	0	6
M-UC2-sph4-B	S.capillifolium	0	0	0	8	0	0	2	74	0	2	7	20	34	20	0	0	2	0	0	18	0	1	2	7
M-UC3-sph1-B	S.capillifolium	0	0	0	19	0	0	0	17	0	2	4	2	48	62	0	0	6	0	0	7	0	0	3	1
M-UC3-sph2-B	S.capillifolium	0	0	0	5	0	0	0	31	0	3	11	23	17	52	0	0	10	0	0	3	0	0	2	2
M-UC3-sph3-B	S.capillifolium	0	0	0	23	0	0	9	23	0	0	9	6	38	28	0	0	10	0	0	21	1	0	2	5
M-UC3-sph4-B	S.capillifolium	1	0	2	10	7	0	0	8	0	5	6	10	24	43	0	0	17	8	0	0	1	0	0	1
M-UC3-sph5-B	S.capillifolium	0	0	0	30	0	1	2	18	2	0	3	13	37	9	0	0	16	0	0	5	39	0	0	1
M-UC4-sph1-B	S.capillifolium	0	0	0	9	0	0	1	27	2	3	2	32	12	37	0	1	2	0	0	29	12	0	0	3
M-UC4-sph2-B	S.capillifolium	0	0	0	14	0	0	6	38	2	0	1	6	28	12	0	0	8	0	0	29	14	0	2	0
M-UC4-sph3-B	S.fallax	0	0	0	2	0	0	0	12	0	0	0	18	32	36	0	0	29	3	0	13	1	0	0	0
M-UC4-sph4-B	S.fallax	0	0	1	28	0	0	7	26	0	0	9	14	17	21	0	0	19	0	0	10	4	0	1	3
M-UC4-sph5-B	S.fallax	0	1	0	23	0	0	5	31	0	0	6	18	20	24	0	0	28	7	0	6	0	0	0	2
M-UC5-sph1-B	S.palustre	0	0	0	14	0	2	2	51	7	5	11	6	38	8	0	0	13	0	0	22	11	0	3	19
M-UC5-sph2-B	S.capillifolium	0	0	0	18	0	0	11	55	0	1	7	11	23	17	0	0	0	0	0	27	0	0	0	16
M-UC5-sph3-B	S.palustre	0	0	0	12	0	0	0	29	1	8	6	8	23	39	0	0	9	4	0	14	8	0	4	0
M-UC5-sph4-B	S.capillifolium	0	0	0	6	0	0	18	65	2	0	16	7	40	2	0	0	4	0	0	8	0	0	2	7
M-UC5-sph5-B	S.fallax	0	0	0	11	0	1	0	20	2	3	2	4	18	9	0	0	7	0	0	61	17	0	0	7
N-UC1-lit1	Sedge	0	0	0	7	0	0	0	5	9	0	0	55	36	0	7	0	0	0	8	24	3	17	0	0
N-UC1-lit2	Heather	0	0	1	24	1	5	0	3	14	11	3	0	22	55	0	0	5	0	0	3	13	0	0	0
N-UC1-lit3	Moss	0	0	0	16	1	2	1	3	0	4	5	0	21	59	0	0	10	2	0	6	5	2	2	0
N-UC1-lit4	Moss-AUL	0	0	0	21	0	12	0	49	5	1	0	0	32	13	0	0	14	1	0	0	18	0	1	4
N-UC2-lit1	Moss	0	0	0	16	0	9	6	21	2	3	11	2	63	16	0	2	14	2	0	0	13	2	1	0
N-UC2-lit2	Sedge	0	0	0	13	0	0	1	3	1	13	2	0	44	49	0	2	5	0	0	5	31	7	1	1
N-UC2-lit3	Heather	0	0	1	34	0	3	3	27	1	3	8	0	22	30	0	4	1	0	0	3	1	1	2	1
N-UC2-lit4	CAMP	0	0	12	5	0	1	4	15	1	18	2	15	30	35	0	18	2	1	0	3	5	6	3	0
N-UC2-lit5	Moss-2	0	0	5	7	0	2	2	12	2	13	4	0	77	36	0	14	7	0	0	1	8	9	6	0
N-UC3-lit1	Heather	0	0	0	32	0	4	4	33	21	9	10	0	25	21	0	2	0	0	0	10	13	0	1	2
N-UC3-lit2	Moss	0	0	0	20	0	4	1	11	10	25	3	0	31	21	0	10	8	0	0	4	20	10	5	0
N-UC3-lit3	Sedge	0	0	0	16	0	5	3	64	29	2	20	0	49	3	0	0	1	0	0	1	24	1	2	3
N-UC3-lit4	Heather	0	0	0	41	0	19	12	20	16	5	14	1	8	9	0	0	0	0	0	25	26	0	4	3
N-UC4-lit1	Heather	0	0	0	14	0	1	1	25	4	0	9	1	87	2	0	0	0	0	0	2	26	0	0	2
N-UC4-lit2	Sedge	0	0	0	31	0	3	0	15	18	6	9	0	42	6	0	1	0	0	0	14	27	0	7	2
N-UC4-lit3	Moss-HYP	0	0	0	31	0	0	3	17	3	12	11	9	53	20	0	0	5	1	0	26	38	1	0	4
N-UC4-lit4	Moss	0	0	2	18	0	2	0	21	4	4	6	13	64	11	0	2	5	3	0	2	16	6	1	0
N-UC4-lit5	Sedge-E.ang	0	0	0	5	0	1	0	50	0	1	7	70	2	9	0	0	3	7	0	2	0	1	1	3
N-UC5-lit1	Sedge-E.vag	0	0	0	18	0	4	9	96	10	2	2	7	4	1	0	1	0	0	0	1	3	0	0	4
N-UC5-lit2	Heather	0	0	11	18	0	3	3	65	0	0	7	11	11	24	0	0	2	1	0	1	2	0	0	8
N-UC5-lit3	Moss	0	0	0	34	0	3	1	7	9	0	8	0	30	16	0	0	9	0	0	9	49	0	2	0
N-UC5-lit4	Sedge-E.ang	0	0	17	18	0	0	0	24	2	5	5	4	26	39	0	4	3	8	0	1	2	1	7	1
N-UC5-lit5	Moss	0	0	9	4	0	0	2	22	5	5	5	25	14	12	0	12	0	2	0	6	4	2	5	0
M-UC1-lit1	Heather	0	0	0	16	0	0	0	9	12	3	3	0	39	14	0	0	3	11	0	6	4	0	2	0
M-UC1-lit2	Sedge	0	0	1	7	0	1	0	52	0	1	9	32	18	3	0	1	16	6	1	7	3	0	0	0
M-UC1-lit3	Moss-HYP	0	0	0	12	0	0	6	15	3	12	6	0	21	11	0	0	6	5	0	47	8	1	1	1
M-UC1-lit4	Moss-PLEU	0	0	0	28	0	0	6	43	4	3	7	0	23	7	0	1	1	6	0	14	21	0	0	2
M-UC1-lit5	S.fal	0	0	4	4	0	0	0	19	1	1	4	31	45	28	2	1	3	5	0	3	0	0	0	3
M-UC2-lit1	Heather	0	0	0	14	0	5	0	19	13	11	15	3	19	24	0	0	2	0	0	21	9	0	1	0
M-UC2-lit2	Sedge-E.vag	0	0	0	20	0	0	0	112	6	0	4	1	24	0	0	0	1	0	0	0	1	0	0	0
M-UC2-lit3	Moss-HYP	0	0	0	18	0	0	0	20	32	5	9	0	23	10	0	0	0	0	0	7	5	1	1	0
M-UC2-lit4	Moss-PLEU	0	0	0	7	0	0	0	33	0	0	6	3	40	18	0	0	12	0	0	5	21	0	2	4

M-UC2-lit5	S.cap	0	0	2	9	0	0	1	21	0	1	3	4	34	68	0	1	21	5	0	3	21	0	1	3
M-UC3-lit1	Heather	0	0	3	20	0	2	1	31	3	23	10	0	57	20	0	0	2	1	0	2	6	1	3	0
M-UC3-lit2	Sedge-E.vag	0	0	0	8	0	0	5	122	1	13	1	2	25	4	0	0	8	2	0	3	3	0	0	18
M-UC3-lit3	Moss	0	0	0	28	0	3	0	16	0	9	3	0	17	8	0	0	18	0	0	31	40	0	1	1
M-UC3-lit4	Moss-PLEU	0	0	0	19	0	1	2	15	0	6	4	3	25	6	0	0	14	0	0	11	56	0	0	0
M-UC3-lit5	Moss-AUL	0	0	0	29	0	8	4	10	2	23	0	0	26	22	0	0	6	1	0	14	25	0	0	0
M-UC4-lit1	Moss-AUL	0	0	1	20	0	0	0	56	32	6	25	1	11	7	0	0	0	0	0	2	6	0	1	10
M-UC4-lit2	Moss-PLEU	0	0	0	25	0	0	0	59	4	8	11	0	54	7	0	0	1	0	0	11	20	0	5	3
M-UC4-lit3	Heather	0	0	0	47	0	2	0	32	18	4	11	0	10	5	0	0	2	0	0	19	20	0	2	7
M-UC4-lit4	Sedge	0	0	0	35	0	0	0	23	19	9	1	1	29	16	0	0	6	0	0	9	26	3	5	2
M-UC4-lit5	Moss	0	0	0	16	0	1	0	14	6	1	3	3	29	2	0	0	0	0	0	41	39	0	0	4
M-UC5-lit1	Moss-HYP	0	0	0	22	0	1	0	3	0	8	3	1	43	18	0	0	20	2	0	13	23	1	4	1
M-UC5-lit2	Moss-RHY	0	0	0	53	0	3	1	13	3	3	2	0	38	3	0	0	15	0	0	3	29	0	0	0
M-UC5-lit3	Heather	0	0	0	28	5	9	7	11	4	26	5	0	26	25	0	0	3	6	0	3	24	1	0	0
M-UC5-lit4	Sedge	0	0	0	39	0	1	0	9	2	3	2	2	58	17	0	0	16	0	0	10	21	0	1	1
M-UC5-lit5	Sedge	0	0	0	16	0	8	0	10	1	30	1	0	45	2	0	0	18	0	0	3	27	0	2	1
B-Plot1-lit1	Sedge	1	0	0	42	0	0	0	0	1	11	0	0	3	57	0	0	28	11	0	0	0	0	3	0
B-Plot1-lit2	Moss	0	0	0	37	1	0	0	6	7	14	0	1	35	23	0	2	14	38	0	11	6	0	3	0
B-Plot1-lit3	Sedge	22	5	1	33	3	0	0	6	1	1	0	3	2	17	0	0	47	2	0	0	1	0	2	0
B-Plot1-lit4	Heather	0	1	0	28	1	2	0	38	20	5	6	0	13	32	0	0	4	4	0	0	0	0	1	0
B-Plot2-lit1	Sedge	0	7	3	34	1	0	0	2	0	12	0	0	52	15	0	0	3	2	4	2	0	0	2	0
B-Plot2-lit2	Heather	0	0	0	22	1	0	0	9	2	9	1	1	100	12	0	0	2	1	0	1	0	0	3	0
B-Plot2-lit3	Moss-HYP	0	0	0	9	0	0	0	5	9	15	0	0	49	25	0	4	0	7	0	18	5	1	6	0
B-Plot2-lit4	Moss-AUL	0	0	0	35	0	0	0	5	1	10	0	0	26	25	0	3	1	39	0	6	3	4	2	0
B-Plot2-lit5	Moss-Poly	0	0	0	13	0	0	0	5	0	3	2	0	67	18	0	0	13	8	0	5	0	2	8	0
B-Plot3-lit1	Heather	0	0	0	28	2	0	0	27	24	7	2	0	47	2	0	0	3	5	0	11	8	0	6	0
B-Plot3-lit2	Sedge	0	0	1	2	1	0	0	0	7	74	0	0	6	9	0	0	2	19	0	21	0	2	3	0
B-Plot3-lit3	Moss-Liverwort	0	0	0	18	4	0	0	4	5	26	0	0	9	39	0	0	12	8	8	20	2	2	4	0
B-Plot3-lit4	Moss-RHY	0	0	0	7	0	0	0	12	11	0	2	0	31	0	0	0	0	0	0	8	59	1	1	0
B-Plot3-lit5	Moss-Poly	0	0	0	73	0	0	0	27	0	1	7	0	15	1	0	0	0	0	0	1	1	0	8	6
B-Plot4-lit1	Heather	0	0	0	35	0	0	0	17	28	3	10	0	85	3	0	0	6	1	0	6	0	0	0	0
B-Plot4-lit2	Moss-HYP	0	0	0	21	0	0	0	7	0	0	0	0	118	0	0	0	0	0	0	0	0	0	0	0
B-Plot4-lit3	Moss-Poly	0	0	0	69	0	0	0	28	2	0	3	8	30	1	0	0	0	0	0	3	0	1	12	0
B-Plot4-lit4	Moss-Poly	0	0	1	64	0	0	0	11	5	5	0	1	45	8	0	0	2	5	0	2	10	0	20	0
B-Plot4-lit5	Sedge	0	0	0	71	1	0	0	23	1	7	0	0	7	11	0	0	0	3	2	0	0	2	3	0
B-Plot5-lit1	Sedge	0	0	0	25	2	0	0	3	0	41	0	0	38	20	0	0	15	9	1	13	2	1	8	0
B-Plot5-lit2	Heather	0	0	0	32	0	0	0	22	4	1	10	0	44	4	0	0	2	32	0	11	1	0	0	0
B-Plot5-lit3	Moss-HYP	0	0	0	1	0	0	0	11	0	0	0	9	53	1	0	0	0	1	0	0	88	0	1	0
B-Plot5-lit5	Moss-Poly	0	0	0	11	1	0	0	1	4	1	5	0	32	77	0	0	0	0	1	3	1	0	54	0

**Appendix 2 (Chapter 3).** Raw data table of testate amoebae in relation to their seasonal change and vertical distribution on *Sphagnum* mosses in a blanket bog (Mosssdale). T: top 3 cm; M: between top and bottom segment; B: bottom 3 cm. S.cap: *Sphagnum capillifolium*, S.fal: *Sphagnum fallax*, S.pal: *Sphagnum palustre*. L + E: living + ensysted.



Plot	living status	Arc.art	Ass.mus	Cen.arc	Cor.dub	Cyc.arc.s	Eug.rot	Eug.str	Eug.tub	Hel.syl	Neb.co.bo	Neb fla	Neb.mil	Neb.tin	Tri.arc
March-s.cap-T-(44)	L + E	0	12	0	12	0	1	47	0	14	32	1	25	1	0
March-s.cap-M-(44)	L + E	0	2	0	6	0	1	19	5	57	16	2	81	0	1
March-s.cap-B-(44)	L + E	0	1	0	2	1	0	19	4	37	19	3	52	0	1
March-s.fal-T-(44)	L + E	0	15	0	4	0	0	8	3	0	125	1	0	2	0
March-s.fal-M-(44)	L + E	0	6	0	2	0	1	50	0	17	63	1	1	6	0
March-s.fal-B-(44)	L + E	0	2	0	4	0	1	35	1	27	50	5	3	5	0
March-s.pal-T-(44)	L + E	71	1	0	5	0	1	10	0	13	64	2	0	0	0
March-s.pal-M-(44)	L + E	19	0	5	1	0	0	27	1	27	53	5	0	0	0
March-s.pal-B-(44)	L + E	7	0	14	2	0	0	23	3	31	30	15	0	2	0
March-s.cap-T-(37)	L + E	0	9	0	28	0	5	6	0	13	44	0	20	2	3
March-s.cap-M-(37)	L + E	0	1	0	10	0	0	16	1	44	24	1	27	3	1
March-s.cap-B-(37)	L + E	0	0	0	11	0	0	15	1	40	23	1	32	4	1
March-s.fal-T-(37)	L + E	0	9	0	15	0	1	18	1	23	49	0	0	0	0
March-s.fal-M-(37)	L + E	0	9	0	4	0	1	39	0	54	17	0	2	0	2
March-s.fal-B-(37)	L + E	0	1	0	3	0	0	37	0	70	9	2	3	0	0
March-s.pal-T-(37)	L + E	0	25	0	5	0	1	14	0	17	76	2	1	4	0
March-s.pal-M-(37)	L + E	2	4	0	1	1	1	24	1	38	32	3	17	5	0
March-s.pal-B-(37)	L + E	0	3	0	0	4	1	40	1	38	19	5	10	4	1
March-s.cap-T-(27)	L + E	0	6	0	30	0	12	46	0	20	27	0	2	0	2
March-s.cap-M-(27)	L + E	0	2	0	17	0	1	58	2	50	7	0	1	0	0
March-s.cap-B-(27)	L + E	0	3	0	25	3	1	48	2	47	4	0	2	1	0
March-s.fal-T-(27)	L + E	0	5	0	8	0	0	20	0	0	85	0	5	2	0
March-s.fal-M-(27)	L + E	0	0	0	3	0	1	36	0	12	53	0	16	0	0
March-s.fal-B-(27)	L + E	0	1	0	5	2	1	27	0	11	31	0	42	2	0
March-s.pal-T-(27)	L + E	0	17	0	30	0	3	27	0	22	34	0	1	0	0
March-s.pal-M-(27)	L + E	0	5	0	8	1	2	15	0	43	24	0	9	0	0
March-s.pal-B-(27)	L + E	0	1	0	6	0	1	24	0	38	12	0	27	0	0
June-s.cap-T-(44)	L + E	0	10	0	14	0	3	38	0	24	41	1	9	0	0
June-s.cap-M-(44)	L + E	0	1	0	6	0	1	27	0	41	17	0	33	4	0
June-s.cap-B-(44)	L + E	0	1	0	3	0	1	13	0	25	15	5	44	4	0
June-s.fal-T-(44)	L + E	0	15	0	29	0	1	40	0	1	35	2	1	13	0

June-s.fal-M-(44)	L + E	0	2	0	19	0	1	61	0	6	23	8	0	21	0
June-s.fal-B-(44)	L + E	0	1	0	6	1	3	52	0	20	10	10	6	11	0
June-s.pal-T-(44)	L + E	40	9	2	5	0	0	11	0	11	70	3	1	0	0
June-s.pal-M-(44)	L + E	9	2	5	3	0	1	20	0	28	44	9	1	0	0
June-s.pal-B-(44)	L + E	1	0	3	1	0	0	27	0	39	18	16	1	1	2
June-s.cap-T-(37)	L + E	0	7	0	13	0	1	32	0	22	47	0	4	1	0
June-s.cap-M-(37)	L + E	0	3	0	7	0	0	40	0	36	22	5	8	3	0
June-s.cap-B-(37)	L + E	0	0	0	0	1	0	5	6	20	16	1	3	0	0
June-s.fal-T-(37)	L + E	0	30	0	18	0	2	29	1	11	59	0	4	0	1
June-s.fal-M-(37)	L + E	0	3	0	8	0	1	32	0	29	15	0	11	0	0
June-s.fal-B-(37)	L + E	0	0	0	3	0	1	21	0	41	6	2	9	0	1
June-s.pal-T-(37)	L + E	0	3	0	3	0	1	12	0	38	51	0	13	12	0
June-s.pal-M-(37)	L + E	0	0	0	0	3	0	8	0	50	11	0	9	2	1
June-s.pal-B-(37)	L + E	0	2	0	0	6	0	7	2	37	5	1	15	2	2
June-s.cap-T-(27)	L + E	0	5	0	15	0	4	41	0	32	41	0	3	0	0
June-s.cap-M-(27)	L + E	0	1	0	6	0	2	34	0	28	30	0	11	7	0
June-s.cap-B-(27)	L + E	0	2	0	5	2	0	16	4	33	11	0	12	2	0
June-s.fal-T-(27)	L + E	0	7	0	13	0	0	13	1	0	100	0	19	1	0
June-s.fal-M-(27)	L + E	0	1	0	3	0	0	11	3	2	52	0	48	7	1
June-s.fal-B-(27)	L + E	0	0	0	5	0	0	9	3	8	19	0	52	17	0
June-s.pal-T-(27)	L + E	0	14	0	7	4	2	15	0	11	46	0	16	4	4
June-s.pal-M-(27)	L + E	0	1	0	9	3	1	9	1	24	11	1	46	4	4
June-s.pal-B-(27)	L + E	0	1	0	1	5	2	5	0	16	11	0	43	1	5
Sep-s.cap-T-(27)	L + E	0	3	0	18	1	1	52	1	12	42	0	1	11	0
Sep-s.cap-M-(27)	L + E	0	0	0	7	0	0	54	1	25	11	0	9	2	1
Sep-s.cap-B-(27)	L + E	0	1	0	2	1	0	23	2	27	21	0	10	3	1
Sep-s.fal-T-(27)	L + E	0	8	0	33	0	4	43	0	0	49	0	9	2	0
Sep-s.fal-M-(27)	L + E	0	9	0	42	1	2	32	0	1	54	0	15	3	0
Sep-s.fal-B-(27)	L + E	0	3	0	15	2	0	14	2	9	20	0	38	12	0
Sep-s.pal-T-(27)	L + E	0	4	0	8	2	2	15	4	15	34	1	30	5	2
Sep-s.pal-M-(27)	L + E	0	0	0	0	3	0	0	3	17	10	0	74	5	6
Sep-s.pal-B-(27)	L + E	0	0	0	0	2	0	1	2	10	8	0	22	4	3

Sep-s.cap-T-(37)	L + E	0	32	0	10	0	1	32	0	11	45	1	2	1	0
Sep-s.cap-M-(37)	L + E	0	2	0	4	1	1	26	7	35	17	1	5	0	0
Sep-s.cap-B-(37)	L + E	0	1	0	2	5	0	14	5	27	31	3	1	1	3
Sep-s.fal-T-(37)	L + E	0	22	0	43	0	4	22	0	1	37	0	4	1	1
Sep-s.fal-M-(37)	L + E	0	5	0	26	0	2	39	4	7	31	0	16	0	1
Sep-s.fal-B-(37)	L + E	0	2	0	20	0	1	39	12	4	5	0	26	0	0
Sep-s.pal-T-(37)	L + E	0	9	0	4	2	0	16	1	24	72	0	3	9	1
Sep-s.pal-M-(37)	L + E	0	1	0	3	4	0	10	6	39	13	0	5	7	1
Sep-s.pal-B-(37)	L + E	0	0	0	0	7	1	5	2	19	14	1	10	8	2
Sep-s.cap-T-(44)	L + E	0	6	0	19	2	0	28	3	15	27	2	11	0	0
Sep-s.cap-M-(44)	L + E	0	2	0	1	1	0	8	4	13	6	0	14	1	0
Sep-s.cap-B-(44)	L + E	0	1	0	3	5	2	6	7	14	9	2	11	4	0
Sep-s.fal-T-(44)	L + E	0	27	0	38	0	9	32	4	7	22	1	1	0	0
Sep-s.fal-M-(44)	L + E	0	5	1	24	0	1	22	2	4	23	1	1	0	2
Sep-s.fal-B-(44)	L + E	0	3	0	22	0	2	56	1	12	17	5	1	0	1
Sep-s.pal-T-(44)	L + E	0	36	10	4	1	1	32	2	3	25	2	0	0	0
Sep-s.pal-M-(44)	L + E	0	2	17	4	3	0	32	0	9	45	2	0	0	2
Sep-s.pal-B-(44)	L + E	0	2	5	2	16	0	9	0	5	29	2	0	0	8
Jan-s.cap-T-(27)	L + E	0	6	0	20	0	2	80	1	10	24	0	0	6	0
Jan-s.cap-M-(27)	L + E	0	1	0	9	2	0	63	0	47	13	0	4	2	0
Jan-s.cap-B-(27)	L + E	0	0	0	3	1	0	19	1	45	11	0	4	3	0
Jan-s.fal-T-(27)	L + E	0	10	0	49	0	3	26	1	0	29	0	15	9	0
Jan-s.fal-M-(27)	L + E	0	2	0	22	0	1	49	0	1	20	0	45	3	0
Jan-s.fal-B-(27)	L + E	0	3	0	8	1	0	34	4	2	17	0	69	10	0
Jan-s.pal-T-(27)	L + E	0	1	0	17	2	2	26	1	12	40	0	4	5	1
Jan-s.pal-M-(27)	L + E	0	3	0	3	4	1	8	13	22	8	0	70	4	3
Jan-s.pal-B-(27)	L + E	0	0	0	3	6	1	0	8	20	12	0	9	7	5
Jan-s.cap-T-(37)	L + E	0	21	0	35	0	3	41	0	8	38	0	1	2	0
Jan-s.cap-M-(37)	L + E	0	7	0	7	0	1	27	2	33	20	0	0	1	1
Jan-s.cap-B-(37)	L + E	0	0	0	2	2	1	6	5	32	22	6	3	1	0
Jan-s.fal-T-(37)	L + E	0	13	0	22	1	1	48	6	6	47	0	6	0	0
Jan-s.fal-M-(37)	L + E	0	10	0	18	0	0	41	21	7	19	0	20	1	0

Jan-s.fal-B-(37)	L + E	0	4	0	19	0	4	16	16	25	15	0	12	1	0
Jan-s.pal-T-(37)	L + E	0	10	0	20	0	0	15	5	30	45	0	1	1	0
Jan-s.pal-M-(37)	L + E	0	2	0	7	4	0	6	5	34	15	2	6	6	2
Jan-s.pal-B-(37)	L + E	0	2	0	2	9	0	8	6	28	16	1	20	8	6
Jan-s.cap-T-(44)	L + E	0	10	0	8	0	0	49	2	29	15	3	17	0	0
Jan-s.cap-M-(44)	L + E	0	0	0	1	0	0	22	2	41	14	7	27	0	0
Jan-s.cap-B-(44)	L + E	0	1	0	0	3	2	13	5	34	12	9	17	1	1
Jan-s.fal-T-(44)	L + E	0	5	0	75	0	9	37	0	3	20	0	0	0	0
Jan-s.fal-M-(44)	L + E	0	3	0	18	0	3	61	3	1	22	0	0	0	1
Jan-s.fal-B-(44)	L + E	0	3	0	4	2	6	42	15	26	14	0	0	0	1
Jan-s.pal-T-(44)	L + E	0	53	2	15	0	10	16	1	2	27	0	0	0	1
Jan-s.pal-M-(44)	L + E	0	12	5	2	1	1	39	3	17	16	5	0	0	4
Jan-s.pal-B-(44)	L + E	0	8	10	0	5	0	19	10	9	11	3	1	0	6

**Appendix 3 (Chapter 4).** Raw data table of testate amoebae in relation to heather management, the corresponding environmental variables and functional trait values of selected species.

**Appendix 3a.** Raw data table of testate amoebae on *Sphagnum* mosses in relation to heather management (i.e., burning (FI) versus cutting (LB) versus uncut (UC: control). T: top 3 cm; B: bottom 3 cm. **Reference sites:** Butt-Butterburn (border mire), KH-Kield Head, WE-Whitlee English side, WS-Whitlee Scottish side. **Experimental managed sites:** N-Nidderdale, M-Mosssdale, W-Whitendale.

Plot	sph.type	Amp.fla	Amp.wri	Arc.cat	Arc.d.s	Ass.mus	Ass.sem	Bul.ind	Cor.con	Cor.dub	Cry.ovy	Cyc.arcl	Cyc.ar.c.s	Deh.jap	Dif.lei	Eug.rot	Eug.stri	Eug.tub	Hel.pet	Hel.syl	Hya.ele	Hya.pap	Hya.sub	NA.1	Neb.car	Neb.co.bo	Neb.fla	Neb.gri	Neb.mil	Neb.tin	Pse.ful	Sph.len	Tri.arc	Trilin		
Butt-plot1-sph1-T	S.medium	0	0	0	0	22	26	1	0	2	0	0	1	0	0	0	8	17	1	27	61	0	0	0	0	2	0	0	2	0	1	0	0			
Butt-plot1-sph2-T	S.papillosum	97	2	0	0	8	2	0	0	2	0	0	1	0	1	1	7	2	0	7	0	0	0	0	5	17	2	1	0	0	1	0	1	0		
Butt-plot1-sph3-T	S.capillifolium	64	0	0	0	6	17	0	0	1	0	0	0	0	0	0	11	3	2	24	26	0	0	0	1	3	2	2	0	0	14	1	0	0		
Butt-plot1-sph4-T	S.medium	97	12	0	1	5	7	0	0	3	0	1	0	0	0	16	0	3	1	0	4	0	0	0	8	0	0	4	2	0	4	0	0	0		
Butt-plot1-sph5-T	S.medium	25	0	0	0	7	5	0	0	1	0	0	0	0	0	0	14	1	1	78	9	7	0	0	1	31	3	0	0	0	1	0	0	0		
Butt-plot2-sph1-T	S.capillifolium	3	63	0	0	30	10	0	0	7	0	0	1	0	0	1	39	13	0	8	0	0	0	0	0	13	0	0	0	0	1	0	0	0		
Butt-plot2-sph2-T	S.medium	4	1	0	1	22	4	0	0	0	0	0	2	0	0	0	21	37	3	50	0	0	0	0	9	1	0	4	0	10	0	1	0	0		
Butt-plot2-sph3-T	S.papillosum	7	0	0	1	33	20	5	0	2	0	2	3	0	0	0	24	11	3	34	0	0	0	0	2	8	3	0	0	0	11	0	0	0		
Butt-plot2-sph4-T	S.medium	77	27	0	0	12	2	0	0	3	0	1	0	0	8	1	5	2	8	7	0	0	0	6	1	2	0	2	0	0	0	1	0	1	0	
Butt-plot2-sph5-T	S.fallax	1	53	0	1	42	0	0	0	3	0	8	1	0	6	0	26	11	1	3	0	0	0	2	0	9	2	1	0	0	1	0	0	0		
Butt-plot3-sph2-T	S.palustre	2	1	20	0	24	4	0	0	0	0	0	3	0	1	0	15	15	0	22	0	2	0	0	2	62	6	2	0	3	0	0	1	0	0	
Butt-plot3-sph3-T	S.medium	22	1	0	0	8	8	0	0	1	0	0	1	1	0	1	52	9	2	28	24	0	0	0	0	4	1	0	0	0	0	0	0	0	0	
Butt-plot3-sph4-T	S.papillosum	112	6	0	0	12	0	0	0	3	0	1	1	0	0	1	11	6	0	10	1	0	0	0	4	16	0	2	1	0	0	0	0	2	0	0
Butt-plot3-sph5-T	S.fallax	2	13	0	0	30	0	0	0	2	0	0	7	0	0	2	14	33	1	16	0	0	0	1	2	30	6	1	0	0	2	0	4	0	0	
Butt-plot4-sph1-T	S.papillosum	60	0	0	0	19	19	0	0	3	0	1	1	0	0	2	6	3	0	13	44	0	0	0	0	5	0	1	0	0	0	0	1	0	0	0
Butt-plot4-sph2-T	S.papillosum	0	52	0	0	38	2	0	0	16	0	0	1	0	0	2	10	13	0	18	0	0	0	0	12	7	3	0	0	0	0	0	1	0	0	
Butt-plot4-sph3-T	S.papillosum	0	0	0	0	14	5	0	0	0	0	0	3	0	0	0	33	2	2	32	13	0	0	0	5	22	3	0	12	0	2	0	2	0	0	
Butt-plot4-sph4-T	S.papillosum	5	0	0	0	21	8	0	0	1	0	0	0	0	0	1	41	10	1	55	1	0	0	0	1	15	2	0	0	0	1	0	0	0	0	
Butt-plot4-sph5-T	S.medium	1	0	1	0	12	0	2	0	3	0	0	0	1	0	0	20	0	0	69	7	0	0	0	10	10	0	0	0	0	2	0	0	0	0	
Butt-plot5-sph1-T	S.fimbriatum	0	28	0	0	35	0	0	0	27	0	0	1	0	0	2	37	5	0	7	0	0	0	0	8	3	3	0	0	1	0	0	3	0	0	
Butt-plot5-sph2-T	S.capillifolium	76	1	0	0	11	3	0	0	27	0	0	0	0	0	3	30	9	2	4	0	0	0	0	3	0	0	2	0	0	0	0	0	0	0	0
Butt-plot5-sph3-T	S.papillosum	45	0	0	0	9	20	0	0	9	0	0	0	0	0	2	9	21	3	21	0	0	0	0	21	0	0	0	0	0	0	0	0	1	0	0
Butt-plot5-sph4-T	S.medium	29	0	0	0	16	13	0	0	6	0	0	0	0	0	3	20	6	5	32	2	6	0	1	0	27	1	1	2	0	0	0	0	0	0	0
Butt-plot5-sph5-T	S.papillosum	3	3	0	0	28	2	0	0	34	0	0	4	0	0	6	26	8	2	16	0	0	0	0	33	5	4	2	0	0	1	1	2	0	0	0
Butt-plot1-sph1-B	S.medium	0	0	0	0	18	8	0	0	1	0	1	5	3	0	2	6	32	0	40	62	0	0	0	3	0	0	1	0	12	0	0	0	0	0	
Butt-plot1-sph2-B	S.papillosum	82	7	0	1	12	3	0	0	1	0	3	4	0	0	6	10	5	0	11	0	0	0	2	4	0	17	0	0	0	0	0	0	0	0	0
Butt-plot1-sph3-B	S.capillifolium	31	3	0	0	9	12	0	0	0	0	0	0	0	0	9	10	0	30	23	0	0	0	0	4	0	6	3	0	25	0	0	0	0	0	
Butt-plot1-sph4-B	S.medium	64	18	0	0	3	2	0	0	6	0	11	0	0	31	0	5	1	1	3	1	0	0	1	12	0	9	0	0	4	0	0	0	0	0	
Butt-plot1-sph5-B	S.medium	3	0	0	0	10	6	0	0	1	0	9	2	6	0	2	0	21	1	65	25	1	0	0	0	4	1	1	0	0	4	0	1	0	0	
Butt-plot2-sph1-B	S.capillifolium	0	43	0	0	12	1	0	0	1	0	0	0	0	0	2	14	56	1	27	0	0	0	0	5	0	0	0	0	0	0	0	0	1	0	0
Butt-plot2-sph2-B	S.medium	3	12	0	0	19	2	0	0	0	0	0	7	0	0	0	1	54	3	45	0	0	0	0	0	1	1	1	0	25	0	1	0	0	0	
Butt-plot2-sph3-B	S.papillosum	3	0	0	0	17	16	0	0	1	0	3	13	0	0	0	0	9	2	50	0	0	0	0	6	5	2	0	1	34	0	0	0	0	0	
Butt-plot2-sph4-B	S.medium	40	43	0	1	4	1	0	0	5	0	1	0	21	2	8	6	0	7	0	0	0	5	0	0	13	1	0	0	1	0	0	1	0	0	
Butt-plot2-sph5-B	S.fallax	2	27	0	0	49	1	0	0	10	0	15	0	0	1	0	19	25	0	2	0	0	0	2	0	4	1	3	0	1	5	0	0	0	0	
Butt-plot3-sph2-B	S.palustre	0	1	8	1	24	3	0	0	4	0	0	1	1	0	2	4	48	0	53	1	0	0	1	0	4	10	7	1	0	1	0	4	0	0	
Butt-plot3-sph3-B	S.medium	15	1	0	0	11	9	0	0	0	0	0	0	3	0	0	9	23	2	55	22	0	0	0	11	3	0	0	0	9	0	0	1	0	0	
Butt-plot3-sph4-B	S.papillosum	7	3	0	0	32	1	0	0	20	0	0	3	0	0	5	35	15	1	24	0	0	0	2	0	2	3	5	4	0	1	0	1	0	0	
Butt-plot3-sph5-B	S.fallax	0	1	0	1	52	0	0	0	16	0	1	0	0	0	2	17	47	1	16	0	0	0	1	0	11	3	2	1	0	3	0	1	0	1	0
Butt-plot4-sph1-B	S.papillosum	39	0	0	0	7	10	0	0	0	0	1	1	1	0	0	14	0	3	30	45	0	0	0	1	3	5	3	3	1	1	0	1	0	0	
Butt-plot4-sph2-B	S.papillosum	0	40	0	0	41	4	0	1	7	0	0	3	0	0	0	21	11	0	17	0	0	0	0	6	10	0	0	0	0	0	0	0	1	0	0
Butt-plot4-sph3-B	S.papillosum	0	2	1	0	9	4	3	0	0	0	0	2	1	0	0	2	25	4	48	36	0	0	0	3	4	0	24	0	7	0	2	0	0	0	
Butt-plot4-sph4-B	S.papillosum	9	0	0	0	26	1	0	0	12	0	0	2	0	0	4	26	35	1	38	2	0	0	0	3	4	0	1	0	11	0	1	0	1	0	0
Butt-plot4-sph5-B	S.medium	0	0	0	0	15	3	0	0	6	0	0	1	3	0	0	0	25	18	1	41	25	0	0	0	9	6	0	13	0	13	0	0	0	0	0
Butt-plot5-sph1-B	S.fimbriatum	1	6	0	0	25	0	0	1	19	1	0	3	0	0	2	68	15	1	8	0	0	0	0	3	5	5	0	0	0	0	0	2	1	0	1
Butt-plot5-sph2-B	S.capillifolium	33	0	0	0	9	3	0	0	10	0	0	1	0	0	6	12	47	2	7	9	0	0	0	4	1	4	6	0	1	0	0	1	1	1	0
Butt-plot5-sph3-B	S.papillosum	15	1	0	0	9	5	0	0	5	0	0	2	0	0	2	1	89	0	26	0	1	0	0	2	3	1	0	0	3	0	0	0	0	0	0
Butt-plot5-sph4-B	S.medium	31	2	0	0	12	3	0	0	3	0	0	1	0	0	4	10	25	4	31	13	0	0	1	0	8	1	11	1	0	0	2	0	0	0	
Butt-plot5-sph5-B	S.papillosum	9	6	0	0	15	2	0	0	11	0	1	6	0	0	3	17	36	0	33	0	0	0	0	5	9	11	0	1	0	0	2	1	0	0	
KH-plot1-domspH-T	S.capillifolium	0	0	0	0	56	0	0	0	16	0	0	2	0	0	0	27	1	0	17	0	0	3	0	0	5	0	0	3	30	0	0	1	0	0	
KH-plot2-domspH-T	S.capillifolium	1	0	0	0	31	0	0	1	24	0	0	0	0	0	4	17	1	0	60	0	0	2	0	0	13	0	0	1	2	0	0	0	2	0	0
KH-plot3-domspH-T	S.capillifolium	0	0	0	0	13	0	0	2	16	0	3	0	0	0	7	17	1	0	18	0	0	4	0	0	52	0	0								

N-UC1-sph2-T	S.palustre	0	0	1	0	46	0	0	0	8	0	0	0	0	7	1	12	0	42	0	0	2	0	0	74	0	0	0	4	0	0	0	0		
N-UC1-sph3-T	S.capillifolium	0	0	0	0	3	0	0	0	25	0	1	0	0	0	9	0	46	0	43	0	0	0	0	31	0	0	10	0	0	0	0	0		
N-UC1-sph4-T	S.capillifolium	0	0	2	0	21	0	0	1	62	3	0	1	0	0	13	1	21	0	0	0	8	0	1	27	0	0	0	12	1	0	0	4		
N-UC1-sph5-T	S.capillifolium	0	0	0	1	53	0	0	0	14	0	0	0	0	0	9	0	33	0	30	0	0	0	27	0	0	2	1	0	0	0	1			
N-UC2-sph1-T	S.fallax	0	0	0	3	15	0	0	4	30	7	0	0	0	1	3	17	43	0	12	0	0	7	2	0	6	2	0	2	3	1	0	1	1	
N-UC2-sph2-T	S.capillifolium	0	0	0	0	2	0	0	0	61	1	0	0	0	0	23	0	10	0	2	0	0	7	0	39	0	0	0	7	0	0	0	0		
N-UC2-sph3-T	S.fallax	0	1	0	10	11	0	0	2	43	14	0	1	2	0	5	1	47	0	13	0	0	5	4	0	3	3	0	0	1	11	0	0	0	
N-UC2-sph4-T	S.fallax	0	0	0	2	7	0	0	0	38	0	0	0	1	0	8	2	35	0	31	0	0	3	0	30	0	0	1	2	2	0	1	1		
N-UC2-sph5-T	S.fallax	0	0	0	0	9	0	0	0	32	39	0	0	0	0	13	2	49	0	4	0	0	2	0	20	1	0	0	1	0	0	0	0		
N-UC3-sph1-T	S.capillifolium	0	0	0	1	6	0	0	0	31	45	1	0	0	0	10	0	34	0	19	0	0	0	0	53	0	0	0	0	0	0	0	1		
N-UC3-sph2-T	S.capillifolium	0	0	0	0	70	0	0	0	35	0	0	1	0	0	5	0	44	0	4	0	0	0	0	4	0	0	0	2	0	0	0	4		
N-UC3-sph3-T	S.capillifolium	0	0	0	0	26	0	0	0	29	0	0	0	0	0	5	0	63	0	2	0	0	1	0	39	0	0	7	1	0	0	0	0		
N-UC3-sph4-T	S.fallax	0	0	0	0	28	0	0	0	20	0	0	0	0	0	18	15	56	0	3	0	0	0	0	16	0	0	2	5	0	0	0	0		
N-UC3-sph5-T	S.fallax	0	0	0	1	112	0	0	0	8	5	1	0	0	0	1	6	7	0	3	0	0	5	1	4	0	0	3	0	0	0	0	0		
N-UC4-sph1-T	S.fimbriatum	0	1	0	7	1	0	0	0	47	0	0	0	0	0	29	4	59	0	3	0	0	0	0	6	1	0	0	2	0	0	0	0		
N-UC4-sph2-T	S.fallax	0	0	0	0	10	0	0	1	37	1	3	0	1	0	5	30	28	0	5	0	0	0	0	27	1	0	5	31	0	0	0	0		
N-UC4-sph3-T	S.fallax	0	0	0	0	1	1	0	0	44	0	0	0	1	0	9	23	35	0	0	0	0	1	0	25	2	0	2	13	0	0	1	0		
N-UC4-sph4-T	S.fimbriatum	0	0	0	8	13	0	0	0	36	31	1	5	1	1	0	44	3	0	6	0	2	4	1	0	8	3	0	1	0	0	1	2	4	
N-UC4-sph5-T	S.fimbriatum	0	0	0	4	34	0	0	0	47	41	0	1	0	0	4	16	9	0	2	0	2	2	2	0	0	0	0	0	0	0	0	0	3	
N-UC5-sph1-T	S.fallax	0	0	0	3	42	0	0	0	40	2	0	0	0	0	9	0	10	0	3	0	0	2	0	51	2	0	0	2	0	0	0	0	0	
N-UC5-sph2-T	S.fallax	0	0	0	6	42	0	0	1	40	12	1	0	0	0	13	14	11	0	8	0	0	0	0	15	1	0	0	0	0	0	0	0	0	
N-UC5-sph3-T	S.fimbriatum	0	0	0	23	5	0	0	0	18	32	1	3	0	6	9	11	15	0	10	0	0	16	1	0	2	2	0	5	1	7	0	1	0	
N-UC5-sph4-T	S.papillosum	0	0	0	0	4	0	0	0	14	0	0	0	1	1	9	5	20	0	67	0	0	2	0	42	0	0	0	1	0	0	0	0	0	
N-UC5-sph5-T	S.capillifolium	0	0	0	0	8	0	0	0	19	0	0	0	0	0	4	0	28	0	35	0	0	1	0	60	0	0	2	3	0	0	0	0	0	
N-UC1-sph1-B	S.capillifolium	0	0	0	0	23	0	0	1	38	1	0	0	0	0	12	0	61	0	41	0	0	1	0	10	0	0	2	0	0	0	1	0	0	
N-UC1-sph2-B	S.palustre	0	0	0	0	47	0	0	0	21	0	0	0	0	0	6	0	14	0	58	0	0	0	0	20	0	0	0	6	0	0	0	0	2	
N-UC1-sph3-B	S.capillifolium	0	0	0	0	8	0	0	0	16	0	0	0	0	0	11	0	70	0	30	0	0	0	0	21	0	0	12	0	0	0	0	0	3	
N-UC1-sph4-B	S.capillifolium	0	0	5	0	26	0	0	13	17	0	4	1	0	0	19	0	42	0	14	0	0	0	0	12	0	0	2	6	0	0	0	0	2	
N-UC1-sph5-B	S.capillifolium	0	0	1	0	9	0	0	0	12	0	1	0	0	0	7	0	72	0	55	0	0	1	0	12	0	0	5	0	0	0	0	0	0	
N-UC2-sph1-B	S.fallax	0	0	0	3	11	0	0	5	12	0	1	1	5	2	3	2	38	0	54	0	0	7	1	8	2	0	3	1	1	0	0	0	0	
N-UC2-sph2-B	S.capillifolium	0	0	0	2	1	0	0	1	56	1	0	0	10	0	12	0	38	0	29	0	0	2	1	0	11	0	0	1	0	2	0	2	0	
N-UC2-sph3-B	S.fallax	0	0	0	15	14	0	0	0	18	1	5	1	7	5	50	0	41	0	0	0	8	2	0	5	2	0	1	0	14	0	1	0	0	
N-UC2-sph4-B	S.fallax	0	0	0	4	6	0	0	0	23	0	1	0	2	0	11	0	51	0	49	0	0	1	0	17	0	0	1	1	0	0	1	0	0	
N-UC2-sph5-B	S.fallax	0	0	0	1	27	0	0	0	13	0	0	0	3	0	18	0	82	0	28	0	0	0	0	6	0	0	2	1	0	0	0	0	0	
N-UC3-sph1-B	S.capillifolium	0	0	0	0	4	0	0	0	6	0	0	0	0	0	7	0	46	0	83	0	0	0	0	24	1	0	0	0	0	0	0	0	0	
N-UC3-sph2-B	S.capillifolium	0	0	0	0	12	0	0	0	39	0	0	0	0	0	3	0	83	0	14	0	0	0	0	4	0	0	3	0	1	0	0	0	11	
N-UC3-sph3-B	S.capillifolium	0	0	0	0	9	0	0	0	71	0	0	0	0	0	6	0	40	0	4	0	0	0	0	6	0	0	35	0	0	0	0	0	10	
N-UC3-sph4-B	S.fallax	0	0	0	0	15	0	0	0	31	0	0	0	2	0	21	0	83	0	5	0	0	0	0	11	0	0	12	13	0	0	0	0	0	
N-UC3-sph5-B	S.fallax	0	0	0	8	12	0	0	0	10	5	0	0	5	0	3	60	12	0	22	0	0	0	1	0	10	15	0	1	2	1	1	0	6	
N-UC4-sph1-B	S.fimbriatum	0	0	0	7	2	0	0	0	30	0	0	0	0	0	21	2	72	0	20	0	0	0	4	1	6	5	0	2	2	0	0	1	0	
N-UC4-sph2-B	S.fallax	0	0	0	1	11	0	0	0	26	1	0	0	1	0	5	9	52	0	11	0	0	0	0	17	0	0	5	28	2	0	0	0	0	
N-UC4-sph3-B	S.fallax	0	0	0	0	0	0	0	0	9	0	0	0	0	0	3	0	80	0	15	0	0	0	0	25	8	0	7	6	0	0	0	0	0	
N-UC4-sph4-B	S.fimbriatum	0	0	0	13	4	0	0	1	20	5	1	3	0	0	3	38	7	0	22	0	0	14	3	0	2	3	0	1	0	0	2	2	1	0
N-UC4-sph5-B	S.fimbriatum	0	0	0	7	12	0	0	0	54	56	0	1	0	0	2	20	5	0	9	0	0	4	1	0	3	1	0	2	0	0	0	5	1	0
N-UC5-sph1-B	S.fallax	0	0	0	16	20	0	0	0	22	0	1	0	0	0	6	8	40	0	20	0	0	0	2	29	4	0	2	0	0	0	0	0	0	
N-UC5-sph2-B	S.fallax	0	0	0	4	16	0	0	0	43	0	0	0	0	0	13	0	15	0	36	0	0	0	1	0	20	5	0	2	0	0	0	0	0	
N-UC5-sph3-B	S.fimbriatum	0	0	0	31	6	0	0	0	21	22	1	8	0	2	5	13	17	0	13	0	0	13	1	0	2	3	0	4	2	3	4	2	0	0
N-UC5-sph4-B	S.papillosum	0	0	0	0	9	0	0	0	14	0	1	4	1	0	6	0	42	0	53	0	0	0	1	0	27	0	0	4	1	0	1	1	0	0
N-UC5-sph5-B	S.capillifolium	0	0	2	0	7	0	0	0	13	0	0	0	0	0	4	0	46	0	70	0	0	0	0	17	0	0	4	2	0	0	0	0	0	0
M-UC1-sph1-T	S.capillifolium	0	0	0	0	31	0	0	0	10	0	0	0	1	0	3	1	29	5	6	0	0	12	2	0	25	15	0	6	7	0	0	1	0	
M-UC1-sph2-T	S.fallax	1	2	0	45	15	1	0	0	15	1	1	3	0	0	2	3	16	0	17	1	0	4	1	0	11	6	0	3	0	0	1	0	0	0
M-UC1-sph3-T	S.capillifolium	0	0	0	1	10	0	54	0	10	0	0	0	0	0	3	0	26	0	1	0	0	1	0	8	1	0	14	23	0	0	0	3	0	0
M-UC1-sph4-T	S.capillifolium	0	0	0	4	9	0	0	0	29	0	1	0	0	1	7	3	49	3	8	0	1	5	1	0	37	2	0	1	0	1	0	0	0	0
M-UC1-sph5-T	S.fallax	0	1	0	6	18	0	0	1	21	7	0	0	1	0	5	42	8	2	14	0	0	2	0	17	6	2	3	0	1	0	0	0	0	
M-UC2-sph1-T	S.fallax	3	1	0	0	30																													

M-UC5-sph1-T	S.palustre	0	0	0	0	9	0	0	0	65	2	1	1	0	0	4	0	41	0	3	0	0	0	0	22	0	0	3	16	0	0	0	13	
M-UC5-sph2-T	S.capillifolium	1	0	0	0	18	0	0	5	34	0	0	3	0	0	3	0	70	0	3	0	0	0	0	50	0	0	9	2	0	0	0	0	
M-UC5-sph3-T	S.palustre	0	0	0	0	18	0	0	0	23	0	0	1	0	0	4	0	52	0	17	0	0	0	0	19	1	0	50	24	0	0	0	6	
M-UC5-sph4-T	S.capillifolium	0	0	0	1	20	0	3	1	30	0	0	5	0	0	7	1	79	0	0	0	0	2	0	0	24	0	0	1	0	0	0	2	2
M-UC5-sph5-T	S.fallax	0	0	0	0	18	0	0	0	21	0	2	2	0	0	1	1	25	4	1	0	0	0	0	38	0	0	34	19	0	0	0	0	
M-UC1-sph1-B	S.capillifolium	0	0	0	0	3	0	0	0	9	0	0	1	2	0	5	0	61	4	27	0	0	0	0	9	14	0	21	2	0	0	0	1	
M-UC1-sph2-B	S.fallax	0	0	0	36	3	1	0	2	33	1	0	1	0	1	9	37	14	0	7	0	0	0	2	4	3	1	0	0	0	1	1	1	
M-UC1-sph3-B	S.capillifolium	0	0	0	0	8	0	0	4	47	0	0	0	0	0	3	2	35	0	8	0	0	0	0	0	1	0	47	13	0	0	1	4	
M-UC1-sph4-B	S.capillifolium	0	0	0	0	3	8	1	0	19	0	0	0	0	0	14	14	54	2	20	0	0	0	0	9	1	2	1	1	1	0	0	1	
M-UC1-sph5-B	S.fallax	1	1	0	15	1	0	0	3	22	0	0	1	2	0	0	49	5	2	28	3	0	6	0	2	10	2	2	1	0	0	0	0	0
M-UC2-sph1-B	S.fallax	0	0	0	0	6	0	1	0	62	0	0	0	3	0	13	25	4	0	12	0	0	0	0	23	0	0	0	0	0	0	0	14	
M-UC2-sph2-B	S.fallax	0	0	0	0	9	0	0	7	27	0	0	0	0	0	5	60	2	0	5	0	0	1	0	15	1	0	4	8	0	0	0	19	
M-UC2-sph3-B	S.capillifolium	0	0	0	0	10	0	0	3	40	0	0	0	0	0	7	5	27	0	9	0	0	1	0	28	4	0	24	4	0	0	0	6	
M-UC2-sph4-B	S.capillifolium	0	0	0	0	8	0	0	2	74	0	0	2	0	0	7	20	34	0	20	0	0	0	0	2	0	0	18	0	1	0	0	2	7
M-UC3-sph1-B	S.capillifolium	0	0	0	0	19	0	1	0	17	0	0	2	0	0	4	2	48	2	62	0	0	0	0	6	0	0	7	0	0	0	3	1	
M-UC3-sph2-B	S.capillifolium	0	0	0	0	5	0	0	0	31	0	0	3	1	0	11	23	17	0	52	0	0	0	0	10	0	0	3	0	0	0	2	2	
M-UC3-sph3-B	S.capillifolium	0	0	0	0	23	0	0	9	23	0	0	0	0	0	9	6	38	0	28	0	0	0	0	10	0	0	21	1	0	0	2	5	
M-UC3-sph4-B	S.capillifolium	1	0	0	2	10	7	0	0	8	0	0	5	9	0	6	10	24	0	43	0	0	0	0	17	8	0	0	1	0	0	0	1	
M-UC3-sph5-B	S.capillifolium	0	0	0	0	30	0	0	2	18	1	2	0	0	0	3	13	37	0	9	0	0	0	0	16	0	0	5	39	0	0	0	1	
M-UC4-sph1-B	S.capillifolium	0	0	0	0	9	0	0	1	27	0	2	3	0	0	2	32	12	0	37	0	0	1	0	2	0	0	29	12	0	0	0	3	
M-UC4-sph2-B	S.capillifolium	0	0	0	0	14	0	0	6	38	0	2	0	0	0	1	6	28	1	12	0	0	0	8	0	0	29	14	0	0	0	2	0	
M-UC4-sph3-B	S.fallax	0	0	0	0	2	0	1	0	12	0	0	0	0	0	0	18	32	4	36	0	0	0	29	3	0	13	1	0	0	0	0	0	
M-UC4-sph4-B	S.fallax	0	0	0	1	28	0	0	7	26	1	0	0	0	0	9	14	17	1	21	0	0	0	19	0	0	10	4	0	0	0	1	3	
M-UC4-sph5-B	S.fallax	0	1	0	0	23	0	0	5	31	0	0	4	0	0	6	18	20	0	24	0	0	0	28	7	0	6	0	0	0	0	0	2	
M-UC5-sph1-B	S.palustre	0	0	0	0	14	0	0	2	51	0	7	5	0	0	11	6	38	0	8	0	0	0	13	0	0	22	11	0	0	3	19		
M-UC5-sph2-B	S.capillifolium	0	0	0	0	18	0	0	11	55	1	0	1	0	0	7	11	23	0	17	0	0	0	0	0	0	0	27	0	0	0	0	16	
M-UC5-sph3-B	S.palustre	0	0	0	0	12	0	0	0	29	0	1	8	9	0	6	8	23	1	39	0	0	0	9	4	0	14	8	0	0	4	0	0	
M-UC5-sph4-B	S.capillifolium	0	0	0	0	6	0	0	18	65	0	2	0	0	0	16	7	40	0	2	0	0	0	4	0	0	8	0	0	0	2	7	0	0
M-UC5-sph5-B	S.fallax	0	0	1	0	11	0	0	0	20	0	2	3	0	0	2	4	18	9	9	0	0	0	7	0	0	61	17	0	0	0	7	0	0
W-2013-UC1-T	S.palustre	0	0	0	3	1	0	0	0	45	0	0	1	0	0	3	9	12	2	14	0	0	24	0	0	38	1	0	1	0	0	3	0	0
W-2013-UC2-T	S.capillifolium	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	11	0	32	0	0	0	18	0	0	83	0	0	0	8	0	0	1	0
W-2013-UC3-T	S.palustre	0	0	0	0	7	0	0	0	30	0	0	0	0	0	0	12	18	0	7	0	0	4	0	0	74	0	0	3	2	0	0	0	0
W-2013-UC1-B	S.palustre	0	0	0	0	10	0	0	0	70	0	0	0	0	0	4	25	11	3	21	0	0	1	0	0	13	3	0	2	0	0	1	0	0
W-2013-UC2-B	S.capillifolium	0	0	0	0	22	0	0	0	74	0	0	0	0	0	2	0	29	0	38	0	0	0	0	8	0	0	1	1	0	0	8	0	0
W-2013-UC3-B	S.palustre	0	0	0	1	7	0	0	0	23	0	0	2	0	0	3	0	43	0	37	0	0	3	0	0	27	0	0	36	0	0	0	0	0
N-2013-Fldom-sph1-T	S.papillosum	0	0	1	0	20	0	0	4	25	0	14	0	0	0	2	16	41	0	18	0	0	0	30	0	0	0	6	0	1	0	0	0	0
N-2013-Fldom-sph2-T	S.capillifolium	0	0	0	0	12	0	0	0	143	0	0	0	0	0	2	1	5	0	2	0	0	10	0	0	5	0	0	0	0	3	0	0	0
N-2013-Fldom-sph3-T	S.capillifolium	0	0	0	0	1	0	0	0	31	0	0	0	0	0	11	68	5	0	28	0	0	6	0	0	24	0	0	5	5	0	0	0	0
M-2013-FI1-sph(a)-T	S.capillifolium	0	0	3	0	13	1	15	0	5	0	0	2	0	1	3	5	0	5	0	0	5	0	0	56	0	0	1	1	0	0	0	0	0
M-2013-FI1-sph(b)-T	S.palustre	0	0	4	0	16	0	0	0	15	0	0	4	0	0	5	10	8	0	20	0	0	7	0	0	51	5	0	1	8	0	0	1	0
M-2013-FI2-sph-T	S.capillifolium	0	0	0	18	3	25	0	6	30	0	0	0	1	0	0	36	0	0	19	0	0	1	0	0	25	0	0	4	2	0	0	0	0
M-2013-FI3-sph-T	S.capillifolium	0	0	0	0	3	0	0	1	14	2	0	0	0	0	4	24	21	0	33	0	0	3	0	0	11	1	0	25	19	0	0	2	0
W-2013-FI1-T	S.capillifolium	0	0	0	0	48	0	0	0	2	0	0	0	0	0	2	2	13	0	0	0	0	0	0	50	0	0	9	3	0	0	0	0	0
W-2013-FI2-T	S.papillosum	0	0	0	1	0	0	0	0	2	0	0	0	0	0	1	22	6	1	57	0	0	2	3	0	54	14	0	3	4	0	0	0	0
W-2013-FI3-T	S.papillosum	0	0	0	1	1	0	0	0	0	0	0	3	0	0	0	26	9	0	58	0	0	0	0	0	27	4	0	35	3	0	0	1	0
N-2015-Fldom-sph1-T	S.cuspidatum	0	0	1	13	19	0	0	0	32	0	0	0	0	0	1	41	3	0	8	0	0	11	0	0	23	2	0	1	5	0	0	0	5
N-2015-Fldom-sph2-T	S.capillifolium	0	0	0	0	64	0	0	0	12	1	0	0	0	0	2	5	6	0	14	0	0	2	0	0	55	0	0	1	3	0	0	0	0
N-2015-Fldom-sph3-T	S.capillifolium	38	0	0	0	2	1	0	0	5	1	1	2	0	0	1	19	7	0	25	0	0	21	1	0	23	0	0	3	29	0	0	0	0
M-2015-FI1-sph-T	S.capillifolium	0	0	0	0	48	0	11	0	7	3	1	1	0	0	3	0	41	0	11	0	0	2	0	0	31	0	0	1	10	0	0	0	0
M-2015-FI2-sph-T	S.capillifolium	0	0	0	0	25	0	0	0	6	0	0	0	0	0	1	1	23	0	20	0	0	6	0	1	2	0	0	14	47	0	0	1	0
M-2015-FI3-sph-T	S.capillifolium	0	0	0	0	27	5	0	0	2	1	0	1	0	0	0	7	1	1	14	0	0	4	0	0	87	0	0	2	3	0	0	0	0
W-2015-FI1-T	S.capillifolium	0	0	0	2	2	0	0	0	8	0	0	2	0	0	1	20	4	0	10	0	0	15	0	0	83	0	0	8	3	0	0	0	0
W-2015-FI2-T	S.fallax	0	0	0	0	6	0	0	0	26	0	0	0	0	2	6	0	0	11	0	0	3	0	0	85	1	0	9	19	1	0	0	0	0
W-2015-FI3-T	S.capillifolium	0	0	0	0	28	0	0	0	21	0	0	0	0	0	7	23	0	12	0	0	0	0	0	59	0	0	28	0	0	0	1	0	0
N-2018-Fldom-sph																																		



M-2013-FI2-sph-B	S.capillifolium	0	0	3	6	11	0	0	0	15	0	0	0	4	0	6	23	11	0	57	0	0	0	0	0	9	2	0	7	1	0	1	3	3
M-2013-FI3-sph-B	S.capillifolium	0	0	0	4	10	0	0	0	17	0	0	1	2	0	2	32	4	1	47	0	0	1	0	0	15	5	0	10	10	0	0	2	2
W-2013-FI1-B	S.capillifolium	0	0	0	2	31	0	0	0	5	0	1	7	3	0	0	0	24	7	26	0	0	1	0	0	23	0	0	27	4	1	0	2	0
W-2013-FI2-B	S.papulosum	0	3	0	3	8	1	0	0	52	0	0	1	1	0	0	3	23	0	30	0	0	0	6	0	13	18	0	2	0	0	1	0	0
W-2013-FI3-B	S.papulosum	0	0	0	0	2	0	0	0	3	0	0	17	0	0	0	1	22	0	54	0	0	0	0	0	37	5	0	31	5	0	0	1	0
N-2015-Fldom-sph1-B	S.cuspidatum	2	0	0	15	8	2	0	1	21	0	0	0	0	16	2	12	33	0	34	0	0	9	0	0	16	0	0	1	0	0	0	0	2
N-2015-Fldom-sph2-B	S.capillifolium	0	0	0	0	13	0	0	1	11	0	0	0	0	0	3	0	40	0	76	0	0	0	0	0	21	0	0	4	0	0	0	0	0
N-2015-Fldom-sph3-B	S.capillifolium	43	0	0	0	13	0	1	0	5	0	0	0	2	0	7	0	43	0	39	0	0	1	0	0	12	0	0	4	6	0	0	1	0
M-2015-FI1-sph-B	S.capillifolium	0	0	0	0	5	0	12	0	6	0	0	11	0	0	0	7	7	0	26	0	0	0	0	0	34	0	0	0	48	0	0	4	0
M-2015-FI2-sph-B	S.capillifolium	0	0	0	1	13	0	0	0	15	0	0	2	2	0	5	48	3	0	38	0	0	0	0	0	9	0	0	19	14	0	0	2	2
M-2015-FI3-sph-B	S.capillifolium	0	0	0	1	7	6	0	0	17	0	0	1	4	0	3	32	1	1	66	0	0	0	3	1	29	1	0	7	9	4	0	0	1
W-2015-FI1-B	S.capillifolium	0	0	0	0	2	0	0	0	15	0	0	2	0	0	1	1	66	0	59	0	0	0	0	0	8	0	0	18	0	0	0	0	0
W-2015-FI2-B	S.fallax	0	0	0	0	9	0	0	0	14	3	0	2	6	0	1	25	1	0	19	0	0	12	4	0	33	6	0	19	18	1	0	1	0
W-2015-FI3-B	S.capillifolium	0	0	0	0	16	0	0	0	6	0	0	11	0	0	0	2	34	0	68	0	0	0	0	0	13	3	0	40	1	0	0	5	0
N-2018-Fldom-sph1-B	S.papulosum	0	0	0	0	3	0	0	0	10	0	0	3	15	1	4	1	45	0	57	0	0	0	8	0	15	0	0	5	0	0	2	0	0
N-2018-Fldom-sph2-B	S.papulosum	0	0	0	0	8	0	0	0	20	0	0	4	3	1	5	7	22	0	66	0	0	0	5	0	7	7	0	0	2	0	0	1	0
N-2018-Fldom-sph3-B	S.fallax	0	0	0	0	6	0	0	0	14	2	0	0	0	0	12	0	69	0	3	0	0	1	0	0	38	0	0	1	0	0	0	0	0
M-2018-FI1-sph-B	S.capillifolium	0	0	0	0	26	0	1	3	45	1	0	1	0	0	5	23	2	0	34	0	0	0	0	0	10	0	0	12	8	0	0	0	5
M-2018-FI2-sph-B	S.capillifolium	0	0	0	0	5	0	1	1	10	0	12	0	0	0	8	37	22	0	33	0	0	0	0	0	45	0	0	2	5	0	0	2	0
M-2018-FI3-sph-B	S.capillifolium	0	0	0	0	25	0	0	0	19	0	0	2	1	0	15	24	25	0	24	0	0	0	0	0	15	0	0	4	12	0	0	1	1
W-2018-FI1-B	S.papulosum	0	0	0	0	4	0	0	0	2	0	1	0	0	0	1	3	47	5	48	0	0	0	0	0	45	0	0	8	7	0	0	2	0
W-2018-FI2-B	S.capillifolium	0	0	0	0	1	0	0	0	8	0	0	0	0	0	1	21	45	0	5	0	0	0	0	77	0	0	1	0	0	0	1	0	0
W-2018-FI3-B	S.papulosum	0	0	0	0	0	0	0	0	1	0	0	0	2	0	1	0	17	0	38	0	0	0	3	0	71	1	0	37	5	0	0	0	0
N-2013-LB-sph1-T	S.capillifolium	0	0	0	0	5	0	0	0	15	1	0	0	0	0	8	9	0	0	10	0	0	1	0	0	71	0	0	15	18	0	0	0	2
N-2013-LB-sph2-T	S.cuspidatum	0	0	0	12	4	0	0	0	55	0	0	1	11	4	45	7	0	5	0	0	0	3	3	0	1	2	0	1	2	3	0	0	5
N-2013-LB-sph3-T	S.capillifolium	0	0	0	0	0	0	0	0	7	0	0	0	0	0	3	4	2	0	4	0	0	0	0	0	116	0	0	15	1	2	0	0	2
M-2013-LB-sph1-T	S.palustre	0	0	10	0	21	0	0	0	5	0	0	1	0	0	1	0	11	3	20	0	0	0	0	0	83	0	0	0	8	0	0	1	0
M-2013-LB-sph2-T	S.fallax	0	0	0	0	16	0	0	0	23	0	0	0	0	0	10	0	50	2	6	0	0	2	0	0	36	1	0	3	3	1	0	0	0
M-2013-LB-sph3-T	S.capillifolium	0	0	0	0	38	0	0	0	23	0	0	0	0	0	2	0	40	0	15	0	0	0	1	0	36	1	0	5	2	0	0	5	5
W-2013-LB1-T	S.papulosum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	176	0	0	1	0	0	0	0	0
W-2013-LB2-T	S.papulosum	0	0	0	0	3	0	0	0	50	0	0	12	0	0	1	5	13	0	24	0	0	8	9	0	11	11	0	10	0	1	0	1	0
W-2013-LB3-T	S.palustre	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	3	0	4	0	0	5	2	0	136	1	0	4	0	0	0	0	0
N-2015-LB1-T	S.capillifolium	0	0	0	0	2	0	0	0	2	0	0	0	0	0	1	11	1	0	20	0	0	2	0	0	126	0	0	0	3	0	0	1	0
N-2015-LB2-T	S.palustre	0	0	0	0	1	0	0	0	12	0	0	1	1	0	4	20	1	0	0	0	0	1	0	0	122	0	0	0	1	0	0	1	0
N-2015-LB3-T	S.fimbriatum	0	0	0	4	4	0	0	0	55	0	0	2	1	0	1	40	2	0	7	0	0	6	0	0	25	0	0	2	5	1	0	3	0
M-2015-LB1-T	S.palustre	0	0	32	0	5	0	0	0	0	0	0	2	0	0	0	1	2	5	5	0	0	0	0	0	110	1	0	1	0	0	0	0	0
M-2015-LB2-T	S.fallax	0	0	0	0	4	0	0	0	8	0	0	0	0	0	0	8	0	3	4	0	0	0	0	0	119	0	0	0	0	0	0	0	0
M-2015-LB3-T	S.fallax	0	0	0	3	42	0	0	0	9	0	0	0	0	0	1	31	0	2	4	0	0	0	0	0	50	3	0	0	2	0	0	2	0
W-2015-LB1-T	S.capillifolium	0	0	0	0	19	0	2	0	10	0	0	0	0	0	0	2	15	1	10	0	0	0	0	0	87	0	0	8	3	1	0	0	0
W-2015-LB2-T	S.capillifolium	0	0	0	0	2	0	0	0	0	0	0	2	0	0	1	21	6	0	4	0	0	5	0	0	112	2	0	11	0	0	0	0	0
W-2015-LB3-T	S.papulosum	0	0	0	5	5	0	0	0	6	0	0	0	0	0	1	0	6	0	22	0	0	25	0	0	73	0	0	2	0	0	0	1	0
N-2018-LB1-T	S.papulosum	0	1	0	2	7	0	0	0	16	0	0	4	1	22	5	36	2	0	24	0	0	8	8	0	11	4	0	0	2	0	0	4	0
N-2018-LB2-T	S.fimbriatum	0	0	0	1	2	0	0	0	29	0	0	0	0	0	3	82	0	0	2	0	0	0	0	0	30	9	0	1	0	0	0	0	0
N-2018-LB3-T	S.papulosum	0	0	1	0	11	0	0	0	6	1	3	2	0	0	1	9	1	0	41	0	0	0	0	0	76	0	0	0	10	0	0	2	0
M-2018-LB1-T	S.palustre	0	0	0	0	4	0	0	0	4	0	0	1	0	0	1	11	0	5	38	0	0	0	0	0	68	1	0	3	13	0	0	2	0
M-2018-LB2-T	S.capillifolium	0	0	0	0	14	0	0	0	5	0	0	0	1	0	0	16	0	7	58	0	0	0	0	0	34	1	0	13	16	0	0	0	0
M-2018-LB3-T	S.capillifolium	0	0	0	2	33	0	0	0	11	0	0	0	0	0	2	27	0	0	25	0	0	0	0	0	40	0	0	3	8	0	0	3	0
W-2018-LB1-T	S.capillifolium	0	0	0	3	6	0	0	0	8	0	2	4	7	0	0	10	22	0	16	0	0	16	0	0	16	2	0	29	11	7	0	4	0
W-2018-LB2-T	S.papulosum	0	0	0	0	2	0	0	0	23	0	0	1	0	0	0	3	5	0	16	0	0	21	0	0	76	1	0	3	1	3	0	0	0
W-2018-LB3-T	S.palustre	0	0	0	0	71	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	26	0	0	38	1	0	2	0	1	0	1	0
N-2013-LB-sph1-B	S.capillifolium	0	0	0	0	13	0	0	0	4	0	1	6	0	0	1	1	19	0	25	0	0	0	0	0	29	0	0	55	15	0	0	2	0
N-2013-LB-sph2-B	S.cuspidatum	0	0	0	16	4	0	0	0	1	22	0	0	2	2	3	73	4	0	9	0	0	7	0	0	3	5	0	1	0	0	0	0	8
N-2013-LB-sph3-B	S.capillifolium	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	1	37	0	19	0	0	0	0	0	72	0	0	22	0	0	0	2	0
M-2013-LB-sph1-B	S.palustre	0	0																															

N-2018-LB1-B	S.papillosum	0	0	0	5	8	1	0	0	11	0	0	4	2	9	1	41	4	0	29	0	0	3	7	0	15	7	0	0	4	1	0	2	0
N-2018-LB2-B	S.fimbriatum	0	0	0	6	8	0	0	0	3	0	0	1	0	0	1	50	11	0	10	0	0	0	3	0	37	24	0	1	3	0	0	4	0
N-2018-LB3-B	S.papillosum	0	0	0	0	20	0	0	0	26	0	3	0	0	0	4	0	9	0	53	0	0	0	0	0	20	3	0	1	4	0	0	6	0
M-2018-LB1-B	S.palustre	0	0	0	0	4	0	0	0	11	0	0	14	0	0	0	7	0	3	31	0	0	2	0	0	29	2	0	24	25	0	1	2	0
M-2018-LB2-B	S.capillifolium	0	0	0	0	3	0	1	0	30	0	0	13	2	0	6	9	24	0	30	0	1	1	0	0	15	0	0	20	6	0	0	2	0
M-2018-LB3-B	S.capillifolium	0	0	0	0	15	0	0	0	16	0	0	4	0	0	1	7	16	0	50	0	0	0	0	0	28	0	0	18	7	0	0	2	0
W-2018-LB1-B	S.capillifolium	0	0	1	1	6	0	0	0	1	0	0	2	4	0	1	29	24	0	13	0	0	12	0	0	14	8	0	22	34	1	0	0	0
W-2018-LB2-B	S.papillosum	0	0	0	0	4	0	0	0	11	0	0	2	0	0	0	0	14	0	87	0	0	4	0	0	38	1	0	6	6	0	0	0	0
W-2018-LB3-B	S.palustre	0	0	0	3	19	0	1	0	8	0	0	9	0	0	0	4	21	0	46	0	0	12	0	0	7	3	0	16	10	0	0	9	0

**Appendix 3b.** Raw data table of testate amoebae in surface (0-5 cm) and deep (50-55 cm) peat in relation to heather management (i.e., burning (FI) versus cutting (LB) versus uncut (UC: control). **Reference sites:** Butt-Butterburn (border mire), KH-Kield Head, WE-Whitlee English side, WS-Whitlee Scottish side. **Experimental managed sites:** N-Nidderdale, M-Mosssdale, W-Whitendale.

Plot	Amp.fla	Amp.wri	Arc.dis.s	Ass.mus	Ass.sem	Cen.arc	Cen.cas	Cor.dub	Cry.ovl	Cyc.arc.l	Cyc.arc.s	Dif.lei	Dif.pul	Eug.rot	Eug.stri	Eug.tub	Hel.pet	Hel.syl	Hya.pap	Hya.sub	NA.1	Neb.co.bo	Neb.fla	Neb.gri	Neb.mil	Neb.tin	Pse.ful	Tri.arc
Butt-plot1-(50-55cm)	31	36	2	10	4	0	0	1	0	0	3	0	0	0	0	4	3	7	0	0	2	0	4	0	6	2	1	1
Butt-plot2-(50-55cm)	24	12	5	8	5	7	0	2	0	0	17	2	0	1	1	1	8	4	0	0	1	1	0	11	2	2	0	
Butt-plot3-(50-55cm)	11	20	0	2	4	1	0	0	0	0	35	0	4	0	0	1	4	4	0	0	1	0	0	19	1	1	2	
KH-plot1-(50-55cm)	0	1	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	1	0	94	0	0	0	0	0	0	0	
KH-plot2-(50-55cm)	0	1	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	109	0	0	0	0	1	1	0	
KH-plot3-(50-55cm)	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	1	0	0	95	0	0	0	0	0	0	1	
WE-plot1-(50-55cm)	35	4	0	10	4	0	0	0	0	0	5	0	1	0	0	0	1	5	1	21	0	3	0	0	16	6	0	
WE-plot2-(50-55cm)	6	1	0	15	2	0	0	0	0	0	9	0	0	0	2	1	1	3	0	64	0	0	1	0	6	0	2	
WE-plot3-(50-55cm)	2	0	0	9	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	97	0	0	0	0	4	4	0	
WS-plot1-(50-55cm)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	0	0	0	0	1	0	2	
WS-plot2-(50-55cm)	81	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41	0	0	0	0	1	0	0	
WS-plot3-(50-55cm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	110	0	0	0	0	0	0	2	
N-UC1-2013-(50-55cm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	162	0	0	0	0	0	0	0	
N-UC2-2013-(50-55cm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	182	0	1	0	0	1	0	13	
N-UC3-2013-(50-55cm)	1	0	0	1	0	0	0	0	0	0	8	0	0	0	0	0	5	1	15	27	0	0	0	0	15	7	0	
M-UC1-2013-(50-55cm)	12	5	2	3	1	0	0	0	0	0	26	0	4	1	0	11	2	7	8	2	1	7	2	0	32	6	1	
M-LB1-2013-(50-55cm)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	105	0	0	0	0	1	0	0	
W-UC1-2013-(50-55cm)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	102	0	0	0	0	0	0	0	
W-UC2-2013-(50-55cm)	4	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	
W-UC3-2013-(50-55cm)	0	0	0	1	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	95	0	0	0	0	0	0	0	
Butt-plot1-0-5 cm	32	15	4	16	8	1	0	0	0	0	6	1	8	0	0	5	1	34	0	0	4	1	2	2	16	2	0	
Butt-plot2-0-5 cm	11	17	18	9	3	1	0	1	0	1	13	7	4	0	1	10	3	39	1	0	2	2	5	7	4	0	1	
Butt-plot3-0-5 cm	3	3	9	22	0	10	1	7	0	5	65	4	27	0	2	21	0	46	0	0	2	2	2	7	39	4	25	
KH-plot1-0-5 cm	4	2	0	1	0	0	0	2	1	2	23	0	5	0	2	0	25	0	59	0	0	3	1	8	1	17	5	
KH-plot2-0-5 cm	0	0	3	10	0	0	1	0	11	1	27	0	3	0	3	5	1	24	0	27	0	1	4	0	26	3	7	
KH-plot3-0-5 cm	2	2	3	2	1	0	0	0	8	4	23	1	3	0	0	2	0	16	0	40	0	0	1	0	22	5	21	
WE-plot1-0-5 cm	2	7	0	4	1	0	0	0	1	0	33	4	2	1	1	10	0	36	0	1	2	2	5	2	39	1	2	
WE-plot2-0-5 cm	0	1	7	14	1	0	0	5	0	0	14	8	0	0	0	32	0	42	0	3	1	5	4	1	5	9	12	
WE-plot3-0-5 cm	1	0	1	13	1	0	0	1	0	0	6	0	5	1	1	8	1	40	0	5	0	5	0	0	53	11	3	
WS-plot1-0-5 cm	3	1	0	8	3	0	0	0	0	0	33	0	2	2	0	22	0	26	0	4	2	8	2	0	37	5	11	
WS-plot2-0-5 cm	2	0	0	10	0	2	3	0	4	19	0	1	2	0	0	9	0	20	0	26	0	0	0	0	38	14	9	
WS-plot3-0-5 cm	0	0	1	4	2	0	0	2	1	0	24	0	0	0	0	11	0	15	0	4	1	2	1	0	40	2	56	
N-UC1-0-5 cm	0	0	4	14	0	0	1	11	3	0	8	0	0	5	0	30	0	29	0	19	0	6	0	0	1	7	16	
N-UC2-0-5 cm	0	0	5	3	0	0	0	5	5	3	3	0	0	3	0	14	0	4	0	66	0	0	0	0	6	5	28	
N-UC3-0-5 cm	0	0	0	8	0	0	0	14	1	0	8	0	6	7	0	18	0	11	0	58	0	0	0	0	5	24	4	
M-UC1-(0-5cm)	6	2	36	6	0	0	0	9	2	1	3	3	0	1	17	29	0	15	0	5	2	4	1	9	3	1	1	
M-UC2-(0-5cm)	0	0	5	10	0	0	2	8	0	2	11	1	0	7	0	27	0	26	0	50	0	4	3	0	11	5	5	
M-UC3-(0-5cm)	0	0	4	5	3	0	1	4	0	5	38	0	5	1	0	9	0	21	0	35	0	3	6	0	7	10	4	
W-UC1-(0-5cm)	0	0	0	7	0	0	3	4	0	8	14	0	0	2	0	23	0	7	0	107	0	0	1	0	5	12	1	
W-UC2-(0-5cm)	0	0	1	6	0	0	0	1	0	1	29	0	0	1	0	6	0	6	0	104	0	0	1	0	4	7	8	
W-UC3-(0-5cm)	0	0	3	12	0	0	5	2	0	1	10	0	1	2	0	44	0	11	0	50	0	0	2	0	9	15	4	
N-2013-F11-(0-5 cm)	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	138	0	0	0	0	0	0	35	0
N-2013-F12-(0-5 cm)	0	0	11	7	0	0	0	16	12	1	0	0	2	1	0	24	0	4	0	12	0	7	0	0	0	12	54	
N-2013-F13-(0-5 cm)	0	0	0	11	0	0	0	3	4	2	0	0	0	4	0	36	0	24	0	34	1	1	0	0	0	7	23	
M-2013-F11-(0-5cm)	0	0	4	4	0	0	0	0	0	1	11	0	1	1	0	11	0	23	0	60	1	0	5	0	10	9	9	
M-2013-F12-(0-5cm)	0	0	1	4	0	0	1	0	0	3	50	0	2	2	0	8	0	5	0	54	0	0	2	0	7	6	9	
M-2013-F13-(0-5cm)	0	0	1	10	0	0	6	5	0	1	31	0	3	2	0	7	0	22	0	9	0	2	1	0	27	30	1	
W-2013-F11-(0-5cm)	0	0	3	7	0	0	2	2	0	5	19	0	0	1	0	24	0	15	0	76	0	5	0	0	14	2	2	
W-2013-F12-(0-5cm)	0	0	1	10	0	0	3	2	0	2	0	0	0	0	0	14	0	6	0	53	0	0	0	0	15	24	14	
W-2013-F13-(0-5cm)	0	0	0	2	0	0	1	1	0	2	9	0	0	0	0	15	0	10	0	110	0	3	2	0	8	14	3	
N-2015-F11-(0-5 cm)	0	0	0	4	0	0	0	0	0	2	5	0	0	0	0	4	0	3	0	47	0	0	0	0	23	2	47	
N-2015-F12-(0-5 cm)	0	0	0	9	0	0	0	1	1	0	15	0	0	1	0	4	0	8	0	58	0	0	0	0	6	17	22	
N-2015-F13-(0-5 cm)	0	0	4	5	0	0	0	1	0	2	15	4	1	0	0	21	0	18	0	68	0	1	5	0	0	6	9	
M-2015-F11-(0-5cm)	0	0	0	7	0	0	1	1	0	2	14	0	1	1	0	20	0	22	0	52	0	3	0	0	21	17	4	
M-2015-F12-(0-5cm)	0	0	0	6	1	0	1	2	0	3	28	0	1	0	0	26	0	23	0	22	0	2	2	0	10	19	7	
M-2015-F13-(0-5cm)	0	0	3	4	0	0	0	2	0	3	42	0	0	2	0	15	0	25	0	42	17	2	6	14	0	8	8	
W-2015-F11-(0-5cm)	0	0	0	8	0	0	0	0	0	3	21	0	0	0	0	13	0	4	0	91	0	5	1	0	4	6	0	
W-2015-F12-(0-5cm)	0	0	0	12	0	0	9	0	0	13	25	0	0	0	0	11	0	10	0	33	0	0	0	0	20	17	2	
W-2015-F13-(0-5cm)	0	0	0	3	0	0	0	0	0	2	6	0	0	0	0	6	0	4	0	140	0	4	0	0	2	1	1	
N-2018-F11-(0-5 cm)	0	0	0	4	0	0	0	0	0	0	22	0	2	1	0	17	0	20	0	34	0	0	0	0	11	21	9	
N-2018-F12-(0-5 cm)	0	0	2	8	0	0	0	2	7	5	0	0	1	5	0	13	0	8	0	93	0	3	0	0	1	10	13	
N-2018-F13-(0-5 cm)	0	0	10	1	0	0	0	2	59	0	2	0	0	1	0	17	0	15	0	8	0	2	0	0	22	8	24	
M-2018-F11-(0-5cm)	0	0	7	6	1	0	0	3	0	4	31	0	0	1	0	4	0	12	0	70	0	3	2	0	14	2	21	
M-2018-F12-(0-5cm)	0	0	3	9	0	0	11	15	0	7	16	0	0	10	0	4	0	19	0	17	0	0	1	0	16	15	0	
M-2018-F13-(0-5cm)	0	0	0	19</																								

W-2018-FI3-(0-5cm)	0	0	2	6	0	0	3	1	0	1	7	0	0	0	0	21	0	5	0	104	0	4	1	0	5	12	2	10
N-2013-LB1-(0-5)	0	0	0	8	0	0	1	2	0	1	3	0	0	0	0	20	0	11	0	75	0	0	0	0	20	12	1	10
N-2013-LB2-(0-5)	0	0	2	13	1	0	1	4	0	1	21	0	0	0	0	40	0	22	0	21	2	7	3	0	2	6	8	6
N-2013-LB3-(0-5)	0	0	1	9	0	0	1	5	0	10	27	0	0	4	0	22	0	27	0	26	0	4	1	0	0	14	4	6
N-2015-LB1-(0-5)	0	0	0	11	0	0	3	1	0	7	0	0	0	0	1	9	0	6	0	59	0	0	0	0	0	19	3	13
N-2015-LB2-(0-5)	0	0	0	10	0	0	4	0	0	13	26	0	0	0	0	12	0	3	0	39	0	1	0	0	3	3	8	10
N-2015-LB3-(0-5)	0	0	0	10	0	0	3	0	0	5	20	0	0	0	0	4	0	2	0	54	0	0	0	0	5	9	64	2
N-2018-LB1-(0-5)	1	0	8	2	0	0	0	0	0	2	10	0	0	0	0	7	0	9	0	66	0	1	0	0	0	5	52	9
N-2018-LB2-(0-5)	0	0	0	19	0	0	1	1	0	5	7	0	0	0	0	13	0	34	0	17	0	2	0	0	10	21	9	14
N-2018-LB3-(0-5)	0	0	0	4	0	0	2	0	0	7	7	0	0	0	0	6	0	6	0	48	0	3	5	0	0	19	32	9
M-2013-LB1-(0-5)	0	1	4	2	0	3	1	3	0	1	16	0	0	2	4	9	5	31	0	2	2	7	8	0	15	0	1	5
M-2013-LB2-(0-5)	0	0	0	6	0	0	0	0	0	0	2	0	4	0	0	16	0	10	0	100	0	0	2	0	22	4	9	10
M-2013-LB3-(0-5)	0	0	7	7	1	0	0	1	0	7	21	0	0	0	1	12	0	39	0	23	0	11	2	0	6	10	1	17
M-2015-LB1-(0-5)	0	0	2	6	0	0	1	0	0	5	34	0	0	0	0	5	0	14	0	44	0	2	20	0	9	17	5	12
M-2015-LB2-(0-5)	0	0	5	7	1	0	0	3	0	5	17	0	0	0	4	5	1	32	0	24	1	5	9	0	3	12	28	4
M-2015-LB3-(0-5)	0	0	4	11	0	0	0	2	0	4	39	0	0	0	3	13	0	24	0	9	1	12	9	0	8	7	3	6
M-2018-LB1-(0-5)	0	0	0	3	0	0	0	2	0	4	10	0	0	0	2	4	0	7	0	79	0	0	3	0	32	15	7	4
M-2018-LB2-(0-5)	0	0	0	4	0	0	0	1	0	2	7	0	0	0	0	22	0	35	0	26	0	1	4	0	14	12	22	10
M-2018-LB3-(0-5)	0	0	14	0	0	0	0	0	0	2	2	0	0	0	8	5	0	46	0	2	0	7	7	0	44	3	0	6
W-2013-LB1-(0-5)	0	0	1	3	0	0	1	0	0	0	10	0	0	1	0	5	0	16	0	121	0	2	6	0	16	2	4	5
W-2013-LB2-(0-5)	0	0	1	2	2	0	1	0	0	6	5	0	0	0	0	26	0	17	0	51	10	2	3	0	7	12	1	11
W-2013-LB3-(0-5)	0	0	2	0	0	0	1	1	0	0	5	0	0	0	0	1	0	5	0	140	0	0	0	0	3	6	4	5
W-2015-LB1-(0-5)	0	0	2	15	0	0	4	0	0	6	10	0	0	0	0	14	0	5	0	100	0	0	0	0	14	11	1	6
W-2015-LB2-(0-5)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	5	0	6	0	156	0	0	0	0	0	2	29	2
W-2015-LB3-(0-5)	0	0	0	11	0	0	4	0	0	3	16	0	0	0	0	12	0	21	0	61	0	0	0	0	2	30	18	3
W-2018-LB1-(0-5)	0	0	0	8	0	0	2	3	0	2	39	0	0	3	0	19	0	19	0	35	0	0	0	0	16	22	7	3
W-2018-LB2-(0-5)	0	0	2	2	0	0	0	1	0	2	22	0	0	0	0	5	0	8	0	113	0	0	1	0	3	6	16	6
W-2018-LB3-(0-5)	0	0	0	7	0	0	1	0	0	3	8	0	0	0	0	6	0	11	0	90	0	0	0	0	7	19	11	16

**Appendix 3c.** Raw data table of environmental variables from surface peat (0-5 cm) in relation to heather management (i.e., burning (FI) versus cutting (LB) versus uncut (UC: control). **Reference sites:** Butt-Butterburn (border mire), KH-Kield Head, WE-Whitlee English side, WS-Whitlee Scottish side. **Experimental managed sites:** N-Nidderdale, M-Mossdale, W-Whitendale.

Plot	Heather (%)	Moss (%)	Sph (%)	Sedge (%)	Herb (%)	Brush/Litter (%)	Bare (%)	Slope (°)	Elevation (m)	Moss_height (cm)	Sph_height (cm)	Lit_height (cm)	depth (cm)	WC (%)	BD (g/cm³)	pH	N (%)	C (%)	DOC (mg/l)	TbN (mg/l)	Cu (µg/g)	Zn (µg/g)	Pb (µg/g)	Al (µg/g)	Ca (µg/g)	Fe (µg/g)	K (µg/g)	Mg (µg/g)	Na (µg/g)	Mn (µg/g)	Cd (µg/g)	P (µg/g)	Si (µg/g)	As (µg/g)
Burt-plot1-0-5 cm	5	1	80	25	0	2	2	1	123	4	8	1	651	96.18	0.04	4.51	1.19	44.82	8.73	0.81	18.31	269.63	162.16	752.09	1240.31	1036.76	155.02	899.98	197.82	7.44	3.87	359.63	96.64	8.62
Burt-plot2-0-5 cm	6	2	75	45	2	10	0	1	123	4	14	3	651	95.53	0.04	4.62	1.34	43.43	7.06	0.52	7.44	486.12	169.02	783.96	1182.88	2304.06	732.26	1252.95	167.33	22.64	2.82	445.85	78.96	0.00
Burt-plot3-0-5 cm	2	85	40	5	8	1	1	1	117	4	10	3	700	95.08	0.05	4.58	1.92	44.66	13.63	1.96	6.09	242.67	51.73	932.13	1811.00	2423.35	426.20	1111.90	166.35	8.52	1.56	805.09	38.56	0.00
KH-plot1-0-5 cm	65	85	1	8	0	12	1	3	315	5	5	4.5	170	90.46	0.10	4.26	1.60	47.08	26.56	2.11	20.90	92.91	218.63	723.04	549.51	2688.68	459.51	757.33	215.19	14.52	5.56	866.55	50.84	1.87
KH-plot2-0-5 cm	60	40	45	12	0	7	1	2	455	5	7	2	203	89.76	0.09	4.31	1.22	46.00	21.83	2.00	9.91	169.39	91.39	615.14	893.32	2618.56	668.91	700.61	165.12	14.99	3.69	576.03	43.00	0.00
KH-plot3-0-5 cm	28	33	36	25	0	4	1	4	455	6	11	3	180	92.07	0.08	4.35	1.71	45.81	16.30	1.67	19.43	88.88	371.15	815.12	770.92	2328.82	288.09	929.17	205.29	7.38	2.82	627.69	43.60	4.76
WE-plot1-0-5 cm	28	5	92	17	0	3	1	4	570	3	7	3	293	95.03	0.04	4.46	1.11	43.17	17.15	1.69	4.85	221.95	82.83	448.12	1004.01	1853.70	714.63	1472.32	248.41	18.26	4.42	462.31	55.64	5.12
WE-plot2-0-5 cm	16	2	96	24	1	3	1	3	413	3	8	3	293	92.92	0.06	4.53	1.50	45.60	21.06	2.22	17.41	151.77	278.07	1001.24	1274.36	2711.28	311.01	803.14	167.42	8.25	3.82	490.21	45.65	0.85
WE-plot3-0-5 cm	42	33	45	18	0	3	0	3	457	5	8	2.5	287	91.67	0.08	4.45	1.72	46.39	14.28	1.99	17.12	180.45	240.76	845.93	1245.47	1887.96	292.45	1093.33	195.16	12.41	2.37	645.69	44.78	0.00
WS-plot1-0-5 cm	38	3	95	20	8	2	1	4	566	2.5	9	1	184	92.02	0.07	4.37	1.50	45.55	13.30	1.33	9.11	156.50	164.16	679.70	1436.34	2735.96	448.86	1178.40	208.38	23.22	3.24	711.89	47.48	0.00
WS-plot2-0-5 cm	53	26	30	8	0	9	1	6	568	3.5	11	3	173	88.97	0.09	4.37	1.68	47.08	24.41	1.93	33.59	107.56	276.24	981.38	883.95	1865.63	522.26	875.72	194.02	13.14	2.96	846.61	36.76	1.66
WS-plot3-0-5 cm	46	1	98	6	0	2	0	6	567	2	10	1	130	92.27	0.07	4.46	1.86	46.49	21.09	2.30	15.48	80.63	158.16	940.77	752.48	2179.45	471.18	736.11	138.74	10.18	2.60	865.04	36.44	2.44
N-UC1-(0-5 cm)	55	35	8	5	0	2	0	2	482	4	8	3	167	89.91	0.09	4.47	1.80	46.45	32.32	2.89	18.87	248.86	241.70	834.05	1596.20	1785.01	480.82	1175.97	198.89	14.70	5.83	764.80	59.85	0.00
N-UC2-(0-5 cm)	45	15	65	30	0	5	0	3	475	1	6	1.5	154	86.42	0.13	4.09	2.11	50.86	27.31	3.58	48.56	100.81	257.07	1152.21	1175.56	954.60	608.00	730.17	164.98	19.32	3.34	1076.44	47.19	0.19
N-UC3-(0-5 cm)	73	78	12	13	0	7	0	2	476	3	4.5	2	194	85.52	0.14	4.10	1.67	47.92	35.49	3.19	13.94	177.64	128.07	760.26	1445.60	5209.54	516.62	1023.44	218.00	20.93	4.36	654.62	42.14	0.00
M-UC1-(0-5cm)	22	5	75	8	1	15	0	2	309	2.5	5.5	2	141	93.87	0.11	4.51	1.25	44.83	24.46	2.72	9.60	449.76	78.10	295.18	1465.52	5106.85	649.20	782.36	188.76	42.11	5.57	571.33	23.64	3.86
M-UC2-(0-5cm)	45	65	15	5	1	12	0	2	328	5	7	3.5	143	88.73	0.13	4.15	2.02	47.72	33.28	2.17	15.08	138.96	135.54	514.44	1421.67	3123.31	427.85	867.92	178.16	12.21	3.14	544.49	22.71	0.00
M-UC3-(0-5cm)	35	15	60	15	0	5	0	3	374	7	6	2.5	161	89.64	0.11	4.21	1.40	45.78	39.82	3.08	11.89	174.47	74.95	435.12	1930.06	4518.11	457.37	779.07	157.51	12.66	4.28	570.38	27.75	0.00
W-UC1-(0-5cm)	18	12	70	12	2	6	0	2	330	4	7	1	218	87.19	0.16	4.16	2.03	50.93	19.94	2.79	29.01	101.46	178.89	1060.92	1407.87	1693.60	320.81	688.38	145.65	18.14	2.63	688.38	26.37	3.42
W-UC2-(0-5cm)	17	45	12	30	2	9	3	2	376	3.5	12	1	174	86.42	0.18	4.30	2.28	49.04	23.86	3.32	54.33	54.52	304.17	997.56	891.74	649.79	132.95	650.08	131.05	13.87	2.77	467.91	17.85	8.08
W-UC3-(0-5cm)	35	95	0	7	1	5	0	2	365	3	8	2	167	85.23	0.16	4.07	2.49	47.89	30.44	3.46	22.56	69.87	202.17	856.37	1345.73	1407.08	216.05	940.00	146.59	7.29	2.46	574.05	25.77	0.00
N-2013-F11-(0-5 cm)	95	25	0	3	1	5	0	7	450	3	NA	3	118	73.44	0.19	4.08	2.17	52.33	25.11	2.51	23.07	70.77	367.54	1270.69	867.17	1677.80	311.88	485.40	118.48	13.99	2.31	787.51	57.54	0.00
N-2013-F12-(0-5 cm)	70	90	1	22	0	3	0	2	458	1	9	3	150	88.18	0.13	4.28	2.10	49.03	35.07	3.03	11.18	410.21	103.10	757.68	1311.09	2874.21	578.62	1044.41	236.81	27.61	4.53	1054.43	23.35	2.33
N-2013-F13-(0-5 cm)	35	45	30	15	0	2	0	2	480	5	8	1	200	87.08	0.13	4.06	2.07	48.82	25.00	2.38	28.12	147.46	361.09	1254.11	1677.83	1696.78	378.11	981.66	168.71	9.78	3.56	911.86	26.22	1.49
M-2013-F11-(0-5cm)	8	35	45	7	0	12	0	10	336	4.5	11	2	64	89.56	0.13	4.21	1.78	46.60	19.62	2.04	12.41	123.98	139.45	513.03	1845.36	2859.16	382.03	942.14	137.97	19.97	3.36	651.79	25.43	0.00
M-2013-F12-(0-5cm)	12	12	85	10	2	1	0	10	394	4	8.5	1	121	90.01	0.12	4.26	2.03	45.33	17.76	2.85	19.87	110.13	167.37	726.30	1406.45	1776.41	425.72	745.08	152.73	11.01	1.69	919.58	27.68	0.00
M-2013-F13-(0-5cm)	8	9	58	13	1	10	1	12	364	4.5	5.5	2	162	84.88	0.19	4.31	1.67	47.72	18.16	3.42	10.23	95.44	123.07	317.17	2004.21	2035.04	315.32	872.09	113.15	48.95	1.72	545.13	28.75	0.00
W-2013-F11-(0-5cm)	12	7	85	18	3	4	2	3	359	3.5	10	0.5	157	88.54	0.13	4.16	2.24	48.79	26.52	2.80	30.22	261.86	608.39	1028.83	1604.36	1828.57	307.14	916.58	154.23	25.81	2.84	672.75	31.28	0.00
W-2013-F12-(0-5 cm)	20	4	90	8	10	3	0	2	365	5	6.5	1.5	109	87.90	0.13	4.16	2.39	49.54	21.03	2.25	34.44	102.44	840.26	1257.27	1000.95	1026.61	278.49	706.02	154.23	15.76	3.09	630.38	26.29	0.00
W-2013-F13-(0-5cm)	5	7	65	45	15	5	2	2	373	6	7	2.5	111	87.85	0.17	4.09	2.27	48.60	18.51	1.96	45.75	140.29	398.24	1000.85	1163.70	2303.18	280.65	809.55	119.87	23.84	2.36	636.46	26.10	0.00
N-2015-F11-(0-5 cm)	1	89	0	0	3	5	2	14	366	2	10	0.5	107	81.98	0.15	4.30	2.40	49.37	15.52	3.53	34.86	141.21	351.33	1259.42	1362.48	1818.44	506.59	792.33	146.20	28.50	3.70	953.68	48.04	0.47
N-2015-F12-(0-5 cm)	75	90	0	3	0	8	0	5	475	3.5	NA	2.5	126	82.10	0.19	4.21	2.08	47.84	21.82	2.97	16.52	153.17	165.58	716.07	1933.85	2302.31	717.39	886.70	185.63	18.66	3.26	905.90	31.80	0.00
N-2015-F13-(0-5 cm)	25	20	65	12	0	5	0	6	485	5	9	2	247	90.57	0.13	4.35	1.63	48.67	13.71	1.88	10.52	156.27	118.15	767.52	1459.93	4499.34	401.95	1040.08	183.17	61.97	4.03	681.71	27.08	1.47
M-2015-F11-(0-5cm)	7	85	0	4	3	33	2	11	336	3	9	2	61	86.32	0.13	4.28	1.77	46.31	16.62	2.59	11.56	52.46	138.10	516.96	1961.12	4758.56	269.52	1010.90	124.96	41.95	2.47	450.09	21.40	0.00
M-2015-F12-(0-5 cm)	15	25	75	10	0	5	0	14	361	6	8	4	150	88.60	0.11	4.32	1.95	45.95	27.37	3.92	12.54	111.56	163.44	560.29	1759.45	2764.73	387.51	894.69	148.97	21.56	2.69	697.43	21.35	3.14
M-2015-F13-(0-5 cm)	12	22	70	12	0	5	0	14	366	4	9	1	126	81.26	0.12	4.29	2.53	46.11	19.13	3.02	10.16	97.63	131.13	332.47	1482.88	4381.92	341.14	382.20	154.60	19.10	3.33	794.55	30.33	0.59
W-2015-F11-(0-5 cm)	15	35	45	12	0	2	0	6	382	5	5	1	128	86.04	0.17	4.16	2.22	50.40	23.32	3.33	67.18	71.35	390.82	1139.93	1055.99	1785.68	422.38	852.00	161.37	14.40	2.67	855.25	27.36	1.30
W-2015-F12-(0-5 cm)	8	80	0	15	10	6	1	3	423																									

M-2018-LB2-(0-5)	10	55	25	0	0	30	0	6	377	2	5.5	2.5	127	92.02	0.09	4.41	1.19	45.43	39.52	4.00	12.46	124.79	178.71	605.88	1567.71	2567.94	402.50	1125.10	142.84	17.98	2.53	666.81	29.72	2.08
M-2018-LB3-(0-5)	25	15	65	3	0	5	0	4	376	4	9	4	95	93.24	0.07	4.53	1.29	44.69	24.93	3.42	8.58	165.69	24.24	222.01	2005.81	3054.95	477.69	1271.51	171.56	60.58	3.16	434.76	24.27	0.00
W-2018-LB1-(0-5cm)	20	75	0	0	1	8	1	5	423	2	NA	2.5	125	84.38	0.14	4.65	2.32	49.60	29.10	3.91	35.99	79.62	298.48	1105.63	1190.51	1798.20	511.28	1019.11	136.32	40.80	2.93	786.05	26.30	0.00
W-2018-LB2-(0-5cm)	5	40	10	6	3	12	7	2	370	1	6	1.5	123	89.59	0.16	4.72	2.55	47.75	19.49	3.13	18.76	158.50	102.79	775.52	1389.96	1651.49	362.52	936.05	139.32	41.26	2.87	669.26	23.36	0.00
W-2018-LB3-(0-5cm)	12	65	0	0	7	15	3	8	378	1.5	NA	1.5	121	83.39	0.18	4.24	2.13	48.44	20.61	4.73	16.56	75.88	31.20	301.29	1906.14	626.83	368.23	773.71	85.94	58.16	1.88	516.96	28.65	0.00



**Appendix 3d.** Raw data table of functional trait values of selected testate amoebae. ave\_len: average body length, ave\_width: average body width, ape\_len: aperture length, ape\_width: aperture width, ape\_type: 1-lobose; 0-filose, test\_silicon: 1-TA with silica shell; 0-TA with non-silica shell, MTA: mixotrophic testate amoebae; 1-MTA and 0-Non MTA.

Species	biovolume ( $\mu\text{m}^3$ )	ave_len ( $\mu\text{m}$ )	ave_wid ( $\mu\text{m}$ )	ape_len ( $\mu\text{m}$ )	ape_wid ( $\mu\text{m}$ )	ape_type	test_silicon	MTA
Amp.fla	16050.09	60.72	26.79	7.10	5.32	1	0	1
Amp.wri	37193.37	61.89	47.31	8.17	6.94	1	0	1
Arc.cat	245699.77	123.41	109.93	27.27	21.97	1	0	0
Arc.dis.s	49032.47	70.99	68.54	23.53	21.67	1	0	0
Ass.mus	12537.38	45.07	34.34	11.51	5.41	0	1	0
Ass.sem	84850.70	77.62	66.50	22.56	9.70	0	1	0
Bul.ind	894692.56	140.94	160.36	63.63	13.49	1	0	0
Cen.aer	127861.60	75.69	67.84	33.82	18.93	1	0	0
Cen.arc	242972.14	120.46	113.53	40.84	36.90	1	0	0
Cen.cas	188226.99	89.16	74.60	38.88	23.37	1	0	0
Cor.con	15264.64	54.10	32.80	15.30	11.70	0	1	0
Cor.dub	4918.09	35.16	23.51	9.91	7.46	0	1	0
Cry.ovl	3353.52	21.93	16.95	4.44	4.44	0	0	0
Cyc.arc.l	68546.97	63.99	63.99	32.98	32.98	1	0	0
Cyc.arc.s	25391.80	45.95	45.95	23.76	23.76	1	0	0
Deh.jap	58690.37	71.73	53.02	26.79	7.66	0	1	0
Dif.lei	138304.45	86.67	70.82	32.68	23.36	1	0	0
Dif.luc	146186.83	89.12	61.43	29.25	23.09	1	0	0
Dif.pul	9446.20	32.56	23.21	9.07	9.07	1	0	0
Eug.rot	9564.96	40.32	26.36	14.22	9.37	0	1	0
Eug.stri	27681.75	59.31	36.17	15.37	11.99	0	1	0
Eug.tub	35224.07	62.36	40.74	16.23	12.66	0	1	0
Hel.pet	219803.30	110.33	83.77	48.52	5.96	1	1	0
Hel.syl	42966.93	66.11	48.25	20.46	5.29	1	1	0
Hya_pap	141568.78	121.11	73.33	33.59	7.51	1	0	1
Hya.ele	50779.23	91.08	43.75	17.26	5.25	1	0	0
Hya.sub	35132.16	63.30	39.94	11.18	7.23	1	0	0
NA.1	207701.33	102.53	97.46	23.61	12.98	0	1	0
Neb.car	749844.59	193.28	129.20	40.55	18.44	1	1	0
Neb.co.bo	298819.60	123.72	96.07	26.22	14.72	1	1	0
Neb.fla	156032.77	81.82	92.82	21.02	13.25	1	1	0
Neb.gri	73494.69	73.79	56.47	18.87	14.94	1	0	0
Neb.mil	40082.55	73.44	42.80	17.52	6.14	1	1	0
Neb.tin	120689.74	91.95	68.67	19.56	11.32	1	1	0
Pla.lab	120348.71	77.05	77.33	34.11	16.05	1	0	0
Pse.ful	9446.20	32.56	23.21	9.07	9.07	1	0	0
Sph.len	18272.58	53.30	31.80	16.10	2.00	1	1	0
Tri.arc	484644.26	126.31	119.30	29.55	24.69	1	0	0
Tri.lin	3129.69	30.20	13.20	6.30	6.30	1	1	0

**Appendix 4 (Chapter 5).** Raw data table of testate amoebae in relation to peatland restoration and the corresponding environmental variables.

**Appendix 4a.** Raw data table of testate amoebae on *Sphagnum* mosses in relation to peatland restoration (i.e., consisted of degraded (DEG), post\_restoration (RES) and least modified (LM) areas in each restoration site). T: top 3 cm; B: bottom 3 cm.

**Reference sites:** border mire (Butt-Butterburn), KH-Kield Head, WE-Whitlee English side, WS-Whitlee Scottish side. **Restoration managed sites:** Cray-Cray Moss, Fleet-Fleet Moss, Stake-Stake Moss.

Plot	sph_type	Amp fla	Amp wri	Arc cat	Arc dis	Ass mus	Ass sem	Cen arc	Cen cas	Cor dub	Cyc arc	Cyc arc.s	Deh jap	Dif lei	Dif luc	Eug rot	Eug str	Eug tub	Hel pet	Hel syl	Hya ele	Hya pap	Hya sub	Neb car	Neb co.bo	Neb fla	Neb gri	Neb mil	Neb tin	Pse ful	Tri arc	
Butt-plot1-sph1-T	S.medium	0	0	0	0	22	26	0	0	2	0	1	0	0	0	0	8	17	1	27	61	0	0	2	0	0	2	0	1	0		
Butt-plot1-sph2-T	S.papillosum	97	2	0	0	8	2	5	0	2	0	1	0	1	0	1	7	2	0	7	0	0	5	17	2	1	0	0	1	1		
Butt-plot1-sph3-T	S.capillifolium	64	0	0	0	6	17	0	0	1	0	0	0	0	0	11	3	2	24	26	0	0	1	3	2	2	0	0	14	0		
Butt-plot1-sph4-T	S.medium	97	12	0	1	5	7	2	0	3	1	0	0	16	1	0	3	1	0	4	0	0	8	0	0	4	2	0	4	0		
Butt-plot1-sph5-T	S.medium	25	0	0	0	7	5	0	0	1	0	0	0	0	0	14	1	1	78	9	7	0	1	31	3	0	0	0	1	0		
Butt-plot2-sph1-T	S.capillifolium	3	63	0	0	30	10	0	0	7	0	1	0	0	0	1	39	13	0	8	0	0	0	13	0	0	0	0	1	0		
Butt-plot2-sph2-T	S.medium	4	1	0	1	22	4	0	0	0	2	0	0	0	0	21	37	3	50	0	0	0	9	1	0	4	0	10	1			
Butt-plot2-sph3-T	S.papillosum	7	0	0	1	33	20	1	0	2	2	3	0	0	0	24	11	3	34	0	0	0	2	8	3	0	0	0	11	0		
Butt-plot2-sph4-T	S.medium	77	27	0	0	12	2	2	0	3	1	0	0	8	0	1	5	2	8	7	0	0	1	2	0	2	0	0	0	0		
Butt-plot2-sph5-T	S.fallax	1	53	0	1	42	0	1	0	3	8	1	0	6	5	0	26	11	1	3	0	0	0	9	2	1	0	0	1	0		
Butt-plot3-sph2-T	S.palustre	2	1	20	0	24	4	0	0	0	0	3	0	1	0	0	15	15	0	22	0	2	0	2	62	6	2	0	3	0		
Butt-plot3-sph3-T	S.medium	22	1	0	0	8	8	0	0	1	0	1	1	0	0	1	52	9	2	28	24	0	0	4	1	0	0	0	0	0		
Butt-plot3-sph4-T	S.papillosum	112	6	0	0	12	0	0	0	3	1	1	0	0	0	1	11	6	0	10	1	0	0	4	16	0	2	1	0	0		
Butt-plot3-sph5-T	S.fallax	2	13	0	0	30	0	1	0	2	0	7	0	0	0	2	14	33	1	16	0	0	2	30	6	1	0	0	2	4		
Butt-plot4-sph1-T	S.papillosum	60	0	0	0	19	19	0	0	3	1	1	0	0	0	2	6	3	0	13	44	0	0	5	0	1	0	0	0	0		
Butt-plot4-sph2-T	S.papillosum	0	52	0	0	38	2	0	0	16	0	1	0	0	0	2	10	13	0	18	0	0	0	12	7	3	0	0	0	1		
Butt-plot4-sph3-T	S.papillosum	0	0	0	0	14	5	0	0	0	0	3	0	0	0	0	33	2	2	32	13	0	0	5	22	3	0	12	0	2		
Butt-plot4-sph4-T	S.papillosum	5	0	0	0	21	8	0	0	1	0	0	0	0	0	1	41	10	1	55	1	0	0	1	15	2	0	0	0	1	0	
Butt-plot4-sph5-T	S.medium	1	0	1	0	12	0	0	0	3	0	0	1	0	0	0	20	0	0	69	7	0	0	10	10	0	0	0	0	2	0	
Butt-plot5-sph1-T	S.fimbriatum	0	28	0	0	35	0	0	0	27	0	1	0	0	0	2	37	5	0	7	0	0	0	8	3	3	0	0	1	3		
Butt-plot5-sph2-T	S.capillifolium	76	1	0	0	11	3	0	0	27	0	1	0	0	0	3	30	9	2	4	0	0	0	0	3	0	0	2	0	0	0	
Butt-plot5-sph3-T	S.papillosum	45	0	0	0	9	20	0	0	9	0	0	0	0	0	2	9	21	3	21	0	0	0	21	0	0	0	0	0	1	0	
Butt-plot5-sph4-T	S.medium	29	0	0	0	16	13	0	0	6	0	0	0	0	0	3	20	6	5	32	2	6	0	27	1	1	2	0	0	0	0	
Butt-plot5-sph5-T	S.papillosum	3	3	0	0	28	2	0	0	34	0	4	0	0	0	6	26	8	2	16	0	0	0	33	5	4	2	0	0	1	0	
Butt-plot1-sph1-B	S.medium	0	0	0	0	18	8	0	0	1	1	5	3	0	0	2	6	32	0	40	62	0	0	3	0	0	1	0	12	0	0	
Butt-plot1-sph2-B	S.papillosum	82	7	0	1	12	3	3	0	1	3	4	0	6	0	0	10	5	0	11	0	0	0	2	4	0	17	0	0	0	0	
Butt-plot1-sph3-B	S.capillifolium	31	3	0	0	9	12	0	0	0	0	0	0	0	0	9	10	0	30	23	0	0	0	4	0	6	3	0	25	0	0	
Butt-plot1-sph4-B	S.medium	64	18	0	0	3	2	1	0	6	11	0	0	31	0	0	5	1	1	3	1	0	0	12	0	0	9	0	0	4	0	
Butt-plot1-sph5-B	S.medium	3	0	0	0	10	6	2	0	1	9	2	6	0	0	2	0	21	1	65	25	1	0	0	4	1	1	0	0	4	1	
Butt-plot2-sph1-B	S.capillifolium	0	43	0	0	12	1	0	0	1	0	0	0	0	0	2	14	56	1	27	0	0	0	5	0	0	0	0	0	0	1	0
Butt-plot2-sph2-B	S.medium	3	12	0	0	19	2	0	0	0	0	7	0	0	0	0	1	54	3	45	0	0	0	1	1	1	1	0	25	1	0	
Butt-plot2-sph3-B	S.papillosum	3	0	0	0	17	16	3	0	1	3	13	0	0	0	0	0	9	2	50	0	0	0	6	5	2	0	1	34	0	0	
Butt-plot2-sph4-B	S.medium	40	43	0	1	4	1	0	0	5	0	1	0	21	1	2	8	6	0	7	0	0	0	0	0	13	1	0	0	0	0	
Butt-plot2-sph5-B	S.fallax	2	27	0	0	49	1	0	0	10	15	0	0	1	3	0	19	25	0	2	0	0	0	4	1	3	0	1	5	0	0	
Butt-plot3-sph2-B	S.palustre	0	1	8	1	24	3	0	0	4	0	1	1	0	0	2	4	48	0	53	1	0	0	0	4	10	7	1	0	1	4	
Butt-plot3-sph3-B	S.medium	15	1	0	0	11	9	0	0	0	0	0	0	0	0	9	23	2	55	22	0	0	0	11	3	0	0	0	9	1	0	
Butt-plot3-sph4-B	S.papillosum	7	3	0	0	32	1	1	0	20	0	3	0	0	0	5	35	15	1	24	0	0	0	2	3	5	4	0	1	1	0	
Butt-plot3-sph5-B	S.fallax	0	1	0	1	52	0	0	0	16	1	0	0	0	0	2	17	47	1	16	0	0	0	11	3	2	1	0	3	1	0	
Butt-plot4-sph1-B	S.papillosum	39	0	0	0	7	10	2	0	0	1	1	1	0	0	0	14	0	3	30	45	0	0	1	3	5	3	3	1	1	1	
Butt-plot4-sph2-B	S.papillosum	0	40	0	0	41	4	0	0	7	0	3	0	0	0	0	21	11	0	17	0	0	0	6	10	0	0	0	0	0	1	0
Butt-plot4-sph3-B	S.papillosum	0	2	1	0	9	4	0	0	0	0	2	1	0	0	0	2	25	4	48	36	0	0	3	4	0	0	24	0	7	2	0
Butt-plot4-sph4-B	S.papillosum	9	0	0	0	26	1	0	0	12	0	2	0	0	0	4	26	35	1	38	2	0	0	3	4	0	1	0	11	1	0	
Butt-plot4-sph5-B	S.medium	0	0	0	0	15	3	0	0	6	0	1	3	0	0	0	25	18	1	41	25	0	0	9	6	0	13	0	13	0	0	
Butt-plot5-sph1-B	S.fimbriatum	1	6	0	0	25	0	0	0	19	0	3	0	0	0	2	68	15	1	8	0	0	0	3	5	5	0	0	0	0	2	0
Butt-plot5-sph2-B	S.capillifolium	33	0	0	0	9	3	0	0	10	0	1	0	0	0	6	12	47	2	7	9	0	0	4	1	4	6	0	1	1	0	
Butt-plot5-sph3-B	S.papillosum	15	1	0	0	9	5	0	0	5	0	2	0	0	0	2	1	89	0	26	0	1	0	2	3	1	0	0	3	0	0	
Butt-plot5-sph4-B	S.medium	31	2	0	0	12	3	0	0	3	0	1	0	0	0	4	10	25	4	31	13	0	0	8	1	11	1	0	0	0	0	
Butt-plot5-sph5-B	S.papillosum	9	6	0	0	15	2	0	0	11	1	6	0	0	0	3	17	36	0	33	0	0	0	5	9	11	0	1	0	1	0	
KH-plot1-domsph-T	S.capillifolium	0	0	0	0	56	0	0	0	16	0	2	0	0	0	0	27	1	0	17	0	0	3	5	0	0	3	30	0	1	0	
KH-plot2-domsph-T	S.capillifolium	1	0	0	0	31	0	0	0	24	0	0	0	0	0	4	17	1	0	60	0	0	2	0	13	0	0	1	2	0	2	0
KH-plot3-domsph-T	S.capillifolium	0	0	0	0	13	0	0	0	16	3	0	0	0	0	7	17	1	0	18	0	0	4	0	52	0	0	10	18	0	0	0
WE-plot1-domsph-T	S.medium	0	0	5	1	24	3	0	0	1	0	0	0	0	0	2	11	0	3	79	0	0	1	0	15	0	0	6	4	0	1	0
WE-plot2-domsph-T	S.palustre	3	0	26	0	13	0	0	0	2	0	0	0	0	0	2	11	0	0	94	0	0	0	8	0	0	4	1	0	1	0	1
WE-plot3-domsph-T	S.capillifolium	116	0	0	0	5	0	0	0	0	0	0	1	0	0	1	18	2	1	12	0	0	0	1	0	0	1	2	0	0	0	0
WS-plot1-domsph-T	S.capillifolium	1	0	8	0	3	0	0	0	16	0	0	0	0	0	0	84	0	0	22	0	0	0	14	0	0	2	6	0	0	0	0
WS-plot2-domsph-T	S.capillifolium	1	0	0	0	5	0	0	0	10	0	0	0	0	0	2	25	3	0	88	0	0	1	0	4	0	0	5	5	0	3	0
WS-plot3-domsph-T	S.capillifolium	9	0	0	1	22	0	0	0	1	15	0	1	0	0	0	1	18	2	2	20	0	0	22	0	0	25	14	0	2	0	0
KH-plot1-domsph-B	S.capillifolium	1</																														

WS-plot1-domsph-B	S.capillifolium	0	0	1	0	0	0	0	0	3	0	0	10	0	0	0	67	0	0	30	0	0	0	0	17	0	0	13	18	0	1	
WS-plot2-domsph-B	S.capillifolium	1	0	0	0	2	0	0	0	4	0	0	3	0	0	5	29	5	0	31	0	0	1	0	21	0	0	42	25	0	3	
WS-plot3-domsph-B	S.capillifolium	0	0	0	0	20	0	0	0	8	0	0	1	0	0	2	24	5	1	34	0	0	0	0	5	0	0	38	33	0	2	
Cray-LM1-T	S.capillifolium	2	0	1	0	1	0	0	2	2	0	1	0	0	0	1	2	44	0	28	0	0	1	0	53	26	0	7	0	0	0	
Cray-LM2-T	S.papilosum	2	0	11	0	1	0	1	0	1	0	0	0	0	0	1	1	1	28	0	0	0	6	0	103	16	0	0	1	0	0	
Cray-LM3-T	S.palustre	0	0	118	0	11	0	1	1	2	0	0	0	0	0	1	2	7	0	14	0	0	0	1	24	2	0	2	2	0	0	
Cray-RES1-T	S.capillifolium	1	1	0	0	6	0	0	0	2	0	1	0	0	0	1	2	3	0	16	0	0	20	0	121	3	0	3	2	0	0	
Cray-RES2-T	S.papilosum	0	0	0	0	3	3	0	0	2	0	8	0	0	0	0	2	16	1	75	0	0	0	0	56	3	0	10	4	0	4	
Cray-RES3-T	S.capillifolium	0	0	0	0	30	2	2	0	10	0	4	0	0	0	2	1	9	8	35	0	0	1	0	88	0	0	3	3	0	1	
Cray-DEG1-T	S.capillifolium	0	0	28	0	4	0	0	0	2	0	0	0	0	0	0	5	0	19	0	0	0	0	0	29	0	0	5	68	0	1	
Cray-DEG2-T	S.papilosum	3	0	0	1	5	2	0	0	2	1	1	0	0	0	1	1	8	0	54	0	0	2	0	84	7	0	1	0	0	0	
Cray-DEG3-T	S.papilosum	0	1	0	0	11	0	0	0	47	1	3	1	0	0	1	5	18	1	32	0	0	3	0	25	8	0	0	7	0	0	
Cray-LM1-B	S.capillifolium	1	0	3	0	8	0	0	2	7	1	4	0	0	0	2	0	81	1	42	0	0	0	0	9	8	0	15	1	3	2	
Cray-LM2-B	S.papilosum	0	0	0	0	0	0	4	0	5	1	1	0	0	0	16	17	9	4	42	0	0	0	0	22	35	0	9	2	0	1	
Cray-LM3-B	S.palustre	0	0	15	0	12	0	16	1	18	1	0	0	0	7	13	36	7	3	17	0	0	2	0	8	6	0	0	0	1	0	
Cray-RES1-B	S.capillifolium	0	0	0	1	8	1	1	0	24	0	0	0	0	0	2	7	33	2	44	0	0	2	0	39	5	0	16	5	0	3	
Cray-RES2-B	S.papilosum	0	0	0	0	2	1	0	0	2	0	16	0	0	0	2	0	15	3	73	0	0	1	0	28	7	0	21	6	8	6	
Cray-RES3-B	S.capillifolium	5	7	0	0	11	1	0	0	3	0	3	0	0	0	0	2	26	10	60	0	0	0	0	22	7	0	14	7	0	3	
Cray-DEG1-B	S.capillifolium	3	0	3	0	18	0	0	0	4	0	0	0	0	0	1	1	12	0	51	0	0	1	0	13	3	0	16	55	0	0	
Cray-DEG2-B	S.papilosum	4	2	0	3	3	6	0	0	5	1	7	0	7	2	0	2	25	1	59	0	0	0	1	34	1	0	1	0	0	0	
Cray-DEG3-B	S.papilosum	0	0	1	0	11	0	0	0	14	4	2	2	0	0	4	0	50	0	46	0	0	1	0	12	7	0	0	10	0	1	
Fleet-LM1-T	S.papilosum	1	0	0	0	6	0	0	0	11	1	3	0	0	0	2	0	21	4	48	0	0	5	0	54	4	0	3	2	1	0	
Fleet-LM2-T	S.capillifolium	0	0	0	0	0	0	0	1	3	0	1	0	0	0	0	1	12	0	46	0	0	10	0	63	0	0	16	2	0	0	
Fleet-LM3-T	S.papilosum	1	1	0	0	2	2	0	1	18	1	6	0	0	0	2	0	20	3	72	0	0	11	0	7	7	0	9	3	2	0	
Fleet-RES1-T	S.papilosum	0	0	0	0	40	1	0	0	23	3	4	2	0	0	1	2	12	6	12	0	0	1	0	42	2	0	7	0	0	3	
Fleet-RES2-T	S.palustre	0	0	0	0	0	0	0	0	12	0	0	1	0	0	0	0	2	2	116	0	0	0	0	25	0	0	0	0	0	0	
Fleet-RES3-T	S.papilosum	0	0	0	0	0	4	1	1	11	0	2	3	0	0	0	0	30	1	44	0	0	1	0	60	1	0	1	0	0	0	
Fleet-DEG1-T	Aul.palustre	0	0	0	0	49	0	0	0	25	1	0	0	0	0	0	0	62	0	1	0	0	3	0	9	0	0	0	12	0	0	
Fleet-DEG2-T	S.papilosum	1	0	0	0	5	0	0	0	12	0	3	0	0	0	1	0	58	7	21	0	0	2	0	37	2	0	13	1	0	0	
Fleet-DEG2(2)-T(Auc-Pal)	Aul.palustre	0	0	0	0	52	0	0	0	10	5	13	0	0	0	0	0	25	0	4	0	0	3	0	13	0	0	0	26	0	1	
Fleet-DEG3-T	Ple-schreberi	0	0	0	0	77	0	0	0	75	1	0	0	0	0	2	0	41	0	1	0	0	1	0	5	1	0	0	5	0	0	
Fleet-LM1-B	S.papilosum	0	1	1	0	8	0	0	0	9	0	3	3	0	0	1	0	17	3	52	0	0	3	0	11	8	0	4	0	0	7	
Fleet-LM2-B	S.capillifolium	0	0	0	0	1	0	0	0	19	2	1	1	0	0	0	0	8	0	39	0	0	1	0	11	2	0	75	2	0	4	
Fleet-LM3-B	S.papilosum	0	6	0	0	4	0	0	1	11	1	8	2	0	0	0	0	18	8	66	0	0	4	0	10	11	0	13	4	0	1	
Fleet-RES1-B	S.papilosum	0	0	0	0	6	0	0	0	6	10	15	12	0	0	0	5	0	15	30	34	0	0	2	0	21	1	0	18	1	1	8
Fleet-RES2-B	S.palustre	0	0	0	0	0	0	0	0	5	0	3	1	0	1	1	0	9	26	86	0	0	0	0	17	5	0	5	1	0	2	
Fleet-RES3-B	S.papilosum	0	0	0	0	5	8	1	0	3	3	33	9	0	0	2	3	25	14	56	0	0	0	0	18	6	0	10	0	0	4	
Fleet-DEG1-B	Aul.palustre	0	0	0	0	76	0	0	0	30	0	0	0	0	0	0	0	42	0	0	0	0	1	0	14	0	0	0	8	0	0	
Fleet-DEG2-B	S.papilosum	0	0	0	0	3	3	0	0	3	0	12	0	0	0	1	0	23	28	24	0	0	0	0	9	17	0	39	1	2	0	
Fleet-DEG2(2)-B	Aul.palustre	0	0	0	0	56	0	0	0	4	5	3	0	0	0	1	0	14	0	1	0	0	0	0	26	0	0	0	92	0	0	
Fleet-DEG3-B	Ple-schreberi	0	0	0	0	29	0	0	0	38	6	3	1	0	0	1	0	51	0	9	0	0	0	0	14	0	0	8	24	0	0	
Stake-LM1-T	S.papilosum	1	0	1	0	57	0	0	0	4	0	0	0	0	0	4	0	3	6	43	0	0	6	0	41	1	0	0	0	0	0	
Stake-LM2-T	S.capillifolium	82	0	0	0	4	0	0	0	1	0	1	0	0	0	0	0	3	0	20	17	24	0	0	6	0	2	0	10	0	0	
Stake-LM3-T	S.capillifolium	0	0	0	1	3	0	0	0	2	0	0	0	0	0	3	0	24	0	49	0	0	3	0	34	0	0	5	34	0	0	
Stake-RES1-T	S.papilosum	1	0	0	1	16	0	0	0	12	3	3	0	0	0	4	0	10	9	40	0	0	4	0	64	1	0	15	1	1	1	
Stake-RES2-T	S.papilosum	0	0	1	15	24	0	0	0	1	10	0	2	3	0	0	0	13	0	62	0	0	32	0	3	0	0	1	2	4	1	
Stake-RES3-T	S.palustre	0	0	1	1	5	0	0	1	10	0	0	0	0	0	2	0	20	0	69	0	0	4	0	7	30	0	5	8	0	1	
Stake-DEG1-T	Dic.scoparium	0	0	0	0	15	0	0	0	30	3	0	0	0	0	1	0	92	0	2	0	0	5	0	1	0	0	4	7	1	2	
Stake-DEG2-T	Ple.schreberi	0	1	0	0	13	0	0	6	4	16	2	0	0	0	0	0	30	0	2	0	0	7	0	9	0	0	0	85	0	1	
Stake-DEG3-T	Aul.palustre	0	0	0	0	8	0	0	4	66	0	3	0	0	0	0	0	26	0	0	0	0	14	0	0	0	0	1	7	1	9	
Stake-LM1-B	S.papilosum	0	0	0	0	5	0	4	0	1	0	2	0	0	0	1	0	19	14	64	0	0	0	0	22	0	0	0	0	0	0	
Stake-LM2-B	S.capillifolium	80	0	0	0	4	0	0	0	3	0	2	0	2	2	0	1	13	0	7	27	10	0	0	3	3	10	0	3	0	0	
Stake-LM3-B	S.capillifolium	0	0	0	0	5	0	0	0	5	0	1	0	0	0	0	0	28	1	38	0	1	0	0	23	0	0	43	12	0	2	
Stake-RES1-B	S.papilosum	0	0	0	2	15	0	0	0	6	2	20	2	3	1	0	0	8	13	69	0	0	3	0	12	0	0	13	0	7	3	
Stake-RES2-B	S.papilosum	0	0	1	29	9	0	0	0	3	2	3	1	4	1	0	0	32	0	42	0	0	29	0	5	0	0	2	5	2	1	
Stake-RES3-B	S.palustre	0	0	0	0	3	0	0	0	9	0	0	3	0	0	1	0	39	0	64	0	0	0	0	1	16	0	15	10	0	1	
Stake-DEG1-B	Dic.scoparium	0	0	0	0	20	0	0	1	19	7	8	1	0	0	4	0	28	0	21	0	0	2	0	1	0	0	26	52	0	2	
Stake-DEG2-B	Ple.schreberi	2	0	3	0	19	0	0	15	16	49	2	0	0	0	0	0	20	0	1	0	0	13	0	3	0	0	2	37	0	5	
Stake-DEG3-B	Aul.palustre	0	0	0	0	7	0	0	0	7	1	11	0	0	0	1	0	61	0	4	0	0										

**Appendix 4b.** Raw data table of testate amoebae in surface (0-5 cm) and deep (50-55 cm) peat in relation to peatland restoration (i.e., consisted of degraded (DEG), post\_restoration (RES) and least modified (LM) areas in each restoration site).

**Reference sites:** Butt-Butterburn (border mire), KH-Kield Head, WE-Whitlee English side, WS-Whitlee Scottish side. **Restoration managed sites:** Cray-Cray Moss, Fleet-Fleet Moss, Stake-Stake Moss.

Plot	Amp fla	Amp.wri	Arc.dis.s	Ass.mus	Ass.sem	Cen.arc	Cor.dub	Cry.ovl	Cyc.arc.s	Dif.lei	Dif.pul	Eug.stri	Eug.tub	Hel.pet	Hel.syl	Hya.sub	NA.1	Neb.car	Neb.co.bo	Neb fla	Neb.gri	Neb.mil	Neb.tin	Pla.lab	Pse.ful	Tri.arc
Butt-plot1-(50-55cm)	31	36	2	10	4	0	1	0	3	0	0	0	4	3	7	0	2	0	0	4	0	6	2	0	1	1
Butt-plot2-(50-55cm)	24	12	5	8	5	7	2	0	17	2	0	1	1	8	4	0	0	0	1	1	0	11	2	0	2	0
Butt-plot3-(50-55cm)	11	20	0	2	4	1	0	0	35	0	4	0	1	4	4	0	1	0	0	0	0	19	1	0	1	2
HK-plot1-(50-55cm)	0	1	0	0	0	0	0	0	6	0	0	0	0	0	1	94	0	0	0	0	0	0	0	0	0	0
KH-plot2-(50-55cm)	0	1	0	3	0	0	0	0	1	0	0	0	0	0	0	109	0	0	0	0	0	1	1	0	0	1
KH-plot3-(50-55cm)	0	0	0	0	0	0	0	0	6	0	0	0	0	1	0	95	0	0	0	0	0	0	0	0	0	1
WE-plot1-(50-55cm)	35	4	0	10	4	0	0	0	5	0	1	0	0	1	5	21	0	0	3	0	0	16	6	0	0	0
WE-plot2-(50-55cm)	6	1	0	15	2	0	0	0	9	0	0	2	1	1	3	64	0	0	0	1	0	6	0	0	0	2
WE-plot3-(50-55cm)	2	0	0	9	0	0	0	0	5	0	0	0	0	0	0	97	0	0	0	0	0	4	4	0	0	1
WS-plot1-(50-55cm)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	99	0	0	0	0	0	1	0	0	0	2
WS-plot2-(50-55cm)	81	0	0	3	0	0	0	0	0	0	0	0	0	0	0	41	0	0	0	0	0	1	0	0	0	0
WS-plot3-(50-55cm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	110	0	0	0	0	0	0	0	0	0	2
Cray-LM1-(50-55cm)	40	11	0	15	3	0	0	0	10	0	1	0	0	3	0	1	0	0	3	0	0	19	5	0	0	1
Cray-LM2-(50-55cm)	17	15	0	15	0	0	0	0	9	0	0	0	1	0	3	5	0	0	5	2	0	2	22	0	7	4
Cray-LM3-(50-55cm)	19	17	0	11	0	0	0	0	20	0	0	0	3	4	5	2	0	0	0	1	0	3	4	0	4	2
Fleet-LM1-(50-55cm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	157	0	0	0	0	0	0	0	0	0	2
Fleet-LM2-(50-55cm)	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	105	0	0	0	0	0	0	0	0	0	1
Fleet-LM3-(50-55cm)	0	0	0	1	0	0	0	0	6	0	0	0	0	0	0	105	0	0	0	0	0	0	0	0	0	1
Stake-LM1-(50-55cm)	16	12	0	5	1	0	1	0	12	0	0	0	5	1	2	37	0	0	1	0	0	3	10	0	0	5
Stake-LM2-(50-55cm)	32	0	0	7	0	3	1	0	7	0	4	0	6	0	1	20	0	5	12	0	0	2	10	0	0	0
Stake-LM3-(50-55cm)	4	4	0	3	0	2	0	0	35	0	0	0	4	1	2	29	0	0	5	0	0	2	5	0	0	4
Cray-DEG1-(50-55cm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cray-DEG2-(50-55cm)	1	0	0	0	0	0	0	0	7	0	0	0	0	0	0	90	0	0	0	0	0	0	0	0	0	1
Cray-DEG3-(50-55cm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fleet-DEG1-(50-55cm)	0	0	0	15	0	0	0	0	30	0	0	0	0	1	2	60	0	0	0	0	0	0	1	0	0	0
Fleet-DEG2-(50-55cm)	43	20	0	5	0	0	0	0	17	0	1	0	0	1	0	0	0	0	0	0	0	18	2	0	0	0
Fleet-DEG3-(50-55cm)	43	2	0	20	0	0	0	0	9	0	0	0	0	8	0	0	0	0	0	0	0	17	3	0	0	1
Stake-DEG1-(50-55cm)	23	0	0	6	0	0	0	0	1	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	2
Stake-DEG2-(50-55cm)	75	0	0	17	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Stake-DEG3-(50-55cm)	60	22	0	15	0	0	0	0	3	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	1
Butt-plot1-0-5 cm	32	15	4	16	8	1	0	0	6	1	8	0	5	1	34	0	4	2	1	2	2	16	2	0	0	0
Butt-plot2-0-5 cm	11	17	18	9	3	1	1	0	13	7	4	1	10	3	39	0	2	1	2	5	7	4	0	0	0	1
Butt-plot3-0-5 cm	3	3	9	22	0	10	7	0	65	4	27	2	21	0	46	0	2	0	2	21	7	39	4	5	25	15
KH-plot1-0-5 cm	4	2	0	1	0	0	2	1	23	0	5	0	2	0	25	59	0	0	0	3	1	8	1	1	17	5
KH-plot2-0-5 cm	0	0	3	10	0	0	0	11	27	0	3	3	5	1	24	27	0	0	1	4	0	26	3	1	7	11
KH-plot3-0-5 cm	2	2	3	2	1	0	0	8	23	1	3	0	2	0	16	40	0	0	0	1	0	22	5	0	21	12
WE-plot1-0-5 cm	2	7	0	4	1	0	0	1	33	4	2	1	10	0	36	1	2	0	2	5	2	39	1	0	2	5
WE-plot2-0-5 cm	0	1	7	14	1	0	5	0	14	8	0	0	32	0	42	3	1	0	5	4	1	5	9	0	12	0
WE-plot3-0-5 cm	1	0	1	13	1	0	1	0	6	0	5	1	8	1	40	5	0	0	5	0	0	53	11	0	3	14
WS-plot1-0-5 cm	3	1	0	8	3	0	0	0	33	0	2	0	22	0	26	4	2	0	8	2	0	37	5	0	11	2
WS-plot2-0-5 cm	2	0	0	10	0	0	3	0	19	0	1	0	9	0	20	26	0	0	0	0	0	38	14	0	9	9
WS-plot3-0-5 cm	0	0	1	4	2	0	2	1	24	0	0	0	11	0	15	4	1	0	2	1	0	40	2	0	56	1
Cray-LM1-(0-5cm)	0	0	1	5	0	0	0	0	41	0	0	0	9	0	4	41	2	0	0	4	0	13	19	1	2	18
Cray-LM2-(0-5cm)	0	2	6	1	0	4	5	0	23	0	0	6	17	0	26	1	3	0	16	13	0	0	3	0	1	2
Cray-LM3-(0-5cm)	1	0	5	16	0	0	13	0	27	0	0	3	16	0	38	1	3	0	6	14	0	3	3	0	0	3
Fleet-LM1-(0-5cm)	0	0	7	3	0	0	7	0	30	0	0	2	13	0	8	7	2	0	3	33	0	17	22	0	0	1
Fleet-LM2-(0-5cm)	0	0	0	7	0	0	2	0	26	0	0	0	7	0	15	54	0	0	1	2	0	15	15	0	3	10
Fleet-LM3-(0-5cm)	0	0	0	14	0	0	1	0	18	0	0	1	6	0	21	40	0	0	3	0	0	21	21	1	2	15
Stake-LM1-(0-5cm)	0	1	18	2	1	0	2	0	9	0	0	13	20	1	31	1	1	0	9	7	0	9	3	0	0	6
Stake-LM2-(0-5cm)	3	4	17	17	0	0	4	0	8	0	0	1	33	0	17	1	2	0	12	6	0	12	5	0	5	12
Stake-LM3-(0-5cm)	0	7	7	8	0	0	6	0	24	0	0	8	22	0	42	16	3	0	12	10	0	8	4	0	3	6
Cray-RES1-(0-5cm)	3	13	3	6	1	0	2	0	13	1	2	4	17	0	8	36	2	0	5	7	0	1	13	0	18	2

Cray-RES2-(0-5cm)	0	0	11	1	3	0	4	0	40	1	0	4	32	0	3	4	8	0	9	14	0	15	8	0	5	0
Cray-RES3-(0-5cm)	0	0	1	8	0	0	4	0	13	5	0	6	23	0	34	1	0	0	3	6	0	68	15	0	1	4
Fleet-RES1-(0-5cm)	0	0	0	0	0	0	1	0	29	0	1	1	5	0	19	64	0	0	2	4	0	4	8	0	11	7
Fleet-RES2-(0-5cm)	1	0	1	8	0	0	1	0	34	0	0	1	9	0	8	17	2	0	2	22	0	14	14	0	1	8
Fleet-RES3-(0-5cm)	0	0	0	6	0	0	3	0	32	0	0	0	9	0	2	1	0	0	4	43	0	9	32	0	0	8
Stake-RES1-(0-5cm)	0	0	0	5	0	0	0	0	3	0	1	0	12	0	5	59	0	0	0	0	0	8	6	0	13	27
Stake-RES2-(0-5cm)	27	6	1	20	1	0	2	0	3	2	0	0	12	0	3	73	0	0	0	0	0	1	1	0	1	8
Stake-RES3-(0-5cm)	0	0	1	6	0	0	1	0	8	0	3	0	17	0	19	79	0	0	0	0	0	9	15	0	4	1
Cray-DEG1-(0-5cm)	0	0	0	1	0	0	7	0	28	0	0	0	6	0	12	30	1	0	4	1	0	25	15	0	25	3
Cray-DEG2-(0-5cm)	0	0	0	22	0	0	0	1	11	0	0	0	10	0	4	46	0	0	0	1	0	7	14	7	3	9
Cray-DEG3-(0-5cm)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	160	0	0	0	0	0	0	1	0	0	0
Fleet-DEG1-(0-5cm)	0	0	0	6	0	0	0	0	27	0	0	0	4	0	17	71	0	0	0	0	0	14	3	0	23	16
Fleet-DEG2-(0-5cm)	2	0	0	14	0	0	0	0	37	0	0	0	0	0	3	95	0	0	0	0	0	0	3	0	8	3
Fleet-DEG3-(0-5cm)	0	0	0	3	1	0	0	0	26	0	1	0	0	0	12	52	0	0	0	2	0	5	4	0	33	3
Stake-DEG1-(0-5cm)	32	13	0	21	0	0	2	0	7	0	0	0	4	0	17	43	0	0	3	0	0	0	2	0	0	2
Stake-DEG2-(0-5cm)	45	3	0	23	0	0	0	0	10	0	0	5	11	0	7	19	0	0	0	0	0	3	21	0	0	1
Stake-DEG3-(0-5cm)	3	11	0	2	0	0	0	0	1	0	1	0	1	0	3	132	0	0	0	0	0	0	0	0	3	12



**Appendix 4c.** Raw data table of environmental variables from surface peat (0-5 cm) in relation to peatland restoration (i.e., consisted of degraded (DEG), post\_restoration (RES) and least modified (LM) areas in each restoration site). **Reference sites:** Butt-Butterburn (border mire), KH-Kield Head, WE-Whitlee English side, WS-Whitlee Scottish side. **Restoration managed sites:** Cray-Cray Moss, Fleet-Fleet Moss, Stake-Stake Moss.

Plot	Heather (%)	Moss (%)	Sph (%)	Sedge (%)	Herb (%)	Brush/Litter (%)	Bare (%)	Slope (°)	Elevation (m)	Moss_height (cm)	Sph_height (cm)	Lit_height (cm)	Peat_depth (m)	WC (%)	BD (g/cm³)	pH	N (%)	C (%)	DOC (mg/l)	TbN (mg/l)	Cu (ug/g)	Zn (ug/g)	Pb (ug/g)	Al (ug/g)	Ca (ug/g)	Fe (ug/g)	K (ug/g)	Mg (ug/g)	Na (ug/g)	Mn (ug/g)	Cd (ug/g)	P (ug/g)	Si (ug/g)	As (ug/g)
Butt-plot1-0-5 cm	5	1	80	25	0	2	2	1	123	4	8	1	651	96.18	0.04	4.51	1.19	44.82	8.73	0.81	18.31	269.63	162.16	752.09	1240.31	1036.76	155.02	899.98	197.82	7.44	3.87	359.63	96.64	8.62
Butt-plot2-0-5 cm	6	2	75	45	2	10	0	1	123	4	14	3	651	95.53	0.04	4.62	1.34	43.43	7.06	0.52	7.44	486.12	169.02	783.96	1182.88	2334.06	732.66	1262.95	167.33	22.64	2.82	445.85	79.96	0.00
Butt-plot3-0-5 cm	2	8	65	40	5	8	1	1	117	4	10	3	700	95.08	0.05	4.58	1.92	44.66	13.63	1.96	6.09	242.67	51.73	932.13	1811.00	2423.35	426.20	1111.90	166.35	8.62	1.56	805.09	38.56	0.00
KH-plot1-0-5 cm	65	85	1	8	0	12	1	3	315	5	5	4.5	170	90.46	0.10	4.26	1.60	47.08	26.56	2.11	20.90	92.91	218.63	723.04	549.51	2668.68	459.51	757.33	215.19	14.52	2.56	866.55	50.84	1.87
KH-plot2-0-5 cm	60	40	45	12	0	7	1	2	455	5	7	2	203	89.76	0.09	4.31	1.22	46.00	21.83	2.00	9.91	169.39	91.39	615.14	893.32	2618.56	668.91	700.61	165.12	14.99	3.69	576.03	43.00	0.00
KH-plot3-0-5 cm	28	33	36	25	0	4	1	4	455	6	11	3	180	92.07	0.08	4.35	1.71	45.81	16.30	1.67	19.43	88.88	371.15	815.12	770.92	2328.82	288.09	929.17	205.29	7.38	2.82	627.69	43.60	4.76
WE-plot1-0-5 cm	28	5	92	17	0	3	1	4	570	3	7	3	293	95.03	0.04	4.46	1.11	43.17	17.15	1.69	4.85	221.95	82.83	448.12	1004.01	1853.70	714.63	1472.32	248.41	18.26	4.42	462.31	55.64	5.12
WE-plot2-0-5 cm	16	2	96	24	1	3	1	3	413	3	8	3	293	92.92	0.06	4.53	1.50	45.60	21.06	2.22	17.41	151.77	278.07	1001.24	1274.36	2711.28	311.01	803.14	167.42	8.25	3.82	490.21	45.65	0.85
WE-plot3-0-5 cm	42	33	45	18	0	3	0	3	457	5	8	2.5	287	91.67	0.08	4.45	1.72	46.39	14.28	1.39	17.12	180.45	240.76	845.92	1245.47	1887.96	292.45	1093.33	195.16	12.41	2.37	645.69	44.78	0.00
WS-plot1-0-5 cm	38	3	95	20	8	2	1	4	566	2.5	9	1	184	92.02	0.07	4.37	1.50	45.55	13.30	1.33	9.11	156.50	164.16	679.70	1436.34	2735.96	448.86	1178.40	208.38	23.22	3.24	711.89	47.48	0.00
WS-plot2-0-5 cm	53	26	30	8	0	9	1	6	568	3.5	11	3	173	88.97	0.09	4.37	1.68	47.08	24.41	1.93	33.59	107.56	276.24	981.38	883.95	1865.63	522.26	875.72	194.02	13.14	2.96	846.61	36.76	1.66
WS-plot3-0-5 cm	46	1	98	6	0	2	0	6	567	2	10	1	130	92.27	0.07	4.46	1.86	46.49	21.09	2.30	15.48	80.63	158.16	940.77	752.48	2179.45	471.18	736.11	138.74	10.18	2.60	865.04	36.44	2.44
Cray-LM1-(0-5cm)	12	3	65	20	6	10	0	1	581	3	7	2	274	87.83	0.11	4.30	2.12	47.45	36.11	3.69	19.75	391.38	264.67	1109.82	3151.62	5849.33	895.43	1103.63	261.81	11.47	3.16	995.59	26.26	0.00
Cray-LM2-(0-5cm)	0	8	25	65	0	25	0	1	581	3	9	2	360	94.12	0.07	4.46	1.31	42.95	15.22	2.35	14.77	444.75	190.45	577.15	3612.06	2412.65	664.32	1000.22	268.26	16.92	2.67	891.49	56.34	0.00
Cray-LM3-(0-5cm)	0	2	95	52	0	5	0	1	581	2	13	2	450	94.10	0.06	4.58	1.64	44.48	10.24	1.93	10.96	163.37	133.44	464.32	1763.59	1229.98	290.35	400.69	132.18	6.00	2.32	494.65	46.24	0.00
Fleet-LM1-(0-5cm)	8	15	65	18	3	7	0	3	562	3	14	1.5	110	90.35	0.08	4.35	2.16	47.16	19.40	2.16	24.67	434.01	285.96	1179.44	884.82	1862.14	541.89	703.37	168.40	12.58	2.12	1279.38	35.64	3.12
Fleet-LM2-(0-5cm)	5	25	40	5	2	15	0	2	563	4	9	1	147	90.07	0.09	4.61	1.49	46.78	19.04	3.04	16.57	378.45	86.26	539.25	1237.67	2005.19	479.12	961.41	135.42	30.53	2.50	538.80	44.08	0.00
Fleet-LM3-(0-5cm)	5	7	70	8	3	15	0	1	568	1.5	9.5	1.5	168	86.67	0.12	4.72	1.92	48.08	19.97	2.71	34.23	211.16	316.01	1489.37	1943.88	1360.03	439.37	1128.65	174.42	12.05	2.26	841.91	39.36	0.00
Stake-LM1-(0-5cm)	8	4	95	33	0	3	0	1	570	4	10	7	360	95.82	0.05	4.58	0.75	43.24	6.12	1.03	9.70	127.15	140.19	554.49	1576.81	1569.30	250.43	1073.21	201.16	10.82	3.83	473.74	65.04	0.00
Stake-LM2-(0-5cm)	2	1	93	12	2	6	1	1	556	5	17	5	229	91.53	0.08	4.39	1.93	48.32	7.31	1.79	7.01	135.91	50.12	310.48	1371.44	847.33	243.68	838.42	118.57	14.55	2.25	392.31	19.41	0.00
Stake-LM3-(0-5cm)	5	16	60	25	15	12	0	1	559	8	9	2	205	92.48	0.08	4.44	1.74	46.79	12.40	1.74	14.74	126.43	322.17	724.90	1549.52	1379.25	396.02	1137.38	182.13	16.37	3.24	810.55	42.88	0.00
Cray-RES1-(0-5cm)	0	1	95	45	0	3	0	1	569	2	7	3	176	90.93	0.09	4.12	1.52	47.23	9.55	1.98	9.75	206.86	191.58	730.24	1381.66	4427.41	218.45	843.89	147.49	11.28	2.18	633.69	36.84	0.34
Cray-RES2-(0-5cm)	1	65	35	45	5	4	2	1	571	2	9	2	267	88.70	0.12	4.20	2.62	46.74	22.83	2.39	22.61	160.46	324.49	1478.77	638.56	2828.43	480.95	644.34	196.80	9.89	4.26	1069.69	26.99	7.67
Cray-RES3-(0-5cm)	8	2	95	65	0	10	0	1	572	3	6	2	227	92.77	0.07	4.37	1.05	44.15	27.27	2.60	6.98	132.01	35.55	240.25	1405.92	1510.24	351.20	921.09	178.14	18.73	2.25	310.15	50.34	0.00
Fleet-RES1-(0-5cm)	3	75	0	13	1	5	1	2	548	7	11	2.5	182	87.07	0.10	4.50	1.86	48.04	22.13	3.38	50.38	91.25	518.49	1790.96	663.16	3610.45	319.03	650.09	149.12	7.84	3.14	772.50	33.23	2.77
Fleet-RES2-(0-5cm)	0	99	0	30	0	1	0	3	552	7.5	14	1	182	90.14	0.10	4.29	2.04	47.15	17.25	1.92	32.54	119.04	318.89	1273.68	1083.43	1817.89	516.46	590.06	169.43	5.23	2.68	999.76	37.98	12.17
Fleet-RES3-(0-5cm)	0	85	6	45	5	2	0	3	556	5	7	2.5	184	89.02	0.11	4.47	2.49	47.26	12.49	2.46	27.13	77.10	396.92	1195.23	1040.19	1354.81	257.89	427.84	96.59	6.85	2.88	991.74	36.09	0.00
Stake-RES1-(0-5cm)	5	8	0	12	0	6	55	2	569	5	6	0.5	275	81.24	0.19	4.23	1.77	48.87	15.86	2.29	20.48	118.15	352.34	1463.51	1712.13	2890.95	234.36	1050.66	133.06	49.10	3.22	387.24	52.48	0.00
Stake-RES2-(0-5cm)	3	20	18	15	5	10	10	3	571	2	10	1.5	305	90.73	0.11	4.41	1.14	49.03	10.70	0.98	9.73	121.93	90.13	680.82	1594.50	1079.53	118.61	1104.30	195.02	8.90	2.19	306.73	63.96	2.59
Stake-RES3-(0-5cm)	5	60	7	30	10	10	0	1	572	2.5	9	2	240	89.03	0.10	4.28	1.99	48.42	15.51	5.71	16.48	183.33	170.52	888.09	2482.77	4210.47	409.29	1095.62	160.08	67.92	3.46	758.78	44.47	3.34
Cray-DEG1-(0-5cm)	1	85	4	50	10	0	2	565	3.5	3.5	2	67	84.31	0.11	3.92	2.24	47.44	25.80	3.99	12.08	66.62	132.65	956.84	1592.66	3539.48	414.86	736.24	109.41	25.68	2.13	857.79	38.88	0.00	
Cray-DEG2-(0-5cm)	3	85	0	80	0	5	2	1	579	4	13	1	192	84.60	0.15	4.21	2.05	47.08	26.99	2.38	40.96	101.03	544.22	1460.97	1644.14	1467.33	354.05	904.56	166.78	15.11	3.44	730.86	48.00	9.16
Cray-DEG3-(0-5cm)	2	80	0	4	27	2	5	1	566	1	10	0.5	120	82.09	0.17	3.99	1.20	50.68	17.28	2.00	5.81	88.83	84.55	728.46	1046.74	2894.27	143.70	694.25	95.98	16.74	2.06	348.22	50.84	0.00
Fleet-DEG1-(0-5cm)	7	75	0	11	0	17	1	1	567	4	NA	1	282	86.28	0.13	4.68	2.04	47.82	19.38	2.89	37.37	391.86	432.64	1567.98	1155.28	3250.10	357.26	975.41	203.82	16.46	3.08	540.78	32.32	2.06
Fleet-DEG2-(0-5cm)	2	33	0	7	5	13	2	1	566	6	NA	1.5	301	85.46	0.14	4.09	1.97	46.85	26.89	3.21	48.33	114.47	767.53	1921.16	597.89	2976.37	287.94	637.30	152.26	6.24	4.07	697.65	37.05	10.41
Fleet-DEG3-(0-5cm)	5	95	0	14	0	12	0	1	566	7	NA	4	342	85.33	0.14	4.02	2.01	47.50	41.22	3.27	49.49	154.75	567.79	1706.14	510.93	8727.71	412.66	540.19	147.57	12.32	3.67	1016.74	37.17	22.40
Stake-DEG1-(0-5cm)	0	4	0.1	2	0	80	4	5	567	1.5	1	1	211	89.99	0.08	4.71	1.05	50.22	10.20	1.82	3.83	118.56	8.80	389.64	2210.27	515.74	200.91	2133.84	192.22	33.67	1.89	269.55	46.39	0.00
Stake-DEG2-(0-5cm)	0	25	0	0	0	80	3	4	553	2	NA	1	258	89.97	0.09	4.59	0.95	50.75	8.11	0.72	1.95													

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