Dissertationes Forestales 387

Versatile responses of testate amoebae and oxygenic photoautotrophic microbes to drying in boreal peatlands

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Academic dissertation

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ABSTRACT

Testate amoebae are microbial predators and hydrological proxies in peatlands. Their functionality and community dynamics are assessed through functional traits (e.g., test size, aperture size, mixotrophy/ heterotrophy, test material). Photoautotrophic microbes have been recently recognized as potentially important contributors to the peatland carbon sink. The responses of these microbes to the drying and associated succession towards arboreal vegetation, which are threatening peatlands as the climate warms and evaporation increases, remain poorly known.

This thesis comprises three studies examining the responses of testate amoebae and photoautotrophic microbes to long-term drying in three boreal peatland types (rich fen, poor fen, bog). The research utilised Lakkasuo water level drawdown (WLD) experiment, where water level has been lowered for two decades to simulate the impacts of climate change. Samples were collected in summer 2022. Testate amoebae were identified and measured microscopically, photoautotrophic communities were analysed using metabarcoding, and photosynthetic capacity was measured with PhytoPAM. The results were compared between control and WLD areas and among sites.

WLD affected photoautotrophic assemblages regardless of the site fertility, whereas testate amoebae responded the most strongly in the rich fen and the least in the bog. In both ends of the fertility gradient, taxonomic changes were decoupled from functional changes. In the poor fen, the functional structure of testate amoeba assemblages differed between control and WLD, and photosynthetic capacity was higher in the WLD area. The sites differed in taxonomic and functional compositions, WLD responses, and functional stability.

Evidence for functional changes was observed only in the poor fen, likely due to a greater rise in nutrient availability and shading compared to the other sites, and, for testate amoebae, because of adaptation to wet and open conditions and low functional redundancy. In contrast, testate amoebae were resistant to the moderate drying in the bog, whereas functional redundancy buffered against changes in testate amoeba communities' functional structure in the rich fen. Overall, the results highlight that microbial responses to drying cannot be generalised across peatland types or microbial groups.

Keywords: microbial assemblages, protists, cyanobacteria, unicellular algae

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TIIVISTELMÄ

Kuoriamebat ovat mikroskooppisia petoja ja veden pinnan indikaattoreita soilla. Niiden toimintaa ja yhteisörakennetta säätelevіä mekanismeja voidaan tutkia toiminnallisten ominaisuuksien avulla (mm. kuoren ja sen aukon koko, ameban aineenvaihdunnallinen strategia (heterotrofia tai miksotrofia), ja kuoren materiaali). Yhteyttävien mikrobien merkitys soiden hiilinielulle on hiljattain havaittu aiemmin arvioitua suuremmaksi. Näiden mikrobien vasteet kuivumiseen ja sen aiheuttamaan kasvillisuussukkessioon kohti metsälajistoa, joka uhkaa soita ilmaston lämmetessä ja haihdunnan lisääntyessä, ovat huonosti tunnettuja.

Tämä työ koostuu kolmesta osatutkimuksesta, joissa selvitettiin kuoriamebojen ja yhteyttävien mikrobien vasteita pitkäaikaiseen kuivumiseen kolmella boreaalisella suotyypillä, joista ravinteikkain oli ruohoinen saraneva, karuin keidasräme, ja näiden välillä varsinainen saraneva. Tutkimus tehtiin Lakkasuon kuivatuskokeella, jossa alentunutta veden pintaa on ylläpidetty kahden vuosikymmenen ajan ilmastonmuutoksen simuloimiseksi. Näytteet kerättiin kesällä 2022. Kuoriamebat tunnistettiin ja mitattiin mikroskopoimalla, yhteyttävät mikrobit tunnistettiin DNA:n metaviivakoodauksella ja niiden yhteytyspotentiaali mitattiin PhytoPAM-laitteella. Näiden mitattujen vastemuuttujien eroa verrattiin kontrolli- ja käsittelyalojen sekä suotyyppien välillä.

Vedenpinnan lasku vaikutti yhteyttävään mikrobilajistoon koealan ravinteisuudesta riippumatta, kun taas kuoriamebat reagoivat voimakkaimmin ruohoisella saranevalla ja heikoiten keidasrämeellä. Ravinteisuusgradientin ääripäissä lajistomuutokset eivät kytkeytyneet toiminnallisiin muutoksiin. Varsinaisella saranevalla sekä kuoriamebayhteisön toiminnallinen rakenne erosi kuivatun ja kontrollialan välillä, ja yhteytyspotentiaali oli suurempi kuivatulla alalla. Eri suotyyppejä edustavat tutkimusalat erosivat toisistaan lajiston, toiminnallisten ominaisuuksien, vedenpinnan laskun aiheuttamien vasteiden, sekä toiminnallisen vakauden suhteen.

Merkkejä toiminnallisista muutoksista havaittiin vain varsinaisella saranevalla, mikä johtui luultavasti ravinteisuuden ja varjostuksen suuremmasta kasvusta verrattuna kahteen muuhun suohon ja kuoriamebojen osalta sopeutumisesta märkiin ja avoimiin olosuhteisiin sekä matalasta toiminnallisesta redundanssista. Sen sijaan sekä kuoriamebat olivat resistenttejä kohtalaiselle kuivumiselle keidasrämeellä, kun taas ruohoisella saranevalla korkea toiminnallinen redundanssi ylläpiti kuoriamebayhteisön toiminnallista rakennetta. Nämä tulokset tulisi huomioida käytettäessä kuoriameboja biomonitoroinnissa. Kaiken kaikkiaan tuloksissa korostuu, että mikrobien vasteita kuivumiselle ei voida yleistää yli suotyyppien tai mikrobiryhmien.

Asiasanat: microbiyhteisöt, alkueliöt, syanobakteerit, yksisoluiset levät

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Joensuu, February 2026,
Olivia Kuuri-Riutta

LIST OF ORIGINAL ARTICLES

I Kuuri-Riutta O, Palacios Ganoza B, Ylänne H, Mitchell EAD, Väliranta MM, Tuittila E-S (2026). Assessing the value of testate amoebae and their functional traits in detecting climate change-induced peatland drying. Microb Ecol*,* 89, article id 35.
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II Palacios Ganoza B, Kuuri-Riutta O, Laine-Petäjäkangas A, Väliranta M, Mitchell EAD, Tuittila E-S. Functional traits drive community assembly of testate amoebae in drying boreal peatlands. Manuscript.

III Kuuri-Riutta O, Le Geay M, Jassey VEJ, Barel JM, Laine AM, Ylänne H, Tuittila E-S (2025). Microbial and bryospheric photosynthesis of boreal peatlands have peatland-type-specific responses to long-term drying. New Phytol 248: 1336 –1350
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AUTHOR'S CONTRIBUTION

Olivia Kuuri-Riutta is fully responsible for the summary of this doctoral thesis.

I O. Kuuri-Riutta collected the data and led the data analysis and writing process under the supervision of E.-S. Tuittila, M. Väliranta, and E.A.D. Mitchell. B. Palacios Ganoza and H. Ylänne contributed to data analysis. E.-S. Tuittila conceptualized the study. O. Kuuri-Riutta and B. Palacios Ganoza drafted the first manuscript together. All authors contributed to the final manuscript.

II O. Kuuri-Riutta collected the data for this manuscript under the supervision of E.-S. Tuittila, M. Väliranta, and E.A.D. Mitchell. The data analysis was conducted, and the writing process was led by B. Palacios Ganoza. O. Kuuri-Riutta made a substantial contribution to the review and editing of the manuscript. All authors contributed to the final manuscript.

III O. Kuuri-Riutta was the first author of this article. She measured the microbial photosynthesis under the supervision of V. Jassey and ran the Tea Bag Index experiment under the supervision of J. Barel. She conducted the data analysis with help from V. Jassey, H. Ylänne, A. Laine, and E.-S. Tuittila. M. le Geay was responsible for dPCR and metabarcoding. A. Laine calculated the P_{max} of mosses. E.-S. Tuittila conceptualized the study. All authors contributed to the final manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ϕPSII	Quantum yield of photosystem II
ETR_{max}	Maximal electron transport rate
MANOVA	Multivariate analysis of variance
NMDS	Non-metric multidimensional scaling
OTU	Operational taxonomic unit
PCA	Principal component analysis
SEM	Structural Equation Model
WLD	water level drawdown
WT	water table

1. INTRODUCTION

1.1 Boreal peatlands in the warming climate

Peatlands are ecosystems where plant biomass production exceeds decomposition and, as a result, plant residues accumulate as peat. Decomposition rates in peatlands are slow due to waterlogged conditions that create anoxia, cold temperatures, and decay-resistant plant litter (Moore and Basiliko 2006; Rydin et al. 2013). As a result, peatlands have accumulated 436 – 543 GT carbon, most of which during the past 11000 years of the Holocene (Loisel et al. 2014; Jackson et al. 2017). This corresponds to one-third to one-fourth of the global soil organic carbon storage (Köchy et al. 2015; Jackson et al. 2017).

Peatlands are a heterogeneous group of ecosystems. Boreal peatlands are classified into two major peatland types according to their ecohydrology, which is further reflected in their vegetation. Minerotrophic fens receive water and nutrients from the surrounding mineral land, and they can be further classified into oligo-, meso-, and eutrophic peatlands based on nutrient availability. Fens are often characterized by sedges, herbs (mesotrophic and eutrophic), and brown mosses (eutrophic). When the accumulated peat layer is so high that the peatland surface loses connection to minerogenic water sources, a peatland undergoes a fen-bog transition, i.e., it turns into an ombrotrophic bog that receives water and nutrients only from precipitation and atmospheric deposition. In these nutrient-poor conditions, *Sphagnum* mosses thrive and inhibit the growth of many other plants by acidifying its surroundings (Rydin et al., 2013; Quillet et al. 2013).

Peatlands, and their carbon storage function, are facing anthropogenic pressures currently and in the future. Human activities, such as land use, burning, and peat mining change peatlands directly, but humans also affect peatlands through the ongoing climate change (Loisel et al. 2020). Climate is warming particularly fast in northern areas (Rantanen et al. 2022), where the majority of peatlands are located (Xu et al. 2018). In the warming climate, evapotranspiration is increasing particularly in peatlands (Helbig et al. 2020), which has been suggested to result in peatland drying (e.g., Roulet et al. 1992; Gong et al. 2012; Chaudhary et al. 2017; Swindles et al. 2019; Zhang et al. 2022) even though precipitation is expected to increase, too (IPCC 2021). However, the future of peatland hydrology remains an active topic of research (see, e.g., Swindles et al. 2019; Kolari et al. 2021; Piilo et al. 2023; Giese et al. 2025).

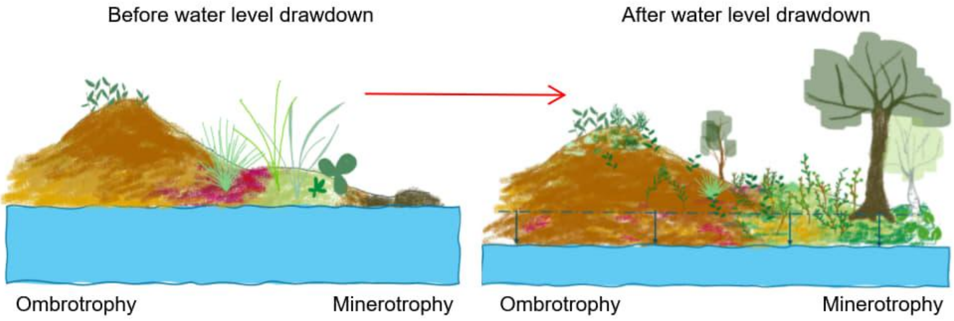

Figure 1. Schematic presentation of vegetation changes typically following water level drawdown along a nutrient gradient in boreal peatlands. The figure is visualising the results of Kokkonen et al. (2019).

A high water table (WT) is not only a crucial prerequisite for peat formation, but it also maintains specific plant communities adapted to wet and often acidic and anoxic conditions (Rydin et al. 2013). Thus, lowering the WT tends to affect peatland communities, often causing a succession where mire specialists are replaced by trees, shrubs, and forest mosses (Fig. 1). However, the rate and magnitude of these changes are highly dependent on the site fertility, as the most growth-limiting resource differs among peatland types. In nutrient-rich fens, trees are able to grow relatively fast when not limited by high WT. In contrast, changes are more subtle in nutrient-limited bogs, where nutrient availability does not support tree growth even when WT is lowered (e.g., Heikurainen and Huikari 1960; Laine et al. 1995; Westman and Laiho 2003; Kokkonen et al. 2019). In addition, abiotic conditions in peatlands are affected by both the drying directly and the drying-associated vegetation succession. As the WT decreases, oxygen concentration in the surface peat increases and oxygen penetrates deeper into the peat, leading to faster mineralisation and, therefore, higher nutrient concentrations in the surface peat (Laiho 2006). Growing trees and shrubs intensify shading to the ground layer, which may decrease soil temperature, and pH tends to decrease (Heikurainen and Seppälä 1963; Minkkinen et al. 1999). Here, drying-induced vegetation succession and following abiotic changes are referred to as secondary changes, as a separation from the immediate impact of drying as such. The net response of peatland carbon storage to drying depends on several complex and interrelated processes, all of which are not well understood. For instance, how persistent drying and the associated secondary changes affect microbial communities and processes remains as a gap in the current knowledge.

1.2 Testate amoebae in boreal peatlands

Testate amoebae are unicellular protists that bear a test (i.e., shell) consisting of protein, siliceous plates, calcite, or xenosomes (particles collected from the environment) (Mitchell et al. 2008). It is a polyphyletic group – in other words, not all testate amoebae are closely related to each other, but test-bearing amoebae have developed independently in multiple lineages throughout evolutionary history (Kosakyan et al. 2016). The first scientific papers that described testate amoebae were published in the 19th century (e.g., Leidy 1879; Penard 1890 as cited by Mitchell 2025). The current knowledge suggests that testate amoebae are crucial in nutrient cycling in soils, even though their role is still not fully understood. However, they are numerous in organic soils (Côuteaux 1972; Warner 1987), feed on a wide range of prey, including decomposer bacteria and fungi (Gilbert et al. 2000; Jassey et al. 2012), thus regulating their community composition and functioning (Antiqueira et al. 2018; Reczuga et al. 2018) and releasing nutrients immobilised to bacterial biomass (Schröter et al. 2003). Moreover, mycorrhizal fungi colonize amoeba tests and use them as nutrient sources (Vohník et al. 2008). They also contribute remarkably to silica cycling (Puppe 2020). Likely due to these important functions, their presence tends to promote plant growth (Bonkowski et al. 2004). In addition, mixotrophic testate amoebae host an endosymbiotic alga (Chlorella spp.) that fixes carbon through photosynthesis (Gomaa et al. 2014).

Testate amoebae are common in peatlands, where they live in the thin water film around mosses. Testate amoeba taxa are sensitive to environmental conditions, especially moisture, in peatlands (e.g., (Harnisch 1927; Steinecke 1927; Harnisch 1948; Jung 1936; Grospietsch 1953; Schönborn 1962 as cited by Mitchell 2025); Meisterfeld 1977; Tolonen et al. 1992; Booth et al. 2025). Building on this fundamental work, testate amoebae are nowadays used as a semi-quantitative proxy to detect past hydrological changes from peat records, in which the tests accumulate in a chronological order and may last millennia (e.g., Warner and Charman 1994; Lamentowicz et al. 2019). This makes testate amoebae a valuable tool, as continuous WT measurements are scarce, and the measurement history is short. In recent decades, testate amoebae have also been applied to monitoring pollution (Kauppila et al. 2006), peatland degradation (McKeown et al. 2024), and restoration success (Creevy et al. 2018; Evans et al. 2025). While the existing modern training sets (e.g., Amesbury et al. 2018; Qin et al. 2021), previous experiments (e.g., Koenig et al. 2018), and comparative field studies (e.g., Zhang et al. 2018; McKeown et al. 2019; Lamentowicz et al. 2020) have documented the WT preferences of testate amoeba taxa, not many studies have considered the effects of secondary changes related to persistent drying, and how they are mediated by peatland types. Especially fens remain underrepresented (Booth et al. 2025).

1.3 Functional traits in testate amoeba research

Functional traits (hereafter abbreviated as "traits") are properties that reflect survival, development, and growth strategies in an individual organism and, thus, the environmental pressures the community is facing (Violle et al. 2007). Traits reflect how the organism affects its surroundings (effect traits), and/or how the organism responds to changes in the environment (response traits). Traits are often compared among communities as community-weighted means, that is, the plot-level or site-level means of trait values weighted by species' abundances (Garnier et al. 2004). Moreover, the functional structure of a community can be assessed via theoretical metrics that take into account both taxonomic identity and traits of the individuals. Such metrics include taxonomic similarity, functional dissimilarity, and beta redundancy. Taxonomic similarity means simply the level of differences in species abundances, functional dissimilarity describes the amount of functionally different species among communities, and beta redundancy quantifies the degree to which these communities share overlapping functions: different species performing the same function enable the site to maintain the function even though some of the species were lost (Ricotta et al. 2020; Ricotta et al.

2021; Ricotta and Pavoine 2024). Thus, traits offer a mechanistic understanding of the processes that shape communities.

The concept of functional traits was originally developed by plant ecologists, but since the first time it was applied to the context of testate amoebae (Fournier et al. 2012), testate amoebae traits have become a common proxy in palaeoecological research (Marcisz et al. 2020). Commonly recorded traits in testate amoebae include the morphological dimensions of the test (i.e., test length and width), biovolume, size of the aperture (opening in the test), test material, test compression, and metabolism (heterotrophy or mixotrophy, where mixotrophy refers to the ability to host a photosynthesising alga as endosymbiont; Fig. 2). According to the current knowledge, adaptation to dry or disturbed conditions is often reflected as small and compressed tests, small and hidden apertures, and a small share of mixotrophs in the community (e.g., Fournier et al. 2012; Koenig et al. 2018; Lamentowicz et al. 2020). However, there are exceptions: e.g., *Trigonopyxis arcula* is a rather large taxon but still highly indicative for dry conditions (Amesbury et al. 2016; Siemensma 2023). The current knowledge gaps include the mechanisms within the testate amoeba community that mediate the responses to drying. These mechanisms can be assessed by analysing traits.

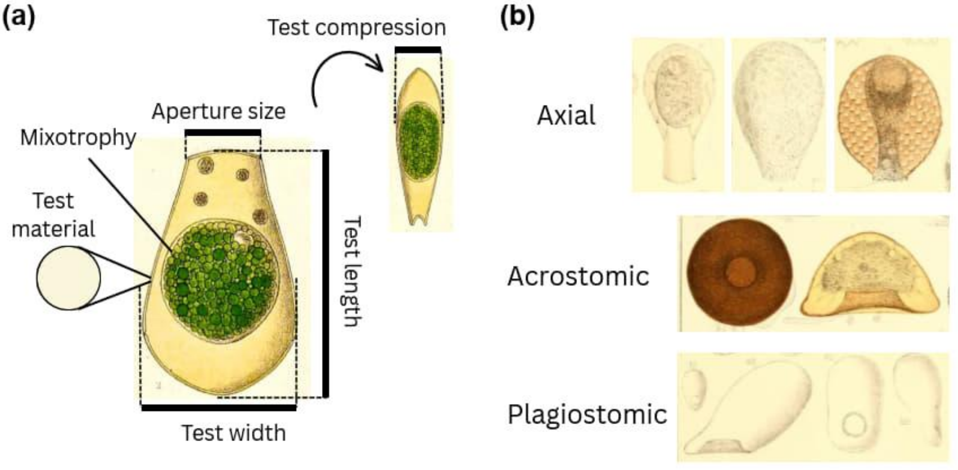

Figure 2. a) Most commonly recorded traits, excluding aperture position, illustrated on *Hyalosphenia papilio* Leidy and **b)** examples of aperture positions of testate amoebae based on original drawing by Joseph Leidy (Leidy 1897).

1.4 Carbon dioxide-fixing microbes in boreal peatlands

Carbon dioxide-fixing microbes in peatlands include oxygenic photoautotrophs, chemoautotrophs, chemolitotrophs, and anoxygenic phototrophic bacteria. Oxygenic photoautotrophs and anoxygenic phototrophic bacteria fix carbon dioxide through photosynthesis, while chemoautotrophs fix it by oxidizing or reducing inorganic molecules or compounds (Grogan 2012). The most common photosynthesizing microbes in peatlands are oxygenic photoautotrophs, including bacteria and unicellular algae (Le Geay et al. 2025). Oxygenic photoautotrophs live in pore water, in the water film around mosses, or within the hyaline cells of *Sphagnum* mosses (Gilbert et al. 1998). Many photoautotrophic microbes, such as Cyanobacteria, are also capable of nitrogen fixation, which makes them an important component in moss microbiome, as nitrogen provision from microbes is known to affect moss growth (Berg et al. 2013; Larmola et al. 2014; Cheng et al. 2024). While photosynthesizing microbes in peatlands have been overlooked until recently, the existing studies suggest that their contribution to peatland carbon fixation is more remarkable than previously assumed, accounting for 4 – 30 % to the carbon fixed by the bryosphere, i.e., mosses and microbes together (Hamard et al. 2021a) and possibly compensating for carbon losses from decomposition (Hamard et al. 2025).

The responses of photoautotrophic microbes to peatland drying are not well understood. Previous research includes observations of both declining abundances and net CO_2 fixation (Payne et al. 2016; Lamentowicz et al. 2020; Jassey et al. 2022) and high microbial photosynthesis rates explained by relatively high microbial biomass, chlorophyll α concentration, electron transport rate, and shade adaptation in the light-harvesting machinery (Perrine et al. 2012; Hamard et al. 2021b). In addition, accelerated mineralisation and dry-tolerant Cyanobacteria may increase soil nitrogen concentrations (Laiho 2006), which promotes microbial photosynthesis in peatlands (Wyatt and Turetsky 2015; DeColibus et al. 2017). This increased microbial photosynthesis could possibly explain why bryospheric photosynthesis tends to be resistant to drying (Hájek et al. 2009; Kangas et al. 2014; Kokkonen et al. 2022) despite declining moss coverage and diversity (Laine et al. 1995; Kokkonen et al. 2019). However, more research is needed to form a reliable picture of how photoautotrophic microbes are responding to climate-induced peatland drying.

1.5 Aims

This study aims to improve our understanding of how testate amoebae and oxygenic photoautotrophic microbes respond to climate-induced water level drawdown (WLD) and the associated secondary changes along a fertility gradient in boreal peatlands (see a summary of the background, knowledge gaps, and research questions corresponding to each aim in Figure 3). Climate-induced WLD is expected to be moderate in intensity and long-term, i.e., persist over decades. The work is divided into three studies (I-III), each of which have their own, specific aim within this context. The specific aims for each study were:

I) To investigate the WLD responses of testate amoeba assemblages and functional traits
II) To investigate the mechanisms through which testate amoebae respond to WLD
III) To investigate the WLD responses of photoautotrophic microbes, microbial carbon fixation, and bryospheric carbon fixation

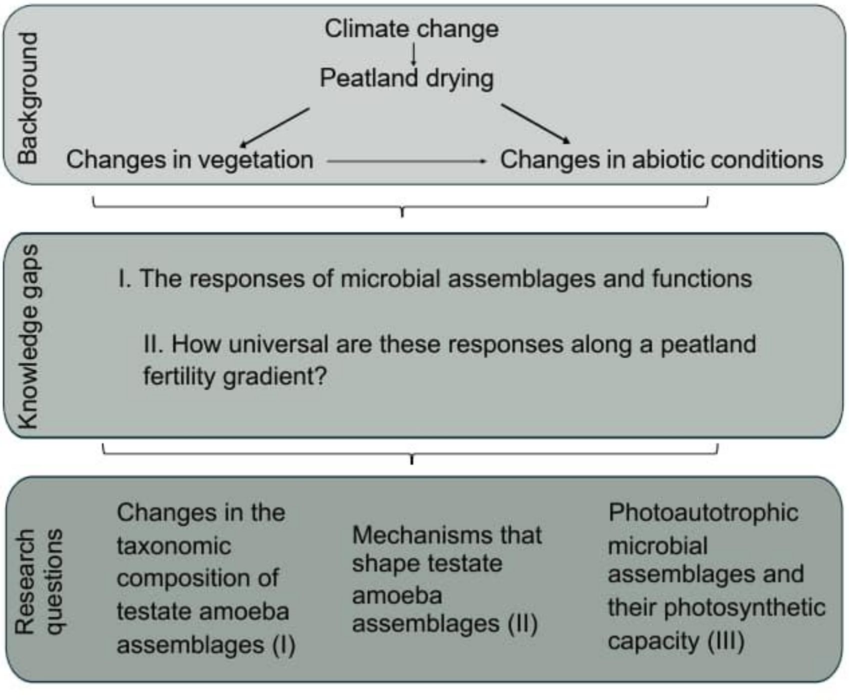

Figure 3. Schematic presentation of the background of this study as well as knowledge gaps and research questions addressed is studies I-III.

2. MATERIALS AND METHODS

2.1 Study site

The study site is an eccentric peatland complex, Lakkasuo peatland (Fig. 4) in the southern boreal vegetation zone, Orivesi, Central Finland (61°47′N, 24°18′E). The annual mean temperature in the area 2000–2022 was 4.32°C, and the average annual precipitation was 687 mm (Finnish Meteorological Institution 2025). Lakkasuo peatland comprises multiple peatland types typical for the boreal zone, including a mesotrophic fen, an oligotrophic fen, and an ombrotrophic bog (hereafter referred to as rich fen, poor fen, and bog).

Since 2001, there has been an ongoing WLD experiment in the rich fen, the poor fen, and the bog in Lakkasuo. In 2000 (for the rich fen and the bog) and 2001 (for the poor fen), an experimental WLD area was established in each site by surrounding it with 30 cm deep ditches. These small ditches were specifically designed to simulate the drying impact of climate change, and they were cleared in 2016. A corresponding control area was established upstream from the experimental WLD area in each site. At the beginning of the experiment, the control area and WLD area within each site did not differ from each other in their vegetation and WT depth. By 2016, vegetation changes had occurred in the WLD areas, with the most notable succession towards arboreal vegetation detected in the rich fen, and the most subtle changes in the bog (Kokkonen et al., 2019). Each study area in the Lakkasuo experiment has 8 – 10 permanent sampling points originally established for greenhouse gas measurements.

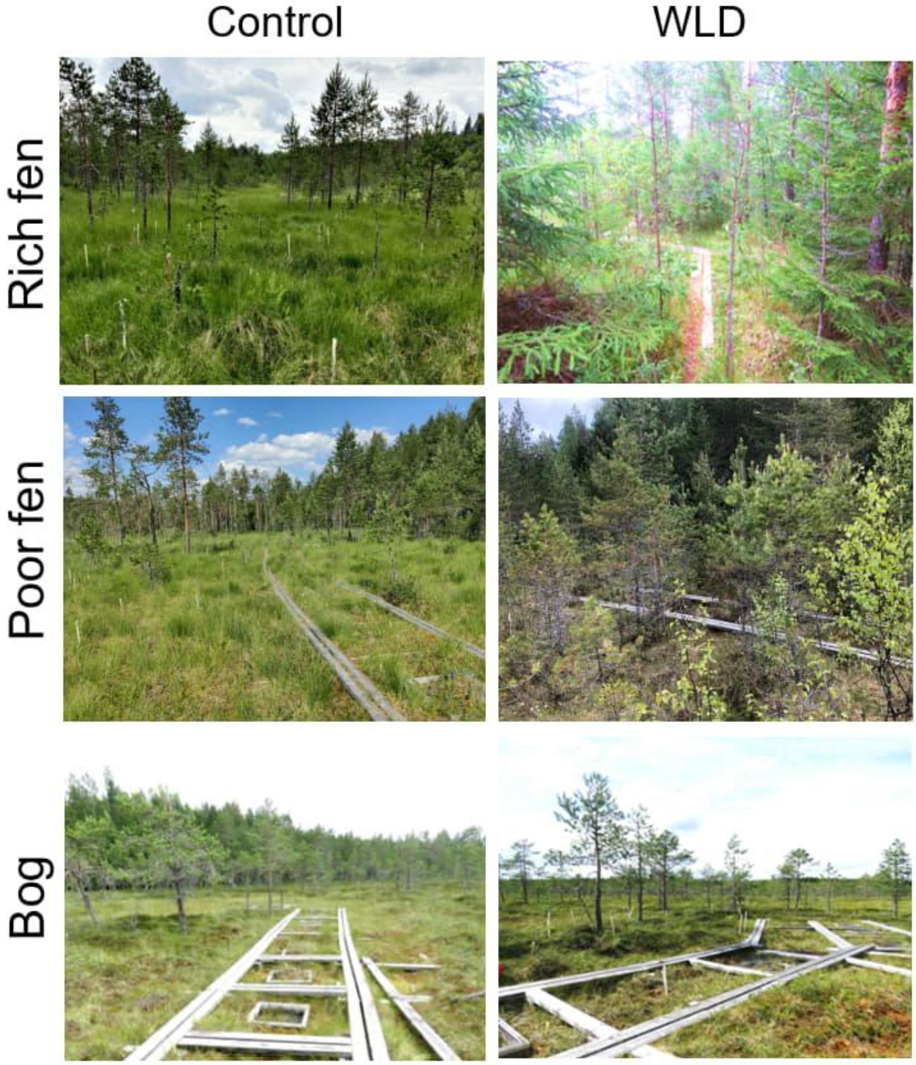

Figure 4. Lakkasuo water level drawdown experiment. Photographs from the study areas by Anna Laine-Petäjäkangas and Olivia Kuuri-Riutta.

2.2 Data collection

The data collection included measuring environmental variables used as background data in this work (I, II, III), and collecting, preparing, and analysing three types of samples: testate amoeba samples (I, II), samples for photoautotrophic community analysis (III), and samples for microbial photosynthesis measurements (III). The overview of the workflow from sample collection to statistical analysis is presented in Fig. 5.

2.2.1 Background data

We utilized nutrient data (I, III) originating from 2016 and previously published in Kokkonen et al. (2019), plant cover data (I, III) published by Köster et al. (2023), and moss photosynthesis data (III) published by Kokkonen et al. (2022). All these data are collected from the permanent sampling points in the Lakkasuo experiment. In addition, we recorded WT (I, III), shading intensity (I, III), and decomposition potential (III) from the same sampling points in summer 2022.

WT was measured relative to moss layer surface, from permanent water wells every second week in summer 2022 (June-August). Negative values indicate WT below the surface and positive WT above the surface. Shading intensity was measured once in summer 2022, by measuring photosynthetically active radiation (PAR) above and below vegetation in each permanent measurement point. It was expressed as % of the initial PAR and calculated as following:

$$100-(\mathrm{PAR}_{\mathrm{below}}/\mathrm{PAR}_{\mathrm{above}}) \tag{I}$$

where $\mathrm{PAR}_{\mathrm{below}}$ is photosynthetically active radiation below the field layer and $\mathrm{PAR}_{\mathrm{above}}$ is photosynthetically active radiation above the field layer. The $\mathrm{PAR}_{\mathrm{above}}$ value represents the ambient PAR intensity, i.e., without any shading, so we used the mean of $\mathrm{PAR}_{\mathrm{above}}$ values measured from the control area for all the points in each site. This way, the ambient value was not affected by trees growing on the WLD areas.

Tea bag index was used to estimate the decomposition potential of each study area. We followed the protocol established by Keuskamp et al. (2013). Briefly, a bag of Lipton's green tea (EAN 8722700055525) and a bag of rooibos (EAN 8711327514348) were buried at an 8 cm depth for three months, starting at the beginning of June 2022. After retrieval, the remaining dry mass of the tea was weighed and tea mass loss, stabilisation factor, and decomposition rate were calculated.

2.2.2 Sample collection and preparation

We collected separate samples for testate amoeba community analysis (I, II), photoautotrophic community analysis (III), and microbial photosynthesis measurements (III) (here referred to as "photosynthesis samples"). All samples were collected from the immediate proximity of the permanent sampling points in the Lakkasuo experiment: 8 from both study areas in the rich fen, 9 from both study areas in the poor fen, and from the bog WLD area, and 10 from the bog control area, totalling 53 samples of each sample type. The samples were collected in summer 2022.

For both testate amoebae (I, II) and photoautotrophic microbes (III), we collected the topmost three cm of 3-10 moss shoots, representing the dominant species in each sampling point. For testate amoebae, the three cm moss sample is unusually short, but due to fast decomposition in the WLD areas, we were not able to reach more than that as the moss layer was compacted already at 3-4 cm. To be consistent, we collected the achievable 3 cm moss sample from all the sampling points. Testate amoeba samples were stored in 4 % formaldehyde, while the photoautotrophic microbe samples were fixed in RNAlater. For photosynthesis samples (III), we collected approximately 5 cm x 5 cm x 3 cm piece of moss and stored it in ziplock bags with some air. The bags were kept in cold till the analysis was performed three days after sample collection.

Microbes from photosynthesis samples as well as testate amoebae were extracted from the mosses following a protocol previously applied by, e.g., Hamard et al. (2021a, b). Photosynthesis samples were first placed in 30 ml of demineralized water. The samples were shaken first by hand and then with a Vortex shaker. After that, they were sieved to remove the plant material using a 100 (photosynthesis samples) or 150 µm (testate amoebae) sieve. After that, the testate amoeba samples were placed in a centrifuge (3000 revolutions per minute) for three minutes, so that the excess liquid could be removed with a pipette, while the photoautotrophs were retained by filtering the solution through GF/F Whatman filters (0.7 µm).

2.2.3 Quantifying testate amoeba communities and functional traits

Testate amoebae were analysed to the morphotype level under a light microscope at 200x and 400x magnifications, using as references Siemensma et al. (2023) and photos in McKeown et al. (2019). We aimed for a total count of 150 individuals in each sample.

The functional traits inspected were biovolume (based on test length, width, and height, and a different formula for each shape of the test as in Fournier et al. 2015), aperture size, mixotrophy, aperture position, test compression, and test material. Test length, test width, and aperture size were measured for ~5 replicates of each taxon in each sample. We aimed to cover at least 80% of the community by the measurements. Categorical traits were converted to a binary format. Finally, the community weighted mean was calculated for each trait in each plot by quantifying the mean trait value of the taxon present in the community, weighted by their relative abundance (Garnier et al. 2004).

2.2.4 Quantifying photoautotrophic microbial communities

Metabarcoding and digital PCR were used to quantify the absolute abundances of photoautotrophic OTUs. The technical details are provided in the methodology section in Kuuri-Riutta et al. (2025). Briefly, DNA was extracted from the moss samples. The DNA samples were sequenced targeting the 23S rRNA gene with the primer pair P23SrV_f1/ P23SrV_r1 (Sherwood and Presting 2007). OTUs were assigned using the μgreen reference database for the 23S rRNA gene (Djemiel et al. 2020). Plant-associated sequences were removed, and a rarefaction was performed for each sample.

Absolute quantification of the 23S rRNA gene was done using digital PCR (dPCR, Qiagen) with the primer pair 23S255f/ P23SrV_r1 (Sherwood and Presting 2007; Le Geay et al. 2024a). The method accounts for the mass of the dry moss sample used for DNA extraction and results in the concentration of target gene copies per gram of dry moss (copies g^{-1} DW). The absolute abundance of different photoautotrophic groups was then calculated using their relative abundance from 23S rRNA sequencing data.

2.2.5 Quantifying photosynthetic capacity of photoautotrophic microbes and bryosphere

The GF/F Whatman filters containing the photoautotrophic microbes (extracted from photosynthesis samples) were exposed to dark for 30 minutes, after which the quantum yield of photosystem II (ϕPSII) and maximal electron transport rate (ETR_{max}) were measured using a PhytoPAM (Phytoplankton Analyzer, Heinz Walz GMBH, Effeltrich, Germany). ϕPSII gives the fraction of the absorbed quanta that are used for photosynthetic electron transport and thus provides a measure of photosynthesis efficiency. ETR_{max} gives the maximum electron transport rate through the photosystem II, thus serving as an indication of the maximal photosynthetic activity (Maxwell and Johnson 2000).

Bryospheric photosynthesis (i.e., the photosynthesis of the moss layer) includes photosynthesis conducted by both mosses and associated microbes. It was calculated as community-weighted mean photosynthetic capacity, using moss species-specific photosynthetic capacity (P_{max}) measured from the same sampling points by Kokkonen et al. (2022), and moss species cover estimates (Köster et al. 2023).

2.3 Statistical analyses

All statistical analyses were conducted in R (R Core Team 2022). The package ggplot2 (Wickham 2016) was used to illustrate the results.

Principal component analysis (PCA) and non-metric multidimensional scaling (NMDS) from the package vegan (Oksanen et al. 2022) were used to analyse and illustrate how site and treatment controlled environmental variables (I, II, III), testate amoeba community composition (I), testate amoeba traits (II), and photoautotrophic microbial communities (III). To test the impact of site, treatment, and site*treatment interaction on the environmental variables and photosynthetic parameters, Analysis of Variance (two-way-ANOVA) was used together with Tukey's post hoc test (III). To test these impacts on testate amoeba communities (I) and traits (I), and photoautotrophic microbes (III), multivariate analysis of variance, MANOVA was run (Wang et al. 2012).

To analyse the functional beta diversity and stability of testate amoeba communities (II), beta redundancy, functional dissimilarity, and taxonomic similarity were calculated (Ricotta and Pavoine 2024) using the package adiv for calculations (Pavoine 2020) and a ternary diagram from the package ade4 for illustration (Dray and Dufour et al. 2007). A distance-based multivariate analysis of variance (db-MANOVA) from the package PERMANOVA (Vicente-Gonzalez and Vicente-Villardon 2022) was used to test functional stability between sites and treatments, betadisper from package vegan (Oksanen et al. 2022) was used to test the variation, and two-way permutational ANOVA from package lmperm (Wheeler and Torchiano 2025) was used to test variation in each index separately.

Linear models with different combinations of explanatory variables were compared using AICc values (Mazerolle 2023) (III) to assess how environmental variables explained microbial photosynthesis. In addition, structural equation models (SEM) (Lefcheck 2016) were built to analyse linkages among environmental variables, the photoautotrophic microbial communities, microbial photosynthetic capacity, and bryospheric photosynthetic capacity (III).

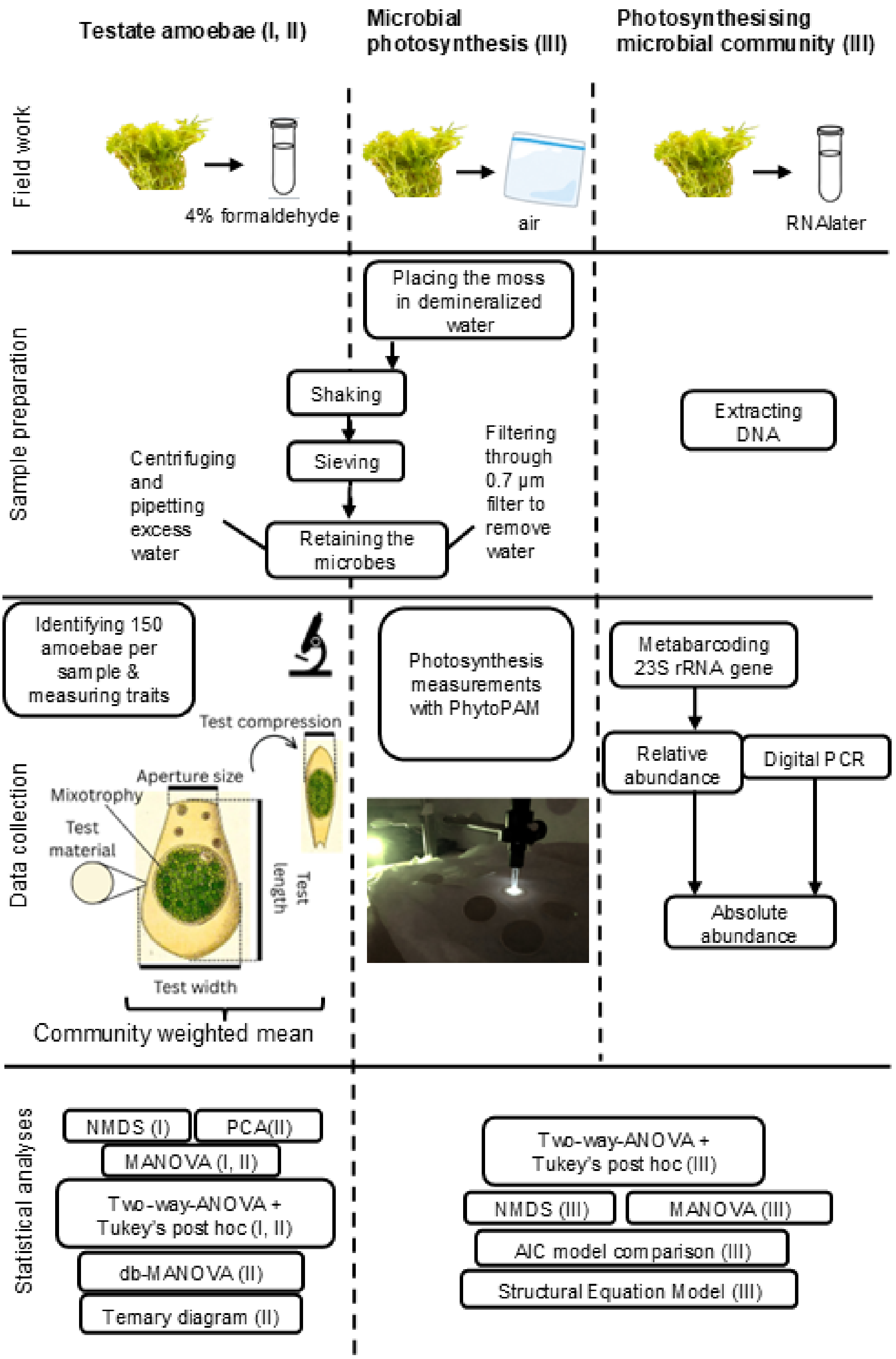

Figure 5. Schematic presentation of the overall workflow from sample collection to statistical analyses.

3. RESULTS

3.1 Environmental variables in the six study areas

After two decades of ongoing water-level drawdown, environmental variables differed among the six study areas (Fig. 6a, b). The most notable differences in environmental variables between WLD and control were recorded in the poor fen (Fig. 6b). The poor fen had shifted towards the richer end of the nutrient gradient, driven by increased nutrient concentrations and shading intensity (Fig. 6a).

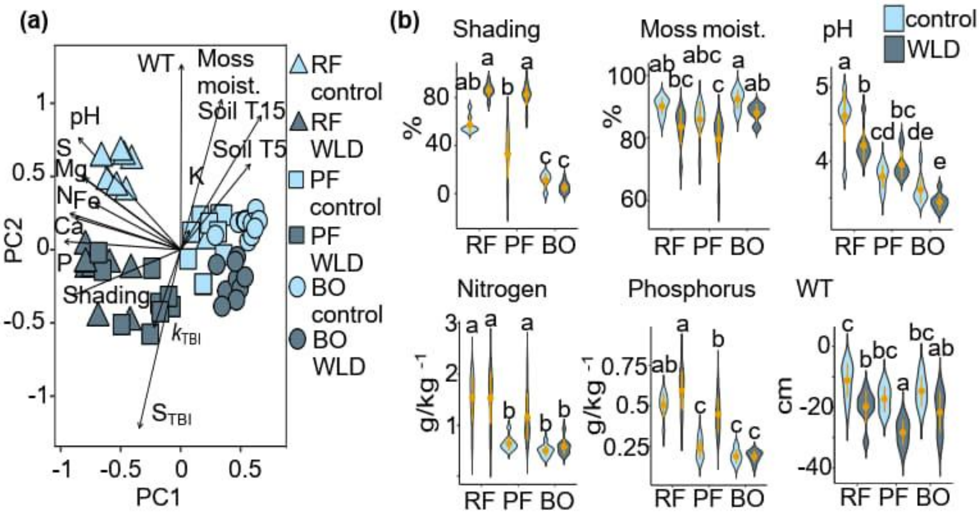

Figure 6. a) Principal component analysis based on environmental variables Lakkasuo water level drawdown experiment. Soil T5(T15) = Soil temperature at 5(15) cm depth. b) Shading intensity, moss moisture content, pH, nitrogen and phosphorus concentratios, and WT (measured as distance between peat surface and WT) in Lakkasuo. RF = rich fen, PF = poor fen, BO = bog, WLD = water level drawdown. The violins illustrate the distribution of the data and the kernel probability density of the data at different values. The yellow dots indicate mean values, and the whiskers show standard deviation. Letters indicate statistically significant differences according to Tukey's pairwise comparison (p-value <0.05). PH and nutrient concentration data originally published in Kokkonen *et al.* (2019).

3.2 Responses of testate amoebae and photoautotrophic microbes to water level drawdown

In the testate amoeba assemblage (Fig. 7a, b) and functional traits (Fig. 7d), the most pronounced shift occurred in the rich fen and the smallest in the bog, while the poor fen was intermediate between the two. On the contrary, the photoautotrophic microbes did not reflect this fertility-mediated pattern, but their community structure differed between all control and WLD areas (Fig. 7c).

Despite the fertility-mediated pattern, MANOVA showed that eleven testate amoeba taxa had a significant preference for either control or WLD areas in the whole dataset: control areas were preferred by *Amphitrema wrightianum* Archer, *Archerella flavum* Archer, *Euglypha compressa* Carter*, Hyalosphenia papilio, Planocarina marginata* Penard*,* and *Phryganella acropodia* Hopkinson, while WLD areas were preferred by *Alabasta militaris* Penard*, Assulina muscorum* Greeff*, Corythion-Trinema* type, *Euglypha strigosa* Ehrenberg*,* and *Valkanovia elegans* Schönborn. In addition, four taxa had a significant preference only in the fens: *Heleopera sylvatica* Penard and *Nebela tincta* type Kosakyan & Lara preferred WLD, while *Hyalosphenia elegans* Leidy and *Trinema enchelys* Ehrenberg preferred control areas. *Nebela tincta* type and *Trinema enchelys* were absent from the bog. For traits, significant differences between the control and WLD areas were detected in pairwise comparisons only in the fens (Fig. 8), where tests and apertures were smaller, and proteinaceous tests and mixotrophy were less common in the WLD areas. In addition, an increase in the share of siliceous tests and plagiostomic apertures, and a decrease in the share of axial apertures and xenosomic tests, were found in WLD areas in the whole data, indicated as a significant main impact in MANOVA. The size was also related to site fertility, richer sites hosting larger amoebae.

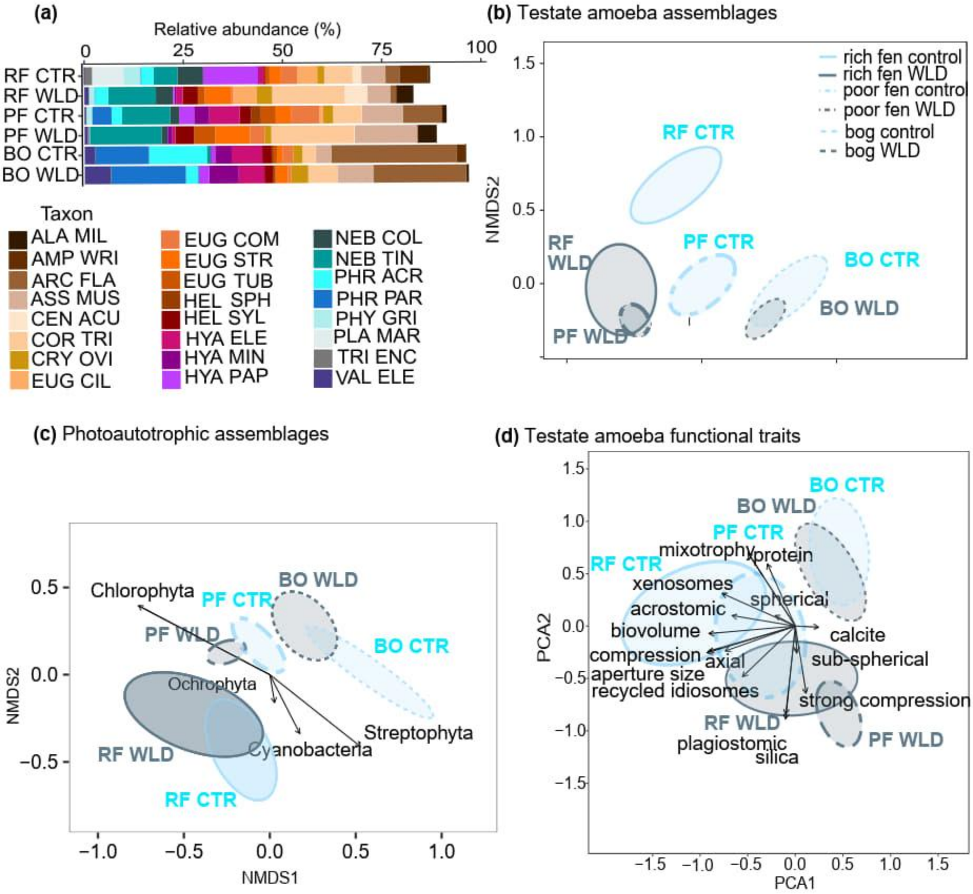

Figure 7. a) Overview of the most common taxa (relative abundance > 10% in at least one plot) in the testate amoeba communities across the WLD experiment in the three peatland types. b) NMDS on testate amoeba community composition. c) NMDS on photoautotrophic microbial phyla. d) NMDS on testate amoeba functional traits, based on original figure produced by Brunella Palacios Ganoza.

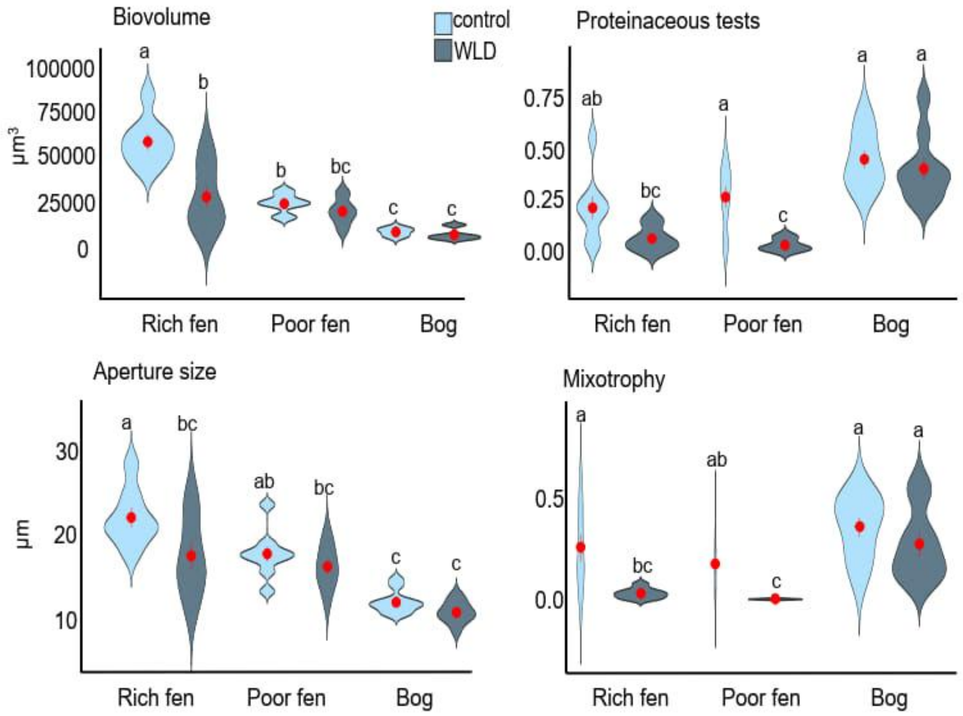

Figure 8. The community-weighted mean of testate amoeba traits that significantly differed between the WT treatments (in Tukey's pairwise comparison): biovolume, aperture size, mixotrophy, and proteinaceous tests. The violins illustrate the distribution of the data and the kernel probability density of the data at different values. The red dots indicate mean values, and the whiskers show standard deviation. Letters indicate statistically significant differences according to Tukey's pairwise comparison (p-value <0.05). Based on original figure produced by Brunella Palacios Ganoza (study II).

3.3 Functional structure of testate amoeba communities in WLD and control areas

The overall functional structure of testate amoeba assemblages (i.e., functional dissimilarity, beta redundancy, and taxonomic similarity inspected together) differed between the WLD and control area in the poor fen (Table 1; Fig. 9), similarly to all the functional diversity indexes when inspected independently (Table 2). In addition, beta redundancy was lower in the rich fen WLD compared to the rich fen control area (Table 2).

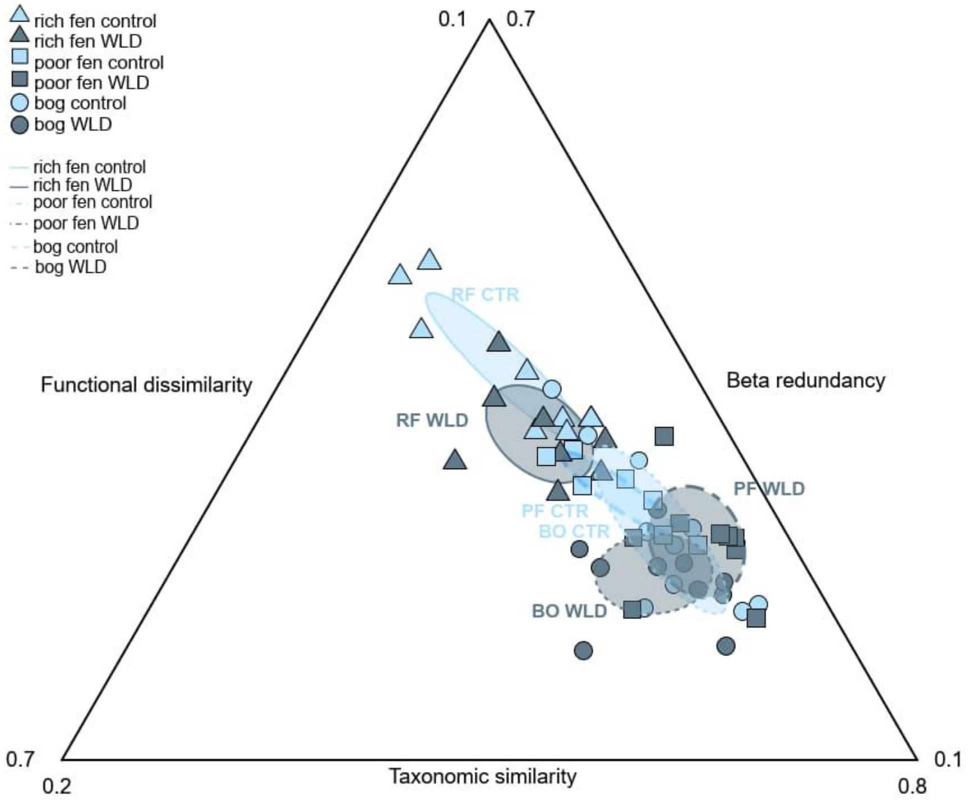

Figure 9. Ternary diagram that illustrates study points based on the beta redundancy, functional dissimilarity, and taxonomic similarity of their testate amoeba assemblages._Based on original figure produced by Brunella Palacios Ganoza (study II).

Table 1. Pair-wise comparison of functional structure (functional dissimilarity, beta redundancy, and taxonomic similarity) variation between treatments. The table shows F-values and adjusted p-values from permutational multivariate ANOVA.

	F	p-value
Rich fen control – Rich fen WLD	2.69	0.11
Poor fen control – Poor fen WLD	9.47	**0.004**
Bog control – Bog WLD	1.28	0.29

Table 2. Mean values of the functional diversity metrics in the study areas. Symbol * indicates a statistically significant ($p<0.05$) deviance from the corresponding control area.

	Rich fen		Poor fen		Bog	
	Control	WLD	Control	WLD	Control	WLD
Taxonomic similarity	0.35	0.4	0.48	0.56*	0.53	0.54
Functional dissimilarity	0.23	0.23	0.2	0.17*	0.19	0.21
Beta redundancy	0.42	0.36*	0.32	0.28*	0.29	0.25

3.4 Photosynthetic capacity of microbes and bryosphere in WLD and control areas

Microbial and bryospheric photosynthetic capacity were higher in the poor WLD compared to the corresponding control area (Fig. 10 a, c). Photosynthetic efficiency (ϕPSII) was generally higher in WLD areas compared to control areas (ANOVA; F = 5.6, p =0.022), but the difference was not significant in any of the pair-wise comparisons (Fig 10b).

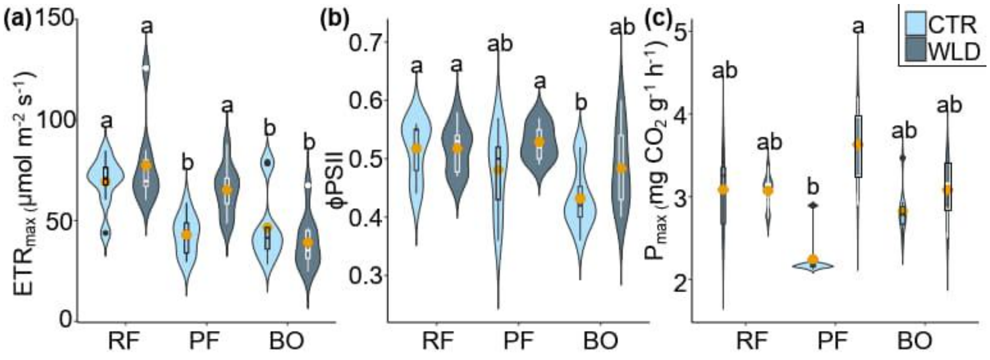

Figure 10. Photosynthetic parameters in the study areas of Lakkasuo water level drawdown experiment (RF = rich fen, PF = poor fen, BO = bog, CTR = control, WLD = water level drawdown). The violins illustrate the distribution of the data and the kernel probability density of the data at different values. The median and interquartile range are shown within the violin distribution as box plots. The red dots show the mean value. Letters indicate significant differences according to Tukey's pairwise comparison (P-value < 0.05). (a) Microbial photosynthetic capacity measured as maximum electron transport rate (ETR_{max}). (b) Microbial photosynthetic efficiency measured as quantum yield of photosystem II (φPSII). (c) Bryospheric photosynthetic capacity measured as P_{max}.

The variation in ETR_{max} was best explained by phosphorus concentration, ϕPSII, and the absolute abundance of Chlorophyta, the variation in ϕPSII by site fertility gradient (represented by PC1 axis, see Fig 6a) and WT, and the variation in P_{max} by the absolute abundance of Cyanobacteria, WT, sulphur concentration, and decomposition potential (see the model details in Table 3). The water level drawdown altered the connections between WT, nitrogen concentration, Cyanobacterial abundance, and photosynthesis – in drying conditions, moss photosynthesis benefitted of deep WT directly, while the role of Cyanobacteria in promoting microbial photosynthesis through nitrogen acquisition was more important in the control areas (Fig. 11).

Table 3. Adjusted r^2, F and p values for models that best explained the variation in ETR_{max}, ϕPSII, and P_{max}, selected by AICc criteria.

Model	adj. r^2	F_{df}	p-value
ETR_{max} ~ P + ϕPSII + Chlorophyta	0.609	26.4	<0.001
ϕPSII ~ PC1 + WT	0.277	10.97	<0.001
P_{max} ~ Cyanobacteria + WT + S + k_{TBI}	0.425	9.87	<0.001

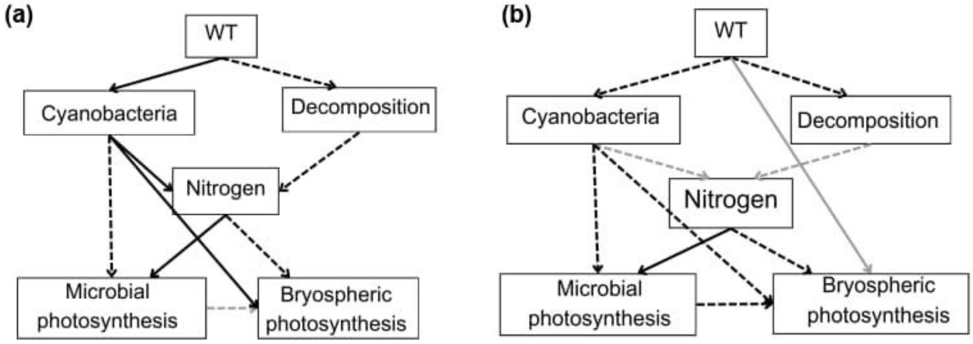

Figure 11. Structural Equation Models illustrating connections between WT, Cyanobacteria, decomposition, nitrogen concentration, microbial and bryospheric photosynthesis in **a)** control areas and **b)** water level drawdown areas. Significant connections in solid arrows and non-significant in dashed arrows, positive connections in black and negative in grey.

4. DISCUSSION

This study examined how testate amoebae and photoautotrophic microbes respond to climate-driven drying and associated secondary changes in boreal peatlands. I compared their community composition, testate amoeba functional traits, and microbial photosynthetic capacity between experimental water-level drawdown (WLD) areas — subjected to moderate, persistent drying — and corresponding ambient control areas. The findings reveal that microbial communities responded to drying differently depending on the site and microbial group: the response of testate amoebae depended on site-fertility more than that of photoautotrophs, which was a taxonomically heterogenic group. Moreover, the site affected the degree to which the taxonomic changes were reflected in the functional structure or the photosynthesis capacity: evidence for these functional changes was observed in the poor fen only.

4.1 Taxonomic changes coupled with functional changes only in the poor fen

This study showed versatile responses of testate amoebae and photoautotrophic microbes to experimental drying. For testate amoeba community composition and traits, the strongest responses were observed in the rich fen and the most subtle in the bog. This supports previous findings (Urbanová and Bárta 2016) and mirrors the patterns reported for vegetation from the same experimental set-up (Kokkonen et al. 2019). In contrast, the photoautotrophic communities responded in all sites without a clear fertility-mediated pattern. For instance, Chlorophyta – the photoautotrophic phylum that was the most strongly associated with high microbial photosynthetic capacity – was more abundant in all WLD areas compared to the control areas.

Despite the taxonomy specific patterns, the functional structure of testate amoeba communities, as well as the microbial and bryospheric photosynthetic capacity, differed between the control and WLD area only in the poor fen. In addition, the analysis of testate amoeba traits showed that both functional dissimilarity and functional beta redundancy were lower in the poor fen WLD area, indicating that testate amoeba communities were functionally less diverse compared to the control area. Accordingly, the share of mixotrophs was near to zero in the poor fen WLD, whereas the co-existence of heterotrophs and mixotrophs indicated more heterogeneous metabolic strategies in the control area. The same has been observed for photoautotrophic microbes by Jassey et al. (2022), who measured constant carbon fixation rates despite species turnover. Microbial and bryospheric photosynthetic capacity, in turn, were increased in the poor fen WLD area.

These findings suggest that the degree to which taxonomic and functional changes are coupled in microbial communities depends on local environmental conditions and the properties of the community. In this experimental set-up, the differences in shading intensity and nutrient concentrations between the control and WLD area were the largest in the poor fen, and the poor fen WLD is also the driest of all study areas. Thus, the environmental pressure on the communities was likely higher in the poor fen WLD compared to the other study areas. High nutrient availability, especially that of phosphorus, was linked to high photosynthetic capacity, possibly explaining the increased photosynthetic capacity in the poor fen. On the other hand, testate amoebae in the poor fen control displayed traits typical for relatively wet and open fens, such as relatively large tests and apertures (Marcisz et al. 2020, and references therein), but also low functional redundancy. This indicates that there were not many functionally similar taxa in the communities, but the taxa lost due to WLD were replaced by functionally different taxa.

In contrast, the functional structure of testate amoeba communities in the rich fen and in the bog did not differ between the control and WLD areas, nor did the photosynthetic capacity. For testate amoebae, this was interpreted to be explained by resistance in the bog and functional resilience in the rich fen. In the bog, testate amoeba community and trait composition were little affected by WLD, likely because of the subtle secondary changes in the ecosystem and the small size of testate amoebae that allows movement in a thin water film and is, therefore, indicative of adaptation to dry and disturbed conditions (Marcisz et al. 2020, and references therein). In the rich fen, in turn, a clear difference in the taxonomic and trait composition was observed between control and WLD, but this was not accompanied by statistically significant differences in the overall functional structure. This was likely due to the high beta redundancy, i.e., multiple functionally similar taxa could compensate for the lost taxa. In contrast to the poor fen, mixotrophic taxa did not completely disappear in the rich fen, even though their proportion declined drastically.

4.2 Implications for the use of testate amoebae as an ecohydrological proxy

The fertility-mediated response of testate amoebae should be taken into account when using them as an ecohydrological proxy (e.g., to reconstruct past WT fluctuations). In drying fens, testate amoebae are affected not only by drying as such but also by multiple secondary changes in the ecosystem, including decreasing moss cover, increasing nutrient concentrations (Kokkonen et al. 2019), and increasing shading from trees and shrubs. The secondary changes added pressure on a community whose trait composition – consisting of large tests and apertures as well as mixotrophic taxa – did not support adaptation to dry or shaded conditions (e.g., Lamentowicz et al. 2020; Marcisz et al. 2020). Thus, the secondary changes following persistent drying may amplify the drying signal in a testate amoeba record in the fens. In contrast, in the bog, the response of testate amoebae may be hindered by the resistance of both vegetation and testate amoebae themselves, as well as the capability of *Sphagnum* mosses to raise water via capillarity (Rydin 1985).

These results support previous studies that recommend cautiousness when running a testate amoeba-based WT reconstruction through a fen-bog transition (Payne 2011; Zhang et al. 2018), when using a modern training set collected from a different peatland type than the paleoprofile (Payne 2011; Amesbury et al. 2016), or using testate amoebae as a single proxy without comparing them to, e.g., plant macrofossils (Tolonen 1986). However, this study also demonstrated the robustness of certain taxa as indicators for either ambient (*Amphitrema wrightianum*, *Archerella flavum, Euglypha compressa, Hyalosphenia papilio, Planocarina marginata,* and *Phryganella acropodia*) or drying conditions (*Alabasta militaris, Assulina muscorum, Corythion-Trinema* type, *Euglypha strigosa,* and *Valkanovia elegans*). In addition, *Heleopera sylvatica* and *Nebela tincta* type preferred WLD, and *Hyalosphenia elegans* and *Trinema enchelys* preferred control areas in the fens. Despite the few exceptional cases where their abundance differed only little between control and WLD area, (i.e., *H. papilio* and *A. flavum* in the bog, *A. muscorum* in the rich fen), these taxa had a significant preference when the whole dataset was inspected, regardless of the fertility-mediated secondary changes. Overall, these results confirmed existing knowledge about the ecology of these taxa (e.g., Tolonen 1986, Koenig et al. 2018; Zhang et al. 2018; Basińska et al. 2020). However, the exceptions to the rule highlight that it is important to assess the entire testate amoeba community rather than rely on a single indicator taxon. This agrees with previous observations in bacterial communities, which have shown that even though “everything is everywhere”, everything does not thrive everywhere, and therefore abundances should be considered rather than presence/absence (Orland et al. 2018). Moreover, this data supports previous papers (e.g., Payne et al. 2016; Lamentowicz et al. 2020) suggesting that the mixotrophic *H. papilio* and *A. flavum* indicate sunlight exposure more strongly than WT.

In this study, biovolume, aperture size, mixotrophy, and the share of proteinaceous tests differed significantly between the control and WLD areas in the fens, and additionally that of siliceous and xenosomic tests as well as plagiostomic and axial apertures in the whole dataset. None of the traits differed between control and WLD in the pairwise comparison in the bog. This is somewhat surprising, as testate amoeba traits have been used as bioindicators also in bogs, even for reconstructing WT depth (van Bellen et al. 2017). However, it is possible that a stronger disturbance, including even deeper WTs and vegetation succession, would trigger a stronger taxonomic turnover accompanied by trait turnover. This suggests that the intensity of a disturbance, rather than persistence, defines whether it is reflected in testate amoeba traits.

4.3 Implications for peatland carbon sink

This study showed that microbial photosynthesis is likely not suppressed by moderate peatland drying, as microbial photosynthetic capacity was increased in the poor fen WLD area, and microbial photosynthetic efficiency, ϕPSII, was generally higher in WLD areas. Similar findings have been reported before by DeColibus et al. (2017), Hamard et al. (2021b), and Le Geay et al. (2024b), but also stable microbial photosynthesis rates across WT regimes (Hamard et al. 2021a) and relatively low microbial photosynthesis in dry mosses (Jassey et al. 2022) have been observed.

ϕPSII reflects the condition of photosystem II – a cell organ crucial in photosynthesis – and it is understood as a reversed stress indicator (Maxwell and Johnson 2000). Thus, the photoautotrophic microbes appeared less stressed in the WLD areas compared to the control areas. This suggests that the light-harvesting cell organs of photoautotrophic microbes function better in these moderately dry and shaded environments compared to wet and open peatlands, supporting earlier research that suggested intracellular adaptation to dry conditions (Perrine et al. 2012). As stated before (Wyatt and Turetsky 2015; DeColibus et al. 2017), nutrient concentration in peat – especially that of phosphorus – was a key driver of microbial photosynthesis. Bryospheric photosynthesis was also higher in the poor fen WLD area compared to the control area, and it benefited from deeper WT directly, whereas the role of Cyanobacteria in regulating moss photosynthesis appeared stronger in the control areas.

Even though this study did not quantify the absolute amount of carbon fixed by microbes, previous research suggests that microbes may contribute to bryospheric carbon fixation remarkably (Hamard et al. 2021a, b). All together, these results suggest that increased microbial photosynthesis may compensate for the predicted carbon losses from drying peat (Hamard et al. 2025), however, as demonstrated here, there is likely spatial variation. In addition, our sampling did not account for the total coverage of mosses, nor their contribution to ecosystem-scale carbon fixation. Kokkonen et al. (2022) have shown that vascular plants are the major component in the carbon-fixing biomass in these study sites, especially in the fens. While they found a similar increase in bryospheric photosynthesis in the poor fen, this was not reflected in the ecosystem-level photosynthesis, which increased the most in the rich fen and was driven by vascular plants.

4.4 Directions for future research

In these studies, the responses of microbial communities and functions to WLD were interpreted by comparing the community assemblages, their functional structure, and measured photosynthetic capacities at one point of time. As it is known that the vegetation and mean WT depth did not differ within each WLD-control pair at the beginning of the experiment (Kokkonen et al. 2019), and the two study areas are situated right next to each other and exposed to similar climatic conditions, it is assumed that the differences observed are mostly caused by the WLD treatment. However, to gain a more detailed understanding of what happens to microbial communities following WLD, it would be beneficial to design experiments in which their microbial succession and functional changes would be followed over time by repeated sampling. Another limitation of this study is the lack of replicates for peatland types. By sampling several replicates of different peatland types, it would be possible to confirm whether the differences observed between the sites are common to all peatlands or more related to local factors. Moreover, the next step should aim for a comprehensive picture of how changes in microbial communities and their functions are reflected in ecosystem-scale processes, such as carbon cycling. This could be achieved by, e.g., combining community and trait analyses with biogeochemical measurements, such as enzyme activity or CO_2 production, and by using molecular methods to consider the entire microbial food web and its functioning (see, e.g., Hultman et al. 2015; Orland et al. 2018; Reczuga et al. 2018). For instance, it would be interesting to compare the observed changes in testate amoebae's functional structure with measured CO_2 production or litter decomposition rates, to quantify the extent to which these observed changes are reflected in decomposition processes. Moreover, while testate amoebae have been shown to be important in regulating the functioning of microbial food webs in peatlands (e.g., Reczuga et al. 2018), we would still benefit of fundamental research aiming to understand, e.g., the feeding habits of different testate amoeba taxa, how they are regulated by functional traits and reflected in the functioning of the rest of the food web.

An interesting discrepancy among the studies within this work was that while microbial photosynthetic capacity benefited from WLD, the share of mixotrophic testate amoebae decreased in both fen WLD areas. This observation suggests that mixotrophic testate amoebae were not major contributors to the microbial photosynthesis. However, this conclusion is in contrast with those of Jassey et al. (2015), who showed experimentally that mixotrophic testate amoebae were a key group among photosynthetic microbes in one peatland. This controversy highlights the need for further research, as photosynthesizing microbes in peatlands remain a novel field. Moreover, as stated before (Hamard et al. 2021a), the fate of carbon fixed by microbes remains unknown – whether it is stored in peat or soon released to the atmosphere determines how effectively photoautotrophic microbes mitigate climate change.

While the drying of peatlands is a likely scenario in many northern areas (e.g., Roulet et al. 1992; Gong et al. 2012; Chaudhary et al. 2017; Swindles et al. 2019; Helbig et al. 2020; Zhang et al. 2022), the vastness of this phenomenon remains uncertain. There is evidence that not only decreased water balance can lead to decreased WTs, but also recent *Sphagnum* expansions or the acceleration of fen-bog transitions in many northern peatlands (Kolari et al. 2022; Magnan et al. 2022; Piilo et al. 2023; Stansfield et al. 2025), could promote the ongoing drop in WTs (Tahvanainen 2011; Kolari et al. 2021). As WT measurements in peatlands are scarce and measurement histories relatively short, testate amoebae are a valuable tool to study these ongoing changes in peatland hydrology. This study shed new light on their responses to moderate but persistent drying in boreal peatlands. This knowledge can be applied in palaeoecological research and peatland monitoring. Combined with other techniques, such as plant macrofossil analysis, vegetation inventories, or remote sensing, it allows us to investigate the past and predict the future of northern peatlands.

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