



# Response of testate amoeba assemblages to peatland drain blocking

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Received: 16 May 2023 / Accepted: 26 August 2023 / Published online: 22 September 2023  
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**Abstract** Peatlands represent globally important habitats and carbon stores. However, human impacts and climate change leave peatlands with a substantial management challenge. Degradation of peatland habitats and their hydrological integrity is increasingly counteracted through the rehabilitation of peatlands including re-wetting and drain blocking. Research into how such management interventions affect peatland microbial assemblages is limited. Here, we investigate the response of testate amoebae (established unicellular amoeboid protist indicators of hydrological conditions in peatlands) to drain blocking on three small lowland raised bogs in Northern Ireland, UK. We sampled *Sphagnum* adjacent to areas of focused flow near sites of damming in addition to control sites away from dam blocking. These restoration measures

show complex but meaningful results after restoration. We observe several key developments following dam blocking: (i) species diversity increases; (ii) unambiguous wet indicator taxa appear in increasing abundance at dammed sites; (iii) and transfer-function reconstructed water-table depths show wetter conditions in the dammed sites. These findings imply wetter conditions after restoration, where routine monitoring presented no clear trend in water-table depths. We found no statistically significant assemblage-level response to experimental or environmental variables, which may be related to antecedent conditions and significant periods of drought during the study period. Thus, caution is advised when utilising testate amoebae for bioindication until their assemblage-level response to restoration is better understood. Nevertheless, this study emphasises the potential of an indicator-taxa based approach to applying testate amoebae as contemporary bioindicators of peatland restoration—particularly on short-term timescales immediately following restoration.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11273-023-09949-w>.

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**Keywords** Peatland · Restoration · Testate amoebae · *Sphagnum* · Biomonitoring

## Introduction

Peatlands represent critically important habitats that anthropogenic pressures are putting at great risk (Auterives et al. 2011; Turner et al. 2014;

Basińska et al. 2020). Predominantly these habitats are threatened by peat extraction, drainage, fire, afforestation, and land-use change (Schaible and Dickson 1990; Cooper et al. 2003; Tomlinson 2010; Douglas et al. 2015). In extreme circumstances, peatlands have been converted wholesale into profitable spaces (e.g., Oil Sands Mining—Rooney et al. 2012). Where peatlands are protected from direct exploitation, climate change and pollution can have major negative impacts on these habitats (e.g., atmospheric ammonia deposition on protected peatlands—Kelleghan et al. 2021). Peatlands provide several important ecosystem services. In the UK, peatlands provide flood relief (Edokpa et al. 2022), cultural heritage/public leisure spaces (Flint and Jennings 2022), and as sites for game bird management (Douglas et al. 2015). Internationally, peatlands prove to be unsustainable yet undeniably valuable agricultural areas (e.g., rice farming on peatland soils—Verhoeven and Setter 2010; Surahman et al. 2018). Most notably, peatlands have global carbon stores exceeding 600 GtC (Yu et al. 2010) and act as carbon sinks when healthy (Schwieger et al. 2021).

Threats from anthropogenic activity leave peatlands with a substantial management challenge. Direct exploitation needs to be opposed and legislated against, while omnipresent forms of large-scale damage such as agricultural pollution and climate change need to be reduced or counteracted. If this is not accomplished, the sizeable global store of carbon in peatlands could potentially be released to the atmosphere at an alarming rate (Ribeiro et al. 2021). Emissions from peatlands are around 23.1 Mt CO<sub>2</sub> equivalent in the UK annually (Parliamentary Office of Science and Technology 2022), as high as 1.91 Gt CO<sub>2</sub> equivalent per year globally (Leifeld and Menichetti 2018), and > 3.26 Mt CO<sub>2</sub> equivalent specifically for the island of Ireland (Wilson et al. 2013; Centre for Ecology & Hydrology 2017). The Intergovernmental Panel on Climate Change (IPCC 2018) reported that a decrease of only 25–30 Gt CO<sub>2</sub> equivalent per year of current global emissions could represent the difference between 1.5 and 2 °C warming by 2030. The effects of 2 °C warming could be extreme; for example, it could cause an increase in permafrost thaw by 2.5 million km<sup>2</sup> (IPCC 2018). International cooperation to see current emissions fall to acceptable levels is perhaps the largest challenge

in generations. Adding 1.91 Gt CO<sub>2</sub> of avoidable carbon equivalents per year from peatlands would thwart these efforts.

Through pervasive anthropogenic pressures, it is thought that > 60% of UK and Irish peatlands are in a degraded state (Wilson et al. 2013; IUCN 2018; Tanneberger et al. 2021). A marked trend towards drying has been observed across European peatlands (Charman et al. 2006; Marcisz et al. 2015), which is linked to direct and indirect anthropogenic pressures on peatlands (Swindles et al. 2019). However, the last two decades have seen ambitious projects to re-wet peatlands across UK and Ireland (e.g., Parry et al. 2014; Holden et al. 2017). Re-wetting through drainage ditch damming can promote carbon sequestration (Beyer et al. 2021; Wilson et al. 2022). Re-wetting inhibits the decomposition of peat by reintroducing anoxic conditions by raising water-tables potentially causing peatlands that have shifted into becoming carbon sources to become carbon sinks again (Nugent et al. 2018). This process leads to peatlands becoming moderate sinks of CO<sub>2</sub> while generally emitting more CH<sub>4</sub>, resulting in overall reductions in total greenhouse gas emissions (Nyberg et al. 2022; Wilson et al. 2022). Vegetation appears to recover in peatlands that undergo re-wetting restoration (Hancock et al. 2018), with important peatland species expanding, such as peat forming *Sphagnum* mosses (Malmer et al. 2003)—though this colonisation can take many years (Anderson and Peace 2017). *Sphagnum* colonisation following re-wetting can occur spontaneously (Graf et al. 2008; Mahmood and Strack 2011). However, practices are being developed to improve peatland re-vegetation, and to reduce greenhouse gas emissions, after re-wetting restoration (e.g., moss layer-transfer technique Lazcano et al. 2018; Purre et al. 2020). Evaluation of restoration success is a complex issue. The succession of vegetation, particularly *Sphagnum* in the case of peatlands, can be used to indicate restoration success (Soini et al. 2010). Different indicator species may have merit in predicting restoration outcomes in early stages post-restoration (González et al. 2013). Despite this, even over multi-year time series it can be difficult to determine the effect of restoration in the hydrology of a site (Green et al. 2017). Furthermore, shifts in greenhouse gas emissions following restoration can be multi-faceted, with reductions in carbon emissions but increases in methane emissions (Strack et al. 2014; Nugent et al. 2018; Nyberg et al.

2022; Wilson et al. 2022). Improved understanding of how to quantify the effectiveness of peatland restoration, which can be used to adapt and improve restoration methods, would be an invaluable tool in future peatland restoration projects.

Testate amoebae are a cosmopolitan group of protists that fulfil the role of dominant microbial consumers in peatlands (Mitchell et al. 2008; Jasey et al. 2013; Kuuri-Riutta et al. 2022). Testate amoebae can be found living on the stems of *Sphagnum* and in the still oxygenated layers of peat found close to the surface (Roe et al. 2017; Kuuri-Riutta et al. 2022). Testate amoebae have been shown to respond to a number of changing peatland conditions (Payne et al. 2012; Marcisz et al. 2020) and provide an established means of inferring past water levels from Holocene peat cores (Hendon and Charman 1997; Booth et al. 2010). The shell or ‘test’ (made from protective autogenous or xenogenous material) of these organisms generally resists decay, allowing them to preserve well in fossil peat (Charman et al. 2000). Assemblage response has been demonstrated to be quite rapid (Koenig et al. 2018a, b), allowing them to be used as contemporary indicators of peatland conditions. Testate amoeba taxa respond to varying peatland conditions differently (e.g., changes in moisture availability; nutrients; light; pH), meaning their response may be useful in inferring subtle changes in these habitats (Marcisz et al. 2020), where measured changes could take much longer to be fully understood. Testate amoebae could prove valuable for organisations trying to secure competitive funding for restoration work, where measured metrics are ambiguous or respond to management intervention too slowly.

Swindles et al. (2016) presented a contemporary time-series approach to testate amoebae assemblage response to changing peatland conditions before and after management interventions on a Welsh blanket bog—prior to this, similar research analysed testate amoebae assemblage changes in peat profiles after restoration (e.g., Davis and Wilkinson 2004; Valentine et al. 2013). Swindles et al. (2016) described a complex assemblage response of testate amoebae following restoration, where diversity increased, and the appearance of key unambiguous wet indicator taxa reflected the observed shift towards wetter peatland conditions. Additionally, Swindles et al. (2016) observed potential interactions between drainage

ditches/treatment sites but could not easily discern this result due to the close proximity of control and treatment sites. For this study, control site locations were carefully considered to avoid obscuring potential interactions between drainage ditches or broader site-wide change. Creevy et al. (2018, 2023) demonstrated promising signs of peatland and testate amoebae assemblage recovery in a forest-to-bog restoration effort. Recent research highlights that further work is needed to provide a clear understanding of testate amoeba response to peatland restoration (Swindles et al. 2016; Creevy et al. 2018, 2023; Kuuri-Riutta et al. 2022). In this study, we investigate the response of testate amoebae to drainage ditch blocking from 2019 to 2021 on a lowland raised bog in Northern Ireland. A site from the Collaborative Action for the Natura Network (CANN) project was selected, which underwent drain blocking restoration in early 2020. This project provided regular water-table monitoring (Fig. 2) which continued for several years to align with restoration. This allowed us to collect *Sphagnum* samples months before the restoration began and have access to routine-monitoring data for the entire duration of the study.

## Hypotheses

We tested the following hypotheses:

- H1** Drain blocking leads to a change in testate amoebae assemblage dynamics.
- H2** Unambiguous wet-indicator taxa abundance increases in response to restoration.
- H3** Increased testate amoebae taxa diversity is observed following restoration.

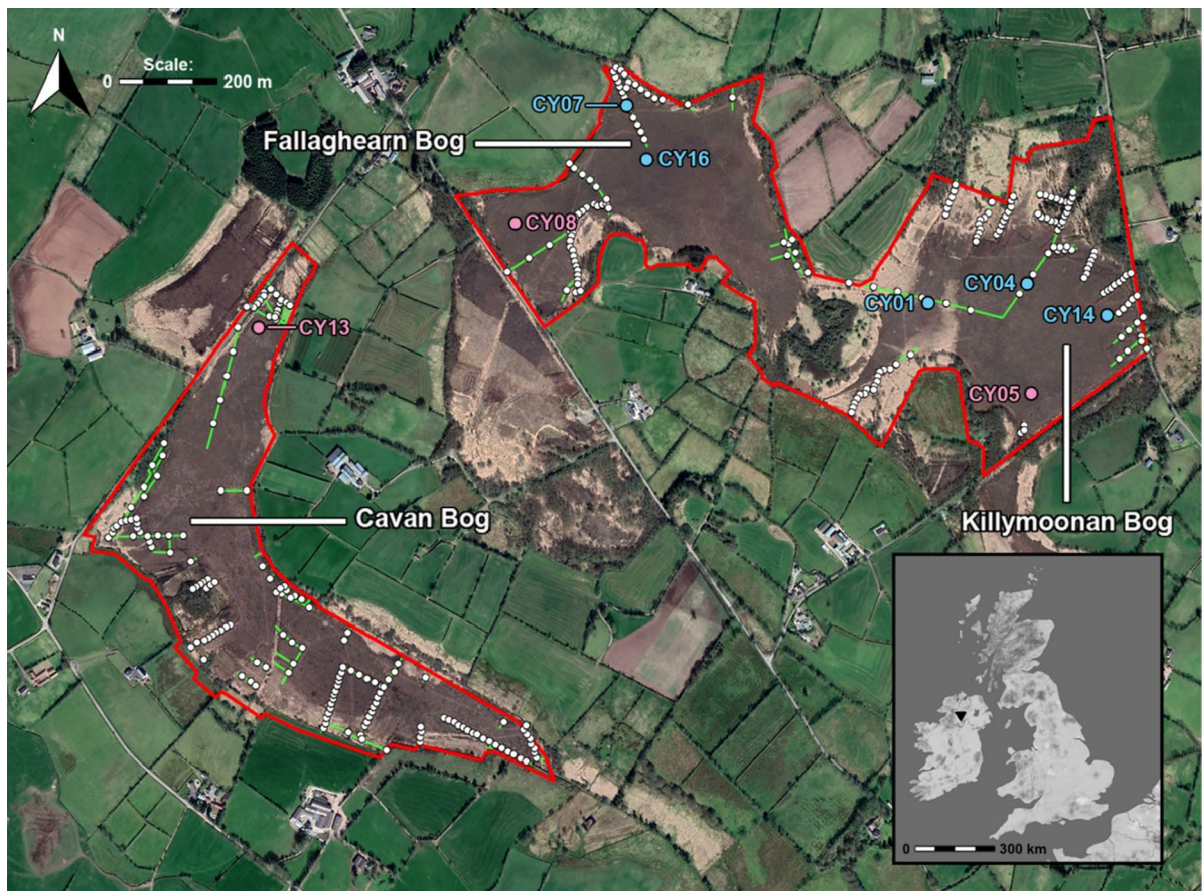
## Methods

### Field site

This study was conducted at Cranny Bogs, a Special Area of Conservation (SAC) close to the town of Fintona in County Tyrone, Northern Ireland (54° 31' 24.0" N, 7° 20' 37.0" W). The site consists of three small, lowland raised bogs: Fallaghearn and

Killymoonan bogs to the east; and Cavan bog to the south-west (Fig. 1). The bogs are a combination of M18 *Erica tetralix-Sphagnum papillosum* raised and blanket bog, and M2 *Sphagnum cuspidatum/recurvum* bog pool communities under the UK National Vegetation Classification (NVC) (Rodwell 2006; DAERA 2015). The bog-myrtle *Myrica gale* can be found across all three bogs with its highest presence felt on Fallaghearn bog and least on Killymoonan bog. Cranny bogs have been predominantly damaged by peat cutting. Although areas of old hand cutting have since begun to regenerate, the historic activity left exposed cut faces as high as 2.5 m. A review of the site in 2015 (DAERA 2015) indicated that it is not known if an extant consent for peat cutting exists and that mechanised peat

extraction has occurred in recent years. All drainage at the site is believed to be associated with this legacy of peat cutting. Sporadic burning is reported on all three bogs with the eastern bogs (Fallaghearn and Killymoonan bogs) having the largest extent of this past damage (DAERA 2015). The site also experiences nitrogen deposition (24.44 kg N/ha/year) above the calculated critical load for raised and blanket bogs (5–10 kg N/ha/year) (DAERA 2015; APIS 2020). The average annual rainfall at the site is between 1050 and 1650 mm year, with average temperatures of 4.2 °C in January and 15.4 °C in July (averages calculated from Edenfel Park and Castlederg MIDAS land surface weather stations for 2019, 2020, and 2021—Met Office 2022a, b) with an elevation of around 110–120 m.



**Fig. 1** Location map of the study site, Cranny Bogs (Cavan, Killymoonan, and Fallaghearn bogs) in County Tyrone, Northern Ireland. *Sphagnum* samples taken approximately 1 m from piezometer locations. Pink=control piezometer and sample

(e.g., CY05); Blue=dammed piezometer and sample (e.g., CY01); White circles=dam locations; Green line=drainage ditches; Red line=SAC boundary (Sources: DAERA 2015; Google Earth 2022)

## Restoration measures

*Sphagnum* samples were collected from eight locations across the three bogs and were selected based on their proximity to the drainage ditches present on the site. The replicates were classified into one of two restoration measures, either ‘dammed’ or ‘control’. The five ‘dammed’ replicates were located in areas of focused hydrological flow near drains whilst the three ‘control’ replicates were collected from areas away from drains and with no contributing hydrological catchment (Fig. 1). All five ‘dammed’ replicates were sampled from the two eastern bogs (Fallaghearn and Killymoonan bogs), whereas the ‘control’ replicates were located in each of the three bogs (Fallaghearn, Killymoonan, and Cavan bogs). The moss species chosen at each sample location were most commonly *Sphagnum capillifolium*. One dammed sample site (CY01) had a mix of *S. capillifolium* and *Sphagnum papillosum*, with a second dammed sample site (CY16) having a mix of *S. capillifolium* and *Sphagnum magellanicum*.

## Routine monitoring

Baseline-monitoring began in the summer of 2018, for the CANN project. Regular water-table monitoring started on 8th July 2019, concluding several months after the final *Sphagnum* samples were collected for this study, on 25th October 2021. Damming of the drainage ditches on the site margins (Fig. 1) began on 30th January 2020 and was complete on all three bogs by 28th February 2020. Dams were constructed using peat, a common technique with smaller width drainage ditches (Armstrong et al. 2009; Parry et al. 2014).

## Measurement of meteorological conditions

The Met Office Integrated Data Archive System (MIDAS) land surface station data was the source of meteorological data used in this study (Fig. 2) (Met Office 2022a, b), including air temperature (°C) and rainfall (mm) which the system reports on daily and hourly intervals. The closest station available for temperature data was situated approximately 8.8 km to the north of the study site in Edenfel Park (54° 35' 38" N, 7° 16' 59" W—Met Office 2022a), with the most suitable station for rainfall data being 24.8 km

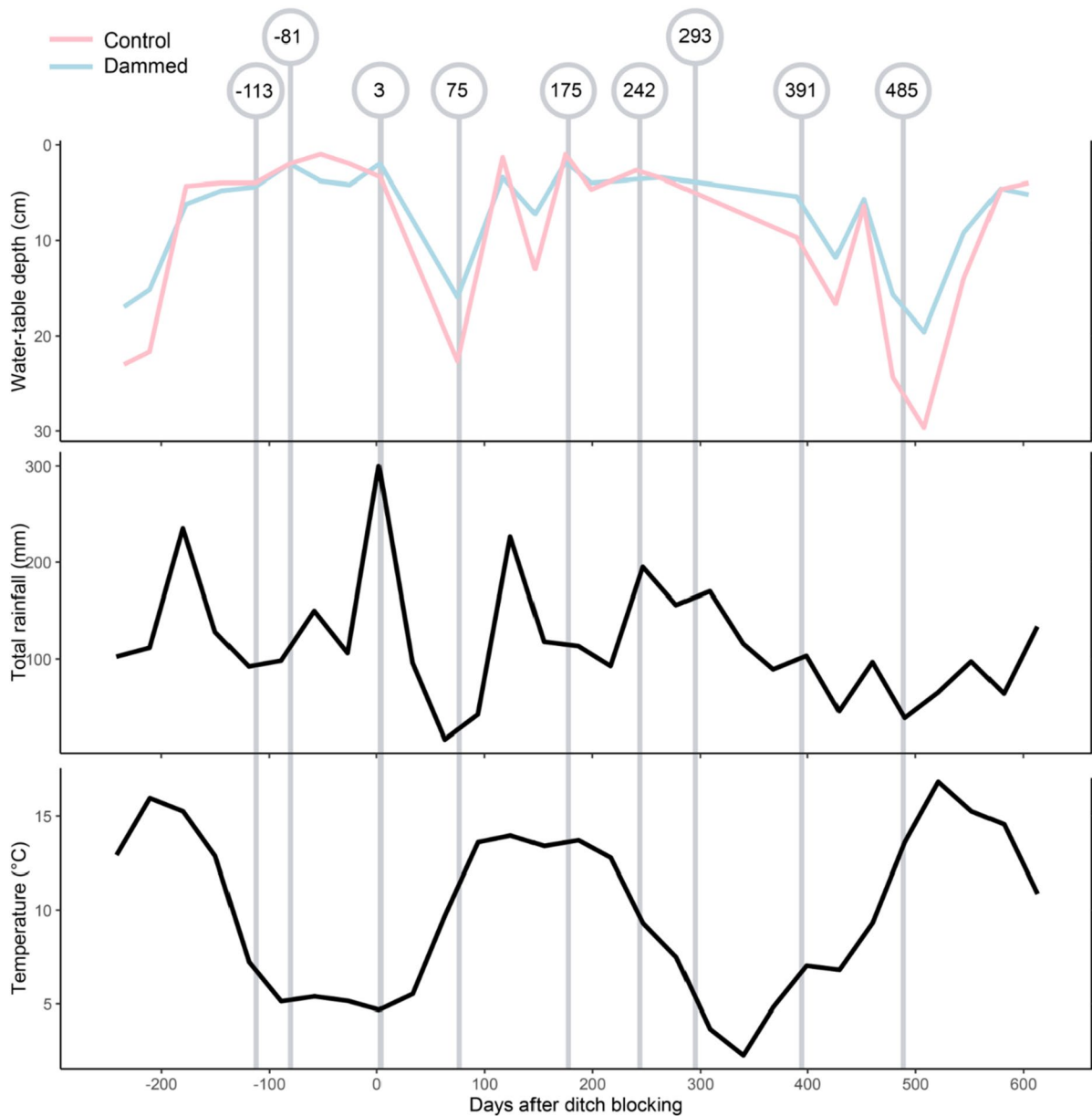
to the north-west of the study site in Castleberg (54° 42' 25" N, 7° 34' 37" W—Met Office 2022b). Other potential weather stations that were considered were further from the site or had incomplete data for the time period required.

## Measurement of water-table depths

Sixteen piezometers were installed across Cranny Bogs in 2019 as part of the CANN project (The CANN Project 2022) to record water-table depth in areas of varying peatland condition (Fig. 2). Twelve piezometers were installed in areas of established raised bog. Of these, eight were located close to drains that were subsequently dammed, and three were placed far from the catchment areas and drains of the peatland. A single piezometer was positioned near catchment areas but away from proposed dams. All eight *Sphagnum* sampling sites (five ‘dammed’ and three ‘control’ restoration measures) were next to these piezometers on the raised bog. The remaining four piezometers were installed in areas of cutover bog are connected to the catchment areas of the peatland.

## Sampling of testate amoebae

Live *Sphagnum* samples were collected 113 (07/11/2019— $t_{.2}$ ) and 81 (09/12/2019— $t_{.1}$ ) days before, and 3 (02/03/2020— $t_0$ ), 75 (13/05/2020— $t_1$ ), 175 (21/08/2020— $t_2$ ), 242 (26/10/2020— $t_3$ ), 293 (16/12/2020— $t_4$ ), 391 (24/03/2021— $t_5$ ), and 485 (28/06/2021— $t_6$ ) days after ditch dam construction was concluded (28/02/2020), corresponding to dates in which routine monitoring occurred at Cranny Bogs. The upper-most part of the *Sphagnum* moss (including a minimum of approximately 2 cm<sup>3</sup> of capitulum, stem, and branches) was selected in the field because the aerobic portions of *Sphagnum* stems have been shown to have the most live testate amoebae (Booth 2002). Each date corresponds with a date of routine water-table monitoring on Cranny Bogs with piezometers near all eight sample sites. *Sphagnum* samples measured greater than 2 cm<sup>3</sup> and were collected from a plot approximately 1 m from a piezometer and kept in individually labelled Ziplock bags. Samples were stored in a freezer until further preparation was carried out. *Sphagnum* samples were prepared using a mostly unmodified version of the



**Fig. 2** Environmental variables over the course of the experiment. Water-table depths were routinely monitored on-site at dammed and control sample locations. Total monthly rainfall

and mean monthly temperature data are from the Met Office Integrated Data Archive System weather stations (Met Office 2022a, b)

standard method (Booth et al. 2010) with the resulting testate amoebae samples being stored at 4 °C in the laboratory for analysis and identification over the following weeks. *Sphagnum* samples in boiling water were passed through a coarse-sieve of 300  $\mu\text{m}$ , then back-sieved at 15  $\mu\text{m}$ . Testate amoebae were then counted and noted as dead (only the test or partial

test present) or ‘live’ (with a clearly visible cytoplasm) under transmitted light microscopy at  $\times 200$ . Identification was performed at this time using illustrated taxonomic guides (Charman et al. 2000; Siemensma 2022). A minimum of 100 testate amoebae were identified and counted per sample (mean = 197). This minimum threshold is recommended by Payne

and Mitchell (2009) for peatland water-table depth studies. Two *Lycopodium* spore tablets were added to samples and were later counted as recommended by the standard method (Booth et al. 2010), and test concentrations were calculated using an established formula (Stockmarr 1971).

### Statistical analysis

Statistical analysis and data presentation was carried out using R version 4.2.1 (R Core Team 2022). The R package ‘vegan’ (version 2.6-2) was used for Non-metric Multidimensional Scaling (NMDS), permutational MANOVA (PERMANOVA), and Analysis of Similarity (ANOSIM), for use in investigating the response of testate amoebae assemblages to several experimental and environmental variables (time, restoration measure, rainfall, temperature, and measured and reconstructed water-table depth). Nonmetric multidimensional scaling (NMDS), PERMANOVA, and ANOSIM analysis were carried out using the Bray–Curtis dissimilarity index. The Shannon Diversity Index (Shannon and Weaver 1949) was calculated for each sample to explore species diversity. Water-table depth reconstructions were generated using the pan-European testate amoeba-based transfer function of Amesbury et al. (2016) using weighted average tolerance downweighting with inverse de-shrinking (WA-Tol). To achieve precision in the testate amoeba-based transfer function reconstructed water-table depths, a minimum count of 100 testate amoebae was strictly adhered to (Payne and Mitchell 2009).

### Results

The most commonly occurring testate amoebae taxa identified at Cranny Bogs were *Nebela tincta*, *Assulina muscorum*, *Archerella flavum*, *Hyalosphe- nia elegans*, *Euglypha ciliata*, and *Heleopera sylvat- ica* (Fig. 6). The six most common taxa represented the vast majority (75%) of the 38 testate amoebae taxa identified. In total, 12,850 individuals were counted (mean 27.65% live testate amoebae—3463 individuals).

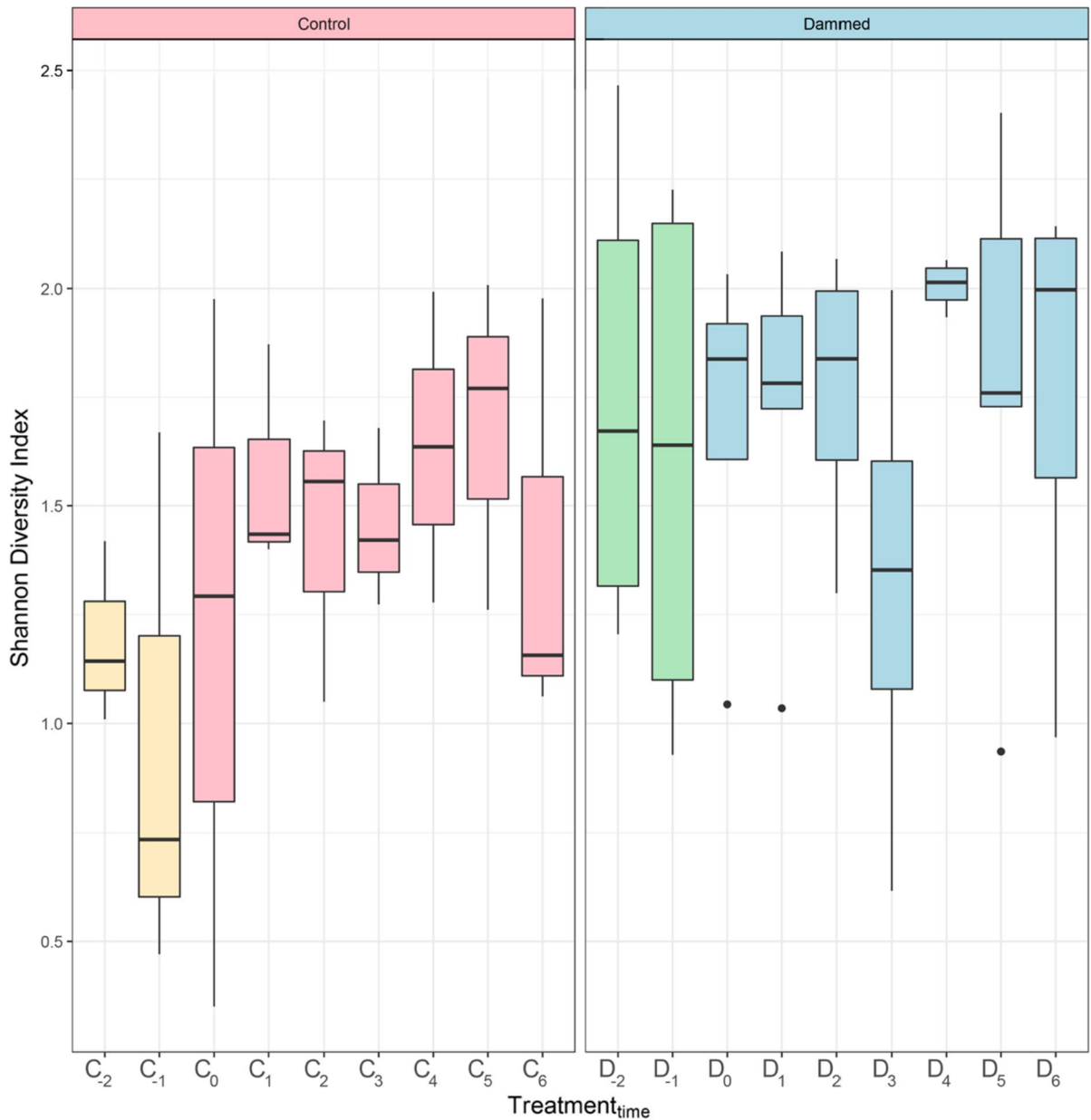
The Shannon Diversity Index (SDI) scores of the assemblages ranged from 0.35 to 2.46 and generally increased both in the control and dammed restoration measures after damming occurred ( $t_0$  onwards)

(Fig. 3). At the dammed sites the SDI scores/values were variable before management occurred, showing a high range and including the largest recorded value (CY07  $t_2$ —2.46) of the study prior to  $t_0$  (Fig. 3). Additionally, the diversity in the control sites was substantially lower than the dammed sites prior to  $t_0$ .

Measured water-table depths at Cranny Bogs (Fig. 4a) appeared to be dominated by seasonality, with the data collected showing a trend towards deeper and drier water-tables in summer 2021 ( $t_6$ )—coinciding with lower observed rainfall during this period. Water-table depths were also reconstructed using a pan-European transfer function (Amesbury et al. 2016) (Fig. 4b) which showed substantial changes to the water-table depths of the dammed restoration measure sites. Reconstructed water-table depths were shallower and wetter in the dammed sites before intervention compared with the control sites.

A number of environmental variables were measured to test against this assemblage dataset. Observed and reconstructed water-table depths, air temperature, rainfall, time, and restoration measures could all be used in association with the testate amoebae assemblages to infer significant effects. Nonmetric multidimensional scaling with ANOSIM and PERMANOVA were used to evaluate the effect these environmental variables might have had on the assemblage composition of testate amoebae (NMDS – ‘stress’ ~ 0.25). All combinations of the dataset were considered, including how the results differed through time (before and after management). None of these variables were found to have a statistically significant effect on the testate amoebae assemblage composition (95% level).

The occurrence of non-ambiguous wet indicator taxa was notable in that prior to installation of drainage ditch dams ( $t_2$  and  $t_1$ ) the only occurrence of these key taxa was a single occurrence (0.43% abundance,  $n = 1$  specimen) of *Amphitrema wrightianum* in a control site (CY13,  $t_1$ ) and a peak of 24.89% abundance of *Archerella flavum* in a dammed site (CY14,  $t_1$ ) (Fig. 5). After management occurred ( $t_0$  onwards) these key taxa did not appear in greater abundance in control sites (Fig. 5), where *A. wrightianum* was never observed again and *A. flavum* appeared in a single occurrence (CY13,  $t_1$ —0.45% abundance). *Amphitrema flavum* was observed in increasing abundance in the dammed sites, peaking at 66.19% relative abundance (CY14,  $t_6$ ) (Fig. 5). Additionally, the key indicator taxa *A.*



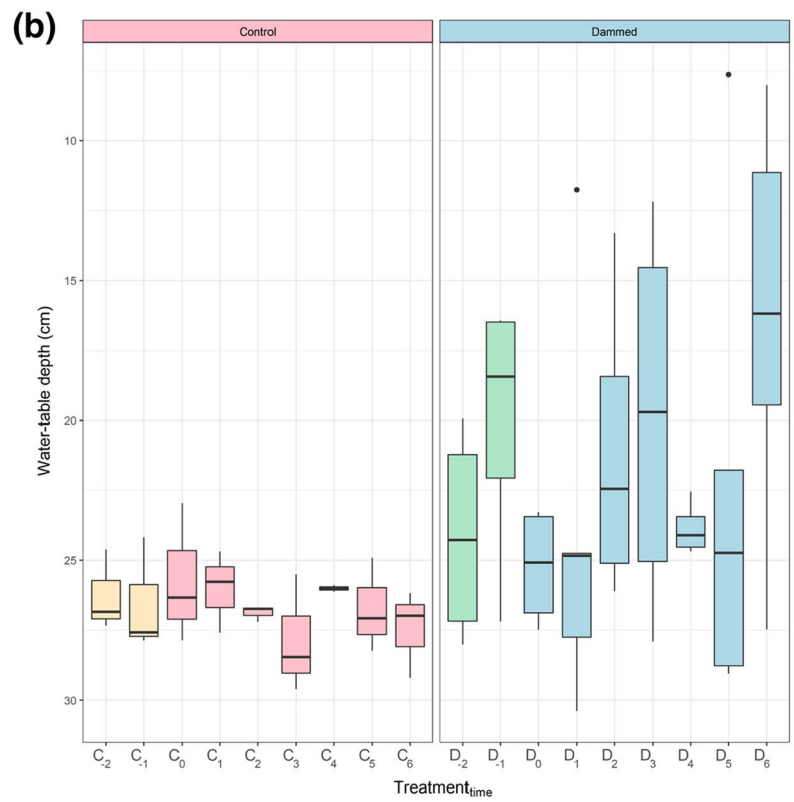
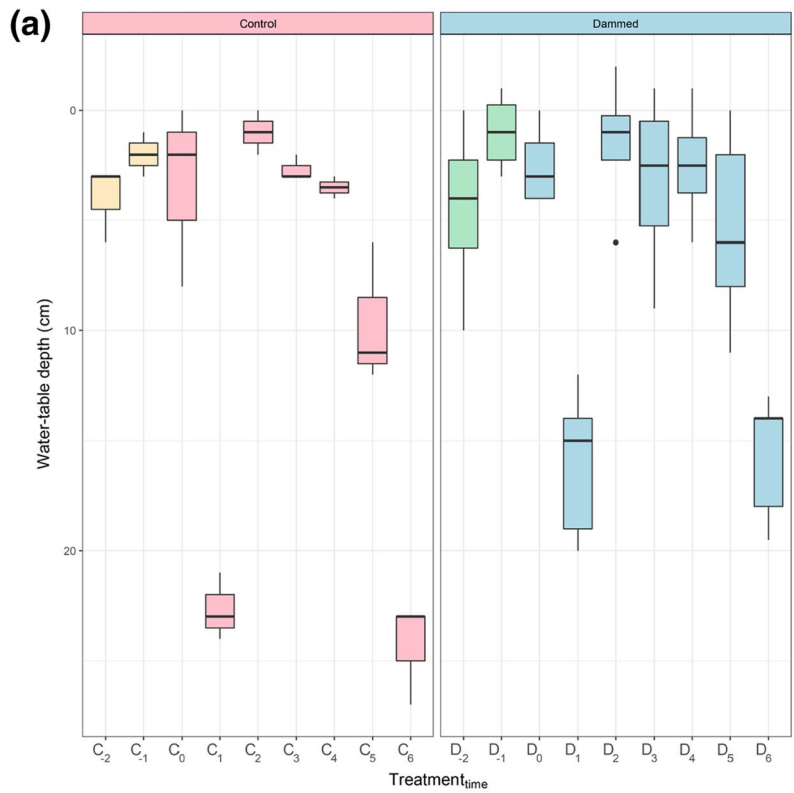
**Fig. 3** Boxplot of testate amoebae assemblage Shannon Diversity Index from control and dammed sample sites. Results prior to restoration (t-2 and t-1) are highlighted

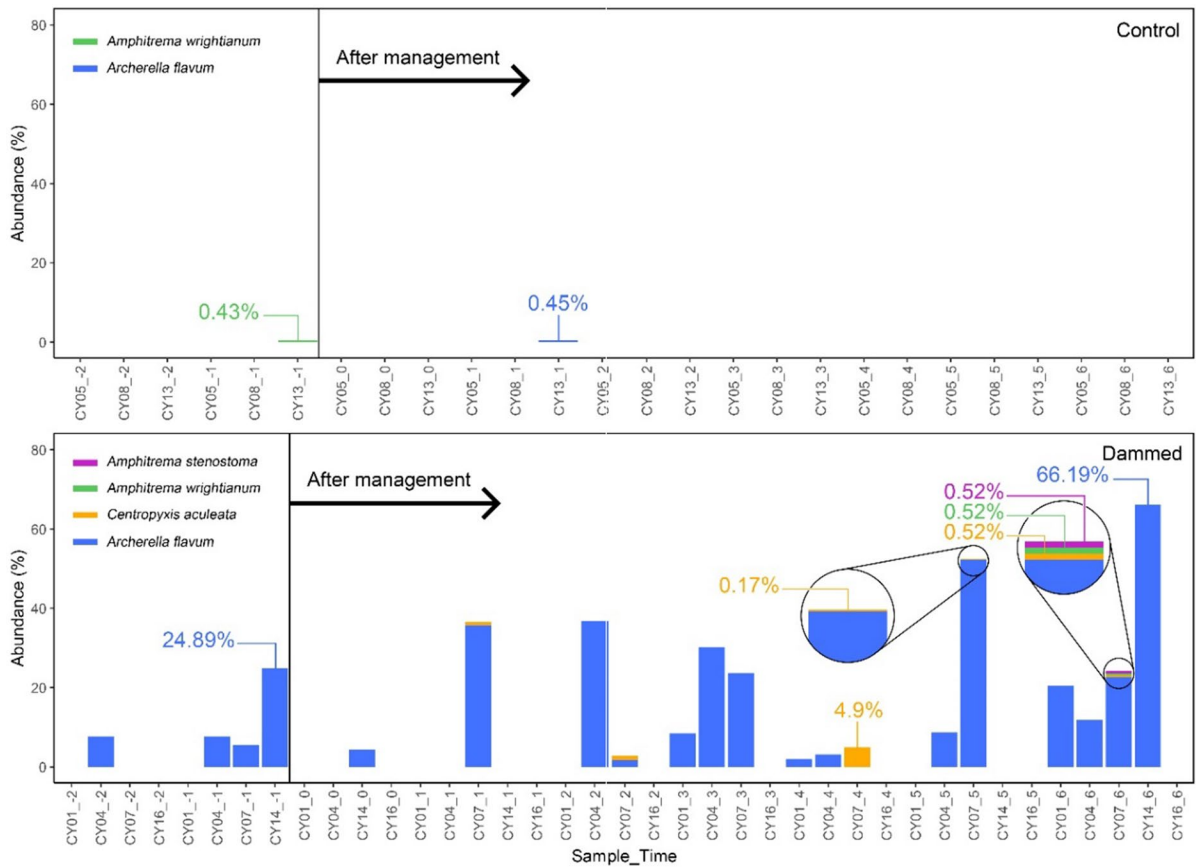
*wrightianum*, *Amphitrema stenostoma*, and *Centropyxys aculeata* type were recorded in a dammed site (CY07) after management occurred (Fig. 5). The single occurrences of *A. wrightianum* and *A. flavum* in the control sites is not likely to be through

interaction between the five dammed sites of this study as these taxa occurred at CY13, the sole sampling location on the southernmost bog, Cavan Bog, where CY13 is located, is separated from the two northern bogs by a road and farmland (Fig. 1).



**Fig. 4 a** Boxplot of measured water-table depths. Results prior to restoration (t-2 and t-1) are highlighted. **b** Boxplot of testate-amoebae based transfer function reconstructed water-table depths. Results prior to restoration (t-2 and t-1) are highlighted





**Fig. 5** Abundance (%) of unambiguous wet indicator taxa before and after restoration ( $t_0$  onwards). Sample site codes (e.g., CY16) are denoted with time of collection (e.g., - 2, - 1, 0 etc.) as seen in Fig. 1

## Discussion

Peatland restoration at Cranny Bogs was characterised by a complex response of testate amoebae. Measured water-table depth indicated no clear response following management intervention ( $t_0$  onwards) (Fig. 4a). Unseasonably dry conditions towards the end of the experiment ( $t_4 - t_6$ ) appear to manifest as a slight trend towards deeper water-table depths (Figs. 2, 4a). Contrary to the measured results, testate-amoeba based transfer function reconstructed water-table depths show a notable trend toward shallower water-table depths in dammed restoration measure sites (Fig. 4). The appearance and proliferation of key unambiguous wet indicator taxa in the dammed sites (e.g., *A. flavum*) (Fig. 5) implies that restoration is having its intended effects. A general increase in SDI across the sites may indicate that management is

having a wider ecohydrological impact across Cranny Bogs (Fig. 3). The large increase in wet indicator taxa specifically in the dammed sites (Fig. 5) suggests that this diversity increase is not caused by interactions between control and dammed sites as seen with similar studies (Swindles et al. 2016). Environmental variables and restoration measures had no statistically significant effect (95% level) on the testate amoebae assemblages. This lack of significance could be in part due to the antecedent conditions of the sites prior to the start of data collection. We believe *N. tinctoria* may have caused the lack of significance in our multivariate analysis. *Nebela tinctoria*, a ubiquitous taxon which is regarded as a poor hydrological indicator (Mitchell and Buttler 1999; Charman et al. 2000; Beaulne et al. 2018), often appears in very wet conditions (Warner 1987) but is commonly interpreted as a dry indicator (Booth 2008; Koenig et al. 2018a, b).

We observed very high abundances of *N. tinctoria* in our samples (Fig. 6), with this taxon even appearing in post-restoration samples at as much as 90% of the testate amoebae identified. The dominance of *N. tinctoria* in this site is problematic given the taxon's indifferent or ambiguous response to hydrological conditions. Nevertheless, the abundance of unambiguous wet indicator taxa increased substantially in the dammed sites (Fig. 5), despite decreased rainfall that could have led to drier conditions in 2021 ( $t_5$  and  $t_6$ ), suggesting improved hydrology at Cranny Bogs following management intervention.

Although the response of testate amoebae to peatland drain blocking in this study has been complex, we can accept two of our three proposed hypotheses. The appearance and proliferation of unambiguous wet-indicator taxa in dammed restoration measure sites and the general increase of SDI on Cranny Bogs allows us to accept our second and third hypotheses ( $H_2$ —Unambiguous wet-indicator taxa abundance increases in response to restoration;  $H_3$ —Increased testate amoebae taxa diversity is observed following restoration). Despite promising signs of testate amoebae assemblage change (e.g., increasingly wet reconstructed water-table depth), the remaining hypothesis ( $H_1$ —Drain blocking leads to a change in testate amoebae assemblage dynamics) must be rejected, as NMDS, PERMANOVA, and ANOSIM multivariate analysis illustrated a lack of statistically significant change at the testate amoebae assemblage-level.

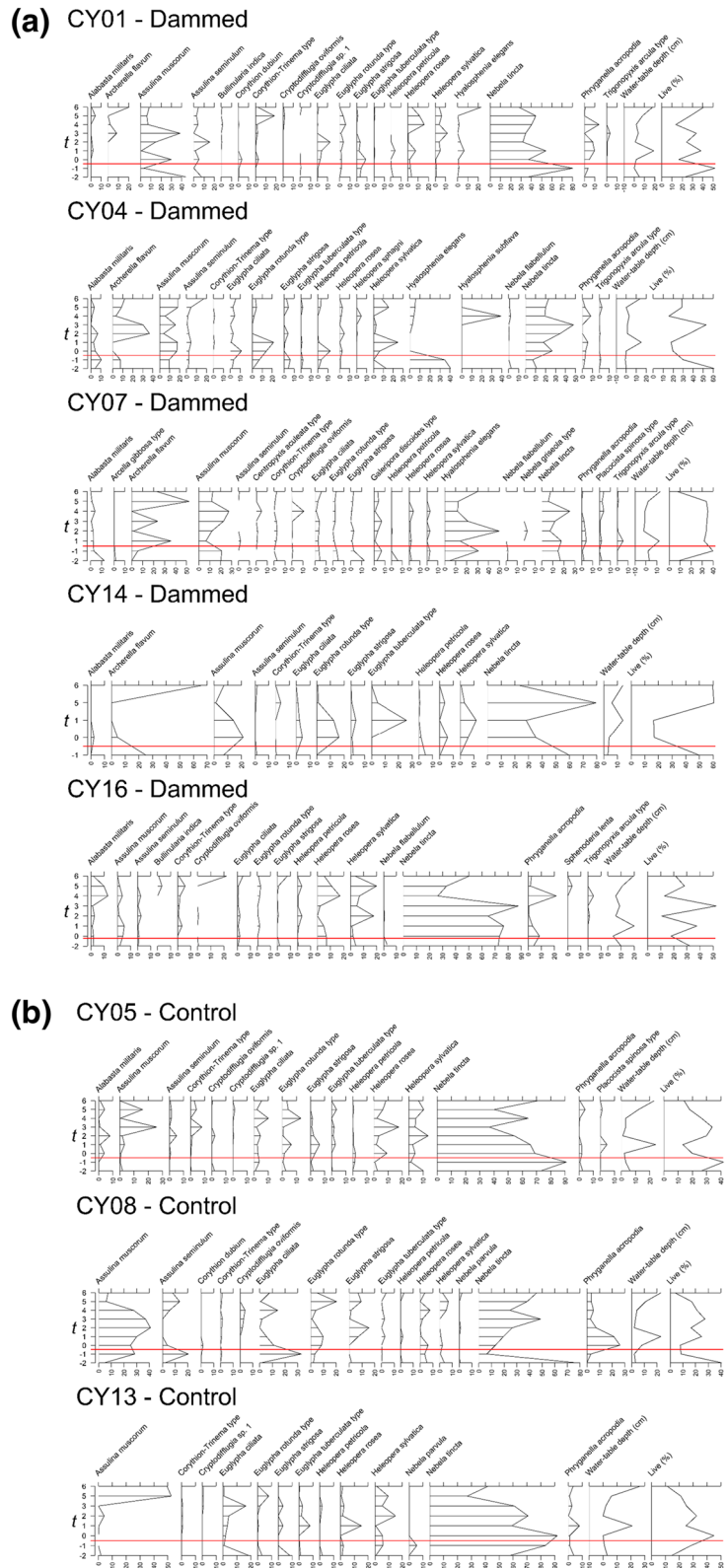
Sample collection at Cranny Bogs began opportunistically, including antecedent conditions that may have caused a lack of significance in our multivariate analysis of testate amoebae assemblages. However, several previous experiments have demonstrated the significant influence environmental factors have at the testate amoebae assemblage-level. Variables associated with anthropogenic pressures have been shown to effect testate amoebae assemblages significantly. Daza Secco et al. (2018) reported that as much as 75% of the difference between testate amoeba assemblages in samples from three raised bogs in Finland were driven by land use. Further understanding of how these assemblages respond to environmental variables may be critical for restoration success. Creevy et al. (2018, 2023) examined forest-to-bog restoration sites where even after 17 years testate amoebae communities had not recovered due to limited expansion of *Sphagnum* after restoration. There is evidence

to suggest that peatlands may be more resistant to hydrological change than previously thought (Lamentowicz et al. 2019), meaning a detailed paleo record at Cranny Bogs could aid in interpreting the sites' ability to recover from present and future disturbance. Further knowledge of the assemblage response of testate amoebae to restoration and critical environmental variables could inform effective restoration efforts. Our results show that these testate amoebae can respond rapidly to restoration, which could avoid decades of stagnant or ineffectual management practice.

Specific methods for testate amoebae sample preparation and analysis can be contentious (e.g., peat/*Sphagnum* sample boiling—Avel and Pensa 2013). Of note to this study, and our employed *Sphagnum* preparation methods (Booth et al. 2010), is the issue of micro-sieving (e.g., 15  $\mu\text{m}$  back-sieve) which has been shown to exclude small testate amoebae taxa (Avel and Pensa 2013; McKeown et al. 2019). Many unambiguous wet indicator taxa, such as those found on Cranny Bogs (*A. flavum*, *C. aculeata* type, *A. stenostoma*, and *A. wrightianum*), are too large (>45  $\mu\text{m}$ ) to be affected by these issues with micro-sieving. However, the multivariate analysis carried out for this study may have been affected by the omission of these small testate amoeba taxa. For instance, the common taxon *Cryptodiffugia oviformis*, was not found to be widespread on Cranny Bogs before or after restoration occurred. Other widespread but notably larger taxa (e.g., *Assulina muscorum*) were observed, which could highlight a loss of small taxa in this study.

At present the use of testate amoebae as bioindicators of peatland restoration success is better understood through an indicator-based approach, where known responses to changing environmental variables by specific taxa are used to infer the condition of the peatland. In this study we demonstrated that this method can be applied relatively simply and can be effective in scenarios where antecedent conditions and climatic events may cause issue with other forms of analysis. Here we used this approach to understand the hydrological response of a raised bog before and after re-wetting occurred. Using key unambiguous wet indicator taxa (*A. flavum*, *C. aculeata* type, *A. stenostoma*, and *A. wrightianum*) we have been able to infer wetter conditions over the course of nearly two years. In this same time frame, direct observation of water-table depths did not clearly indicate wetter

**Fig. 6 a** Percentage testate amoebae data for dammed sample sites (CY01, CY04, CY07, CY14, and CY16—Fig. 1). Measured water-table depth and the percentage of live testate amoebae are included. Red line denotes date of restoration. Samples were collected on the following dates: 07/11/2019 (– 2); 09/12/2019 (– 1); 02/03/2020 (0); 13/05/2020 (1); 21/08/2020 (2); 26/10/2020 (3); 16/12/2020 (4); 24/03/2021 (5); and 28/06/2021 (6). **b** Percentage testate amoebae data for control sample sites (CY05, CY08, and CY13—Fig. 1). Measured water-table depth and the percentage of live testate amoebae are included. Red line denotes date of restoration. Samples were collected on the following dates: 07/11/2019 (– 2); 09/12/2019 (– 1); 02/03/2020 (0); 13/05/2020 (1); 21/08/2020 (2); 26/10/2020 (3); 16/12/2020 (4); 24/03/2021 (5); and 28/06/2021 (6)



conditions due to periods of drought. Koenig et al. (2015) highlighted how straightforward this approach can be, by demonstrating that the use of as few as ten easily identifiable testate amoebae taxa, can be used to outperform bryophytes and vascular plants in ‘bioindication value’. Furthermore, the use of testate amoeba indicator taxa may have applications in monitoring other forms of anthropogenic pressures on peatlands (e.g., ammonia pollution indicators—Payne et al. 2013).

If more is not done to understand the effectiveness of peatland restoration efforts, greenhouse gas emissions from degraded peatland sites could worsen. Emissions estimates from degraded peatlands are contentious (Leifeld and Menichetti 2018; Xu et al. 2018) with estimates of global total emissions and carbon stores of peatlands consistently uncertain (e.g., Congo Basin peatland extent; Dargie et al. 2017). Degraded UK peatland emissions were only formally included in the national greenhouse gas inventory as late as 2019 (Parliamentary Office of Science and Technology 2022). Official estimates for the Republic of Ireland have historically had considerable error ranges (Wilson et al. 2013; Donlan et al. 2016). Tropical peatlands account for the majority of peatland greenhouse gas emissions at this time (Leifeld and Menichetti 2018; Ribeiro et al. 2021), though notably they account for a major part of the global store of peatland carbon (Xu et al. 2018; Ribeiro et al. 2021). The response of tropical peatlands to restoration is poorly understood, particularly in terms of greenhouse gas emissions changes following management intervention (Kumar et al. 2020). Uncertain estimates for global peatland greenhouse gas emissions already suggest that as much as 1.91 Gt of CO<sub>2</sub> equivalents are released per year (Leifeld and Menichetti 2018). If global emissions are actually much higher, the case for understanding and implementing improved peatland restoration is urgent. Testate amoebae could be a vital part of these future restoration efforts, informing management practice and aiding in accurate and effective monitoring. However, the use of testate amoebae in this manner is not well understood in tropical peatlands (Swindles et al. 2014; Liu et al. 2019; Krashevskaya et al. 2020) so we extend recommendations of further research on testate amoebae and restoration effectiveness in these regions, and investigations into the use of testate amoebae as bioindicators in agriculture and forestry on drained peatlands. In Germany

greenhouse gas emissions from drained peatlands make up the largest part of emissions from agriculture (Tiemeyer et al. 2020), nevertheless a growing body of research suggests that agriculture and forestry on drained peatlands could potentially remain productive while greatly reducing emissions (Tanneberger et al. 2020; Evans et al. 2021). However, study of testate amoebae assemblage response to agricultural systems and forestry on drained peatlands is limited: Daza Secco et al. (2018) demonstrated the use of testate amoebae assemblages as bioindicators on drained peatlands for forestry; and Qin et al. (2020) presented their use on drained peatlands used for agricultural. In this study we have noted that restoration at Cranny Bogs has likely influenced the hydrology of the site—creating wetter conditions that favour these wet-indicator taxon (e.g., *Archerella flavum* and *Centropyxis aculeata* type). However, testate amoebae assemblages also respond to a number of ecological controls (Arrieira et al. 2015; Roe et al. 2017) such as: food availability; temperature; light; oxygen; and access to minerals for ‘test’ construction. The ways in which re-wetting influences these controls should be studied in greater detail.

Swindles et al. (2016) advised caution when using testate amoebae for biomonitoring of peatland restoration, especially when trying to analyse their assemblage-level response, due to this method needing further and more robust experimentation. Here we extend that recommendation of caution and highlight that more research needs to be targeted at understanding the assemblage-level response of testate amoebae following restoration. However, we note that the use of unambiguous wet indicator taxa has merit in rapid bioindication for early stages of peatland restoration.

## Conclusions

This study investigated the use of testate amoebae as contemporary bioindicators of peatland hydrology following restoration on three small lowland raised bogs in Northern Ireland. Unambiguous wet indicator taxa (*A. flavum*, *C. aculeata* type, *A. stenostoma*, and *A. wrightianum*) were observed in increasing abundance on dammed restoration measure sites after management intervention ( $t_0$  onward)—with some taxa appearing for the first time. These unambiguous wet indicator taxa were not observed in

increasing abundance on any of our control sites. Diversity increased in both the control and dammed sites. This increase of unambiguous wet indicator taxa on dammed sites, lack of proliferation of these taxa in control sites (despite single occurrences of *A. flavum* and *A. wrightianum*), and a general site-wide diversity increase, suggest management has led to wetter conditions in and around former drainage ditches. Multivariate analysis was conducted to evaluate the assemblage-level response of testate amoebae in relation to experimental and environmental variables. Drought and the antecedent conditions of the study site appear to have affected the findings of this analysis, and as such we observed a lack of statistically significant assemblage-level change in response to these variables. Though complex, the findings of this investigation contribute to the growing body of research illustrating the value of testate amoebae as contemporary bioindicators of peatland restoration. An indicator-taxa based approach remains the clearest way of utilising testate amoebae for bioindication, with their assemblage-level response remaining complex and in need of further study at this time.

**Acknowledgements** We thank Ronald Surgenor from Ulster Wildlife for assistance in the field and helping us gain access to Cranny Bogs.

**Author contributions** CRCE, GTS, and PMF contributed to the study conception and design. Material preparation, data collection and analysis were performed by CRCE, DJM, and SG. The first draft of the manuscript was written by CRCE. GTS, HMR, and DJM commented on previous version of the manuscript.

**Funding** This study was undertaken as part of a PhD project at Queen's University Belfast, Northern Ireland, UK. A PhD stipend and research training support grant was provided by the Department for the Economy Co-operative Awards in Science and Technology (DFE CAST). Sphagnum samples were collected by Ulster Wildlife staff working on the Collaborative Actions for the Natura Network (CANN) project, an Interreg Europe project managed by the Special European Union's Programme Body. G.T. Swindles acknowledges financial support from the Dutch Foundation for the Conservation of Irish Bogs, the Quaternary Research Association and the Leverhulme Trust (RPG-2021-354). All other authors have no relevant financial or non-financial interests to disclose. Graeme T Swindles, Donal J Mullan, and Helen M Roe were all employed by Queen's University Belfast during the study period. Patricia M Fox and Simon Gray were both employed by Ulster Wildlife during the study period.

**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

#### Declarations

**Competing interests** The authors declare that they have no conflict of interest.

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