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# Testate amoebae are informative bioindicators of critically high ammonia deposition on peatlands

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ABSTRACT

The global nitrogen cycle has been majorly disrupted by anthropogenic activity. While nitrogen emissions in the UK and Ireland are declining, ammonia ($NH_3$) remains a significant exception. $NH_3$ emissions are mostly agriculturally sourced and deposited on nearby habitats at high rates in both countries. Peatlands are globally important wetlands that are vulnerable to $NH_3$ deposition. Essential peatland restoration risks being diminished by excessive $NH_3$ deposition, leading to the loss of valuable ecosystem services. This study investigates testate amoebae (indicators of contemporary and historic peatland conditions) as bioindicators of seasonal $NH_3$ deposition on six peatlands across Northern Ireland, UK. *Sphagnum*, an $NH_3$-sensitive bryophyte, was sampled adjacent to $NH_3$ monitoring sites once per season for a year. When $NH_3$ deposition was critically high, multivariate analysis demonstrates a link between $NH_3$ and testate amoebae assemblage change. Similarly, at high $NH_3$ deposition sites, testate amoebae taxa diversity is observed to be significantly reduced in springtime, when it is expected to be highest. Although, in response to high $NH_3$ deposition large algivorous taxa do not proliferate as was anticipated, and mixotrophic taxa abundance decreases could not be linked primarily to $NH_3$. This research demonstrates the continued potential of testate amoebae as highly informative peatland bioindicators.

## 1. Introduction

Since the development of the Haber-Bosch process (industrial fixation of ammonia ($NH_3$) from atmospheric nitrogen) and the increasing use of manure and synthetic fertilisers in agriculture, anthropogenic activity has heavily disrupted the global nitrogen cycle (Galloway et al., 2008; Stein and Klotz, 2016). In many countries, limited access to nitrogen is contributing to acute food insecurity (Masso et al., 2017). Elsewhere, excess nitrogen can lead to reduced water quality, increases in greenhouse gas emissions, and widespread damage to biodiversity (Guo et al., 2022; Huisman et al., 2018; Midolo et al., 2019). The UK and Ireland have nitrogen in excess (Kelleghan et al., 2021; Woodward et al., 2022), although, both nations have reduced a large part of their nitrogen emissions; excluding $NH_3$ which has remained high for decades (Department of Agriculture, Environment and Rural Affairs, 2024a, 2024b; Environmental Protection Agency, 2024a, 2024b).

Agricultural activity produces most $NH_3$ emissions (> 80 % in the EU) (Leip et al., 2015), which is deposited rapidly on adjacent land and to wider areas through precipitation (Barry et al., 1993; Goss et al., 1995; Loubet et al., 2009). This has led to annual $NH_3$ emissions in the UK and Ireland to exceed 380 Kt, which are overwhelmingly sourced from agriculture (Department of Agriculture, Environment and Rural Affairs, 2024c; Environmental Protection Agency, 2024b; Loubet et al., 2009). A major component of this agriculturally-sourced $NH_3$ deposition is from the storage and field-application of livestock manure slurries (Amon et al., 2001; McGinn and Janzen, 1998). Stored manure can be an acute point-source of $NH_3$ emissions (Amon et al., 2001) and is often applied to agricultural land to improve productivity, replenish soils after crop harvests, and ameliorate grasslands for livestock grazing (Araji et al., 2001; Kumar et al., 2013). If $NH_3$ emissions are not reduced, vulnerable habitats and species could be irreparably damaged, contributing to biodiversity loss and climate change (Behera et al., 2013; Kelleghan et al., 2021).

Peatlands are internationally important habitats, that are threatened by climate change and global biodiversity loss (IPCC, 2023; WWF, 2020). These habitats account for carbon stores in excess of 600 Pg globally (Yu et al., 2010) and are uniquely biodiverse (Bullock et al., 2012; Spitzer and Danks, 2006). However, widespread drainage and industrial peat extraction threaten their carbon storage and contribute to greenhouse gas emissions as high as 1.91 Pg $CO_2$ equivalents per year

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from peatlands (Chapman et al., 2003; Leifeld and Menichetti, 2018; Salm et al., 2012). Additionally, peatland land-use change into agriculture and forestry can severely impact peatland biodiversity (Graham et al., 2017; Heikkinen et al., 2023). Efforts to reduce the impact of these anthropogenic pressures are increasingly being pursued, and peatland restoration measures have been implemented for decades (e.g. Fox, 1986; Meade, 1992). Likewise, governments have begun to adopt country-scale peatland protection schemes (e.g. National Peatland Plan (Scotland), National Peatland Strategy (Republic of Ireland), and the Northern Ireland Peatland Strategy) (Nordbeck and Högl, 2024).

Global peatland restoration research has seen an exponential increase in recent years (Apori et al., 2022). However, peatland restoration risks being curtailed by $NH_3$ deposition, as these ombrotrophic and oligotrophic habitats are especially sensitive to atmospheric pollutants; receiving all their nutrients through the atmosphere (Bobbink et al., 1998; Payne et al., 2013; Levy et al., 2018). Critical levels of $NH_3$, defined as concentrations of $NH_3$ 'above which direct adverse effects may occur', exist to protect vulnerable habitats like peatlands (Posthumus, 1988, cited in Cape et al., 2009, p. 1033). This is because bryophytes, such as *Sphagnum* which are integral in the formation of peat (Moore, 1987; Rydin and Jeglum, 2006), are easily damaged by $NH_3$ deposition (e.g. tissue/foliar damage/death) (Cape et al., 2009; Krupa, 2003; Sheppard et al., 2011). As such, the annual mean critical level of $NH_3$ deposition for lichens and bryophytes is 1 μg $NH_3$ $m^{-3}$, a third of the rate for more resilient higher plants (3 μg $NH_3$ $m^{-3}$) (Cape et al., 2009). Critical levels for $NH_3$ deposition are derived from field observations to produce ecologically relevant recommendations to protect vulnerable habitats (Cape et al., 2009). Likewise, the techniques used to define these critical levels can be adapted by practitioners in the field (e.g. Kapusta et al., 2014; Yang et al., 2017).

Testate amoebae are a polyphyletic group of unicellular amoeboid protists found in most freshwater and terrestrial habitats (Smith et al., 2008). The well-preserving shells (tests) of testate amoebae mean they have been used in palaeoenvironmental studies for over a century (e.g. Lindberg, 1899, cited in Tolonen, 1986, p. 645). More recently, palaeohydrological reconstruction techniques have been adapted to enable testate amoebae to function as contemporary bioindicators (Mitchell et al., 1999; Warner and Chmielewski, 1992). Additionally, testate amoebae functional traits and specific tolerances can be informative in determining near real-time peatland changes (e.g. Marcisz et al., 2020; McKeown et al., 2024). Specifically, large testate amoebae taxa (i.e. large pseudostome/test), which have previously been suggested as an $NH_3$ deposition indicator (Jassey et al., 2012; Payne et al., 2013), and mixotrophic taxa, which host endosymbiotic algae that may respond to changes in nutrient availability (Herbert et al., 2019; Marcisz et al., 2014; Payne et al., 2016), could be particularly valuable in detecting the effects of nutrient inputs like $NH_3$ on peatland ecosystems. Additionally, unlike surface vegetation such as *Sphagnum*, which is central to peatland functioning but may show slow or delayed responses to changes in nutrient levels (e.g. Vitt et al., 2003), testate amoebae can exhibit rapid, sensitive reactions to shifts in environmental conditions such as $NH_3$ deposition (e.g. Payne et al., 2016). Their quick response to subtle alterations in water chemistry, nutrient availability, and hydrological conditions makes them an ideal tool for monitoring and detecting early-stage changes in peatland ecosystems (e.g. Evans et al., 2024; Marcisz et al., 2020; Swindles et al., 2016). This sensitivity allows them to serve as valuable bioindicators in tracking the impacts of atmospheric pollutants like $NH_3$ on peatland health, which might not be immediately apparent through other methods, such as assessing *Sphagnum* physiology alone.

There is a growing body of research on testate amoebae as contemporary peatland bioindicators, especially concerning peatland hydrological conditions; study of other environmental factors is comparatively limited (Silva et al., 2022). Although, there have been numerous experiments considering testate amoebae responses to nitrogen input on peatlands (e.g. Gilbert et al., 1998; Jiroušek et al., 2013; Mitchell and Gilbert, 2004; Mitchell et al., 2003), and similar experiments conducted on heathland, urban/industrial areas, and arid soil crusts (Meyer et al., 2012; Payne et al., 2012; Pérez-Uz et al., 2023). However, only one study considers testate amoeba response to $NH_3$ deposition on peatlands specifically (Payne et al., 2013), and to date, no studies examine the response of testate amoebae to agricultural activity-linked seasonal cycles of $NH_3$ deposition on peatlands. In this study, the effects of $NH_3$ deposition on six peatlands across Northern Ireland are examined. *Sphagnum* moss was sampled adjacent to an established $NH_3$ monitoring program once per season for a year, coinciding with expected seasonal cycles of $NH_3$ deposition. The assemblage-level change and functional traits of testate amoebae were analysed to determine if testate amoebae have merit as bioindicators of critically high $NH_3$ deposition.

The following hypotheses were tested:

[$H_1$] Testate amoeba assemblage composition is altered in response to $NH_3$ deposition.

[$H_2$] Abundance of mixotrophic taxa decreases in response to $NH_3$ deposition.

[$H_3$] High $NH_3$ deposition causes an increase in abundances of large testate amoebae taxa.

[$H_4$] Testate amoebae taxa diversity decreases in response to increased $NH_3$ deposition.

## 2. Methods

### 2.1. Field sites

This experiment involved sampling *Sphagnum* and determining $NH_3$ deposition at six peatlands across Northern Ireland (Fig. 1). Samples were collected at Ballynahone Bog (54°49′21.4″ N, 6°39′45.4″ W) (Fig. 1B), Curran Bog (54°47′58.0″ N, 6°38′44.4″ W) (Fig. 1B), Garry Bog (55°06′47.0″ N, 6°31′39.1″ W) (Fig. 1A), Moneygal Bog (54°44′27.0″ N, 7°37′50.1″ W) (Fig. 1E), Peatlands Park (54°29′18.8″ N, 6°35′58.1″ W) (Fig. 1C), and Slieve Beagh (54°20′48.4″ N, 7°10'40.8″ W) (Fig. 1D). Slieve Beagh is an upland blanket bog, with all remaining sites being lowland raised bogs; and all sites are Special Areas of Conservation (SAC). As part of the EU INTERREG VA funded Collaborative Action for the Natura Network (CANN) project, all six sites in this experiment had some rewetting restoration and invasive species removal conducted between 2017 and 2022.

#### 2.1.1. Ballynahone bog

Ballynahone Bog (Fig. 1B) has roughly equal areas of active and degraded raised bog (Table 1). The largest portion of the bog is owned by the Northern Ireland Environment Agency (NIEA) (98 ha) with more than forty individuals owning the remainder of the site. The site has been degraded by industrial peat extraction, hand cutting, burning, and significant drainage, which have not occurred since SAC designation in 2005 (Department of the Environment, 2015a). However, fifty large drains were partly blocked when the site became an ASSI (Northern Ireland Areas of Special Scientific Interest, equivalent to UK Sites of Special Scientific Interest/SSSI) in 1994 (Department of the Environment, 2015a); with many more dams installed in 2021 as part of the CANN project. NIEA and UK Centre for Ecology and Hydrology (UKCEH) $NH_3$ monitoring at the Ballynahone Bog identified two close-proximity farms to the site that could be sources of $NH_3$ (Suppl. Fig. A.1) (van Dijk et al., 2020).

#### 2.1.2. Curran bog

Curran Bog (Fig. 1B) has mostly degraded raised bog, with a small portion of active and intact raised bog (Table 1). The site is owned by forty-three individuals, and has a history of extensive hand cutting, industrialised peat extraction, burning, and drainage (Department of the Environment, 2015b). Peat cutting and extraction have reduced since SAC designation, but it is not known if extant rights for peat cutting exist at the site (Department of the Environment, 2015b). Drainage at Curran

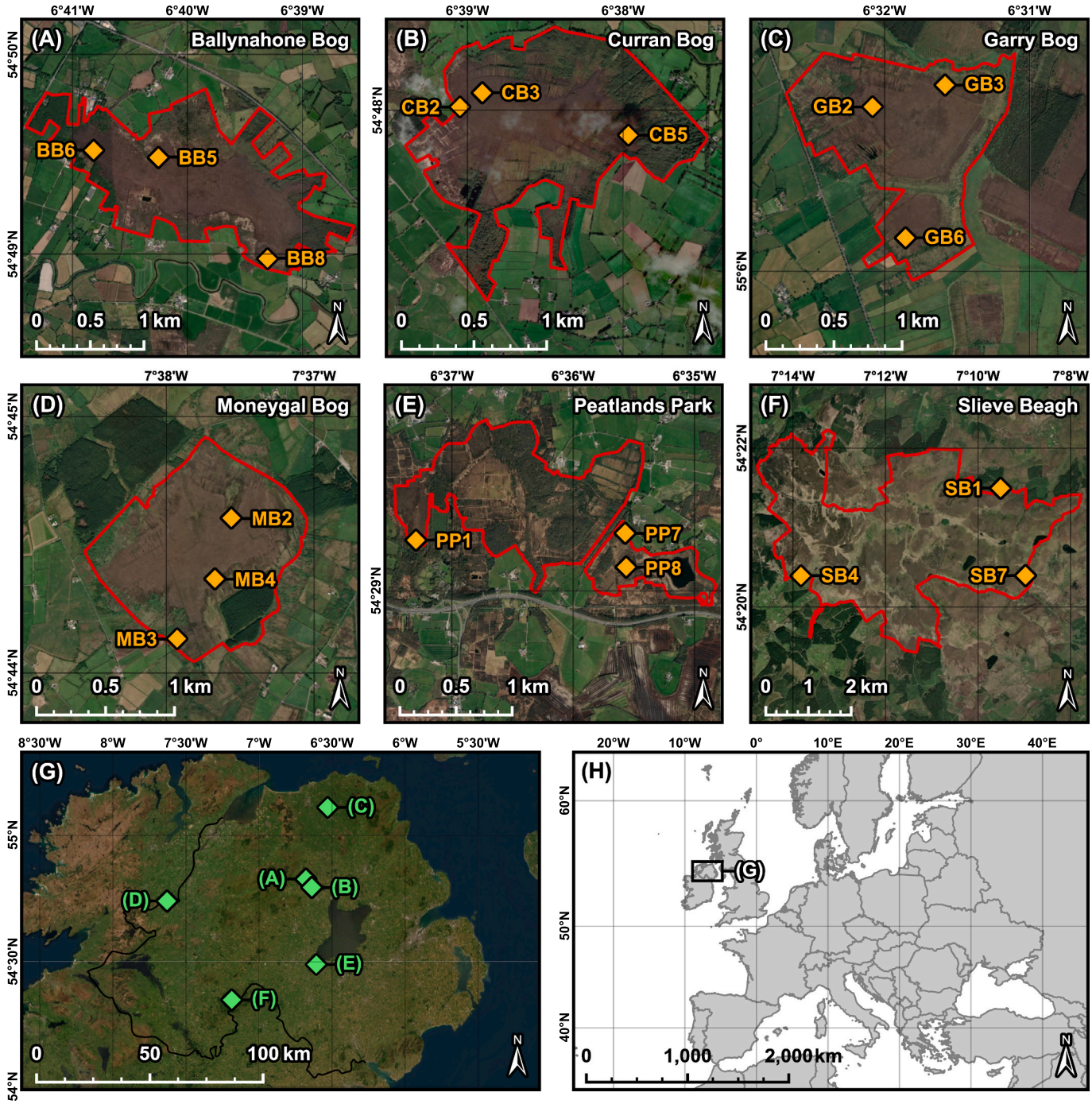


**Fig. 1.** The six study sites: Ballynahone Bog (**A**), Curran Bog (**B**), Garry Bog (**C**), Moneygal Bog (**D**), Peatlands Park (**E**), and Slieve Beagh (**F**). Shown on a wider map of Northern Ireland (**G**) and its position in Europe (**H**). Orange diamonds denote $NH_3$ monitoring and *Sphagnum* sample locations (e.g. GB2, SB1, & MB3); Green diamonds denote locations of each peatland site in Northern Ireland; Red line marks SAC boundaries (Map sources: Esri, 2024a, 2024b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Bog does not cross the intact surface at the centre of the raised bog but is extensive at the borders of the study sites. Drainage ditch dams were installed at Curran Bog as part of the CANN project, though restoration plans were never fully fulfilled due to landowner permission issues. NIEA and UKCEH $NH_3$ monitoring identified three large industrial farms within 5 km of the site, and twenty-four possible animal housing/slurry storage sites within 2 km, that could both represent sources of $NH_3$ (Suppl. Fig. A.1) (Tang et al., 2022, 2023).

### 2.1.3. *Garry bog*

Garry Bog (Fig. 1A) is mostly active intact raised bog, with small areas of degraded bog (Table 1). The site is mostly owned by the Forest Service with remaining areas owned by two or three individuals. Garry Bog has a long-standing management agreement with the Forest Service but has previously been subject to hand cutting and mechanised extraction of peat across the fringes of the site (Department of the Environment, 2015c). No drains dissect either of the main peat domes, though old drainage is evident at the edges of the site, with many being dammed as part of the CANN project in 2022. NIEA and UKCEH $NH_3$

**Table 1**
Characterization of field sites studied.

| Site | Active bog (ha)[a] | Degraded bog (ha)[a] | SAC (ha)[a] | Elevation (m) | Temperature (°C)[b] | Rainfall (mm)[c] |
|---|---|---|---|---|---|---|
| Ballynahone Bog | 131 | 111 | 244 | 40–45 | 5.1–14.5 | 1233 |
| Curran Bog | 25.48 | 126.86 | 183.3 | 38–43 | 5.1–14.5 | 1233 |
| Garry Bog | 142.7 | 10.2 | 154.76 | 30–35 | 5.9–14.6 | 1016 |
| Moneygal Bog | 114.2 | 38 | 155.79 | 125–135 | 5.0–14.6 | 1372 |
| Peatlands Park | 21.8 | 117.2 | 207.5 | 15–20 | 5.4–15.0 | 1056 |
| Slieve Beagh | 1112 | – | 1900 | 200–380 | 5.1–14.4 | 1406 |

[a] Data obtained from Department of the Environment (2015a, 2015b, 2015c, 2015d, 2015e, 2015f).
[b] Data on temperature (mean January and July) obtained from Met Office (2024b).
[c] Data on annual rainfall obtained from Met Office (2024a).

monitoring at the site identified three large industrial farms within 5 km, and eighteen potential animal housing/slurry storage sites within 2 km, that could both be sources of $NH_3$ (Suppl. Fig. A.1) (Tang et al., 2022, 2023).

#### 2.1.4. Moneygal bog

Moneygal Bog (Fig. 1E) is mostly an active raised bog, with large degraded areas (Table 1). The Forest Service owns most of the site, with a small northern parcel owned by an individual (Department of the Environment, 2015d). Moneygal Bog has been extensively hand cut for peat, with some mechanised extraction occurring (Department of the Environment, 2015d). Past burning is evident and can be clearly identified on the site (Department of the Environment, 2015d). The site has areas of forestry plantation (~ 14 ha) which have been suggested to have a drying effect on the surrounding peat surface (Department of the Environment, 2015d). Drainage is mostly at the edges of the site, with some old drains crossing the intact dome (Department of the Environment, 2015d). Some drains have since been dammed as part of the CANN project between 2020 and 2022. NIEA and UKCEH $NH_3$ monitoring at the site identified nine possible animal housing/slurry storage sites within 2 km, that could be sources of $NH_3$ (Suppl. Fig. A.1) (Tang et al., 2022, 2023).

#### 2.1.5. Peatlands Park

Peatlands Park (Fig. 1C) is a large area of degraded raised bog, and a small area of active raised bog (Table 1), adjacent to significant Oak woodland (> 40 ha) (Department of the Environment, 2015e). The site is mostly owned by NIEA with four individuals owning small sections. Past cutting, drainage, and burning have left the site at risk of invasive species encroachment (Department of the Environment, 2015e). As part of the CANN project, many drains were dammed and invasive species (particularly *Rhododendron*) were cut-back. NIEA and UKCEH $NH_3$ monitoring at the site identified three large industrial farms within 5 km, and twenty potential animal housing/slurry storage sites within 2 km, that could both be sources of $NH_3$ (Suppl. Fig. A.1) (Tang et al., 2022, 2023).

#### 2.1.6. Slieve Beagh

Slieve Beagh (Fig. 1D) is the third largest intact peatland in Northern Ireland and has a very large area of active blanket bog, with significant natural lakes (> 15 ha), and dry heath (80 ha) (Table 1) (Department of the Environment, 2015f). The Forest Service own approximately 600 ha of the site, with twenty individuals owning sections, and as many as sixty-five others who have turbary rights to cut peat for fuel (Department of the Environment, 2015f). Peat cutting at the perimeter of Slieve Beagh has occurred in the recent past (Department of the Environment, 2015f), and heathland management-connected burning is evident across the entire site (Department of the Environment, 2015f). Drainage is limited to the edge of the site which was dammed in 2021 as part of the CANN project. NIEA and UKCEH $NH_3$ monitoring at the site identified two large industrial farms within 5 km, several more within 10 km, and three possible animal housing/slurry storage sites within 2 km, that could be sources of $NH_3$ (Suppl. Fig. A.1) (Tang et al., 2022, 2023).

### 2.2. Ammonia and meteorological data

Atmospheric $NH_3$ gas concentrations were monitored by NIEA and the UKCEH at all six sites (Fig. 2) using adapted low-cost passive high absorption (ALPHA) samplers (Tang et al., 2001) that were installed on most sites in 2020. ALPHA samplers have been collecting atmospheric $NH_3$ gas concentrations at Ballynahone Bog since 2014 (van Dijk et al., 2020). ALPHA samplers were prepared using a standard UKCEH methodology that provides a representative of atmospheric $NH_3$ concentrations (Stephens et al., 2021; Tang et al., 2001). Additionally, plastic bird spikes were installed on top of ALPHA sampler supports to prevent bird perching and soiling, which can impact $NH_3$ concentrations. Atmospheric $NH_3$ concentrations were collected once per month, from ALPHA samplers at a range of locations across each bog and chemically analysed by UKCEH in Edinburgh. Issues surrounding data collection at Slieve Beagh led to 2023 $NH_3$ deposition data for this site to be incomplete. Most data for ALPHA sampler SB7 was present for 2023, whilst more than half of the planned 2023 data for SB1 and SB4 was missing. Mean $NH_3$ deposition data from Slieve Beagh in 2020, 2021, and 2022 was used to supplement the missing periods from 2023 data collection (Fig. 2F). At all other sites 2020–2022 data was comparable year-on-year to $NH_3$ deposition data collected in 2023 (Tang et al., 2022, 2023; van Dijk et al., 2020).

To compare total monthly rainfall (mm) and mean monthly temperatures (°C) at each site, the Met Office Integrated Data Archive System (MIDAS) was utilised, sourcing data from MIDAS land surface weather stations through 2023 (Fig. 3) (Met Office, 2024a, 2024b). The nearest suitable MIDAS station to each site was: Portglenone (54°51′54.0″ N, 6°27′28.8″ W) 14 km northeast of Ballynahone Bog and Curran Bog; Giant's Causeway (55°14′02.4″ N, 6°30′43.2″ W) 14 km north of Garry Bog; Castlederg (54°42′25.2″ N, 7°34′37.2″ W) 5 km southeast of Moneygal Bog; Armagh (54°21′07.2″ N, 6°39′00.0″ W) 15 km south of Peatlands Park; and Thomastown (54°19′48.0″ N, 7°35′42.0″ W) between 23 and 29 km east of Slieve Beagh (Met Office, 2024a, 2024b).

### 2.3. Field sampling

At each site, three ALPHA sampler locations were sampled once per season for a year (31/03/2023–13/01/2024). Three replicates of *Sphagnum capillifolium* (Ehrh.) Hedw. were collected at each ALPHA sampler in Spring 2023 (Table 2). Each replicate of *S. capillifolium* was taken within a radius of ~5 m of each ALPHA sampler. Replicates were chosen to be as far apart from one another as could be achieved, given the available *S. capillifolium* near each ALPHA sampler. Replicates were repeated in Summer, Autumn, and Winter (Table 2) from the same individual areas of *S. capillifolium*. Compass directions, distances, and handheld GPS were used to relocate the same area of *S. capillifolium* each season, in relation to permanent ALPHA samplers. Samples were selected from a single *Sphagnum* species to avoid discrepancies in sensitivity to $NH_3$, as differing tolerance to nitrogen deposition has been

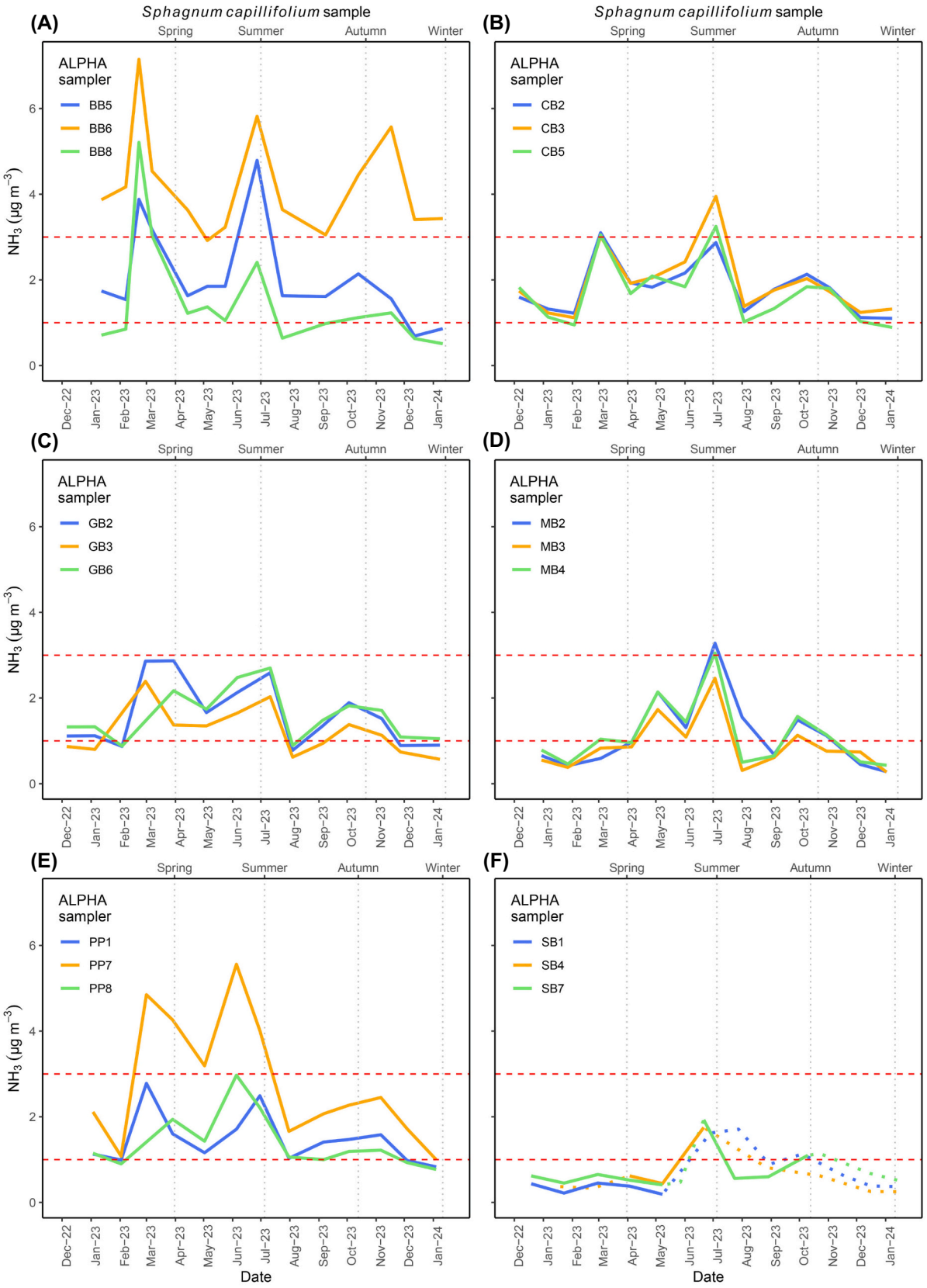


**Fig. 2.** $NH_3$ deposition rates at Garry Bog (**A**), Ballynahone Bog (**B**), Curran Bog (**C**), Moneygal Bog (**D**), Peatlands Park (**E**), and Slieve Beagh (**F**). Red dashed line denotes critical $NH_3$ deposition rates for bryophytes and lichens (1 $NH_3$ µg $m^{-3}$), and higher plants (3 $NH_3$ µg $m^{-3}$) (Cape et al., 2009). Grey dotted line marks *Sphagnum capillifolium* sample collection dates. ALPHA samplers SB1 (blue), SB4 (orange), and SB7 (green) for Slieve Beagh (F) have supplemented $NH_3$ deposition data from 2020 to 2022 (Tang et al., 2023, 2023), represented by broken lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

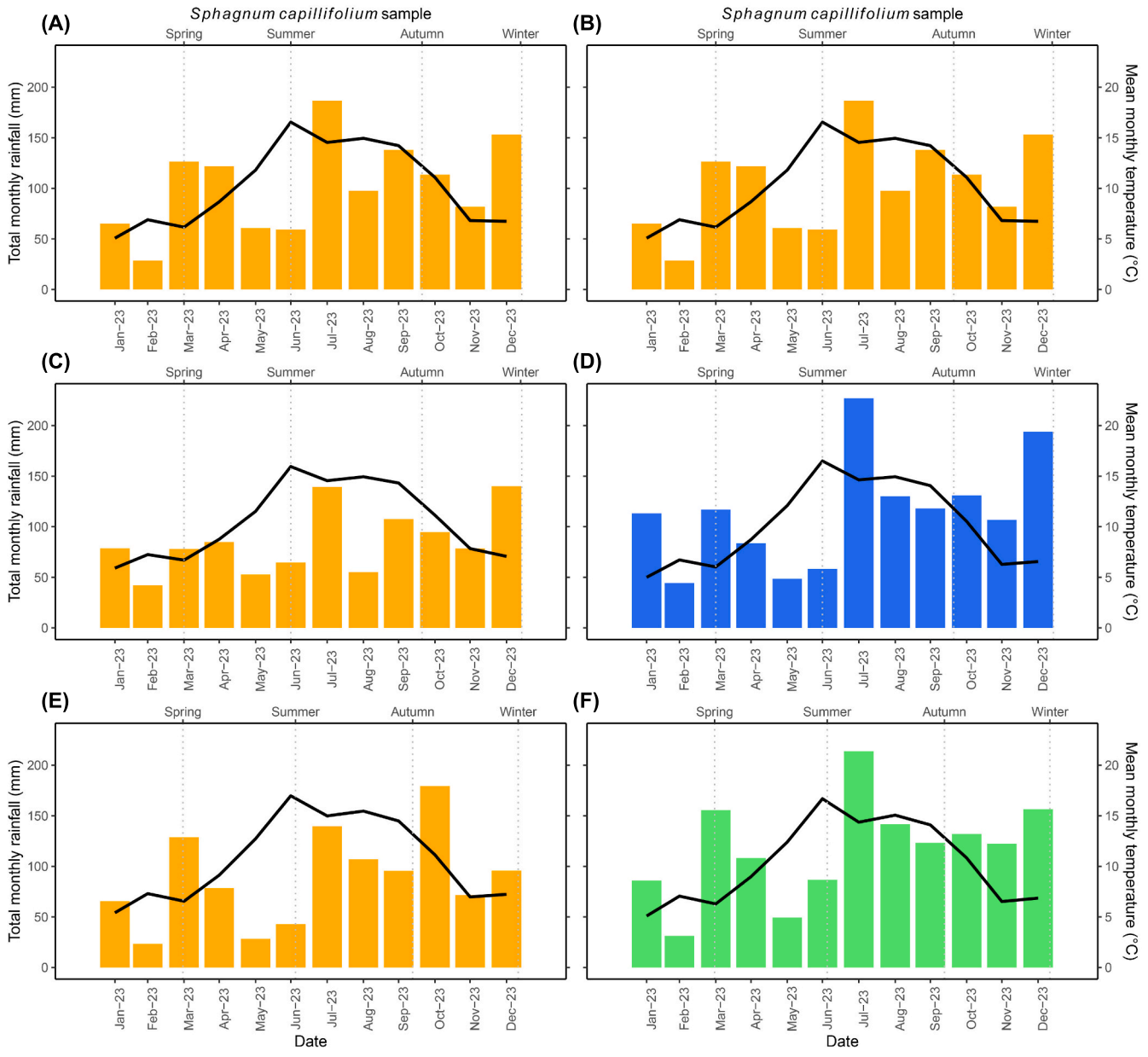


**Fig. 3.** Total monthly rainfall (mm) and mean monthly temperature (°C) data from the Met Office Integrated Data Archive System weather stations (Met Office, 2024a, 2024b) for Ballynahone Bog (**A**), Curran Bog (**B**), Garry Bog (**C**), Moneygal Bog (**D**), Peatlands Park (**E**), and Slieve Beagh (**F**). Site $NH_3$ deposition rate highlighted in orange (high; mean > 1 $NH_3$ μg $m^{-3}$); blue (intermediate; mean = 1 $NH_3$ μg $m^{-3}$); and green (low; mean < 1 $NH_3$ μg $m^{-3}$). Grey dotted line denotes *Sphagnum capillifolium* sample collection dates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

demonstrated in *Sphagnum* species (Jauhiainen et al., 1998). Additionally, *S. capillifolium* was chosen due to its abundance at each site, meaning samples were not taken at extreme distances from ALPHA samplers.

NIEA/UKCEH monitoring in previous years indicated differing $NH_3$ deposition rates at each peatland (Tang et al., 2022, 2023). Ballynahone Bog, Curran Bog, Garry Bog, and Peatlands Park all had high $NH_3$ deposition rates (mean > 1 $NH_3$ μg $m^{-3}$), often exceeding critical levels of lichens/bryophytes (1 μg $NH_3$ $m^{-3}$) and vascular plants (3 μg $NH_3$ $m^{-3}$) (Cape et al., 2009). Moneygal Bog had intermediate $NH_3$ deposition rates (mean 1 $NH_3$ μg $m^{-3}$), and Slieve Beagh had low $NH_3$ deposition rates (mean < 1 $NH_3$ μg $m^{-3}$).

**Table 2**
*Sphagnum capillifolium* replicate collection dates for spring, summer, autumn, and winter at all six sites.

| Site | Spring | Summer | Autumn | Winter |
|---|---|---|---|---|
| Ballynahone Bog | 01/04/2023 | 01/07/2023 | 21/10/2023 | 13/01/2024 |
| Curran Bog | 01/04/2023 | 01/07/2023 | 21/10/2023 | 13/01/2024 |
| Garry Bog | 01/04/2023 | 01/07/2023 | 21/10/2023 | 13/01/2024 |
| Moneygal Bog | 01/04/2023 | 01/07/2023 | 21/10/2023 | 13/01/2024 |
| Peatlands Park | 31/03/2023 | 05/07/2023 | 13/10/2023 | 11/01/2024 |
| Slieve Beagh | 31/03/2023 | 05/07/2023 | 13/10/2023 | 11/01/2024 |

### 2.4. Laboratory analysis

Three replicates of the uppermost portions (5–10 $cm^3$) of *S. capillifolium* capitula, stems, and branches, which have higher

concentrations of live testate amoebae (Booth, 2002; Booth et al., 2010; Roe et al., 2017), were collected near three ALPHA samplers at each site. A standard method (Booth et al., 2010) involves coarse-sieving (300 μm) and back-sieving (15 μm) 2 $cm^3$ of *Sphagnum* in boiling water which is later refrigerated at 4 °C. It is notable that micro-sieving (15 μm back-sieve) for *Sphagnum* sample preparation has been shown to remove very small testate amoeba taxa (Avel and Pensa, 2013). However, small taxa were not the focus of this study. Larger taxa potentially respond to high $NH_3$ deposition rates (e.g. Jassey et al., 2012; Payne et al., 2013) and were therefore more important to this study.

Using dichotomous keys/illustrated taxonomic guides (Charman et al., 2000; Siemensma, 2024), testate amoebae species level identification was conducted at 200× under transmitted light microscopy. The status of testate amoebae was noted as 'dead' for individuals where only a test/partial test was apparent, and as 'alive/recently alive' for individuals where a clear cytoplasm was visible. To maintain ecologically relevant minor testate amoebae assemblage changes, a minimum sample size of > 150 individuals was adhered to in all replicates (Payne and Mitchell, 2009). The testate amoebae functional traits: biovolume ($\mu m^3$); mixotrophy (not-mixotrophic; mixotrophic); test material (agglutinated mineral; proteinaceous; siliceous); pseudopodia type (filose; lobose); and pseudostome size (μm), were allocated to all identified taxa after analysis was complete using taxa-specific functional trait data from published literature (Fournier et al., 2015; Krashevska et al., 2020; van Bellen et al., 2017).

### 2.5. Statistical analysis

Non-metric multidimensional scaling (NMDS) analysis used the Bray–Curtis dissimilarity index method to visualise the similarity of testate amoebae assemblages at all six sites for spring, summer, autumn, and winter. Additionally, environmental and experimental variables ($NH_3$ deposition; season; ALPHA sampler; rainfall; temperature) were fitted through envfit (999 permutations). Permutational multivariate analysis of variance (PERMANOVA) enabled significance testing between replicates, rainfall, temperature, season, ALPHA samplers, and $NH_3$ deposition rates. Threshold Indicator Taxa Analysis (TITAN) was performed to assess the response of testate amoebae taxa to an $NH_3$ deposition gradient (Baker and King, 2010). This analysis identified taxa that exhibited significant declines ('decreasers'; z-) or increases ('increasers'; z+) along an $NH_3$ gradient and determined community-level threshold responses. Bootstrapping (500 resamples; 500 permutations) was used to estimate uncertainty around taxon-specific thresholds, and purity and reliability scores were used to assess the consistency and significance of species responses. Peaks in sum(z-) and sum(z+) values were used to identify community-level change points along the $NH_3$ deposition gradient. Biovolume and pseudostome size community-weighted means were calculated for each replicate at all six sites (sum of the products of taxa functional trait values, weighted by their percentage abundance). SDI (Shannon and Weaver, 1949) values were calculated for each sample so species diversity could be understood throughout the experiment. Friedman rank sum test was performed to assess significant differences between seasons for testate amoebae functional traits and SDI values. All statistical analysis and data visualisation was carried out using R ver. 4.3.3 (R Core Team, 2024), using the R package 'vegan' ver. 2.6–4 (Oksanen et al., 2022) for all multivariate analyses, and 'TITAN2' ver. 2.4.3 (Baker and King, 2010) for TITAN analysis.

## 3. Results

### 3.1. Ammonia deposition

In this experiment, $NH_3$ deposition rates for all six sites (Fig. 2) broadly followed the expected trends observed in previous years (Tang et al., 2022, 2023). At sites defined to have 'high $NH_3$ deposition' measured $NH_3$ deposition for this experiment increased substantially one month before spring samples were collected (mean = 3.53 $NH_3$ μg $m^{-3}$) and again around the time of summer sample collection (mean = 3.45 $NH_3$ μg $m^{-3}$). Additionally, a third smaller increase in $NH_3$ deposition rates was observed at these sites approximately around the time of autumn sample collection (mean = 2.01 $NH_3$ μg $m^{-3}$). At Moneygal Bog a pre-spring peak of $NH_3$ deposition was not observed, though $NH_3$ deposition rates increase at the time of summer sampling (mean = 2.93 $NH_3$ μg $m^{-3}$) and at the time of autumn sampling (mean = 1.4 $NH_3$ μg $m^{-3}$). Moneygal Bog was the only lowland raised bog site to not experience a clear increase of $NH_3$ deposition just before spring sampling; however, in late April, the site was characterised by a sharp increase in $NH_3$ deposition rates (mean = 2 $NH_3$ μg $m^{-3}$). Finally, at Slieve Beagh a single obvious increase of $NH_3$ deposition occurred around the time of summer sampling (mean = 1.8 $NH_3$ μg $m^{-3}$); however, no other distinct increases in $NH_3$ deposition were observed at Slieve Beagh. All six sites are characterised by reduced $NH_3$ deposition rates at the time of winter sampling, although, mean $NH_3$ deposition rates were still higher in high $NH_3$ deposition sites (mean = 1.42 $NH_3$ μg $m^{-3}$), compared to 'low/intermediate $NH_3$ deposition' sites (mean = 0.49 $NH_3$ μg $m^{-3}$). Finally, for the entire experimental period, mean rates of $NH_3$ deposition are higher in high $NH_3$ deposition sites (mean = 2 $NH_3$ μg $m^{-3}$) than at low/intermediate sites (Slieve Beagh = 0.7 $NH_3$ μg $m^{-3}$; Moneygal Bog = 1.09 $NH_3$ μg $m^{-3}$).

### 3.2. Testate amoeba assemblages

At all six sites (Table 3), just five taxa (*Assulina muscorum*, *Nebela tincta*, *Euglypha rotunda* type, *Euglypha ciliata*, and *Euglypha strigosa*) accounted for the majority (56.9 %) of individual testate amoebae counted (Figs. A.2–A.7). Specifically, four taxa (*A. muscorum*, *N. tincta*, *E. rotunda* type, and *Archerella flavum*) accounted for more than half (51.6 %) of individuals counted at Ballynahone Bog (Suppl. Fig. A.2). Likewise, four taxa (*A. muscorum*, *N. tincta*, *E. rotunda* type, and *E. strigosa*) accounted for greater than half (58 %) of individuals counted at Curran Bog (Suppl. Fig. A.3). At Garry Bog, five taxa (*A. muscorum*, *N. tincta*, *E. rotunda* type, *E. strigosa*, and *E. ciliata*) accounted for half (50.1 %) of individuals counted (Suppl. Fig. A.4). Only three taxa (*N. tincta*, *A. muscorum*, and *E. rotunda* type) accounted for half (50.6 %) of individuals counted at Moneygal Bog (Suppl. Fig. A.5). Five taxa (*N. tincta*, *A. muscorum*, *A. flavum*, *Cryptodifflugia oviformis*, and *E. rotunda* type) accounted for half (50.6 %) of individuals counted at Peatlands Park (Suppl. Fig. A.6). Finally, at Slieve Beagh, four taxa (*N. tincta*, *A. muscorum*, *E. ciliata*, and *E. rotunda* type) accounted for over half (52.7 %) of individuals counted (Suppl. Fig. A.7). On average, SDI values (Figs. A.8 and A.9; Table 3) were lower in spring samples at bogs with high $NH_3$ deposition (Ballynahone Bog, Curran Bog, Garry Bog, and Peatlands Park), when compared to summer ($p = 0.034$); where at sites with low/intermediate $NH_3$ deposition (Slieve Beagh and Moneygal Bog) there was no statistically significant difference in SDI values between seasons.

### 3.3. Multivariate analysis

NMDS ordination of testate amoebae assemblages and key environmental variables from all six sites were plotted using the Bray–Curtis dissimilarity index on two dimensions (Fig. 4); resulting in varying 'stress' at each site; wherein stress < 0.2 is a good fit, and stress > 0.2 is a poor fit and means NMDS ordination is harder to interpret (Ballynahone Bog = 0.28; Curran Bog = 0.26; Garry Bog = 0.21; Moneygal Bog = 0.2; Peatlands Park = 0.16; Slieve Beagh = 0.24). Replicates from different seasons at Ballynahone Bog (Fig. 4A), Curran Bog (Fig. 4B), and Garry Bog (Fig. 4C) are clustered separately in the NMDS ordination space. Whereas Moneygal Bog (Fig. 4D), Peatlands Park (Fig. 4E), and Slieve Beagh (Fig. 4F) seasonal replicates are not clearly distinct and are clustered together in the centre of each biplot. Environmental variables

**Table 3**
Summary of total samples, individual counted testate amoebae, identified taxa, and Shannon–Wiener diversity index (SDI) values at all six sites.

| Site | Samples | Individuals | Taxa | Spring SDI | Summer SDI | Autumn SDI | Winter SDI |
|---|---|---|---|---|---|---|---|
| Ballynahone Bog | 36 | 7923 | 32 | 2.0782 | 2.1963 | 2.1343 | 2.1382 |
| Curran Bog | 36 | 7800 | 33 | 2.1992 | 2.4111 | 2.2223 | 2.1646 |
| Garry Bog | 36 | 7680 | 42 | 2.1249 | 2.257 | 2.5082 | 2.3341 |
| Moneygal Bog | 36 | 7944 | 39 | 1.9321 | 1.996 | 2.0754 | 1.9079 |
| Peatlands Park | 36 | 7849 | 41 | 2.0908 | 2.2095 | 2.1221 | 2.125 |
| Slieve Beagh | 36 | 8032 | 35 | 2.116 | 2.3202 | 2.0572 | 1.9962 |
| All sites | 216 | 47,228 | 51 | 2.0902 | 2.2317 | 2.1866 | 2.111 |

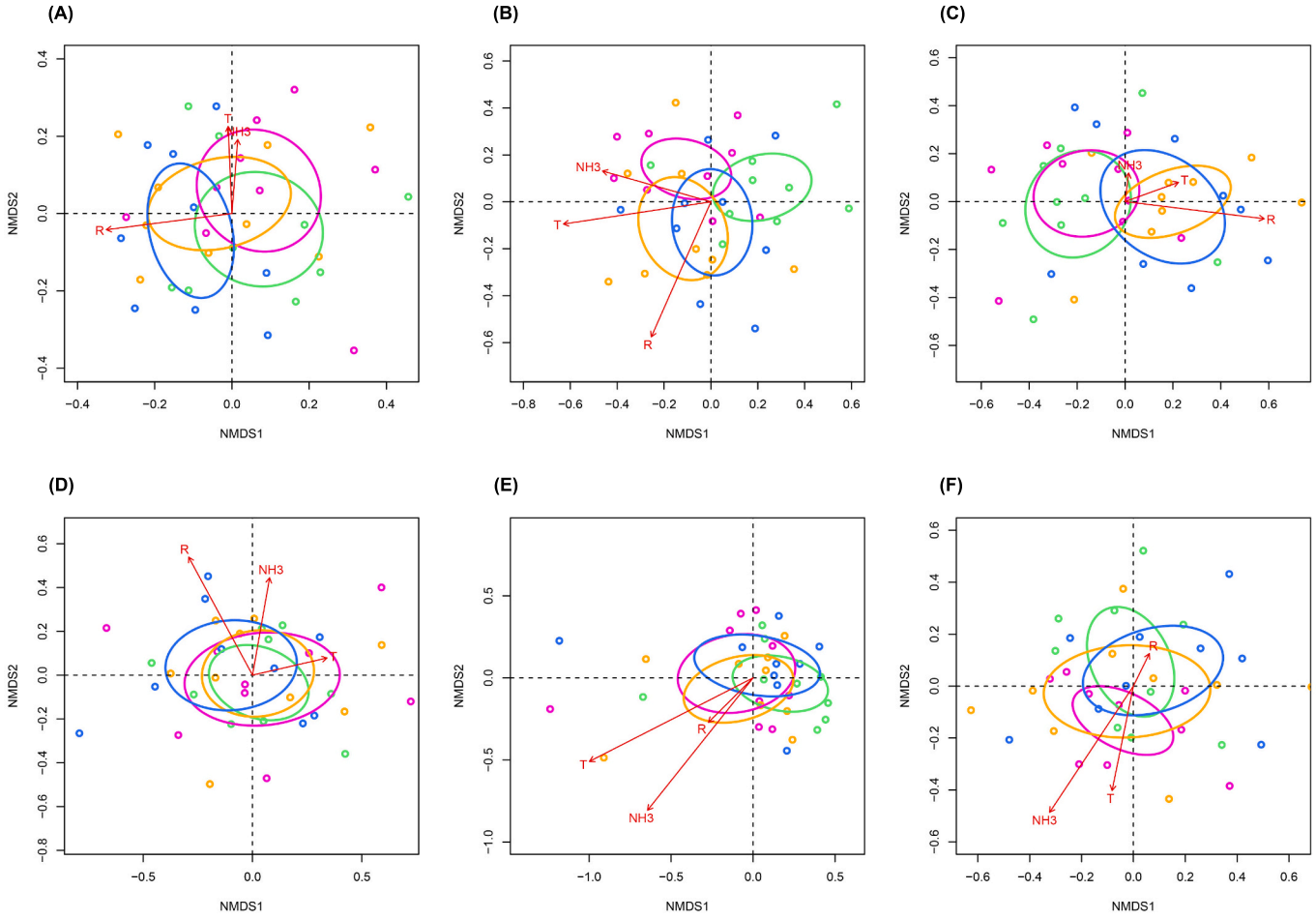


**Fig. 4.** Non-metric multidimensional scaling (NMDS) ordination biplots for testate amoebae assemblages at Ballynahone Bog (**A**), Curran Bog (**B**), Garry Bog (**C**), Moneygal Bog (**D**), Peatlands Park (**E**), and Slieve Beagh (**F**). Centroids are plotted as coloured ellipses for replicates from each season: spring (green); summer (purple); autumn (orange); and winter (blue). Red arrows represent 'envfit' environmental variables for $NH_3$ deposition, rainfall (R), and temperature (T). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fitted through 'envfit' to the assemblage dataset (Fig. 4; Table 4) show that $NH_3$ deposition significantly explained the ordination results with a low goodness of fit in half of the high $NH_3$ deposition sites (Table 4). ALPHA samplers (three locations across each site where replicates and $NH_3$ were sampled) significantly explain the ordination with a more moderate goodness of fit in five out of six sites (Table 4). Season and temperature each only significantly explained the ordination of Curran Bog with a low goodness of fit (Table 4), and rainfall significantly explained the ordination of Curran Bog and Garry Bog with a similarly low goodness of fit (Table 4). Increasing $NH_3$ deposition was most closely associated with spring and summer replicates at Ballynahone Bog (Fig. 4A), Curran Bog (Fig. 4B), and Garry Bog (Fig. 4C). Due to being clustered around 0 on both NMDS axes 1 and 2, it was not possible to discern the relationship between $NH_3$ deposition and particular seasonal replicates at Moneygal Bog (Fig. 4D), Peatlands Park (Fig. 4E), and Slieve Beagh (Fig. 4F).

Mixotrophic taxa appeared to be commonly associated with increasing $NH_3$ deposition on one or both NMDS ordination axes (Suppl. Fig. A10). Specifically, *Amphitrema stenostoma* was associated with increasing $NH_3$ deposition at three of the four sites this taxon was observed to occur at, *Amphitrema wrightianum* at two of three sites, *Heleopera sphagni* at four of six sites, and *Placocista spinosa* type at all five sites it was observed to occur at. However, *Hyalosphenia papilio* was only associated with increasing $NH_3$ deposition at two of four sites. Furthermore, *A. flavum*, which constituted the vast majority of individual mixotrophic taxa occurrences across all six sites (87 %), was only associated with increasing $NH_3$ deposition in three of the total six sites. Only half of the mixotrophic taxa observed at Moneygal Bog (2/4) and

**Table 4**
NMDS ordination environmental variable goodness of fit results at all six sites. Significant *p*-values (< 0.05) are marked by an asterisk (*). ALPHA samplers represent three locations at each site where *S. capillifolium* replicates and $NH_3$ were sampled.

| Site | Variable | *p* | $R^2$ |
|---|---|---|---|
| Ballynahone Bog | ALPHA sampler | 0.007 * | 0.1821 |
| | Season | 0.139 | 0.1315 |
| | $NH_3$ | 0.253 | 0.081 |
| | Rainfall | 0.094 | 0.1384 |
| | Temperature | 0.183 | 0.1011 |
| Curran Bog | ALPHA sampler | 0.241 | 0.0761 |
| | Season | 0.002 * | 0.2577 |
| | $NH_3$ | 0.069 | 0.1394 |
| | Rainfall | 0.007 * | 0.2519 |
| | Temperature | 0.008 * | 0.2379 |
| Garry Bog | ALPHA sampler | 0.04 * | 0.1348 |
| | Season | 0.051 | 0.1828 |
| | $NH_3$ | 0.017 * | 0.2264 |
| | Rainfall | 0.006 * | 0.2516 |
| | Temperature | 0.404 | 0.0551 |
| Moneygal Bog | ALPHA sampler | 0.001 * | 0.4446 |
| | Season | 0.941 | 0.0246 |
| | $NH_3$ | 0.821 | 0.0117 |
| | Rainfall | 0.744 | 0.017 |
| | Temperature | 0.921 | 0.0046 |
| Peatlands Park | ALPHA sampler | 0.001 * | 0.3513 |
| | Season | 0.617 | 0.0642 |
| | $NH_3$ | 0.029 * | 0.1997 |
| | Rainfall | 0.995 | 0.0006 |
| | Temperature | 0.319 | 0.0705 |
| Slieve Beagh | ALPHA sampler | 0.002 * | 0.2554 |
| | Season | 0.314 | 0.1041 |
| | $NH_3$ | 0.018 * | 0.2074 |
| | Rainfall | 0.82 | 0.0118 |
| | Temperature | 0.169 | 0.1026 |

**Table 5**
PERMANOVA results for environmental variables at all six sites. Significant *p*-values (< 0.05) are marked by an asterisk (*). ALPHA samplers represent three locations at each site where *S. capillifolium* replicates and $NH_3$ were sampled.

| Site | Variable | *p* | *F* |
|---|---|---|---|
| Ballynahone Bog | ALPHA sampler | 0.011 * | 2.6576 |
| | Season | 0.019 * | 2.8154 |
| | $NH_3$ | 0.019 * | 2.6738 |
| | Rainfall | 0.468 | 0.8989 |
| | Temperature | 0.19 | 1.3638 |
| Curran Bog | ALPHA sampler | 0.011 * | 2.05 |
| | Season | 0.539 | 0.9011 |
| | $NH_3$ | 0.083 | 1.8107 |
| | Rainfall | 0.004 * | 3.3432 |
| | Temperature | 0.028 * | 2.26 |
| Garry Bog | ALPHA sampler | 0.001 * | 2.7791 |
| | Season | 0.121 | 1.5672 |
| | $NH_3$ | 0.004 * | 3.012 |
| | Rainfall | 0.026 * | 2.0533 |
| | Temperature | 0.375 | 1.0416 |
| Moneygal Bog | ALPHA sampler | 0.001 * | 3.5058 |
| | Season | 0.002 * | 2.8951 |
| | $NH_3$ | 0.195 | 1.3317 |
| | Rainfall | 0.001 * | 3.5886 |
| | Temperature | 0.012 * | 2.7747 |
| Peatlands Park | ALPHA sampler | 0.001 * | 4.507 |
| | Season | 0.329 | 1.1426 |
| | $NH_3$ | 0.004 * | 3.9505 |
| | Rainfall | 0.067 | 2.0507 |
| | Temperature | 0.009 * | 3.3233 |
| Slieve Beagh | ALPHA sampler | 0.001 * | 3.001 |
| | Season | 0.538 | 0.8874 |
| | $NH_3$ | 0.057 | 1.7965 |
| | Rainfall | 0.002 * | 3.0978 |
| | Temperature | 0.125 | 1.5057 |

Peatlands Park (3/6) were associated with increasing $NH_3$ deposition. More than half of each mixotrophic taxa observed at Ballynahone Bog (2/3), Garry Bog (3/5), and Slieve Beagh (5/6) were associated with increasing $NH_3$ deposition. Finally, all mixotrophic taxa observed at Curran Bog (4/4) were found to be associated with increasing $NH_3$ deposition along one or both NMDS axes.

PERMANOVA analysis (Table 5) showed that $NH_3$ deposition explained a statistically significant amount of the variance in the testate amoebae assemblages of the majority of the high $NH_3$ deposition sites, although not at Curran Bog, or either of the low/intermediate sites (Table 5). PERMANOVA analysis also indicated that testate amoebae replicates were significantly different between each ALPHA samplers at all six sites (Table 5), rainfall explained a moderate degree of the variance in testate amoebae assemblages at four of the total six sites (Table 5), and temperature explained a similar degree of the variance at three sites (Table 5).

### 3.4. TITAN analysis

TITAN analysis was conducted on a combined dataset of all six sites to identify significant thresholds in the response of testate amoeba taxa in relation to $NH_3$ deposition gradients. Rare (< 3 total occurrences) and ubiquitous (appearing in all samples) taxa were filtered prior to analysis, which included the rare taxa *Arcella hemispherica*, *Centropyxis aerophile*, *Centropyxis platystoma* type, *Difflugia bacillariarum*, *Galeripora artocrea*, *Nebela carinata*, *Plagiopyxis callida*, and *Quadrulella symmetrica*, and the ubiquitous taxon *A. muscorum*.

TITAN analysis identified eleven taxa with robust responses to $NH_3$ deposition, comprising five taxa that significantly declined (decreasers; z-) and six taxa that significantly increased (increasers; z+) along the gradient. The decreasers included *Centropyxis aculeata*, *Corythion–Trinema type*, *C. oviformis*, *Galeripora catinus*, and *N. tincta*, while the increasers consisted of *Assulina seminulum*, *Cryptodifflugia* sp. 1, *E. rotunda*, *Euglypha tuberculata*, *Nebela flabellulum*, and *Pseudodifflugia fulva* (Fig. 5). These taxa exhibited strong indicator responses with high purity (> 0.95) and reliability (> 0.95) scores, signifying clear ecological thresholds in relation to $NH_3$ deposition.

Peak sum(z-) (decreasers) and sum(z+) (increasers) for all eleven identified taxa occurred above the critical level for lichens and bryophytes (1 μg $NH_3$ $m^{-3}$) but below that of higher plants (3 μg $NH_3$ $m^{-3}$) (Fig. 6). However, the 95 % confidence interval for decreasers does span the lichen and bryophyte critical level threshold, whereas the 95 % confidence interval for increasers clearly falls above the same critical level threshold (Fig. 6). Specific decreaser and increaser taxa vary in the strength of their response (z-score) to increasing $NH_3$ deposition, with *G. catinus* being the most responsive decreaser, while *A. seminulum* is the most responsive increaser (Fig. 5). Additionally, of the eleven identified taxa, most decreasers have lobose pseudopodia (4 of 5), whereas the majority of the increasers have filose pseudopodia (4 of 6) (Fig. 5).

### 3.5. Functional traits

Community-weighted mean (CWM) biovolume and pseudostome size were not found to change significantly through seasons at most ALPHA samplers at all six sites. However, across all sites combined, CWM biovolume was found to be significantly lower in winter when compared to summer ($p = 0.008$) and autumn ($p = 0.02$), but to not be significantly different between seasons when high or low/intermediate $NH_3$ deposition sites were grouped. CWM pseudostome was not found to be significantly different between any season, or when grouping sites by $NH_3$ deposition levels.

Test material construction at all six sites had no immediately obvious trends between seasons. However, testate amoeba taxa with silicious tests were the most abundant at all six sites, with a mean abundance of 83.9 % across the entire study. Additionally, mean abundances of taxa with siliceous tests were relatively stable across seasons at all sites

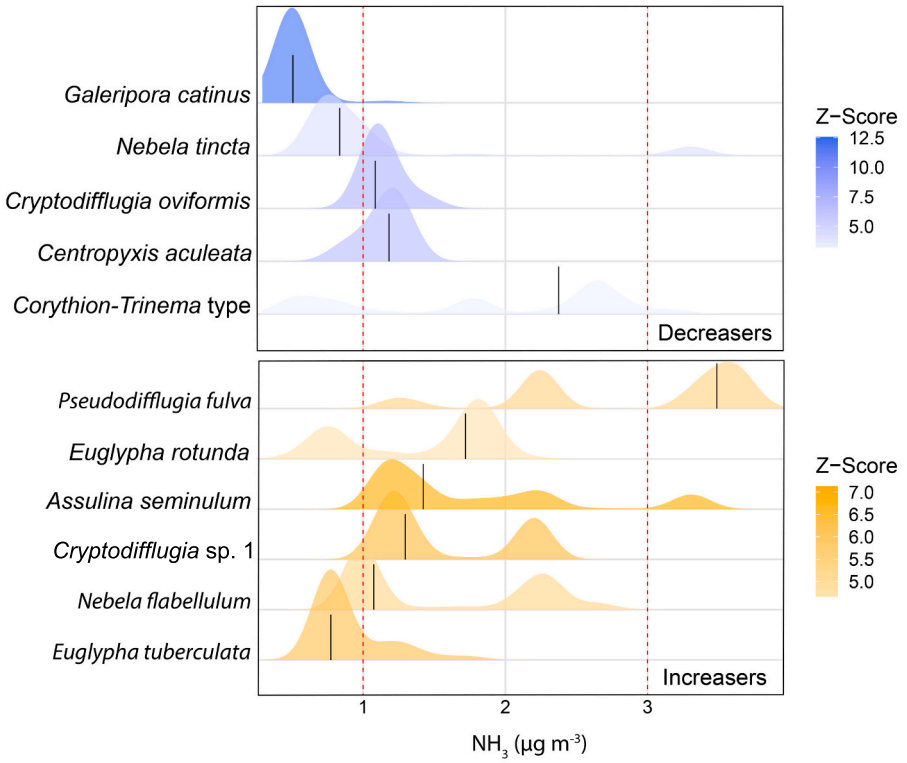


**Fig. 5.** TITAN analysis 'ridge plot' showing the response of testate amoebae taxa to increasing $NH_3$ deposition (µg $m^{-3}$). Each density curve represents the distribution of $NH_3$ values associated with a specific taxon, coloured by z-score values indicating the strength of response. Taxa responding negatively to $NH_3$ are shown in blue (top panel), while those responding positively are in orange (bottom panel). Black vertical lines indicate the $NH_3$ deposition at which each taxon shows a significant change in response. Red dashed lines denote $NH_3$ deposition critical levels for lichens/bryophytes (1 µg $m^{-3}$) and higher plants (3 µg $m^{-3}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(spring = 85.2 %; summer = 88.1 %; autumn = 81.9 %; winter = 80.3 %). Taxa with proteinaceous tests were the next most common (mean abundance = 14.1 %), and taxa with agglutinated mineral tests were the least common (mean abundance = 2 %). Most sites had relatively similar taxa abundances of different test materials at samples taken near each ALPHA sampler; a notable exception was all 3 ALPHA samplers at Curran Bog, which had much lower mean abundances of taxa with proteinaceous tests (mean = 4.5 %). However, sites with high $NH_3$ deposition had notably lower mean proteinaceous test taxa abundances in summer (9.86 %) when compared with all other seasons (mean = 15.61 %) (Fig. 7B). Sites with low/intermediate $NH_3$ deposition proteinaceous test taxa abundances increased throughout the year, peaking in winter (Fig. 7B).

Mixotrophic testate amoebae taxa varied considerably between sites and samples in this experiment, and 7 of the 18 total ALPHA samplers had very low abundances of mixotrophic taxa across all seasons (maximum abundance < 2 %). Across all replicates at all six sites, the mean abundance of mixotrophic taxa was 93 % in spring, 4.42 % in summer, 9.03 % in autumn, and 8.91 % in winter (Fig. 7A). In total, six mixotrophic taxa were identified across all six sites (*A. stenostoma*, *A. wrightianum*, *A. flavum*, *H. sphagni*, *H. papilio*, and *P. spinosa* type). The overwhelming majority of the mixotrophic taxa occurrences observed throughout this experiment, were *A. flavum*, at 87 %. All other mixotrophic taxa abundances were roughly equal (2–4 %), with *A. wrightianum* being the least abundant mixotrophic taxa observed at all six sites (0.52 %). Curran Bog was observed to have by far the lowest overall mean mixotrophic taxa abundances (mean = 1.13 %). Per season, high $NH_3$ deposition rate sites had similar mixotrophic taxa abundances in all seasons; except summer which was notably lower (Fig. 7A). In low/intermediate $NH_3$ deposition rate sites, mean mixotrophic taxa abundances increase season-on-season to a peak abundance in autumn (Fig. 7A).

The ratio of testate amoebae with lobose or filose pseudopodia (L/F ratio) was not characterised by any obvious trends during the study period. The mean L/F ratio of all six sites for each season was stable (spring = 0.36; summer = 0.35; autumn = 0.35; winter = 0.33). Likewise, between sites, the mean L/F ratio was relatively similar (Ballynahone Bog = 0.32; Curran Bog = 0.27; Garry Bog = 0.3; Moneygal Bog = 0.4; Peatlands Park = 0.39; Slieve Beagh = 0.4). In all replicates at all six sites, the L/F ratio rarely exceeded 0.5 (taxa with lobose pseudopodia outnumbering taxa with filose pseudopodia), only occurring 31 times in the 216 total replicates of this experiment (Ballynahone Bog = 3; Curran Bog = 0; Garry Bog = 1; Moneygal Bog = 12; Peatlands Park = 7; Slieve Beagh = 8).

## 4. Discussion

### 4.1. Ammonia deposition

At Ballynahone Bog, Curran Bog, Garry Bog, and Peatlands Park $NH_3$ deposition was on average much higher than the critical level of bryophytes and lichens (1 µg $NH_3$ $m^{-3}$) (Cape et al., 2009) (Fig. 2). Whereas Moneygal Bog was unique among lowland raised bog sites in not having an increase of $NH_3$ deposition just before spring sampling, and overall having lower mean $NH_3$ deposition rates for the year (equal to the critical level of bryophytes and lichens) (Fig. 2D). Finally, at Slieve Beagh, rates of $NH_3$ deposition only exceeded the critical level of bryophytes and lichens in a summer spike of deposition, with a mean $NH_3$ deposition rate lower than this critical level throughout the year (Fig. 2F). Observing testate amoeba assemblage dynamics in relation to changing and often critically high $NH_3$ deposition onto peatlands, appears to have merit in the biomonitoring of this pollutant; although their

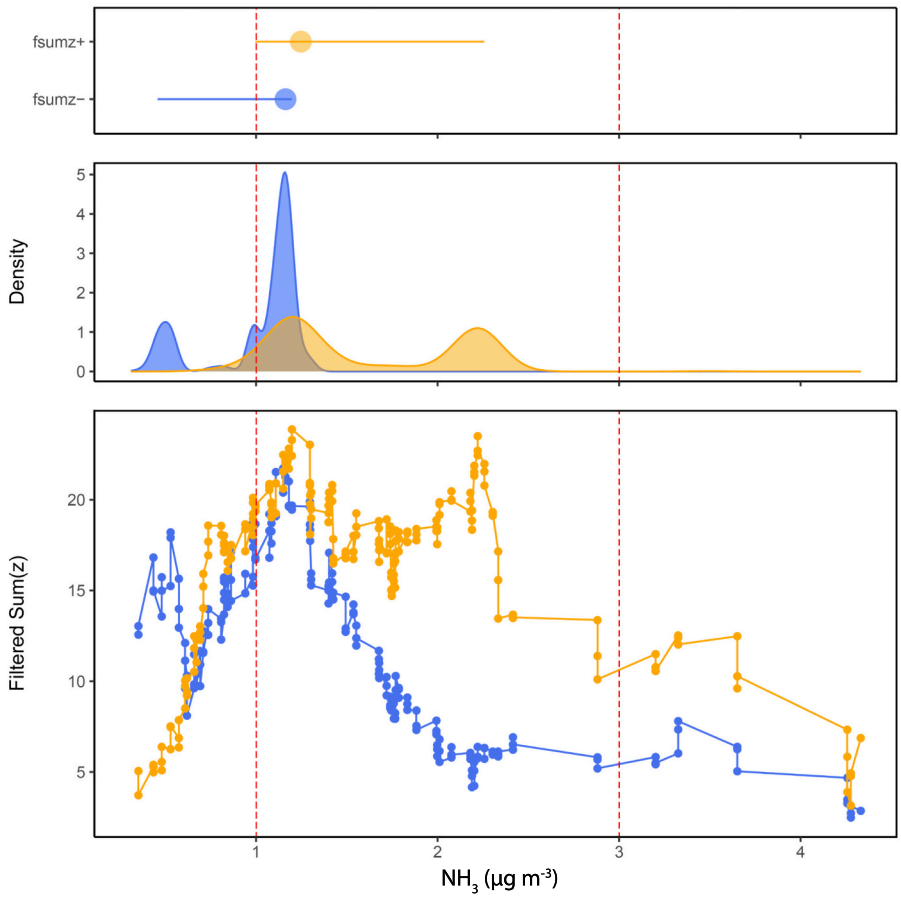


**Fig. 6.** TITAN summary plot showing community-level responses of testate amoebae to increasing $NH_3$ deposition (µg $m^{-3}$). The top panel displays the reliability and strength of change points for negatively (blue; decreasers; fsumz−) and positively (orange; increasers; fsumz+) responding taxa. The middle panel shows kernel density estimates of $NH_3$ deposition rates associated with each response group. The bottom panel presents filtered sum(z) scores for decreaser (blue) and increaser (orange) taxa. Red dashed lines denote $NH_3$ deposition critical levels for lichens/bryophytes (1 µg $m^{-3}$) and higher plants (3 µg $m^{-3}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

response at each site was variable, and more research is required to further elucidate this.

Observed increases in $NH_3$ deposition throughout the year were likely related to agricultural activity. At Ballynahone Bog, Curran Bog, Garry Bog, and Peatlands Park, dramatic increases in $NH_3$ deposition were measured in spring, summer, and autumn (Fig. 2). At Moneygal Bog a spike of $NH_3$ deposition was detected in late April, in summer, and autumn (Fig. 2D). Slieve Beagh alone had only one obvious $NH_3$ deposition spike, in mid-June before summer samples were collected (Fig. 2F). In previous years, similar increases of $NH_3$ deposition at these sites were suggested to be the result of nearby application of agricultural manure as slurry (Tang et al., 2022, 2023). Furthermore, other studies have reported that this agricultural practice increases $NH_3$ deposition rates (Jordan et al., 2007; Pedersen et al., 2024; Uwizeye et al., 2020). Low rates of $NH_3$ deposition across-the-board in winter during this experiment are related to the Nutrients Action Programme, which prohibits slurry spreading during a 'closed period' in Northern Ireland, beginning in autumn (15th September – 31st October), ending in mid-winter (31st January) (Department of Agriculture, Environment and Rural Affairs, 2024c; The Nutrient Action Programme Regulations (Northern Ireland), 2019). Outside of this period spreading is likely to occur in early spring, summer, and sometimes autumn (Lewis et al., 2003; Tang et al., 2022, 2023; Webb et al., 2010); which aligns closely with observed trends in this study.

Deposition rates at each site are likely a direct result of agricultural activity near each site (Suppl. Fig. A.1) (Tang et al., 2022, 2023; van Dijk et al., 2020). Likewise, though exact information on local animal housing/slurry store or large industrial farming activity was not available for Ballynahone Bog; two large farms directly border the site, which could account for its very high mean $NH_3$ deposition levels (Suppl. Fig. A.1) (van Dijk et al., 2020). Slieve Beagh was observed in previous years (and in the intermittent data collected for this experiment) to have very low $NH_3$ deposition rates; related to the size of this large upland blanket bog. Distance from deposition sources is known to strongly control $NH_3$ deposition (Leith et al., 2004; Zapletal and Mikuška, 2019), and due to the site characteristics of Slieve Beagh most agricultural activity was more distant from the sites ALPHA samplers, compared to all other sites of this study.

### 4.2. Multivariate analysis

Multivariate results suggest that higher rates of $NH_3$ deposition have detectable effects on testate amoebae assemblages. Particularly, PERMANOVA analysis showed that testate amoebae assemblage variance could be explained by $NH_3$ deposition at a majority of high $NH_3$ deposition sites, but not at sites with low/intermediate rates of $NH_3$ deposition. Likewise, NMDS ordination biplots (Fig. 4) only showed seasonality between replicates in high $NH_3$ deposition sites, which often appeared to be associated with increasing $NH_3$ deposition (Fig. 4A–C). However, this analysis alone only identifies that known high $NH_3$ deposition significantly controlled testate amoebae assemblage variance in this experiment; further analysis is needed if this method can be used as a bioindication tool for monitoring peatlands for this pollutant, wherein measurements of $NH_3$ would not be taken.

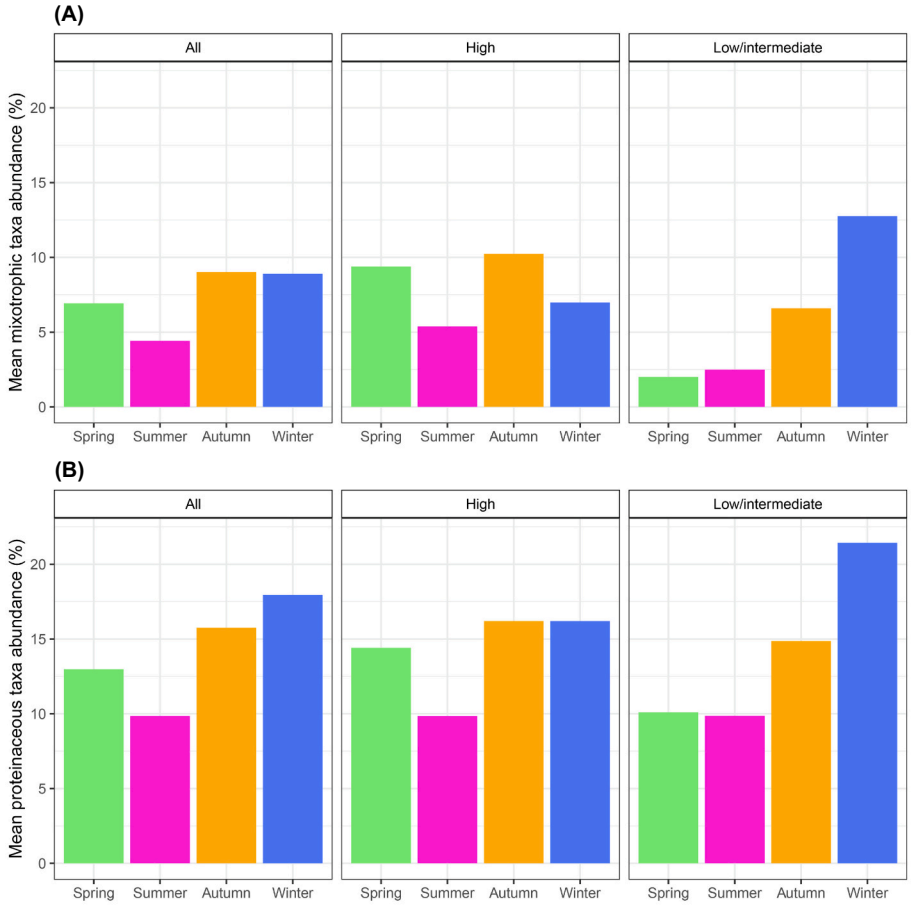


**Fig. 7.** Mean abundances (%) of mixotrophic testate amoebae taxa (**A**) and taxa with proteinaceous test material construction (**B**) at all six sites, at high $NH_3$ deposition sites (Ballynahone Bog, Curran Bog, Garry Bog, and Peatlands Park) (mean $>$ 1 $NH_3$ μg $m^{-3}$), and low/intermediate $NH_3$ deposition rates (Slieve Beagh/Moneygal Bog) (mean $\leq$ 1 $NH_3$ μg $m^{-3}$).

### 4.3. TITAN analysis

Our findings highlight a clear ecological threshold in the responses of certain testate amoeba taxa to $NH_3$ deposition, with decreasers peaking around the important lichen and bryophyte critical level (1 μg $NH_3$ $m^{-3}$) and increasers showing significant changes above this level (Figs. 5 and 6). While these thresholds align with critical $NH_3$ levels for lichens and bryophytes, the confidence intervals suggest some overlap, indicating potential variability in species sensitivity, particularly for decreasers (taxa that respond negatively to increasing $NH_3$ deposition). The strong indicator responses of taxa (Fig. 5) reinforce the utility of TITAN analysis in identifying $NH_3$-sensitive bioindicators.

Although we observed a distinction in pseudopodium type, with most decreasers having lobose pseudopodia and most increasers possessing filose pseudopodia (Fig. 5), our broader functional trait analysis suggests this pattern is not conclusive. Further study is needed to determine whether pseudopodium type could serve as a meaningful indicator of $NH_3$ deposition severity on peatlands.

Overall, our results demonstrate that TITAN analysis, particularly when integrated with NMDS and other multivariate techniques, provides a powerful approach for assessing testate amoebae responses to $NH_3$ deposition. Further research incorporating additional environmental gradients and functional trait-based analyses will be essential to refining our understanding of these relationships.

### 4.4. Community-weighted functional traits

$NH_3$ deposition in peatlands may promote algal biomass increases that affect testate amoeba assemblage dynamics (Payne et al., 2013). This study suggested larger testate amoebae may increase in abundance when $NH_3$ deposition is critically high, potentially a response to increased algal food availability for these dominant microbial predators (Jassey et al., 2012; Payne et al., 2013). In this experiment, it was expected that the abundance of larger (potentially algivorous) testate amoebae taxa would increase in line with $NH_3$ deposition rates. Additionally, it was anticipated that these taxa would be more abundant in sites with generally higher rates of $NH_3$ deposition. To evaluate this, testate amoeba CWM biovolumes and pseudostome sizes were examined. It was found that a detectable change in CWM biovolume occurred when all six sites were grouped; wherein winter CWM biovolumes were found to be ~17 % smaller than that of the previous sampling period. Grouped in the same way, no CWM pseudostome size difference was found. The mean reduction of CWM biovolumes during winter at all sites of this study could have been evidence that increased $NH_3$ deposition in the other periods of this experiment equated to increased abundances of larger testate amoebae taxa. Although having not detected a statistically significant change in CWM pseudostome size for the same period, suggests this size disparity may not be related to large algivorous taxa. Furthermore, a change in CWM biovolume was not observed specifically in high $NH_3$ deposition sites; suggesting CWM biovolumes were on average smaller in winter at all sites for some other reason. Testate amoebae biovolume and pseudostome size have been linked to changing hydrological conditions, trophic status, and type of vegetation (Fournier et al., 2012; Marcisz et al., 2020; Zhang et al., 2020a); any which could have caused a reduction in CWM biovolumes observed in this experiment.

### 4.5. Taxa diversity

Overall SDI values did not change much in the study period, although SDI values from all sites combined were lower in spring than the next sampling period (Suppl. Fig. A.8). However, no difference was observed for low/intermediate $NH_3$ deposition rate, and when high $NH_3$ deposition sites were grouped, lower spring SDI values were still evident (Suppl. Fig. A.8). Reduced taxa diversity in spring, specifically in sites with high $NH_3$ deposition rates, directly contrasts with previous research, where testate amoebae taxa diversity is expected to be highest in spring, and little evidence exists for nitrogen pollutants influencing diversity (Lamentowicz et al., 2013; Marcisz et al., 2014; Mitchell, 2004; Mitchell and Gilbert, 2004). A possible explanation for the reduced SDI values observed in spring during this study could be due to a dominant few taxa having greater success during this period. Payne et al. (2013) suggest that algivorous taxa may have greater success in peatlands with higher $NH_3$ deposition. However, in this study, it was not clear whether algivorous taxa were more successful at times of higher $NH_3$ deposition, with no taxa responding consistently to changes in $NH_3$ deposition throughout the year. Additionally, without a longer study period, it is not possible to eliminate the possibility that SDI values were lower in the previous months/years and some other effect is driving the increase in SDI values observed after spring in this study (i.e. CANN project rewetting restoration).

### 4.6. Mixotrophic taxa

Mixotrophic taxa and their endosymbiotic algae should be more abundant in the summer period due to increased light availability (Herbert et al., 2019; Marcisz et al., 2014; Payne et al., 2016). However, mixotrophic taxa abundances have been shown to reduce in abundance with increasing nitrogen (Mitchell, 2004). In this experiment, mixotrophic taxa had a complicated response to different $NH_3$ deposition rates (Suppl. Fig. A.11). Broadly, in high $NH_3$ deposition rate sites, mean mixotrophic taxa abundances were relatively similar across seasons, with a clear overall reduction in mixotrophs in summer and winter (Fig. 7A). Conversely, in low/intermediate $NH_3$ deposition rate sites mean mixotrophic taxa abundance was lowest in spring and summer, higher in autumn, and highest in winter (Fig. 7A). Across-the-board increases of summer $NH_3$ deposition observed in this study could explain reduced mixotrophic taxa abundance during this period (Fig. 2). However, NMDS ordination (Suppl. Fig. A.10) showed most individual mixotrophic taxa being associated with increasing $NH_3$ deposition; with *P. spinosa* type observed to always be associated with increasing $NH_3$ deposition in this study. Most other mixotrophic taxa had opposite responses to $NH_3$ deposition at some sites compared to others; overall being associated with increasing $NH_3$ deposition in most sites they appeared in. These results are difficult to interpret, particularly the response of specific mixotrophic taxa to often critically high $NH_3$.

If $NH_3$ deposition was driving mixotrophic taxa abundance declines in this study, it would stand to reason that these taxa would also be less successful in spring and autumn; particularly at high $NH_3$ sites, where spring, summer, and autumn deposition rates were similar (Fig. 2). Furthermore, summer should be the single most affected sampling period at low/intermediate $NH_3$ sites, because $NH_3$ deposition rates were not as high in spring and autumn at these sites. Instead, spring had the lowest abundance of mixotrophic taxa at these sites (Fig. 7A). Moreover, mean mixotrophic taxa abundance was highest in winter at low/intermediate $NH_3$ sites, directly countering the most favourable conditions of these taxa (Fig. 7A). Increased temperature and reduced rainfall have been demonstrated to decrease mixotrophic testate amoebae taxa abundances and biomass (Basińska et al., 2020; Jassey et al., 2015; Zhang et al., 2020b), and the warmest mean temperatures and some of the lowest total monthly rainfalls were recorded in the months preceding summer sampling at all six sites (Fig. 2). Furthermore, global climate records were consistently broken, month-on-month, throughout 2023 (e.g. global land and sea surface temperatures) (Ripple et al., 2023). Though not simple to determine outright given the study parameters, observed low rainfall and high temperatures at the time of summer sampling at all six sites could be driving mixotrophic taxa declines (Fig. 3); potentially adding to the negative pressure of $NH_3$ deposition at this time. PERMANOVA analysis supports this, as Ballynahone Bog alone was the only site where either rainfall, temperature, or a combination of the two was not found to significantly explain some of the testate amoebae assemblage variance alongside $NH_3$ deposition. The methods of this study expanded over multiple years, with on-site measured meteorological conditions, might be able to better separate temperature, rainfall, and $NH_3$ deposition effects on mixotrophic taxa abundances.

A trend of increasing abundance of taxa with proteinaceous tests was observed in low/intermediate $NH_3$ deposition sites over the course of the experiment, with the highest abundance of these taxa being from winter replicates at these sites (Fig. 7B). Of the six mixotrophic taxa observed across the six sites, only two do not have proteinaceous tests (*H. sphagni* and *P. spinosa*). Furthermore, these two taxa, which have a siliceous plate test construction, account for only 5.56 % of all mixotrophic taxa occurrences overall. Therefore, changes in test material appear to be overwhelmingly driven by increasing mixotrophic taxa abundances in this experiment (Fig. 7).

### 4.7. Other environmental influences

Testate amoebae have often been shown to be affected directly or indirectly by rainfall and temperature, particularly in degraded peatlands (Lamentowicz et al., 2013; Marcisz et al., 2014; Swindles et al., 2016), with these being important climatic variables on ombrotrophic bogs (Rydin and Jeglum, 2006; Siegel and Glaser, 2006; Tsyganov et al., 2013). Ballynahone Bog was the only site where both rainfall and temperature did not appear to have some statistically significant effect on the testate amoebae assemblage (Table 5). Despite their proximity to one another ($< 3$ km) (Fig. 1B), testate amoebae assemblages at Ballynahone Bog appear resilient to changing rainfall and temperature, where these meteorological variables impacted the testate amoebae assemblages at Curran Bog significantly (Table 5). Although both sites have a history of extensive drainage, fire, and peat extraction (Department of the Environment, 2015a, 2015b), Ballynahone Bog remains a large area of active raised bog (Table 1); a counterpoint to the majority degraded raised bog area of Curran Bog (Table 1). The disparity in their overall condition may have led to this evident diverging response in testate amoebae assemblages. Likewise, the poor condition of Curran Bog may have influenced why high $NH_3$ deposition rates were not found to significantly explain testate amoebae assemblage variation at this site (Table 5). Peatlands Park also has significant areas of degraded raised bog (Table 1); however, only temperature, and not rainfall, was found to explain a degree of the testate amoebae assemblage variance for this site ($p = 0.009$; $F = 3.23$) (Table 5). However, the weather station near Curran Bog recorded almost 200 mm more annual rainfall than the station near Peatlands Park (Fig. 3; Table 1). It is not clear if higher rainfall at Curran Bog or comparatively low rainfall at Peatlands Park led to differences in the effect of $NH_3$ deposition at these sites; a longer study might aid in interpreting the complex interactions of these environmental variables.

### 4.8. Limitations

It was not possible to select a lowland raised bog site with low $NH_3$ deposition rates in place of Slieve Beagh. If possible, this would have ensured all sites were lowland raised bogs eliminating additional considerations when comparing sites. As an upland blanket bog, Slieve Beagh has key differences to all other sites in this experiment, including peat depth and age, land area, annual rainfall, and elevation (Joosten, 2016; Minayeva et al., 2016; Rydin and Jeglum, 2006). Furthermore, as

an upland site, Slieve Beagh will receive more wet nitrogen deposition than all other sites of this study (Fowler et al., 1995); which may negatively influence the site's intended role as a low $NH_3$ deposition comparison to the intermediate and high $NH_3$ deposition rates of the other sites. Nevertheless, upland blanket bogs and lowland raised bogs are both ombrotrophic, oligotrophic, and share much of the same vegetation making both types of peatlands similarly vulnerable to atmospheric deposition (Bobbink et al., 1998; Levy et al., 2018; Rydin and Jeglum, 2006).

$NH_3$ data for Slieve Beagh had to be supplemented with data from previous years (Fig. 2F) (Tang et al., 2022, 2023). While this measure was deemed appropriate based on data from most other sites in this study, it includes $NH_3$ data from during the height of the COVID-19 pandemic. COVID-19 has widely been linked to disrupted agricultural activity (Gruère and Brooks, 2021; Kuttippurath et al., 2024; Tang et al., 2022, 2023), which could have affected the applicability of these previous years as substitutes for missing 2023 data; however, no other data was available for $NH_3$ deposition at Slieve Beagh. Nevertheless, as the only monitored peatland in Northern Ireland with low $NH_3$ deposition year-on-year, Slieve Beagh is likely still useful as a counterpoint to the moderate or critically high $NH_3$ deposition rates of all other sites; although, comparisons and conclusions drawn from the site need to be taken with caution.

In this study, nearby landowners were not contacted to determine the exact timing and location of agricultural slurry spreading or storage near each study site, as $NH_3$ deposition data collection and land access rights were facilitated by NIEA and UKCEH. As a result, there was limited direct dialogue with landowners regarding $NH_3$ data or land access rights. Future research should engage with landowners to better identify the timing and location of $NH_3$ point sources, which would improve the interpretation of $NH_3$ deposition data, help validate seasonal measurements and provide a clearer understanding of the relationship between agricultural activity, peatland pollution, and the response of testate amoebae.

### 4.9. Implications

Agricultural activity-linked seasonal $NH_3$ deposition at Ballynahone Bog, Curran Bog, Garry Bog, Moneygal Bog, Peatlands Park, and Slieve Beagh was characterised by a varied testate amoebae assemblage response. Multivariate analysis was leveraged to demonstrate complex testate amoebae assemblage-level changes, specifically at sites where $NH_3$ deposition was on average critically high; allowing the first hypothesis of this study to be accepted ($H_1$ – Testate amoebae assemblage dynamics are altered in response to $NH_3$ deposition). The fourth hypothesis is accepted ($H_4$ – Testate amoebae taxa diversity changes in response to increased $NH_3$ deposition) due to observing SDI values that were statistically significantly lower in the spring at sites with high $NH_3$ deposition rates. Conversely, the final two hypotheses must be rejected ($H_2$ – Mixotrophic testate amoebae abundances decrease in response to $NH_3$ deposition; $H_3$ – High $NH_3$ deposition causes increases in abundances of large testate amoebae taxa). No compelling evidence of increased abundance of larger testate amoebae taxa was found, and while mixotrophic taxa abundances did decrease at most ALPHA samplers in summer, it was apparent that low rainfall and high temperatures at this time were likely involved in this decline. Further study will be needed to untangle broad environmental factors and the specific effects of $NH_3$ deposition, particularly in regard to the abundances of mixotrophic taxa.

This experiment was designed to aid peatland restoration efforts by illuminating the potentially substantial impact of anthropogenic $NH_3$ deposition on these habitats through testate amoebae analysis. Failing to consider $NH_3$ deposition when managing peatlands could lead to ineffectual restoration (Krupa, 2003; Zhou et al., 2021). In Northern Ireland, where $NH_3$ deposition is particularly acute and increasing (Dore et al., 2020; Garland et al., 2023; Jordan et al., 2007; Tang et al., 2018) its consideration is vitally important. However, future research must focus on expanding the methods of this experiment over multiple years to ensure these effects are consistent over longer timescales; especially as this might allow for separating the complex effects of relevant environmental variables. Furthermore, it would be beneficial to test these methods with a wider range of environmental variables, such as: water-table depth; pH; vegetation abundance; light availability; and conductivity, to ensure $NH_3$ deposition affects testate amoebae to the degree reported here. Notwithstanding these considerations, this study presents testate amoebae assemblages as a promising bioindicator of $NH_3$ deposition and highlights the widespread nature of this pollutant on peatlands in Northern Ireland.

## 5. Conclusions

This study investigated the response of testate amoebae assemblages to seasonally variable $NH_3$ deposition across six peatlands across Northern Ireland. The key findings are as follows:

1. Seasonal peaks in $NH_3$ deposition, linked to agricultural activity, frequently exceeded critical thresholds for bryophytes and lichens at most study sites.
2. Multivariate analysis revealed distinct seasonal shifts in testate amoebae assemblages at sites experiencing critically high $NH_3$ deposition, demonstrating their potential as indicators of this peatland pollutant.
3. TITAN analysis identified key $NH_3$-sensitive testate amoebae taxa, suggesting their potential use in biomonitoring $NH_3$ pollution in peatlands.
4. Contrary to expectations, large testate amoebae taxa did not show increased abundance in response to elevated $NH_3$ deposition. Further research is needed to assess this hypothesized response.
5. Sites with critically high $NH_3$ deposition exhibited lower abundances of mixotrophic testate amoebae taxa during typically favourable months, indicating the potential of this functional trait as a bioindicator of $NH_3$ pollution.
6. Testate amoebae diversity was lowest in spring at high $NH_3$ deposition sites, diverging from previous findings that nitrogen deposition does not affect diversity and that taxa richness should peak in spring. While $NH_3$ may contribute to this decline, further experimental studies are needed to disentangle its effects from other environmental factors.

These findings highlight the value of testate amoebae as bioindicators of $NH_3$ pollution and suggest further research is needed to refine their application in peatland monitoring and conservation efforts.

## CRediT authorship contribution statement

**Callum R.C. Evans:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David A. Hatton:** Writing – review & editing, Project administration, Investigation. **Graeme T. Swindles:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejop.2025.126147.

## Data availability

Data will be made available on request.

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