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Emmanuela Daza Secco

Assessing Ecological Effects of Peatland Use with Testate Amoebae



UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
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ABSTRACT

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Due to their ability to store carbon in the form of peat, peatlands play a key role in the carbon cycle. Besides carbon accumulation, peatlands release dissolved organic matter to surface waters in their catchments, emit methane, and nitrous oxide. Hence, they can strongly affect the global climate. In addition, peatlands provide habitat for wildlife, help in water regulation, and store valuable palaeo-environmental information. As most ecosystems, peatlands are affected by anthropogenic activities such as mining, peat extraction, forestry and agriculture. Due to the increasing peatland degradation, monitoring methods have been developed in order to evaluate their ecological state. Here, the usefulness of testate amoebae (TA) as biological indicators of peatland disturbance and restoration actions success, their use as surrogates of plant community responses to peatland restoration, as well as indicators of disturbances caused by peat extraction in surrounding lakes was assessed. To better understand the response of TA to human-induced changes in peatlands, environmental variables broadly known to affect TA such as temperature, water chemistry, and water table depth were also analysed. TA appeared to be an excellent tool to evaluate disturbance in Finnish peatlands as their responses to human-induced changes overrode natural variation caused by spatiality and seasonality. On the other hand, TA showed to respond faster to peatland restoration actions than plant communities. Also, lake TA communities from sediments did not reflect effects of peat extraction in lakes. Overall, the study showed promising results when considering terrestrial TA as indicators of peatland land use changes, including the evaluation of restoration success. However, more studies are required to evaluate the usefulness of TA lake sediment communities as indicators of the effects of peat extraction on receiving lakes. The results also highlighted the need for future research on TA combining molecular tools and morphological analysis to fully understand their potential use as indicators of peatland changes and to further comprehend their complexity and biodiversity.

Keywords: Bioindicators, boreal catchments, community concordance, peatland hydrology, restoration, seasonal variation, spatial variation.

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TIIVISTELMÄ

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Soiden käytön ekologisten vaikutusten arvioiminen kuoriamebojen avulla

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Koska suot sitovat hiiltä turpeeseen, niillä on tärkeä merkitys hiilen kierrossa. Toisaalta soilta vapautuu liuennutta orgaanista ainetta vesistöihin. Suot tuottavat myös metaania ja typpioksiduulia, joten ne voivat vaikuttaa merkittävästi ilmastoon. Lisäksi suot ovat rikkaan eliöstön elinympäristöjä, säätelevät veden kiertokulkua ja varastoivat paleolimnologista tietoa. Kuten useimpiin ekosysteemeihin myös soihin vaikuttaa ihmisen toiminta, mm. kaivosteollisuus, turvetuotanto sekä metsä- ja maatalous. Soiden ekologinen tila on jatkuvasti huonontunut, ja tilan seurantaan on kehitetty erilaisia menetelmiä. Tässä tutkimuksessa arvioitiin kuoriamebojen käyttökelpoisuutta soiden käytön ja ennallistamistoimien vaikutusten bioindikaattoreina sekä soveltuvuutta soiden kasviyhteisöjen vasteiden ja turvetuotannon alapuolisissa järvissä aiheuttamien muutosten kuvaamiseen. Lisäksi tutkittiin kuoriameboihin vaikuttavia ympäristötekijöitä, kuten lämpötilaa, veden kemiallisia ominaisuuksia ja suoveden tasoa. Kuoriamebat osoittautuivat erinomaiseksi mittariksi arvioitaessa häiriöiden vaikutuksia suomalaisilla soilla, koska niiden vasteet ihmistoimintaan ylittivät luonnollisesta ajallisesta ja paikallisesta vaihtelusta johtuvat vasteet. Toisaalta kuoriamebat reagoivat ennallistamistoimiin kasviyhteisöjä nopeammin. Sen sijaan järvisedimenttien kuoriamebayhteisöt eivät ilmentäneet turvetuotannon vaikutuksia alapuolisiin järviin. Kaiken kaikkiaan tutkimus osoitti kuoriamebojen käyttökelpoisuuden soiden käytön muutosten indikaattoreina, mukaan lukien ennallistamisen onnistumisen arvioiminen. Lisää tutkimuksia kuitenkin tarvitaan järvisedimenttien kuoriamebayhteisöjen soveltuvuudesta turvetuotannon vaikutusten arvioimiseen alapuolisissa järvissä. Tulokset korostavat myös tarvetta yhdistää molekulaarisia ja morfologisia tutkimusmenetelmiä, jotta kuoriamebayhteisöjen potentiaali suoluonnon muutosten indikaattorina sekä yhteisöjen monimuotoisuus ja kompleksisuus voidaan kokonaisuudessaan ymmärtää.

Avainsanat: Ajallinen vaihtelu; bioindikaattorit; boreaalinen valuma-alue; ennallistaminen; paikallinen vaihtelu; soiden hydrologia; yhteisöjen konkordanssi.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–III.

- I Daza Secco E., Haapalehto T., Haimi J., Meissner K. & Tahvanainen T. 2016. Do testate amoebae communities recover in concordance with vegetation after restoration of drained peatlands? *Mires and Peat* 18 (12):1–14.
- II Daza Secco E., Haimi, J., Högmander H., Taskinen S., Niku, J. & Meissner K. 2018. Testate amoebae community analysis as a tool to assess biological impacts of peatland use. *Wetlands Ecology and Management* 26: 597–611.
- III Daza Secco E., Haimi J., Vähäkuopus T., Ojala A., Valpola S. & Meissner K. 2019. Testate amoebae as a potential tracer of organic matter dislodged from peat extraction areas. Submitted manuscript.

Contributions to the original papers by the authors are presented in Table 1. Other contributions are stated in the acknowledgments of the papers.

TABLE 1 Author contributions to original articles. EDS= Emmanuela Daza Secco, KM= Kristian Meissner, JH= Jari Haimi, HH= Harri Högmander, ST= Sara Taskinen, JN= Jenni Niku, TV= Tuija Vähäkuopus, AO= Antti Ojala, SV= Samu Valpola, TH= Tuomas Haapalehto, TT= Teemu Tahvanainen.

	I	II	III
Original Idea	EDS, KM, TT	KM, HH	KM, SV
Data	EDS, TH, TT	EDS	EDS, AO,TV
Analyses	EDS, KM	EDS, HH, ST,JN	EDS, KM, AO,TV
Writing	EDS, KM, JH	EDS, KM, JH	EDS, KM, JH, OA, TV

1 INTRODUCTION

1.1 Peatland management, monitoring and assessment

1.1.1 Peatland land uses

Peatlands are key ecosystems in the northern latitudes as they cover large areas and play a fundamental role in the carbon cycle due to their high accumulation rate of organic matter in the form of peat (Clymio 1984). Besides their role in carbon accumulation, peatlands are key ecosystems as they provide habitat for important wildlife, help in water regulation, recreation, and are important archives of palaeo-environmental information (Bonn et al. 2004). In general, pristine peatlands consist of two layers: an upper aerobic layer (acrotelm) where the decomposition rate is relatively high, and a lower anaerobic layer (catotelm) where the decomposition rate is much lower (Clymio 1984). During the Holocene the climate has facilitated the long-term peat accumulation that maintains an important carbon sink in the northern hemisphere. Recent calculations of carbon stored in peatlands above the 45°N latitude estimate this stock to be 436 Gt (Loisel *et al.* 2014). Besides carbon accumulation, peatlands release dissolved organic matter (DOM) to surface waters in their catchments (Tranvik and Jansson 2002), and emit methane (CH₄) and nitrous oxide (N₂O), substances, that strongly affect global climate (Gong *et al.* 2013). The mentioned functions are mainly controlled by different processes at large scales such as nutrient dynamics, evapotranspiration, and water table level (Waddington *et al.* 2010).

As most ecosystems nowadays, peatlands are affected by direct and indirect anthropogenic land use activities (e.g. peat harvesting, forestry, grazing, farming, etc.), as well as soil and air pollution (Mikkuta and Rothwell 2016). In Finland, about 30 % of the total surface area is classified as peatlands (Tomppo 2000). In Southern Finland, bogs are the dominating peatland type, while fens are found mainly in the north. Bogs are characterized by dwarf shrub dominated vegetation and ombrotrophic *Sphagnum* mosses and a high production of decomposition-resistant litter. Fens are more nutrient rich, dominated by sedge vegetation and minerotrophic *Sphagnum* species with higher decomposition rates (Straková *et al.*

2011). Worldwide, Finland is the biggest peat producer, and in 2013 ca. 4 % of the total national energy was produced by burning peat, mainly for district heating (Anon. 2013). Peatland use is mainly concentrated to Finland's central and southern raised-bog region, west coast, and north-ostrobothnian area, where the peat accumulation is more efficient and hence, most disturbed peatland ecosystems are located in these regions (Tomppo 2000, Turunen *et al.* 2002).

Peatland draining strongly increases aerobic decomposition of the uppermost peat layer by disturbing the acrotelm-catotelm stratification (Niedermeier and Robinson 2007). Also, it negatively affects rivers by causing eutrophication and increased transport of suspended solids which in turn, causes siltation and changes in stream bed conditions. Draining also elevates concentrations of nitrogen and phosphorus by increased organic matter decomposition rates, and decreases concentrations of potassium, calcium, and magnesium in the surface peat (Sundström *et al.* 2000). Additionally, the resulting change in vegetation from slowly decomposed *Sphagnum* to easily decaying vascular plants increases the decomposition rate and nitrogen mineralization which in turn reduces peat formation and the carbon storage capacity (Berendse *et al.* 2001).

In Finland, the high percentage of land covered by peatlands causes inland waters to naturally receive high amounts of organic matter. This release of materials from peatlands into surface waters plays an important role in nutrient cycling, photochemical processes, and aquatic communities (Stevenson 1994, Klavins *et al.* 2012). Peatland drainage has been found to increase the input of allochthonous organic matter into receiving waters, which in turn causes shifts in lake nutrient dynamics (Klöve 2001) affecting the whole food web and ecosystem functioning. In most parts of the world the current use of peatlands and their status is not sustainable. Peatland use results in the release of stored carbon, and the decrease in biodiversity and ecosystem functions (Parish *et al.* 2008). Peatland degradation directly affects millions of people around the globe e.g. by promoting floods and water shortages, degrading soils, and causing wildfires (Parish *et al.* 2008, Glina *et al.* 2017). Due to the aforementioned important characteristics of peatlands, and the large range of consequences following their drainage, projects around peatland conservation and restoration are growing in both scope and scale (Parry *et al.* 2014). Ecological restoration broadly targets to decrease the negative impacts of land degradation (Hobbs and Cramer 2008) and hence, can potentially reverse its effects, and reinstate important ecosystem services (Wortley *et al.* 2013).

1.1.2 Peatland restoration

In general, peatland restoration projects aim to totally, or at least partially, restore the most important ecosystem services and the biodiversity (Lunn and Burlton 2013). These restoration goals are usually pursued by blocking ditches, altering the surface microtopography to reinstate surface water retention (Aapala *et al.* 2009), and by rewetting the degraded areas (Gorham and Rochefort 2003). In bog restoration, often trees are also removed mainly to increase water table (Vasander

et al. 2003) in order to restore the *Sphagnum* carpet, the original vegetation, and to reinstate the peat accumulation process (Price *et al.* 1998). However, due to habitat degradation, specialized species typically found in peatlands are becoming rare (Haapalehto *et al.* 2011). Additionally, *Sphagnum* recolonization may be difficult as bare peat surfaces can undergo cracking and crust formation processes, limiting the establishment of plant propagules (Salonen 1987). According to Price and Whitehead (2001), *Sphagnum* has naturally recolonized areas only under certain specific hydrologic conditions; i.e. when soil moisture is higher than 50 %, soil-water pressure is below 100 cm, and the average water table is -24.9 cm (SD = ± 14.3 cm). However, the use of mulches has been proposed to help the recovery of the vegetation cover (Wheeler and Shaw 1995) as it has been shown to lower evaporation (Hares and Novak 1992) improving in turn, soil moisture and temperature conditions. In the long term, peatland restoration aims at: i) reinstating the biogeochemical cycles, hydrology, productivity and decomposition rates to allow peat accumulation, ii) reconstructing food webs that resemble the trophic structure of the ones prior to the disturbance, and iii) the full re-establishment of biodiversity at its different levels such as species, communities, ecosystems and landscapes (Gorham and Rochefort 2003).

1.1.3 Evaluation of restoration success

Restoration is a relatively novel practice, but already widely introduced into natural resources sustainable management strategies with high expectations: by 2020 15 % of the degraded ecosystems should be restored (Anon. 2010). However, restored ecosystems often bear a lower value of both biodiversity and ecosystem services compared to the targeted non-impacted ecosystems (Benayas *et al.* 2009, Wortley *et al.* 2013). The degree to which the current restoration methods work to re-establish carbon storing (e.g. Moreno-Mateos *et al.* 2012, Strack and Zuback 2013) and how the recovery of vegetation relates to restoration in boreal peatlands is still understudied (Haapalehto 2014). In general, it is suggested that the recovery of the ecosystem functions follows the re-establishment of the structure of biological communities (Moreno-Mateos *et al.* 2012, Kareksela *et al.* 2015). In this sense, functional restoration of peatland ecosystems must be based on a scientific understanding of the core feedback mechanisms and interactions between biological communities, climate and hydrology (Rochefort & Andersen 2017).

The evaluation of restoration success is neither an easy nor a straightforward task (Wortley *et al.* 2013). Monitoring and assessing the ecological state is a fundamental part of peatland conservation and restoration programmes (Trepel 2007) and has been used in the evaluation of restoration success. For example, peatland assessments have provided evidence that relatively simple changes in peatland management are able to limit or even reverse many negative, peatland use induced impacts (Parish *et al.* 2008). However, once severely disturbed, certain types of peatlands will be difficult or even impossible to restore to their original state (Gorham and Rochefort 2003).

An important part of the basic information about the release of organic carbon, vegetation changes, and transformation of physical properties of the soil can be obtained with the study of peatland characteristics following restoration processes (Grand-Clement *et al.* 2015).

In general, a positive relationship between the recovery of ecosystem structure and its function is expected (Dobson *et al.* 1997). Shantz and Prize (2006) for example, found an 88 % decrease in runoff and a higher seasonal mean water table after three years of restoration which in turn, facilitated *Sphagnum* recolonization. Additionally, pore water chemistry has been found to change after peatland restoration measures have been implemented, where the responses vary according to the intensity of the re-wetting processes, the peatland class and trophic level (vegetation gradients), the initial water table level, and the soil and air temperature (Menberu *et al.* 2017).

However, in practice often no significant relationships or even negative correlations have been observed (Cortina *et al.* 2006). Further, the relationships between recovery of ecosystem structure and its function may change over time. In peatlands, restoration involving rewetting has proven to be a good strategy to maintain ecosystem functioning in terms of surface peat accumulation rate, despite a delayed recovery of ecosystem structure (Haapalehto 2014). Hence, the selection of an appropriate monitoring method to evaluate peatland restoration success must primarily take into account the restoration goals.

Most studies have focused on the consequences of restoring peatlands previously used for agriculture (e.g. Schimelpfenig *et al.* 2014) or forestry (e.g. Haapalehto *et al.* 2011, Nicia *et al.* 2017). However, in most cases, data from monitoring and assessment prior to ecosystem disturbance are very scarce or even totally missing. In this sense, the restoration goals become generalized to reinstate the ecosystem to the “type” to which it originally belonged (Gorham and Rochefort 2003). Large-scale restoration projects rarely last longer than 2–5 years mainly due to funding. Therefore, different approaches such as bioindication and/or palaeoecology can be used to detect changes that might not be observable using only biochemical or hydrological data (Swindles *et al.* 2018). Palaeoecology has greatly advanced during the past decades, and its advances can be applied to cost-effectively evaluate restoration success and to assess and monitor ecosystem conditions (e.g. Smol 1992). Hence, biomonitoring and paleolimnological assessment of key biological communities and ecosystem functions must be included in monitoring programs if the aim is to truly understand ecosystem changes caused by peatland management. Additionally, the time needed to observe restoration success of peatland structure and composition might easily surpass ten years (Haapalehto 2014). Thus, the selection of quick-response bioindicators able to detect and track changes during shorter observation periods, and which could also accurately detect and predict long-term changes if continuous monitoring is implemented would be useful.

1.2 Bioindication

Organisms are adapted to a specific type of ecosystem and its environmental conditions (e.g. type of substrate, temperature, biological interactions, light availability, etc.). Evolutionarily, populations primarily adapt to a particular range of abiotic factors in order to maximize growth and reproduction rates. When the values of these factors fall out of the populations' optimum range, its overall fitness will be reduced, affecting population dynamics and in turn, affecting the whole community (Holt and Miller 2011). Consequently, the development of populations and ecosystems as a whole is determined by the ability of the organisms to cope with environmental stressors, (Schüürmann and Markert 1998) making environmental stress a driver of evolution.

Environmental factors have been under constant change due to climate changes over millions of years. However, in recent centuries the type, frequency and dimension of such changes have been greatly altered due to human activities such as: habitat fragmentation and loss, resource depletion, intensification of agriculture, climate change, and the introduction of new substances (Markert *et al.* 2003). The new, human-induced, stressors can also have additive or synergistic effects with natural stressors which may surpass the tolerance level of organisms (Markert *et al.* 2003).

The total number of eukaryotic species world-wide is estimated to be ca. 9 million (Mora *et al.* 2011), and due to the effects the aforementioned human-induced stressors on organisms, their number is dramatically decreasing (e.g. Sánchez and Wyckhuys 2019). Hence, the overall outcome of human-induced stress can manifest as either a change in the number, or a limitation in the distribution of species, along with changes in the viability of populations, and ultimately, the composition and function of ecosystems (Kappelle *et al.* 1999). Such changes do not only have ecological consequences but can also affect economic processes and human well-being (Markert *et al.* 2003).

These alarming environmental changes, observed mostly during the last decades, represent a major challenge to the scientific community and have focused efforts on finding management solutions and adequate tools for ecological monitoring. Monitoring methods able to detect ecological changes over the long term as well as at an early stage, are amongst the most widespread strategies used for change detection (Siddig *et al.* 2016). For example, monitoring tools such as easy-to-identify biological indicators are of high importance when assessing the short-term success of ecological restoration (Herrick *et al.* 2006). The concept of biological indicators was brought to the scientific context by Hall and Grinnell (1919) as a result of associating different plant and animal taxa to large geographical zones that shared structural and compositional features, i.e. "life zones". Since then, the concept has been used in a wide range of studies including assessment of habitat quality, monitoring of ecological integrity, verification of compliance of industries to specific environmental laws, and has been included in policies and regulations for environmental protection (Carignan and Villard 2002).

There are different factors that could be regarded as bioindicators such as biological processes, abundance, presence and/or distribution of single species, or whole communities/assemblages that potentially reflect the ecological quality of an ecosystem and its changes over a period of time (Holt and Miller 2011). The use of bioindicators has proven to be a powerful method to study environmental changes caused by ecosystem perturbation. Studying all biotic communities in a particular ecosystem is highly difficult if not impossible. Thus, it is of high importance to identify which taxa or groups of taxa should be studied and what kind of information they can provide (Koenig *et al.* 2015). Focus is often put on indicators of ecosystem health i.e. the ecosystem's ability to maintain its structure and function when facing external stress (Costanza & Mageau 1999).

Since the 1960's the use of bioindicators has spread worldwide (Holt and Miller 2011), and new techniques have arisen to meet the need to study different anthropogenic stressors. Nowadays, the biological indicators used in assessment and monitoring of ecosystem health, are usually well-studied taxonomic groups. Research on bioindicators has focused mainly on identifying those species or taxonomic groups that reliably reflect responses of other species (or the overall biodiversity) to disturbance, to indicate changes in the environment (McGeoch 1998), and in general to cost-effectively assess ecosystem integrity by sampling a determined number of organism groups (Dolph *et al.* 2011). The use of bioindicators is based on the premise that the current status or trends of community structure and composition, growth rate, and reproductive success of one taxon or group of taxa reflect cumulative environmental changes (Burger 2006).

However, not all organisms are suitable bioindicators. In general, a good indicator species is expected to reflect environmental changes due to its limited tolerance to abrupt changes in the environmental factors, in contrast to rare species that are infrequently found, or ubiquitous species that often display a weak response to environmental change (Fig. 1).

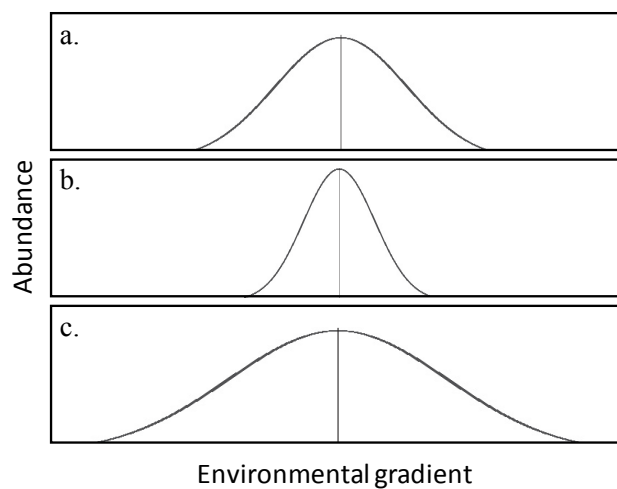


FIGURE 1 Environmental ranges of (a) proper bioindicators, (b) rare species, and (c) ubiquitous species. Image modified from Holt and Miller (2011).

In general terms, good bioindicators are organisms that possess specific hallmark traits such as: i) a lifespan long enough to reflect changes in environmental conditions through time and space, ii) being easily and cost-effectively identifiable, and iii) having a ubiquitous distribution (Holt and Miller 2011).

Although the selection of a proper bioindicator should be based on the species' known response to environmental change, an appropriate bioindicator is not necessarily a single species. Actually, the most common bioindication techniques include the study of a whole community that can encompass a wide range of tolerances to environmental disturbance allowing a multi-metric approach (Holt and Miller 2011). In addition, bioindicators must be selected for the specific aim of the study taking into account the organizational levels that encompasses species, habitat, and ecosystem (Carignan and Villard 2002; Fig. 2).

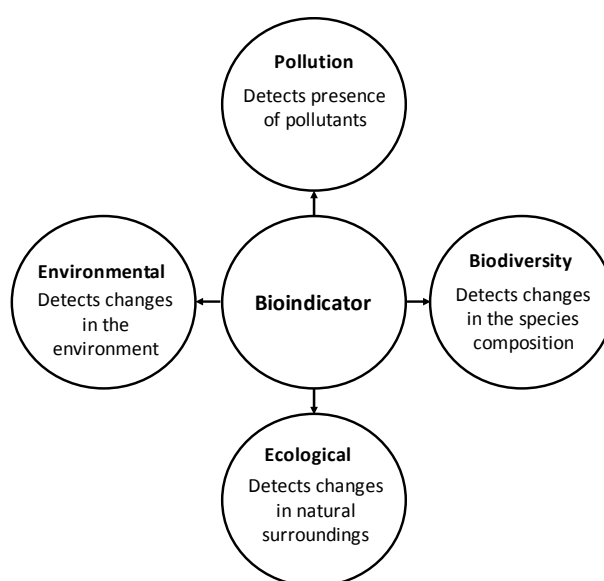


FIGURE 2 Types of bioindicators classified according to the aim of the study. Image modified from Carignan and Villard (2001).

The aforementioned categories of bioindicators do not necessarily exclude each other but, depending on the aim and scope of the studies, they require specific approaches (Stewart *et al.* 2007). Even though the types of bioindicators are not mutually exclusive, the classification is useful as the types differ in the extent in which they can be correctly measured. For example, environmental and ecological bioindicators might appear very similar however, environmental bioindication focuses more on specific abiotic characteristics (e.g pH, temperature, chemical concentrations), whilst ecological bioindication is more complex as it aims to quantify for example habitat disturbance or climate change (Stewart *et al.* 2007). According to the scope of the study, different taxa could be suitable depending on their lifespan, specific adaptations to certain conditions, reproduction rate, and distribution:

- *Microorganisms:*
Microorganisms are a major component of the biomass in soils and water, and are mainly responsible for the nutrient cycling controlling the productivity and energy flows within an ecosystem. Their high growth rate can reflect even rapid physicochemical changes, providing insights into short lived environmental disturbance (Hosmani 2014).
- *Plants:*
Plants are commonly used as indicators of environmental, ecological and pollution disturbance either by analysing the structure and composition of the communities or by studying their photosynthetic or growth rates. They are often a good tool to reflect the condition of the environment because of their sessile nature and their system stabilizing ability when favourable conditions are met (Jain *et al.* 2010).
- *Animals:*
Animals are one of the most widely used groups of bioindicators. Animal tissues can indicate contamination by pollutants, especially for those that bioconcentrate (Khatri and Tyagi 2015). Thanks to their heterotrophic nature, they also reflect changes in lower trophic levels (Jain *et al.* 2010).

One of the most common organisms regarded as good indicators in a broad range of ecosystems are invertebrates, as their presence has been associated more strongly to environmental factors than to biological interactions such as competition, predation, or parasitism (Schoener 1986). However, it is always important to consider the spatial scales observed as local level stressors could mask effects of global scale stressors. Hence, local pressures should be accounted for in order to properly identify the effects of the stressors under study. This can be related to the fact that there are differences in the population growth rates, habitat specificity and generation times between species (Murphy *et al.* 1990).

Additionally, with the multiple human activities taking place close to certain habitats, ecosystems are not exclusively affected by one single factor but instead, different stressors are degrading their ecological status. In Europe for example, more than 40 % of surface water bodies are affected by at least two stressors (Anon. 2018). When multiple stressors are affecting an ecosystem, the outcomes can be either: i) additive: the effect of multi-stressors equal the sum of single stressor effects, ii) synergistic: the effects of multi-stressors surpass the sum of single stressor effects, or iii) antagonistic: the effects of multi-stressors fall below the sum of single stressor effects (Fig. 3; Anon. 2018). Therefore, single species cannot be considered good bioindicators and arguably, a single group of taxa alone cannot either, as the results might be susceptible to misinterpretations.

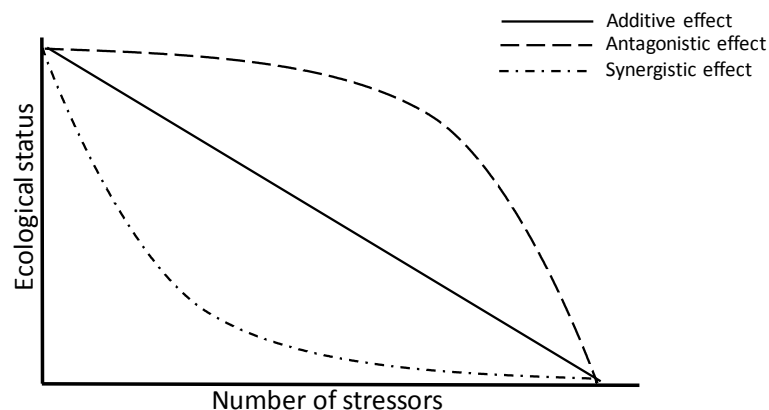


FIGURE 3 Outcomes of additive, antagonistic and synergistic effects of multi-stressors on ecological status of surface waters. Image modified from Dunne (2009).

Despite the extensive use of bioindicators, their many limitations have been pointed out (e.g. U.S EPA 2008, Lindenmayer and Likens 2011). These include: i) a high taxa richness and/or habitat diversity does not necessarily indicate the presence of other particular species (Pearson and Cassola 1992), ii) selection criteria of bioindicator taxa tend to be subjective, iii) ambiguous taxonomic terminology, iv) limited understanding on the association between the bioindicator and the specific scope of the study, v) the influence of different biological interactions, and most importantly vi) a single taxon rarely reflects the complexity of an entire ecosystem (Siddig *et al.* 2016). Additionally, despite some success at large scales, results have suggested that due to lack of concordance no single taxonomic group can accurately reflect changes in other groups in the general biodiversity (Heino 2010), or in the complexity of the whole environment (Lindenmayer and Likens 2011). This is related to the niche concept (Gause 1934) which states that two species cannot occupy the same niche. Hence, it makes it highly unlikely that there could be a perfect correspondence between a single indicator species and the rest of the taxa occupying an ecosystem (Hutto 1998).

To evaluate the effectiveness, i.e. ability to easily reflect changes in the environment of indicator taxa, different criteria have been proposed. On the one hand, differences in the frequency of occurrence of species in relation to a gradient of human disturbance should be considered. In other words, if a species only appears in pristine environments, it could be regarded as an indicator of ecological integrity. If in contrast, a species is mostly associated with disturbed environments, it could be regarded as an indicator of disturbance (Carignan and Villard 2002). Another factor to be considered is the habitat specialization as for example, species restricted to specific habitats are more likely to be susceptible to habitat degradation resulting from human activities (Carignan and Villard 2002).

On the other hand, a growing number of studies are aiming to quantify the degree of concordance between different species assemblages (e.g. Heino 2010, Johnson and Hering 2010). Community concordance assesses the extent to which different taxonomic groups exhibit similar distributions and abundances across a

region due to their similar responses to environmental changes (Jackson & Harvey 1993). As concordance is based on species' identities, it provides a better view of similarity between communities than species richness alone (Pawar *et al.* 2017). Different factors affect community concordance between assemblages such as strong biotic interactions (Johnson and Hering 2010), similar responses to the same environmental factors (Heino 2010), and co-loss of sensitive species as a result of environmental stress (Yates and Bailey 2010). But when concordance is not observed, it is most probably due to differential response to environmental gradients, or to the fact that organisms of different sizes (e.g. microorganisms, macroinvertebrates, fishes, etc.) perceive changes at different scales, such that covariation does not arise (Heino 2010). In summary, the main prerequisite to accurately use one taxon as a bioindicator for the condition of another taxon, is that concordance occurs at the same scale at which the biotic indices are designed (Paavola *et al.* 2006).

1.3 Testate amoebae as bioindicators of peatland ecosystem changes and peatland use impacts on lakes

The use of bioindicators to evaluate the ecological status of aquatic environments is a broadly used practice. Siddig *et al.* (2016) found that 50 % of the literature dealing with the use of bioindicators has focused on marine and wetland environments. The most commonly used organism groups are animals (46 %) while the least used are microorganisms (10 %). The possibility to evaluate peatland status changes resulting from land use such as afforestation, has been well documented for macroscopic organisms as birds and plants (e.g. Lachance *et al.* 2005). Additionally, plant communities have also been proven to be good bioindicators when evaluating peatland restoration success (e.g. Haapalehto *et al.* 2011, Poulin *et al.* 2013). However, to date, below-ground communities have been largely disregarded despite their well-known role in nutrient cycling, and the fact that they represent the majority of taxa in peatland ecosystems (Creevy *et al.* 2018).

Testate amoebae (TA) are a polyphyletic assemblage of unicellular eukaryotes that belong to the groups Euglyphida and Arcellinida (Meisterfeld 2002). TA are characterized by the possession of pseudopodia and the formation of an external shell (test), and they are usually found in wet environments (Charman 1999). TA are especially abundant in *Sphagnum* dominated peatlands where they are one of the dominant organism groups in terms of biomass with a density as high as 16×10^3 individuals m^{-2} (Sleigh 1989). TA feed on a wide variety of microorganisms such as fungi, microalgae, bacteria, other Protista, and decaying organic matter (Mitchell *et al.* 2000a). Hence, they are a key component of the below-ground food web and play a fundamental role in nutrient and carbon cycling. TA diversity and distribution are mainly driven by hydrological variables such as substrate moisture and water table depth. However, chemical

conditions of the peat layer such as oxygen concentration, pH and in general, chemical peat composition, have been found to be also important variables affecting the TA community structure (Charman 1997, Bobrov *et al.* 1999, Booth and Zygmunt 2005). TA are present in peatlands throughout the year and seem to be less affected by weather variations than other microorganisms due to their encysting capacity during unfavourable conditions. Because of such characteristics, several studies have recently used TA as bioindicators of human-induced environmental changes in peatlands (e.g. Jauhiainen 2002, Koenig 2015, Swindles *et al.* 2016, Creevy *et al.* 2018).

In most ecosystems, pre-disturbance information on biological communities is scarce or completely unavailable. In lakes for example, the use of paleolimnological methods has been critical to analyse long-term trends and to design restoration and conservation strategies (Kowalewski *et al.* 1997) especially in the absence of comparable reference state systems (Kauppila *et al.* 2012). TA outer tests preserve well in lake sediments and peat deposits (Mitchell *et al.* 2008), even where low pH values affect the preservation of other fossil records (Beyens and Meisterfeld 2001). Further, due to their short generation times, TA tests facilitate the collection of high-resolution data (Medioli and Scott 1988) making them ideal paleolimnological bioindicators (Dallimore *et al.* 2000). Because of their bottom-dwelling habits, TA are particularly subtle to respond to variations in the ecological conditions of the sediment-water interface such as metal contamination (Kihlman and Kauppila 2012, Nasser *et al.* 2016), pH (Patterson *et al.* 2013), dissolved oxygen (Drljepan *et al.* 2014), salinity (Roe and Patterson 2014), and alterations of substrate characteristics (Kihlman and Kauppila 2012). However, as their taxonomic identification is mainly based on test characteristics and as morphological variability has been found within taxa (Charman 2001), accurate identification can be difficult. As a result, among TA researchers, diverse taxonomic approaches are commonly used (Kosakyan *et al.* 2016). Thus, direct comparisons of diversity studies must be carried out with caution (Prentice *et al.* 2017).

Finnish surface waters receive naturally large amounts of organic matter due to the high proportion of peatland dominated catchments (Skjelkvale *et al.* 2001, Mattson *et al.* 2005). Increases in the amount of organic matter load to surface waters are increasingly drawing attention, especially because organic matter load has been related to ecological changes caused by human activities such as climate change, hydrology alterations (e.g. Räike *et al.* 2012) and land use changes (Armstrong *et al.* 2010, Yallop *et al.* 2010). As the size range of TA falls within the size of the organic matter typically transported from peatlands to downstream water bodies (Marttila & Kløve 2010), TA could be a suitable particle tracer of matter transportation from peatlands to e.g. downstream lakes. Additionally, TA could be a faster tool to obtain valuable ecological information in the assessment of peatland status than plants (I), even in cases when no TA species level identification is achieved (Koenig *et al.* 2015).

Microorganisms such as TA respond immediately to environmental changes, which make them good short-term bioindicators of disturbance in certain ecosystems such as peatlands (Gilbert and Mitchell 2006). They could be a

cost-effective and simple tool because they require only point-time samplings of mosses, soils, or water. Further when combined with a paleoecological approach they could be used to compare communities of past and present conditions (Gilbert and Mitchell 2006). Because of their small size, the spatial-temporal environmental scale that affects TA is clearly a challenge for ecological studies. However, a deeper knowledge about the microorganism world in peatlands is fundamental for a full understanding of peatland functioning (Gilbert and Mitchell 2006).

TA are divided into two main groups: Arcellinida and Eupglyphida. Arcellinida (lobose TA) possess lobulated pseudopodia, while Eupglyphida (filose TA) possess a filamentous pseudopodium (Fournier *et al.* 2012). The presence of both reproductive strategies and the fact that different TA species display different adaptations to specific environmental gradients, highlight the importance of the study of TA as a community instead of analysing single species. For example, some species of the genus *Hyalosphenia* (e.g. *H. elegans* and *H. papilo*) are mainly related to very wet environments while *Bullinularia indica* and *Arcella catinus* are species commonly found in drier environments (Mitchell *et al.* 2000b), and *Trinema lineare* which is associated to forested, and considerably dryer sites (Creevy *et al.* 2018). Species that possess adaptations such as spine-shells, e.g. *Euglypha spp.*, are considered to be especially well adapted to waterlogged environments as the spines help to restrict their sinking and overall movement (Bobrov *et al.* 2002).

TA taxa found in lentic sediments differ from the taxa found in mineral soils and also from taxa found in organic, water-logged soils such as peatlands. Hence, taxonomic keys used to identify TA are usually selected depending on the scope of the study. For paleolimnological studies of sediment samples, Kumar and Dalby (1998) designed a taxonomic key to separate infra-subspecific variants (or strains) exclusively reported in lake sediment samples. Strains of *Diffugia oblonga* such as "*oblonga*", "*glans*", "*lanceolata*", etc., are associated to lake sediments and display a ubiquitous distribution as far as there is sufficient organic material in the substrate (Patterson *et al.* 1985). Hence, they can be used to reflect trends in sedimentation and sediment quality as suggested by Kihlman and Kauppila (2012).

However, limitations exist when classifying or associating TA to certain habitats. Species formerly reported to be associated to dry conditions such as *A. catinus* have lately been found to have broad moisture tolerances (I, II). However, widely used taxonomic keys usually cluster different species into a single group which can broaden tolerance ranges and may lead to misinterpretations. Additionally, synonyms, reclassification of species, and possible phenotypic differences within single species are commonly known issues (for details see Oliverio *et al.* 2014). A recent study by Oliveiro *et al.* (2014) applying molecular tools to study TA biodiversity showed that TA are a group much more complex than mere morphological analyses would suggest, with considerable plasticity in their forms and several cryptic species. Such observations together with findings by Fournier *et al.* (2012) who suggested that morphological traits are related to environmental conditions, highlight the future need for combining morphology

and molecular approaches when studying TA communities to avoid misinterpretations (Oliveiro *et al.* 2014).

Lastly, sample collecting and processing techniques, for example the mesh size used to retain TA might also affect the observations as for example, *T. lineare* and other small species such as *Diffflugia pulex* which were commonly associated only with soils, have also been found in lakes sediments when using smaller mesh sizes (III).

1.4 Aims of the thesis

This study proposes the use of testate amoebae as bioindicators for routine monitoring of the ecological state of peatlands under different land uses. Additionally, it explores the possibility to use testate amoebae communities as a tool to differentiate between allochthonous organic matter loading from peat extraction areas and peatlands under different land uses to surrounding lakes. The present study explored whether testate amoebae are a suitable tool to time-effectively explore human-induced environmental changes in peatlands and whether TA accumulate in lake sediments when sediment load increases from peatlands to lakes due to peatland ditching and drying for peat extraction. Further, here was investigated whether TA are better indicators of peatland environmental changes than plants, and whether they display concordant responses to one another. Responses of TA to environmental changes, their concordance with plants, and their responses to anthropogenic disturbances are assessed by studying TA communities at peatlands from: restored, natural, and forestry affected peatland areas. Special attention was paid to the magnitude of TA responses to human-induced disturbances compared to responses to random natural environmental changes due to seasonality, or community variation within sites. More specifically, the following questions were addressed:

1. Are TA and plant communities concordant in their responses to environmental changes in peatlands? (I)
2. Are TA more efficient, or faster indicators of peatland restoration success than plants? (I)
3. Do the TA community responses to anthropogenic disturbances in peatlands override changes caused by seasonality? (II)
4. Do within site differences in TA communities override responses caused by changes in peatland land use? (II)
5. Do TA communities reflect differences between organic matter loads from peat extraction areas and peatlands drained for other purposes? (III)
6. Are TA a good paleoecological tool for finding reference (pre-disturbance) conditions in lakes affected by peat extraction loads? (III)

2 MATERIALS AND METHODS

2.1 Study sites and set-ups

2.1.1 Site locations

Sampling sites were located mostly in southern and central Finland (Fig. 4). The area is part of the boreal region characterized by abundant lakes, *Sphagnum*-dominated peatlands, and coniferous forests. The most common peat formations are raised bogs which are characterized by minerotrophic edges and an ombrotrophic centre, sustaining mesotrophic vegetation (Euroala *et al.* 1984), composed mainly of *Sphagnum* mosses and dwarf shrubs (Straková *et al.* 2011).

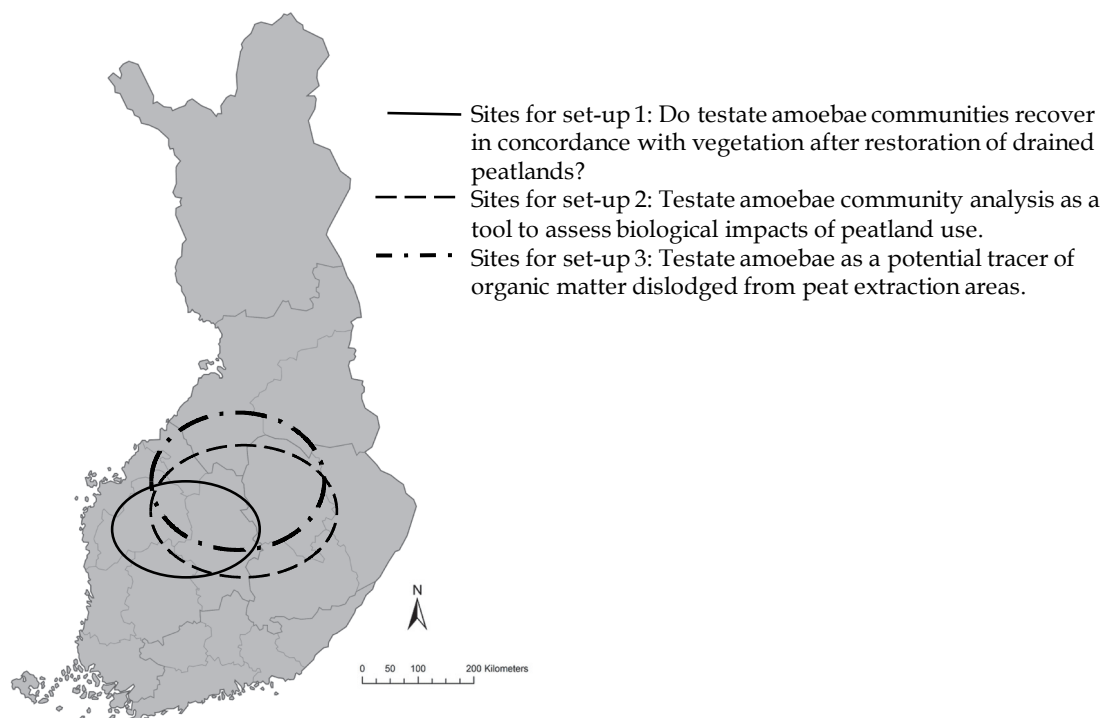


FIGURE 4 Locations of study sites in central and southern Finland.

In Europe as well as globally, Finland has the largest peatland area relative to its total area, which in turn, provides for the amount of dissolved organic matter exported to surface waters (Forsius *et al.* 2017, Arvola *et al.* 2016). Additionally, the amount of organic matter exported from drainage areas increases proportionally to increasing peatland area (Mattsson *et al.* 2005). Due to human activities (e.g. forestry, peat extraction, agriculture, etc.) in southern Finland, most lakes have been altered from their original ecological state.

2.1.2 Set-up 1: Analysing concordance between testate amoebae and plant communities' responses to environmental changes

The first set of study sites to identify the degree of concordance of testate amoebae and plant communities in response to restoration procedures after peatland drainage consisted of 19 study sites divided into four land use management classes: i) natural, ii) ditched, iii) ditched and restored 3–7 years before sampling, and iv) ditched and restored 9–12 years before sampling (I). For brevity, the restored sites will be referred to as Res05 and Res10, respectively. Sampling sites were located between 61°53'–62°51'N and 22°53'–25°26'E in South-Boreal region in southern Finland. The region has a mean annual temperature of 4 °C and mean annual precipitation of 650 mm. One criterion for site selection was the hydrological independency determined by topographic data and field observations. Ditched sites were used most likely during the 1960s and 1970s for forestry purposes, and some of the ditches have been cleared again during the 1990s while the restored sites were reserved for conservation during the 1980s.

Sampling was done using a grid of twenty 1 m² plots with two polypropylene pipe wells for water table depth measurements, and an extra pipe well for taking water chemistry samples at three selected plots. Selection of the plots at ditched and restored sites was based on their perpendicular position to the ditch on a transect of 15 m from the ditch. At natural sites, plots were similarly arranged, but the location of the first plot was randomised. At each site, redox potential, electrical conductivity, and pH were measured at the same time of biological sampling (see 2.2.1). Additionally, water samples were taken for cation concentration analysis and water table depth was measured using the polypropylene pipe wells (for details see Haapalehto *et al.* 2014, I).

2.1.3 Set-up 2: Testate amoebae community analysis as a tool to assess biological impacts of peatland use

Two peatlands were included to represent each land use type: i) natural: Riihineva and Aitosuo, ii) forestry: Lahnanen and Ruuskanlampi, and iii) restored: Aitoneva60 and Aitoneva80 (II). At each site, fifteen 100 cm² sampling plots were placed in three lines of semicircle shapes starting randomly. At the top-left corner of each sampling plot, 2 cm diameter polypropylene pipe wells with 2 mm slits at every 3 cm were placed for water table depth (WTD)

measurements (Fig. 5). At each sampling plot, oxygen and pH were recorded (For details see I).

Vegetation cover at natural and restored sites were fairly similar, dominated by mats of mosses, shrubs and scarce tree coverage, and with a high water table. Forestry sites were considerably dryer, with only few plots where the water table reached the top of the soil.



FIGURE 5 Distribution of sampling plots and installation of polypropylene pipe wells at the forestry site Lahnanen (left), restored site Aitoneva60 (middle), and natural site Aitosuo (right).

Aitoneva restored sites were previously drained for peat extraction and are currently monitored by the peat extraction company VAPO. The sites were restored ca. 60 (Aitoneva60) and 80 (Aitoneva80) years ago by filling ditches and re-wetting. At restored sites, vegetation re-establishment was passive, allowing natural recolonization by plants (mosses).

2.1.4 Set-up 3: Testate amoebae as a potential tracer of organic matter dislodged from peat extraction areas

A total of 36 lakes were selected to sample sediments (III). Study lakes were divided into two groups: i) possibly affected by peat extraction (impact lakes) and ii) non-affected by peat extraction (control lakes). Other activities such as forestry and agriculture have taken place in the catchment areas where both types of lakes (impact and control) are located. Lakes were included in the impact group if peat extraction areas were present in the upstream catchment area (i.e. lakes receiving water from the extraction area). Lakes used as controls were either located upstream from extraction areas or did not receive waters from extraction areas. However, due to the high incidence of human activities on peatlands in southern Finland, all lakes (impact and control) are located in catchments with large ditched peatland areas. One of the most important factors taken into account to select the impacted lakes was the close location of a control (having in some cases both impact and control lake within the same catchment areas), the peatland area cover percentage, and the proportion of lake area vs catchment area.

2.2 Data collection

2.2.1 Testate amoebae collection

TA extraction from peatlands (I and II) was done using the upper photosynthetic part of the mosses following the protocol proposed by Booth *et al.* (2010). TA from lakes sediments (III) were sampled using a Limnos Sediment Corer (for details see Kansanen *et al.* 1991) during the summer of 2013 and 2014. Sediment samples were homogenized inside the bags and ca. 2 g of the sample was separated to obtain TA.

To analyse concordance between TA and plant communities (I), sampling was done during July and August 2007. Plant and TA communities were sampled from plots where water table depth and water chemistry were analysed. Relative abundance of plants at each plot was estimated as percentage cover of each taxon of vascular plants, bryophytes and lichens (I). To assess differences between the effects of seasonality, intermediate scale spatial variation and land uses on TA (II), samples were taken during spring (May), summer (July), and autumn (September) between 2013 and 2015 (II).

All TA samples were analysed using the protocol proposed by Booth *et al.* (2010). Samples were boiled in distilled water with *Lycopodium clavatum* spore tablets (batch 1031) standard preparation from Lund University (Sweden) for density estimations. Separation of TA was done using 21 µm mesh size sieves. Retained material was transferred into Eppendorf tubes and centrifuged at 3.000 rpm for five minutes. Identification was mainly done to species or taxa groups based only on characteristics of the shells using different taxonomic keys (e.g. Kumar & Dalby 1998, Charman *et al.* 2000, Meisterfeld 2002, Clark 2003, Mitchell 2003, Mazei and Tsyganov 2006). As only current communities were taken into account, no empty shells were counted.

2.2.2 Measurements of water table depth and environmental parameters

Polypropylene pipe wells set in the sampling plots were used to measure water table depth (WTD; I and II). When the WTD was below ground, i.e. below the top of the moss layer, values were recorded as negative and positive when plots were flooded. WTD measurements were done in May, June, July, August and September during 2008 (I) and in May, July and September from 2013 to 2015 (II).

Water samples were taken from the polypropylene pipe wells for water chemistry analysis (I). At natural sites, pipe wells located in the central part of the sampling grid were used while at ditched and restored sites the sampling location was 15 m from the ditch and 10 m apart. Water samples were collected in August 2007. Conductivity (EC), pH and redox potential (Eh7) were measured shortly (i.e. within hours) after sample collection with a Consort SP50X meter with SK10T, SP10B and SP50X electrodes. Cation concentrations (Al, Ca, Fe, K, Mg, and Na) were obtained by filtering water samples through 0.45 µm pore size filters and analysed with a Perkin Elmer Optima 4300 DV inductively coupled

plasma spectrometer. Water temperature and pH (II) were measured in situ during TA sampling.

2.2.3 Lake sediment sampling and core dating

Lake echo sounding studies were conducted during the spring and summer 2013 and 2014 and the sediment sampling points were selected from the optimal sedimentation areas identified in an echo sounding study. Sediment samples were taken using a Limnos Sediment Corer (Fig. 6, for details see Kansanen *et al.* 1991). The first layer (current TA communities) and 15th (i.e. 15 cm depth) of sediment were stored in plastic bags for TA extraction. The 15th depth layer was selected as the pre-peat extraction sample taking into account the annual sedimentation rate for a lake located in a peatland catchment.

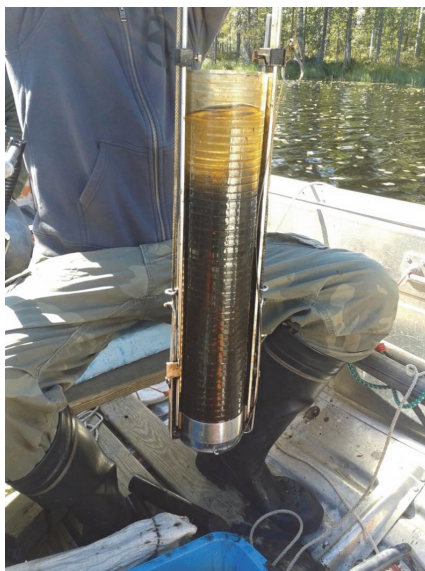


FIGURE 6 Limnos Sediment Corer used to sample the 1st (1 cm) and 15th (15 cm) sediment layer (current and pre-peat extraction samples, respectively). Photo: Emmanuela Daza Secco.

Sediment cores were dated (III) by analysing ¹³⁷Cs concentration peaks. Analyses were performed at GTK (Geological Survey of Finland) using two different gamma spectrometers, an older EG&G Ortec ACETM-2K equipped with a four-inch NaI(Tl) detector and a new fully digital BrightSpec bMCA-USB pulse height analyser coupled to a well-type NaI(Tl) detector. Concentrations of ¹³⁷Cs from the 36 studied lakes were measured using a 1 cm resolution. Some analyses were repeated on parallel sediment sequences to control the quality of ¹³⁷Cs estimations and to allow sediment subsampling.

2.3 Statistical analyses

Plot averages of taxa richness, Shannon–Wiener diversity, and relative abundances or estimated densities of TA and plants were calculated for each site (I, II). TA density (I, II, III) was estimated using the *Lycopodium* counts as an external marker (Stockmarr 1971). Given the differences in pore space, mass, and water content among moss species, habitats and collection times, the volume of sample is hardly accurately calculated. Therefore, TA densities are used only as semi-quantitative data estimations and results and discussion focus more on TA taxa richness and diversity. Differences in community structures were identified using a Non-Metric Multidimensional Scaling (NMDS) based on Sørensen's (Bray–Curtis) distance. Depending on whether the richness, diversity and TA density data did not deviate significantly from normality and homocedasticity assumptions, comparisons between groups (sites or sediment layers) were done using either ANOVA and *t*-tests or perMANOVA and Kruskal–Wallis. To avoid noise, rare TA taxa and sediment samples with less than 30 tests counted were removed from the analyses. To visualize the main patterns between different sampling sites in terms of the taxa composition, a model-based ordination method as suggested in Hui *et al.* (2015) and Warton *et al.* (2015) was applied (II). A latent variable model with two latent variables was fitted to the data assuming a negative binomial distribution of the TA density to produce an ordination plot based on the bivariate latent variables (II). Latent variable models were fitted to data sets separately for different seasons. Finally, to identify drivers of TA community composition, a latent variable model with covariates was applied (II). All calculations were done using R (version 3.0.2) and the vegan package for NMDS analysis (Oksanen *et al.* 2015).

3 RESULTS

3.1 Testate amoebae community changes in relationship to peatland land uses

The efficiency of TA communities as indicators of short term success of peatland restoration (question 2, in chapter 1.4) and the responses of the TA communities to environmental changes caused by peatland uses (questions 3 and 4) were studied in the experimental set-ups 1 and 2. In general, NMDS ordinations showed that the structure and composition of TA communities differed between land uses (I, II). In the set-up 1, TA communities were clearly grouped along the x-axis, and the largest distances were observed between natural and ditched sites. Restored sites were mainly grouped in the centre of the ordination overlapping with all other land use classes. However, restored sites Res05 were more closely grouped to the ditched sites while Res10 grouped closer to the natural sites (I). In set-up 2, TA communities also grouped by land uses (natural, forestry, and restored) although their separation was not clear (II).

In general, most TA taxa were broadly found at all sites regardless of the land use but their densities varied. Some species such as *Arcella catinus* showed a high tolerance to changes caused by peatland land uses, and were found in I and II abundantly at all sites regardless of the land use. In contrast, species from the genus *Hyalosphenia* were mostly found on floating bog mats and at natural and restored sites, and were found in low densities at ditched and forestry sites. In both studies, natural sites tended to have the highest taxa richness and Shannon diversity index values (Table 2). Regarding TA densities, no differences between natural, restored and drained/forestry sites (I, II) were found.

TABLE 2 Average values of testate amoebae communities' Shannon diversity index, taxa richness, and density. Values of restored sites in I include sites restored 3–7 and 9–12 years before sampling (Res05 and Res10, respectively). Values are averaged by land uses. In II, means were calculated over years and seasons. Highest values are in bold. Values in parenthesis correspond to S.E.

Study	Land use	Diversity	Richness	Density 10 cm ⁻³
I	Natural	2.27 (0.10)	17.73 (0.71)	15283 (2998)
	Ditched	1.94 (0.07)	13.09 (0.70)	6767 (936)
	Restored	1.66 (0.08)	12.27 (0.44)	20776 (2202)
II	Natural	2.09 (0.01)	16.28 (0.20)	31861 (1435)
	Forestry	1.43 (0.03)	9.58 (0.22)	22424 (1600)
	Restored	1.93 (0.01)	13.97 (0.19)	28117 (1236)

The overall effects caused by land uses were also contrasted with the effects caused by seasonality (question 3) and with the differences due to within site variations (question 4, II). Despite some overlap by seasons (spring, summer, autumn), ordination results showed clear clustering by land use. Ordinations using the entire data set (all seasons and years together) showed a similar pattern where all communities grouped by land use regardless of site, season, and year.

3.2 Concordance between testate amoebae communities and plant communities after peatland restoration

To answer study questions 1 and 2, testate amoebae and plant communities from restored, ditched and natural sites were compared. A total of 44 TA taxa and 45 plant taxa were recorded at all study sites. No significant differences were found between TA and plant taxa richness between management classes. However, TA richness tended to be higher at natural sites compared to plants. When comparing Shannon diversity indices, differences between TA and plant communities were observed only at natural and ditched sites (being higher for TA), whereas no differences were found at restored Res05 sites and Res10 sites.

For TA communities, taxa richness was higher at natural sites, while no differences in plant taxa richness were observed between management classes. TA taxa Shannon diversity differed only between restored Res05 and Res10 sites, while no differences of plant diversity were observed between management classes. In general, both richness and diversity of TA showed a tendency for the

highest values at natural sites and the lowest at Res10 sites. No clear patterns were observed for plant communities.

The structure and composition of TA communities differed between all management classes except between Res05 and Res10 while for plant communities only natural sites were different from other land uses (I). NMDS ordination showed a separation of TA communities by management classes, most clearly between ditched and natural peatlands. A similar pattern was observed for plants communities but the grouping was not as clear.

As bryophytes and vascular plants differ in their biological processes including growth rate, community concordance was analysed separately comparing TA first to mosses and then to vascular plants. However, because no differences in results were found, mosses and vascular plants were grouped together. There was a weak correlation between TA and plant communities when analysing communities from all management classes together (I). When concordance was analysed within management classes, TA and plant communities showed a weak concordance only at Res05 sites (I).

3.3 Hydrology and water chemistry in peatlands under different land use management and their relationship with testate amoebae communities

Differences in environmental conditions between land uses (or management classes) and their relationship with the biotic communities were analysed (I, II). In general, water chemistry did not show significant differences between the management classes. For some parameters (Al and Mg) the concentrations were below detectable limits. No differences between the management classes were found for environmental parameters measured in set-up 2 either, except for pH which was found to differ between Aitoneva60 and Aitoneva80, being lower in Aitoneva80.

Expected variation was observed in water table depth between management classes and between months. When land uses were compared, the lowest values were recorded at ditched sites and the highest at Res05 sites. Overall, lowest values for water table depth in all management classes were observed in June (set-up 1).

Ca and K concentrations were the only two parameters that significantly correlated with TA community variability (set-up 1) while none of the three measured parameters in the set-up 2 appeared to be related to TA ordination. pH alone explained 14.8 %, temperature 2.6 % and water table depth 2.3 % of the total covariation between environmental parameters and TA communities. It is important to highlight that in set-up 1, the hydrological parameters were not paired with TA samples which can constitute a barrier for accurate interpretations. When analysing seasonality and variation between years (II), results showed that these natural variations did not affect TA communities.

However, when analysing land use as a covariate with the TA ordination it explained 71.5 % of the covariation across taxa.

3.4 Testate amoebae as particle tracers of organic material dislodged from peat extraction areas and their applicability as a paleoecological tool to identify pre-peat extraction conditions

Current (surface) and pre-peat extraction (bottom) TA communities from lakes downstream of peat extraction (impact lakes) and lakes non-affected by peat extraction but affected by other human activities (control lakes) were compared to answer study questions 5 and 6 (III). When comparing taxa richness, diversity and density of TA communities, no differences were found between the impact and control group. NMDS ordinations showed similar results. No clear grouping was observed for TA communities by either sediment layer or catchment land use. When catchment parameters such as lake area, catchment area, peatland area, peat production area, percentage of ditched area, percentage of peatland, and percentage of production area were fitted into the ordination, no association between any of the parameters and TA communities was observed.

Among the total 54 taxa found in III (Fig. 7), the most abundant species was *Trinema lineare*. In general, the most common and abundant taxa across all lakes belonged to the genus *Diffflugia*. The most common *Diffflugia* species were *D. pulex*, *D. oblonga* varieties “oblonga”, “glans”, and “bryophyla”, *D. urceolata* varieties “urceolata” and “elongata”, and *D. protaeiformis* variety “acuminata”. All observed taxa are commonly reported as typical taxa of lake sediment TA communities except for *T. lineare*.

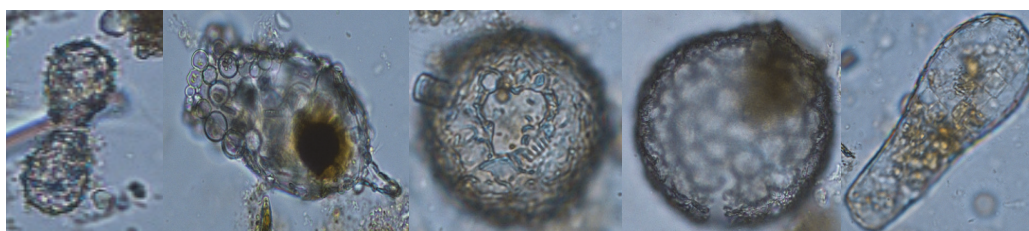


FIGURE 7 Photos of some common taxa found in current and pre-peat extraction sediment samples. From left to right: *Diffflugia pulex*, *Diffflugia protaeiformes* “amphoralis”, *Cucurbitella tricuspis*, *Diffflugia tuberculata*, and *Quadrudella symmetrica*. Photos: Emmanuela Daza Secco.

3.5 Results summary

The responses of TA communities to changes in land use and restoration, and the evaluation of their effectiveness as a quick-response ecological bioindicators, are summarized in figure 8.

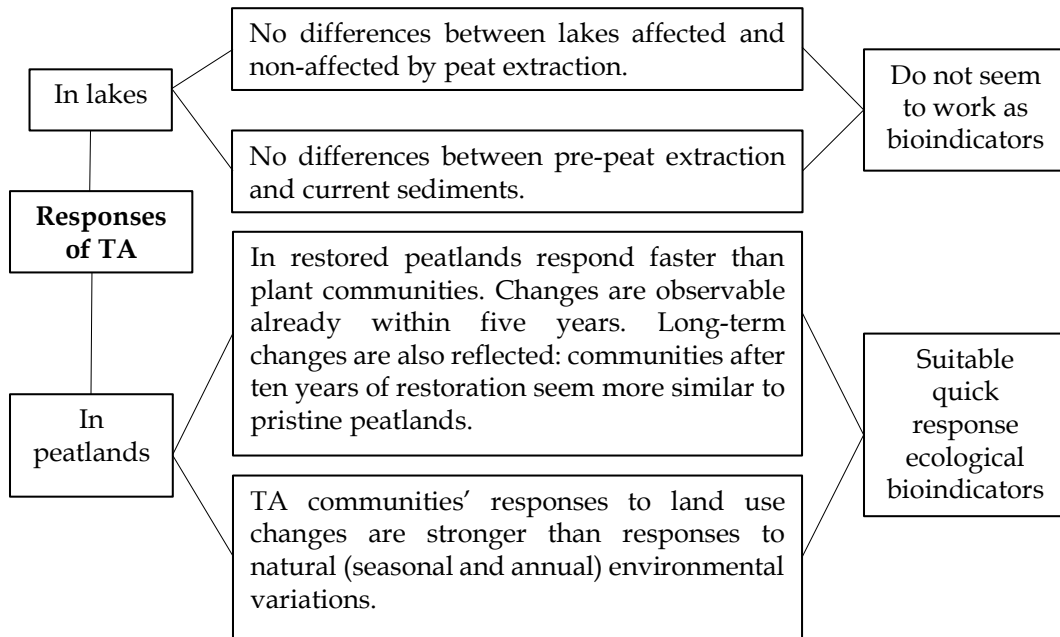


FIGURE 8 Flow chart summarizing the main findings of the suitability of testate amoebae (TA) communities as quick response ecological indicators.

4 DISCUSSION

4.1 Changes in water table depth, water chemistry, and testate amoebae communities in response to different peatland land uses and restoration actions

Analysed together, the results from set ups 1 and 2 confirm that while there is a clearly lowered water table depth, pH, conductivity, redox potential, and chemical concentrations did not significantly change in ditched peatlands. Following peatland restoration, water table depth increases due to ditch blocking but again, chemical parameters do not appear to change. Among the measured environmental variables in this study, hydrology appears to be the most important in shaping peatland ecosystems. Peatland drainage for purposes other than peat extraction (e.g. forestry, peat extraction, agriculture) lowers the water table depth, reducing substrate moisture (Laine and Vanha-Majamaa 1992) and resulting in high within site gradients of water table level and the exposure of bare peat at the surface (Price *et al.* 2003).

The lowest and most variable water table levels are frequently found closer to the ditch where also vegetation is almost completely destroyed, and erosion increases due to bare peat exposure (Haapalehto 2014). The disruption between the acrothelm and catothelm stratification due to the lowering of water table increases the rate of vegetation decomposition mainly in the surface which reduces the accumulation of peat (Niedermeier and Robinson 2007). It is expected that the reduction in peat accumulation reduces the ability of peatlands to store carbon which is one of the most important ecosystem functions of peatlands. However, previous findings are contradictory in this respect since both negative and positive carbon balances have been observed in peatland ecosystems after ditching (e.g. Lohila *et al.* 2011, Simola *et al.* 2012, Pitkänen *et al.* 2013).

Additionally, it has been observed that lowering water table depth causes significant changes in the chemical composition of the surface peatland water such as elevated concentrations of nitrogen (N) and phosphorus (P) and reductions in potassium (K), calcium (Ca), and magnesium (Mg) (Sundström *et*

al. 2000). For example, pore water pH and other elements' concentrations have been reported to increase shortly after peatland drainage (Moore *et al.* 2013). However, findings seem also to be contradictory as changes can also be driven by other factors such as peatland class and trophic level, water table level, and temperature, among others (Menberu *et al.* 2017). In the present study, such changes were not observed. It is also important to highlight that due to the extensive sampling, data collection methods were slightly modified in order to facilitate sampling therefore, water samples in this study were not directly measured from pore water but instead, samples were taken from pipe wells which might have masked possible changes in water chemistry observations.

On the other hand, here TA, plants, water chemistry, and water table depth were recorded many years after drainage was implemented which might explain the lack of differences observed between drained, natural and restored peatlands. These observations are supported by the findings of Holden *et al.* (2004) who concluded that the long-term changes in water chemistry of pore water after peatland drainage are not well understood and seem to not follow a particular pattern.

Ditch blocking in peatland restoration aims to recover ecosystem functions and structure by attempting to recreate key hydrological properties such as original water table level and flow paths (Aapala *et al.* 2009). Such restoration efforts have resulted in both successes and failures of recovering water table depth (e.g. Worrall *et al.* 2007, Laine *et al.* 2011, Wilson *et al.* 2011a, b) and water chemistry (Höll *et al.* 2009, Wilson *et al.* 2011a, b).

Despite some successful results after peatland restoration in terms of hydrological recovery, the effective restoration of their plant community composition is still a concern (Moreno-Mateos *et al.* 2012). In some cases, when uneven hydrological recovery is observed due to insufficient ditch blocking, water flow is redirected into the artificial paths that formed after ditching (Haapalehto 2014). Hence, the recovery of biological communities during peatland restoration might be affected by the prevailing within site gradient of degradation, with the worse conditions usually observed close to the ditches. Additionally, bare peat surfaces also cause cracking and crust formations that limit the recolonization of original vegetation by restricting the establishment of plant propagules (Salonen 1987).

TA respond mainly to hydrological conditions such as peat moisture. Taking into account the above mentioned observations on within-site variations in hydrological conditions, the findings of the present study suggest that neither within-site variations mask the differences in TA community composition and structure between peatland land uses, nor do natural changes in water table depth caused by seasonality or yearly climatological variations as observed in set-up 2. However, a reliable conclusion regarding the relationship between TA and environmental variables in set-up 2 might be difficult as the water samples for chemical analysis and the water table depth measurements were not paired with the TA sampling (data was not always taken from the exact same points). This issue has an important repercussion when interpreting the results as even in natural peatlands, surface moisture conditions can be highly variable.

This study showed that TA communities do not seem to respond to single environmental variables such as water table depth, pH, or temperature. However, ordination patterns showed a clear grouping of TA communities by land uses (I and II). This suggests that TA might be acting more as ecological than environmental bioindicators as the differences did not seem to be explained by single variables but instead, by the whole ecosystem disturbance.

NMDS ordinations with fitted water chemistry variables showed an association between TA communities and Ca and K concentrations. Such observations have been reported also previously (e.g. Lamentowicz *et al.* 2010, Hájková *et al.* 2011, Raabe *et al.* 2012, Jassey *et al.* 2014) and could be explained by the fact that TA require Ca for shell-building (Lamentowicz *et al.* 2011). Relationships between TA communities and other nutrients have been also reported (e.g. Mitchell *et al.* 2000a, Jauhiainen 2002, Mitchell *et al.* 2004) but remain to be poorly understood. One of the most plausible explanations for the observed relationship is based on how the nutrient availability indirectly affect TA by controlling the amount of their prey such as bacteria, fungi, and other protists and micrometazoa (Mitchell *et al.* 2004). However, despite the associations observed between TA community composition and structure with Ca and K, the observed differences in the studies of this thesis appeared to be more related to peatland land use than to any single environmental parameter.

TA density estimates were generally higher at natural and restored sites compared to the drained sites. This suggests that restoration actions aimed to increase water table level will generally result in an increase in TA densities making the communities more similar to natural communities in terms of abundance. Such patterns were also observed when comparing TA taxa richness and diversity at natural and restored sites to that at the drained sites. These observations are supported by Fournier *et al.* (2012) who found higher TA densities in soils with higher water holding capacity and expected higher moisture content. Hence, after successful restoration actions, TA communities seem to increase in density, taxa diversity and richness over time.

Despite an increase in TA taxa richness and diversity after restoration measures, the highest taxa richness and diversity values were observed at natural sites. This pattern cannot be explained exclusively by water table level as at some particular restored sites, water table levels were even higher compared to that at natural sites. However, at undisturbed sites, there is more time for cumulative colonization of taxa with little species replacement (Wanner and Xylander 2005) which might result in higher overall taxa richness and diversity when compared to restored sites. Some taxa (e.g. *Arcella artocrea*, *Centropyxis ecornis*, *Diffflugia leidyi*, *Diffflugia lithophila*, *Hyalosphenia minuta*, and *Pseudodiffflugia fascicularis*) were exclusively found at natural sites which suggests that such taxa are either not able to recolonize, or require very long time for recolonization. Such findings emphasize the need to reduce or if possible, avoid peatland habitat degradation in order to preserve peatland biodiversity.

Some species such as *Arcella catinus* and *Bullinularia indica* were abundantly found at all sites. This contradicts other findings that have classified both species as indicators of dry conditions. On the other hand, the mixotrophic taxa

Hyalosphenia papilio and *Heleoptera sphagni* were found broadly distributed across all sites however, the lowest relative abundance was observed at both ditched sites (I). In the case of *H. papilio* the highest abundances were found on floating mats which might be related to the importance of the relative hydrological stability for this taxon distribution (Sullivan and Booth, 2011). Given the different trophic status found between TA taxa, other factors besides hydrological conditions might affect their distribution. For example, light availability plays a key role in the distribution and abundance of mixotrophic taxa (Mitchell and Gilbert 2005). In this sense, the presence of higher vegetation as bushes and trees at ditched and forestry sites, might limit the amount of light reaching the moss carpet reducing in turn, the abundance of mixotrophic taxa.

This might suggest that when dealing with TA as bioindicators, the use of a single taxa's presence and relative abundance to reflect gradients of environmental variables may not be appropriate, but instead, whole TA communities may give a more reliable view of habitat disturbance, summarizing overall changes in environmental conditions. It is also important to highlight the caveats when interpreting relative abundance alone. Compositional data can pose challenges for example when mistakenly treating two communities having the same relative abundances equally, despite obvious differences in total abundances (Chong and Spencer, 2018).

Additionally, when analysing TA community structure and composition, differences in taxa composition caused by seasonality, annual variation, and within site variation did not seem to mask the effects caused by land uses. Hence, it can be suggested that even though particular TA taxa may respond to changes in specific conditions, TA community parameters such as taxa richness, diversity and density are more likely a result of the interaction of different environmental factors and biological interactions directly related to land uses (i.e. disturbance). It should also be highlighted that the taxonomic keys used for identification in this study, group some morphologically similar species into a single taxon which may be inaccurate (Oliveiro *et al.* 2014) and increase the range of environmental tolerance of each taxa group (see details in Booth 2001).

4.2 Effectiveness of testate amoebae as short-term bioindicators of peatland restoration success

In general, peatland restoration actions aim to reinstate the ecosystem structure and its most important services by blocking ditches to re-wet sites. In some cases, plant species reestablishment is actively helped by reintroducing plant propagules but in many cases, it is passive and relies on natural recolonization. It has been observed that restoration towards the target community can be successful during the first ten years regarding abundance of certain species but not in terms of species richness (Haapalehto 2014). The differences in plant communities between peatlands restored 9–12 years ago and pristine

communities might be due to poor seed dispersal of some species or competition between plants specially adapted to peatlands and more generalist plant species (Seabloom and Valk 2003). Additionally, it is expected that after restoration of peatlands, both vertebrates and invertebrate species recover faster than the plant community (Moreno-Mateos *et al.* 2012). Whatever the case, it has widely been observed that total recovery of the original peatland conditions and ecosystem structure is seldom achieved after habitat degradation (for details see Haapalehto 2014).

Additionally, while clearly TA communities from recently restored sites (Res05) were closer associated to ditched sites, TA communities from sites that were restored 10 years ago (Res10) were already closer to natural sites, no such patterns were observed for plant communities. This can be explained by the faster response of TA to hydrological variations compared to plants as reported by Warner and Chmielewski (1992), who observed that changes in TA communities can be observable during the first 2.5 years after drainage, suggesting that TA communities are a quickly responding bioindicator of changes in peatlands. The observed time differences in TA and plant community responses to restoration actions might also be explained by the differences in their turnover, dispersal abilities, adaptation strategies, resistance, and resilience (Wardle 2002). It has also been suggested that spatial limitation is less likely to occur in soils compared to above ground habitats (Wanner and Xylander 2005). Hence, TA species that were able to recolonize might be facing less competition pressure for space and resources compared to plant communities.

TA are an important component of belowground peatland biota where they can account for more than half of the microbiota community biomass. Therefore, TA play an important role in nutrient cycling and predator-prey relationships as they feed on bacteria and smaller protists, and are an important source of food for soil animals. In general, protists and mainly TA can account for 20–40 % of nitrogen mineralization as they feed on bacteria hence, excrete bacterial nitrogen to the soil (Griffiths 1994). Nitrogen is a limiting nutrient for plant growth, and this observation could suggest that the presence of TA can be an important factor for successful plant recolonization as TA improve nitrogen availability and thus may reduce plant competition for resources.

On the other hand, observations suggest that TA might behave similarly to plants, i.e. that the TA community recovers in terms of density of certain species but not necessarily in species composition. Average species densities were higher at restored sites and indeed, a high dominance of the species *Arcella catinus* at sites restored ten years ago was observed where it accounted for more than 70 % of the whole TA community (Res10).

Other studies using TA as indicators of peatland restoration success (e.g. Davis and Wilkinson 2004, Swindles *et al.* 2016) have found that certain indicator species of wet conditions reflect differences in water content of peat after restoration. This study showed that, despite reflecting differences in community structure and composition between ditched and restored sites, species such as *Arcella catinus* and *Bullinularia indica*, were found in a broad moisture range. Other species such as *Arcella artocrea*, *Centropyxis ecornis*, *Diffflugia leidyi*, *Diffflugia*

lithophila, *Hyalosphenia minuta*, and *Pseudodifflugia fascicularis* were restricted to natural sites, and *Hyalosphenia elegans* and *Physochila griseola* were only found at Res10 and natural sites. Hence, this suggests that despite the recovery of favourable moisture conditions, certain TA taxa might require at least a decade for recolonization, or might not be able to recolonize restored sites at all. These difficulties to recolonize by both peatland plant and TA taxa highlights the need to avoid peatland habitat degradation, as to recover original biodiversity, costly re-introduction programs would be required (see also Moreno-Mateos *et al.* 2012).

Finally, the rapid response showed by TA communities in five years after restoration management suggests that TA can be a good indicator of early restoration success. These findings support the factors that have been identified to make TA suitable early indicators of restoration of degraded peatlands: i) good preservation of empty shells in peat sediments allowing the reconstruction of former environmental conditions (Davis and Wilkinson 2004), ii) different taxa or group of taxa respond differently to moisture conditions (Charman 2001), and iii) their small size and different reproduction strategies which allow rapid colonization when environmental conditions become favourable again (Wilkinson 2001).

4.3 Testate amoebae as bioindicators of disturbance in lakes receiving discharge from peat extraction areas and determination of reference conditions

The use of TA as indicators of past and present environmental conditions of lakes has broadly been acknowledged. As TA size (20–300 μm) falls within the size range of particles dislodged from peatlands (Marttila and Kløve 2010), this study aimed to use TA as particle tracers of sediments dislodged from peatlands that accumulate in lakes sediments, as well as to establish a reference of previous TA communities. However, when comparing the current and pre-peat extraction TA communities from lakes receiving discharge from peat extraction areas (impact lakes) and lakes receiving discharge from areas under other land uses (control lakes) no differences were found.

Different studies using TA as indicators of lake conditions (e.g. Roe *et al.* 2010, Killman and Kauppila 2012) have found a relationship between TA and ecological gradients, e.g. nutrient and metal concentrations. For example, *Centropyxis spp.* has been reported to display a broad tolerance range for different conditions such as oxygen concentrations (Killman and Kauppila 2012). On the other hand, the different strains of *D. oblonga* seem to respond to sediment quality preferring organic sediments (Killman and Kauppila 2012). The lack of differences between TA communities from peat extraction impact and control lakes suggest that in the case of central Finland, peat extraction is not significantly changing lake sediment properties for TA when compared to lakes affected by other peatland land uses. On the other hand, comparisons between

pre-peat extraction and current TA communities also suggest that in terms of TA sediment biota, different peatland land uses do not seem to exert a significant stress on TA as also found by Kihlman and Kauppila (2012) when studying the effects of mining.

As surface waters are rarely affected by a single stressor, sediment TA communities might be responding also to other factors such as the trophic status of the lakes (Roe *et al.* 2010). This could imply that the movement of nutrients from peat extraction areas to lakes is similar to other peatland uses resulting in no differences in lake trophic status that could be reflected by sediment TA. Indeed, the TA communities in lake sediments pre-dating peatland use do not seem to be affected by the type of peatland use.

Results showed that TA communities were always composed of the typical taxa found in lake sediments with the exception of few taxa small in size such as *Diffflugia pulex*, *Trinema-Corythion* and *Trinema lineare* that suggest that there is no colonization of peat soil associated species coming from peatland areas. The presence of *D. pulex*, *Trinema-Corythion* and *T. lineare* found in the samples, might be explained by the mesh size used to retain TA. In the present study, a 21 μm mesh instead of the commonly applied $> 30 \mu\text{m}$ mesh was used. As the size of *D. pulex*, *Trinema-Corythion* and *T. lineare* is around 30 μm they are not commonly retained and reported in lake studies (e.g. Boudreau *et al.* 2005, Burdíkova *et al.* 2012). It is, however, difficult to determine precise taxa-habitat associations as observed species are depending on the collection techniques, the taxonomic keys used for identification, and the difficulties to identify certain taxa morphologically which consequently might not always be reported in a particular environment.

When tracking particles from land ecosystems to lakes, the distance travelled by particles, the slope, and a variety of abiotic factors and biological interactions play an important role. Characteristics of the channels of the connecting running waters between the peatlands and the receiving lakes together with biological processes occurring along the way will then determine the type and amount of particles reaching the receiving lakes. For example, the accumulation of woody debris (Chergui *et al.* 1993), and the presence submerged macrophytes (Petticrew and Kalff 1992), and cobbles (Chergui *et al.* 1993) may actively retain travelling particles, including TA. In addition, in headwaters a large part of the organic matter that is consumed by aquatic fauna, comes from allochthonous inputs. Thus, TA entering streams from peatlands might serve as food for filter feeders such as blackfly larvae that capture small seston (Hershey *et al.* 1996). However, if the peat extraction significantly increased the amount of particles dislodged compared to other peatland uses, the increment might still be observable in receiving lakes due to the time difference between the establishment of macroinvertebrate filter feeder populations and the onset of the high runoff periods from production areas. On the other hand, particle aggregation is a common process likely to take place in aquatic environments. Thus, if TA are aggregating with other particles during their transport from peatlands to lakes, they may be sinking before reaching lakes (see also Black *et al.* 2007). Furthermore, differences in TA community composition and structure

between forested and natural peatlands have been observed (I) and consequently, such differences were expected to be reflected in TA communities found in impact and control sediments. Hence, as also observed by Kauppila *et al.* (2016) there might not be an actual increment in the amount of organic matter dislodged from peat extraction areas when compared to other peatland land uses.

The present results also showed that TA community composition and structure of all the studied sediments corresponded to those of typical lakes located in peatland-dominated catchments. Despite the fact that no environmental variables were recorded, the presence of peatlands composed by *Sphagnum* and *Carex* species is expected to acidify the water running to the lakes. This was reflected by the sediment TA communities where the acidic-sensitive species *Centropyxis aculeata* was present in most samples (including pre-peat extraction) in low densities. This observation also supports the idea that peat extraction is not causing a significant impact in the environmental conditions that control TA communities such as water pH. Additionally, as TA have been observed to respond to lake trophic status, a significant difference in the organic material input (e.g. sedimentary phosphorus) originating from peat extraction compared to other peatland use practices should have been reflected in the composition and structure of TA communities (see Roe *et al.* 2010).

5 CONCLUSIONS

This thesis aimed to evaluate the effectiveness of testate amoebae as bioindicators of impacts caused by different peatland land uses. Keeping in mind the complexity involved in the selection of proper bioindicators, the results suggest that TA are a promising tool to monitor environmental changes in peatland ecosystems. When it comes to the most important characteristics of a good bioindicator, TA communities can generally be considered a suitable tool for monitoring peatland changes. However, I point out also some recommendations for the use of TA as bioindicators of peatland ecosystem changes.

First, it is important to have comparable reference TA communities from the same sites under study by using fossilized samples. When using TA, this is not an impossible task given the good preservation of the shells in peat deposits, allowing the study of fossilized remains. It is important to note that each site is the result of the specific local environmental conditions and allows a wide range of different biological interactions. Hence, even if comparable near-pristine ecosystems are available, same-site fossil communities might be more accurate than using similar, closely located pristine peatlands as reference, despite the effects of previous different climate conditions that might affect the fossilized TA that lived at that site.

Second, when analysing different peatland or lake ecosystems within the same study, it is important to use the same sampling techniques and identification keys throughout the study. On occasions, comparisons between different studies might lead to misinterpretations when different procedures are used and not all taxa are necessarily caught in the samples. Additionally, in future studies, molecular techniques will improve the identification of TA in monitoring studies and could also be combined with morphological characterizations as certain morphological traits are also indicative of environmental conditions.

Third and lastly, I recommend to study TA community composition and structure as a whole, as different taxa display a variety of responses to changing environmental conditions, and as observed in this study, neither a single taxon, or taxa richness alone showed significant differences in response to peatland land

uses. Such patterns were only observed when contrasting community responses against peatland land use categories.

In summary, based on the present results I conclude that TA are a promising easy-to-use tool for biomonitoring the ecological status of peatlands. For example, TA communities appear to respond during the short time-scale, usually time available to monitor ecosystem changes in restoration studies. Studies I and II further demonstrated that TA communities tend to develop towards the target communities after successful restoration measures have been implemented. TA are also good indicators of restored peatland biodiversity recovery progress because certain taxa seem unable to recolonize restored sites at all or will require a very long recolonization time.

Responses of plant and TA communities should not be used as surrogates of one another. Due to their shorter generation time, TA appear to respond faster than vegetation making them a faster indicator of changes. However, TA cannot fully summarize the changes displayed by other organism communities such as plants. Hence, to obtain a broader knowledge of peatland dynamics and a holistic understanding of changes caused by land uses, it is important to study both TA and plant communities. Biomonitoring strategies for restoration should take into account that TA will reflect changes in hydrological conditions of peatlands but might not adequately reflect changes in ecosystem functions despite the fact that the re-establishment of TA communities may be needed for recovery of plant communities and the subsequent recovery of associated ecosystem functions.

Another important characteristic of TA communities found in the present study is their property to respond to human-induced environmental changes despite their responses to natural spatial and seasonal fluctuations. This is an important finding when monitoring long term changes of peatland ecosystems as it corroborates the idea that TA are suitable indicators of human disturbance in boreal environments where seasonal changes are drastic enough to change the whole biotic community. Hence, I suggest that TA are a good biomonitoring tool that can be used throughout the year and without the need for extensive and expensive sampling strategies.

Successful peatland restoration requires the re-establishment of vegetation and consequent animal communities that are sustained through crucial processes such as primary and secondary production, which in turn highly depend on the availability of macro- and micronutrients. The results thus suggest the importance of studying soil communities in peatlands as the belowground community dynamics are not only different from those aboveground, but also due to the importance of microorganisms in different key ecosystem processes such as decomposition and nutrient cycling that help sustain peatland food webs, primary production and carbon fixation.

Besides the importance of continuing research on finding suitable bioindicators, it is important to consider that each peatland is unique and will require different monitoring techniques. I propose TA as an excellent tool for routine monitoring of peatland changes as TA respond to one of the main factors driving peatland ecosystems structure and function: hydrological variations.

However, when relationships between peatlands and downstream lakes are the focus and specifically the effects of peatland use on lake sedimentation processes, TA do not seem best suited as particle tracers or bioindicators. Such observations may have different underlying causes. First, TA communities might just not reflect the potential increment in particles dislodged from peatlands to lakes. This conclusion is supported by two particular observations: i) TA taxa usually associated to peatlands but not to lake sediments were found in both current and pre-peat extraction samples and ii) even though TA are suggested to be sensitive to changes in the trophic status of a lake, no such responses were observed. Hence, even though there is an increase in the amount of materials dislodged from peatlands due to peat extraction, lentic sediment TA communities do not seem to respond to this change.

Second, differences might not be observable because of the treatment methods used in the peat extraction areas that specifically aim to retain particle dislodging after ditching processes. Third, despite the increase of materials dislodged in peat extraction areas, biotic and abiotic processes along the way to the lake may function as retaining mechanisms.

Lastly, the observed lack of differences between current TA communities in peat extraction impacted lakes compared to predating communities from the same lakes could suggest that even before large scale peat extraction practices, other human-induced disturbances, such as agriculture and forestry, were already taking place in the study areas. These disturbances might have produced effects that are not significantly reflected by lake TA biota or have already permanently changed the TA communities.

In summary, when monitoring changes caused by peatland land use and restoration success, TA structure and composition seem to be an excellent easy to use ecological bioindicator as they seem to respond more to human-induced changes than to natural fluctuations (seasonal and annual). Additionally, compared to frequently used indicators (e.g. plant communities) TA reflect changes in a shorter period of time which suggest their applicability as quickly responding bioindicators.

Further studies combining morphology and molecular techniques will be required to establish a base line for using TA as a monitoring tool. More research is also required on organic matter movement between peatlands and surface waters including for example sediment traps, to fully understand biological interactions affecting the transport of nutrients and organic particles from peatlands to receiving lakes. Finally, continued studies relating interactions between belowground and aboveground biotic communities are required to fully comprehend peatlands structure and ecosystem function in order to determine the best indicators of peatland disturbance and subsequent restoration actions.

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ORIGINAL PAPERS

I

DO TESTATE AMOEBAE COMMUNITIES RECOVER IN CONCORDANCE WITH VEGETATION AFTER RESTORATION OF DRAINED PEATLANDS?

by

Emmanuela Daza Secco, Tuomas Haapalehto, Jari Haimi, Kristian Meissner &
Teemu Tahvanainen 2016

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Do testate amoebae communities recover in concordance with vegetation after restoration of drained peatlands?

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SUMMARY

The environmental importance of peatlands has stimulated efforts to restore their specific ecosystem structure and functions. Monitoring and assessment of the ecological state of the peatland is fundamental in restoration programmes. Most studies have focused on the responses of vegetation and, to a lesser extent, on testate amoebae (TA). To our knowledge, none have investigated whether these two groups show concordance in the context of restoration of drained peatland. Here we assess community concordance between TA and vegetation in boreal peatlands belonging to four different land use management classes (natural, drained, restored 3–7 years ago, and restored 9–12 years ago). TA and vegetation communities were concordant when all of the studied sites were compared. However, there was no concordance within management classes except for sites restored 3–7 years ago. We found that TA and vegetation communities are not surrogates of one another when measuring the success of restoration, and that thorough studies of both communities are required to build a holistic understanding of the changes during restoration from an ecosystem perspective. TA seemed to respond faster to changes caused by restoration and, hence, could be better early indicators of restoration success than plants. Furthermore, studies of the relationships between TA and plant communities could provide important insights to aid understanding of the link between the recovery of ecosystem structure and the reinstatement of ecosystem functions.

KEY WORDS: assessment, ecological state, monitoring, indicators of restoration, taxa surrogates

INTRODUCTION

Peatlands of the boreal and subarctic regions cover only 3 % of the global land area. Due to the accumulation of organic matter as peat during hundreds to thousands of years, they store *ca.* 500 Gt of C corresponding to one third of the global soil C (Yu 2011, Yu 2012). Like many other ecosystems, peatlands have been heavily exploited. In Finland, more than 50 % of the total peatland area has been drained for forestry, 2.6 % for agriculture and 0.6 % for peat mining (Lappalainen 1996, Vasander *et al.* 2003). Peatland use is mainly concentrated in central and southern Finland where natural peatlands now occupy less than 25 % of their original area (Aapala *et al.* 1996). The increasing threat of environmental degradation has raised awareness of ecological restoration as a component of conservation programmes (Dobson *et al.* 1997). The environmental importance of peatlands has led to a growing number of attempts

to restore or partially reinstate surface water retention, the carbon sink, and specific flora and fauna (Lunn & Burlton 2013).

Monitoring and assessment of ecological state is a fundamental part of peatland conservation and restoration programmes (Trepel 2007). Studies based on the assessment of plant community composition after restoration have shown promising results (e.g. Haapalehto *et al.* 2011, Laine *et al.* 2011, Hedberg *et al.* 2012, Poulin *et al.* 2013). However, little is known about changes in the microorganism communities of restored peatlands. Testate amoebae (TA) are a polyphyletic group of shell-building unicellular protists (Meisterfeld 2002), commonly associated with peatland plants, and especially abundant in *Sphagnum* mosses (Tolonen 1986). In peatlands, the number of TA can be as high as 16×10^6 individuals per m², making them a significant component of the heterotrophic soil community (Sleigh 1989). TA diversity and distribution in bogs is mainly controlled by

hydrological variables (e.g. moisture content and water table depth), and in fens by water pH, oxygen concentration and peat composition (Charman 1997, Bobrov *et al.* 1999). Depending on taxa and environmental conditions, their generation time ranges from days to weeks, while that of bryophytes and vascular plants is much longer (Schönborn 1986). Under natural conditions, TA communities are stable between seasons (Warner *et al.* 2007), but when conditions change, they can change within months (Marcisz *et al.* 2014).

Koenig *et al.* (2015) found that TA provide more accurate information on microenvironmental conditions than vegetation, and that valuable ecological information can be obtained without knowing all of the TA taxa. Thus, TA communities have been proposed as a tool for monitoring and assessment of peatland conditions, but to date TA have been employed in relatively few studies (e.g. Jauhainen 2002, Davis & Wilkinson 2004, Raabe & Lamentowicz 2012, Turner & Swindles 2012, Koenig *et al.* 2015).

The biological indicators that are used in assessment and monitoring of ecosystem health, i.e. the ecosystem's ability to maintain its structure and function when facing external stress (Costanza & Mageau 1999), are usually well-known taxonomic groups. However, the degree to which certain taxonomic groups actually mirror trends among other groups and can be used as surrogates is still unknown, and studies based on the surrogate-taxa approach have repeatedly been questioned (e.g. Paavola *et al.* 2003, Bilton *et al.* 2006). Most bioassessment studies focus on single taxonomic group responses to environmental factors while few have addressed parallel group responses (i.e. concordance). Community concordance describes the degree of similarity in distributions and abundances of different taxonomic groups across a region and emerges when different communities show similar responses to environmental changes (Infante *et al.* 2009). Studies that have investigated concordance have mostly focused on lakes and fluvial systems (e.g. Heino 2002, Mykrä *et al.* 2008, Infante *et al.* 2009, Jyväsjärvi *et al.* 2014) and very few have examined such patterns for peatland ecosystems (Mitchell *et al.* 2000b, Lamentowicz *et al.* 2010, Hájek *et al.* 2014, Koenig *et al.* 2015, Hunter Jr *et al.* 2016).

The factors that drive community concordance include strong species interactions within and between communities (Heino 2002) and co-losses of species in response to environmental stress, including that caused by anthropic stressors (Mykrä

et al. 2008, Yates & Bailey 2010). Life histories and reproductive and dispersal capabilities of taxa could influence the extent to which different environmental factors and biotic interactions drive spatial patterns of community composition and, subsequently, community concordance (Shurin *et al.* 2009). Because it is based on species identities rather than only the number of species, community concordance provides a wider understanding of similarities between communities than richness measures alone (Pawar *et al.* 2007). Furthermore, when concordance among taxonomic groups does not occur at a specific geographical scale, the use of a single group as an indicator for the state of the ecosystem may not be appropriate (Paavola *et al.* 2006).

Studies of the success of peatland restoration generally focus on the recovery of vegetation, but whether important microorganisms such as TA are concordant in their responses to restoration and how these responses are linked to general ecosystem functions remains an open question. Because TA are especially common in *Sphagnum* and other mosses, they are potentially concordant with peatland vegetation. Therefore, we assessed concordance of the changes in TA and plant community structures among natural, ditched and restored boreal peatlands. We specifically aimed i) to analyse changes and concordance between TA and plant communities in response to restoration processes, and ii) to determine which environmental variables drive the changes in these two communities.

METHODS

Study sites

The study sites were located in the south boreal climatic-phytogeographical zone of southern Finland (latitude 61° 53'–62° 51' N, longitude 22° 53'–25° 26' E). The larger peatland formations in this region are mainly raised bogs, while small weakly minerotrophic *Sphagnum*-dominated mires are typically found in a landscape mosaic with coniferous forests. Sampling sites were located ca. 150 m above sea level with mean annual temperature +4 °C and precipitation ca. 650 mm. We selected 19 study sites divided in four land use management classes: i) natural ($n=5$), ii) drained ($n=4$), iii) drained and restored 3–7 years before sampling ($n=5$), and iv) drained and restored 9–12 years before sampling ($n=5$). For brevity, the management classes will hereafter be referred to as Natural, Ditched, Res05 and Res10, respectively.

Pine fen sites of low minerotrophy were selected on the basis of field observations, and historic aerial photographs were used to ensure that the original tree stands of the drained and restored sites were similar. Sites with naturally similar vegetation were located in a mosaic of ombro-mesotrophic peatland vegetation where *Sphagnum* was the main peat-forming plant genus. Surface water flow was considered independently for each management class, on the basis of topographic data and field observations. The thickness of the peat layer ranged from 95 cm to more than 200 cm and the underlying soil was till or sand.

Sites whose hydrology has not been altered by ditches or other direct measures represent the least impacted management class and are subsequently referred to as “Natural”. We acknowledge that forestry measures in distant parts of Natural site catchments may have slight impacts on their hydrology (Tahvanainen 2011), but such effects were not obvious. Ditched and Restored sites were drained for forestry purposes during the 1960s and 1970s. During the 1980s, peatlands were restored by filling in the ditches and removing trees from areas where drainage had significantly increased tree growth. More detailed information about the studied peatlands is given in Haapalehto *et al.* (2014).

Field sampling and sample processing

A grid of $20 \times 1\text{m}^2$ plots was established at each site (Figure 1). Plots at the Ditched and Restored sites were arranged in five parallel transects, spaced four metres apart, running perpendicular to the ditch. Within each transect there were plots at 5, 10 and 15 metres from the ditch. A $15\text{m} \times 20\text{m}$ grid of plots was used at each Natural reference site. The location of the first plot at each study site was randomised.

For water chemistry analyses at the Natural sites, three 32 mm diameter polypropylene pipe wells with 2 mm slits spaced at 2–3 cm intervals and polypropylene filter gauges were distributed in the central part of the sampling grid. At the Ditched and Restored sites filter gauges were set at a distance of 15 m from the ditch and 10 m apart (Figure 1). Water samples were collected in August 2007. Redox potential (E_{h}), electrical conductivity (EC) and pH were measured 2–5 hours after sample collection using a Consort SP50X meter with SP50X, SK10T and SP10B electrodes, respectively. The samples were then stored in darkness at $+4\text{ }^\circ\text{C}$ until further analysis. Cation concentrations (Al, Ca, Fe, K, Mg, Na) were obtained after filtration with $0.45\text{ }\mu\text{m}$ pore size filters and analysed with a Perkin Elmer Optima 4300 DV inductively coupled plasma

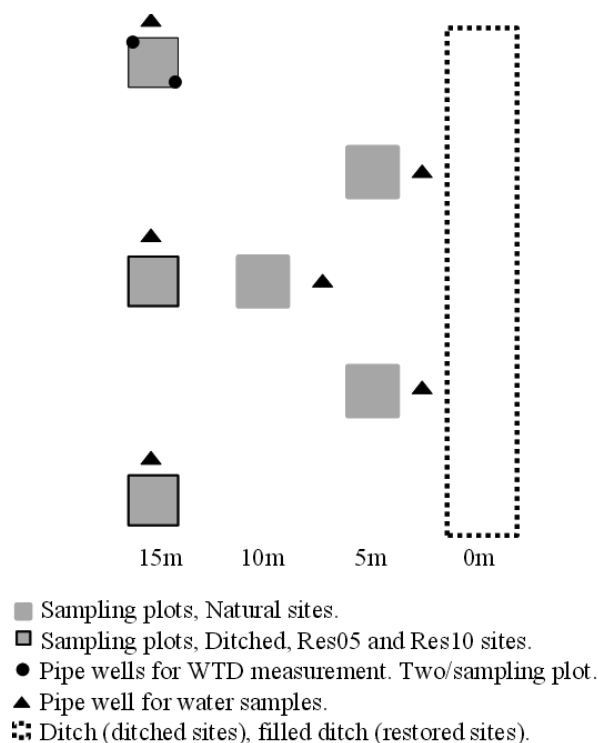


Figure 1. Sampling design for testate amoebae, plants and environmental variables. Distances (m) refer to distances of the 1m^2 plots from ditches at the Ditched and Restored sites. At Natural sites, a similar arrangement of plots was used. Res05: peatlands restored 3–7 years ago; Res10: peatlands restored 9–12 years ago.

optical emission spectrometer. Water table depth (WTD) was measured five times (in May, June, July, August and September) during 2008 in two permanent polypropylene pipe wells at selected plots. WTD values were corrected for the natural slope (see Haapalehto *et al.* 2014) and averaged to absolute levels throughout the site. WTD is usually recorded as negative values, but to facilitate readability, inverse positive values are used here.

Plants and TA were sampled at the plots located adjacent to the polypropylene pipe wells (three plots at each site). We estimated the relative abundance (percentage cover) of each taxon of vascular plants, bryophytes and some lichens for each sampled plot. Sampling was done during July and August 2007. To obtain TA, approximately 10cm^3 of bryophyte mass was extracted (see also Booth *et al.* 2010). Samples were stored in plastic Ziploc bags, frozen, defrosted and oven dried, stored in paper bags and analysed in 2013 using the protocol proposed by Booth *et al.* (2010). To retain TA, each sample was

boiled for ten minutes in distilled water with one tablet of *Lycopodium clavatum* spores (batch 1031) standard preparation from Lund University (Sweden), and sieved through a 300 µm mesh to remove coarse materials and onto a 7 µm mesh. Retained TA were centrifuged at 3000 rpm for five minutes and stored in distilled water. At least 150 TA were counted and identified using 40× magnification (Olympus BX41 microscope). As the standing plant community is the result of several years of development, non-living TA were also included in the analysis to integrate the accumulation of tests over the entire time period. TA identification was based on characteristics of the shell following several different taxonomic keys (e.g. Charman *et al.* 2000 with modifications by Booth 2008, Meisterfeld 2002, Clarke 2003, Mitchell 2002, Mitchell 2003, Mazei & Tsyganov 2006).

Data analysis

We calculated species richness, Shannon diversity index and relative abundances of TA and plant communities for each site (using plot averages), and averaged by management classes. For TA communities, density was estimated using the *Lycopodium* count as an external marker (see Stockmarr 1971 for details) and relative abundance was calculated as a percentage of the total counted. ANOVA and Tukey's (HSD) post hoc test were performed to assess differences in species richness and Shannon diversity index between management classes. Due to the unbalanced design, permutational MANOVA (PerMANOVA) was used to test the significance of the differences in structure and composition among the communities in the four management classes. Differences in water chemistry and WTD were analysed using ANOVA or Kruskal-Wallis depending on whether the data met the normality and homoscedasticity assumptions. To summarise variability in the communities, a Non-metric Multidimensional Scaling (NMDS) based on Sørensen's (Bray-Curtis) distance was performed. We used NMDS to examine the degree of concordance of water chemistry variables with community ordinations. WTD was not included in the community ordinations, as these data were not collected at exactly the same time as the biological samples. A two dimensional NMDS solution achieved a moderate stress level for plant communities but not for TA communities; however, for simplicity of comparison we retained the two dimensional TA solution. Concordance between TA and plant communities was tested using the PROTEST analysis (Peres-Neto & Jackson 2001).

All calculations were done using R version 3.0.2 (R Core Team 2013) and the vegan package for NMDS and PROTEST analyses (Oksanen *et al.* 2015).

RESULTS

Community data

Altogether, 44 TA and 45 plant taxa were found in our study. TA taxon richness at Natural sites ($\bar{X} = 17.7$ range 14–24, SE = 0.71) was higher than at Ditched, Res05, and Res10 sites (ANOVA $F_{[1,3]} = 15.79$, $p < 0.001$) whereas no differences were found among Ditched, Res05, and Res10 sites (Table 1, Figure 2a). The Shannon diversity index for TA was generally higher at Natural sites, where the highest value was 2.56, while the lowest values were found at Res05 sites (Figure 2c). We observed differences in TA diversity only between Natural-Res05 and Natural-Res10 sites (Table 1). The highest cell density was found at Res10 sites ($\bar{X} = 24953$ cells/10cm³) and the lowest at Ditched sites ($\bar{X} = 6767$ cells/10cm³).

No differences were found in the mean number of plant taxa between management classes (Figure 2b). In general, plant diversity tended to be higher at Res05 sites but, overall, there were no significant differences between classes ($F = 0.9$, $p = 0.46$; Table 1, Figure 2d). TA community structure and composition differed between classes except between Res05 and Res10 (Table 2). For plant communities, only Natural sites differed from all other classes (Table 2, see also Haapalehto 2014).

On average, TA communities in Natural, Res05, and Res10 sites were mainly dominated by *Arcella catinus* and *Bullinularia indica*. At Ditched sites, *Trigonopyxis arcula* was the most widely dominant taxon followed by *A. catinus*. More than 50 % of the TA species were found across all sites regardless of management class, but some species displayed more restricted distributions. For example, *Arcella artocrea*, *Centropyxis ecornis*, *Diffflugia leidyi*, *Diffflugia lithophila*, *Hyalosphenia minuta* and *Pseudodiffflugia fascicularis* were present at Natural sites only; while *Hyalosphenia elegans* and *Physochila griseola* were absent from Ditched and Res05 sites (Table 3). The plant communities at all sites were dominated by *Sphagnum angustifolium* regardless of management class. However, densities were highly variable. *Pleurozium schreberi* was the second most dominant species at Res05 and Ditched sites, while at Res10 and Natural sites *S. magellanicum* and *S. fuscum* were more abundant (see Haapalehto 2014).

Table 1. Tukey’s (HDS) results for TA taxon richness and Shannon diversity. Res05: peatlands restored 3–7 years ago; Res10: peatlands restored 9–12 years ago.

		Richness				Shannon Diversity			
		Difference	lower bound	Upper bound	<i>p</i>	Difference	lower bound	Upper bound	<i>p</i>
Natural	Res05	5.47	3.00	7.94	<0.001	0.64	0.23	1.04	0.002
	Res10	-5.47	-7.94	-3.00	<0.001	-0.60	-1.00	-0.19	0.003
	Ditched	4.64	1.96	7.33	<0.001	0.31	-0.12	0.74	0.205
Res05	Res10	0.00	-2.47	2.47	1.000	0.04	-0.37	0.44	0.993
	Ditched	-0.82	-3.51	1.86	0.847	-0.33	-0.76	0.10	0.171
Res10	Ditched	-0.82	-3.51	1.86	0.847	-0.29	-0.72	0.14	0.253

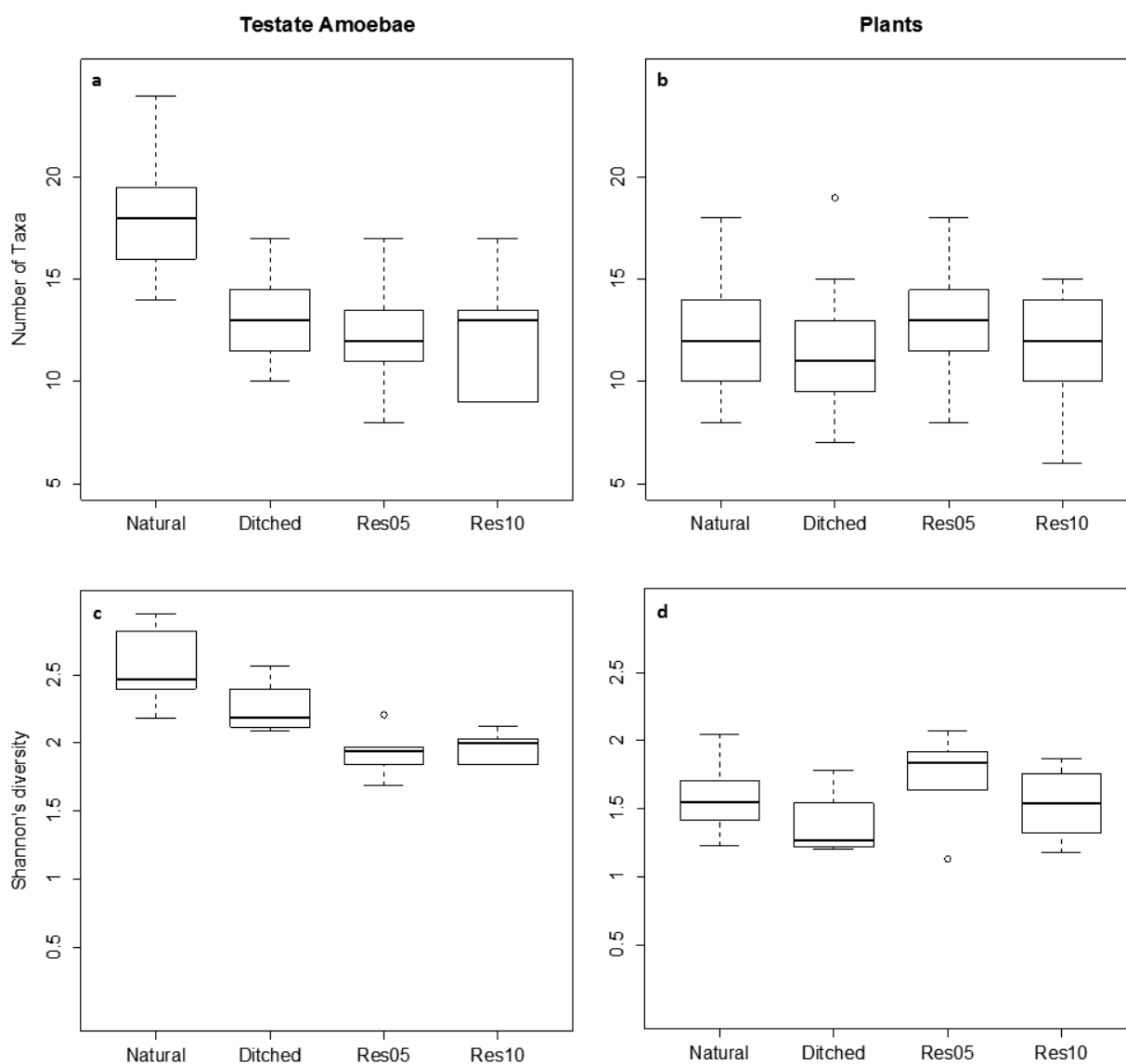


Figure 2. Taxon richness boxplots for testate amoebae (a) and vegetation (b). Shannon’s diversity boxplots for testate amoebae (c) and vegetation (d) for each management class. Circles: outliers, upper whisker: maximum value excluding outliers, upper box line: upper quartile, middle line inside box: median, lower box line: lower quartile, lower whisker: minimum value excluding outliers. Res05: peatlands restored 3-7 years ago; Res10: peatlands restored 9-12 years ago.

Table 2. PerMANOVA pairwise comparisons for TA and plant community structure and composition. Res05: peatlands restored 3-7 years ago; Res10: peatlands restored 9-12 years ago.

		TA						Plants					
		df	SS	MS	Pseudo-F	r ²	p	df	SS	MS	Pseudo-F	r ²	p
Natural	Res05	1	0.28	0.28	2.74	0.25	0.021	1	0.44	0.44	3.23	0.29	0.042
	Res10	1	0.29	0.29	4.02	0.33	0.016	1	0.15	0.15	1.83	0.19	0.047
	Ditched	1	0.48	0.48	4.90	0.41	0.006	1	0.46	0.46	4.11	0.37	0.016
Res05	Res10	1	0.09	0.09	1.36	0.15	0.259	1	0.33	0.33	2.35	0.23	0.104
	Ditched	1	0.36	0.36	3.90	0.36	0.036	1	0.12	0.12	0.66	0.09	0.499
Res10	Ditched	1	0.59	0.59	10.17	0.59	0.010	1	0.35	0.35	2.98	0.30	0.093

Table 3. TA average relative abundances in percentage and standard deviations for each management class. (*) Taxa found just at natural sites; (**) taxa found at Natural and in sites restored 10 years ago; (***) taxa found at all but Ditched sites; (****) taxa found just at Ditched sites. Res05: peatlands restored 3-7 years ago; Res10: peatlands restored 9-12 years ago.

Taxa	Treatments							
	Natural		Ditched		Res05		Res10	
	X̄	SD	X̄	SD	X̄	SD	X̄	SD
* <i>Arcella artocrea</i>	0.78	1.56	0	0	0	0	0	0
* <i>Centropyxis ecornis</i>	0.09	0.33	0	0	0	0	0	0
* <i>Diffugia leidy</i>	0.05	0.17	0	0	0	0	0	0
* <i>Diffugia lithophila</i>	0.04	0.17	0	0	0	0	0	0
* <i>Hyalosphenia minuta</i>	0.53	0.85	0	0	0	0	0	0
* <i>Pseudodiffugia fascicularis</i>	0.04	0.16	0	0	0	0	0	0
** <i>Hyalosphenia elegans</i>	3.60	3.93	0	0	0	0	0.22	0.48
** <i>Physochila griseola</i>	0.67	1.03	0	0	0	0	0.09	0.35
*** <i>Heleopera petricola</i>	2.56	3.76	0	0	0.34	0.63	0.12	0.34
**** <i>Arcella vulgaris</i>	0	0	0.11	0.37	0	0	0	0
**** <i>Euglypha compressa</i>	0	0	0.11	0.37	0	0	0	0
**** <i>Hyalosphenia subflava</i>	0	0	0.12	0.41	0	0	0	0
**** <i>Phryganella acropodia</i>	0	0	0.06	0.19	0	0	0	0
**** <i>Trigonopyxis minuta</i>	0	0	1.14	3.79	0	0	0	0
**** <i>Unidentified</i>	0	0	0.34	0.81	0	0	0	0
<i>Archerella flavum</i>	6.02	6.27	0.06	0.19	0	0	0.53	1.71
<i>Arcella catinus</i>	20.96	20.17	14.05	16.46	37.44	17.27	44.95	30.18
<i>Arcella discoides</i>	0.13	0.49	0.41	1.03	0.48	1.17	0.16	0.63
<i>Argynnia vitrea</i>	0.18	0.52	0	0	0.04	0.16	0	0
<i>Assulina muscorum</i>	4.86	4.54	4.92	2.85	3.62	2.62	3.03	3.18
<i>Assulina seminulum</i>	0.56	0.89	0.29	0.62	0.09	0.24	0.04	0.17
<i>Bullinularia indica</i>	8.43	5.41	13.08	10.94	16.81	19.60	10.21	11.62
<i>Centropyxis aculeata</i>	1.18	2.90	2.29	4.92	0.29	1.11	1.93	4.17
<i>Centropyxis cassis</i>	0.05	0.17	6.61	6.38	2.02	5.41	0.24	0.65
<i>Centropyxis platystoma</i>	0	0	0	0	0.04	0.17	0	0
<i>Corythion spp.</i>	1.95	2.93	3.47	3.71	4.23	3.67	3.93	5.70
<i>Cyclopyxis arcelloides</i>	0.48	0.66	6.01	5.82	0	0	0.35	0.66
<i>Diffugia globulosa</i>	0.04	0.16	2.33	5.04	0	0	0.13	0.51
<i>Diffugia lucida</i>	0.11	0.30	0.06	0.20	0.04	0.17	0	0
<i>Euglypha rotunda</i>	3.55	4.16	0.97	1.61	0.83	1.04	3.00	3.39
<i>Euglypha strigosa</i>	3.79	3.30	1.23	1.60	3.01	2.86	2.36	2.31
<i>Euglypha tuberculata</i>	1.23	2.07	3.97	6.65	3.65	3.29	2.07	4.09
<i>Heleopera rosea</i>	1.23	1.81	0.18	0.31	0.45	1.23	0.95	1.36
<i>Heleopera sphagni</i>	5.86	9.24	0.28	0.74	0.68	1.24	0.22	0.48
<i>Heleopera sylvatica</i>	3.02	4.12	0.18	0.43	0.68	1.28	0.47	0.56
<i>Hyalosphenia papilio</i>	7.99	8.01	0.29	0.61	4.43	9.75	8.97	10.84
<i>Nebela bohémica</i>	0	0	0	0	0.04	0.15	0.71	2.06
<i>Nebela flabellulum</i>	0	0	0	0	0.04	0.16	0	0
<i>Nebela militaris</i>	4.21	3.74	2.03	3.49	3.15	3.99	1.07	1.11
<i>Nebela parvula</i>	1.12	1.86	0.17	0.57	0.22	0.86	0.27	0.55
<i>Nebela tincta</i>	5.34	3.42	5.75	6.01	5.15	4.61	4.65	3.41
<i>Pseudodiffugia fulva</i>	0.04	0.17	0.43	1.43	0	0	0	0
<i>Trigonopyxis arcuata</i>	5.13	5.80	26.61	15.36	7.24	5.86	3.22	3.63
<i>Trinema lineare</i>	4.17	6.44	2.43	3.23	4.99	4.59	6.10	7.17

Community-environment relationships and community concordance

Water chemistry did not vary significantly between sites (Table 4). However, in some cases (mainly for Al and Mg) chemical concentrations were below the detection limits and were recorded as 0. In general, the highest mean values for studied elements apart from Fe were recorded at Ditched sites (Figure 3). WTD varied between the management classes (Table 4) and between months, with the lowest depths recorded at the Ditched sites (Figure 4).

A two-dimensional solution NMDS ordination of the TA communities produced the lowest stress=0.207. Ca and K were significantly correlated with TA community variability ($p=0.02$ and $p=0.01$, respectively; Figure 5a). The ordination of the plant community resulted in a two-dimensional solution (final stress=0.168) with significant relation to Fe ($p<0.01$), K ($p<0.01$) and Na ($p=0.04$; Figure 5b).

Table 4. ANOVA (*) and Kruskal-Wallis (**) for water chemistry variables. Calculations are based on average values for each site over all management classes.

	<i>df</i>	χ^2/F	<i>p</i>
*Al	3	2.47	0.481
*Fe	3	2.97	0.397
*Mg	3	4.72	0.194
*Ca	3	7.30	0.063
*K	3	5.14	0.162
**Na	3	2.75	0.079
**EC	3	0.27	0.843
**pH	3	0.89	0.470
**Eh ₇	3	2.06	0.148
**WTD	3	4.16	0.025

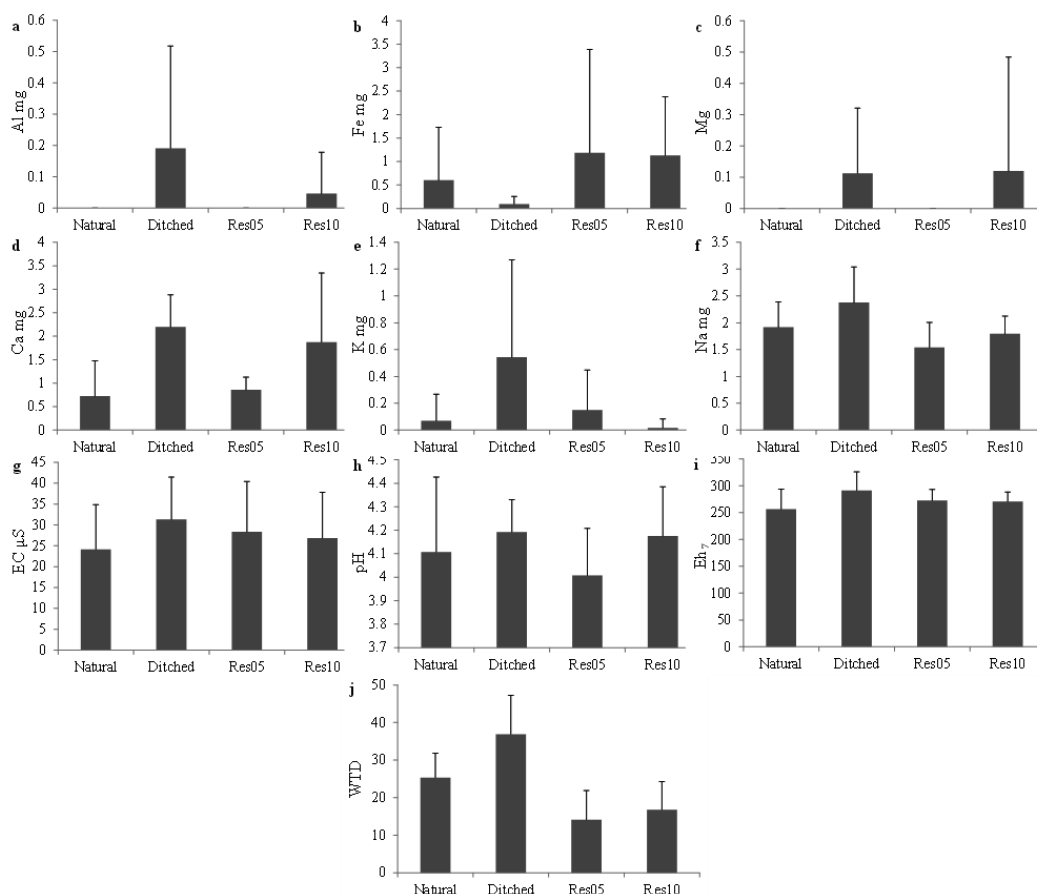


Figure 3. Average values and standard deviations of the environmental variables measured for each land use management class: (a) aluminum, (b) iron, (c) magnesium, (d) calcium, (e) potassium, (f) sodium, (g) conductivity, (h) pH, (i) redox potential, (j) water table depth. Res05: peatlands restored 3–7 years ago; Res10: peatlands restored 9–12 years ago.

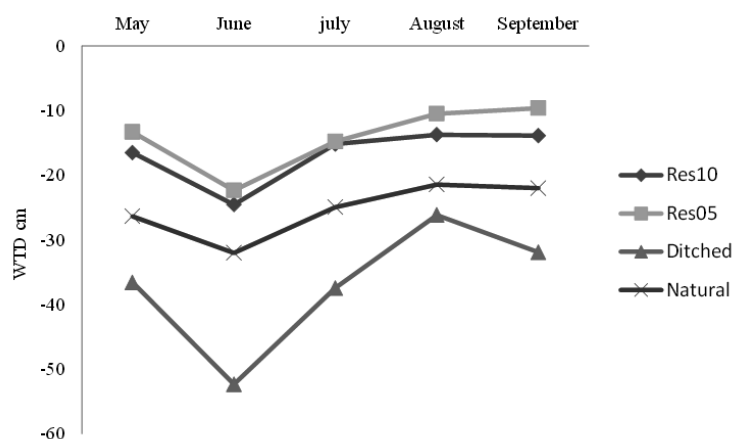


Figure 4. Monthly water table depth variation in 2007. Res05: peatlands restored 3–7 years ago; Res10: peatlands restored 9–12 years ago.

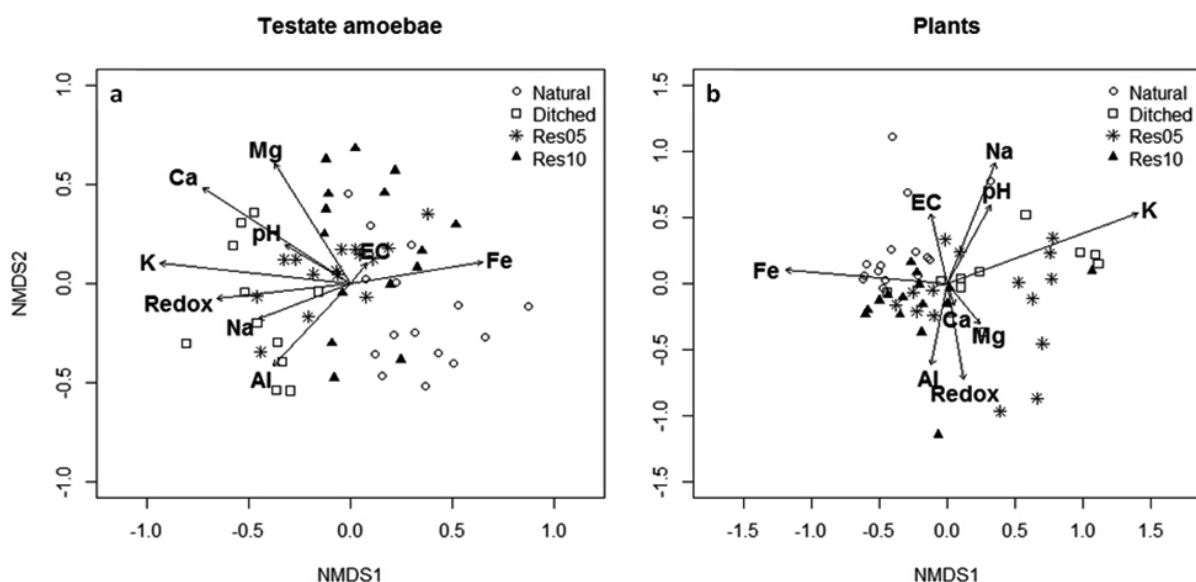


Figure 5. NMDS ordinations of testate amoebae (a) and plant community (b) relationships among management classes. Distance measure: Sørensen (Bray-Curtis). Arrow lengths represent the vector loadings of environmental variables. Res05: peatlands restored 3–7 years ago; Res10: peatlands restored 9–12 years ago.

NMDS plots grouped TA communities by management classes. A separation for communities between the Ditched and Natural sites was observed on NMDS1. Res05 sites were mainly grouped in the centre of the ordination and overlapped with all other management classes, while Res10 sites were fully separated from Ditched sites and more closely grouped to Natural sites (Figure 5a). Ordination of plant communities showed greater variation compared to TA and less clear separation along

NMDS1; however, group separation was observed for Natural and Ditched sites (upper left and centre right in the ordination, respectively). Res05 was mostly grouped closer to Ditched sites overlapping only partly with Res10, whereas Res10 sites clustered closer to Natural sites (Figure 5b).

To look for concordance between plant and TA communities we first ran separate analyses to compare TA with moss species and TA with vascular plant species (results not shown). However,

as no differences were found, both moss and vascular plants were grouped to form a plant community in all subsequent analyses. We found a significant but weak concordance between TA and plant communities (PROTEST $r=0.44$, $m^2=0.79$, $p=0.001$). To assess whether TA and plant communities were concordant within classes, we ran PROTEST separately for each. Results indicated that only communities at Res05 sites were concordant, but even this relationship was weak (PROTEST $r=0.54$, $m^2=0.70$, $p=0.01$).

DISCUSSION

Natural sites showed higher TA richness with more diverse communities than other management classes. However, we did not find similar results for plant species richness. Res10 sites showed the lowest TA richness and diversity, which could be attributed to the high dominance of *Arcella catinus* at many of the sites, where this species accounted for more than 70 % of the community. Spatial limitation is less likely to occur in soils than in above-ground environments (Wanner & Xylander 2005). Hence, TA might face less competitive pressure as different taxa may simply occupy different ecological niches, whereas plant species tend to compete for the same space and nutrients. As Natural sites have not undergone extreme environmental changes compared to the other management classes, their higher taxon richness could be attributed to a longer time for cumulative colonisation without a significant TA taxon replacement. This supports the observations by Wanner & Xylander (2005), who studied TA colonisation and successional processes and found temporal changes in TA species composition but very little or no species replacement.

Restoration efforts to rehabilitate peatlands focus mainly on raising the water table to recreate natural habitat conditions. Substratum moisture is often found to be the main factor controlling TA communities (Tolonen 1986), particularly in *Sphagnum* dominated peatlands (Booth 2001), with higher densities of TA cells usually found in soils with high water holding capacity (Fournier *et al.* 2012). Despite the lack of corresponding moisture data, we assume that restoration measures increased peat moisture due to elevated water table levels. As a consequence, increasing TA cell densities could be expected at restored sites over time. We did observe such patterns, as the highest TA densities were found at Res10 sites and the lowest at Ditched sites.

Testate amoebae communities at the wettest sites (Natural, Res10 and Res05) were mostly dominated by *A. catinus* and *B. indica*, while taxon dominance at Ditched sites shifted towards *T. arcuata*. However, both *A. catinus* and *B. indica* were highly abundant across all sites suggesting that, although these species are considered indicators of dry conditions, they can display a broad moisture tolerance. Some less abundant species such as *A. artocrea*, *C. ecornis*, *D. leidyi*, *D. lithophila*, *H. minuta* and *P. fascicularis* were found exclusively at Natural sites. This suggests that not all species recolonise even within a decade after restoration. Poor recolonisation by some typical plant species after restoration (Haapalehto *et al.* 2011, Hedberg *et al.* 2012) highlights the need to avoid degradation of natural habitats, as species that disappear from a site may be very difficult to reinstate without costly and uncertain re-introduction programmes (see also Moreno-Mateos *et al.* 2015). *H. elegans* and *Ph. griseola* occurred only at Natural sites and sites restored ten years ago, suggesting either that they have a longer recolonisation time or that disturbed habitats are just not appropriate for those species. Among plant species, *S. angustifolium* remained dominant across all sites. This species has a wide ecological niche and is known to survive in drained peatlands. However, *S. angustifolium* densities increased rapidly following restoration measures (Haapalehto *et al.* 2011).

The ordinations showed significant relationships of TA communities with Ca and K. Strong relationships between Ca and TA communities have been reported before (e.g. Lamentowicz *et al.* 2010, Hájková *et al.* 2011, Raabe & Lamentowicz 2012, Jassey *et al.* 2014). These could be due to the importance of Ca for shell building in some TA species (Lamentowicz *et al.* 2011). TA relationships with other nutrients have also been observed (e.g. Mitchell *et al.* 2000b, Jauhiainen 2002, Mitchell *et al.* 2004) but their direct effects on TA are not fully understood. Such correlations could arise when water chemistry and nutrient availability indirectly affect TA communities by controlling their prey organisms such as bacteria, fungi, other Protista and micrometazoa (Mitchell *et al.* 2004).

Plants were strongly related to levels of K and Fe, indicating the vital importance of pore water chemistry. The highest K concentrations were found at Res05 sites, and the lowest in Natural and Res10. As an easily leachable cation, K may be released from dying biomass and rewetted organic material after hydrological restoration. Hence, the lower concentrations of K in pore water at Res10 sites

might indicate that K is efficiently taken up during the regrowth of *Sphagnum* and other peatland species. Additionally, after ditch filling small ponds (i.e. topographically lower areas) are formed at restored sites enabling the establishment of anaerobic and reducing conditions that could favour reduction of Fe³⁺ to Fe²⁺ (Haapalehto *et al.* 2014). The reduced form of Fe is readily available for uptake by plants and facilitates plant regrowth.

In NMDS ordination vegetation communities displayed more within-class variation than did TA communities. While TA communities differed between Ditched & Res5 (PerMANOVA), plants did not show differences. These results suggest that TA taxa may be better suited than plants as indicators of early restoration success, on account of their rapid response to hydrological change. Indeed, this was observed by Warner & Chmielewski (1992), who found changes in TA taxa composition within 2½ years of peatland drainage. Talbot *et al.* (2010), who studied peat cores from drained peatlands, also found that TA species indicative of dry conditions reached their highest percentages around the time of drainage, suggesting a very quick response potential of the TA communities to environmental disturbance. Our results highlight the severity of hydrological disturbance by drainage as TA communities still displayed clear differences from those at Natural sites even a decade after restoration. Observed long-term drainage impacts on TA are paralleled by changes in plant communities after drainage (Laine *et al.* 1995, Kareksela *et al.* 2015). From visual inspection of NMDS, plant communities ten years after restoration appeared more similar to those of Natural sites. Maanaviilja *et al.* (2014) suggested that only a large rise in water table level would induce significant changes in vegetation communities. In our study plants were sampled 15 m from the ditch where water table changes due to drainage and restoration are smallest and where plant community compositions still largely overlap (Haapalehto *et al.* 2014, Kareksela *et al.* 2015). Finally, Wardle (2002) pointed out that aboveground vegetation communities and soil microorganisms differ in their resistance, resilience, adaptation strategies and dispersal abilities. These differences might also relate to the observed time lags in responses of TA and plant communities to restoration measures.

When comparing all sites, TA and vegetation communities were concordant although this relationship was weak. When each management class was separately analysed we found no community concordance within the management

classes except in the case of Res05 sites. Some studies on stream community concordance (e.g. Infante *et al.* 2009) have suggested scale dependency, which could explain the patterns observed. However, the fact that concordance between TA and plant community was found to be weak, and within-class community concordance apparently lacking, suggests that TA and vegetation community composition are determined in different ways except during the early post-restoration period. Thus, our results suggest that, overall, communities i) respond differently to the same set of environmental factors, or ii) are affected by different environmental factors over timespans exceeding five years, or iii) have different timescales of succession processes, and/or iv) display low overall interaction. Additionally, from their comparison of vascular plants, bryophytes, fungi, diatoms, desmids and TA, Hájek *et al.* (2014) suggested that body size and lifespan play key roles in determining concordance between communities.

In peatland ecosystems, microtopographical transitions can occur at scales of a few centimetres and it is important to avoid the associated ecological gradients, as far as possible, when designing sampling schemes. Gradients at sub-centimetre scales may also be relevant in the context of TA (Mitchell *et al.* 2000a). Vertical variation in habitat conditions is caused mainly by differences in chemical composition between groundwater and rainwater, and is enhanced by cation exchange in the presence of *Sphagnum*. Mitchell *et al.* (2000b) suggest that, because moss and TA species are totally dependent on microsite conditions, they might show different responses than vascular plants which access ‘soil’ water *via* roots at various depths. However, in separate analyses to compare TA with moss species and TA with vascular plant species, we found no differences in concordance patterns (results not shown).

Restoration strategies such as rewetting of drained peatlands aim primarily to restore vegetation cover (e.g. Similä *et al.* 2014), and this makes plant communities the obvious indicators for restoration success. Our results suggest that the effects of restoration on other components of the peatland ecosystem (e.g. TA communities) may not be properly reflected by the use of vegetation-based indicators alone. Studies of the microbial loop in peatlands by Gilbert *et al.* (1998) revealed that TA, using a wide range of organisms as prey, can account for almost half of the microbial community. Through their feeding TA regulate bacterial biomass and contribute to nutrient mineralisation, nutrition

of soil animals, and plant growth. Griffiths (1994) found that Protista (mainly TA) can account for 20–40 % of N mineralisation as they excrete bacterial N into the soil. Because the TA communities of Res05 sites were more similar than those of Ditched sites to the TA communities of the targeted original ecosystem, restoration by rewetting appears to be efficient in inducing rapid recovery of an important structural component of the ecosystem.

While the relationship is still poorly understood, previous studies suggest that recovery of some important ecosystem functions (e.g. surface peat accumulation) precedes the recovery of ecosystem structure (Kareksela *et al.* 2015). In accordance with that study, our results suggest that recovery of the community structure of TA precedes that of plants and may even be a prerequisite for the recovery of higher plants and ecosystem functions. Thus, TA could play a fundamental role in facilitating plant succession by providing nutrients and consolidating the assembly process on newly exposed land surfaces (Hodkinson *et al.* 2002).

Despite some similarities in early responses, we conclude that TA and vegetation communities cannot be used as surrogates for one another when measuring restoration success. To gain a holistic understanding of the changes in important ecosystem components during restoration from an ecosystem perspective requires the use of both vegetation and TA communities, as also suggested by Raabe *et al.* (2012). However, the shorter generation time of TA enables them to respond more rapidly to environmental changes than vegetation, which makes them better early indicators of restoration success - particularly because post-restoration successional changes in TA communities appear to be targeted towards re-establishment of the site's original community composition. Our results also suggest that further studies on the relationship between TA and plant communities could provide important insights into understanding how the crucial link between ecosystem structure and functions operates during recovery. Better assessment of the dynamics and interplay of post-restoration recolonisation processes for plant and TA communities in the boreal region will require longer-term studies than were achievable here.

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II

TESTATE AMOEBAE COMMUNITY ANALYSIS AS A TOOL TO ASSESS BIOLOGICAL IMPACTS OF PEATLAND USE

by

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ABSTRACT

As most ecosystems, peatlands have been heavily exploited for different human purposes. For example, in Finland the majority is under forestry, agriculture or peat mining use. Peatlands play an important role in carbon storage, water cycle, and are a unique habitat for rare organisms. Such properties highlight their environmental importance and the need for their restoration. To monitor the success of peatland restoration sensitive indicators are needed. Here we test whether testate amoebae can be used as a reliable bioindicator for assessing peatland condition. To qualify as reliable indicators, responses in testate amoebae community structure to ecological changes must be stronger than random spatial and temporal variation.

In this study, we simultaneously assessed differences between the effects of seasonality, intermediate scale spatial variation and land uses on living testate amoebae assemblages in natural, forested and restored peatlands. We expected the effects of seasonality on testate amoebae communities to be less pronounced than those of land use and within site variation.

On average, natural sites harboured the highest richness and density, while the lowest numbers were found at forestry sites. Despite small changes observed in taxa dominance and differences in TA community structure between seasons and years at some sites, spatial heterogeneity, temperature, pH, nor water table depth seemed to significantly affect testate amoebae communities. Instead, observed differences were related to type of land use, which explained 75% of the community variation. Our results showed that testate amoebae community monitoring is a useful tool to evaluate impacts of human land use on boreal peatlands.

Keywords: Bioindicators, boreal peatlands, forestry, land uses, peatland restoration.

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INTRODUCTION

While peatlands of the boreal and subarctic regions cover only 3% of the global area, they store ca. 500Gt of C corresponding to one third of the terrestrial carbon storage (Yu 2011, 2012). Roughly 50

% of the total Finnish peatland area has been used for forestry, followed by 2.6% for agriculture, and 0.6% for peat mining (Lappalainen 1996; Vasander et al. 2003). Peatland use in Finland is mainly concentrated to the central and southern parts of the country where less than 25% of the peatland area is pristine (Aapala et al. 1996).

Besides their importance as carbon storage, peatlands play an important role in balancing the water cycle. They are also a unique habitat for many organisms including many rare and endangered species and form repositories of paleontological information through the accumulation and storage of remains of flora, fauna, and atmospheric particles (Gorham 1991; Barber 1993). Thanks to these properties, there is an increased attention on the environmental importance of peatlands and the need for restoration of impacted areas to regain lost ecosystem services (e. g. specific biodiversity, carbon sink, etc.; Lunn and Burlton 2013). To accurately determine whether ecosystem structure and functioning are indeed moving towards near-pristine state following restoration attempts, sensitive, yet robust indicators are needed.

To date, there is no evidence that any specific indicator (be it biological, chemical, or physical) outperforms others in indicating changes in peatlands or their restoration success (Chapman et al. 2003). Indeed, in the case of biological indicators, it is well known that different taxa respond differently to ecosystem dynamics and environmental gradients (Francez et al. 2000). However, assessments of peatland plant community composition to study restoration success have shown some promising results (e. g. Haapalehto et al. 2011; Laine et al. 2011; Hedberg et al. 2012; Poulin et al. 2013) but little is known about responses of other important components of peatland ecosystems. Recently, the search for efficient indicators has directed the focus on testate amoebae because they possess several beneficial qualities (e. g. Mitchell et al. 1999; Charman 2001; Koenig et al. 2015; Daza Secco et al. 2016). First, testate amoebae (TA) are shell-building protists (Charman 1999), found in a wide range of habitats e.g. soils, lakes, rivers, they are very strongly associated with peatland plants and especially abundant in *Sphagnum* mosses (Tolonen 1986). Second, TA density can be as high as 16×10^6 individuals per m^2 and even in boreal environments TA can produce several generations per year (Sleigh 1989). Third, TA are a vital component of the microorganism community in *Sphagnum*-dominated peatlands where they account for almost half of the community in terms of biomass (Gilbert et al. 1998). Lastly seasonality is generally not expected to significantly affect the TA communities since they can survive throughout the year through their encysting capacity (Gilbert and Mitchell 2006). These factors could make TA a valuable

tool in the assessment of short -and long- term responses of peatlands to land use changes (e. g. Koenig et al. 2015; Daza Secco et al. 2016). However peatland microorganisms can exhibit microtopographic transitions at scales of few centimetres (Mitchell et al. 2000b) and previous studies on temporal (Warner et al. 2007) and spatial TA community variation (Mitchell et al. 2000b) highlight the need to account for spatial variation when trying to assess seasonal community changes. Thus, to which degree spatial or temporal variation in community structure may confound the use of TA in routine monitoring of peatlands is yet unknown and warrants study.

In this study, we simultaneously assess differences between the effects of seasonality, intermediate scale spatial variation and land uses on living TA assemblages in i) natural peatlands, ii) forested peatlands and iii) restored peatlands. We expected the effects of seasonality on TA communities to be less pronounced than those of land use and within site variation.

MATERIALS AND METHODS

Study sites

Study sites were chosen to represent three stages of peatland use: pristine, forested, and restored. We assumed that if restoration was successful, sites restored many decades ago were likely to resemble natural sites more than sites actively forested ones.

All the studied peatlands are raised bogs, which represent the prevalent peatland type within a mosaic of *Sphagnum*-dominated peatlands, coniferous forests and lakes in the boreal zone of central Finland. Sites were chosen based on their similar characteristics such as elevation, mean annual temperature, and mean annual precipitation (Table 1). Each land use was represented by two peatlands: Riihineva and Aittosuo (natural), Lahnanen and Ruuskanlampi (forestry), and Aitoneva60 and Aitoneva80 (restored).

Table 1. Sampling sites coordinates and elevation by land use. Elevation is given in meters above sea level. Land use refers to: Natural: peatlands not under direct human influence, Forestry: peatlands used for forestry, Restored: peatlands previously under human use but restored either 60 or 80 years ago.

Land use	Coordinates		Elevation (m.a.s.l.)	Temperature (mean annual °C)	Precipitation (mean annual mm)
	Longitude	Latitude			
Natural	25° 28' 9-24° 37' 53" E	61° 50' 43"-62° 45' 15" N	ca. 150	3	600
Forestry	25° 28' 9-24° 37' 53" E	61° 50' 43"-62° 45' 15" N	ca. 150	3	600
Restored	23° 18' 19.3248" E	62° 10' 52.1544" N	ca. 100	4	650

While *Sphagnum* mosses mainly dominated at natural sites, Aittosuo had a higher coverage of shrubs and trees whereas mosses almost exclusively covered Riihineva. At the forestry sites Lahnanen and Ruuskanlampi, vegetation was mainly composed of brown and *Sphagnum* mosses with high presence of trees and bushes. Lahnanen was mainly a dry site surrounded by ditches while Ruuskanlampi displayed a patchwork of flooded and very dry spots and highly diversified microhabitats. Restored sites Aitoneva were previously used for peat extraction and were restored either 60 (Aitoneva60) or 80 (Aitoneva80) years ago by blocking ditches and rewetting the sites to restore previous hydrological conditions in order to allow natural peatland species to recolonise. Aitoneva60 particularly, displayed the highest water table of all sites, and it was flooded most of the year with small streams crossing the site. On average, the highest water table depths were found at restored sites while lowest at forestry sites (Fig. 1).

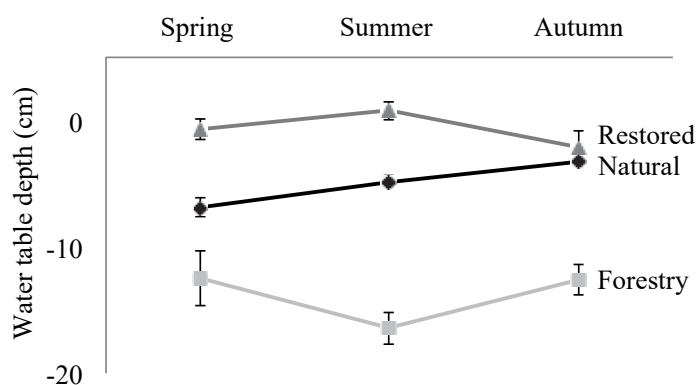


Fig. 1 Average water table depth by season for the three land uses. Values correspond to measurements during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling plots/site at each sampling time. Restored: peatlands previously under human use but restored either 60 or 80 years ago, Natural: peatlands not under human use, Forestry: peatlands used for forestry. Y-axis:

water table depth given in centimetres below ground level. Points represent mean values; bars represent SE (± 1).

Field sampling and sample processing

Starting from a randomly selected point, fifteen 100 cm²-sampling plots were placed at each site and distributed in three concentric semicircles of five plots each with ca. 1m spacing (Fig. 2). For water table depth measurements (WTD), 2cm diameter polypropylene pipe wells with 2mm slits at every 3cm were placed at the top-left corner of each sampling plot. Water table depth was recorded as negative values when the water level was below ground (top of moss layer), and as positive values when it was above ground (flooded plots). Water temperature and water pH were measured in the middle of each sampling plot using a VWR pH meter1000H. For TA samples, bryophyte mass was collected using a knife, including only the stem and capitulum of the mosses (ca. 10 cm) in order to obtain mainly living TA (see Booth et al. 2010). Samples were stored in Ziploc bags at 5°C before further analysis. TA samples were taken during spring (May), summer (July), and autumn (September) in three consecutive years (2013, 2014 and 2015). WTD, pH and water temperature from the peat layer were measured during each TA sampling.

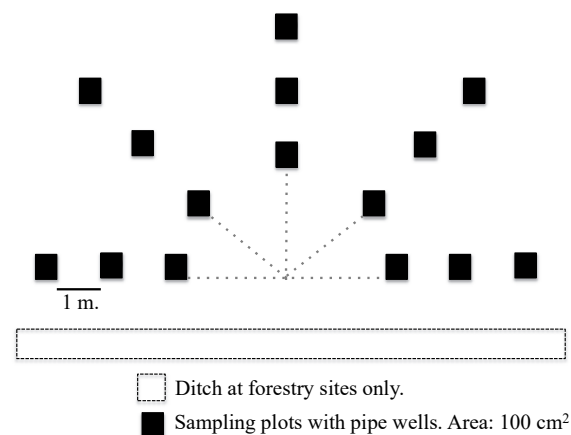


Fig. 2 Schema of the sampling design for testate amoebae, pH, temperature and water table depth.

TA samples were analysed during the following days immediately after sampling in order to account only for the living TA. Sample processing was carried using the protocol proposed by Booth et al. (2010). Each sample was boiled for ca. 10 minutes in distilled water with one tablet of *Lycopodium clavatum* spores (batch 1031) standard preparation from Lund University (Sweden). To remove coarse materials, samples were sieved through 300 μ m mesh and further filtered onto a 7 μ m mesh to retain TA

and transferred into a centrifuge tube. TA were centrifuged at 3000rpm for five minutes and stored in distilled water. Counting and identification of living TA (empty shells were not taken into account) was done using a 40X magnification (Olympus BX41 microscope). TA were identified mainly to species or species groups based on characteristics of the shell following a number of different taxonomic keys (e.g. Charman et al. 2000; Meisterfeld 2002; Clark 2003; Mazei and Tsyganov 2006).

Data analysis

TA number of taxa and relative abundances were calculated for each site (using plot averages), and averaged by land use. Concentrations of TA in 10 cm³ of fresh *Sphagnum* were estimated using the *Lycopodium* counts as an external marker (Stockmarr 1971). The Shannon Wiener diversity index (Shannon and Weaver 1963) was used to estimate TA diversity and a non-parametric Kruskal-Wallis H analysis (Van Hecke 2013) was used to check for temporal differences in TA richness and diversity. TA taxa not frequently found were excluded from ordination analysis to avoid noise. We applied a model-based ordination method as suggested in Hui et al. (2015) and Warton et al. (2015) to visualize the main patterns between different sampling sites in terms of the taxa composition. A model-based ordination approach offers several advantages over traditional distance-based ordination methods such as non-metric multidimensional scaling (NMDS). The main advantage is that models can be used to account for important features such as the mean-variance relationship. For a thorough comparison of model-based and traditional ordination methods, see Hui et al. (2015). A latent variable model with two latent variables was fitted to the data assuming a negative binomial distribution for the TA density. An ordination plot was then produced based on the bivariate latent variables. As the samples were collected in three seasons, we first fitted latent variable models to data sets corresponding to different seasons separately. Lastly, a latent variable model with covariates was fitted in order to identify drivers of amoeba community composition.

RESULTS

Environmental variables

Environmental variables generally displayed clear patterns during all study years. On average, pH was highest at restored sites, especially at Aitoneva60. Surface water temperature of the peatlands was less variable between sites, but values were slightly higher at the natural site Riihineva (Fig. 3).

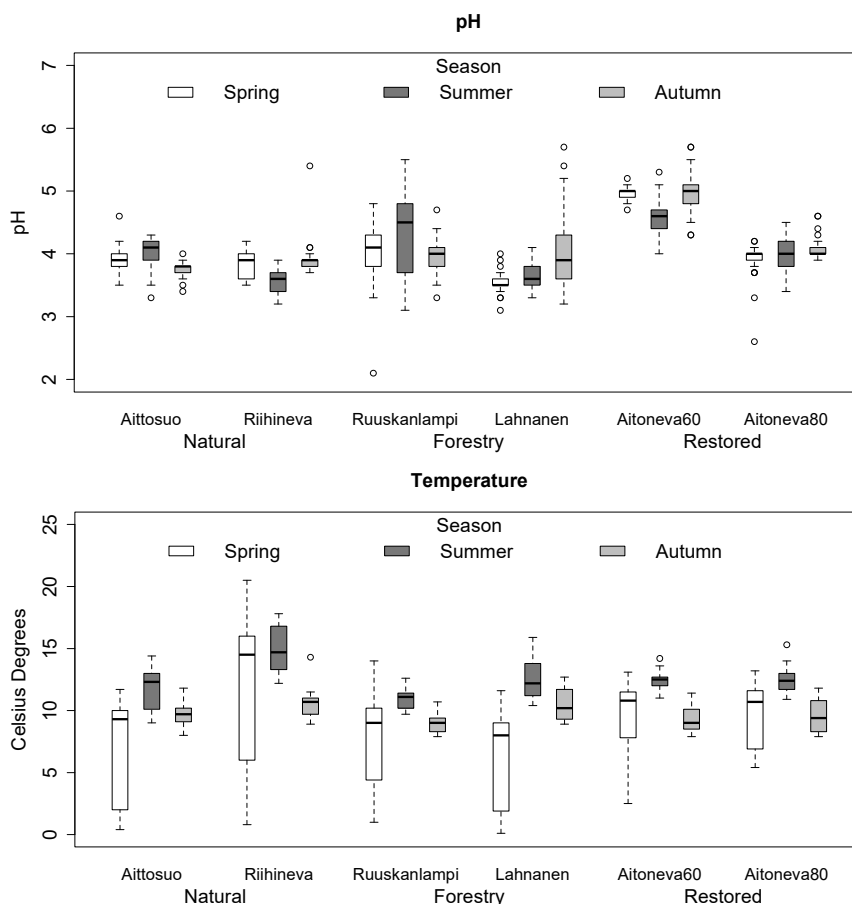


Fig. 3 Environmental variables (surface water pH and temperature) in relation to land use and season of sampling. Values correspond to measurements during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling plots/site at each sampling time. Natural: peatlands not under human use, Forestry: peatlands used for forestry, Restored: peatlands previously under human use but restored either 60 or 80 years ago. Circles: outliers, upper whisker: maximum value excluding outliers, upper box line: upper quartile, middle line inside box: median, lower box line: lower quartile, lower whisker: minimum value excluding outliers.

TA community data

We found altogether 62 TA taxa in our study. The highest number of taxa was found at natural sites except in spring 2013 when number of taxa was highest at restored sites (Fig. 4). On average, forestry sites harboured the lowest number of taxa (Fig. 4). TA densities showed a less distinct pattern. Some particularly high densities ($>150,000/10\text{cm}^3$) of TA were recorded in autumn at Aittosuo and Ruuskanlampi (natural and forestry, respectively; Fig. 4).

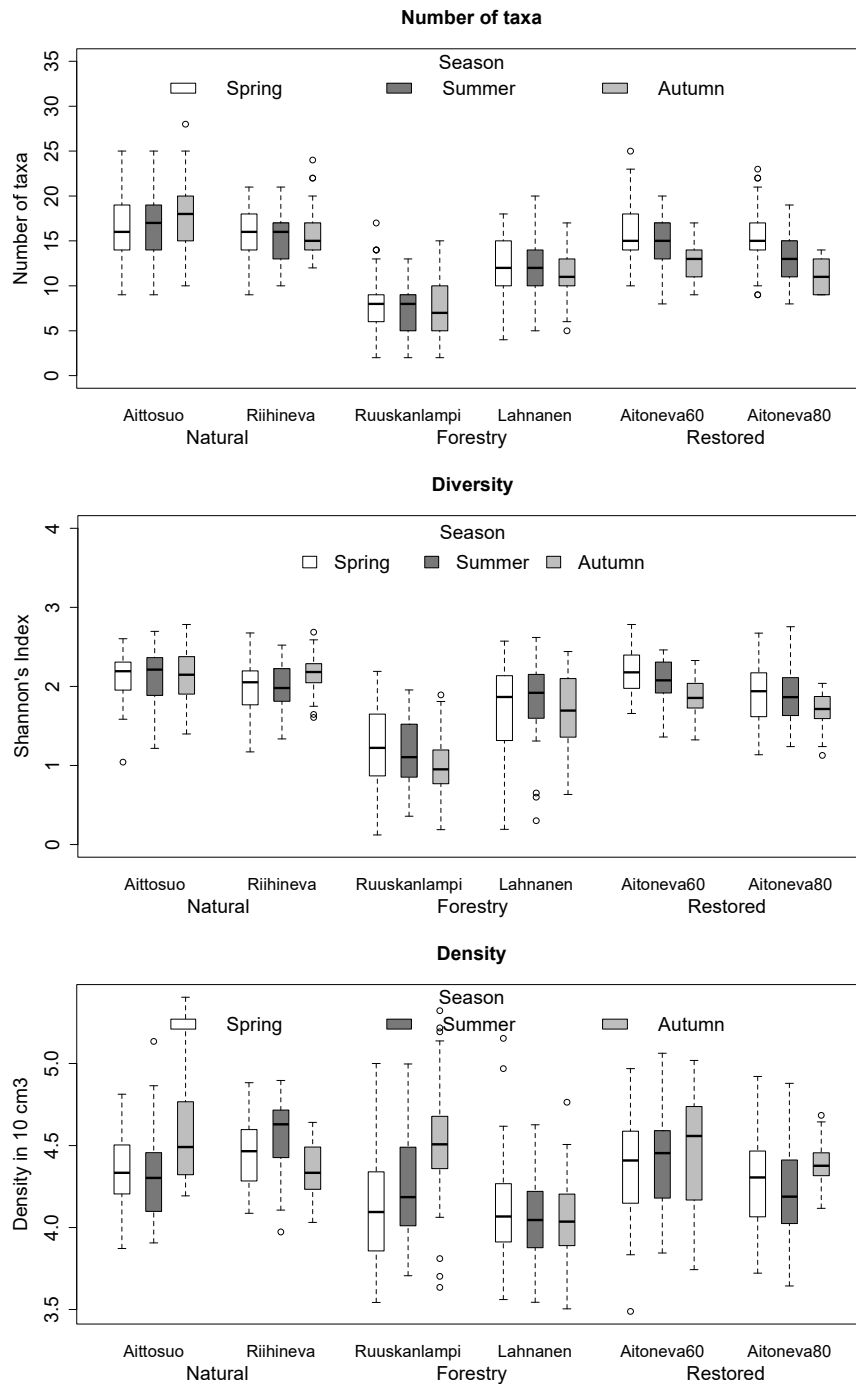


Fig. 4 Number of taxa, Shannon Wiener diversity, and density, whisker boxplots for testate amoebae in relation to land use and season of sampling. Values take into account correspond to measurements during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling plots/site at each sampling time. Natural: peatlands not under human use, Forestry: peatlands used for forestry extraction, Restored: Peatlands previously under human use but restored either 60 or 80 years ago. Circles: outliers, upper whisker: maximum value excluding outliers, upper box line: upper quartile, middle line inside box: median, lower box line: lower quartile, lower whisker: minimum value excluding

outliers. Density values were transformed to logarithmic scale, original values ranged between 3081,87 and 253383,38 testate amoebae/10 cm³ of fresh *Sphagnum*.

Number of TA taxa and diversity were significantly affected by variations in environmental variables between years at Aittosuo (natural) and Lahnanen (forestry) sites, while at both restored sites the differences were related to seasonality. At Riihineva (natural) significant differences were observed just in taxa diversity between seasons, while at Ruuskanlampi (forestry) richness varied between years and diversity between seasons (Table 2).

Table 2 Non-parametric Kruskal-Wallis H tests results of testate amoebae taxa richness and Shannon's diversity index differences between years and seasons ($df=2$ for all comparisons). Significant differences in richness and diversity are marked in bold. Values take into account correspond to measurements during three years (2013-2015) at each site, during spring, summer and autumn for a total of 15 sampling plots/site at each sampling time. The ranges shown in the table refer to the site variation of the annual and seasonal means. Natural: peatlands not under human use, Forestry: peatlands used for forestry extraction, Restored: Peatlands previously under human use but restored either 60 or 80 years ago.

Land use	site	Variation between years						Variation between seasons					
		richness			Shannon Wiener diversity			richness			Shannon Wiener diversity		
		range	H	p	range	H	p	range	H	p	range	H	p
Natural	Riihineva	15.3-15.9	1.3	0.540	1.9-2.1	1.2	0.540	15.5-15.8	0.2	0.910	1.9-2.2	11	0.005
	Aittosuo	14.2-18.9	41	<0.001	1.9-2.2	31	<0.001	16.5-17.6	2.3	0.310	2.1-2.2	0.2	0.900
	Lahnanen	10.6-13.2	18	<0.001	1.5-1.9	9.6	0.018	11.1-11.8	2.2	0.330	1.7-1.8	2.6	0.280
Forestry	Ruuskanlampi	6.7-8.6	7.0	0.030	1.1-1.2	1.5	0.480	7.3-8.1	1.1	0.560	1-1.3	6.2	0.045
	Aitoneva60	14.5-14.8	0.1	0.960	2-2.1	1.7	0.440	12.8-16.1	26	<0.001	1.9-2.2	27	<0.001
Restored	Aitoneva80	12.6-13.7	2.9	0.230	1.8-1.9	4.2	0.120	11.2-15.6	44	<0.001	1.7-1.9	11	0.003

At natural sites, *Arcella catinus*, *Hyalosphenia papilio* and *Centropyxis aculeata* were the most abundant taxa representing 16.3%, 15.7% and 9.9% of the total average TA numbers, respectively. *A. catinus* represented up to 40% of communities at forestry sites. Other common taxa at forestry sites were *C. aculeata* (14%) and *Trigonopyxis arcuata* (9.5%). At restored sites dominant taxa differed from those at the other types of peatlands, and showed the highest variability between seasons. Overall *Euglypha compressa*, *C. aculeata* and *Diffflugia globulosa* were the most abundant taxa representing on average 41% of the communities at restored sites (appendix 1).

Community-environment relationships and community ordinations

In the ordination, the different land uses clustered separately, suggesting that they differed in their TA taxa composition but seasonality did not seem to affect TA communities (Fig. 5).

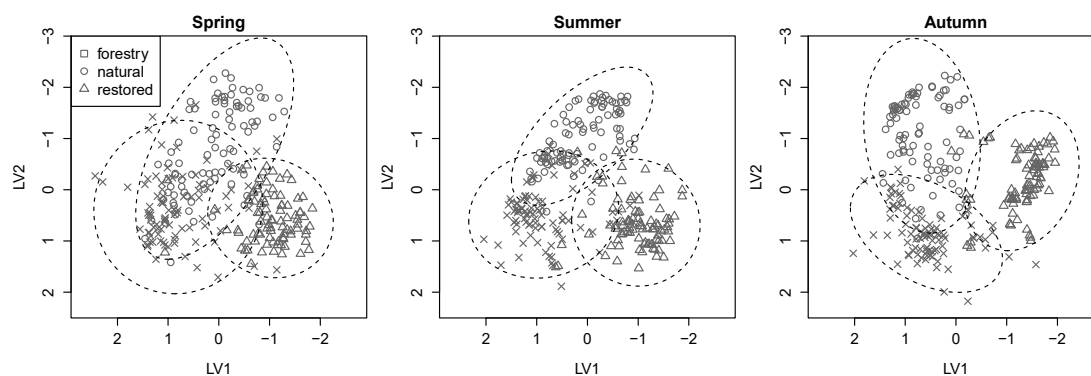


Fig. 5 The ordination of $n=270$ sites based on generalized linear latent variable model without any covariates assuming negative binomial distributed concentrations. The measurements were taken during the years 2013-2015 and three different seasons. Here the ordinations are shown separately for spring, summer and autumn. The sites in ordination are labelled according to the land use (Natural: peatlands not under human use, Forestry: peatlands used for forestry, Restored: Peatlands previously under human use but restored either 60 or 80 years ago).

As the seasons did not affect the ordination, we fitted a latent variable model to the whole dataset (Fig. 6) resulting in a similar pattern as in Fig. 5. When plotting by sites instead of land use, sites still mainly grouped by land use. The natural site Riihineva displayed a different pattern, where all the plots grouped separately from the other sites (Fig. 6).

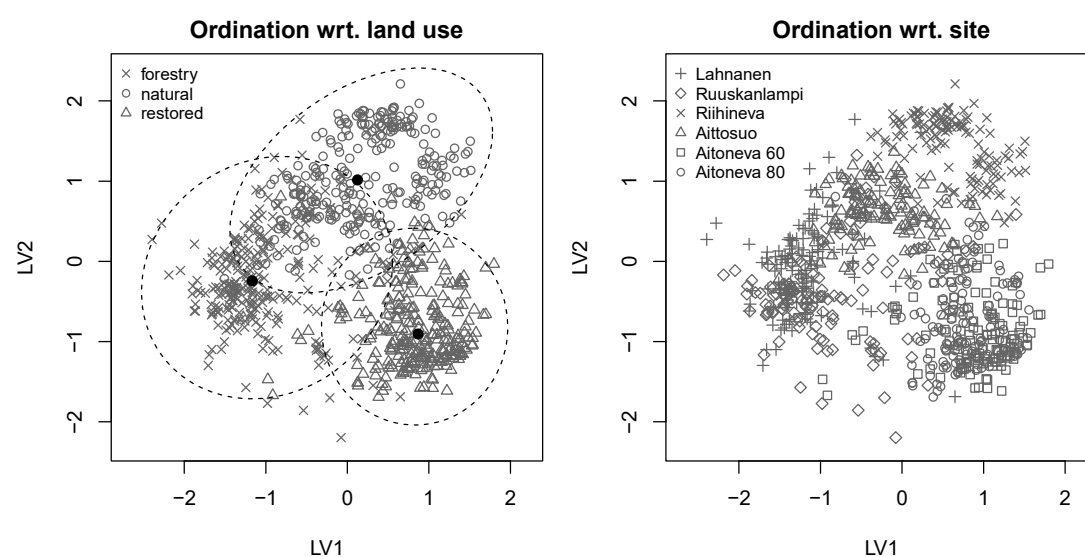


Fig. 6 The ordination of $n=810$ sites based on generalized linear latent variable model without any covariates assuming negative binomial distributed concentrations. The measurements were taken during

the years 2013-2015 and three different seasons. The sites in left ordination plot are labeled according to the land use (Natural: peatlands not under human use, Forestry: peatlands used for forestry, Restored: Peatlands previously under human use but restored either 60 or 80 years ago), and in right ordination plot according to the sampling site. In left ordination plot, the spatial medians for ordination points corresponding to each land use are shown as black dots.

To visualize whether the sites align along any of the measured gradients we plotted the sites with shadings corresponding to their respective pH, temperature values, and WTD (Appendix 2). However, none of the variables seemed to affect the ordination i.e. any of the covariates did not affect testate amoebae community structure and composition. To evaluate the amount of variation in testate amoebae taxa caused by different predictors, we used ratios of traces of residual covariance matrices from generalized linear latent variable models as a measure for total variation (Warton et al. 2015). Adding the land use as a covariate to the null model reduced the trace from 497 to 142. Thus, the land use alone explained approximately 71.5% of the covariation across species. Further, the pH alone explained 14.8%, the temperature 2.6% and water table depth 2.3% of the total covariation. To compare the locations of the scatterplots related to different land uses we determined the spatial medians for the sets (see Fig. 6) and tested for differences in spatial medians. The locations differed significantly (Spatial signs test, $p < 0.0001$; Oja and Randles 2004).

DISCUSSION

Overall, our results did not show large variations in environmental variables between the land uses except that the highest values of water table depth were recorded at restored and the lowest at forestry sites. Testate amoebae community structure and composition differed between seasons only at the restored sites. However, when all data were analysed together, differences seemed unaffected by seasons or years. Additionally, testate amoebae community structure and composition did not respond to the measured environmental variable gradients (pH, temperature and WTD). Instead, the land use seemed to explain most of the variation between TA communities.

On average, natural peatlands Riihineva and Aittosuo harboured the highest number of taxa, and displayed highest diversity and TA density, while the lowest values were found at forestry sites. Wanner

and Xylander (2005) found in mineral soils that higher TA taxa richness might be result of a longer time for cumulative colonisation without substantial species replacement, which can also be the explanation for the higher taxa richness found at our natural sites. Additionally, TA commonly occur in highest numbers in wet mosses coverage (Charman 2001), habitat more frequently found at natural sites compared to forestry and restored sites.

Forested sites are inherently different from *Sphagnum*-dominated peatlands, thus such differences together with the disturbances caused by forestry practices may have restricted the colonisation and survival of drought intolerant taxa, reducing both the TA taxa richness and their densities. The lowest water tables were in general recorded at forestry sites, where peat was also mainly drier. In *Sphagnum* dominated peatlands, peat moisture is considered the main factor affecting TA communities (Tolonen 1986; Booth 2001) and wetter habitats have been found to harbour higher densities of TA (Fournier et al. 2012). Our results generally supported these findings as natural and restored sites showed both higher water table levels and TA densities. However, we found particularly high TA densities in autumn samples at natural and forestry sites. These high TA densities at individual plots were obviously not related to any of the measured environmental variables as these plots were particularly dry (WTD as low as -22cm in some cases) at the time of sampling. While we cannot entirely rule out human errors in sample processing which might have led to such high observed numbers, we feel that observed densities are more likely the result of other favourable environmental factors, such as nutrient concentrations that can either affect TA directly (Lamentowicz et al. 2011) or indirectly by controlling their food sources (Mitchell et al. 2004).

Taxa dominance was relatively constant among study years and seasons at natural and forestry sites. The high abundance of *A. catinus* at forestry sites and *H. papilio* at natural sites for example, agrees with common findings suggesting that these taxa are potential indicators of dry and wet conditions, respectively (e.g. Charman and Warner 1992; Mitchell et al. 1999; Bobrov et al. 2002; Galka et al. 2012).

The highest abundances of *A. catinus* at natural sites were recorded at the driest plots at Aittosuo where they represented more than 50% of the TA communities. Plots in Aittosuo varied in their hydrological characteristics, some plots being very dry (water level as low as 30cm below ground) compared to the average water level found in this study. However, *A. catinus* was commonly found at all sites, even at the flooded restored sites, but in much lower abundances. This suggests that *A. catinus* could display broad moisture tolerance in Finnish peatlands, as also found by Daza Secco et al. (2016).

It should be also noted that the taxonomic keys used in this study group some morphologically similar species into one taxon, which may increase its range of environmental tolerance (for more details see Booth 2001). The highest abundances of *H. papilio* were recorded at Riihineva site where they represented more than 40% of the TA community. Riihineva is a site particularly different in its plant composition. The most remarkable characteristic of this site is the absence of vascular plants while mosses are dominating and creating a homogeneous mat. In fact, in a study by Booth and Zygmunt (2005) *H. papilio* was restricted to floating peat mats, suggesting that hydrological stability of such peat mats is of importance for this taxon. These habitat characteristics were unique to Riihineva and might also explain why TA communities from Riihineva sites grouped particularly separated from the other sites.

Compared to forestry and natural sites, taxa dominance was more variable between seasons at restored sites where the two dominant taxa were *E. compressa* and *D. globulosa*. These findings agree with other studies (e.g. Bobrov et al. 1999; Booth 2002) that have found the spiny shells of the spined forms of *Euglypha* help them to restrict their sinking and movements during interstitial water flows to in the wet habitats they are commonly associated with (Bobrov et al. 2002). Both *E. compressa* and *D. globulosa* were common and generally abundant also at natural sites, but very scarce or totally absent from forestry sites, suggesting a low tolerance of dry conditions. In contrast, *C. aculeata* was abundant at all sampling sites regardless of their hydrological conditions. Taxa such as *C. aculeata*, with an intermediate moisture optimum, are common along a wide range of the moisture gradient (Booth 2001). It should be pointed that also *C. aculeata* may be similar as *A. catinus*, grouping different species together, and thus influencing its observed tolerance range.

Although pH, temperature and WTD are often the most important variables driving TA communities, their effect was not able to mask the effect of land use in our study. Hence, it is possible that TA community structure is driven by other variables such as nutrient concentrations in peat water or biotic interactions more directly linked to the land use. Some studies have found an important relationship between TA and nutrients (e. g. Mitchell et al. 2000a; Jauhiainen 2002; Mitchell et al. 2004). Calcium for example, directly affects some TA species due to its importance in the shell building process (Lamentowicz et al. 2011). While the direct role of other nutrients in the regulation of TA communities is still poorly understood, Mitchell et al. (2004) suggested that nutrients indirectly affect TA via affecting the presence and abundance of their prey organisms.

After restoration measurements, it is often inherently expected that both environmental variables and biological communities move towards the pristine conditions of a site, recreating ecosystems that preceded human activities (Choi 2004). However, in our ordinations, sites clustered separately by land use, and natural and restored sites did not show higher similarities between them than they did to forestry sites. A combination of random forces such as percolating rainwater, convective transport into atmosphere, burrowing and other moving efforts, may greatly determine the spatial distributions of dormant protists such as TA (Finlay et al. 2001). Hence, the high similarities in taxa composition between natural and forestry sites might be explained by their geographical proximity, as they were more closely located to each other than to restored sites.

A commonly expected main outcome of most ecological restoration programs is that the restored ecosystem reverts back to its pristine conditions; however, the validity of this expectation has recently been openly debated (Wortley et al. 2013). In some wetlands and peatland studies that evaluated restoration success, no conclusive evidence for changes towards a target community after restoration was found (e.g. Zedler and Callaway 1999; Moreno-Mateos et al. 2012). Given such results, evaluations of restoration success should account for: i) the unpredictability of ecological succession, ii) the difficulties to determine the pre-disturbance state of the ecosystem, and the fact that iii) ecological change might be irreversible (Choi 2004). Irreversibility of ecological damage has been previously observed in both diversity and ecosystem services that usually remain lower in the restored than in the reference ecosystems (Wortley et al. 2013; Gałka et al 2017). These findings also highlight the importance of studies including peat core sampling in order to recognise the TA community structure and composition before disturbance, and evaluate whether the observed changes following restoration truly move towards the original states of sites (Gałka 2017).

The importance of discriminating between the large-scale climatic effects i.e. seasonal variation, and the local-scale changes caused by environmental disturbance (e.g. drainage) on TA communities has been pointed out earlier (e. g. Mitchell et al. 2000b; Warner et al. 2007; Talbot et al. 2010). Here, we found that despite small changes in taxa dominance (especially at restored sites), changes in TA community structure and composition related to land use strongly overrode effects of seasonality and random spatial variation, explaining 75% of the differences between the TA communities. Land use has been previously reported to greatly affect the microorganisms' communities in wetland environments. For example, Hartman et al. 2008 found that wetland restoration significantly influenced the bacterial

community composition when comparing restored vs. reference wetlands even when taking into account soil chemistry and wetland type. The lack of influence of seasonal variation has been previously reported for TA in peatlands (e. g. Gilbert et al. 1998) and TA in other habitat types (e.g. Schönborn 1986).

Low temporal variation has been attributed to the trophic diversity behaviour of TA and their capacity to resist changes in temperature and water content through encysting (e.g. Gilbert et al. 1998). On the other hand, studies of soil TA communities (e.g. Finlay and Fenchel 2004; Tysganov et al. 2013) have shown that climate-independent drivers of change i.e. local conditions such as soil moisture, either related to topography or geology (Tysganov et al. 2013) greatly affect the taxa number and abundance of soil TA. The differences in the responses of TA communities to large vs. local-scale environmental variation may also be related to the role of vegetation in regulating microclimatic conditions through shading, precipitation interception, etc. (Wookey et al. 2009; Graae et al. 2012). Other studies on the influence of seasonal fluctuations on TA community structure and composition (e.g. Lamentowicz et al. 2013; Marcisz et al. 2014) have found differences mainly in TA density between seasons with highest values during spring. However, such a pattern was not observed in our results.

In conclusion, our results showed that in boreal peatlands, the magnitude of the response of TA communities to human-induced environmental changes is higher than their response to local spatial and seasonal environmental variation. Additionally, other studies have also shown the advantages of using not only TA community structure and composition (e. g. Koenig et al. 2015; Daza Secco et al. 2016) but also their functional traits (Marcisz et al. 2016) as bioindicators of peatland disturbance. Here, we suggest the use of TA communities as an efficient tool for assessment and monitoring of ecological changes in boreal peatlands caused by human disturbances. Further, TA can and should be used over broad spatial and temporal scales commonly applied in routine monitoring. An especially attractive property of TA is their robustness against short-term climatic events. This greatly expands the timeframe over which samples from the same year can be combined in analyses, which is an especially helpful feature in the boreal region, where weather conditions from spring to autumn can change drastically.

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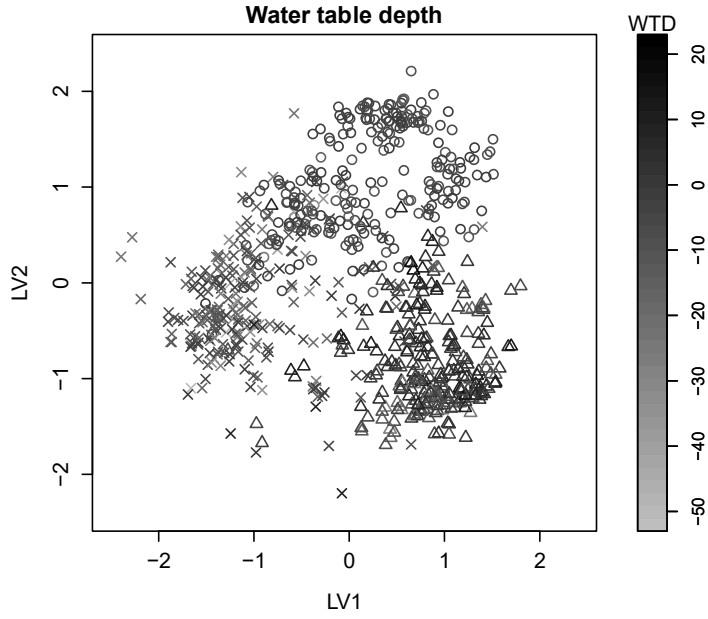
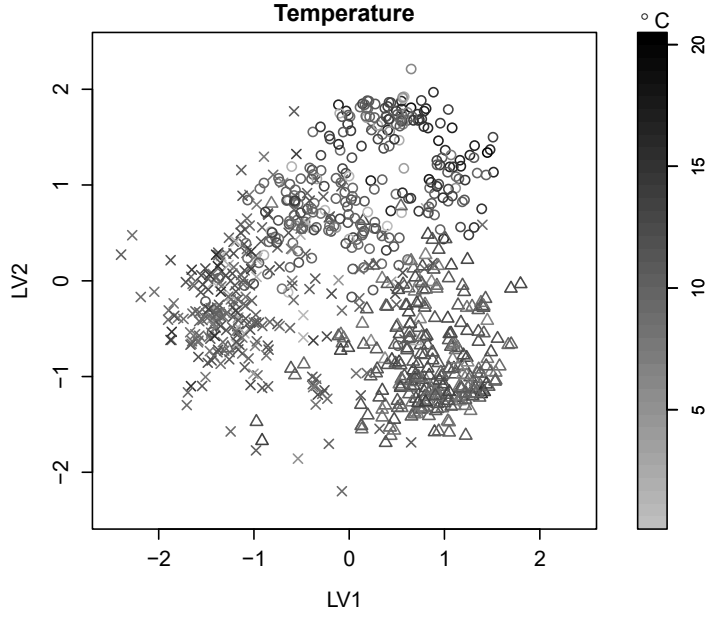
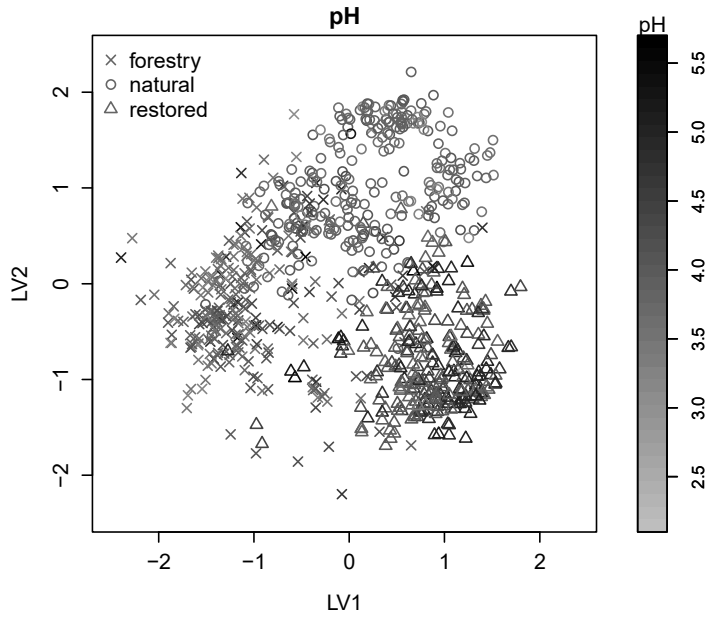
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III

TESTATE AMOEBAE AS A POTENTIAL TRACER OF ORGANIC MATTER DISLODGED FROM PEAT EXTRACTION AREAS

by

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