

Literature review on testate amoebae as environmental indicators and as a functional part of the microbial community in northern peatlands

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SUMMARY

In this review we assess past and current trends in the use of testate amoebae in peatland science, concentrating mainly on studies conducted in northern peatlands. We also discuss the potential of testate amoebae for future research. Testate amoebae are unicellular protists that are covered by protective tests which are easily identifiable and are stored in peat over millennia. Testate amoeba species are specialised to live in a thin water film coating the mosses that creates different microhabitats for species varying in size and shape. They are therefore considered good indicators for hydrology, in particular. In peatlands they represent dominant consumers in the microbial food web, but mixotrophic species also contribute to photosynthesis. Due to their functional role in peatland microbial communities, and as related to challenges in identification and taxonomy, there has been increasing interest towards understanding their functional traits. We suggest that more fundamental research about testate amoeba taxonomy, autecology and functional ecology is needed, but at the same time we can conclude that testate amoebae are a useful tool for contemporary topics in peatland science, including climate-induced changes in peatland functioning and peatland restoration.

KEY WORDS: climate responses, functional ecology, mixotrophy, peatland food webs

INTRODUCTION

Testate amoebae are free-living unicellular protists that thrive in several ecosystems, and they are particularly plentiful in peatlands (e.g., Mitchell *et al.* 2008). As the name suggests, all testate amoebae form a protective shell which is called a test (Charman 2001). The test may be proteinaceous, agglutinated or idiosomic (Charman *et al.* 2000, Tsyganov *et al.* 2016). Proteinaceous tests are completely organic and usually without any covering material. Agglutinated tests are covered with foreign material (xenosomes) such as mineral particles and pieces of diatoms, and idiosomic tests are covered with endogenously synthesised plates composed of silica or calcium (Tsyganov *et al.* 2016). The identification of testate amoeba species is based on the shape, size and ornamentation of the test that are characteristic to the species (Charman 2001, Mitchell *et al.* 2008). However, as testate amoebae reproduce mainly asexually, the definition of a species in the proper sense of the word is challenging. Thus, scientists often speak about morphospecies instead of species (Charman *et al.* 2000).

It is well established that the distribution of testate amoeba taxa within peatlands is controlled mainly by the prevailing hydrological conditions, i.e., by water table depth, which has promoted their use as a

hydrological proxy (e.g., (Harnisch 1927) as cited in Tolonen 1986, Tolonen 1986, Tolonen *et al.* 1994; Table 1). In peat, the tests are archived over millennia, and as peat is deposited *in situ*, it forms a stratigraphic archive from which it is possible to compare past species assemblages in chronological order and to reconstruct past hydrological changes (Charman 2001, Mitchell *et al.* 2008). Hydrological reconstructions have been one of the major research fields since the early 20th century (e.g., (Harnisch 1927, Steinecke 1927) as cited in Tolonen 1986, Warner & Charman 1994, Amesbury *et al.* 2016, 2018). Recently, there has been increasing interest in the role of testate amoebae as an active part of the microbial community (e.g., Fournier *et al.* 2012, 2015; Jasey *et al.* 2015, M. Lamentowicz *et al.* 2020). In peatlands, testate amoebae are the most abundant single group of microorganisms (half of the microbial biomass) (Gilbert *et al.* 1998) and the dominant microbial consumers, which occupy several trophic positions from primary producers to the top of the food web (Gilbert *et al.* 2000, Jasey *et al.* 2012, 2013). Their role in primary production is to act as a host for a photosynthesising, endosymbiotic alga. The ability to participate in photosynthesis in addition to consumption makes them mixotrophic ((Schönborn 1965) as cited in Tolonen 1986, Gilbert *et al.* 2000). Their high

Table 1. Environmental preferences of selected testate amoeba taxa. Nomenclature according to Siemsen (2022).

Species	Synonyms	WT indicator	Other information	References
<i>Alabasta militaris</i>		Dry, wetter in high pH environments	Acid, pH ~ 4.5, stable conditions	Amesbury <i>et al.</i> 2016, Booth 2002, Ł. Lamentowicz <i>et al.</i> 2011, Sullivan & Booth 2011, Zhang <i>et al.</i> 2018a
<i>Amphitrema stenostoma</i>		Wet	<i>Sphagnum</i> -habitats, acid	M. Lamentowicz & Mitchell 2005
<i>Amphitrema wrightianum</i>		Wet	<i>Sphagnum</i> -habitats, acid	Amesbury <i>et al.</i> 2016, Booth 2002, M. Lamentowicz & Mitchell 2005, Swindles <i>et al.</i> 2008, Tolonen <i>et al.</i> 1994
<i>Archerella flavum</i>	<i>Arcella flavum</i>		Acid, stable conditions	Ł. Lamentowicz <i>et al.</i> 2011, Sullivan & Booth 2011
<i>Assulina muscorum</i>		Dry, wetter in high pH environments	Acid, more common in bogs	Amesbury <i>et al.</i> 2016, M. Lamentowicz & Mitchell 2005, Ł. Lamentowicz <i>et al.</i> 2011, Tolonen <i>et al.</i> 1994, Zhang <i>et al.</i> 2018a
<i>Assulina seminulum</i>		Dry, wetter in high pH environments	Acid	Ł. Lamentowicz <i>et al.</i> 2011, Zhang <i>et al.</i> 2018a
<i>Bullinularia indica</i>	<i>Bulinella indica</i>	Dry	Stable conditions	Booth 2002, Sullivan & Booth 2011, Swindles <i>et al.</i> 2008, Zhang <i>et al.</i> 2018a
<i>Centropyxis acuelata</i>		Moderate/ indifferent	Considerable amounts of mineral soil, high pH	M. Lamentowicz & Mitchell 2005, Zhang <i>et al.</i> 2018a
<i>Centropyxis aerophila</i>	<i>Centropyxis cassis</i>	Moderate	Considerable amounts of mineral soil; minerotrophy, optima in eutrophy	Opravilová & Hájek 2006, Zhang <i>et al.</i> 2018a
<i>Centropyxis platystoma</i>	<i>Cyclopyxis platystoma</i>		Unstable conditions	Sullivan & Booth 2011
<i>Centropyxis</i> spp.			Pioneering taxon	Zhang <i>et al.</i> 2018a
<i>Corythron-trinema</i> type		Dry, wetter in high pH environments	Pioneering taxon	Amesbury <i>et al.</i> 2016, Koenig <i>et al.</i> 2018, Swindles <i>et al.</i> 2008, Tolonen <i>et al.</i> 1994, Zhang <i>et al.</i> 2018a
<i>Cryptodiffugia oviformis</i>		Dry		Amesbury <i>et al.</i> 2016; Zhang <i>et al.</i> 2018a
<i>Cyclopyxis arcelloides</i> type		Dry	Considerable amounts of mineral soil	Booth 2002, Zhang <i>et al.</i> 2018a
<i>Diffugia bacillifera</i>		Wet		Swindles <i>et al.</i> 2008
<i>Diffugia globulosa</i> type			Unstable conditions	Sullivan & Booth 2011
<i>Diffugia leidy</i>		Wet	Acid	Amesbury <i>et al.</i> 2016, M. Lamentowicz & Mitchell 2005, Ł. Lamentowicz <i>et al.</i> 2011
<i>Diffugia lucida</i>			High minerotrophy, unstable conditions	Ł. Lamentowicz <i>et al.</i> 2011, Sullivan & Booth 2011
<i>Diffugia oblonga</i> type	<i>Diffugia pyriformis</i>	Wet		Amesbury <i>et al.</i> 2016
<i>Diffugia pulex</i>		Indifferent / mixed results	Unstable conditions	Sullivan & Booth 2011, Zhang <i>et al.</i> 2018a

Species	Synonyms	WT indicator	Other information	References
<i>Diffflugia</i> spp.			Mineral particles / diatoms available, abundant in calcareous environments	Ł. Lamentowicz <i>et al.</i> 2011, Opravilová & Hájek 2006
<i>Euglypha compressa</i>		Dry / indifferent	Considerable amounts of mineral soil	Koenig <i>et al.</i> 2018, Zhang <i>et al.</i> 2018a
<i>Euglypha filifera</i>			Calcium-rich	Ł. Lamentowicz <i>et al.</i> 2011
<i>Euglypha rotunda</i>		Dry / indifferent	Acid, considerable amounts of mineral soil	M. Lamentowicz & Mitchell 2005, Zhang <i>et al.</i> 2018a
<i>Euglypha tuberculata</i>		Dry / indifferent	Acid, considerable amounts of mineral soil	M. Lamentowicz & Mitchell 2005, Zhang <i>et al.</i> 2018a
<i>Galeripora catinus</i>	<i>Arcella catinus</i>	Indifferent		Zhang <i>et al.</i> 2018a
<i>Galeripora discoides</i>	<i>Arcella discoides</i>	Wet	Unstable conditions, acid	Swindles <i>et al.</i> 2008, Sullivan & Booth 2011, M. Lamentowicz & Mitchell 2005
<i>Heleopera rosea</i>			Stable conditions	Sullivan & Booth 2011
<i>Heleopera sphagni</i>	<i>Heleopera picta</i>	Wet	Acid, <i>Sphagnum</i> -habitats	Booth 2002, Ł. Lamentowicz <i>et al.</i> 2011
<i>Heleopera sylvatica</i>		Dry	Acid	Booth 2002, M. Lamentowicz & Mitchell 2005
<i>Hyalosphenia elegans</i>		Wet	Acid	M. Lamentowicz & Mitchell 2005
<i>Hyalosphenia papilio</i>		Moderate / indifferent	Acid	Koenig <i>et al.</i> 2018, Zhang <i>et al.</i> 2018a
<i>Hyalosphenia subflava</i>		Dry		Booth 2002, Swindles <i>et al.</i> 2008
<i>Nebela bohémica</i>		Moderate	High pH	M. Lamentowicz & Mitchell 2005
<i>Nebela</i> spp.			More common in bogs	Zhang <i>et al.</i> 2018a
<i>Nebela tinctoria</i>		Dry	Acid, considerable amounts of mineral soil	Booth 2002, Koenig <i>et al.</i> 2018, Ł. Lamentowicz <i>et al.</i> 2011, Tolonen <i>et al.</i> 1994, Zhang <i>et al.</i> 2018a
<i>Padaungiella lageniformis</i>	<i>Nebela lageniformis</i>		Calcium-rich, high minerotrophy	Ł. Lamentowicz <i>et al.</i> 2011
<i>Paraquadrula irregularis</i>		Moderate; wet	Calcium-rich, high minerotrophy, neutral/ alkaline	Amesbury <i>et al.</i> 2016, Ł. Lamentowicz <i>et al.</i> 2011
<i>Planocarina carinata</i>	<i>Nebela carinata</i>	Wet	<i>Sphagnum</i> -habitats, acid	Amesbury <i>et al.</i> 2016, Booth 2002, M. Lamentowicz & Mitchell 2005
<i>Planocarina marginata</i>	<i>Nebela marginata</i>	Wet	<i>Sphagnum</i> -habitats	Booth 2002
<i>Pseudodiffflugia fulva</i>			Calcium-rich, high minerotrophy, unstable conditions	Ł. Lamentowicz <i>et al.</i> 2011, Sullivan & Booth 2011
<i>Sphenodaria lenta</i>			Pioneering taxon, considerable amounts of mineral soil, minerotrophy, unstable conditions	Sullivan & Booth 2011, Zhang <i>et al.</i> 2018a
<i>Trigonopyxis arcuata</i>		Dry	Associated with <i>Rhododendron tomentosum</i>	Booth 2002, Ł. Lamentowicz <i>et al.</i> 2011, Swindles <i>et al.</i> 2008, Wilkinson & Mitchell 2010

abundance and the multiple roles make testate amoebae important players in several ecosystem functions including carbon and nutrient cycling. The interest towards the functional role of testate amoebae has promoted novel applications and approaches (e.g., Fournier *et al.* 2012, 2015; M. Lamentowicz *et al.* 2015, Zhang *et al.* 2020a, 2020b).

In this review we summarise and discuss past and current trends in using testate amoebae in peatland science, with emphasis on northern peatlands. We reflect on and summarise the latest advances in both the traditional transfer function approach and testate amoeba functional ecology, which has become popular only recently. Finally, we recognise directions for future research.

TESTATE AMOEBEA COMMUNITIES AS HYDROLOGICAL PROXIES IN PEATLANDS

The foundations for testate amoeba research were established in the late 19th and early 20th centuries by pioneers who described several testate amoeba taxa from various environments (e.g., (Leidy 1879, Penard 1891) as cited in Corliss 2001, (Lindberg 1899) as cited in Tolonen 1986, (Lagerheim 1902) as cited in Charman *et al.* 2000). The first testate amoeba based studies in peatlands soon followed, often focusing on population ecology (e.g., (Steinecke 1927, Harnisch 1927, Grospietsch 1953) as cited in Tolonen 1986, Heal 1961, 1964). Early on, scientists had already named hydrology as the main ecological controller of testate amoeba distribution, especially on ombrotrophic peatlands (Tolonen 1986 and references therein). Until today, one of the major research trends around the topic has been the use of testate amoeba records (often referred to as Rhizopod analysis in older literature) as a hydrological proxy (e.g., Tolonen 1966, 1986; Tolonen *et al.* 1994, Warner & Charman 1994, Amesbury *et al.* 2016, 2018). Compared to plants, which show a considerable lag in their response, testate amoebae can be used as a proxy on a fine temporal scale (Väliranta *et al.* 2012) as the life cycle and reproduction of testate amoebae are rapid: populations double in approximately one month (Heal 1964), and hydrological changes have been reported to cause change in testate amoeba community composition within a few months (Koenig *et al.* 2017) or even within weeks (Marcisz *et al.* 2014).

Quantitative peatland water table depth reconstruction using testate amoebae was first introduced by Warner & Charman (1994) who used a

mathematical model (transfer function) to reconstruct the past water table fluctuations based on fossil testate amoeba assemblages. In the transfer function procedure, knowledge of the optimal water table depth for living taxa, i.e., a modern training set, is applied to fossil assemblages. The assumption is that the water table depth preferences have remained similar throughout the timespan covered. The method has since become widely used among peatland scientists. Local and regional transfer functions have been developed for several regions in Europe (e.g., Woodland *et al.* 1998, Payne & Mitchell 2007, Ł. Lamentowicz *et al.* 2008) and North America (e.g., Booth 2002, Lamarre *et al.* 2013), and most recently for permafrost peatlands (Swindles *et al.* 2015, Zhang *et al.* 2017, Taylor *et al.* 2019). Local transfer functions may sometimes lack modern analogies, i.e., not all species found in the fossil samples are present in the training set, or vice versa (Charman & Blundell 2007). Because applying a transfer function from another location may lead to a false reconstruction outcome, it is recommended to use a training set that is as local as possible (Turner *et al.* 2013). However, local transfer functions are not available everywhere, which has promoted the development of continent-wide transfer functions for Europe and North America (Charman & Blundell 2007, Amesbury *et al.* 2016, 2018).

There are certain issues and limitations to be considered when applying the transfer function method. Often inconsistent and sometimes inaccurate testate amoeba taxonomy reduces transfer function performance. However, major wet/dry shifts should be detectable even if the lowest possible taxonomic level is not reached (Payne *et al.* 2011, Mitchell *et al.* 2014, Kosakyan *et al.* 2016). There have been concerns about poor preservation of certain, i.e., idiosomic taxa (e.g., Tolonen 1986), which may cause bias in the reconstructions, as these taxa are rarely found in deeper peat layers but are commonly met near the peatland surface. In most cases, this is thought to have only a minor effect on model performance (Mitchell *et al.* 2007, Swindles *et al.* 2020). However, in some cases the bias may be significant, and therefore Swindles *et al.* (2020) recommend excluding weak idiosomic siliceous tests when a transfer function is applied. Statistical issues recognised in transfer functions include spatial autocorrelation (Telford & Birks 2005), uneven sampling of an environmental gradient (Telford & Birks 2011), and clustered samples (Payne *et al.* 2012). Leave-one-out cross-validation has been a much-used method for model performance testing (e.g., Charman & Blundell 2007), however, a comparison by Amesbury *et al.* (2013) shows that

leave-one-out cross-validation often produces overly optimistic estimates of model performance. Thus, more advanced statistical testing is needed, such as leave-one-site-out (Payne *et al.* 2012), h-block cross-validations (Telford & Birks 2009), and segment-wise root mean square error of predictions (Telford & Birks 2011).

Most transfer functions are developed for ombrotrophic bog conditions, and are thus not directly applicable to fens, which differ from bogs in their water quality (Heal 1961). Aside from water table depth, the distribution of testate amoeba taxa is known to be affected by water chemistry: pH (Heal 1964, Ł. Lamentowicz *et al.* 2007), water-soluble phenols (Jassey *et al.* 2011), sulphates (Ł. Lamentowicz *et al.* 2007), and available minerals (Opravilová & Hájek 2006, Ł. Lamentowicz *et al.* 2011). To date, most research has been carried out on ombrotrophic bogs, whereas fens have received less attention (see, however: Opravilová & Hájek 2006, Payne 2011, Ł. Lamentowicz *et al.* 2011, Zhang *et al.* 2018a). In a recent study, Zhang *et al.* (2018a) concluded that the successional stage of a peatland, which intergrades the different aspects of ecohydrology (i.e., quantity and quality of water), explained most of the variation in testate amoeba communities. Calcium concentration and pH, and consequent differences in the vegetation, seem to explain the largest part of the variation in distribution across eutrophic and mesotrophic fens, whereas water table depth is more important in acidic oligotrophic fens and the main driving factor in ombrotrophic bogs (Charman 2001 and references therein, Opravilová & Hájek 2006, Jassey *et al.* 2014, Zhang *et al.* 2018a; Table 1). Furthermore, the tolerance range of testate amoeba species for water table depth may be different in bogs and fens - so much so that the same taxon has a narrower tolerance range in acidic bogs environments than in more nutrient-rich environments (Zhang *et al.* 2018a). This poses challenges for water table depth reconstructions in fen environments.

Even though testate amoeba based transfer functions are widely used for quantitative water table reconstructions, the common understanding is that this method does not yield the absolute magnitude (cm) of the changes (Turner *et al.* 2013, Swindles *et al.* 2015). Accordingly, the results should be considered as “semi-quantitative”, merely indicating the timing and direction of change (Amesbury *et al.* 2016). However, so far it is the most-used method to gain information about past hydrological conditions on peatlands. As climate change is expected to alter peatland hydrology, which is tightly connected to peatland carbon dynamics (Loisel *et al.* 2021), the

interest in hydrological reconstructions on peatlands remains high, as knowing how the hydrology has changed through different climate phases in the past will allow us to predict the hydrological conditions in the future. Even though the testate amoeba based transfer function method has limitations, it is still a relevant tool for scientists trying to understand how peatlands will react to the ongoing warming. The most reliable results are obtained when several proxies and methods, e.g., testate amoebae and plant macrofossils, are combined (e.g., Väiliranta *et al.* 2012).

INCREASING INTEREST IN FUNCTIONAL ECOLOGY OF THE TESTATE AMOEBAE

Since the beginning of the 21st century, increasing concern about the fate of peatland carbon storage has extended the interest in testate amoebae beyond their use as a proxy, to include their role in peatland functioning and particularly in carbon dynamics. In general, microbes regulate the organic carbon pool in soils via decomposition, carbon fixation and the formation of stable organic matter (Trivedi *et al.* 2013 and references therein). During the past few decades the role of testate amoebae in the microbial community has been clarified, and it seems clear that they play an important role in the microbial food web, occupying a large number of trophic positions. As consumers they prey on microalgae, bacteria, fungi, ciliates, other testate amoebae and rotifers. Their consumption preferences depend on the testate amoeba species and the availability of prey (Gilbert *et al.* 2000, Jassey *et al.* 2012, 2013). Also, the season seems to play a role. For example, during a relatively dry summer period when a thinner water film may reduce the mobility of the prey species, testate amoebae consumed more ciliates and rotifers than in spring and autumn (Gilbert *et al.* 2003). Mixotrophic testate amoebae adjust their strategy according to prevailing conditions; they prefer heterotrophic energy sources whenever those are highly available, whereas when such prey is scarce, endosymbionts become their primary energy source and give them a competitive advantage (Jassey *et al.* 2013). This is an interesting finding in the global scale because phototrophic microbes, including mixotrophic testate amoebae, contribute 10–30 % of the total carbon fixation in peatlands (Hamard *et al.* 2021).

While it is known that testate amoebae which consume bacteria and other small soil microbes affect the composition of the whole microbial community and, therefore, the carbon and nutrient cycling through decomposition of organic matter (Bonkowski 2004, Van der Heijden *et al.* 2008),

quantitative estimations focusing solely on them remain scarce (Wilkinson & Mitchell 2010, Reczuga *et al.* 2018). It has been shown that testate amoebae contribute up to 7–13 % of total carbon and nitrogen mineralisation in upland forest soils (Schröter *et al.* 2003) but, to date, such estimations have not been presented for peatlands. In addition, testate amoebae contribute to the soil silica cycle by synthesising biogenic silica for test construction material (= biosilification). The rate of biosilification by testate amoebae in forest ecosystems is comparable to, and sometimes exceeds, that of trees (Aoki *et al.* 2007, Puppe 2020, and references therein). Just recently, biosilification of 13–97 ng of Si per 150 tests has been reported in peatlands (Qin *et al.* 2022) and, based on the estimated testate amoeba abundances (Heal 1964) and generation rates (Foissner 1999), that implies annual biosilification of 0.56–58.2 kg ha⁻¹. This range is wide but comparable to the results reported from forest ecosystems; e.g., Puppe *et al.* (2015) reported a testate amoeba biosilification rate of 0.5–80 kg ha⁻¹ yr⁻¹ in a temperate forest. Interestingly, biosilification rates were higher on peatlands that were exposed to strong human influence, i.e., to land-use changes and drainage (Qin *et al.* 2022).

One of the main methods for studying the functional role of testate amoebae is the functional trait approach, which provides an additional proxy for both environmental conditions and ecosystem functioning (Fournier *et al.* 2015). Functional traits have been defined as “any morphological, physiological or phenological feature measurable at the individual level” (Violle *et al.* 2007), and even more precisely by Kearney *et al.* (2021) as “properties of individual organisms that have a connection to organismal performance in terms of survival, development, growth, and reproduction”. Functional traits are further classified into two sub-categories: response traits and effect traits. Response traits reflect the response of a species to environmental change, and effect traits show the effect of a species on ecosystem functions (Fournier *et al.* 2015). This separation highlights the dual role of the traits, which provide information on the prevailing environmental conditions as well as on how species may affect the ecosystem functions.

Traits are seen as a solution to overcome some of the issues related to taxonomy-based approaches, as certain traits exhibit a similar response to environmental conditions regardless of the species or the peatland type, which means that the approach is independent of the possible taxonomic bias and equally applicable to bogs and fens (Marcisz *et al.* 2020). For investigation of the effects of current

climate change on peatland carbon cycling, testate amoeba functional traits may not only provide key information regarding the microbial carbon cycle, but also document the changes in moisture regimes and thus the functioning of the whole ecosystem. Recently, the first functional trait based transfer function for water table reconstruction was developed, and the results were similar to those obtained using a species based transfer function (Van Bellen *et al.* 2017). The most relevant functional traits of testate amoebae are described in the following sub-sections.

Test size

Test length and width are measured manually under a light microscope and the test biovolume can be calculated based on those measurements, using a different formula for each test shape (Figure 1; Fournier *et al.* 2015). A small size increases the survival potential in a thin film of water, whereas large taxa thrive in wet conditions (Tolonen 1986, Koenig *et al.* 2017, Koenig *et al.* 2018, Marcisz *et al.* 2020). Thus, a shift from the dominance of large taxa to that of small taxa is generally considered a drying signal (Fournier *et al.* 2015, Marcisz *et al.* 2016, Koenig *et al.* 2018, Marcisz *et al.* 2020). However, small taxa do not necessarily disappear during wet



Figure 1. Test length, test width and aperture size in *Hyalosphenia elegans*.

phases - actually, several small taxa, e.g., *Diffflugia pulex* and *Pseudodiffflugia fulva*, seem to thrive in highly variable hydrological environments, and it has been suggested that these taxa are indicative of a strongly fluctuating water table (Sullivan & Booth 2011). McKeown *et al.* (2019) report contrasting results from New Zealand, associating small taxa with wet locations. This may indicate that local geography and biotic interactions affect the responses of small testate amoebae. Moreover, small taxa often dominate forested sites (Payne *et al.* 2016, Creevy *et al.* 2018, M. Lamentowicz *et al.* 2020), and a decrease in large taxa as a response to hard frost events has also been reported (Jassey *et al.* 2016).

Test compression

Testate amoebae are often classified according to test compression on a semi-continuous scale from compressed to hemispherical, sub-spheric and spheric; compressed referring to tests with height less than $\frac{1}{4}$ of the width (Fournier *et al.* 2015, Marcisz *et al.* 2020). Compressed test increases fitness in a thin water film and thus is more common in dry conditions (Fournier *et al.* 2015, Koenig *et al.* 2017, Krashevskaya *et al.* 2020).

Aperture size and position

Aperture size correlates with prey type. Testate amoebae with small apertures feed mainly on bacteria, fungi, algae and small protists, whereas those with larger apertures prefer larger prey and act as top consumers (Jassey *et al.* 2012, 2013). Changes in aperture size may be used to interpret changes in functioning of the microbial food web; the presence of top consumers is considered to be a sign of a complex food web (Jassey *et al.* 2013, Marcisz *et al.* 2020).

Both aperture size and position have also been associated with hydrological changes. Smaller apertures that are hidden or ventral seem to be beneficial in dry conditions (M. Lamentowicz *et al.* 2015, Koenig *et al.* 2017, Van Bellen *et al.* 2017), whereas terminal apertures are more common in wet environments (Marcisz *et al.* 2016). Furthermore, small or hidden apertures are thought to be an adaptation to disturbed conditions, as they have been observed to become more common after fires, peat extraction, and atmospheric deposition of dust and pollution (Fiałkiewicz-Kozieł *et al.* 2015, Marcisz *et al.* 2016).

Mixotrophy

The proportion of mixotrophs in the testate amoeba community can be estimated using a microscope. Mixotrophy can be detected directly from living

testate amoeba communities as the green algae are visible inside the test, and the relative abundance of mixotrophic species can be used as an estimation for palaeocommunities.

The relative abundance of mixotrophs indicates the role of testate amoebae in the functioning of the microbial community; a higher proportion of mixotrophs indicates a higher carbon fixation potential. The biomass of mixotrophic testate amoebae has been shown to decrease in response to experimental warming, and this led to decreased bryosphere carbon fixation rates (Jassey *et al.* 2015). The loss of mixotrophy has also been associated with drought and disturbances such as fire (Marcisz *et al.* 2016, Koenig *et al.* 2017, Marcisz *et al.* 2020, Zhang *et al.* 2020b). Mixotrophic testate amoebae have been reported to be abundant in open peatlands, whereas they are nearly absent from forested sites (Payne *et al.* 2016, Creevy *et al.* 2018, M. Lamentowicz *et al.* 2020). Shading has been suggested as one explanation but, as hydrology often changes along an “openness” gradient, it remains uncertain which of these is the main driver of the observed pattern. Although more research is needed before the fundamental driving factor can be confirmed, it seems clear that mixotrophic taxa decrease with increasing tree cover. This knowledge may be useful in interpreting palaeorecords.

Test construction

Test construction can be determined visually under a microscope. Test material has been associated with both hydrology and atmospheric dust deposition. Koenig *et al.* (2017, 2018) have shown that, as a response to water table drawdown, both agglutinated and idiosomic tests (tests covered by mineral particles or plates) increase at the cost of proteinaceous tests. Similarly, proteinaceous tests are more common in open peatlands - which also tend to be wetter - whereas the share of idiosomic and agglutinated tests increases with forest cover (M. Lamentowicz *et al.* 2020). However, Van Bellen *et al.* (2017) report a strong positive correlation between mineral presence in the tests and water table level, i.e., they found more agglutinated tests in wetter hollows compared to dry hummocks. They suggest that this may be due to the distribution of dust deposition, which varies within a site so that either wind or water flow leads to a build-up of dust in hollows. As the results are not unequivocal, current knowledge is insufficient to support strong conclusions about the relationship between test construction and hydrology.

Fiałkiewicz-Kozieł *et al.* (2015) studied the response of testate amoebae to atmospheric pollution

and found an increase in agglutinated taxa after atmospheric deposition, and were even able to identify anthropogenically generated particles from the tests. Thus, test construction may be a potential tool for estimating the anthropogenic impact on ecosystems.

FUTURE DIRECTIONS IN TESTATE AMOEBA RESEARCH IN PEATLANDS

Taxonomy and ecology

Despite the development of novel tools independent of taxonomy, accurate taxonomy and ecological knowledge remain the basis for much testate amoeba research (Payne *et al.* 2011). Testate amoeba taxonomy is based mostly on morphology, and there has been confusion as to which features are sufficiently distinctive to define a species, especially as there is also intraspecific variation caused by different environmental conditions and, on the other hand, certain taxa lack visible differences (Charman *et al.* 2000, Kosakyan *et al.* 2016). Recent molecular studies, i.e., DNA barcoding, have shown that morphology-based identification often fails to capture some of the diversity among testate amoebae (Kosakyan *et al.* 2012, Singer *et al.* 2015) and the taxonomy has been revised based on novel information about the phylogenetic position of certain species - e.g., former *Nebela militaris* has recently been placed in a new genus *Alabasta* (Duckert *et al.* 2018), and the *Nebela collaris* complex was split into eight species based on genetic differences even though only three are commonly identified based on morphology (Singer *et al.* 2015). Because of this rapid development in the taxonomy, the use of nomenclature among scientists is sometimes inconsistent (Kosakyan *et al.* 2016). Identification of small taxa is often a particular challenge, which is one reason why they are often grouped into morphotypes. However, it has been shown that the identification of small taxa to the lowest possible taxonomic level increases the reliability of hydrological reconstructions, which highlights the need for taxonomic work that concentrates specifically on small taxa (McKeown *et al.* 2019). Kosakyan *et al.* (2016) express serious concerns about the future of the field, as taxonomy - which represents the basic research - is generally poorly funded and, consequently, a new generation of scientists no longer specialise in this research field.

Testate amoeba autecology has a long research tradition (e.g., Tolonen 1986, Tolonen *et al.* 1992, Charman & Warner 1997, M. Lamentowicz & Mitchell 2005, Ł. Lamentowicz *et al.* 2007). In spite of that, the ecological preferences of all relevant taxa,

especially many small species, are not well established (McKeown *et al.* 2019). Particular attention should be given to wetlands other than ombrotrophic bogs, which have received most of the attention until recently (see, however, Opravilová & Hájek 2006, Ł. Lamentowicz *et al.* 2011, Payne 2011, Zhang *et al.* 2018a).

Functional ecology of testate amoebae

Despite the evident importance of testate amoebae for the soil carbon, nutrient and silica cycles (Bonkowski 2004, Aoki *et al.* 2007, Van der Heijden *et al.* 2008, Hamard *et al.* 2021), their exact contribution is still not comprehensively understood, particularly in peatlands. Moreover, the interactions between soil microbes and plants are complex, and plant-microbe links seem to respond to changing conditions more rapidly than the structures of the respective communities (Robroek *et al.* 2021). As stated by Robroek *et al.* (2021), these interactions should be more carefully studied in peatland environments to assess potential feedbacks between the peatland vegetation and microbial communities.

While some of the functional traits described above are already widely used in assessing both the responses of testate amoeba communities to environmental changes and the functionality of the community, there are functional traits that are less well understood but worthy of further investigation as potential environmental indicators. These traits include test shape, test construction and lobose-filose index. Test shape has been shown to vary in lake environments according to nutrient gradient and oxygen availability (Macumber *et al.* 2020). To the best of our knowledge, well-established trends for test shape variation in relation to environmental gradients in peatlands do not exist. However, Krashevskaya *et al.* (2020) found oviformed/elongated tests to be typical of dry microhabitats in a peatland, which implies that test shape is a potential additional proxy for peatland hydrology. Test construction responds to several environmental variables, such as hydrology and atmospheric dust deposition, and it may also affect the soil silica cycle (Aoki *et al.* 2007, Puppe 2020). Test construction appears to vary according to hydrological conditions, although the results are contradictory. The proportion of both agglutinated and idiosomic tests has been shown to increase as a response to drought (Koenig *et al.* 2017, 2018), but agglutinated tests have also been shown to be more abundant in wetter microhabitats (Van Bellen *et al.* 2017). In addition to hydrology, high mineral matter input increases the proportion of agglutinated and proposedly also idiosomic taxa, as it ensures the presence of mineral particles and silica



that are used to produce these types of tests. Proteinaceous tests, in turn, are most abundant in *Sphagnum*-dominated environments with low mineral matter input (Fiałkiewicz-Kozieł *et al.* 2015, Van Bellen *et al.* 2017, Marcisz *et al.* 2021). The lobose/filose index, which refers to the relative proportion of lobose (e.g., *Arcella*, *Nebela*, *Centropyxis*, *Diffugia*) and filose (e.g., *Euglypha*, *Corythron*, *Trinema*) testate amoebae, has been suggested as a tool to describe the successional stage of testate amoeba communities. The index is based on the assumption that filose testate amoebae are r-strategists with shorter generation times than lobose amoebae, which have the longer generation times typical for k-strategists (Bonnet 1976). However, as stated by Marcisz *et al.* (2020), this has not been reliably confirmed, and more research on the life strategies of testate amoebae is needed. If better understood, information on life strategies could be used to interpret ecosystem stability.

Environmental monitoring

Vast peatland areas have been affected by land-use change either in the surrounding catchment or through direct conversion into agricultural land, forest stands or cutover peatland, as well as by atmospheric deposition of pollutants or dust. This creates a need for peatland restoration and conservation which, in turn, has created a need for tools to evaluate the ecological status of a peatland. Testate amoebae show great potential as a biomonitoring tool (e.g., Koenig *et al.* 2015). Their communities respond readily to environmental variability, even on a short time scale (Sullivan & Booth 2011, Väiliranta *et al.* 2012), which allows their use as early warning signals.

Koenig *et al.* (2015) concluded that testate amoebae are more accurate indicators of local microtopography, water table depth and *Sphagnum* C:N ratio than plants, which are currently the most frequently used bioindicators. The potential of testate amoebae has been recognised, for instance, in monitoring peatland restoration success (Swindles *et al.* 2016, Creevy *et al.* 2018), identifying atmospheric pollution (Fiałkiewicz-Kozieł *et al.* 2015), and the evaluation of human impacts on peatlands (Carballeira & Pontevedra-Pombal 2021). High species diversity and wide abundance of mixotrophic taxa are considered as signals of low human impact or successful restoration (Swindles *et al.* 2016, Creevy *et al.* 2018, Carballeira & Pontevedra-Pombal 2021). Successful restoration is also implied by the reappearance of wet indicators (Swindles *et al.* 2016). A shift to small and hidden apertures has been associated with post-disturbance testate amoeba

communities (Marcisz *et al.* 2016). While Swindles *et al.* (2016) note that there is a need for caution when interpreting the changes in testate amoeba communities because they are affected by several factors, Koenig *et al.* (2015) are more optimistic. They list nine easily identifiable testate amoebae species (i.e., *Alabasta militaris*, *Archerella flavum*, *Amphitrema wrightianum*, *Assulina muscorum*, *Assulina seminulum*, *Heleopera rosea*, *Hyalosphenia elegans*, *Hyalosphenia papilio* and *Nebela tinctoria*) that could provide sufficient information for biomonitoring purposes, making testate analysis a more commonly available tool.

Peatland climate interactions

Multiple interacting changes occur simultaneously in both climate and peatlands, and peatland climate response patterns remain uncertain (Loisel *et al.* 2021). Assessing climate-induced changes in peatland hydrology and carbon cycle is currently one of the most widely used applications of testate amoebae: changes in both the community structure and functional traits are used to interpret peatland climate responses and possible feedback mechanisms (e.g., Jassey *et al.* 2015, Zhang *et al.* 2020a). Climate warming is expected to lead to drying in boreal and temperate ecosystems due to increased evapotranspiration (Helbig *et al.* 2020), and drying signals have already been observed in European peatlands (Swindles *et al.* 2019). Drying may trigger changes in vegetation, such as tree encroachment (Kokkonen *et al.* 2019), and in carbon dynamics (e.g., Laine *et al.* 2019). In subarctic and arctic peatlands, permafrost thaw is projected to cause increasing greenhouse gas emissions as a result of active layer deepening and formation of thermokarst lakes and fens (Hugelius *et al.* 2020). As discussed above, testate amoeba species composition and functional traits can be used as a hydrological proxy, which enables the quantification of recent hydrological changes in peatlands. Predictions of the effects of ongoing climate change can be assessed by studying the recent changes and current testate communities but also by searching for analogies from the past. This highlights the need for further development and application of the transfer function approach. Northern peatlands offer archives that cover a millennia-long (up to post-glacial) deposition history and enable reconstructions of past peatland hydrology and associated climate links, as well as testate amoeba community structure and functioning (Zhang *et al.* 2018b, M. Lamentowicz *et al.* 2019).

The importance of testate amoebae in peatland carbon cycling has been recognised only recently (e.g., Jassey *et al.* 2015, Hamard *et al.* 2021) and is

not yet fully understood, which is why there is a clear need for further research. For instance, how much of the carbon stored in peatlands was originally fixed by microbes remains unknown (Hamard *et al.* 2021). Expected changes in climatic conditions, hydrology and vegetation are likely to affect the functioning of microbial communities (Peltoniemi *et al.* 2015), but there are still many open questions related to both the environmental changes and the functioning of peatland microbial communities, and these call for more research before any predictions can be made. A decrease in the overall microbial biomass as a response to warming has been observed in northern fens (Peltoniemi *et al.* 2015), whereas drought reduces testate amoebae biomass but not bacterial or fungal biomass (Reczuga *et al.* 2018). This is likely to alter the resistance of organic matter. If peatland surface drying were to become a truly widespread phenomenon, a shift to large-scale dominance of smaller testate amoeba taxa with smaller apertures seems possible (Fournier *et al.* 2015, Koenig *et al.* 2017, 2018; Marcisz *et al.* 2020). The disappearance of large testate amoebae may have cascading effects on lower trophic levels, thereby affecting the functioning of the microbial food web and, consequently, carbon and nutrient cycling in peatlands (Reczuga *et al.* 2018). In addition, both warm and dry conditions are known to favour heterotrophy at the cost of mixotrophy (Wilken *et al.* 2013, Jassey *et al.* 2015, Koenig *et al.* 2018), and possible tree encroachment might increase shading, which has been associated with the loss of mixotrophic microbes as well (Payne *et al.* 2016, M. Lamentowicz *et al.* 2020). Also, the effect of other vegetation changes, such as increasing shrub cover, on mixotrophic testate amoebae is worthy of further investigation. As microbial photosynthesis is known to be responsible for approximately 10 % of the total carbon fixation in a peatland, the loss of mixotrophic testate amoebae could reduce the climate mitigation potential of peatlands (Jassey *et al.* 2015, Hamard *et al.* 2021). Both the basic functioning of soil microbes in the peatland carbon cycle and the changes they face should not be ignored when the aim is to predict the future of the peatland carbon store.

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AUTHOR CONTRIBUTIONS

OK-R led the writing process with contributions from the others.

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