Faculty of Sciences
Institute of Biology

Using testate amoeba community structure and functional diversity to assess the impact of climate changes on ecosystem functioning: finding relevant and convenient tools for bio-monitoring of *Sphagnum* peatlands evolution or recovery.

Ph.D thesis

Isabelle Koenig

Thesis committee:
Prof. Edward A. D. Mitchell (Supervisor)
Dr. Renée-Claire Le Bayon (Co-supervisor)
Dr. Marco Moretti
Dr. Vincent Jassey

University of Neuchâtel
04/07/2017

UNIVERSITÉ DE NEUCHÂTEL

Faculté des Sciences
Secrétariat-décanat de Faculté
Rue Emile-Argand 11
2000 Neuchâtel – Suisse
Tél : + 41 (0)32 718 21 00
E-mail : secretariat.sciences@unine.ch

IMPRIMATUR POUR THESE DE DOCTORAT

La Faculté des sciences de l'Université de Neuchâtel autorise l'impression de la présente thèse soutenue par

Madame Isabelle KOENIG

Titre:

“Using testate amoeba community structure and functional diversity to assess the impact of climate changes on ecosystem functioning: finding relevant and convenient tools for bio-monitoring of *Sphagnum* peatlands evolution or recovery”

sur le rapport des membres du jury composé comme suit:

- Prof. Edward Mitchell, directeur de thèse, UniNE
- Dr Renée-Claire Le Bayon, co-directrice de thèse, UniNE
- Dr Marco Moretti, WSL, Birmensdorf, Suisse
- Dr Vincent Jassey, CNRS, Université Toulouse III, France

Neuchâtel, le 13 juillet 2017

Le Doyen, Prof. R. Bshary

"On aime ce qui nous a émerveillé, et on protège ce que l’on aime"

Jacques-Yves Cousteau

Table of contents

Abstract

Human activity and ongoing climate changes have an increasing impact on ecosystems and lead to the loss of several natural habitats. Ecosystems provide functions contributing to human well-being and these functions could be estimated based on a monetary value. Among these ecosystems, wetlands and peatlands are of primary importance, by hosting a specific flora and acting as a carbon sink. Their waterlogged soils are anoxic, and the mineralisation of the organic matter is slow. Organic matter accumulates in the peat and thus fixes atmospheric carbon in soil. But this function is directly linked to the humidity content, which depends on precipitation rate and local climate. With ongoing climate changes and human pressure on peatlands, these ecosystems are regressing all over the world. In Switzerland for example only 10% of the wetlands, that still existed in the 19$^{\text{th}}$ century, remained until now. Henceforth, developing tools that allow the monitoring of peatlands has become a key priority.

The aim of this thesis was to develop bio-indicators that can be used to monitor the status and functioning of *Sphagnum* peatlands. Until now, monitoring of *Sphagnum* peatlands was mainly conducted through vegetation surveys. However, as vegetation responds to perturbation with a delay, its power to assess short term changes is limited. Particularly, it is difficult to estimate the impact of revitalisation actions (i.e. manipulation of water table, introduction of vegetation, etc.) on the global functioning of the peatland. While vegetation takes years to reflect changes, micro-organismal communities will react sooner. Among these micro-organisms, testate amoebae, a group of unicellular protists building a shell (test), play an important role in food web functioning in *Sphagnum* peatland's soils and are well correlated to local changes. They are now used to monitor various gradients like humidity, pH, nutrients contents and pollutants. The shell is characteristic and allows the identification to species level. Recently morphological traits were correlated to ecological preferences of taxa and studies use them to underline environmental gradients. However, studies in control conditions are needed to define functional traits i.e. traits that are correlated with environmental filters. Therefore a clear definition of the correlation between morphological traits and ecosystem functioning is necessary.

In this thesis we showed that a group of selected testate amoeba taxa is more precisely correlated to micro-gradient (humidity content) than vegetation along an altitudinal gradient of *Sphagnum* peatlands in Switzerland. We demonstrated that these ten selected taxa, which were easy to determine, allow to monitor the water table depth along the pool – hummock gradient at each elevation while vegetation (global or vascular plant and bryophytes separately) was better correlated to elevation or autocorrelated to the site itself. Henceforth, these ten testate amoeba taxa describe the status and functioning of the bogs more precisely. In addition we compared the structure of testate amoeba community and community weighted mean of functional traits between an artificial peatbog (covering an area of 100m^2) set up in the Botanical Garden in Neuchâtel and a regenerated bog in Bois des Lattes (NE). Rebuilding a fully functional *Sphagnum* peatbog at low elevation is challenging, especially with long warm and dry periods during the first year. Our results showed that testate amoeba community structure changed drastically during this first year and seemed to indicate that the bog functioning was deeply disturbed. Henceforth, the comparison between the community structure found on bare peat in Bois des Lattes and the living community structure found in both samplings (2015 and 2016) in the Botanical Garden was significantly different. In the Botanical Garden, *Corythion dubium* represented ca. 20% to 30% of the whole living population while the *Difflugia* genus and *Phryganella acropodia* dominated sampling in Bois des Lattes (ca. 25%). On the other hand, the global functioning of both sites appeared to be quite similar based on functional traits and indicated a water stressed situation. Thus, even if the community structure was relatively different between the Botanical Garden bog and Bois des Lattes, the global functioning remained in the same range in both sites.

We also monitored the changes in testate amoeba community structure and correlated functional traits with water table fluctuations in a controlled experiment i.e. mesocosms. We assessed the response of testate amoeba community structure and weighted mean of traits to three levels of water tables, wet (-4cm), intermediate (-15cm, taken as control) and dry (-25cm). In wet and intermediate plots, little changes occurred over 18 months and the community stayed largely dominated by *Hyalosphenia papilio*, a mixotrophic taxa common in *Sphagnum* peatlands and with a relatively wide tolerance to fluctuation in water level depths. In dry plots, the shift in community structure occurred rapidly and taxa related to dryer conditions dominated the community (*Nebela tincta* group, *Corythion dubium*). In these plots, the shift in community weighted mean of functional traits went towards small taxa, with a small hidden pseudostome (ventral and ventral-central) and the loss of mixotrophy. In the follow-up of this experiment, we simulated a regeneration situation by putting the water level at -10cm in all plots. Four samplings were taken into account, the first and the last of the previous experiment and two additional samplings were done, two and six months after the rewetting. Our results showed that, during the recovery period, the community structure of testate amoebae reflected the change in water level, in the former dry treatment. Taxa living in more humid conditions like *H. papilio*, recovered whereas *N. tincta*, and *C. dubium*, taxa related to dry conditions, regressed in community structure. The community weighted mean of traits shifted and indicated that the environmental constraints were less drastic in theses plots. Interestingly, the community structure and the weighted mean of traits in some former wet and intermediate plots indicated that the local conditions should be dryer and, indeed, this reflected the state of *Sphagnum* stems which was very dry in these plots.

This thesis confirms the bio-indication potential of testate amoeba communities and functional traits in *Sphagnum* peatlands monitoring. This can be used either for a follow- up of the revitalisation measures or to diagnose their functioning. The trait based approach, by circumventing the difficulties of precise identification and by highlighting ongoing processes, provide cues to build bio-indication tools based on functional trait classes and proportion without the need of complex statistics. Moreover, assessing the success of restoration measures is important to assess the role of peatlands in the carbon cycle. In this context, easy to handle bio-monitoring tools better correlated to very local hydrologic conditions are needed and testate amoeba community structure and community weighted mean of functional traits could play this role.

Keywords: Peatlands, bio-monitoring, testate amoebae, functional traits, revitalisation, ecosystem functioning, mesocosm studies

Résumé

L'impact grandissant de l'homme sur les écosystèmes et les changements climatiques en cours font disparaître nombre de milieux naturels dans le monde. Il est aujourd'hui acquis que les écosystèmes naturels et la biodiversité ont un rôle important dans le bien-être de l'humanité et que les services qu'ils rendent ont une valeur monétaire qui peut être estimée (services écosystémiques). Parmi ces écosystèmes, les zones humides et les tourbières ont une importance particulière, liée autant à la flore très spécifique qui y vit qu'à leur rôle de puits de carbone. En effet, leurs sols, détrempés une grande partie de l'année, sont anoxiques et la matière organique s'y décompose très lentement. Elle s'y accumule, permettant de fixer dans le sol (la tourbe) du carbone atmosphérique. Mais cette fonction est liée directement à l'engorgement en eau, qui dépend du régime des précipitations et donc du climat. Dans un contexte de changement climatiques, mais aussi à une pression anthropique croissante, les tourbières sont partout dans le monde en voie de régression. En Suisse, par exemple, il ne reste actuellement que 10% des zones humides existantes au XIX$^{\text{ème}}$ siècle. Dès lors, il est urgent de développer des outils qui permettent de suivre leur évolution.

L'objectif de cette thèse est de mettre au point des indicateurs permettant de suivre l'état des tourbières ainsi que leur fonctionnement en tant qu'écosystème. Les relevés de végétation utilisés depuis longtemps permettent de suivre les changements sur le long à très long terme ; cependant, cet outil est insuffisant pour documenter des changements rapides de l'ordre de quelques mois. En particulier, lors de travaux de revitalisation, il est difficile d'estimer l'impact des mesures prises (comme par exemple : manipulation de la nappe d'eau, réintroduction d'espèces végétales, etc.) sur le fonctionnement général de la tourbière. Si la végétation prendrait des années à se modifier, les communautés de micro-organismes vont réagir en effet beaucoup plus rapidement. Parmi ces micro-organismes, les amibes à thèques, un groupe de protistes unicellulaires se protégeant dans une coquille (appelé thèque), jouent un rôle prépondérant dans le réseau trophique des sols de tourbière et sont aujourd'hui reconnues comme étant de bonnes indicatrices de divers gradients tel l'humidité, le pH, les nutriments ou les polluants. Les thèques de ces organismes ont une forme caractéristique qui permet leur identification au niveau de l'espèce. Des traits morphologiques ont été corrélés aux préférences écologiques des espèces, et il est possible, en les observant, de mettre en évidence des filtres environnementaux. La définition des traits fonctionnels nécessite des expérimentations en milieu contrôlé pour définir clairement les corrélations entre les traits morphologiques et le fonctionnement de l'écosystème.

Le premier article (chapitre 2) a permis de montrer qu'une sélection d'espèces d'amibes à thèque est plus finement corrélée aux changements de micro-gradients (humidité) que la végétation dans un gradient altitudinal de tourbières naturelles relativement préservées. En effet, dans cette étude, nous montrons qu'un pool de dix espèces d'amibes à thèque faciles à identifier, permettent de suivre la profondeur de la nappe le long du gradient gouille – buttes, à diverses altitudes alors que la végétation (globale ou séparée entre plantes vasculaires et bryophytes) est plutôt mieux corrélée à l'altitude et autocorrélée au site lui-même. Dès lors, ces dix espèces d'amibes sont de meilleures indicatrices de l'état et du fonctionnement de la tourbière.

Dans le second article (chapitre 3), nous comparons la structure de la communauté d'amibes ainsi que la moyenne pondérée des traits fonctionnels entre une tourbière artificielle créée au Jardin Botanique de Neuchâtel (d'une surface d'environ 100m^2) avec une tourbière naturelle en cours de régénération (Bois des Lattes, NE). Recréer une tourbière fonctionnelle à basse altitude est un solide défi, surtout lorsque la météo impose une longue sécheresse la première année. Nos résultats indiquent que la population d'amibes à thèque à fortement changé durant cette première année et semble indiquer que le fonctionnement de la tourbière a été profondément perturbé. En effet, la comparaison entre les communautés d'amibes sur le site de référence et les deux échantillonnages du jardin botanique (2015 et 2016) montrent des différences significatives ; alors que *Corythion dubium* représente environ1/5-1/3 de tous les individus vivants rencontrés au jardin botanique, cette communauté est dominée par *Difflugia* sp. et *Phryganella acropodia* sur le site de référence (Bois des Lattes, NE). Par contre, sur la base des traits fonctionnels, le fonctionnement semble assez proche entre les sites, avec des signes indiquant un stress hydrique important mais comparable entre le site de référence (sphaignes sur tourbe nue) et le jardin botanique.

La troisième partie de cette thèse nous a permis de corréler les traits fonctionnels avec la fluctuation de la nappe d'eau dans une situation contrôlée en mésocosmes. Dans la première phase de l'expérimentation (article 3, chapitre 4) le niveau d'eau a été réglé à −4cm (humide), −15cm (contrôle) et −25cm (sec) depuis le sommet des capitulums et plusieurs échantillonnages ont été réalisés durant 18 mois. Les traitements mouillés et contrôle ont peu changé durant cette phase, restant largement dominés par *Hyalosphenia papilio*, une espèce mixotrophe fréquente en tourbière et avec une bonne tolérance aux variations de la nappe. Dans les réplicas secs, nos résultats montrent que la population d'amibes à thèque répond rapidement au traitement en basculant vers des espèces connues pour mieux tolérer les conditions sèches (groupe de *Nebela tincta*, *Corythion dubium*). Les traits fonctionnels mis en évidence dans le traitement sec sont un biovolume plus petit, un pseudostome caché (ventral ou ventral-central) et étroit ainsi que la perte de la mixotrophie. Dans la seconde phase (papier 4, chapitre 5), nous avons simulé une situation de régénération en remontant le niveau d'eau à −10cm de la surface de la sphaigne. Quatre échantillonnages ont été pris en compte, le premier et le dernier de la première phase, ainsi que deux échantillons 2 et 6 mois après l'égalisation du niveau d'eau. Dans le traitement sec, la population d'amibes à thèque revient progressivement à son état du début de l'expérimentation avec une diminution des taxa adaptés aux conditions sèches et la réapparition de taxa corrélés à des conditions humides (et en particulier *Hyalosphenia papilio*). La moyenne pondérée des traits fonctionnels se modifie indiquant des contraintes environnementales moins drastiques. De façon intéressante, certains échantillons des traitements humides et contrôle ont vu leur communauté d'amibes ainsi que leur moyenne pondérée de traits indiquer que la situation locale était plus sèche et reflétait ainsi l'état de la sphaigne dans laquelle l'échantillonnage s'est fait.

Cette thèse permet de montrer le potentiel de bio-indication des amibes à thèque et de leurs traits fonctionnels dans le suivi des tourbières, que ce soit pour suivre des mesures de revitalisation ou pour faire un diagnostic de leur état et de leur fonctionnement. L'approche par les traits, en contournant la difficulté d'une identification des taxa très précise et en mettant en évidence les mécanismes en cours, permettrait de créer un outil de bio-indication basé sur des classes de traits et des proportions, ne nécessitant pas l'utilisation de statistiques complexes.

Mots-clé : Tourbières, bio-monitoring, testate amoebae, traits fonctionnels, revitalisation, fonctionnement des écosystèmes, études en mésocosmes

Acknowledgement

©freedesignfile

A Ph.D thesis is a tedious work which implies a complete investment, and which would not have been possible to carry out without the support and guidance I received from many people.

Firstly, I would like to thank my supervisor, Prof. Edward Mitchell, for the opportunity given to undertake this journey, for supporting me during hard times, and for the confidence given. I would also like to thank my co-supervisor, Dr. Claire Le Bayon, for helping me to get back on track, for supporting me as well during hard times, and for the valuable advices provided. I am also grateful to the members of my committee, Dr. Marco Moretti and Dr. Vincent Jassey, for accepting to read and comment on my thesis, as well as for their useful advices.

As this thesis was partly done in collaboration with another project (Climpeat project), I would like to warmly thank Dr. Matthieu Mulot for all his work done on the mesocosms, for all the discussion we had, and for his friendship. I would also like to take the opportunity to thank Prof. Mariucz Lamentowicz and his team in Poznan for the warm welcome they gave me, and for the many trips and visits we did in Poland.

Then, I would like to thank all the team of the laboratory of soil biodiversity, and particularly Dr. E. Lara, Dr. C. Seppey, Dr. I. Szelecz (who also proofread), A. Pillonel, F. Schwendener, Q. Blandenier, and C. Duckert for all the discussions held, for the ideas shared, and for the laughs and parties we had.

This work was mainly based on free softwares (R, RStudio, LaTeX, TeXnicCenter, Inkscape, Sumatra, etc.), therefore, I would like to thank all the people involved in these projects, as well as the person managing the help forums, which were truly helpful, if not indispensable, and which allowed me to make the best out of them.

I would also thank the University of Neuchâtel and Climpeat project who both funded this work, the Botanical Garden of Neuchâtel, all the owners of natural parks, in which I took samples, and the Environment Offices of Bern and Neuchâtel for granting access to the sites. Last but not least, I would like to thank my family and friends, and particularly my partner Sylvette, Aude, her daughter, who partly proofread, and my parents, who all confidently followed me on this late professional path.

This journey was an amazing experiment, sailing from enjoyment to dissatisfaction, from time out to stress, from strong confidence to (profound) discouragement. However, embarking on this thesis has provided me with the opportunity to meet great people from all over the world. It was indeed a worthwhile journey, which I will never regret having undertaken.

Chapter 1

Introduction to the thesis: Using testate amoeba community structure and functional diversity to assess the impact of climate changes on ecosystem functioning: finding relevant and convenient tools for bio-monitoring of *Sphagnum* peatlands evolution or recovery.

1.1 Introduction

Although providing key ecosystem services, wetlands are regressing all over the world, having lost more than 50% of their surface in North America, Europe, Australia, and China during the 20th century (Costanza et al., 1998; Mitsch and Gosselink, 2000). In 1971, countries and non-governmental organisations agreed upon the importance of protecting wetlands, in order to ensure their effective management, and promoted an international cooperation on these ecosystems (Ramsar, 2013). Understanding their functioning, in particular in relation to climate changes will likely give sound tools to better protect or restore them.

1.1.1 Ecosystems

Ecosystem services

Since the end of 20th century, the impact of humankind was demonstrated, both on climate changes (IPCC, 2014) and ecosystems (Millenium Ecosystem Assessment, MEA, 2005; Vitousek, 1997). In addition to the changes in ecosystem functioning through land conversion, water cycle modification or massive inputs of chemicals, fertilizers and pollutants, the standardisation of crops, farming methods, and landscapes has lead to a global biodiversity loss (Cardinale et al., 2012; MEA, 2005). Since mid-1990, several studies have demonstrated that biodiversity, ecosystem functioning, and ecosystem services were correlated (Cardinale et al., 2012; Chapin III et al., 2000; Hooper et al., 2005; Loreau et al., 2001). Ecosystem services provide an approximative value of an ecosystem for human well-being (MEA, 2005; Wallace, 2007). Estimating ecosystem services based on monetary value is controversial but it exerts a leverage on a wider audience, including political and economic authorities (Boyd and Banzhaf, 2007; Costanza et al., 1998; Fisher et al., 2008; Gómez-Baggethun et al., 2010). To achieve this accordingly, indices and quantitative measurements of key "ecosystem service providers" are necessary (Costanza et al., 2014, 1998; Kremen and Ostfeld, 2005; Vačkář et al., 2012). Well known indices such as species richness, species evenness, $\alpha-$, $\beta-$ and $\gamma-$diversity, etc., which are based on community species assemblage, are commonly used. Nevertheless they might lead to controversy, with contradictory results (Colwell, 2009; Loreau et al., 2001). Beside these indexes, new tools have emerged, such as functional diversity, which is based on the assumption that species do not react equally to environmental constraints (Keddy, 1992; Woodward and Diament, 1991).

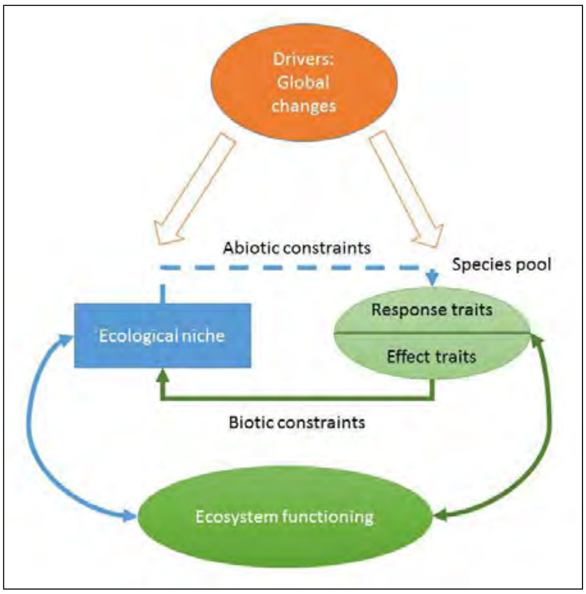


Figure 1.1 – Simplified scheme of ecosystem dynamics. Ecological niche and species pool are in constant interactions by action and feedback (biotic and abiotic contraints). Global drivers impact on physico-chemical dimensions of local environment (humidity rate, temperature, nutrient availability, etc.) and or on the species community structure (change in species richness, composition, interaction, etc.). Species will respond to the new constraints by selecting traits that are better adapted to the situation (response traits) and, in return, will impact on niche dimension (effect traits). Ultimately, ecosystem functioning and ecosystem services will evolve in response of niche and species community changes.

Functional diversity

Functional diversity provides powerful tools, which are well correlated with the functioning of the ecosystem (Cadotte et al., 2011, 2009; Díaz et al., 2007; Petchey and Gaston, 2002). Environmental drivers (climate, land use changes, pollutant deposition, etc.) affect ecological niches by inducing physico-chemical changes. Species pool responds by shifting towardss taxa which are better adapted to the new situation (habitat filtering theory, Woodward and Diament, 1991, Figure 1.1), and therefore, by feedback, the new community modifies the dimension of the niche (biotic constraints, Chapin III et al., 2000; Lavorel and Garnier, 2002). At the ecosystem level, the function and services provided will be impacted (Cadotte et al., 2011; Costanza et al., 2014, 1998). At community level, these changes are reflected by modification in the range traits of the species living in, therefore traits can be related to ecosystem functioning (Cadotte et al., 2011; Diaz et al., 2007; Gravel et al., 2016; Lavorel and Garnier, 2002). Functional traits are supposed to be directly related to environmental filtering, either by responding to ecological changes – response traits – or by impacting ecosystem processes – effect traits (Chapin III et al., 2000; Lavorel and Garnier, 2002). Defined by analogy to the Hutchinsonian niche, the functional niche is a multidimensional space where axes are related to functions or processes rather than to environmental factors (Rosenfeld, 2002). In this space, the mean trait value of a pool of species allows to model the response of the community to environmental drivers (Ackerly and Cornwell, 2007). The kind, range and relative abundance of functional traits of taxa involved in response to environmental filters (rather than the taxonomic diversity itself) can be used to estimate ecosystem functioning (Cadotte et al., 2011; Loreau et al., 2001; Pavoine et al., 2011). Functional diversity is now a well-recognized tool to monitor impacts and responses to changes in ecosystem functioning and of ecosystem services (Cornwell, W.K. et al., 2008; Díaz et al., 2013; Hedberg et al., 2013; Schnoor et al., 2015), and begins to be applied to animals and micro-eukaryotes (Arrieira et al., 2015; Gagic et al., 2015; Gerisch et al., 2012; McGill et al., 2006; Mouillot et al., 2007).

According to Violle et al. (2007), functional traits are measurable with morphological, physiological or phenological characteristics at the individual level of organisms. Selected traits must have a significant impact on organisms' fitness (Cadotte et al., 2011; Diaz et al., 2007; Mlambo, 2014; Violle et al., 2007). For instance, plant functional traits are correlated to water and nutrient access (growth form, rooting depth), fire survival (basal area, leaf dry matter content), and to life and growth strategies (seeds size, specific leaf area) (Kattge et al., 2011; Lavorel and Garnier, 2002; Silva et al., 2013; Violle et al., 2007). Animal functional traits reflect the capacity of transporting nutrients (body size, mobility-related traits, behaviour, mouth parts), predation ability (hunting tools, mouth dimension), or adaptation to hunting (body size, reproductive rate, elusiveness; Díaz et al., 2013; BETSI, 2012). Micro-eukaryote functional traits are related to life strategy (encystment, phylogenetic groups), nutrient availability (biovolume, mixotrophy), adaptation to water restriction (test compression, position of aperture; Arrieira et al., 2015; Fournier et al., 2012; Litchman et al., 2007). However, functional diversity alone cannot explain all biodiversity-related effects. Further research is needed, either to consolidate and widen this approach or to collect and measure functional traits within various ecosystems and regions in order to have standardized lists and protocols (Blaum et al., 2011; Díaz et al., 2007).

1.1.2 Stresses and disturbances

Stresses and disturbances are major drivers of community assembly and ecosystem processes (Hooper et al., 2005). Both vary in intensity, frequency, regularity and predictability (e.g. seasonal or catastrophic). Accordingly, community responses range from slow turnover in community composition to total die-out and recolonization (Parsons, 2005). Disturbances result in on-off, nearly extreme, perturbations that affect directly or indirectly organisms. They strongly impact the community structure by killing, displacing or damaging members of one or more taxa and create opportunities for new taxa to become established (Sousa, 1984) thus introducing heterogeneity in ecosystems. Disturbances are part of the cycle of all ecosystems by renewing them, and/or favouring r-organisms over K-organisms (Frontier et al., 2008; Rykiel, 1985). On the other hand, stresses create conditions that put more pressure on organisms by restricting production of the ecosystem and impacting species fitness. Stresses are generally not lethal for organisms. Therefore, they involve adaptive strategies and trade-offs to respond to chemical, physical or biological constraints but without, in most cases, radically changing the community structure (Frontier et al., 2008; Grime, 1977). Both stress and disturbance also have a temporal dimension, and depending on their duration, the response of the ecosystem may not be the same. In case of a sudden, brutal disturbance, the community could recover after a period of time, either by itself (i.e. phenotypic plasticity, encystment, persistent seeds) or through immigration from the less disturbed patches around. When perturbation is more severe, new species, that are better adapted to the local conditions and favoured by the lower competition potential of the former community, settle down (Frontier et al., 2008; White and Pickett, 1985).

Ongoing climate changes and growing human impact increase the level and the variety of perturbations on a wide range of ecosystems (MEA, 2005). If some ecosystems are extending, most of them are declining or are under high pressure (MEA, 2005). Among them, wetlands, as highly complex ecosystems, are particularly affected (Euliss Jr

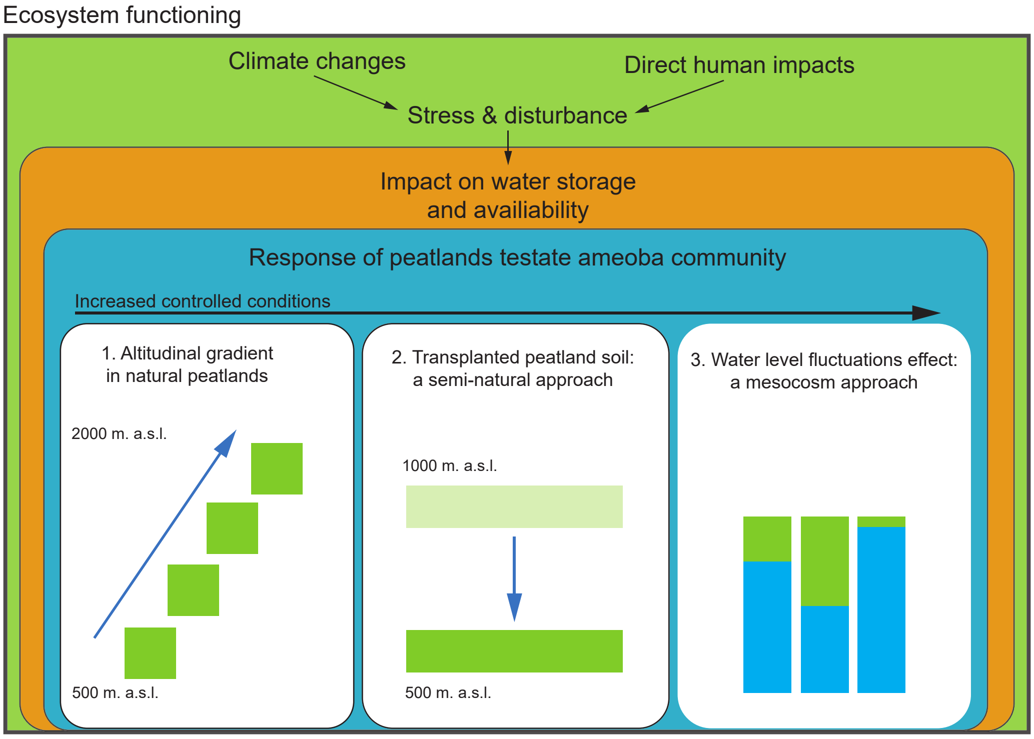


Figure 1.2 – General structure of the thesis. This work will investigate the impact of global perturbation on *Sphagnum* peatlands. As peatlands are very sensitive to changes in water content, we will investigate three different situations, with an increased control on water availability: 1) the impact of climate through an altitudinal gradient in natural peatlands, in Switzerland; 2) the evolution of a transplanted peatland at a lower altitude; 3) the adaptation of *Sphagnum* peatland to strictly controlled water level in a mesocosm experiment, with two phases: the stressed phase with three contrasted water levels (Climpeat experiment), followed by a recovery experiment with equalised water level.

et al., 2013; MEA, 2005). Since wetlands provide a wide range of services including atmospheric carbon storage, regulation of hydrological cycles, nutrient cycling, erosion regulation, etc., a better understanding of their functioning would improve protection and effective measures of recovery (Mitsch and Gosselink, 2000; Ramsar, 2013).

1.1.3 *Sphagnum* peatlands

Sphagnum peatlands (also called mires, peat bogs or raised bogs; Moore and Bellamy, 1974) are particular wetlands characterised by high water content, low nutrient availability and high acidity. Due to water saturation of soil, mineralisation rates are very low and favour organic matter accumulation (Gobat et al., 2010; Goodall, 1983; Mitsch and Gosselink, 2000). Mainly (but not restrictively) distributed in mid to high latitudes of both hemispheres, peatlands cover 3-5% of Earth's land area (Bridgham et al., 2006; Maltby and Immirzi, 1993) and stock an estimate 500 ± 100 Gt carbon, equivalent of 50-75% of total atmospheric carbon (Solomon et al., 2007; Yu, 2012). Acting as an important carbon sink – when actively accumulating organic matter – they contain one third of total soil organic carbon (Turunen et al., 2002). Beside their role in the carbon cycle, peatlands are also hotspots for biodiversity, since they are hosting highly specialised fauna and flora (Barber, 1993; Chapman et al., 2003; Littlewood et al., 2010). They also play an important role in water regulation and purification, educational opportunities, etc. (MEA, 2005). Nevertheless peatlands, and wetlands in general, are highly sensitive to perturbations. With ongoing climate changes, land use conversion and peat extraction, peatlands are endangered (Belyea and Malmer, 2004; Bragazza et al., 2016; Bridgham et al., 2006; Junk et al., 2012). These perturbations, mainly related to lowering water level, turn them in less anoxic ecosystems, accelerating plant material decomposition, and shifting these ecosystems from carbon sink to carbon source, thus acting as positive feedback on global warming (Arneth et al., 2010; Belyea and Malmer, 2004; Davidson and Janssens, 2006). Since the Ramsar Conference in 1971, a growing interest in protecting and restoring wetlands in general, has arisen worldwide.

Whilst it is mainly based on vegetation surveys, birds or invertebrates community structures, microorganisms based measurement (community structure, respiration rates, microbial biomass), peat chemical analyses, hydrology studies, etc., peatlands restoration monitoring is often limited by a lack of funding, a lack of knowledge about the large scale functioning of peatlands, and a lack of international consensus about monitoring tools, etc. (Bonnett et al., 2009). In addition, recovery of damaged peatlands is often slow and incomplete and the fully biochemical functioning may occur only after 50-100 years relative to untouched peatlands (Moreno-Mateos et al., 2012). Several research groups of research have studied the relationships between peatlands and climate changes but the magnitude of the response of peatlands in continental climate setting is not really clear yet (Bonnett et al., 2009). Among the parameters involved in peatland functioning, the water content appeared to be one of the most important (Mitsch and Gosselink, 2000; Zerbe et al., 2013). Microorganisms have significant ecosystem functions in peatlands (Gilbert and Mitchell, 2006; Laggoun-Defarge et al., 2008). They are involved in nutrient cycling and respond quickly and specifically to environmental fluctuations. Therefore, using the change in microbial community structure is relevant to point out the ecological state of a peatlands (Anderson, 2006; Gilbert and Mitchell, 2006; Jassey et al., 2013a; Laggoun-Defarge et al., 2008; Lamentowicz et al., 2013).

1.1.4 Testate amoeba community

Testate amoebae are unicellular protists protecting themselves in a shell – called test – made either by auto-production or by accretion of particles found in the ecosystem (Meisterfeld, 2002a,b; Smith et al., 2008). Regarded as being quite ubiquitous and widely distributed and abundant (even if still discussed today, see Finlay and Clarke, 1999; Foissner, 2006; Wilkinson, 2001), they preferentially live in wet ecosystems (lakes, rivers, mosses and humid soils), and in estuarine environments (Meisterfeld, 2002a; Mitchell et al., 2008a; Warner, 1990). Belonging to three phylogenetic groups (Arcellinida, Euglyphida and Amphitrematidae), they experience several life strategies like r- and K-strategies, mixotrophy, encystment, etc. (Coûteaux and Darbyshire, 1998; Vincke et al., 2006; Wanner et al., 2008). Their role in the soil food web occurs at different levels, either as predators – bacteria, fungi (Hausmann et al., 2003) or even nematodes (Mitchell, 2015) – or as prey. They contribute to the turnover of carbon, nitrogen and silica and thus are key organisms in soil nutrient cycles (Jassey et al., 2015; Schröter et al., 2003; Wilkinson, 2008). This is the case in peatlands and forest humus in particular, in which testate amoeba is the main group of protozoan (Gilbert et al., 2003; Schröter et al., 2003; Gilbert et al., 1998).

The tests (shell) of testate amoebae are preserved in soils and sediments for years and allow the determination at a high taxonomic level (Mitchell et al., 2008b). The community structure of testate amoebae is well correlated with pH, humidity, nutrient content (Bobrov et al., 1999; Booth et al., 2008; Charman and Warner, 1992; Lamentowicz et al., 2010; Tolonen et al., 1992). They are used in paleo-ecological reconstruction of past environments (Charman, 2001; Warner and Charman, 1994) and biomonitoring, particularly in wetlands (Booth, 2001; Laggoun-Defarge et al., 2008; Qin et al., 2013). For few years, testate amoebae have integrated in functional diversity approaches, for example for the restoration follow-up of a floodplain in Switzerland (Fournier et al., 2012), in the upper Paranà River in Brazil (Arrieira et al., 2015) or in peatlands (Fournier et al., 2016, 2015; Jassey et al., 2016; Lamentowicz et al., 2015).

1.2 Working hypothesis

A better understanding of the response of peatland to stress and disturbance is important to protect or restore them, in particular with ongoing climate changes (Belyea and Malmer, 2004; Bridgham et al., 2006; IPCC, 2014). As optimal functioning of peatlands is related to water-logged conditions (Quinty and Rochefort, 2003), finding relevant proxies that are able to show trends in water level fluctuation or able to follow rewetting of damaged peatlands is essential (Bonnett et al., 2011; Trepel, 2007). Peatlands were traditionally monitored based on vegetation surveys (Bonnett et al., 2009; Graf et al., 2010; Hajkova et al., 2011) but vegetation structure is expected to change over few years and need trained staff or specialists to reliably monitor them (Bonnett et al., 2009; Vittoz and Guisan, 2007). In addition, recent works show that multi-proxy approaches are more powerful in ecological studies to highlight pattern and evolution (Bonnett et al., 2009, 2011; Vačkář et al., 2012). Testate amoeba community structure responds rapidly (days to month) to changes in water content (Amesbury et al., 2013; Charman and Warner, 1992; Marcisz et al., 2014; Woodland et al., 1998). Since the beginning of the century, they have been used to monitor peatlands, e.g. to show the restoration rate, the water level depth or the response to environmental drivers (Gilbert and Mitchell, 2006; Laggoun-Defarge et al., 2008; Lamentowicz et al., 2013). In this thesis,

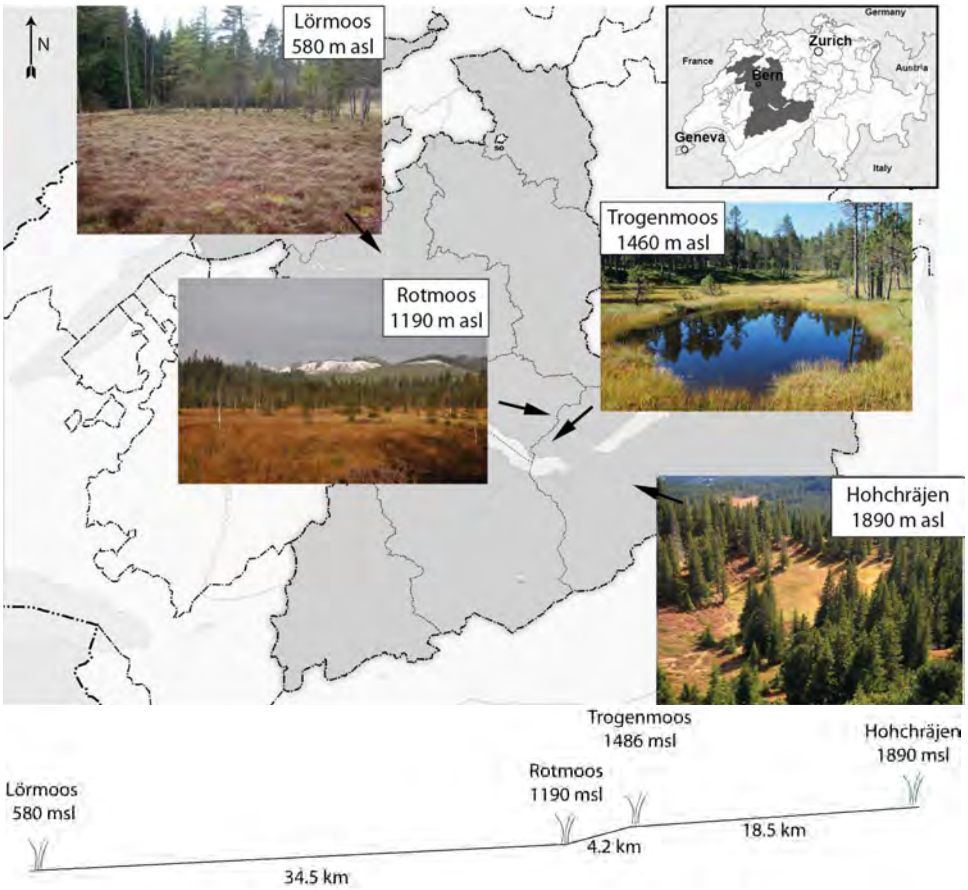


Figure 1.3 – Location, altitude and pictures of the four selected *Sphagnum* peatlands in the Bern Alps. All sites are protected by law since late 1980s and are registered as raised bogs in the Swiss Federal bogs and mires inventory. Below the overview map stands the graphical representation of the distance between sites (1/2 scale – horizontal to vertical).

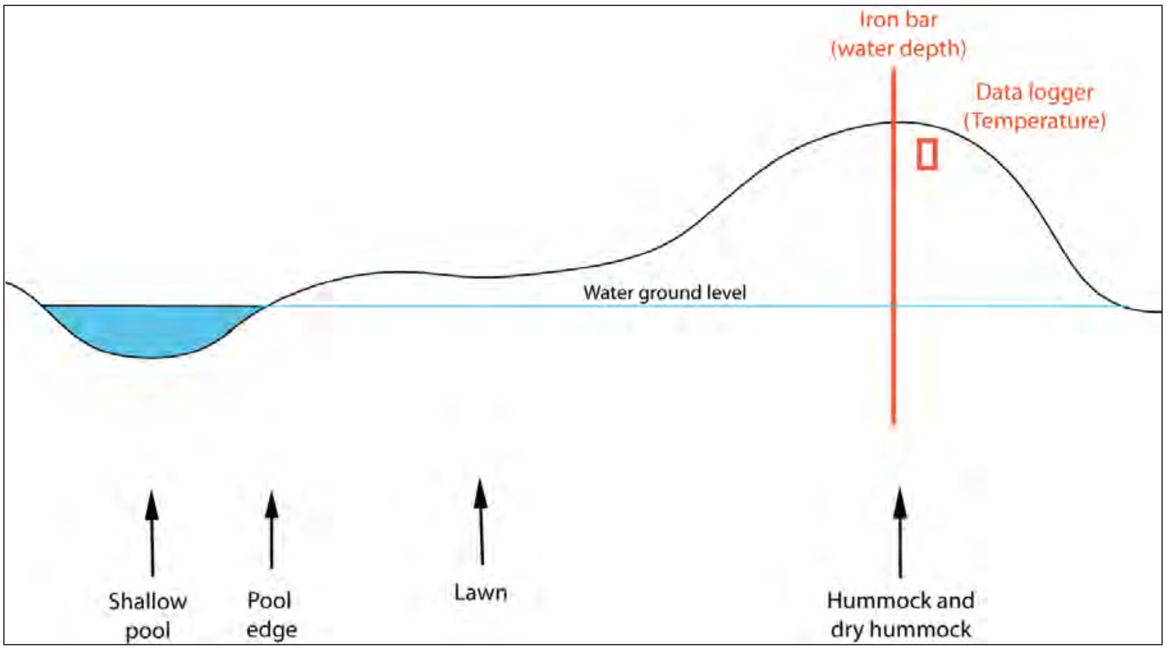


Figure 1.4 – Experimental design of the altitudinal comparison of response of vegetation and testate amoeba community structure to environmental variables (see text for details). In each site, vegetation relevés and *Sphagnum* stems (for extraction of testate amoebae) were collected along the humidity gradient, from pool to hummock (see below). Water table depth was monitored over one year (summer 2010 to summer 2011) and temperature was taken into account during growing season (ca. March to July 2011).

we will use multi-proxy approaches, between vegetation composition, testate amoeba community structure and testate amoeba functional diversity under controlled conditions in peatlands (Figure 1.2).

Working hypotheses:

1. Testate amoeba community structure shows finer micro-environmental gradients in peatlands compared to global vegetation surveys.
2. Functional diversity of testate amoeba community is complementary to testate amoeba community structure to understand ecosystem functioning of peatlands.
3. This double approach (functional diversity and taxonomy based structure) allows to monitor changes in ecosystem functioning, particularly by showing the current ecosystem functioning during revitalisation or restoration of peatlands.

To test our hypotheses involving testate amoebae, bio-monitoring, *Sphagnum* peatlands and functional diversity, we applied a progressive method going from natural peatlands to strictly controlled water table level in a mesocosm study. The topics are: 1) the response of testate amoeba community along an altitudinal gradient of natural *Sphagnum* peatlands in Switzerland; 2) the evolution of testate amoeba community after the transplantation of *Sphagnum* carpets at lower altitude, in a 100m^2 artificial peatland (Neuchâtel Botanical Garden); 3) the response of testate amoeba community to water level changes in a mesocosm experiment, in Neuchâtel Botanical Garden.

1.3 Study sites

1.3.1 Altitudinal gradient in northern Swiss Alps

The first study compared the structure of vegetation (vascular plants and bryophytes) and testate amoeba community in four *Sphagnum* peatlands along an altitudinal gradient, from the Swiss plateau to pre-Alps (Figure 1.3). We compared the response of a selection of ten testate amoeba morphotaxa (i.e. species or species-complexes) – selected according to their sensibility to water table depth – and the whole plant community to soil temperature, water table depth, and carbon and nitrogen content of *Sphagnum* mosses along the moisture gradient, from pool to hummock (Figure 1.4). The selection of peatlands was made in order to compare sites as similar as possible in Switzerland. All four peatlands are categorized as raised bogs in the Swiss Federal bogs and mires inventory (Ecker et al., 2008).

In each site, vegetation relevés were made along the humidity gradient observed in the field, from pool to hummock (micro-habitat), with five replicates for every micro-habitat. Simultaneously, *Sphagnum* stems were collected in each sampling plot for extraction of testate amoebae. Environmental variables were recorded all along the vegetation growing season (from about March to July 2011). Indirect and direct gradient ordinations, multiple factor analysis (MFA) and transfer function models for inferring water table depth were expected to show that a selection of ten testate amoeba taxa should be more powerful (% variance explained in RDA) and accurate (discrimination among habitats) indicators of local conditions (micro-habitat type, water table depth and *Sphagnum* C/N ratio) than the vegetation (vascular plants and bryophytes either individually or combined and considering the full diversity).

1.3.2 Artificial peatland

In 2014, an artificial raised bog was built in the Botanical Garden of Neuchâtel (Mulhauser et al., accepted). Covering a surface of 100 m^2 and with a maximal depth of 1.5 meter, it aimed to: 1) show this ecosystem to the public and use it as an informative and teaching tool, 2) favour the conservation of rare and endangered species *ex-situ*, 3) study the challenges of peatlands restoration in challenging climatic conditions. The bog was divided in two parts, (with 0.5 m and 1.5 m peat depth), each one being sub-shared in six sections (Figure 1.5). Four treatments were applied: bare peat with and without elimination of weeds (NP-W versus NP-NW); vegetalized surface (peatlands specific vegetation) with and without weeding (P-W versus P-NW; Figure 1.5). The vegetation is monitored three times a year (spring, summer and autumn) and no-peatlands plants are removed following each survey. Five piezometric tubes allowed to measure water table depths all along the year, and were measured every two weeks.

Summer 2015 was very dry and warm and the bog suffered from severe drought from June to December with rainfall events only in September. This unintentional experiment allows us to test the resistance of the testate amoeba community in *Sphagnum* patches according to their size and vascular plant cover (end-of-school project partly included here). For this project, we selected 27 patches of living *Sphagnum* with a diameter from 6 to 44cm and

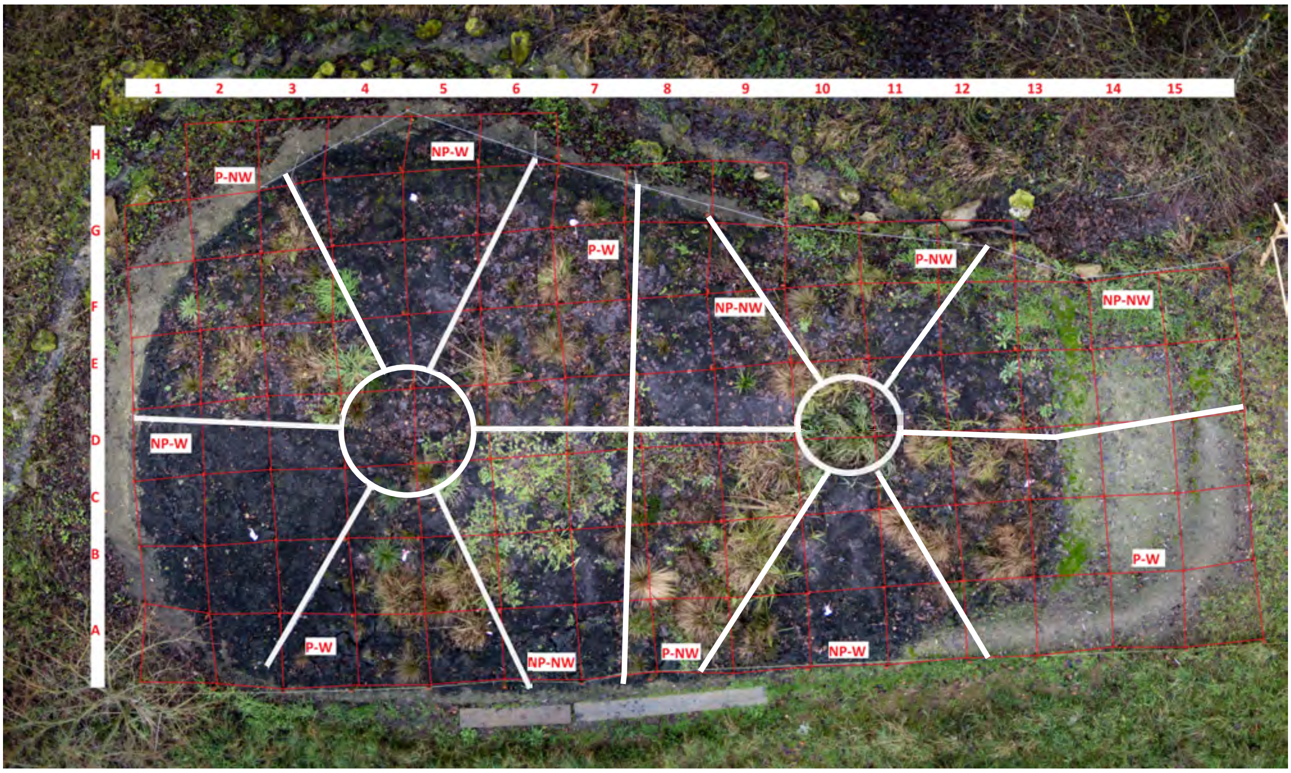


Figure 1.5 – Aerial image of the artificial peatbog, in the Botanical Garden of Neuchâtel. The bog is divided in two parts, with 0.5m (on the right hand) and 1.5m peat depth (on the left hand). Each part is divided in six sections, with contrasting treatments: naked peat (NP) or with peatland specific vegetation (P), both with (W) or without (NW) weeding (photo December 2015, ©Botanical Garden of Neuchâtel).

a cover from 0% to 90% (marked as permanent plots for future works). The first sampling campaign was conducted in September 2015, and preliminary results suggested that the community survived better in most shaded plots, at least in small and medium sized ones, with a shift towards most tolerant species. In autumn 2016, two additional samplings were made, the first one in permanent plots in Botanical Garden, and the second one in a regeneration area of a natural peatland in Bois des Lattes, Switzerland (1000 m a.s.l.), to have a reference point (i.e. natural reference). Environmental data include *Sphagnum* taxa and vitality, size and vascular cover of *Sphagnum* patches.

We followed the community of testate amoeba through taxonomic and functional diversity, with the structure of testate amoeba community from Le Bois des Lattes as a reference (Ruiz-Jaen and Mitchell Aide, 2005). We expected that the functional diversity approach will provide informations about the evolution of the niche constraints in the artificial peatland in response to water stress, in addition to taxonomic based interpretation. In comparison with the functional diversity in Bois des Lattes, we expect to have a comprehensive view of the functional niche we must favour to assess the success of the restoration (Hedberg et al., 2013; Laughlin, 2014).

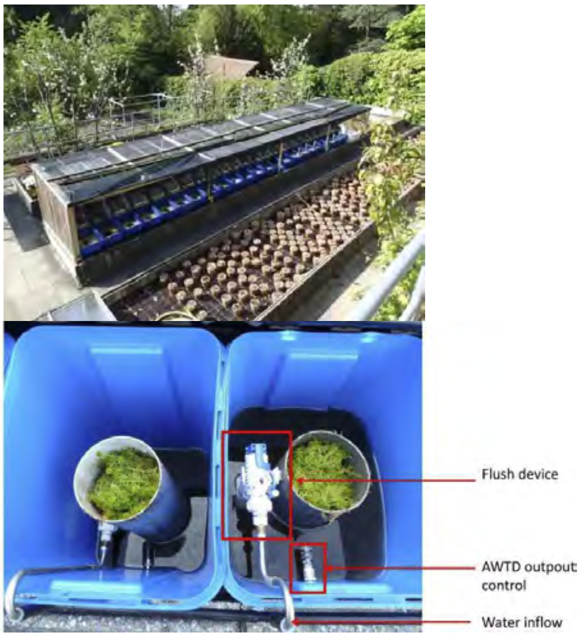


Figure 1.6 – Right hand: Installation of mesocosms in the Neuchâtel Botanical Garden. The peat cores were fitted in the blues tins and protected against direct sunlight. Left picture: detail of the mesocosm installation. The grey tubes contain a peat core with a *Sphagnum fallax* carpet on top. The minimum water level was controlled using a pump. The maximum water level was controlled by an overflow. The intermediate water level was controlled by a slow-drip (pictures M. Mulot).

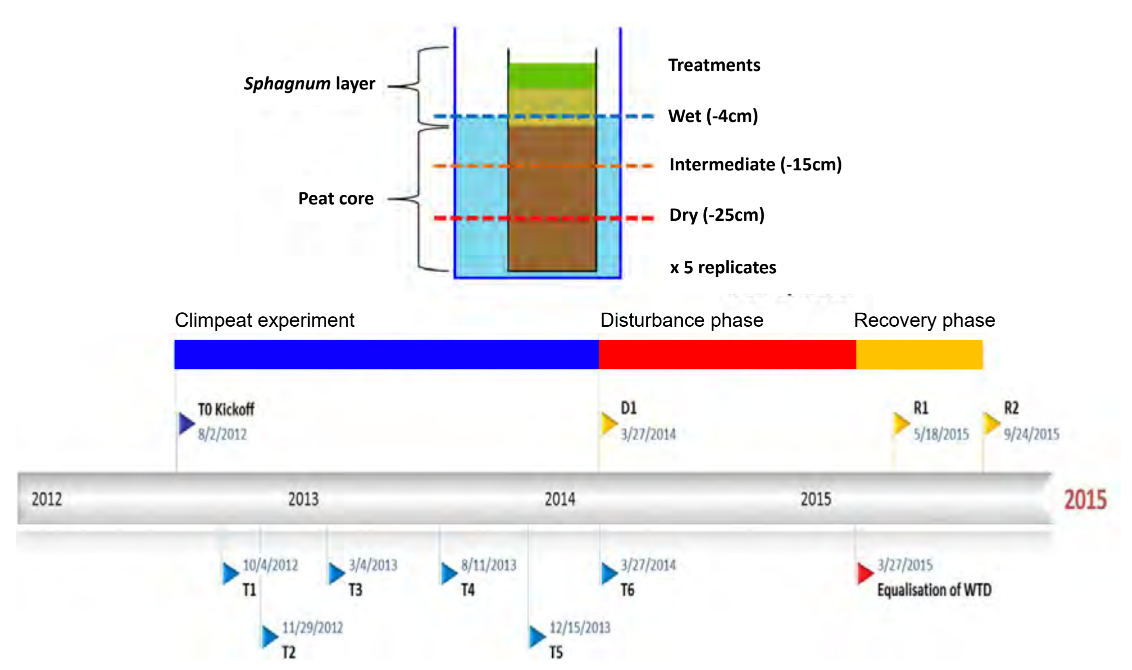


Figure 1.7 – top: Schematic representation of the mesocosm; a layer of *Sphagnum fallax* covers the top of the peat core. The water table depth (WTD) was defined from the bottom part of living *Sphagnum*. The can was filled up with water coming from a pool-to-hummock gradient with the whole microbial community, allowing all plots to begin with the whole range of expected community of testate amoebae. Bottom: Timeline of the Climpeat experiment (blue) and the recovery experiment (red: disturbance phase, yellow: recovery phase), only T0 and T6/D1 were the same in both experiments.

1.3.3 Mesocosms study

Modelling the complexity of relationships and feedback between organisms and physico-chemical variables is problematic. Disentangling links in natural ecosystems by changing selected variables is not really possible (Keddy, 1992; Macarthur and Levins, 1967). Studies in mesocosms, albeit simplistic, allow overcoming some of these limitations by isolating the effect of only one variable, whilst all other variables are being controlled (Benton et al., 2007; Cadotte et al., 2011; Fraser and Keddy, 1997).

In a collaborative project with Poland (Climpeat project, funded by the Polish-Swiss Research Programme), we built mesocosms in Neuchâtel and conducted a study with acute control on water table depth (Figure 1.6, Mulot et al., 2015). This mesocosm approach allowed us to manipulate the water table depth. We used three specific cases: draught (dry treatment), no change (control treatment), and flood event (wet treatment). We followed the evolution of testate amoeba community composition through taxonomic and functional diversity approaches, over a period of two and a half years. *Sphagnum* sampling for testate amoeba extraction was made seven times during the experimental period (Figure 1.7, covering ca. 2 years), together with the measurements of environmental variables (temperature of peat, soil moisture, air moisture and temperature, rain gauge, and solar radiation). Multivariate approaches, both taxonomic and functional diversities will improve the comprehension of the evolution of niche characteristics in response to the variation of a single variable. We also expect to highlight the most meaningful traits of testate amoeba taxa in response to drought or flooding aiming to develop tools for environmental monitoring of peatlands.

Following this experiment, in 2015, we equalized the water level in all mesocosms to monitor recovery of testate amoeba community structure. We made two additional samplings in May and September 2015 and compared the evolution of the community structure with the first experiment.

Chapter 2

Comparative ecology of vascular plant, bryophyte and testate amoeba communities in four *Sphagnum* peatlands along an altitudinal gradient in Switzerland

Isabelle Koenig[a], Elizabeth Feldmeyer-Christe[b], Edward A. D. Mitchell[a,c]

Abstract

Monitoring tools are needed to assess changes in peatland biotic communities and ecosystem functions in response to on-going climate and other environmental changes. Although the responses of soil organisms and plants to ecological gradients and perturbations do not always correlate, peatland monitoring is mainly based on vegetation surveys. Testate amoebae, a group of protists, are important contributors to carbon and nitrogen cycling in organic soils and are useful bioindicators in peatland ecology and paleoecology. There is however little comparative data on the value of testate amoebae, vascular plants and bryophytes as bioindicators of micro-environmental gradients in peatlands.

We compared the relationships of testate amoebae, bryophytes, and vascular plants with soil temperature, water table depth, micro-habitats and the carbon and nitrogen content of *Sphagnum* mosses in four peatlands along a 1300m altitudinal gradient in Switzerland. We used the full diversity of vascular plants and bryophytes but only a selection of ten easily identifiable testate amoeba morpho-taxa (i.e. species or species-complexes).

Indirect and direct gradient ordinations, multiple factor analysis (MFA) and transfer function models for inferring water table depth showed that a selection of ten testate amoeba taxa are more powerful (% of variance explained in RDA) and accurate (discrimination among habitats) indicators of local conditions (micro-habitat type, water table depth and *Sphagnum* C/N ratio) than vegetation (vascular plants and bryophytes either individually or combined and considering the full diversity).

Our study showed that a limited list of ten easily identifiable testate amoeba taxa have a higher bioindication value than the full bryophytes and vascular plants. Furthermore, testate amoebae can be analysed on samples collected at any season (accessibility allowed and if precise sampling sites are well marked) – a clear advantage for biomonitoring and can be used to infer past changes from the peat record at the same taxonomic resolution. This simple approach could therefore be very useful for biomonitoring of peatlands.

Keywords: peatland ecology; bioindication; testate amoebae; vascular plants; bryophytes; community ecology; elevation gradient

[a] Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland

[b] Biodiversity and Conservation Biology, Ecosystem Dynamics, Swiss Federal Research Institute WSL, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland

[c] Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

Ecological Indicators, 2015, 54, 48-59

doi:10.1016/j.ecolind.2015.01.043

2.1 Introduction

Ongoing rapid climate change, largely attributed to the increase in greenhouse gas emissions (Pachauri et al., 2014), has stimulated a considerable research effort on how biotic communities and related processes respond to these changes especially with respect to carbon (C) dynamics. Peatlands stock approximately the equivalent of 75% of total atmospheric C (Gorham et al., 2003). If actively growing they represent a significant C sink, but, when mineralization surpasses accumulation (e.g. owing to drainage or climate-related drought) they become a C source (Moore and Knowles, 1989). Climate warming or precipitation decrease may reduce C sequestration even of pristine peatlands and limit the regeneration potential of damaged peatlands (Jong et al., 2010; Samaritani et al., 2011), thus feeding back positively on warming (Arneth et al., 2010). It is therefore important to assess how peatlands respond to environmental change both in their structure (e.g. communities) and function (e.g. hydrology, C-balance; Davidson and Janssens, 2006).

A powerful approach to understanding the effects of environmental perturbation is to use bioindicators. As it is clearly not possible to study all living organisms in detail the question then is to determine which group of organism should be studied and what information they can provide. To answer this question comparative studies of different functional and taxonomic groups are required. Research on peatland ecology has historically focused primarily on vegetation and many assumptions on ecological gradients or functioning are made on the basis of vegetation patterns.

Some groups of peatland soil micro-organisms have been quite well studied in the last few decades (Gilbert and Mitchell, 2006), and especially the testate amoebae, a group of protists building a shell (called test). The shell allows relatively easy identification and is preserved in peat and in lake sediments thus making it possible to use them for paleoenvironmental reconstruction. Testate amoebae are well correlated to the main ecological gradients in *Sphagnum* peatlands, especially soil moisture (generally measured as water table depth), pH and nutrients (Booth and Zygmunt, 2005; Mieczan, 2009; Swindles et al., 2009). This has led to their use in the study of primary (e.g. palaeoecological records, Chambers et al., 2012; Charman, 2001; McMullen et al., 2004) and secondary succession (Talbot et al., 2010). Testate amoebae are increasingly considered as a useful tool for peatland bio-monitoring and management (Davis and Wilkinson, 2004; Mitchell et al., 2008b; Turner and Swindles, 2012). Although the taxonomy of these organisms is not fully satisfactory, recent studies have also shown that they could provide valuable ecological information even at low taxonomic resolution (Mitchell et al., 2014).

Few studies have compared the community patterns of testate amoebae (or other soil microbes) and plants or their respective potential as bioindicators (see for example Hajek et al., 2011; Hajkova et al., 2011). The existing ones (in peatlands and proglacial succession) show some discrepancy between the community-environment relationships (Carlson et al., 2010; Lamentowicz et al., 2010) and transfer function model performance (Mitchell et al., 2013) of testate amoebae vs. bryophytes and/or vascular plants. In *Sphagnum*-peatlands, higher species-environment correlations were found for testate amoebae than for bryophytes, vascular plants or both combined (Lamentowicz et al., 2010), while transfer function performance (on raw data) was higher for bryophytes for DWT but not for pH (Mitchell et al., 2013). Here we address this question in four *Sphagnum* peatlands located along elevation gradient from the Swiss lowlands (ca. 600m a.s.l.) to the subalpine-alpine limit (ca. 1900m a.s.l.).

To our knowledge, there are only three published studies of testate amoeba communities along altitudinal gradients. In forests soils and mosses, between $400m$ and $2500m$ a.s.l., in Bulgaria, Todorov (1998) showed that species richness decreased with altitude and that there was a significant difference among the biotopes. Mitchell et al. (2004) studied testate amoeba communities in a single moss species (*Hylocomiums splendens* (Hedw.) W.P. Schimp.) in the northern Italian Alps between 1000 and 2000m a.s.l.. Community structure was found to be more strongly correlated to soil chemical composition (silica vs. calcareous) than to altitude and no significant decrease in species richness was found with altitude. Krashevska et al. (2007) studied the testate amoeba communities in litter and upper soil horizons of three sites in a tropical mountain forest at 1000, 2000 and 3000m a.s.l. in Ecuador. Testate amoeba density was lowest at the mid altitude and highest at 3000m a.s.l., with an opposite pattern for specific richness. However part of these differences may be due to soil type, which varied with altitude (alumic acrisol, gley cambisol and podzol, respectively from the lowest to the highest site) as well as vegetation. Indeed, testate amoeba diversity and community structure were shown to vary in relation to factors such as plant functional richness (Ledeganck et al., 2003) and soil

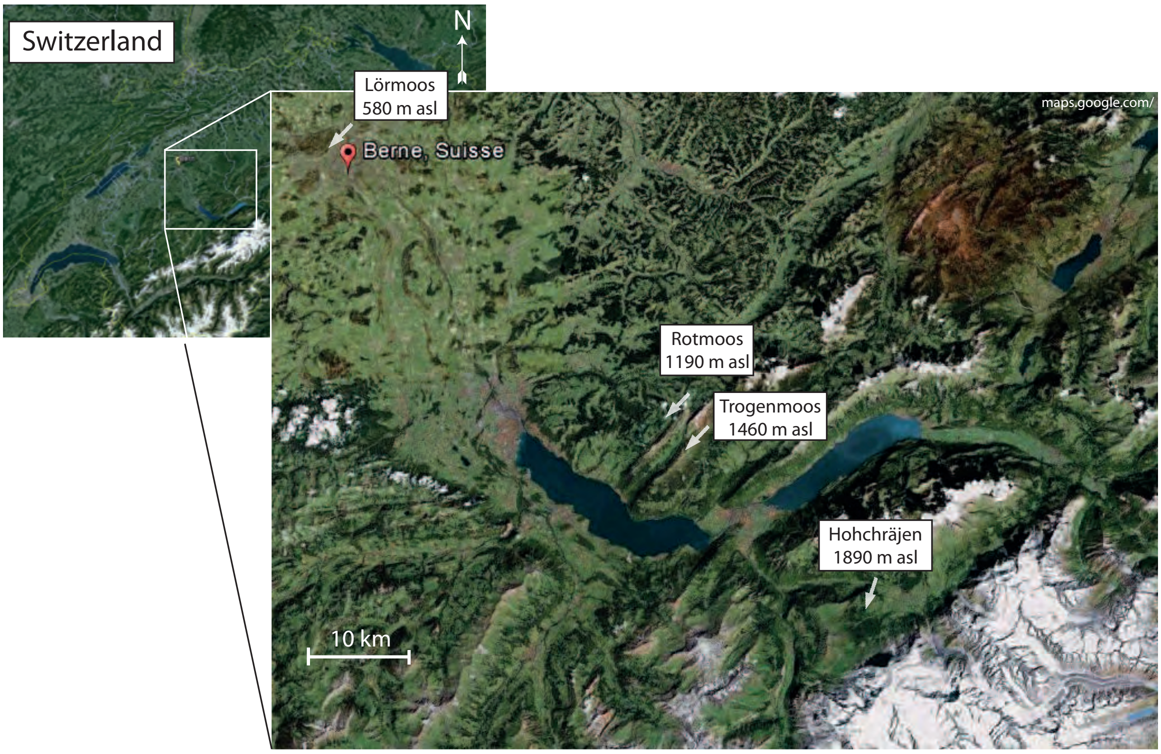


Figure 2.1 – Location of the four studied *Sphagnum* peatlands in Switzerland

type (Bonnet, 1964). These factors should therefore either be accounted for in numerical analyses, or excluded using appropriate sampling design.

Our goal here was not to specifically address diversity or ecological patterns along this elevation gradient but by studying an altitudinal gradient, we aimed to simulate a change in temperature (synchronic approach) while focusing on the same specific microhabitats of *Sphagnum*-dominated peatlands. We covered a broader range of habitats at each elevation than Mitchell et al. (2004) while still remaining in comparable biotopes at each study site, unlike the other two cited elevation gradient studies. We aimed to answer two main questions: 1) How do the relationships between testate amoebae, bryophytes and vascular plants and the main micro- and macro-ecological gradients compare and thus what are their respective predictive power for these variables? 2) How powerful are these three groups for discriminating among different micro-habitats along the whole humidity gradient (pool to hummock) within sites? As developing taxonomy expertise represents a true limitation for the use of protists in biomonitoring, rather than using the full community we addressed these questions using a selection of 10 morpho-taxa that can easily be identified by untrained analysts after a short learning period.

2.2 Methods

2.2.1 Study sites

We selected four sites in the same meso-climatic region (transition from Swiss Plateau to pre-Alps), in

Table 2.1 – Geographical and climatic characteristics of the four studied *Sphagnum*-dominated peatlands in Switzerland.

Site	Coordinates		Altitude	Temperature	Precipitation	Study area taken in account
	North	East	m a.s.l.	°C [a]	mm [a]	m^2 [c]
Lörmoos	7°17′39″	46°58′54″	580	7.9	1028	1600
Rotmoos	7°50′30″	46°47′39″	1190	4.7	≈ 1300 to 1500[b]	2200
Trogenmoos	7°51′48″	46°45′39″	1460	3.1	≈ 1300 to 1500[b]	1900
Hohchräjen	7°58′10″	46°36′41″	1890	1.0	1575	1800

[a] Mean annual average for 1961–1990.

[b] Estimated from MeteoSwiss general cartsmaps.

[c] Estimated using the geoportal of canton Bern (https://www.geo.apps.be.ch/fr/).

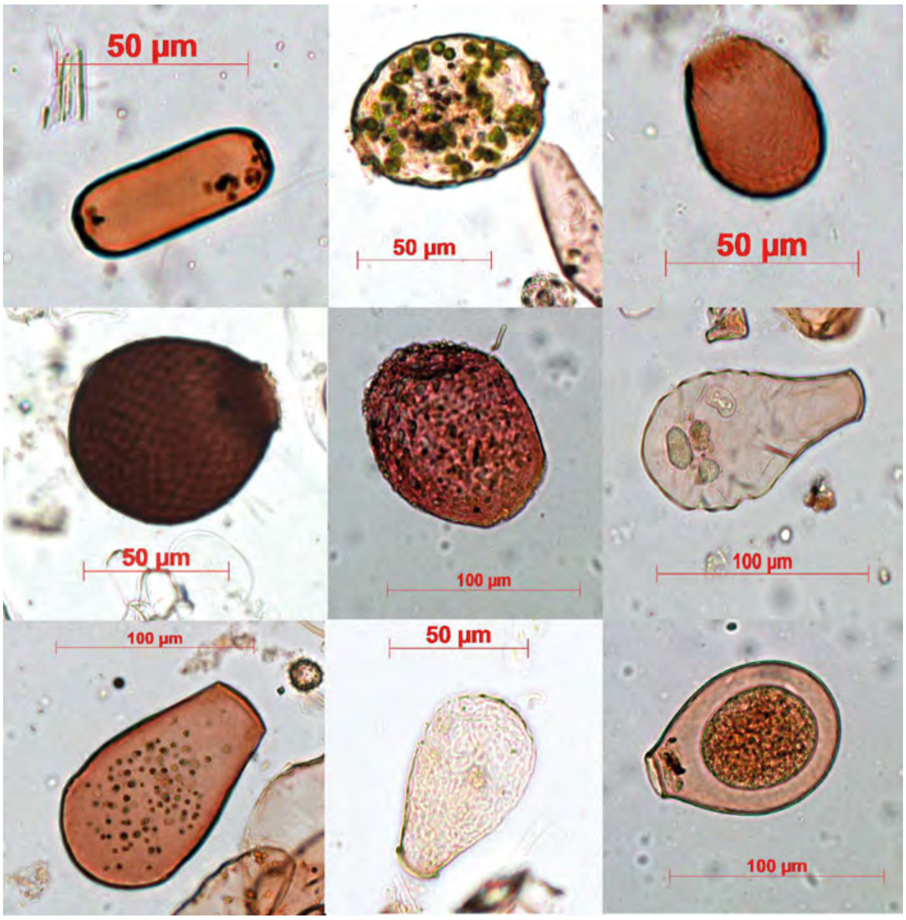


Figure 2.2 – Pictures of the selected testate amoeba morpho-taxa: (a) *Archerella flavum*, (b) *Amphitrema wrightianum*, (c) *Assulina muscorum*, (d) *Assulina seminulum*, (e) *Heleoperarosea*, (f) *Hyalosphenia elegans*, (g) *Hyalosphenia papilio*, (h) *Nebela militaris*, and (i) *Nebela tincta s.s.l.*.

the northern Swiss Alps (canton Bern, Fig. 2.1). All sites are legally protected at least since 1987 and are categorized as open bogs in the Swiss Federal bogs and mires inventory (Ecker et al., 2008). The four study sites are located along an altitudinal gradient from 580 m a.s.l. to 1890 m a.s.l. The regional climate is temperate continental; only the lowest site (Lörmoos, Table 2.1) is slightly drier and warmer than the other three, being less influenced by higher rainfall associated to mountains. General climatic data was obtained from MeteoSwiss, the Swiss Federal Office of Meteorology and Climatology. The two lower sites have been impacted by peat harvesting and/or cattle grazing, but the upper two are considered to be relatively pristine (i.e. as close as can be in Switzerland).

2.2.2 Sampling design and fieldwork

Plots were selected in autumn 2010 in the main micro-habitats within each study site: pool, pool edge, dry hollow, lawn, hummock, and dry hummock, with five replicates per micro-habitat in most cases and three at the minimum (total: 19 to 25 plots per site and 84 overall). Pools were missing in Lörmoos owing to the general dryness of the site. As Lörmoos was particularly dry in autumn 2010 and no pools with standing water existed, we selected the lowest microtopography habitats and called them "hollows" to differentiate them with the pools with standing water that existed in the other sites. These hollows were indeed the wettest microsites in Lörmoos. In Rotmoos the "pool edge" microhabitat was clearly defined, with specific vegetation, owing to the steeper slope between the lawns and the pools. Percentage cover of vascular plants and bryophytes were estimated according to the Londo scale (Londo, 1976). Nomenclature follows for vascular plants and Jahns (2007) for bryophytes. *Sphagnum* species were identified, first in the field and identifications were confirmed by microscopy following Daniels and Eddy (1990).

An iron bar was inserted in each plot to obtain a time-integrated measurement of the depth of water table (DWT). The bars were left for ten to twelve months (September to November 2010 – August 2011). The DWT was estimated as the vertical

Table 2.2 – Summary of environmental variables measured at four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland. DWT: depth to water table; GDD: growing-degree day; T_{Med}: median soil temperature; T_{Range}: soil temperature range; Amp_{Tm}: mean of soil daily temperature range (the last four variables were measured at 7.5cm depth from the beginning of the growing season to July 2011). Different letters indicate significant differences (Tukey Honest Significant Differences calculated on transformed data; see text for further details).

	Lörmoos 580m a.s.l.		Rotmoos 1190m a.s.l.		Trogenmoos 1460m a.s.l.		Hochräjen 1890m a.s.l.	
	Mean	se	Mean	se	Mean	se	Mean	se
N [%]	0.540^{a}	0.021	0.692^{b}	0.042	$0.564^{a,b}$	0.049	0.549^{a}	0.05
C [%]	43.1^{a}	0.3	43.0^{a}	0.1	$42.8^{a,b}$	0.2	42.2^{b}	0.1
C /N	$81.6^{a,b}$	2.6	67.4^{a}	3.7	84.8^{b}	5.6	84.9^{b}	5.7
DWT [cm]	21.5^{a}	3	12.2^{b}	1.3	8.1^{b}	1	10.5^{b}	1.5
GDD	1908.7^{a}	33.6	1499.7^{b}	7.3	1481.3^{b}	46.8	923.4^{c}	70.3
T_{Med} [°C]	13.6^{a}	0.2	12.7^{b}	0.1	$13.1^{a,b}$	0.3	12.7^{b}	0.2
T_{Range} [°C]	22.0^{a}	0.8	20.5^{a}	0.8	23.1^{a}	0.7	12.5^{b}	0.5
$AmpT_{m}$ [°C]	3.3^{a}	0.7	4.0^{a}	0.6	4.8^{a}	0.5	2.8^{a}	0.2

distance between the top of the moss carpet and the limit between the rusted and non-rusted parts of the bars. Though more approximate than direct regular or continuous WTD monitoring, it allowed us to estimate the maximum water level in each plot. This method was adapted from the PVC discoloration method (Booth et al., 2005) since the PVC tapes we experimented with did not discolour under anoxic conditions (Mitchell unpublished). Soil temperature was measured in three micro-habitats and three replicates per site using HOBO Pendant®Temperature/Alarm Data Logger (8K-UA-001-08, precision 0.14°C at 25°C). The selection of plots to be monitored was made in order to cover the whole variability inside each micro-habitat. Therefore at each site one or two micro-habitat(s) did not have any measured temperature data. The data loggers were inserted at 7.5cm depth, corresponding to the maximal density of testate amoeba shells (Buttler et al., 1996; Mitchell and Gilbert, 2004) and programmed for one measurement every 15 minutes from October 2010 to August 2011, except for the highest site, which could not be accessed until May 2011.

2.2.3 Testate amoebae and moss elemental chemistry

Five or six *Sphagnum* mosses were collected in each plot and pooled to constitute a representative sample of each plot. The section between 5 and $10cm$ under the top of the capitulum was used for testate amoeba extraction, thus avoiding the vertical micro-distribution of species in the upper centimetres (Mitchell and Gilbert, 2004). Testate amoebae were extracted using a standard sieving (200μm) and back-sieving (15μm) method (Booth et al., 2010) using deionised water. The shells were concentrated by centrifugation and stored in 2mL tubes with three drops of glycerol ($C_3H_8O_3$) and one tablet of *Lycopodium clavatum* (batch N° 938934, 10679 ±426 spores per tab) standard preparation from Lund University (Sweden) according to Stockmarr (1971). *Sphagnum* C and N contents were measured by pyrolysis on the moss remaining after testate amoeba extraction and the C/N ratio calculated from these data.

Testate amoebae were identified and counted up to a minimum of 150 shells but any slide was counted entirely to avoid possible bias due to the uneven distribution of shells depending on size or shape (i.e. smaller taxa being more likely to move closer to the edges of the coverslip). To assess the bioindicator potential of testate amoebae even with minimal taxonomic knowledge, we selected ten taxa which are abundant in *Sphagnum* peatlands, known to be well correlated with humidity and pH gradients and are commonly used in paleo reconstruction (Charman et al., 2004; Mitchell, 2003; Opravilova and Hajek, 2006): acid pools taxa: *Archerella flavum*, *Amphitrema wrightianum*, *Hyalosphenia papilio*; acid lawns: *Heleopera rosea*, *Hyalosphenia elegans*; acid hummocks: *Nebela militaris*, *Assulina muscorum*, *Assulina seminulum*, *Nebela tincta s.l.* and *Nebela collaris*, Figure 2.2. The taxonomic resolution we used corresponds to the approach used in many palaeoecological studies (Charman, 2001). Testate amoeba taxa that did not correspond to the ten selected taxa were also counted to reach the total of 150 shells. This enabled us to calculate the contribution of the selected taxa to the overall community.

Using a limited subset of ten taxa and a relatively coarse taxonomic resolution has, potentially, two main advantages: 1) it makes it easier for non-specialists to conduct similar analyses as only minimal training will be required to be able to identify the ten selected morpho-types and 2) it strongly reduces the risk of confusion among analysts making comparisons among studies much easier. As we tend to calibrate a tool to monitor peatlands, the choice of easily identified taxa is a strong advantage with respect to the potential use of this method by peatland managers or for high school and undergraduate student projects. The potential disadvantage however is that a given species complex may include taxa with contrasted ecological preferences in which case the accuracy of the ecological inference would be lower. This approach represents an alternative to other approaches such as using genus-level identification (Wilkinson and Davis, 2000) or using all taxa but pooling morphologically similar taxa regardless of taxonomic affiliation (Mitchell et al., 2014). Results were expressed in relative abundance (percentage of the total population) for multivariate numerical analyses.

2.3 Results

2.3.1 Environmental data

The environmental data included depth to water table (DWT), C and N content and the C/N ratio of *Sphagnum*, altitude, micro-habitat, growing degree days (GDD) calculated from the temperature data using the software HOBO®Ware Pro 3.2 (actual temperature method, no cut-off, threshold: 0°C), median temperature (TMed), temperature range (TRange) and the mean of daily temperature range ($AmpT_{Med}$) during the main vegetation period. The vegetation period was calculated from the beginning of increase of GDD until July 31st 2011 (peak biomass). Environmental data were used either globally or split in two sub-matrices: micro-environmental (DWT, C, N, C/N, AmpTm) and macro-environmental (Altitude, GDD, TMed, TRange). Measured data for *Sphagnum* total N, C and C/N and DWT (n=84) and temperature (GDD, TMed, TRange and $AmpT_{Med}$, n=34) are given in Table 2.2.

As only 34 from the 84 plots were equipped with temperature data loggers, we completed the environmental dataset in order to work with the full vegetation and testate amoeba data. For the microhabitats for which temperature data was available we calculated the mean of the three measured values within the micro-habitat and used these values for the two missing values. For the microhabitats lacking temperature data we replaced the missing values with predicted values based on the observed data and the estimated joint distribution of the temperature and all the other environmental variables. We used a multiple imputation based on a bootstrapped EM (expectation maximization) algorithm implemented in the Amelia II (package Amelia II for R Project for Statistical Computing, 2.14.0, (Honaker et al., 2011)). Amelia fills data gaps using a predictive model based on principal component analyses (PCA) on all data including samples for which all variables were measured and those which had data gaps. The missing values are calculated so as maintain the multidimensional structure of the PCA. Furthermore the model takes into consideration gradients or differences among data subsets, in our case these were the local (pool/hollow to hummock) humidity gradient and the four sites. Further information on the algorithm is given in Honaker and King (2010). The model was run five times and for each missing value the average value of the five iterations was used.

2.3.2 Numerical analyses

A three-pronged approach was used to explore the data:

A) We first compared the environmental variables among sites using ANOVAs and Tukey HSD (Honest Significant Differences) tests with logarithm transformed data (natural log) for N, C, DWT and AmpTm; square-root transformation for GDD and TRange. We assessed how the environmental variables, the testate amoeba communities and the vegetation (i.e. vascular plants + bryophytes) varied in relation to 1) micro-habitat and 2) altitude using between class analysis (BCA). BCA can be seen as a particular case of a principal component analysis with respect to instrumental variables (PCAIV), in which a single factor is used as instrumental variable. These analyses were performed using R (R Core Team, 2016) and the packages ade4 for R (Dolédec and Chessel, 1987) and vegan for R (Oksanen, 2015).

B) We then modeled the correlation between each community and the environmental variables using redundancy analyses (RDA, vegan for R Project, version 2.0-5, (Oksanen, 2015)) with Hellinger-transformed community data and assessed using transfer function how each data set performed to infer DWT. We used four data sets for these analyses: 1) vegetation (i.e. vascular plants + bryophytes), 2) vascular

Table 2.3 – Percentage cover of vascular plants and bryophytes at four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland. Rare species with an overall mean $< 1\%$ are not shown.

	Lörmoos 580m a.s.l.		Rotmoos 1190m a.s.l.		Trogenmoos 1460m a.s.l.		Hochräjen 1890m a.s.l.	
	Average	se	Average	se	Average	se	Average	se
Vascular plants								
Andromeda polifolia	0	0	1.91	0.57	4.04	1.28	0	0
Calluna vulgaris	1.16	0.71	8.73	2.84	3.03	1.27	0.99	0.57
Carex limosa	0	0	0.63	0.63	5.11	2.39	11.37	3.08
Carex rostrata	0.47	0.2	6.56	2.86	0.33	0.33	11.72	2.42
Drosera sp.	1.39	0.58	1.64	0.6	3.35	1	0	0
Eriophorum vaginatum	5.55	1.69	2.23	0.93	1.92	1.07	9.98	3.16
Juncus filiformis	0	0	0	0	0	0	1.29	0.67
Menyanthes trifoliata	0	0	1.82	1.26	0	0	0	0
Molinia caerulea	3.27	1.55	3.78	0.84	0.03	0.03	0	0
Rhynchospora alba	2.66	0.75	0	0	0.08	0.08	0	0
Scheuchzeria palustris	0	0	0	0	2.01	1.12	0	0
Trichophorum cespitosum	0	0	13.11	2.45	18.18	3.16	0	0
Vaccinium oxycoccos	23.23	4.25	4.56	0.88	1.56	0.51	0	0
Vaccinium uliginosum	0	0	0	0	1.21	0.68	4.14	1.55
Bryophytes								
Aulacomnium palustre	0.77	0.5	1.42	0.69	0	0	0.04	0.04
Polytrichum strictum	0.84	0.55	3.4	1.14	0.03	0.03	5.94	2.21
Sphagnum capillifolium s.l.	25.78	5.19	4.04	2.14	0	0	9.52	3.4
Sphagnum cuspidatum	0	0	3.37	1.99	5.51	3.24	0	0
Sphagnum fallax	17.92	6.25	0.26	0.26	22.85	4.88	21.24	5.23
Sphagnum magellanicum	14.16	5.48	17.6	4.33	22.91	5.74	19.29	5.04
Sphagnum majus	0	0	0	0	2.08	1.54	0	0
Sphagnum papillosum	1.49	0.87	24	4.6	0.96	0.71	0	0
Sphagnum russowii	0	0	0	0	0	0	2.17	1.5
Sphagnum subsecundum	0	0	0	0	0	0	1.16	0.96
Sphagnum tenellum	0	0	0.32	0.32	3.73	1.86	0	0

Rare species not shown: *Carex pauciflora*, *Eriophorum angustifolium*, *Nardus stricta*, *Phalaris arundinacea*, *Picea abies*, *Pinus mugo*, *P. sylvestris*, *Vaccinium myrtillus*, *Viola palustris*, *Pleurozium schreberi*, and *Polytrichum commune.*

plants, 3) bryophytes and 4) testate amoebae. The environmental data were used as explanatory variables in the RDA. We tested each explanatory variable independently as well as the global model. Transfer function were built using weighted averaging (wa), with tolerance down-weighting, using the R package "rioja" (Juggins, 2012). Prediction error was estimated by a "leave-one out" cross-validation and the performance of the models assess by the correlation between observed and predicted values and the root mean squared error of prediction (RMSEP).

C) Finally we explored the correlative structures among the six data matrices: 1) vascular plants, 2) bryophytes, 3) testate amoebae, 4) macro-environment, 5) micro-environment, 6) site name and Humidity code (1 – pool to 5 for dry hummock) projected passively in the analysis) using multiple factor analysis (MFA, FactomineR for R Project, version 1.21, (Lê et al., 2008). We quantified the correlation between each pair of matrices using the RV coefficient.

All analyses were conducted on both the extended data set (i.e. including estimated missing temperature data) as well as on the samples for which direct measurements were available.

2.3.3 Macro and micro-environmental characteristics of the four sites

Sphagnum carbon content decreased from 43.1% to 42.2% from the lowest to the highest site. *Sphagnum* nitrogen content was highest at Rotmoos and similar at the other three sites. As a result, *Sphagnum* C/N ratio was significantly higher at Rotmoss than at the other three sites (Table 2.2, only measured data were taken in account). Average water table was lowest at the low elevation site Lörmoos (DWT $= 21.5$cm) and never reached the surface at that site. The other three sites were wetter (DWT $= 8.1$ to 12.2cm) and all included pools. GDD decreased significantly with elevation. However, T_{Med},

Table 2.4 – Average density and relative abundance of ten testate amoeba morpho-taxa at four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland.

	Lörmoos 580m a.s.l.		Rotmoos 1190m a.s.l.		Trogenmoos 1460m a.s.l.		Hochräjen 1890m a.s.l.		All sites
Taxon	Average	se	Average	se	Average	se	Average	se	Average
Density [10^3 **ind/g]**									
Amphitrema wrightianum	0	0	26.7	9.0	16.5	4.8	191.9	93.8	55.3
Archerella flavum	3.3	1.4	5.6	2.5	28.9	13.6	63.0	21.4	23.6
Assulina muscorum	5.8	1.4	12.8	2.1	13.9	4.0	10.7	4.5	10.9
Assulina seminulum	1.9	0.3	1.4	0.3	1.3	0.3	4.8	2.2	2.3
Heleopera rosea	0.8	0.3	1.1	0.3	0.2	0.1	3.6	2.0	1.4
Hyalosphenia elegans	24.0	4.9	20.3	4.1	23.7	6.9	0	0	17.4
Hyalosphenia papilio	1.1	0.5	1.4	0.4	6.1	1.6	47.3	10.8	12.8
Nebela collaris	3.7	1.2	3.6	0.9	1.1	0.4	6.2	1.7	3.6
Nebela militaris	2.4	1.5	1.8	0.5	1.2	0.9	1.2	0.6	1.7
Nebela tincta	5.3	1.2	5.0	1.3	1.3	0.3	9.1	3.6	5.1
Total community	82.3	13.3	191.8	30.4	184.2	30.1	392.8	109.3	209.4
% of total community	58.7		41.5		51.1		86.0		64.0
Relative abundance [% **of total count]**									
Amphitrema wrightianum	0	0	8.8	2.4	7.0	1.9	26.0	6.3	10.2
Archerella flavum	2.4	1.0	2.6	1.2	9.0	2.8	17.9	5.9	7.5
Assulina muscorum	8.1	1.6	7.5	0.9	5.7	0.9	2.8	0.9	6.1
Assulina seminulum	3.7	0.7	1.3	0.3	1.1	0.3	1.8	0.8	1.9
Heleopera rosea	1.2	0.4	0.8	0.2	0.3	0.2	2.3	1.2	1.1
Hyalosphenia elegans	30.3	5.9	15.0	3.0	19.3	4.5	0	0	16.3
Hyalosphenia papilio	1.0	0.4	1.0	0.3	3.2	0.7	16.0	2.3	4.9
Nebela collaris	6.6	2.1	3.5	1.2	1.1	0.4	5.2	1.7	4.0
Nebela militaris	2.5	1.4	1.4	0.4	1.1	0.8	0.7	0.2	1.4
Nebela tincta	8.0	2.0	4.7	1.2	1.3	0.4	5.8	2.2	5.0

T_{Range} and $AmpT_{Med}$ - measured at a depth of 7.5cm - did not show any significant altitudinal trend.

2.3.4 Vegetation

The vegetation of the four sites included characteristic *Sphagnum* peatland taxa such as *Calluna vulgaris*, *Carex rostrata*, *Drosera sp.*, *Eriophorum vaginatum* and *Vaccinium oxycoccos* for vascular plants and *Aulacomnium palustre*, *Polytrichum strictum*, *Sphagnum magellanicum* and *S. capillifolium* for bryophytes (Table 2.3). *Andromeda polifolia* and *Sphagnum cuspidatum* were found only at the two mid-elevation sites. The vegetation at Lörmoos was dominated by *V. oxycoccos* while species characteristic for very wet conditions were sparse (*C. rostrata*, *Rhynchospora alba*). Rotmoos included several species characteristic for slightly minerotrophic conditions (e.g. *Menyanthes trifoliata*, *Trichophorum cespitosum* and *Sphagnum papillosum*, Delarze et al., 2008). Trogenmoos contained both rare species such as *Scheuchzeria palustris* and *Sphagnum majus*, which are characteristic for *Caricion lasiocarpae*, a rare vegetation type only occurring in some of the best preserved mires (Delarze et al., 2008), as well as *T. cespitosum* and *Sphagnum fallax*, two taxa often associated with disturbance (Grosvernier et al., 1997). The highest site, Hochräjen, was characterized by some montane-alpine taxa such as *Juncus filiformis*, *Nardus stricta* (not showed in the table) and *Vaccinium uliginosum* (foothills).

2.3.5 Testate amoebae

Testate amoeba density averaged 209 x 10^3 individuals per gram dry weight of *Sphagnum* and increased almost five-fold (82.3 - 393 x 10^3 ind /g) from the lower to the higher site (Table 2.4). The ten selected taxa accounted for 64% of the total community on average over the four sites. This percentage was lowest at Rotmoos (41.5%) and highest at Hochräjen (86%), mostly due to the high abundance of three mixotrophic species characteristic for wet habitats *Amphitrema wrightianum* (26% of the total community), *Archerella flavum* (17.9%) and *Hyalosphenia papilio* (16%) at the highest site (Table 2.4). Noteworthy was also the absence of *Hyalosphenia elegans* from the highest site (Hochräjen) while it was the most abundant taxon at the other three sites (15 – 30.3%). By contrast *A. wrightianum* was absent from the lowest site (Lörmoos) while it was quite abundant (ranking 1st to 3rd in proportion of the whole community) at the other three sites (Table 2.4).

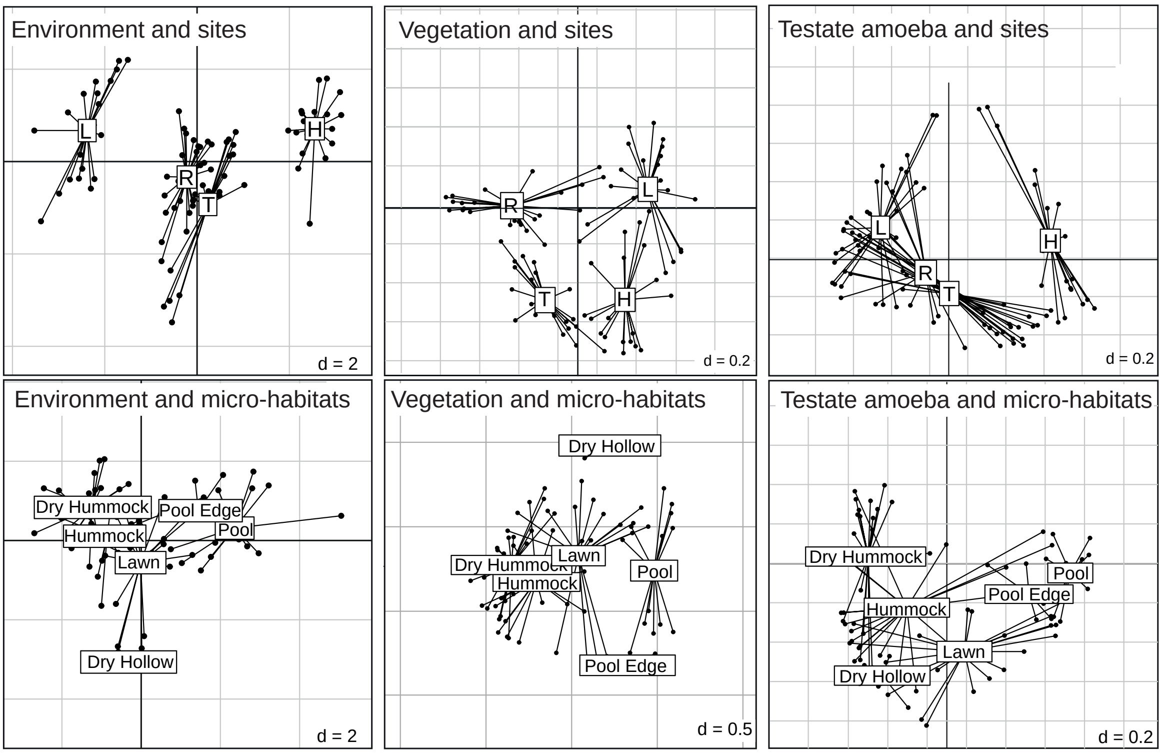


Figure 2.3 – Between class analysis of environmental data (left), vegetation (centre), and testate amoeba communities (right) from four *Sphagnum*-dominated peatlands in relation to sites (top row) and micro-habitats (bottom row). L: Lörmoos (alt: 580 m); R: Rotmoos (1190 m); T: Trogenmoos (1460 m); H: Hochräjen (1890 m).

2.3.6 Between class analyses

The first set of between class analyses (BCAs), revealed contrasted altitudinal patterns of environmental characteristics, vegetation and testate amoeba communities (Figure 2.3, boxes 1-3). The BCA on environmental data showed the altitudinal gradient and a high similarity between the two intermediate sites. In the vegetation BCA the four sites were well separated, but the pattern did not correspond to the altitudinal gradient. The elevation gradient was also visible on the testate amoeba BCA although the lowest site partly overlapped with the two intermediate sites and the overall spread within each site was higher than for the environmental data. In the BCAs with micro-habitats as explanatory factor the humidity gradient was well correlated to axis 1 (Figure 2.3, boxes 4-6). In the vegetation BCA the hollows and pool edges were clearly separated while drier micro-habitats overlapped. Micro-habitats were better separated based on testate amoebae than on the environmental data. The unusual (rather dry) hollows of Lörmoos stood out in the BCAs on environmental as well as on vegetation data and in the testate amoeba BCA it's position was close to hummocks and lawns. The pool edges (only defined in Rotmoos) also stood apart in the vegetation BCA, while this was not the case for the analyses based on environmental characteristics and testate amoeba, where pool edges were projected close to pools.

2.3.7 Direct gradient analyses of the correlation of each community with the soil dataset and performance of transfer function models

All eleven variables tested individually in the RDA significantly explained some part of variance in the testate amoebae and vascular plant data sets while for bryophytes two variables were not significant (TRange and AmpTm). Highest correlations were found with testate amoebae for eight variables, with vascular plants with two (GDD and TRange) and with bryophytes with one (T_{Med}, Table 2.5, upper part).

Vascular plants showed higher correlation than bryophytes for all variables related to altitude (macro-env), except for T_{Med}. This agrees with the presence or higher abundance of some vascular plant species either at high elevation (*J. filiformis* or *V. uliginosum*) or low elevation (*V. oxycoccos*). Likewise the similar pattern observed for testate amoebae agrees with the distribution of *H. elegans* (absent from the highest site) and *A. wrightianum* (ab-

Table 2.5 – Summary results of redundancy analyses (RDAs) on vascular plant, bryophyte and testate amoeba community data, macro- and micro-environmental variables from four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland. Percentage of variance explained by individual macro- and micro environmental variables and associated p-value from RDAs on vegetation (vascular plants + bryophytes), vascular plants, bryophytes and testate amoeba data. Highest scores for each variable are underlined and highest scores per data set are bolded. Upper table: whole dataset with calculated missing values (n = 84), lower table: variance explained with sample directly measured (n = 34).

Whole dataset (n = 84)	Bryophytes + Vasc.		Vascular plants		Bryophytes		Testate amoebae	
	%	p-Value	%	p-Value	%	p-Value	%	p-Value
Macro-environment								
Altitude	9.3	0.005	**17.6**	0.005	4.6	0.005	19.0	0.005
GDD	7.5	0.005	14.1	0.005	3.9	0.010	13.8	0.005
T_{Range}	5.5	0.005	10.5	0.005	1.2	0.360	8.0	0.005
T_{Med}	5.5	0.005	5.4	0.005	6.0	0.005	4.0	0.010
All macro-env.	**22.8**	**0.005**	**33.0**	**0.005**	**16.6**	**0.005**	**32.1**	**0.005**
Micro-environment								
DWT	13.1	0.005	14.4	0.005	11.6	0.005	**26.0**	0.005
C	9.3	0.005	7.3	0.005	**12.2**	0.005	17.5	0.005
N	6.0	0.005	3.8	0.005	6.9	0.005	10.3	0.005
C/N	5.0	0.005	3.0	0.024	5.8	0.005	8.7	0.005
$AmpT_{Med}$	4.6	0.005	5.4	0.005	2.5	0.071	5.7	0.005
All micro-env.	**28.9**	**0.005**	**30.4**	**0.005**	**27.4**	**0.005**	**47.1**	**0.005**
Descriptive variables								
Micro-habitat	28.6	0.005	27.4	0.005	27.8	0.005	38.9	0.005
Site	28.1	0.005	39.1	0.005	22.6	0.005	26.6	0.005

Raw dataset (n = 34)	Bryophytes + Vasc.		Vascular plants		Bryophytes		Testate amoebae	
	%	p-Value	%	p-Value	%	p-Value	%	p-Value
Macro-environment								
Altitude	12.2	0.005	**20.6**	0.005	6.3	0.077	23.5	0.005
GDD	9.3	0.015	16.2	0.005	5.1	0.140	16.2	0.005
T_{Range}	9.4	0.005	13.5	0.005	3.3	0.400	9.6	0.015
T_{Med}	6.0	0.033	7.3	0.017	6.5	0.059	4.1	0.240
All macro-env.	**29.5**	**0.005**	**42.5**	**0.005**	**18.7**	**0.046**	**39.1**	**0.005**
Micro-environment								
DWT	14.2	0.005	14.7	0.005	**11.6**	0.010	**24.3**	0.005
C	6.7	0.015	6.6	0.024	8.2	0.024	15.7	0.005
N	6.5	0.013	4.0	0.260	7.3	0.036	10.4	0.013
C/N	6.0	0.028	2.9	0.350	7.6	0.017	9.0	0.027
$AmpT_{Med}$	6.2	0.025	8.0	0.013	3.2	0.370	9.1	0.020
All micro-env.	**33.9**	**0.005**	**35.6**	**0.005**	**28.7**	**0.005**	**50.7**	**0.005**
Descriptive variables								
Micro-habitat	31.7	0.005	31.9	0.005	27.6	0.005	45.0	0.005
Site	33.3	0.005	44.4	0.005	27.1	0.005	32.4	0.005

sent from the lowest site). Correlations with all micro-env variables (related more to micro-habitat variation) were highest for testate amoebae. Correlations with bryophytes were higher than with vascular plants for moss elemental chemistry variables (C, N, and C/N) while the opposite was true for DWT and $AmpT_{Med}$, two variables measured below the moss carpet. In the variance partitioning (based on RDA) the whole macro-env dataset explained a similar proportion of variance of the vascular plants and testate amoeba data (respectively 33.0% for vascular plants and 32.1% for testate amoeba, Table 2.6), but a lower proportion of the bryophyte data (16.6%). Micro-env variables explained a higher proportion of the testate amoeba than the vascular plant and bryophyte data (respectively 47.1%, 30.4% and 27.4%).

The descriptive variables were most strongly correlated with vascular plants for the site (39.1%) and with testate amoebae for micro-habitats (38.9%), in agreement with the BCA analysis. In line with the RDA results with micro-environmental variables, the performance of DWT transfer function models was highest for testate amoebae, somewhat lower for vascular plants and whole vegetation and lowest for bryophytes (Table 2.6).

Table 2.6 – Performance of testate amoebae-based transfer function for DWT based on data from four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland: leave-one out cross-validated tolerance down-weighted [inverse (WA.inv.tol) and classical (WA.cla.tol)] weighted average models.

	Whole vegetation		Vascular plants		Bryophytes		Testate amoebae	
	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2
WA.inv.tol	7.00	0.46	6.66	0.51	7.84	0.32	**5.91**	0.61
WA.cla.tol	7.60	0.47	6.91	0.52	11.88	0.33	6.77	**0.62**

RMSEP: root mean square error of prediction; R^2: correlation between observed and predicted values.

2.3.8 Combined analysis of testate amoeba, vegetation and environmental data

The position of samples in the multiple factor analysis (MFA, Figure 2.4) showed quite clearly the altitudinal and humidity gradients. All Hochräjen plots were well separated from the three other sites, which partly overlapped in the ordination space. Rotmoos and Trogenmoos overlapped with respect to the altitudinal gradient except for the wettest micro-habitats. The position of samples from the lowest site suggested drier conditions than for the other three sites and the micro-habitat described as hollow appeared to be more similar to the lawns than to the pools of the other sites.

In the projection of species and variables, pools were associated to relatively high N content and higher median soil temperature (Figure 2.5). Hummocks and dry hummocks were correlated with low water table (i.e. high DWT) and high C/N ratio. The altitudinal gradient was correlated with T_{Range} and GDD. Altitude was best correlated with two testate amoeba taxa, *H. papilio* for the higher site and *H. elegans* for the lowest one, together with two vascular plants, *Vaccinium oxycoccos* and *Molinia caerulea.* The pools of the higher site were characterized by *A. wrightianum* and *A. flavum* for the testate amoebae, *Carex limosa* and *C. rostrata* for the vascular plants and *Sphagnum fallax* for the mosses. The same micro-habitats in Trogenmoos and Rotmoos were correlated with *A. muscorum* for the testate amoebae, *T. cespitosum* for the vascular plants and

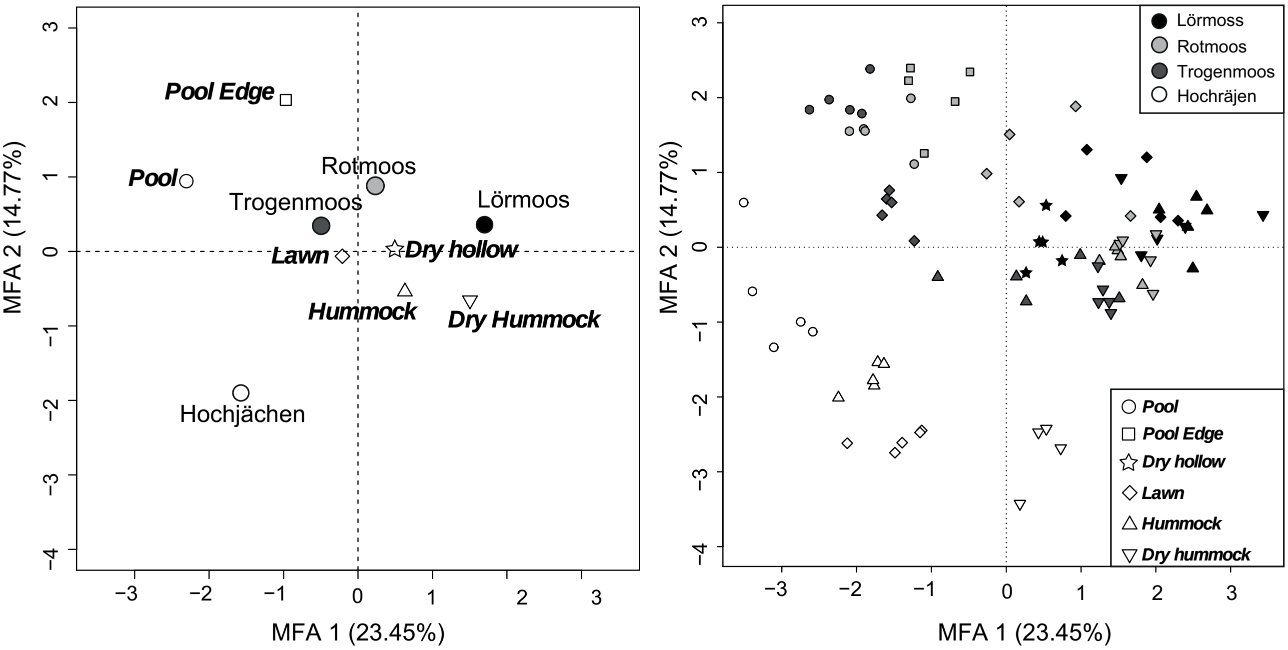


Figure 2.4 – Multiple factor analysis (MFA) of vascular plant, bryophyte and testate amoeba community data, macro- and micro-environmental variables from four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland. Left: projection of centroids of qualitative variables (site names and micro-habitats) projected passively on the MFA. Right: Individual factor map, showing the position of samples in the MFA. Humidity gradient go from top left part of the graph to bottom right angle and altitude gradient go from bottom left part to top right part.

Table 2.7 – RV coefficients from the multifactor analysis (MFA) on vascular plants, bryophytes, testate amoebae, micro-environmental variables, macro-environmental variables from four *Sphagnum*-peatlands along a 1300 m elevation gradient in Switzerland. The descriptive variables were passively projected in the analysis. Upper half matrix: p-values; lower half matrix: RV coefficients. MFA indicates the correlation and p-value with the overall combined ordination.

	Vasc. plant	Bryophytes	Amoebae	Micro-env.	Macro-env.	MFA
Vascular plants	1	<0.001	<0.001	<0.001	<0.001	0.769
Bryophytes	0.248	1	<0.001	<0.001	0.001	0.594
Amoebae	0.543	0.257	1	<0.001	<0.001	0.747
Micro-env	0.324	0.263	0.431	1	<0.001	0.661
Macro-env	0.375	0.115	0.296	0.164	1	0.542
MFA	0.769	0.594	0.747	0.661	0.542	1

S. papillosum and *S. cuspidatum* for the mosses. The position of the Lörmoos hollow samples clearly suggested that they were drier than pools at the other sites.

The RV coefficients of the MFA (Table 2.7), which correspond to a squared Pearson correlation coefficient showed that micro-environmental variables were more strongly correlated to testate amoebae while macro-environmental variables were more strongly correlated to vascular plants. The analysis of the reduced data set yielded similar results (details not shown).

2.4 Discussion

2.4.1 Macro- and micro-environmental characteristics of the four sites

We aimed to select four comparable sites while covering a range of climatic conditions found along the elevation gradient and other factors potentially influencing peatland ecosystems that may also vary along this gradient. Indeed, several factors are associated to altitudinal gradients including differences in the degree of human impact, precipitation rates, duration of snow cover and pollution levels (Körner, 2007). The soil chemistry data measured at the four sites were within the range expected for *Sphagnum*-dominated peatlands (Bragazza and Gerdol, 2002; Waughman, 1980) and with the exception of the lowest site all micro-sites could be found at each site. Inter-site differences reflect climatic gradients (e.g. drier lower site), ombrotrophy-minerotrophy gradient (somewhat higher minerotrophic influence at the Rotmoos site), and history of human impact (drainage at the lowest site). As such these four sites therefore provide a reasonable range of general characteristics to allow the comparative study of species-environment relationships for vascular plants, bryophytes and testate amoebae.

2.4.2 Community & environment relationships – micro-habitats and DWT

As expected community structure of vascular plants, bryophytes and testate amoebae all reflected well the pool-hummock gradient as shown by significant correlations to micro-habitat type (explaining 27.4% to 38.9% of the species data in the RDA) and the depth to the water table (explaining $11.6to26\%$ of the data). However, a striking result of this study is that ten testate amoeba taxa were more strongly correlated than vascular plants, bryophytes, or both combined to micro-habitats (38.9% vs. 27.4 to 28.6%) and to the humidity gradient (26% vs. 11.6 to 14.4%). With the exception of temperature-related variables and sites the correlation of all variables were about two times higher with the ten selected testate amoeba taxa than with all bryophytes, all vascular plants or both groups combined. The performance of transfer functions also logically performed better with the testate amoeba data than the vascular plants and bryophytes. Furthermore the performance of the models is comparable to that of models based on the full diversity of testate amoebae.

It may appear surprising that bryophytes are not better related to micro-environmental variables. Indeed, they could be expected to respond in a similar way than testate amoebae to humidity and nutrients gradients. Some variables not taken in account like niche competition, sensitivity to microclimatic variations, interstitial water conductivity or nutrients may be more important for bryophytes than for testate amoebae and could explain the relative weakness of the relationships observed here (Bragazza, 1997; Buttler et al., 1998; Malmer et al., 1992; Mitchell et al., 2000). However our results agree with a recent comparative ecological study of testate amoebae, bryophytes and vascular plants in fens and bogs, which showed that testate amoeba communities were more strongly correlated to all measured variables (Conductivity, pH, magnesium, calcium and DWT) than bryophytes or vascular plants or both combined (Lamentowicz et al., 2010).

The absence of *Amphitrema wrightianum* at the lowest site is in line with its association with bog pools, which were absent from that site. *Archerella flavum*, a species characteristic for wet and oligotrophic lawns, was rare at the two lower sites, in line with average drier (Lörmoos) or more minerotrophic (Rotmoos) conditions. Both taxa were abundant at the upper two sites, and especially at the highest one. However, these two taxa may respond to site history (e.g. peat harvesting and subsequent revitalization, Buttler et al., 1996; Laggoun-Defarge et al., 2008) as much if not more than to differences in climate, soil moisture or nutrient content.

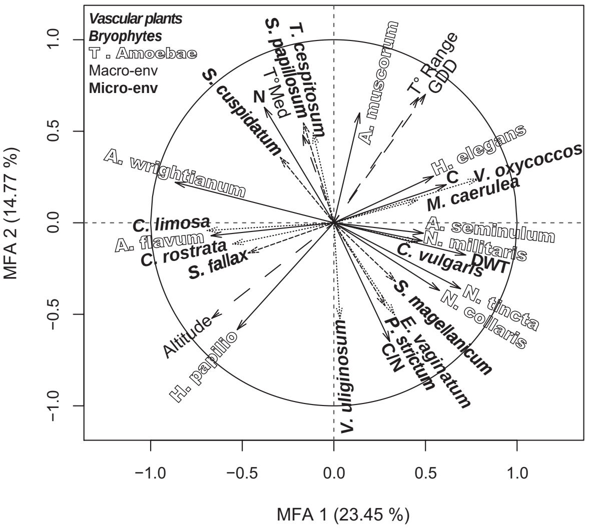


Figure 2.5 – Correlation circle map of the MFA of vascular plant, bryophyte and testate amoeba community data, macro- and micro-environmental variables from four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland (see Figure 2.4). For clarity, only variables with a score over $\cos^2=0.2$ are represented. T Amoebae: testate amoebae, Micro-env: micro-environmental variables (N, C, C/N, $\mathrm{AmpT_{Med}}$), Macro-env: macro-environmental variable (Alt, GDD, $\mathrm{T_{Med}}$, $\mathrm{T_{Range}}$).

2.4.3 Community patterns in relation to sites

The four sites were best defined by the vegetation data (explaining 39.1% of variance in the RDA vs. 22.6% and 26.6% for bryophytes and testate amoebae respectively). This difference seems partly due to the presence of characteristic subalpine plant species at the highest site (e.g. *Juncus filiformis*, *Nardus stricta*), to differences in trophic status among sites (*Menyanthes trifoliata* indicating more minerotrophic conditions at Rotmoos), and to the absence of some taxa at some sites in part due to the absence of pools at the lowest site: *Carex limosa* and *C. rostrata* both require very wet conditions that were rare or absent at the lowest, driest site (Lauber et al., 2007). These patterns may, however, also relate to the history of the sites. For example *Vaccinium oxycoccos* was shown to be favoured by bog revitalization measures (Graf et al., 2010) and this is in line with its high abundance at the lowest site.

2.4.4 Community patterns in relation to elevation and temperature

Testate amoeba communities were correlated to altitude and GDD gradient in the same range as vascular plants, but bryophytes were only weakly correlated to these two variables. The correlation of vascular plants along the altitude gradient fits with the known distribution of plants along elevation gradients and could thus be expected despite the fact that this study was focused on a very specific ecosystem and a limited potential list of plant species. By contrast, such a result was not expected for testate amoebae, which are usually primarily correlated to micro-environmental conditions such as DWT (Booth et al., 2008).

All other factors being equal, species such as *Carex limosa*, *C. rostrata*, *Archerella flavum* and *Amphitrema wrightianum* which are associated with wetter conditions can be expected to be more abundant at higher elevation where the summer hydric stress is shorter and less intense than at lower elevation. A recent study showed that testate amoeba communities are correlated not only to DWT but also to moisture variability (Sullivan and Booth, 2011). Moisture variability is likely to be lower at higher elevation and this could partly explain altitudinal patterns. Such differences may also have implications for the functioning of microbial food webs (Lamentowicz et al., 2013).

A recent study using a similar approach (i.e. four *Sphagnum*-dominated peatlands along an elevation gradient in Switzerland, two of which – the highest and lowest sites - were identical as in this study) showed that altitude explained a third of the variance in testate amoeba community data while DWT only explained a marginal fraction (Lamentowicz et al., 2013). This result was explained by the fact that only a single habitat (lawns) was sampled and thus the DWT gradient was very short. In our study we sampled the full DWT gradient within each site and

DWT emerged as the strongest variable explaining testate amoeba community structure. Altitude explained a smaller fraction (19% vs. 33%) of variance than in the study of Lamentowicz et al. (2013). This difference between the two studies may be due to the different taxonomic resolution, to the fact that altitude explains a different proportion of variance depending on the microhabitat, or to the fact that only two of the four sites were shared between the two studies.

The distribution pattern of the two *Hyalosphenia* species in our study and the one of Lamentowicz et al. (2013) suggests a possible response to elevation with *H. elegans* being limited to warmer conditions and *H. papilio* to colder sites. However, the absence of *H. elegans* in Hochräjen, the highest site, is most likely not due to severe weather conditions as it was found near the Arctic Circle (Beyens and Chardez, 1995; Payne et al., 2006) and it was abundant in a *Sphagnum magellanicum* sample from the Northwest Territories, Canada (Mitchell, unpublished data). A possible alternative explanation is that *H. elegans* tolerates moisture fluctuations somewhat better than *H. papilio* (Sullivan and Booth, 2011). The absence of *H. elegans* from the highest site could also be due to slightly more minerotrophic conditions at this site (e.g. runoff influencing hydrochemistry during snowmelt). In line with this, *H. elegans* was shown to respond very strongly (100%) to N & P enrichment (Mitchell, 2004). However, this species did not show a clear response to N addition (Mitchell, 2004).

With only four sites it is clearly impossible to generalise such results. Further studies, ideally including a higher number of sites and experimental manipulations of climate DWT and other environmental factors are clearly needed to explore in more depth the relationship between *Sphagnum* peatland testate amoeba communities and altitude.

2.4.5 Implications for bioindication

Although vegetation analysis is a well-established method for the peatland monitoring our study showed that the analysis of testate amoebae provides more accurate information on micro-environmental conditions than vascular plants and/or bryophytes. Furthermore we show that it is not necessary to know all testate amoeba taxa to obtain valuable ecological information; a carefully selected list of a limited number of easily identifiable taxa suffices for bioindication purposes. The generation time of testate amoebae ranges from a few days to weeks depending on taxa and environmental conditions is much faster than that of bryophytes and vascular plants (Schönborn, 1986). Communities are rather stable under natural conditions between seasons (Warner et al., 2007), but they can change over a period of several months when conditions change (Marcisz et al., 2014). Thus testate amoebae respond to environmental changes at an appropriate speed for being used in monitoring programs.

An additional advantage of testate amoeba analysis is that samples can be collected at all seasons (providing sites are accessible and sampling sites are permanently marked – e.g. with long sticks – to be found beneath the snow). Sampling is much faster than the time required for vegetation relevés and thus more site can be studied in a given field campaign. The time needed for microscopic analyses is reasonable once the species are known (and learning this is quick for the selected taxa). Testate amoeba analysis for peatland bioindication purposes is therefore a potentially very powerful and economically interesting approach.

2.5 Conclusion

Although testate amoebae are regularly used for paleoenvironmental reconstruction, their usefulness in biomonitoring is not yet fully recognized beyond academic research. Our study shows how a selection of only ten, easily identifiable testate amoeba morpho-taxa can provide valuable ecological information on micro-environmental conditions in *Sphagnum*-dominated peatlands. An untrained analyst can learn to identify these ten taxa in a few hours and only a simple microscope is needed for the analyses. This method therefore has the potential to become part of the standard toolkit of peatland managers.

2.6 Acknowledgements

We thank Swiss Federal Office of Meteorology and Climatology MeteoSwiss for access to climatic data, Radu Slobodeanu for statistical help, the municipalities of Itramen and Habkern and the canton of Bern for granting access to the sites and the University of Neuchâtel for funding.

Chapter 3

Impact of two hot and dry summers on testate amoeba community structure and functional diversity of an artificial bog

Isabelle Koenig[a], Kjetill Christinat[a], Mirko d'Inverno[a], Edward A. D. Mitchell[a,b]

Abstract

Central Europe peatlands have been strongly damaged by human impacts and conservation efforts now aim to preserving the remaining sites and restoring damaged ones. But such efforts are potentially jeopardized by on-going climate warming. A small (100m^2) artificial peatland was built in the Botanical Garden of Neuchâtel in the autumn of 2014 with the aim to study peatland restoration in a climatically challenging context (i.e. the Swiss lowlands 550m a.s.l.). The very hot and dry summers of 2015 and 2016 provided an unintentional experiment opportunity to test the resistance of biotic communities to unfavourable conditions. We focus on testate amoebae, a key functional group in microbial food webs, commonly used as indicators of current and past ecological conditions (e.g. water table depth, pH). We assessed the changes in testate amoeba community structure and functional traits in *Sphagnum* patches of contrasted sizes and shading by vascular plants collected in the experimental bog in the autumns of 2015 and 2016. We also collected analogous samples in a naturally regenerating cutover peatland in the Jura Mountains at 1000m a.s.l. as a reference. Living and dead assemblages were counted separately to assess the recent (ca. 1 year) shifts in communities.

The comparison of living and dead assemblages showed an increasing representation of testate amoeba taxa indicative for dry conditions (*Corythion dubium*, small *Euglypha* sp.) in 2015 and a further weaker shift in 2016. This shift was mirrored by changes in traits with an increased proportion of smaller taxa with a small pseudostome and a loss of mixotrophy, coherent with the draughts of 2015 and 2016. Contrary to our hypothesis the shift to dry indicators was more marked in the larger and more shaded moss patches, due to the better survival, and hence potential for community shifts in these patches while the communities in the smaller/less shaded barely survived. Communities in the reference site contained a lower proportion of wet indicator species than the initial communities in the artificial bog, reflecting the origins of the transplanted vegetation, but this pattern reversed by the end of the second year.

On-going climate warming is likely threatening the survival of bogs at the warm/dry limit of their distribution, and even more the restoration of damaged bogs. However, change in the vegetation can be slow and managers need early indicators of change. Our results illustrate the usefulness of testate amoebae as bioindicators of hydrological conditions in secondary bogs. Assessing the success of restoration measures is essential, but often not done by lack of financial resources. The analysis of testate amoebae is an economical option for rapid assessment of restoration success and could easily be implemented in the toolkit of peatland managers.

Keywords: Peatlands, testate amoebae, functional traits, artificial bog, botanical garden, reference site, regeneration.

[a]Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland
[b]Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

In preparation

3.1 Introduction

Sphagnum-dominated peatlands are ecosystems with permanent waterlogged soils, low nutrient availability and high acidity (Mitsch and Gosselink, 2007). Anoxic conditions hamper organic matter mineralisation leading to carbon accumulation. Northern peatlands represent an important carbon stock of 500±100Gt (gigatons), representing ca. 1/3 of all soil C trapped in ca. 3% of total land area (Gorham, 1991; Yu, 2012). Peatlands are threatened by direct impact such as peat mining (Ramsar, 2013) and ongoing climate changes, resulting in the release of C and thus feeding back positively to warming (Davidson and Janssens, 2006; Fleischer et al., 2016). Peatlands contain a relatively small number of highly specialised taxa, well-adapted to the characteristic drastic conditions. These species are however poor competitors (MacArthur and Wilson, 1967) and when conditions change (e.g. due to nutrients input, drought, or drainage) less-specialised and more competitive species colonise, leading to a loss of biodiversity and C-sequestration function (Chapman et al., 2003). Peatlands also represent invaluable archives of past environmental changes and human history (Buckland, 1993). Understanding global change effects on peatlands is therefore relevant at the global (C-cycling) and local/regional (biodiversity conservation, preservation of peat archives) scales. To achieve this a combination of observational, experimental and modelling approaches are used. Our focus here is on an experimental and observational study making use of an artificial peatland built in a botanical garden for outreach, conservation and research purposes.

In 2014, a small (ca. 100m^2) raised bog was constructed in the Botanical Garden of Neuchâtel, Switzerland (Figure 3.1) and planted with characteristic species collected from regional pristine and regenerating cutover bogs. As high moisture and low nutrient contents are needed to ensure the viability of specific raised bog plants and associated microbial communities (Andersen et al., 2013), recreating a lowland peatbog is challenging, because the local climate at ca. 530m a.s.l. is warmer and drier than in the mountains at ca. 1000m a.s.l.. In 2015, extended periods of drought occurred in Switzerland (MétéoSuisse, 2016) followed by a humid winter 2015-16 and spring in 2016 with more than 750m of rain for the first semester in Neuchâtel (75% of mean annual amount), followed by a short but intensive heatwave in the summer of 2016 (MétéoSuisse, 2017). In the fall of 2015, the bog was very dry with deep cracks in the peat and a drastic reduction of the water table (lowest average of five measurement points on August 8th 2015: -59.7cm; Sup. Figure 3.A1). The rainy spring of 2016 provided suitable conditions for the recovery of the bog vegetation. A vegetation survey indeed showed that most of the plants and bryophytes survived (Mitchell et al., unpublished data). However, the high water level of spring 2015 was never again reached for any extended period since (Sup. Figure 3.A1), suggesting that appropriate water level for the long-term survival of the bog cannot be maintained in the long-term. These meteorological conditions of 2015 and 2016 therefore represented a first real challenge for this experimental bog, as well as a valuable unintentional experiment opportunity to test the resistance of characteristic bog communities to unfavourable conditions.

Figure 3.1 – The experimental raised bog in Botanical Garden of Neuchâtel in spring 2015. Top: global view on May the 4th during the very wet spring period, right insert: *Vaccinium myrtillus*, left insert: *Vaccinium oxycoccos*. Left pictures, gradual drying of "fen" area (top to bottom: May the 4th, May the 13th, June the 2nd), during that period, the water table dropped from 19cm above ground level to 8cm under ground level. Right pictures (top to bottom and left to right): *Eriophorum vaginatum*, *Betula nana*, *Sphagnum* patches with *Drosera rotundifolia* and apparition of first cracks on bare peat; *Polytrichum strictum*, *Pinus mugo* subsp. *uncinata*; *Drosera rotundifolia*.

To assess the impact of these two climatically challenging years on the bog, we analysed the patterns of testate amoeba communities in *Sphagnum* patches of different size, and covering a broad range of shading from vascular plants in 2015 and 2016. Testate amoebae are unicellular protists building a shell (named test). They represent the dominant micro-eukaryotes in *Sphagnum* peatlands (Gilbert and Mitchell, 2006). Owing to the relatively short generation times (weeks to months – allowing communities to adapt to changing conditions), their community turnover along environmental gradients, and the decay-resistant tests they produce, testate amoebae are useful indicators to monitor changes in environmental conditions (Mitchell et al., 2008b). Testate amoebae are widely used as paleohydrological indicators (Booth, 2002; Lamentowicz et al., 2015; Payne et al., 2008; Swindles et al., 2009; Qin et al., 2013) and increasingly also as biomonitors in peatland monitoring and restoration (Booth, 2008; Davis and Wilkinson, 2004; Daza Secco et al., 2016; Laggoun-Defarge et al., 2008; Sullivan and Booth, 2011). They have indeed been shown to reflect water table depth and hydrochemistry more accurately than vegetation in *Sphagnum* peatlands (Koenig et al., 2015) and to respond rapidly to changes in hydrology and peatland restoration (Buttler et al., 1996; Daza Secco et al., 2016; Lamentowicz et al., 2013; Marcisz et al., 2016).

Testate amoebae can be used as bioindicators using the classical taxonomical approach as well as the functional traits approach. Here we explore both. Functional diversity and community weighted mean of traits are meaningful tools to measure and estimate ecosystem functioning (Laliberté et al., 2014; Lavorel and Garnier, 2002; Ricotta and Moretti, 2011). The assumption of the functional diversity approach is that species morphology and life history traits reflect and affect niche dimensions (Kearney et al., 2010; Mouillot et al., 2007; Villéger et al., 2011). Well-selected traits can be used to assess the impact of stress or disturbance on communities by revealing changes in niche dimensions (Fournier et al., 2015; Moretti and Legg, 2009; Mouillot et al., 2013), and, through these effect on communities, inform on ongoing ecological processes (e.g. changes C cycling due to alterations of the food web structure). The selection of traits should be done carefully and the relation between traits and environmental filtering assessed or at least strongly suspected (Mlambo, 2014; Petchey and Gaston, 2006). This approach was first developed for plants and animals and is now being explored for microorganisms such as ciliates and testate amoebae (Fournier et al., 2015, 2016; van Bellen et al., 2016). The functional traits approach allows partly overcoming identification difficulties and provides more directly ecologically informative information than change in taxonomic composition (Booth, 2008; Daza Secco et al., 2016; Fournier et al., 2015; Koenig et al., 2015; Lamentowicz et al., 2013, 2015). For example, Fournier et al. (2012) demonstrated that testate amoeba functional traits were related to soil moisture in a floodplain. Marcisz et al. (2016) showed that the proportion of mixotrophic taxa decreased and the proportion of small taxa with a hidden (plagiostomic) aperture increased in response to fire and peat extraction in two paleaoecological datasets in Poland. In the mesocosm study (Chapter 4), we observed a decrease of mixotrophic taxa and an increase in small taxa with a ventral or ventral-central aperture in response to a lowering of the water level.

We hypothesised that 1) the community structure and community weighted mean of functional traits (CWM) of testate amoebae from *Sphagnum* patches growing on bare peat would change in response to the severe droughts of 2015 and 2016 and 2) that the magnitude of this effect would be highest in small *Sphagnum* patches with low shading by vascular plants as compared to larger, more shaded patches. We expected shifts in CWM to reflect change in moisture condition and to inform on change in microbial food web structure. We compared these temporal patterns with the testate amoeba communities of similar *Sphagnum* patches sampled in a naturally recovering peatland located at 1000m in the Jura Mountains, used as a reference and expected to see clearer difference in the lowland experimental site.

3.2 Materiel and methods

3.2.1 Experimental bog and study design

The experimental bog was built in autumn 2014 in the Botanical Garden of Neuchâtel at 530m a.s.l.. A ca. 60cm layer of clay-rich sediments (marls) was overlain with 0 5 - 1 5m of peat collected from a former peatland in the Jura Mountains near Neuchâtel. This bog aims to show to the public an example of these threatened ecosystems [90% of the surfaces have been lost to peat harvesting and conversion to agricultural land in Switzerland and 90% of the remaining 10% surfaces are degraded (Grünig, 1994)] and use it as an educational tool. A

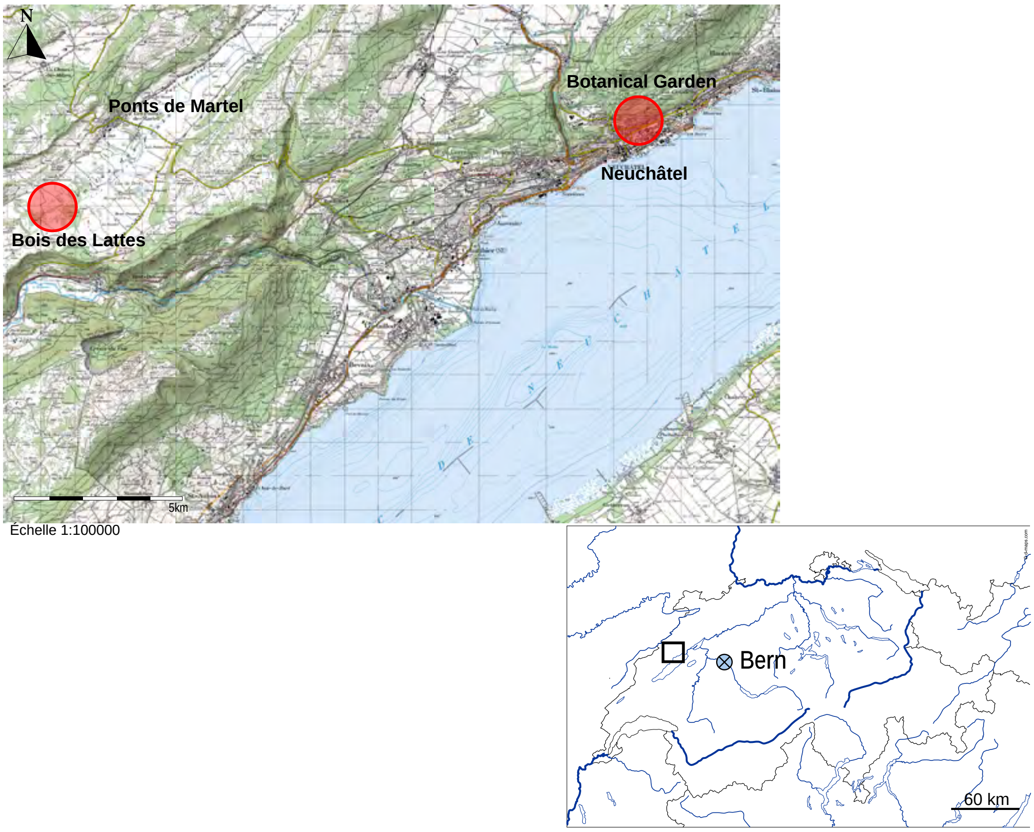


Figure 3.2 – Localisation of the Botanical Garden in Neuchâtel and the Bois des Lattes peatland, Vallée des Ponts de Martel (Neuchâtel), Switzerland. Maps are taken from the geoportail of Neuchâtel, on July 10th 2017, http://sitn.ne.ch/theme/main

second aim is to maintain in ex-situ some regionally rare and endangered plant species such as *Betula nana* L.. The third aim is to conduct experimental studies on aspects of peatland restoration in challenging climatic conditions (Mulhauser et al., accepted). The water level depth is measured weekly in five piezometers tubes inserted in different parts of the bog (Mulhauser et al., accepted) and vegetation surveys are conducted yearly.

To compare our experimental results with a realistic situation we selected as a reference site the Bois des Lattes (BdL) peatland, the largest remaining peatland of Switzerland (Vallée des Ponts de Martel, 1000m a.s.l., Table 3.1, Figure 2). The site was intensively exploited until the mid-20th century for peat extraction and is now protected by law. In 2015, drainage ditches were filled in to increase the level of water table in a formerly exploited zone. This allowed spontaneous recolonization by bog vegetation in patches of different sizes. On September 16th 2016 we collected 12 samples at this site.

In order to assess how the size of patches and shading by vascular plants influenced the survival of testate amoebae during a drought period, we selected *Sphagnum* carpets with various sizes and degrees of vascular cover. We measured the diameter of each selected patch (average of several measurements if the patch was of irregular shape) and estimated vascular plant cover using the Londo semi-quantitative scale (Londo, 1976). *Sphagnum* patches were selected in a large former cutover peat surface (ca. 8 ha) to cover the whole range of potential vascular cover ($10 - 100\%$ in both sites) and patch diameters ($6 - 35$cm, – i.e. available range in BG and $13 - 45.5$cm in BdL – i.e. patches smaller than 13cm could not be found).

We estimated a *Sphagnum* vitality index differentiating “healthy” mosses (i.e. green or red depending on the species) and “dead or dying” mosses (i.e. dry and yellowish). *Sphagnum* taxa were identified macroscopically. About ten randomly picked *Sphagnum* mosses were sampled in each patch, on September 16th 2015 (BG-2015), and on October 27th 2016 (BG-2016) for testate amoeba extraction. The top 3cm of the mosses (including the capitulum) was fixed with a 4% solution of glutaraldehyde ($C_5H_8O_2$), allowing to differentiate the living and recently dead amoeba assemblages (Mitchell and Gilbert, 2004).

Table 3.1 – Location and long-term (1981-2010) climatic characteristics (MétéoSuisse, 2016, 2017) of the study sites.

Site	GPS		Altitude[a]	Global radiation[a]	Mean annual temperature[b]	Average precipitation[b]	Total amount of snow[b]	Days with snow[b]
	North	East	m a.s.l.	kWh/m^2/year	°C	mm	cm/year	days /year
Botanical Garden	46°59′59″	6°56′8″	529	375	10.2	978	45.6	13.4
Bois des Lattes	46°58′32″	6°42′25″	1000	1073	5.7[c]	1500[c]	300[c]	45.5[c]

[a] Geoportal of Service d'Information du Territoire Neuchâtelois (SITN)

[b] Federal Office of Meteorology and Climatology MeteoSwiss, mean value 1981-2010.

[c] Rounded mean value between La Brévine, NE, 11 km West of Bois des Lattes and la Chaux-de-Fonds, NE, 12 km Nord-East

3.2.2 Testate amoebae

Testate amoebae were extracted by standard sieving (15µm) and back sieving (150µm – Botanical Garden –, 250µm – Bois des Lattes –, Booth et al., 2010; Charman, 2001). Rose Bengal was added to stain the cytoplasm and differentiate living from dead individuals. One tablet of *Lycopodium clavatum* (standard preparation of Dept. of Quaternary Geology, Lund University – Sweden – batch No. 938934, 10679 ±400 spores per tab) was added for density calculation (Booth et al., 2010; Stockmarr, 1971). Testate amoebae were identified at morpho-species level under light microscopy at 200x and 400x magnification. This work was partly done in the frame of undergraduate projects and identification of some taxa was challenging. In order to ensure consistency and avoid possible bias due to taxonomic confusion (Heger et al., 2009; Payne et al., 2011) the following grouping of taxa was applied. *Centropyxis aerophila* includes *C. aerophila*, *C. cassis*, *C. platystoma* and *Cyclopyxis kahli*; *Corythion dubium* includes *C. delamarei*; *Cryptodifflugia* sp. includes several other small taxa (*C. oviformis*, *C. minuta*); *Difflugia lucida* type includes all *Difflugia* larger than 50µm and *Difflugia pulex* type includes *Difflugia* taxa smaller than 50µm, with a shell made of xenosomes (e.g. *Cryptodifflugia sacculus*, *Schoenbornia humicola* and *Pseudodifflugia* sp.); *Euglypha* taxa were split into two groups, large taxa (>60µm) and small taxa (<60µm). Taxa from the *Nebela tincta-collaris* group (Kosakyan et al., 2012) were split between *N. tincta* (<95µm, *N. tincta s.str.*, *N. gimlii*, *N. guttata*, *N. rotunda*) and *N. collaris* (>95µm; *N. collaris*, *N. bohemica*); *Phryganella acropodia* includes *Cyclopyxis eurystoma*. Morphotaxa were grouped by water table depth optima, in three classes, <15cm, 15–30cm and >30cm, based on several datasets (Poland, Petchora – Russia, Jura – Switzerland; Lamentowicz and Mitchell, 2005; Mitchell et al., 1999; Lemonis, 2012). We counted a total of 100 individuals from the Bois des Lattes samples. In the Botanical Garden, only limited amounts of *Sphagnum* could be sampled to avoid significantly impacting the already stressed vegetation. As a result, in these samples we aimed for minimum total counts of 50 individuals (Payne and Mitchell, 2008). In all cases counting was stopped after a maximum of two hours.

3.2.3 Functional diversity

The community weighted mean (CWM) of functional traits was calculated separately on both living and dead assemblages. Morphometric traits were measured during counting under light microscopy, randomly. As test length and biovolume were correlated (R^2 adjusted: 69%, calculated on data from a previous study, Koenig unpublished), we used the test length as a proxy for biovolume, as length is easier to measure. Community weighted mean of traits represent the mean value of each trait weighted by the species' relative abundance (Dray and Legendre, 2008; Lavorel and Garnier, 2002; Suding et al., 2008). Functional traits are assumed to reflect the local ecological conditions and can be used to estimate the niche dimension through the functional niche (multi-dimensional space in which traits are axes Villéger et al., 2011). We selected traits expected to respond to changes in humidity:

1. Mixotrophy: binary (1 = mixotrophy, 0 = heterotrophy). The presence of photosymbionts is interpreted as an adaptation to wet oligotrophic conditions such as existing in bog pools. Mixotrophy is both a response trait [mixotrophs being indicative of wet conditions (Mitchell et al., 1999)] and an effect trait [mixotrophs were shown to enhance carbon fixation (Jassey et al., 2015)].

2. Test compression: binary (1 = compressed; 0 = round in cross-section). Compressed tests are expected to favour the survival in thin water film but at the cost of increasing material relatively to amoeba biovolume (Bonnet, 1964).

3. Origin of test material: factor with four levels (protein, idiosomes, xenosome_prey, xenosome_particles). Testate amoeba shells are composed of organic material (protein test), secreted silica plates (idiosomes), or made of particles found in the environment (xenosomes, either prey skeleton reused in test construction or inorganic particules). The presence of taxa is related to the availability of test material (Gilbert and Mitchell, 2006; Schwind et al., 2016)

4. Pseudostome position: factor with three levels (terminal, ventral, ventral-central). The position of the pseudostome could represent an adaptation to moisture as exposed aperture (terminal) is better adapted to wet environments and a hidden one to drier conditions (Bonnet, 1964).

5. Pseudostome size: quantitative (range: 4.5 to 55.8µm). The aperture size is an effect trait as it determines the range of prey: taxa with a large aperture have a higher trophic position in microbial food-webs (i.e. predators of other protists and micro-eukaryotes) while taxa with a small aperture are mainly bacterivorous (Jassey et al., 2013b).

6. Test length: quantitative (range: 18.2 to 153.7µm). Used as a proxy for biovolume. This is a response trait: larger taxa have longer generation times than small taxa and are thus expected to both be more sensitive to perturbation and to recover more slowly from perturbations. As larger taxa generally also have large aperture, this is also to some extent an effect trait.

3.2.4 Numerical analyses

Rare taxa (i.e. mean relative abundance $< 1\%$) were excluded from the data set (Table 3.A2). All analyses were made on relative abundance after removal of these taxa, or on density. Mean, standard error, median, minimum and maximum were calculated on relative abundance and on density, on the whole dataset, for each site separately, and / or with respect to living and dead assemblage. Difference in mean values between sites or between living and dead assemblages were tested using Wilcoxon rank sum test as the data neither followed normality nor homoscedasticity criteria. Statistical tests between living and dead assemblages were considered as repeated measurements. The same applied to the BG-2015 and BG-2016 data sets given that the same patches were sampled. Pairwise comparison for CWM of each trait between sites where tested by Wilcoxon rank test, with Holm correction and exact p-value. For ordinations, species data were square root-transformed (i.e. Hellinger transformation, Legendre and Gallagher, 2001) to balance the influence of rare and abundant taxa and the community weighted mean of traits data were standardised. We computed indirect ordinations (principal component analyses) on taxonomic and CWM to assess the community patterns. Finally, we assessed the significance of selected environmental variables (sites, *Sphagnum* vitality index, vascular cover, patch size and *Sphagnum* taxon) using an analysis of variance based on redundancy analysis (variance partitioning).

We performed β-diversity analyses between all pairs of testate amoeba assemblages (relative abundance data), using the Bray-Curtis index, which is equivalent to the Sørensen-Dice diversity index for abundance data (Baselga and Orme, 2012; Jari Oksanen et al., 2016). We performed a multivariate homogeneity of groups dispersion analysis, based on a principal coordinates analysis with spatial median type (Baselga and Orme, 2012). As the density of living assemblages in 2015 was very low, we applied the bias adjustment for calculating groups dispersion, the null hypothesis (H0) for the analysis of variance (anova) being that there was more variance between sites than between groups. Finally, to test our hypothesis that shade (provided by vascular cover) and patch size should protect the testate amoeba community against the effect of drought, we plotted the β-diversity between 1) living and dead assemblages for each site, 2) living assemblages in BG-2015 and BG-2016, versus the gradient of 1) vascular cover as percentage of the maximum for the site, 2) patch size as percentage of the maximum for the site, 3) the average of both.

All analyses were carried out with the R statistical software (R Core Team, 2016). Ordination were computed using the package vegan (Jari Oksanen et al., 2016), β-diversity indexes were calculated with the package betapart (Baselga and Orme, 2012) and community weighted mean of func-

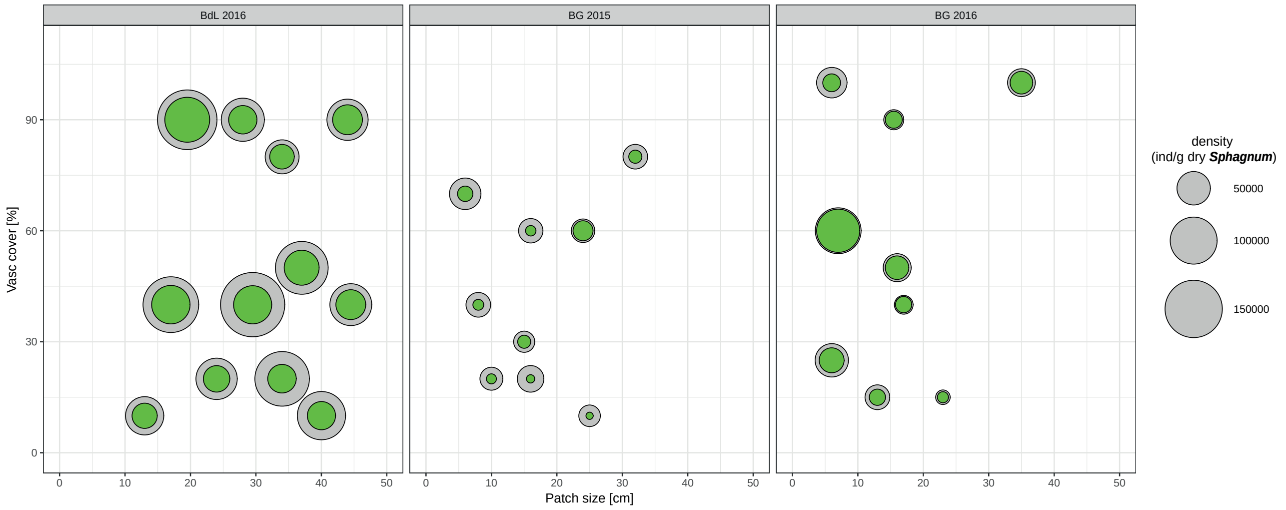


Figure 3.3 – Biplots of overall density of testate amoebae assemblages in the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL) in relation to *Sphagnum* patch diameter (x axis) and percentage vascular plant cover (y axis). The surface of each circle is proportional to density: light grey background: total density of dead + living assemblages; green: density of living assemblages. Differences between all pairs of data sets were significant (p-value < 0.05, data not shown). In BG-2015 the percentage of living testate amoebae was positively correlated to the vascular cover for patches < 20cm. However, no such pattern was observed in BG-2016 and BdL.

tional traits was calculated using the package FD (Laliberté et al., 2014).

3.3 Results

3.3.1 Climate and sampled moss patches at the two sites

The average climate (mean value for 1981-2010) is sunnier, colder and wetter in the reference site (Bois des Lattes – BdL) than in the Botanical Garden (BG, Table 3.1): solar radiation: Δ BG-BdL: −700 kWh/m^2/year, annual temperature: Δ BG-BdL: 4.5°C, average precipitation: Δ BG-BdL: 540mm/year, cumulative amount of snow and number of days with snow cover: Δ BG-BdL: -250cm/year and -32days/year, respectively (MétéoSuisse, 2017). The years 2015 and 2016 were hotter and drier than the long-term average in both BG and BdL. At BG-2015 was the hottest year since 1864 (1.29°C above the mean of 1981-2010, in Neuchâtel) and the second hottest summer after 2003. In North-western Switzerland, total hours of sunlight were 125% and precipitations 73% of the mean for 1981-2010 (MétéoSuisse, 2016). The situation in 2016 was less extreme: average temperature was above the mean for 1981-2010 (+0.6°C), but total hours of sunlight and precipitation were within the norm of 1981-2010. However, this lack of overall difference was due to the very wet and cold spring. The second half of the year was warmer with a short but record heatwave in summer and an extremely warm September (+2.7°C in Neuchâtel, since the beginning of measurements, in 1864; MétéoSuisse, 2017). Overall the BdL site was slightly less impacted than Neuchâtel during the climate anomaly in 2015 and 2016 (Sup. Figure 3.A2).

Vascular cover was not significantly different between the two sites (Student t-test = -0.072, p-value = 0.943, Sup. Table 3.A1) but *Sphagnum* patches were on average significantly larger in BdL (t-test = 3.903, p-value = < 0.001, Sup. Table 3.A1 and Sup. Figure 3.A3). Sampling in BdL was done mostly in *Sphagnum magellanicum* patches (66.6%), and to a lesser extent in *S. fallax* and *S. rubellum* (16.6% each, Sup. Table 3.A1). The BG samples were mainly taken in *S. fallax* (44.4%) and *S. magellanicum* (33.3%), and the remaining in other *Sphagnum* taxa. Overall most sampled *Sphagnum* mosses were alive (BG-2015: 77.8%, BG-2016: 91.7%, and BdL: 83.3%), the remaining being half dry and possibly already dead respectively.

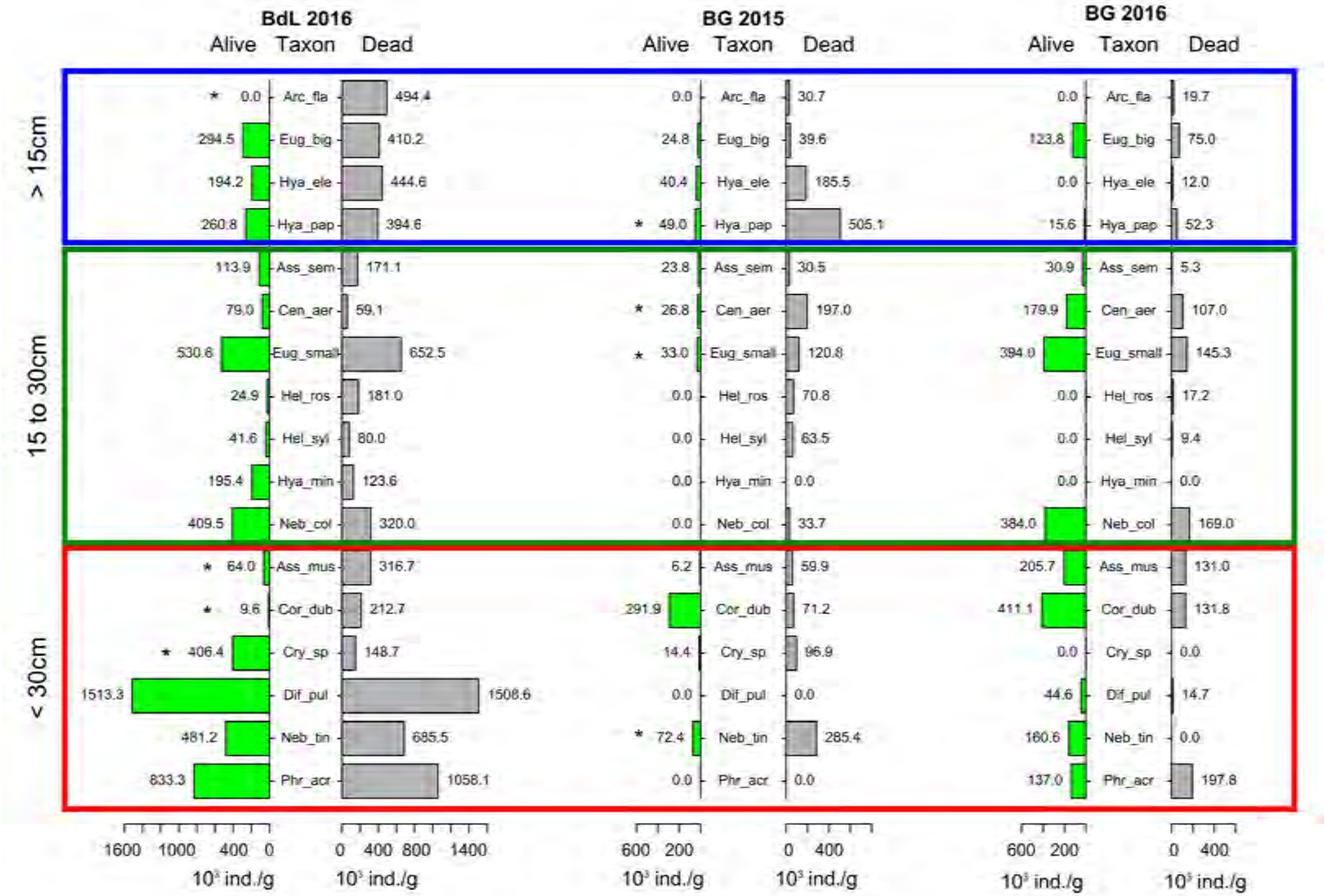


Figure 3.4 – Density of testate amoeba taxa in the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL). For each data set, the density of each morphotaxon is split between the living (left, green) and the dead (right, light grey). Morphotaxa are arranged according to three groups of water table depth optima: < 15cm (top frame), $15 - 30$cm (middle frame) and > 30cm (bottom frame). Stars indicate significant differences between the density of living and dead populations (Wilcoxon rank sum test, p-value < 0.05). Morphotaxa abbreviation: Arc_fla: *Archerella flavum*; Ass_mus: *Assulina muscorum*; Ass_sem: *Assulina seminulum*; Cen_aer: *Centropyxis aerophila* type; Cor_dub: *Corythion dubium*; Cry_sp: *Cryptodifflugia* sp.; Dif_pul: *Difflugia pulex* type; Eug_big: *Euglypha* “big taxa”; Eug_small: *Euglypha* “small taxa”; Hel_ros: *Heleopera rosea*; Hel_syl: *Heleopera sylvatica*; Hya_min: *Hyalosphenia minuta*; Hya_ele: *Hyalosphenia elegans*; Hya_pap: *Hyalosphenia papilio*; Neb_col: *Nebela collaris*; Neb_tin: *Nebela tincta*; Phr_acr: *Phryganella acropodia* type.

Density (ind/g)	All sites	BdL					BG-2015					BG-2016				
Morphotaxa	WTD	mean	se	median	min	max	mean	se	median	min	max	mean	se	median	min	max
Archerella flavum	<15cm	4120.3	1107.9	3638	0	13074	341.2	111.9	481	0	794	219.1	148.4	0	0	1177
Euglypha "big taxa"	<15cm	5872.8	1456.5	3397.5	998	14517	715.3	355.1	0	0	3116	2208.0	928.8	1239	0	7525
Hyalosphenia elegans	<15cm	5323.5	2465.3	2192	0	29714	2510.0	707.3	2353	0	6037	133.1	103.8	0	0	929
Hyalosphenia papilio	<15cm	5461.7	3484.6	499	0	42864	6156.8	2136.2	2886	0	18733	754.4	530.3	0	0	4767
Assulina seminulum	15-30cm	2375.2	638.6	1413	0	6049	603.2	341.4	0	0	3064	402.6	209.0	0	0	1559
Centropyxis aerophila	15-30cm	1151.0	499.8	0	0	4840	2486.4	755.0	1677	0	6232	3187.2	1754.5	0	0	12713
Euglypha "small taxa"	15-30cm	9859.3	2308.4	6877	1497	27361	1709.2	568.4	1022	335	5608	5993.2	2563.1	2477	0	22362
Heleopera rosea	15-30cm	1715.4	1328.3	0	0	16168	787.1	553.6	0	0	4986	191.6	127.2	0	0	929
Heleopera sylvatica	15-30cm	1013.3	411.2	645	0	4975	705.6	546.0	0	0	4986	104.6	104.6	0	0	941
Hyalosphenia minuta	15-30cm	2658.9	1162.7	0	0	11007	0.0	0.0	0	0	0	0.0	0.0	0	0	0
Nebela collaris	15-30cm	6079.3	1742.0	4132.5	0	20283	374.1	374.1	0	0	3367	6144.9	3793.4	1881	0	35868
Assulina muscorum	>30cm	3171.9	1284.9	1448	0	15084	734.3	544.7	0	0	4985	3741.0	1925.1	808	0	17872
Corythion dubium	>30cm	1853.2	551.3	1000.5	0	5788	4034.6	1757.8	1004	0	15282	6032.0	2266.6	3179	0	21832
Cryptodifflugia sp	>30cm	4626.2	1063.2	4618.5	0	13074	1237.4	863.3	0	0	7215	0.0	0.0	0	0	0
Difflugia pulex	>30cm	25182.7	5926.5	19069.5	0	78445	0.0	0.0	0	0	0	658.4	413.4	0	0	3531
Nebela tincta	>30cm	9722.6	2388.2	9076.5	499	25982	3976.0	1012.9	3689	0	10198	1784.3	1727.1	0	0	15595
Phryganella acropodia	>30cm	15762.3	3731.9	11082	2935	52157	0.0	0.0	0	0	0	3720.4	1280.5	1881	0	9535

Relative abundance	All sites	BdL					BG-2015					BG-2016				
Morphotaxa	WTD	mean	se	median	min	max	mean	se	median	min	max	mean	se	median	min	max
Archerella flavum	<15cm	3.9%	1.0%	3.2%	0.0%	10.9%	1.3%	0.4%	1.4%	0.0%	3.4%	0.6%	0.4%	0.0%	0.0%	3.4%
Euglypha "big taxa"	<15cm	5.9%	1.5%	4.1%	0.6%	18.0%	2.3%	1.0%	0.0%	0.0%	7.0%	4.8%	1.6%	3.8%	0.0%	15.1%
Hyalosphenia elegans	<15cm	4.5%	1.6%	2.3%	0.0%	15.7%	10.4%	3.2%	9.5%	0.0%	31.0%	0.6%	0.4%	0.0%	0.0%	3.4%
Hyalosphenia papilio	<15cm	4.7%	2.8%	0.8%	0.0%	33.8%	24.1%	8.3%	14.3%	0.0%	71.8%	1.7%	1.3%	0.0%	0.0%	11.5%
Assulina seminulum	15-30cm	2.2%	0.6%	1.4%	0.0%	6.7%	2.1%	1.3%	0.0%	0.0%	11.5%	1.7%	1.1%	0.0%	0.0%	10.0%
Centropyxis aerophila	15-30cm	1.2%	0.5%	0.0%	0.0%	6.1%	8.4%	2.1%	8.6%	0.0%	19.1%	9.1%	4.6%	0.0%	0.0%	33.7%
Euglypha "small taxa"	15-30cm	9.1%	1.6%	7.4%	2.3%	20.2%	5.7%	1.4%	3.8%	1.3%	12.7%	16.0%	6.7%	12.8%	0.0%	65.5%
Heleopera rosea	15-30cm	1.5%	1.0%	0.0%	0.0%	11.9%	2.2%	1.4%	0.0%	0.0%	11.3%	0.6%	0.4%	0.0%	0.0%	3.4%
Heleopera sylvatica	15-30cm	0.9%	0.3%	0.8%	0.0%	3.7%	2.0%	1.3%	0.0%	0.0%	11.3%	0.2%	0.2%	0.0%	0.0%	1.9%
Hyalosphenia minuta	15-30cm	2.9%	1.4%	0.0%	0.0%	14.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Nebela collaris	15-30cm	5.9%	1.5%	4.9%	0.0%	18.3%	1.9%	1.9%	0.0%	0.0%	16.7%	12.2%	4.0%	8.0%	0.0%	37.7%
Assulina muscorum	>30cm	3.2%	1.2%	2.2%	0.0%	14.3%	2.0%	1.3%	0.0%	0.0%	11.3%	15.4%	6.2%	3.6%	0.0%	46.0%
Corythion dubium	>30cm	2.1%	0.9%	0.9%	0.0%	11.2%	15.3%	7.0%	3.8%	0.0%	63.3%	18.1%	5.9%	12.0%	0.0%	58.9%
Cryptodifflugia sp	>30cm	4.1%	0.8%	4.4%	0.0%	7.6%	5.6%	4.1%	0.0%	0.0%	35.7%	0.0%	0.0%	0.0%	0.0%	0.0%
Difflugia pulex	>30cm	22.0%	3.7%	19.9%	0.0%	41.7%	0.0%	0.0%	0.0%	0.0%	0.0%	2.3%	1.3%	0.0%	0.0%	10.3%
Nebela tincta	>30cm	9.9%	2.8%	10.1%	0.8%	35.0%	15.2%	3.7%	15.4%	0.0%	38.2%	2.0%	1.8%	0.0%	0.0%	16.4%
Phryganella acropodia	>30cm	14.2%	2.4%	12.6%	3.8%	32.1%	0.0%	0.0%	0.0%	0.0%	0.0%	11.9%	3.5%	12.5%	0.0%	29.3%

Table 3.2 – Descriptive statistics of testate amoeba communities (dead and living pooled together), with density (above) and relative abundance (below) for each site separately. Morphotaxa are ordered according to water table depth class (WTD, estimated based on previous studies in Europe, Lamentowicz and Mitchell, 2005; Mitchell et al., 1999; Lemonis, 2012, ; se: standard error.)

3.3.2 Testate amoeba density and community structure

The density of testate amoebae was significantly different between site and sampling date being higher in BdL (10.8 ± 1.2 x 10^4 ind/g dry *Sphagnum*), lower in BG-2016 (3.59 ± 0.86 x 10^4 ind/g), and lower in BG-2015 (2.68 ± 0.25 x 10^4 ind/g, Figure 3.3, details on Sup. Table 3.A3). The proportion of living individual was highest in BG-2016 (62% ± 6%), lower in BdL, (43% ± 2.7%), and lowest in BG-2015 (25% ± 0.7%, Figure 3.4 and Sup. Table 3.A3).

We identified a total of 24 morphotaxa, and after removing rare taxa (mean relative abundance < 1%), 17 morphotaxa remained for further analyses (Sup. Table 3.A2). Overall, the most abundant taxa were *Corythion dubium* (10.9%), small *Euglypha* sp. (10.2%), *Hyalosphenia papilio* (9.6%), *Diffugia pulex* group (9.5%), *Phryganella acropodia* (9.3%) and *Nebela tincta* (9.1%), Sup. Table 3.A2). Large *Euglypha* sp., were the most frequent (found in 93.3% of samples) but were not abundant (4.5%). The other frequent taxa were *C. dubium*, small *Euglypha* sp., *Nebela tincta*, *N. colaris*, *P. acropodia*, *Assulina muscorum* and *H. papilio* (60 − 83.3%). The smallest taxa were the most abundant (*D. pulex*, *P. acropodia*, small *Euglypha* and *N. tincta*, $5.6 - 10.3$ x 10^3 ind/g). *Archerella flavum* was never found alive and could be a contamination from the adjacent bare peat.

In BG-2015 only one species was found mostly alive: *C. dubium* (292 x 10^3% of living individuals/gram, relative abundance: 15.3% Figure 3.4 and Table 3.2). *Hyalosphenia papilio* was the most abundant taxon (24.1%, Table 3.2), but only 8.6% of individuals were alive (Wilcoxon test, p-value: 0.03, Sup. Table 3.A4). The next most abundant taxa were *N. tincta* (15.2%, of which 20.2% alive) and *Hyalosphenia elegans* (10.4%, of which 17.7% alive). In BG-2016, no taxon showed significant difference between the proportion of living and dead individuals. The overall community composition was different from that of 2015. The most abundant taxon was *C. dubium* (18.1%, of which 78.1% alive) followed by small taxa of *Euglypha* sp. (16%, of which 73.1% alive), *A. muscorum* (15.4%, of which 61% alive), *Nebela collaris* (12.2%, of which 69.4% alive), *P. acropodia* (11.9%, of which 41% alive), and *Centropyxis aerophila* (9.1%, of which 62.7% alive). Other taxa accounted for less than 5% of the community. Except for *P. acropodia*, each of the most abundant taxum (i.e. relative abundance > 10%) had a higher proportion of living than dead individuals. In BdL, the most abundant morphotaxa were *Difflugia pulex* (22%, 50.1% of which alive), *P. acropodia* (14.2%, of which 44.1% alive), *N. tincta* (9.9%, of which 41.2% alive) and large *Euglypha* "small taxa" (9.1%, of which 44.8% alive). All other taxa each accounted for less than 7% of the total community (Table 3.2, Figure 3.4, Sup. Table 3.A4).

3.3.3 Community weighted mean of traits

The CWM of BG-2015 shifted clearly between the dead and living assemblages towards smaller taxa (Wilcoxon p-value: 0.044), more compressed (p-value: 0.021), with a smaller pseudostome (p-value: 0.013), an increased proportion of tests made of agglutinated particles (xenosomes, p-value: 0.013), and a loss of mixotrophy (p-value: 0.013). By contrast, there was no significant difference in CWM between living and dead assemblages in BG-2016 (Figure 3.5). The CWM of traits of BG-2016 (dead and living assemblages) were similar to the living assemblage of BG-2015. In BdL, the CWM did not differ significantly between living and dead assemblages, except for mixotrophy which was significantly lower in the living assemblage (Wilcoxon rank test p-value: 0.006; Figure 3.5; Sup. Table 3.A5). The CWM of BG-2016 showed few differences with either BdL 2016 (living and dead assemblages) and living assemblage of the BG-2015 samples (Figure 3.5 and Sup. Table 3.A5).

3.3.4 Multivariate analyses of taxonomic and CWM data

The principal component analysis (PCA) of species data showed a clear separation among the three data sets (Fig. 3.6a), in agreement with the abovementioned clear contrasts in taxonomic composition. The distance between living and dead assemblages in the ordination space, which reflects the temporal shift in community structure, was highest in BG-2015 and lowest in BdL.

The PCA on CWM of traits also showed a contrast among the three data sets, although not as clear as for the taxonomic data (Fig. 6b). The BdL samples appear as intermediate between BG-2015 and BG-2016 on the biplot of the first two axes. Mixotrophy, large taxa, taxa with a large pseudostome and tests made of protein were highly correlated and characterised some samples of the dead assemblage of BG-2015 and BdL. The large *Sphagnum* patches of BG-2016 were correlated with idiosome tests, a ventral pseudostome, small taxa, and taxa with a small aperture, but anti-correlated with mixotrophy. By contrast, small patches of BG-2016 had a similar CWM pattern as the small patches of BdL, with a test

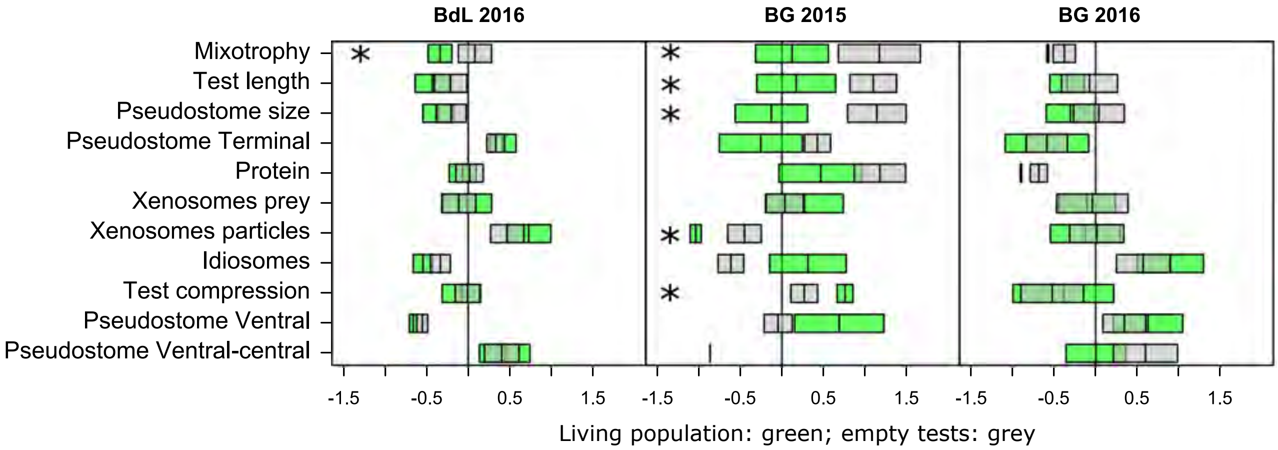


Figure 3.5 – Boxplots of community weighted mean of traits in living and dead testate amoeba assemblages in the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL). Boxes represent standard error and vertical middle black line the mean values of CWM for the site (z-scored). Traits are arranged in the order of decreasing humidity, with traits expected to decrease with water stress at the top, and traits expected to increase with water stress at the bottom. Stars indicate significant trait differences between living and dead assemblages, based on a Wilcoxon rank test for paired value and exact p-value when available.

made of xenosomes, a ventral-central pseudosome, a low degree of compression and a low proportion of mixotrophs.

In the redundancy analyses, the selected variables (i.e. sites – BdL, BG-2015, BG-2016 –, living vs. dead assemblages, vascular cover, patch size and *Sphagnum* taxon) jointly explained 37.3% of the variance of the testate amoeba community data, and 36.1% of the CWM data (Table 3.3). Site alone significantly explained (p-value: 0.001 in both cases) the highest part of the variance: 21.2% (community data) and 21.5% (CWM). For the community data, three other variables were significant: *Sphagnum* taxon (7.5%, p-value: 0.002), living vs. dead assemblages (3.8%, p-value: 0.002) and vascular cover (2.9%, p-value: 0.006). For the RDA on CWM data, only one additional variable was significant: *Sphagnum* patch size (3.15% of variance explained; p-value: 0.017).

3.3.5 How well do patch size and shading explain the shifts in communities?

The projection of principal coordinates analysis of β-diversity between living population at each sampling site and date (Figure 3.6) indicated that although all sites were relatively well separated, the β-diversity was significantly higher only between BdL-2016 and BG-2015. β-diversity did not differ between living and dead assemblages in BdL and in BG-2016 and neither vascular cover nor patch size seemed to have an impact on the testate amoeba assemblages (Figure 3.8). Contrary to our hypothesis, in BG-2015, β-diversity between living and dead assemblages increased with shading, and to a lesser extent with increasing patch size (Figure 3.8).

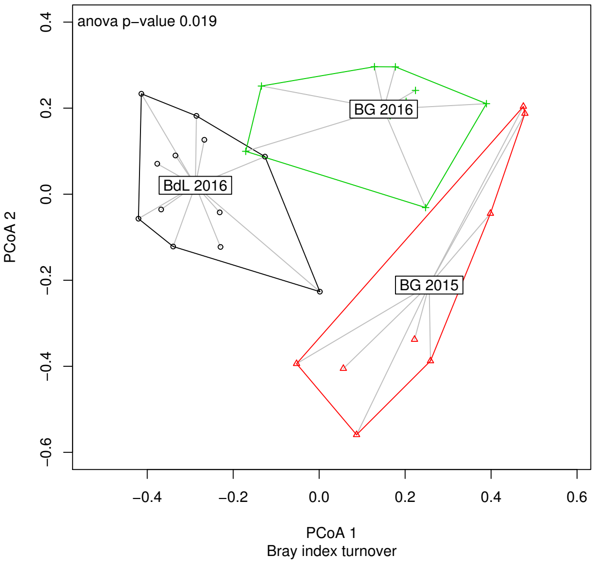


Figure 3.6 – Principal coordinate projection of β-diversity (Bray-Curtis index) calculated between all pairs of living testate amoeba assemblages from the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL). β-diversity was significantly higher between BG-2015 and BdL than inside each of these two groups or between BG-2015 or BdL and BG-2016. The analysis takes into account the adjustment for small sample bias.

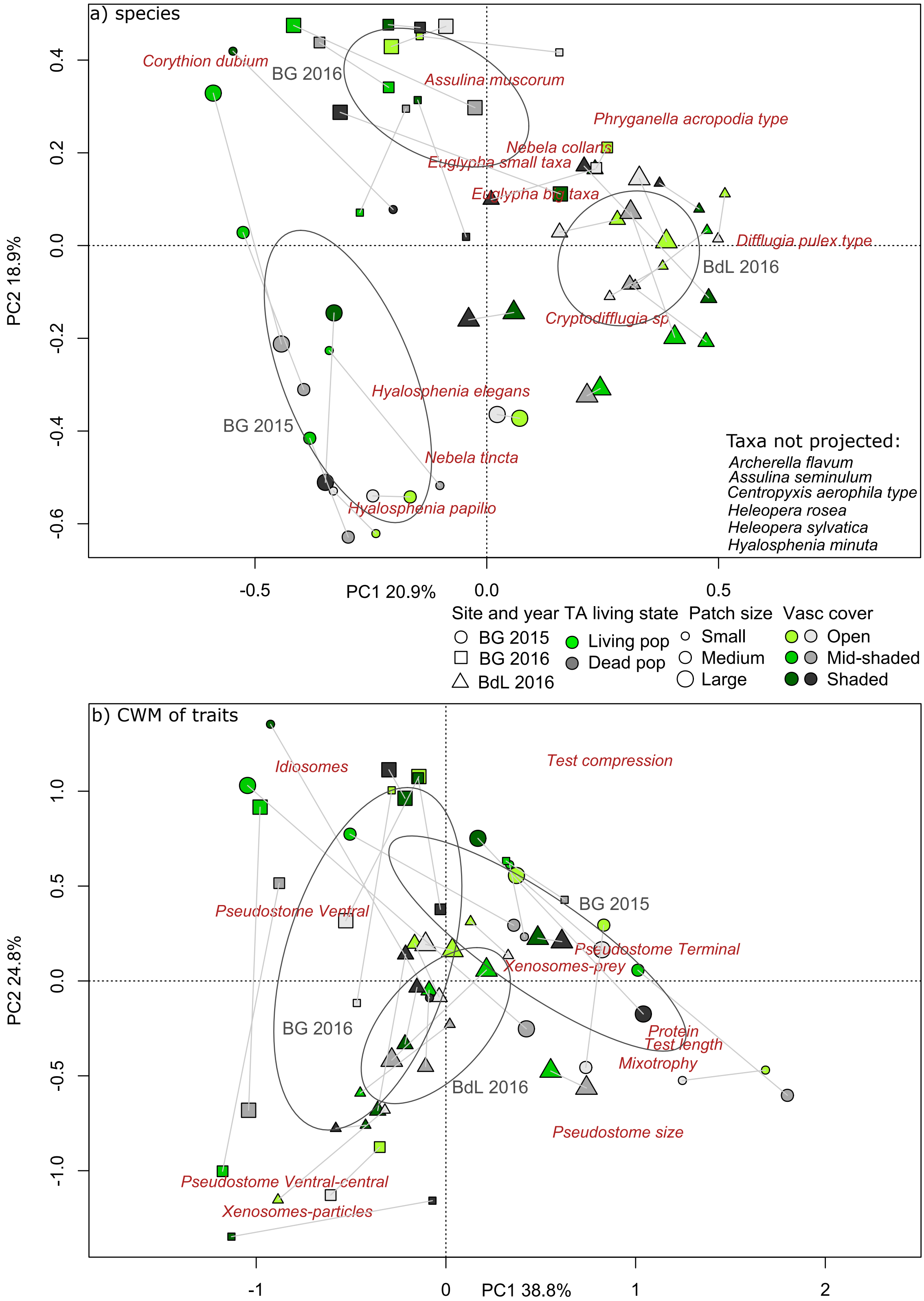


Figure 3.7 – Principal component analyses of amoeba assemblages in the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL): a) Hellinger-transformed community data and b) standardized community weighted mean of functional traits. For better readability, some taxa with low scores on both axes were not projected on the biplot. Axes 1 and 2 were significant in the PCA on community data and jointly explained 39.8% of the variance. The four first axis were significant in the PCA on CWM data and together explained 63.8% of the data (axes 3 and 4 are shown in Sup. Figure 3.A2). Ellipses indicate the standard deviation around the centroid for each data set (living and dead assemblages combined). The six data sub-sets are shown by different symbols: BdL alive: green triangles, BdL dead: grey triangles, BG-2015 alive: green squares, BG-2015 dead: grey squares, BG-2016 alive: green circles, BG-2016 dead: grey circles. Symbol sizes indicate patch size and shading indicate the % of vascular plant cover.

3.4 Discussion

3.4.1 Testate amoeba density and community composition in the two sites

Testate amoeba density was ca. three time higher in the Bois des Lattes peatland (BdL), a naturally regenerating bog in the Jura Mountains used as a reference, than in the artificial peat bog in the Botanical Garden (BG) of Neuchâtel (Figure 3.3). But both were within the range reported from *Sphagnum* peatlands (Mitchell and Gilbert, 2004; Gilbert and Mitchell, 2006).

Testate amoeba communities differed among the three sites (Figure 3.9 & 3.10) but generally corresponded to communities reported from relatively dry *Sphagnum* peatland (Lamentowicz et al., 2013; Mitchell et al., 1999; Opravilova and Hajek, 2006). In BG-2015, some taxa were indicative for wet or relatively wet oligotrophic conditions (*H. papilio*, *H. elegans*; Mitchell et al., 1999), but the overall (living + dead) community was nevertheless dominated by *C. dubium*. This taxon, which was dominant in both BG-2015 and BG-2016, is frequently reported from dry bog habitats (Mitchell et al., 1999) as well as from perturbed or stressed environments (e.g. dry mosses on walls and trees, tundra, etc.; Beyens et al., 1986; Bonnet, 1991). The rest of the community in 2016 indicated relatively dry conditions. In BdL, the dominant taxa (*Difflugia pulex* group, *Phryganella acropodia* group, small *Euglypha* and *Nebela tincta*) mostly indicated low water table (i.e. > -25cm; Bobrov et al., 2002; Lamentowicz et al., 2011; Mitchell et al., 1999). The taxonomic composition of living and dead assemblages were quite similar. Only three taxa showed significant differences between living and dead population, but these taxa were not dominant (i.e. $< 5\%$ of relative abundance).

3.4.2 Magnitude of the two summer droughts and effects on testate amoeba communities

By sampling the top 3cm of *Sphagnum* mosses and counting the dead and living assemblages separately we were able to assess the recent changes in community structure (i.e. ca. one year), and specifically how the communities and associated functional traits responded to two years of drought.

Although not identical, both sites experienced extreme climatic conditions in 2016, the summer of 2015 being more extreme. Thus, we hypothesised that these conditions impacted the testate amoeba communities. The expected impact should logically be highest in BG-2015 for two reasons: The first reason is that the moss patches planted in the experimental bog originated from both secondary and pristine bogs and some were kept in the Botanical Garden for a few years in very wet conditions prior to building the bog. This is indeed reflected by the high abundance of taxa such as *Hyalosphenia papilio* in the dead assemblage of BG-2015. The second reason is that the drought and heat wave was most extreme in 2015. Thus, the contrast between the "original" conditions experienced by the communities and the conditions to which they were exposed to was strongest in 2015. In 2016, the climate was less extreme and communities had already partly adapted to the drier conditions and thus a lesser shift in community structure could be expected.

In agreement with this, our results showed that the testate amoeba communities from the Botanical Garden clearly shifted in 2015, as shown by the contrast between dead and living assemblages and the high mortality (ca. 75%). The community structure shifted towards taxa related to drier conditions such as *C. dubium* (alive: 80.5%) together with the loss of taxa related to humid conditions (*H. papilio* alive: 8.6%, *H. elegans* alive: 15.6%, *H. rosea* alive: 0%, *N. collaris* alive: 0%, Figure 3.3; Bobrov et al., 2002; Booth, 2002; Lamentowicz et al., 2011; Mitchell et al., 1999).

By contrast, communities from the Botanical Garden bog did not change much more in 2016, likely because their structure corresponded to the dry conditions. Indeed, the most abundant taxa in living population in 2016 were *C. dubium* (20.1%), small *Euglypha* (18.1%) and *A. muscorum* (16.6%, Figure 3.9), all of which tolerating relatively dry conditions and nutrient inputs (Booth, 2001; Gilbert and Mitchell, 2006; Mitchell et al., 1999; Opravilova and Hajek, 2006). Nevertheless, the percentage of living individuals increased markedly ($+232\%$). The adaptation to new conditions was therefore mostly achieved during the year 2015.

Despite the 2016 drought the communities of BdL remained quite stable (Jassey et al., 2011; Mitchell and Gilbert, 2004). It however remains possible that, like in the Botanical Garden, more marked shift in communities took place in BdL in 2015 as compared to 2016.

The CWM of the BG-2016 living and dead assemblages and the BG-2015 living assemblages were more similar to the living and dead assemblages of the BdL reference site. These results suggest that,

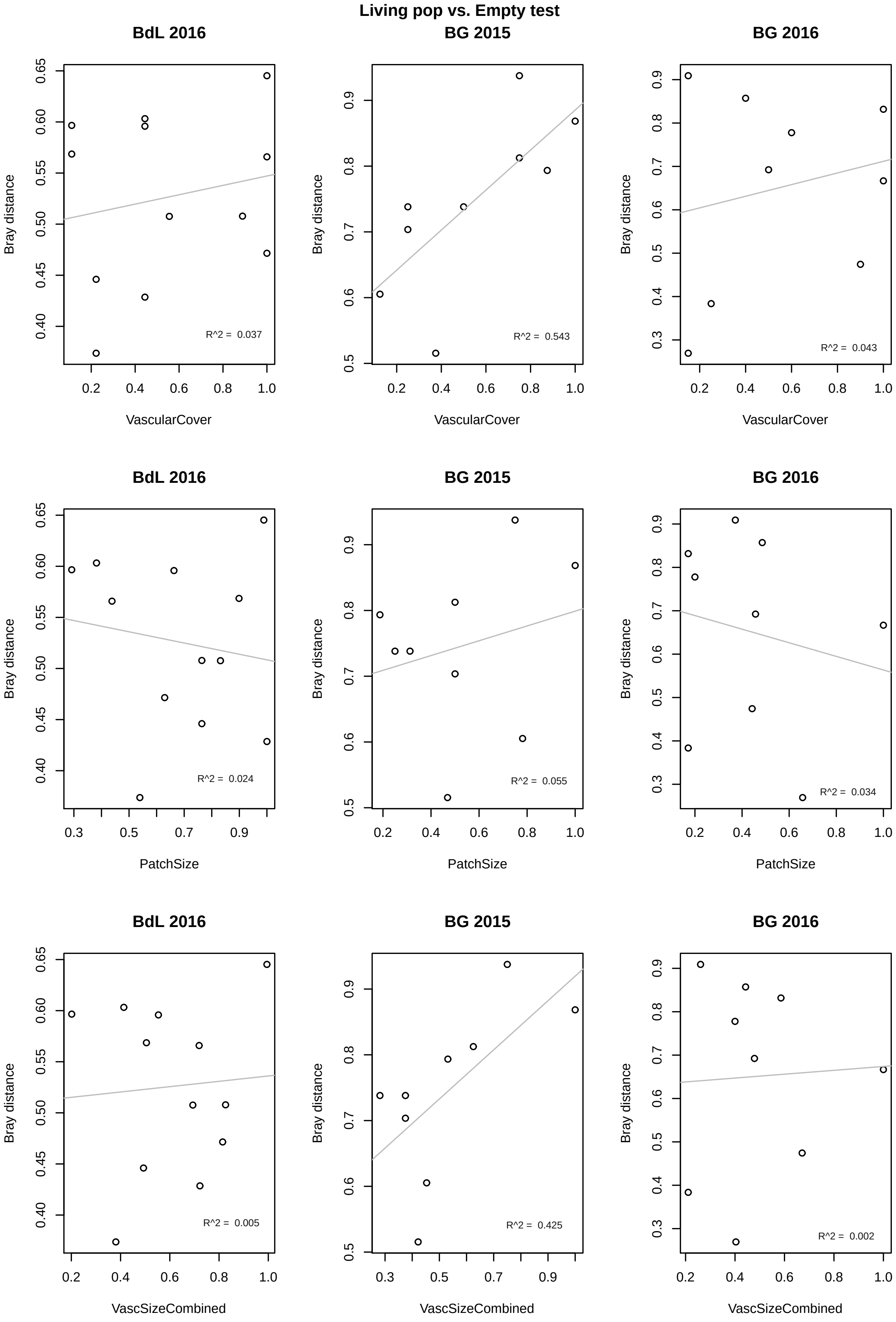


Figure 3.8 – Principal coordinate projection of β-diversity (Bray-Curtis index) calculated between all pairs of living testate amoeba assemblages from the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL). β-diversity was significantly higher between BG-2015 and BdL than inside each of these two groups or between BG-2015 or BdL and BG-2016. The analysis takes the adjustment for small sample bias into account.

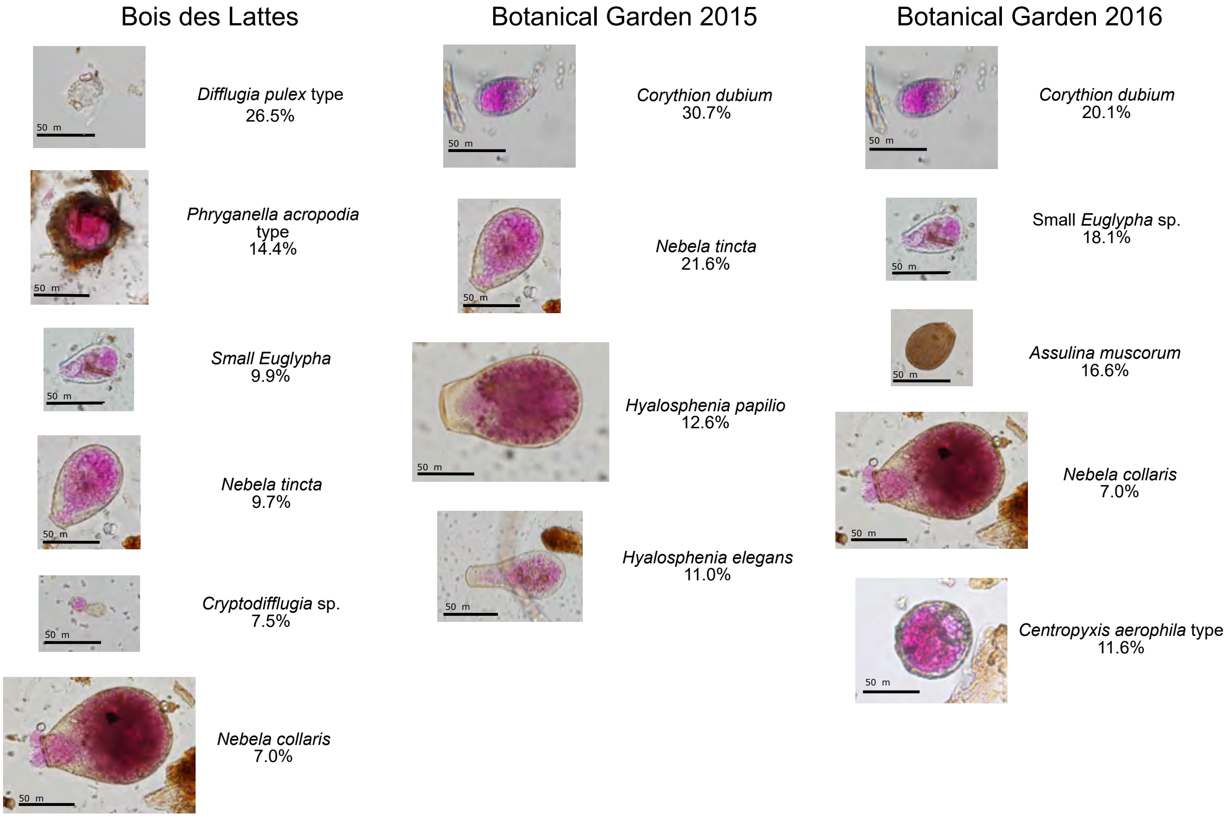


Figure 3.9 – Illustrations of the dominant testate amoeba taxa from the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL; living and dead assemblages combined). All taxa are shown at the same scale. The illustrated morphotaxa jointly accounted for ca. 75% of the assemblage. Rose Bengal was used to differentiate living/encysted (with pink cytoplasm) from dead individuals.

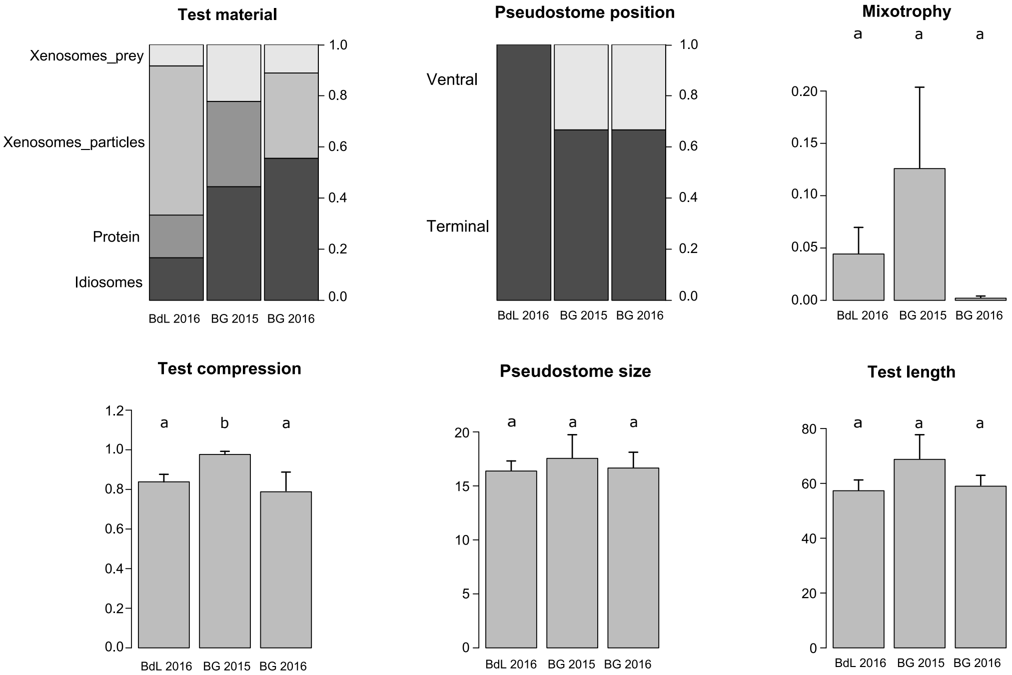


Figure 3.10 – Distribution of functional traits for living testate amoeba assemblages from the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL). The first two graphs show the proportion of each categorical traits as selected by the functional composition of FD package (funcomp, type="All"). The last four graphs indicate the mean and standard error (error bars) for continuous and binary traits. As some samples had only few individuals in each category we did not test the different sites statistically.

although the taxonomic composition of communities differed among the three sample sets, the abiotic conditions (e.g. moisture content) assumed to control communities through ecological filtering were comparable to a regenerated bare peat bog. By contrast, the CWM of traits from BG-2015 indicated that the original testate amoeba community was adapted to more humid conditions than the ones found in *Sphagnum* patches in Bois des Lattes. The strong contrast between these initially much wetter conditions and the drought experienced during the summer 2015 had a dramatic effect on testate amoeba communities. Shifts in testate amoeba communities in response to experimental manipulation of water table depth were previously observed at a similar speed (Mulot et al., 2017; Marcisz et al., 2014).

3.4.3 Testate amoeba community shifts in the two study sites vs. patch size and shading

We hypothesised that the testate amoeba community would survive better in large patches with high vascular cover as this combination would a) maximise the potential storage of water (due to higher overall volume of the mosses) and b) minimise water loss. Reduced water moss is due to the lower surface/volume ratio of the larger moss carpets and to the reduced water vapour gradient between the moss surface and the air directly above the mosses under the shade of vascular plants. To assess how patch size and shading influenced the magnitude of the shift in testate amoeba communities, we calculated the β-diversity between living and dead assemblages as a measure of changes in population structure. We did not find any significant correlation in BdL and BG-2016 between β-diversity and patch size and/or shading. However, in BG-2015, contrary to our hypothesis, β-diversity was lower in open patches than in shade ones (Figure 3.8). We explain this apparent contradiction by the fact that in the less shaded patches the communities barely survived while in the shaded patches the amoebae could remain active; nevertheless, the dry-adapted taxa (e.g. *Corythion*, *Euglypha*) thrived better and increased their relative abundance in the community. As a result, the community shift measured as β-diversity was higher in the more shaded moss patches. This is further supported by the fact that very few living individuals remained in open patches (Figure 3.3). The living population thus only corresponded to the remains of the original community, without any (or only very little) replacement of taxa.

3.4.4 Evolution of functional niche

Functional traits were selected in relation to water stress. When conditions become drier, the thickness of the water film becomes thinner and nutrient content and prey availability change. A compressed and small test with a ventral or ventral-central aperture theoretically allows the amoeba to stay active even in a thin water film (Fournier et al., 2012; Marcisz et al., 2016; Payne et al., 2016b). Under drier conditions, bacteria and fungi populations increase and this is reflected in smaller pseudostome size (Bragazza et al., 2016; Jassey et al., 2013a,b; Gavazov et al., 2017). Finally, mixotrophy is expected to decrease as nutrient availability increases due to higher mineralisation, thus reducing the competitive advantage mixotrophs have over heterotrophs (Jassey et al., 2015; Stoecker, 1998). In BG-2015 the functional niche clearly differed between dead and living assemblages, indicating a shift towards drier conditions with a decrease in mixotrophy, test length and pseudostome size and an increase in shell compression (Fournier et al., 2015; Jassey et al., 2013a,b; Marcisz et al., 2016).

The relationship between pseudostome position and water level has so far been explored using mesocosm experiments, field experiments and observational studies along humidity gradients in natural peatlands (Fournier et al., 2016; Marcisz et al., 2016; Payne et al., 2016b). Our results suggest that it could be interesting to monitor specifically functional traits in regenerated bogs to assess adaptations to local constrains. As adaptations are trade-off between niche filters, competition, access to nutrient, etc. (Holyoak et al., 2005; Kearney et al., 2010; Tilman, 1982; Vandermeer, 1972), long term studies could highlight temporal patterns from the stressed stage to the stable community weighted mean of traits.

The shift in test material is more explainable as protein test was mainly related, in the Botanical Garden, to the presence of *Hyalosphenia* ssp. which disappeared completely between 2015 and 2016. In BdL, the dominance in CWM of xenosome tests might be related to a broader presence of mineral particles and to a more complex community structure, including the presence of testate amoebae occupying different trophic levels including bacterivorous, fungivorous but also microeukaryotic predators (Geisen et al., 2015; Gilbert and Mitchell, 2006; Jassey et al., 2012).

The CWM of mixotrophy calculated on living assemblages was highest in BG-2015, almost nil in BG-2016 and intermediate in BdL. Mixotrophy was

Table 3.3 – Variance partitioning of testate amoeba assemblage and CWM of functional traits data based on redundancy analyses. “Site” is a categorical variable for the three sampling sites grouped together, and explained the highest fraction of variance in both datasets. TA living state corresponds to living vs. empty assemblages, Vascular cover (% estimated), patch size (average diameter of the patch), *Sphagnum* sp. categorical variable for the four sampled taxa (*S. magellanicum*, *S. fallax*, *S. rubellum*, and other *Sphagnum* taxa). Significant variable are in bold (p-value < 0.05)

	Testate amoeba community					CWM of traits				
	Df	Variance	%	F	p-value	Df	Variance	%	F	p-value
Site	**2**	**3.61**	**21.2%**	**8.63**	**0.001**	**2**	**2.37**	**21.5%**	**8.58**	**0.001**
TA living state	**1**	**0.65**	**3.8%**	**3.10**	**0.002**	1	0.29	2.7%	2.11	0.074
Vascular cover	**1**	**0.50**	**2.9%**	**2.38**	**0.006**	1	0.18	1.6%	1.28	0.27
Patch size	1	0.31	1.8%	1.48	0.119	**1**	**0.43**	**3.9%**	**3.15**	**0.017**
Sphagnum sp.	**3**	**1.28**	**7.5%**	**2.04**	**0.002**	3	0.70	6.3%	1.68	0.069
Residual	51	10.66	62.7%			51	7.03	63.9%		

shown to vary seasonally (Marcisz et al., 2016) and in relation to sunlight (Schönborn, 1965). In our case as we sampled during the same season (fall) and after long sunny period, mixotrophy should be high if it was locally suitable (Marcisz et al., 2016; Schönborn, 1965). Here we have the opposite and this is in line with dryer and less oligotrophic local conditions where mixotrophy gave no additional advantage (Jassey et al., 2013b).

The CWM of test length and pseudostome size were small in both BG and BdL, indicating that water stress was high in both sites and that the community was mainly dominated by bacterivorous testate amoebae (Fournier et al., 2015; Marcisz et al., 2016; van Bellen et al., 2016).

The dead assemblages were expected to reflect the shape of the original community (Mitchell et al., 2008b). But so far, we do not know if this “old” community was viable in the climatic context of the Botanical Garden regardless of the unexpected drought. The comparison between all three sites and particularly when considering only living populations indicated few differences (Figure 10) in CWM, illustrating the rapidity of community shifts and the pertinence of separating living and dead assemblages.

These results indicate a high mineralisation rate and a low capacity for C fixation and indeed likely an indication that these peatlands are C sources (Bragazza et al., 2016; Jassey et al., 2013a, 2015). The comparison with the BdL reference site revealed that the structure of the community, based on functional traits indicated comparable drivers, i.e. hydric stress, in both BdL and BG.

3.4.5 Global evolution of the bog in Botanical Garden and future challenges

When we sampled the Botanical Garden in 2015, the bog showed clear signs of impact from the long and dry summer, with deep cracks in the peat. The very low density of living amoebae in BG-2015 and the shift in testate amoeba community structure suggested that the peatland was in a very critical condition. Even with the heavy rains of the following winter / spring, the water level in the bog barely reached the surface again and the “pools” remained dry. Despite this, the specific bog vegetation generally reminded alive until 2016 and most of the *Sphagnum* patches survived, although they tended to become smaller and drier than in 2015 (data not shown). The evolution of testate amoeba communities reflected the macroscopic state of *Sphagnum* patches and confirmed that the humidity was lower.

The community structure was clearly different between the three data sets (PCA, Figure 3.7), which was also confirmed by the 21.2% of variance explained only based on sites (Table 3.3). On the other hand, the community weighted mean of traits indicated that the functional niche was similar between BG and BdL. Therefore, in BG, the shift in community structure and functional traits space stayed within the range of a community living in drier parts of oligotrophic and acidic bogs (Booth, 2001; Lamentowicz et al., 2013; Lamentowicz and Mitchell, 2005). The important shift in community structure found in BG-2015 could be due to the drought, to the transplantation of the *Sphagnum* carpets to lower altitude or to a change in humidity between the original peatbog and the artificial one, more likely a mix of all.

Our results show that it will be very challenging to maintain the artificial bog in the Botanical Garden.

A key condition to keep a bog active is maintaining waterlogged conditions during long periods (Mitsch and Gosselink, 2000). In the experimental bog, the water level almost never reached the soil surface again after the 2015 drought. These low conditions stimulate peat mineralisation, releasing nutrients for plant growth and favouring non-bog vegetation including plants species from the surrounding meadows and forests. Without intensive weeding, several specific peatland plant species may not remain (e.g. *Drosera rotundifolia* seems to have disappeared), although some characteristic bog species can grow well on relatively dry bogs, including *Vaccinium oxycoccos* (Ecker et al., 2008; Graf et al., 2010).

This study encounters some limitations. The sampling in BG was made with parsimony to preserve the bog as much as possible. As the density of testate amoebae was very low, some samples had only few living individuals (ca. 10). Secondly, we compared the community structure to BdL. This choice was coherent as some *Sphagnum* patches came from this peatland. However, some *Sphagnum* patches also came from other sites and from more natural settings. Our results nevertheless show that such comparative studies would be extremely valuable, ideally comparing *Sphagnum* patches from the same site (BdL) some of which transplanted in the experimental site.

Nevertheless, the testate amoeba community structure and the community weighted mean of traits gave coherent results and allowed us to interpret the ongoing processes. Moreover, the selected traits indeed seem pertinent to assess the impact of drought on the *Sphagnum*–inhabiting microorganisms and are coherent with the macroscopic state of the experimental bog. By developing a set of traits that is easy to measure for non-specialists, like presence of photosymbionts (mixotrophy), class of test length and/or position of the pseudostome, monitoring tools could be developed to follow the acclimation of such ecosystems along with vegetation relevés, which are expected to react on longer time (Artz et al., 2008; Daza Secco et al., 2016; Mitchell et al., 2000).

3.5 Conclusions

Botanical gardens are privileged places for plant conservation and public information. By integrating also experimental ecosystems such as peatlands, even if they are not as spectacular as tropical greenhouses, they could increase public awareness for the challenges of biodiversity and ecosystem conservation and restoration. Moreover, by giving an easy access to environments like peatlands, they bring some rare ecosystems which are worth to be protected worldwide to the attention of a wide public. Using soil microorganisms as indicators for the management of these ecosystems also nicely illustrates how different spatial and temporal scales should be integrated in ecology.

3.6 Acknowledgment

We would like to thank the Service Faune Forêt Nature (SFFN) of Neuchâtel and in particular Sébastien Tchantz for granting access to the protected site of Bois des Lattes. We would like also to thank master students (Biogeosciences Master's program of the Universities of Neuchâtel and Lausanne) who helped collect samples.

Appendix

Supplementary tables

Table 3.A1 – Characteristics of sampled patches in the Neuchâtel Botanical Garden experimental peatland and Bois des Lattes: patch size (average diameter) and vascular cover (% estimated). Differences were tested with a Student t-test as the data followed assumptions for normality and homoscedasticity.

	Botanical Garden		Bois des Lattes		Student t-test	
	Mean (se)	Range	Mean (se)	Range	t	p-value
Patch size [cm]	16.1(2.1)	6.0 to 35.0	30.4 (3.0)	13.0 to 44.5	3.903	<0.001
Vascular cover [%]	49.2 (7.1)	10 to 100	48.3 (9.1)	10 to 90	-0.072	0.943
Sphagnum fallax	44.4%		16.7%			
Sphagnum magellanicum	33.3%		66.7%			
Sphagnum rubellum	-		16.7%			
Sphagnum sp.	22.2%		-			
Sphagnum vitality	BG-2015	BG-2016	BdL			
Alive	77.8%	91.7%	83.3%			
Half dry	22.2%	8.3%	16.7%			

Table 3.A2 – Descriptive statistics for the overall testate amoeba community data (living individuals and empty tests in the Neuchâtel Botanical Garden experimental peatland – 2015 and 2016 – and Bois des Lattes reference site). Morphotaxa are ordered by decreased abundance and taxa in grey were discarded before the analyses. n: number of sampling plots were the taxa occurred, N: total number of sampling plots (30).

	Frequency		Relative abundance					Density (ind /g dry *Sphagnum*)				
Morphotaxa	absolute N	relative N/n	mean %	se %	median %	min %	max %	mean	se	median	min	max
Corythion dubium	25	83.3%	10.9	3.0	3.7	0	63.3	3761	912	1968	0	21832
Euglypha “small taxa”	22	73.3%	10.2	2.2	7.4	0	65.5	6254	1331	4597	0	27361
Hyalosphenia papilio	17	56.7%	9.6	3.2	1.7	0	71.8	4258	1558	731	0	42864
Difflugia pulex	14	46.7%	9.5	2.4	0.0	0	41.7	10271	3234	0	0	78445
Phryganella acropodia	19	63.3%	9.3	1.8	8.1	0	32.1	7421	1980	3416	0	52157
Nebela tincta	22	73.3%	9.1	1.9	6.1	0	38.2	5617	1269	2401	0	25982
Nebela collaris	20	66.7%	6.6	1.6	3.7	0	37.7	4387	1379	1663	0	35868
Assulina muscorum	18	60.0%	6.5	2.2	1.8	0	46.0	2611	797	885	0	17872
Centropyxis aerophila	17	56.7%	5.7	1.6	1.6	0	33.7	2163	605	725	0	12713
Hyalosphenia elegans	18	60.0%	5.1	1.3	1.5	0	31.0	2922	1061	707	0	29714
Euglypha “big taxa”	28	93.3%	4.5	0.8	3.6	0	18.0	3226	759	1561	0	14517
Cryptodifflugia sp.	13	43.3%	3.3	1.3	0.0	0	35.7	2222	612	0	0	13074
Archerella flavum	17	56.7%	2.1	0.5	1.2	0	10.9	1816	558	567	0	13074
Assulina seminulum	17	56.7%	2.0	0.5	0.9	0	11.5	1252	323	608	0	6049
Heleopera rosea	9	30.0%	1.5	0.6	0.0	0	11.9	980	556	0	0	16168
Hyalosphenia minuta	5	16.7%	1.2	0.6	0.0	0	14.3	1064	513	0	0	11007
Heleopera sylvatica	12	40.0%	1.0	0.4	0.0	0	11.3	648	237	0	0	4986
Trinema lineare	6	20.0%	0.7	0.4	0.0	0	10.7	223	101	0	0	2155
Difflugia lucida	7	23.3%	0.4	0.1	0.0	0	2.9	257	103	0	0	2420
Trigonopyxis arcula	6	20.0%	0.3	0.1	0.0	0	1.9	217	101	0	0	2487
Nebela militaris	5	16.7%	0.2	0.1	0.0	0	2.0	228	100	0	0	2155
Arcella catinus	2	6.7%	0.2	0.2	0.0	0	4.3	65	48	0	0	1324
Bullinularia indica	2	6.7%	0.1	0.1	0.0	0	2.1	53	37	0	0	914
Amphitrema wrightianum	2	6.7%	0.1	0.0	0.0	0	0.9	82	57	0	0	1244

Table 3.A3 – Density [10^3 ind /g dry *Sphagnum*] and proportion [%] of living testate amoebae amoebae in each sample of the Neuchâtel Botanical Garden experimental peatland (2015 and 2016) and Bois des Lattes reference site. Mean values are significantly different between all pairs of sites, based on a Wilcoxon rank test (results not included).

BdL 2016			BG 2015			BG 2016		
Sample	Density	Prop. alive	Sample	Density	Prop. alive	Sample	Density	Prop. alive
BdL_L0_1	135.56	26.6%	BG_L0_A	20.20	9.5%	BG_L0_A	8.87	56.0%
BdL_L0_2	105.59	33.3%	BG_L2_B	26.56	26.9%	BG_L2_B	34.13	65.5%
BdL_L1_3	126.77	43.2%	BG_M0_A	31.11	8.5%	BG_M0_A	26.93	41.4%
BdL_L1_5	79.19	50.0%	BG_M0_B	19.45	36.2%	BG_M0_B	15.05	75.0%
BdL_L2_1	51.60	51.4%	BG_M1_A	26.10	16.7%	BG_M1_A	17.34	71.4%
BdL_L2_2	76.65	51.7%	BG_M1_B	24.13	71.7%	BG_M1_B	34.76	69.8%
BdL_M0_2	77.05	40.0%	BG_S0_B	23.02	17.2%	BG_S0_B	41.32	32.7%
BdL_M1_2	188.99	34.6%	BG_S1_B	26.67	17.6%	BG_S1_B	95125	88.5%
BdL_M2_3	84.10	42.4%	BG_S2_B	44.25	22.5%	BG_S2_B	49.85	54.7%
BdL_S0_3	65.86	42.4%						
BdL_S1_2	141.55	47.0%						
BdL_S2_1	162.27	56.2%						
Mean	**107.92**	**43.0%**		**26.83**	**25%**		**35.93**	**62.0%**
se	**12.32**	**2.7%**		**2.48**	**6.7%**		**8.60**	**6.0%**

Table 3.A4 – Wilcoxon rank test value for density of living testate amoebae, each taxon between the living population and the empty tests in the Neuchâtel Botanical Garden experimental peatland (2015 and 2016) and Bois des Lattes reference site. NA (not available) correspond to taxon not present in the selected site.

	BdL		BG-2015		BG-2016	
Taxon	V	p-value	V	p-value	V	p-value
Archerella flavum	55	0.006	15	0.059	3	0.371
Assulina muscorum	35	0.021	6	0.181	5	0.590
Assulina seminulum	20	0.059	6	0.855	1	0.423
Centropyxis aerophila	3	0.584	33	0.042	4	0.855
Corythion dubium	55	0.006	3	0.142	5	0.151
Cryptodifugia sp.	4	0.019	3	0.371	0	NA
Difugia pulex	27	1.000	0	NA	1	1.000
Euglypha “small taxa”	54	0.529	33	0.042	4	0.108
Euglypha “big taxa”	45	0.205	4	0.855	3	0.584
Heleopera rosea	10	0.059	60	0.181	3	0.371
Heleopera sylvatica	23	0.529	6	0.181	1	1.000
Hyalosphenia elegans	22	0.205	32	0.059	3	0.371
Hyalosphenia minuta	0	0.059	0	NA	0	NA
Hyalosphenia papilio	14	0.529	36	0.014	4	0.789
Nebela collaris	18	0.636	1	1.000	11	0.673
Nebela tincta	42	0.450	36	0.014	0	0.371
Phryganella acropodia	48	0.519	0	NA	21	0.272

Table 3.A5 – Wilcoxon rank test for each functional trait between living and dead testate amoeba assemblages in the Neuchâtel Botanical Garden experimental peatland (2015 and 2016) and Bois des Lattes reference site.

	BdL		BG-2015		BG-2016	
CWM	V	p-value	V	p-value	V	p-value
Mixotrophy	55	0.006	44	0.008	9	0.201
Test length	63	0.064	40	0.039	30	0.426
Pseudostome size	55	0.233	44	0.008	33	0.25
Pseudostome Terminal	27	0.38	27	0.234	22	1
Test Protein	47	0.569	35	0.164	14	0.106
Xenosomes prey	32	0.622	19	0.944	22	0.624
Xenosomes particles	20	0.151	44	0.008	21	0.726
Idiosomes	61	0.092	6	0.055	13	0.301
Test compression	38	0.97	1	0.021	12	0.8
Pseudostome Ventral-central	43	0.791	0	NA	23	0.151
Pseudostome Ventral	59	0.129	9	0.234	15	0.426

Table 3.A6 – Pairwise comparison (Wilcoxon rank test with “Holm” correction for the p-value) community weighted mean (CWM) of selected traits between pairs of data sets (Bois des Lattes: BdL, Neuchâtel Botanical Garden: BG-2015 and BG-2016) and living assemblages (_A) / dead assemblages (_D)

Mixotrophy	BdL_A	BdL_D	BG_T1_A	BG_T1_D	BG_T2_A
BdL_D	0.271	-	-	-	-
BG_T1_A	1	1	-	-	-
BG_T1_D	0.032	0.758	0.031	-	-
BG_T2_A	1	0.020	0.604	0.023	-
BG_T2_D	1	0.429	0.604	0.023	0.604
Test length					
BdL_D	1	-	-	-	-
BG_T1_A	1	1	-	-	-
BG_T1_D	0.007	0.013	0.195	-	-
BG_T2_A	1	1	1	0.047	-
BG_T2_D	1	1	1	0.219	1
Pseudostome size					
BdL_D	1	-	-	-	-
BG_T1_A	1	1	-	-	-
BG_T1_D	0.014	0.014	0.047	-	-
BG_T2_A	1	1	1	0.047	-
BG_T2_D	1	1	1	0.156	0.750
Pseudostome Terminal					
BdL_D	1	-	-	-	-
BG_T1_A	1	1	-	-	-
BG_T1_D	1	1	0.936	-	-
BG_T2_A	1	1	1	0.195	-
BG_T2_D	0.034	0.02	1	0.023	1
Test Protein					
BdL_D	1	-	-	-	-
BG_T1_A	1	1	-	-	-
BG_T1_D	0.045	0.066	0.211	-	-
BG_T2_A	0.001	0.001	0.09	0.045	-
BG_T2_D	0.047	0.04	0.104	0.023	0.211
Xenosomes prey					
BdL_D	1	-	-	-	-
BG_T1_A	1	1	-	-	-
BG_T1_D	1	1	1	-	-
BG_T2_A	1	1	1	1	-
BG_T2_D	1	1	1	1	1
Xenosomes particles					
BdL_D	1	-	-	-	-
BG_T1_A	0.003	0.003	-	-	-
BG_T1_D	0.056	0.080	0.047	-	-
BG_T2_A	0.875	0.992	0.237	1	-
BG_T2_D	0.875	1	0.112	1	1
Idiosomes					
BdL_D	1	-	-	-	-
BG_T1_A	1	1	-	-	-
BG_T1_D	1	1	0.219	-	-
BG_T2_A	0.094	0.373	0.387	0.039	-
BG_T2_D	0.084	0.373	0.734	0.023	0.602
Test compression					
BdL_D	1	-	-	-	-
BG_T1_A	0.023	0.011	-	-	-
BG_T1_D	1	1	0.13	-	-
BG_T2_A	1	1	0.32	1	-
BG_T2_D	1	1	0.13	0.3	1
Pseudostome Ventral-central					
BdL_D	0.254	-	-	-	-
BG_T1_A	0.211	1	-	-	-
BG_T1_D	0.031	0.148	0.98	-	-
BG_T2_A	0.015	0.052	1	0.98	-
BG_T2_D	0.015	0.052	1	0.98	1
Pseudostome Ventral					
BdL_D	1	-	-	-	-
BG_T1_A	0.001	0.001	-	-	-
BG_T1_D	0.001	0.001	-	-	-
BG_T2_A	0.922	0.936	0.18	0.18	-
BG_T2_D	1	1	0.11	0.11	0.18

Supplementary figures

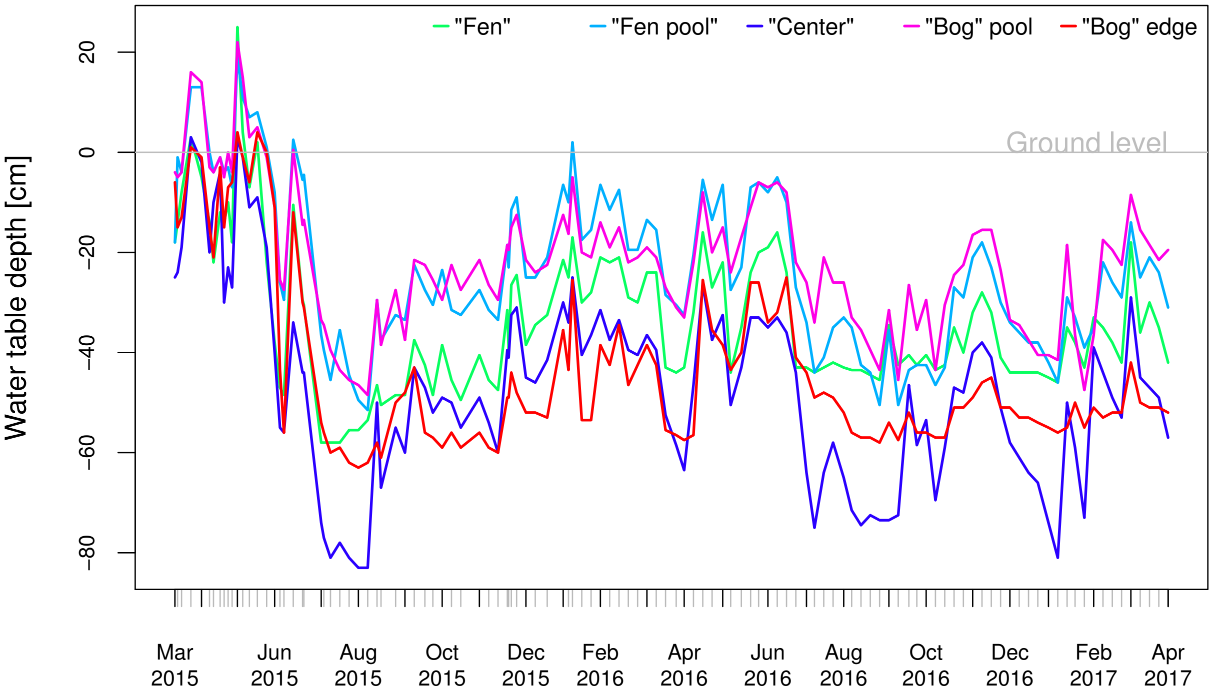


Figure 3.A1 – Fluctuation of water table depth measured in five piezometers in the Neuchâtel Botanical Garden experimental peatland between March 2015 and April 2017. The horizontal line represents the ground level (i.e. peat surface or top of moss carpet). The peatland was very wet during the spring of 2015 and then the water table dropped sharply during the summer drought of 2015. The water level then increased again during the winter and spring 2016 but then dropped again in the summer 2016 and did recover since.

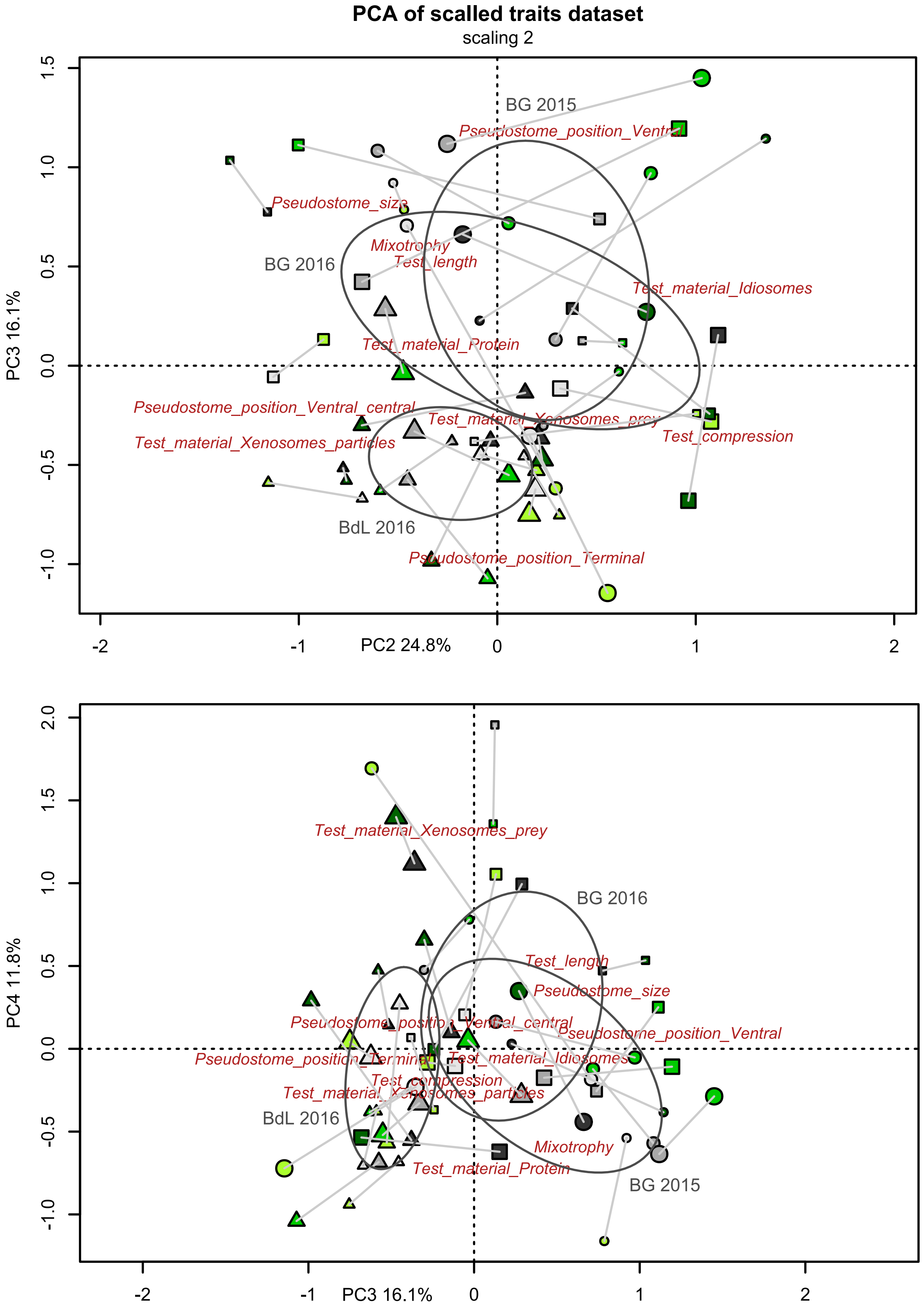


Figure 3.A2 – : Principal component analyses of standardized community weighted mean of testate amoeba functional traits in the Neuchâtel Botanical Garden experimental peatland (BG-2015 and BG-2016) and the Bois des Lattes reference peatland (BdL): Top: axes 2 and 3, bottom: axes 3 and 4. The six data sub-sets are shown by different symbols: BdL alive: green triangles, BdL dead: grey triangles, BG-2015 alive: green squares, BG-2015 dead: grey squares, BG-2016 alive: green circles, BG-2016 dead: grey circles. Symbol sizes indicate patch size and shading indicate the % of vascular plant cover.

Chapter 4

Taxonomic and functional traits responses of *Sphagnum* peatland testate amoebae to experimentally manipulated water table

Isabelle Koenig[a, *], Matthieu Mulot[a,c, *], Edward A. D. Mitchell[a,b]

Abstract

Biomonitoring tools are useful to assess the impact of environmental changes on the functioning of ecosystems. Existing tools mostly require species identification, thus allowing to estimating changes in biodiversity, and possibly inferring ecosystem functioning, using functional diversity and traits based approaches.

Testate amoebae are good indicators of surface moisture conditions in *Sphagnum* peatlands and are routinely used in palaeoecology. Their shells (tests), on which identification is based, can also be used to define functional traits and thus to infer changes in ecosystem functioning.

We investigated the response of testate amoeba communities to manipulated water table depth (wet: -4cm, intermediate: -15cm, and dry: -25cm) over time (seven time points, 19 months) using mesocosms by comparing two approaches: community composition and functional composition responses, using a combination of morphological (biovolume, length, aperture size and position) and physiological (mixotrophy/heterotrophy, shell material) traits.

This is the first study investigating the temporal dynamics of environmental filtering on testate amoeba assemblages. Taxonomical and functional approaches showed similar response patterns, confirming that water level acted as a strong environmental filter. After one year *Hyalosphenia papilio* decreased in the dry treatment, and the community structure shifted towards a dominance of dry indicators (*Nebela tincta* complex, *Corythion dubium*, *Euglypha compressa*) and the selected functional traits (smaller, heterotrophic, compressed species, with a ventral aperture) corresponded to drought adaptations.

In line with recent observational and transfer function studies exploring the use of testate amoebae functional traits, our experimental results illustrate how well-selected traits could be used to monitor the impact of present and past climatic changes on *Sphagnum* peatlands.

Keywords: Testate amoebae, mesocosm experiment, functional diversity, functional traits, *Sphagnum* peatland, biomonitoring, water table depth

[a] Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland

[b] Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

[c] Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 7144, Adaptation et Diversité en Milieu Marin, Equipe EPEP, Station Biologique de Roscoff, F-29680 Roscoff, France

[*] Joint first authors (equal overall contribution)

Ecological Indicators, accepted

4.1 Introduction

Biomonitoring tools are useful to assess the impact of environmental changes on the functioning of ecosystems. Finding and testing relevant proxies to monitor the evolution of ecosystems are therefore priorities in applied ecological research (Bartkowski et al., 2015; Laurila-Pant et al., 2015). In addition to describing biodiversity itself, good bioindicators should also inform about ecosystem services. Biomonitoring approaches are now accepted as standard tools to assess environmental quality (European Commission, 1999; Finlayson, 1994).

Functional diversity (FD) is a useful tool to model ecosystem functioning. Based on the relationship between environmental constraints and community structure, through functional traits, FD allows assessing the impact of environmental changes on both community structure and ecosystem functioning (Bockstael et al., 2000; Cadotte et al., 2011; Keddy, 1992; Woodward and Diament, 1991). Species traits may explain both the effects of environmental changes on community structure, (response traits) and the feedback from species presence on ecosystem functioning (effect traits, Lavorel and Garnier, 2002). Traits used for FD analysis should ideally relate to niche characteristics and/or directly or indirectly to ecosystem processes (Mlambo, 2014; Violle et al., 2007). The FD approach was initially developed for macroscopic organisms such as plants, fishes and soil invertebrates, for which different sets of traits have been validated and more recently for microorganisms (Arrieira et al., 2015; Barnett et al., 2013; Fournier et al., 2016, 2015, 2012; Jassey et al., 2016; Lamentowicz et al., 2015; Marcisz et al., 2016; van Bellen et al., 2016; Violle et al., 2011). Our focus here is on the impact of climate change on testate amoebae living in *Sphagnum* peatlands.

Sphagnum-dominated peatlands are key ecosystems in the global Carbon cycle as C stores and sinks (Frolking and Roulet, 2007; Turunen et al., 2002), but are threatened by direct human impact (peat harvesting, drainage) and climatic change (warming, drought) (Belyea and Malmer, 2004; Bonn et al., 2016; MEA, 2005; Briones et al., 2014). The key to peat accumulation is the low decomposition rate, which is driven by the anoxic conditions in the usually water-logged soils (Belyea and Malmer, 2004; Rydin et al., 2013). *Sphagnum*-dominated peatlands, are primarily situated in high latitudes where documented and predicted climate warming are greatest (IPCC, 2013; Hassol, 2004). Understanding their response to climatic change is therefore a research priority (Belyea and Malmer, 2004; Pachauri et al., 2014; Rydin et al., 2013).

As the vegetation of peatlands is dominated by perennial plants, shifts in vegetation in response to environmental changes are slow. By contrast, soil microorganisms, including protists such as testate amoebae, have short generation times and are thus well adapted to being used as early warning indicators of environmental changes (Buttler et al., 1996; Gilbert et al., 1998; Laggoun-Defarge et al., 2008). Even within stable conditions, testate amoebae were shown to be better indicators of micro-environmental gradients than plants (Koenig et al., 2015). Testate amoebae are a polyphyletic group of protists building a shell (i.e. "test"). They are known to be well correlated with environmental gradients like soil moisture, water table depth, pH, or nutrients content (Booth and Zygmunt, 2005; Jassey et al., 2011; Swindles et al., 2009). Their decay-resistant shell, well preserved in peat and sediments after the organisms death, allows past climates to be reconstructed by inferring ancient local conditions through testate amoeba community structures (Chambers et al., 2012; Talbot et al., 2010). In addition, testate amoebae functional traits can also be defined in accordance with functional diversity assumptions and be used for biomonitoring or paleoreconstruction (Fournier et al., 2015; Jassey et al., 2016; Kajukało et al., 2016; van Bellen et al., 2016).

The structure of testate amoeba communities living in peatlands has long been shown to be well correlated to factors such as water table depth and pH (Amesbury et al. 2013; Jassey et al. 2011; Marcisz et al. 2014; Mazei et al. 2007; Mieczan 2009; Swindles et al. 2009; Wilken et al. 2013). The informative power of testate amoebae relies strongly on the accuracy of identification. However, due to taxonomic uncertainties (Booth, 2008; Mitchell et al., 2013, 2008b; Payne et al., 2012), a significant yet unknown fraction of the data used in ecological studies contains errors. Although numerical experiments showed that such taxonomic inconsistencies generally have little effect on ecological interpretation, (Payne et al., 2011), it is nevertheless desirable to develop bioindication tools that are robust to potential identification biases.

The mechanistic approach to community assembly states that community membership is constrained to those species with the appropriate functional traits to reach a site (i.e., are able to overcome a dispersal filter) and are able to spread under the circumstances set by the environment and other organisms (i.e., the abiotic and biotic filters Belyea and Lancaster, 1999; Lebrija-Trejos et al., 2010). The role of environmental filtering in shaping testate amoeba communities and diversity patterns has been studied in tundra and taiga ecosystems (Mazei, 2008; Mazei et al., 2009, 2011; Tsyganov et al., 2015) and in primary succession of mine tailing (Dunger et al., 2001). Testate amoeba functional traits (phylogenetic grouping, origin of shell material, shell size and compression) were shown to correlate to environmental conditions in a floodplain (Fournier et al., 2012). In *Sphagnum* peatlands, mixotrophic testate amoebae (i.e. species harbouring endosymbiotic algae), which dominate micro-eukaryotic communities under wet conditions such as bog pools, were shown to significantly contribute to the C sink function (Jassey et al., 2013a, 2015). Understanding the functional responses of peatland testate amoebae to environmental filtering would therefore be useful to better assess the effect of water level on peatland functioning through its impact on a key functional group of soil microorganisms. From a bioindication perspective, it is essential to understand the dynamic processes involved in environmental filtering in order to test the causality of relationships and determine at which temporal scale organisms can be used as bioindicators – and this can only be achieved using experimental approaches (Marcisz et al., 2014).

We monitored the temporal pattern of the response of testate amoeba communities and corresponding variations in traits (Ackerly and Cornwell, 2007) using a *Sphagnum* peatland mesocosm experiment with artificially manipulated water table (Mulot et al., 2015). Our hypothesis was that the testate amoeba community weighted mean of traits (CWM: the abundance-weighted mean of the species trait values within a community) and community structure would exhibit similar responses to water level manipulation, but that the CWM would be more directly ecologically informative than community structure in understanding ecosystem functioning.

4.2 Material and methods

4.2.1 Experimental design

The mesocosms were plastic tanks filled with water (Annexe 1). In the centre of each tank a PVC tube ($45\mathrm{cm}$ high, $12\mathrm{cm}$ diameter with regularly-spaced lateral holes to allow water movement) contained a peat core topped with a carpet of *Sphagnum fallax* collected. The water level in each mesocosm was adjusted to maintain three average water table depths ($-4\mathrm{cm}$,$-15\mathrm{cm}$, and $-25\mathrm{cm}$, hereafter referred to as "wet", "intermediate" and "dry" treatments). Each treatment was replicated five times, giving a total of 15 mesocosms. The *Sphagnum* carpets were collected in a peatland in the Jura Mountains (Creux de l'Epral, 47°12′18.3″N; 6°56′5.83″E; altitude: 990 m). Each carpet was seeded with a water extract from pool, hummock, and lawn ("bog microbial cocktail") to provide the full community potential (Mulot et al., 2015). Therefore, the communities should be undistinguishable at T0 in all mesocosms. For the extraction of testate amoebae, the top three centimetres of Sphagnum mosses were collected on 02/08/2012 (T0), 04/10/2012 (T1), 29/11/2012 (T2), 04/03/2013 (T3), 11/08/2013 (T4), 15/12/2013 (T5), and 27/03/2014 (T6). Water levels were recorded twice a day using automatic custom piezometers as described in Mulot et al. (2015).

4.2.2 Testate amoeba isolation and characterization

Testate amoebae were extracted by sieving and back-sieving through mesh filters (Booth et al., 2010; Jassey et al., 2011). A minimum of 100 individuals (living + dead) per sample were counted. The rationale for including living as well as dead individuals was that this corresponds to the community that eventually will be preserved in the peat and recovered in palaeoecological studies. Our focus here is thus not specifically on the living community and thus a potential limitation inherent to this choice is that by including the dead amoebae we expect some lag in the response to the experimental treatment. In some late dry samples, testate amoeba density was very low and only 50 individuals could be found. As the diversity in these samples was low due to the strong dominance of one species (*Hyalosphenia papilio*), and given that we had five replicates per treatment, having only 50 specimens in a few samples was considered as acceptable (Payne and Pates, 2009). Identification of morpho-species followed Charman et al. (2004) and Mitchell (2003). While this clearly underestimates diversity, it corresponds to the standard generally used in palaeoecological studies and that retains most ecological signal (Mitchell et al., 2014). Some taxa were thus lumped into broad morphological groups: *Nebela tincta* complex (as define by Kosakyan et al., 2012), *Euglypha ciliata* and *E. compressa*, and *Cyclopyxis eurystoma* and *C. kahli* to overcome likely identifica-

Table 4.1 – Description, ecological meaning and references for the six functional traits selected for testate amoeba taxa.

	Unit	Description	Type of trait	Ecological meaning	References
Biovolume	μm^3	Volume of shell (90% occupied by the living amoeba)	Response	Related to the metabolic rate and the capacity of the food web to process energy.	Fournier et al. (2012) Laggoun-Defarge et al. (2008) Makarieva et al. (2008)
Test length	µm	Maximal length of the shell	Response	Proxy for biovolume (assuming isometry).	
Aperture position	Factor	From a terminal aperture to the completely central one.	Response	Represents the ability to survive in thin water film and thus the ability to remain active and contribute to the food web in dry conditions.	Fournier et al. (2012) Lamentowicz et al. (2015)
Test material	Factor	Protein, silica or agglutinated made (organic debris, diatom frustules, mineral particles)	Response	Availability of material and / or prey to construct the test. Source of material appear to be a major regulator of abundance and repartition of testate amoebae along Sphagnum parts.	Gilbert and Mitchell (2006)
Test compression	Binary	0: not compressed 1: compressed	Response	Survival potential in drier situations and thus potential contribution to the food web in dry conditions.	Fournier et al. (2012)
Mixotrophy	Binary	Presence (1) or not (0) of photosynthetic endosymbionts	Effect	Mixotrophy is a key factor in oligotrophic conditions and plays a role in peatland C cycling.	Fournier et al. (2015) Jassey et al. (2015, 2011)
Aperture size	µm	Width of the shell aperture	Effect	Related to prey size and food web functioning	Jassey et al. (2013a)

tion biases when data from different analysts is combined (Amesbury et al., 2016; Mitchell et al., 2014). Seven samples were discarded because of disturbances in the mesocosm or technical problems during sampling or preparation, resulting in 98 final samples.

The chosen traits and their ecological meaning, as defined by Lavorel and Garnier (2002), Messier et al. (2010), Mlambo (2014) are given in Table 4.1. Test length was also used as a proxy for biovolume; as these two variables were strongly correlated ($R^2 = 0.69$) only biovolume was kept for further analyses. Biovolume is also ecologically more relevant as it is known to indicate the metabolic rate and the capacity of the food web to process energy (Fournier et al., 2012; Jassey et al., 2013a; Laggoun-Defarge et al., 2008; Makarieva et al., 2008; Tsyganov et al., 2012). All traits were measured on the observed material. The morphological dimensions were measured directly at 400x magnification using an inverted IX-81 Olympus microscope, and the *Olympus cellSens Dimension* software. Biovolume was calculated according to the general shape of the shell, applying geometrical formulas as in Fournier et al. (2015).

4.2.3 Numerical analyses

We first assessed how the experimentally-induced changes in testate amoeba community structure translated into inferred water table depth as if these communities were used in a palaeoecological study. To this aim, we inferred the theoretical water table depth (WTD) based on the observed community structure of each sampling date with a transfer function. To build this transfer function we used a training set from the Jura Mountains (Mitchell et al., 1999), but keeping only the species common to both data sets and samples with a depth to water table lower than $40\mathrm{cm}$, corresponding to the possible range of water levels in the mesocosm experiment. We used the weighted average (WA) model, with a leave-one-out cross validation (Juggins, 2015). The significance of similarity between communities throughout the experiment was assessed by an analysis of similarity (anosim, vegan package), with a Bray-Curtis dissimilarity measure and 999 permutations. ANOSIM is a non-parametric analyse allowing us to test the difference between and within groups for two or more groups, using the rank of dissimilarity values (Clarke, 1993). We then computed the community weighted mean (CWM, funcomp, FD package, Laliberté et al., 2014) of selected traits (biovolume, test length – only for linear regression with biovolume – , aperture position, test compression, mixotrophy and aperture size) in each sample. The CWM represented the average of each trait value weighted by the relative abundance of each species, based on the fourth-corner approach (Dray and Legendre, 2008). To compare the response of community traits and community taxonomic composition to water level through time, we finally computed a principal response curves (PRC) analysis (prc, vegan) on Hellinger transformed species dataset and standardized CWM of traits. PRC is a multivariate tool based on eigenvector ordination (special case of redundancy analysis) suitable for time series with contrasted treatment (Van den Brink and Ter Braak, 1999).

All analyses were carried out on R statistical software (R Core Team, 2016). Community based measures were computed using the R package vegan (Oksanen, 2015), while functional diversity measures were calculated using the R package FD (Laliberté et al., 2014) and mean water table depth was assessed with transfer function using R package rioja (Juggins, 2015).

4.3 Results

4.3.1 Testate amoeba diversity and community structure and inferred water table depth

A total of 17 morpho-species were recorded in the samples (Table 4.2). The richest samples were the dry plots at T4-T5 with a species richness of 10-11 and the poorest sample (intermediate, T5) showed only two species. *Hyalosphenia papilio* was the most abundant taxon, representing $78\% \pm 2.2\%$ of the total count on average. The two next most abundant taxa were *Nebela tincta* s.l. ($5.5\% \pm 0.9\%$) and *Arcella catinus* ($4.4\% \pm 0.5\%$; Table 4.2).

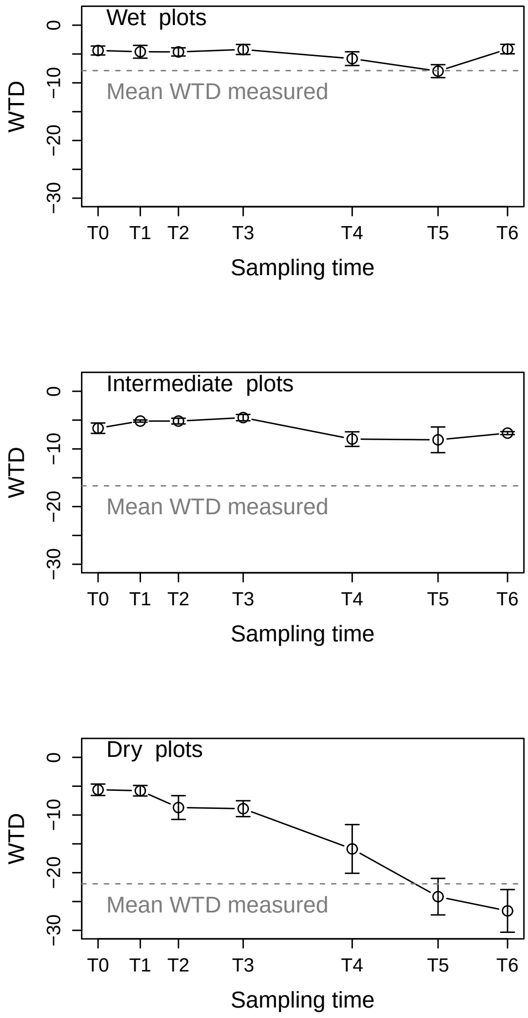


Figure 4.1 – Inferred water table depth (WTD) based on the testate amoeba community structure in *Sphagnum fallax* mesocosms with controlled water table depth. For each sampling point the mean inferred WTD and standard error is shown for each treatment (black dots and lines). The mean water table depth measured in each mesocosm is shown as a grey dotted line. The community structure at the beginning of the experiment was dominated by indicator species of wet and the inferred water table depth was $-5.5 \pm 0.5\mathrm{cm}$ (se)

The communities were similar in all treatments at the beginning of the experiment (analysis of similarities at T0: $R = 0.05$, $p = 0.242$, Table 4.3) and subsequently diverged among treatments (maximum at T5, $R = 0.62$, $p = 0.001$, Table 4.3). *Hyalosphenia papilio* strongly dominated the communities in the wet and intermediate treatments throughout the duration of the experiment (min: 78.4% wet at T5, max: 92.9% intermediate at T3; Table 4.4). In wet plots, some taxa expanded slightly by the end of the experiment (*Amphitrema wrightianum*, *Phryganella accropodia*), but otherwise, the community structure

Table 4.2 – Overall relative abundance of testate amoeba taxa over the whole duration of the experiment ($n = 98$ samples), mean, median, standard error (se), minimal (min) and maximal (max). Taxa are ordered by decreasing mean relative abundance.

	n	mean	median	se	min	max
Hyalosphenia papilio	98	78.08%	85.37%	2.23%	0.57%	98.61%
Nebela tincta s.l.	98	5.46%	2.05%	0.91%	0.00%	43.25%
Arcella catinus	98	4.36%	3.13%	0.52%	0.00%	38.36%
Corythion dubium	98	2.87%	0.00%	1.21%	0.00%	83.43%
Centropyxis aculeata	98	1.80%	0.31%	0.49%	0.00%	34.78%
Phryganella acropodia	98	1.50%	0.00%	0.45%	0.00%	27.87%
Heleopera rosea	98	1.40%	0.00%	0.36%	0.00%	25.16%
Euglypha compressa/ciliata	98	1.31%	0.48%	0.21%	0.00%	10.42%
Assulina muscorum	98	0.90%	0.00%	0.14%	0.00%	5.83%
Hyalosphenia elegans	98	0.74%	0.00%	0.13%	0.00%	8.23%
Archerella flavum	98	0.62%	0.00%	0.23%	0.00%	16.36%
Physochila griseola	98	0.27%	0.00%	0.13%	0.00%	8.94%
Cyclopyxis eurystoma/kahli	98	0.17%	0.00%	0.08%	0.00%	7.69%
Trinema lineare	98	0.17%	0.00%	0.06%	0.00%	4.48%
Amphitrema wrightianum	98	0.16%	0.00%	0.07%	0.00%	3.92%
Argynnia dentistoma	98	0.12%	0.00%	0.05%	0.00%	3.73%
Assulina seminulum	98	0.08%	0.00%	0.03%	0.00%	2.30%

was rather stable over the course of the experiment. By contrast, in the dry plots, *H. papilio* decreased strongly over time from 81.3% to 16.9%, and some taxa indicative for high water table, disappeared (e.g. *Archerella flavum*, *Cyclopyxis eurystoma/kahli*), while dry indicators increased (e.g. *Corythion dubium*, *Nebela tincta* s.l., *Assulina muscorum*).

In wet plots, the inferred water table depth was constant at around $-2\mathrm{cm}$ throughout the experiment, increasing to $0\mathrm{cm}$ at T6 (Figure 4.1). In intermediate plots, the trend was very similar to that of wet plots, with a slight lowering of the water table depth after T3 until T6 to $-4\mathrm{cm}$. In dry plots, the inferred water table depth lowered to $-20\mathrm{cm}$ by T6.

Table 4.3 – Analysis of similarity among treatments for each sampling time (T0-T6) based on a Bray-Curtis dissimilarity index and 999 permutations.

	R Statistic	Sig
T0	0.05	0.242
T1	0.15	0.087
T2	0.18	0.030
T3	0.33	0.006
T4	0.51	0.009
T5	0.62	0.001
T6	0.56	0.001

4.3.2 Change in traits space over time

The community weighted mean of traits illustrates how the space defined by selected traits (related to the ecological niche) was filled and how the traits space evolved over the experiment (Figure 4.2). Aperture position, test material and aperture size remained similar in the wet and intermediate plots over the entire course of the experiment, while a clear shift occurred in the dry plots. After T4 a shift in test material was observed in dry plots from protein to silica and agglutinated (Figure 4.2c). At T6 the community in dry plots was dominated by ovoid, agglutinated or silica test, and a ventral position of aperture (Figure 4.2a). The proportion of mixotrophic species stayed around 60% over the course of the experiment in the wet and intermediate plots (Figure 4.2d), but decreased gradually in the dry plots from to less than 20% by T6. By contrast, test compression did not vary in the dry and intermediate plots but decreased between T3 and T5 in the wet plots (Figure 4.2e), and was tended to increase again at T6. Pseudostome (aperture) size decreased in dry plots from T3 onwards (Figure 4.2f). Test length and biovolume decreased slightly in dry plots from T3 onwards and remained stable in wet and intermediate plots (Figure 4.2 g&h).

4.3.3 Principal response curves

The two principal response curves (PRC) analyses on 1) Hellinger-transformed species data and 2) CWM of Gower distance and standardized traits data, with treatment and time as explanatory variables, were significant ($p < 0.01$, based on the analysis of variance of the analysis). These two analyses explained 61.9% and 61.0%, respectively of the variance: specifically, the conditional, constrained and unconstrained fraction of variance in the Spe-

Table 4.4 – Relative abundance of testate amoeba taxa per treatment (wet, intermediate – inter – and dry plots) and sampling time (T0 to T6), with number of plots counted (n), mean (%) and standard error (se %). In order to show the pattern, species are ordered in increasing optimal water table depth (WTD, Optima and tolerance – Tol) as inferred using a transfer function from the Jura Mountains peatlands (Mitchell et al., 1999). Grey bars are proportional to average relative abundance in the time/treatment to highlight the pattern in the dataset (conditional colouring in MS Excel). In dry plots, the shift of community structure towards an increasing dominance of dry adapted species appears significantly.

	WTD (cm)			**T0**			**T1**			**T2**			**T3**			**T4**			**T5**			**T6**		
Taxa	**Optima**	**Tol.**	**Treatment**	**n**	**mean**	**se**	**n**	**mean**	**se**	**n**	**mean**	**se**	**n**	**mean**	**se**	**n**	**mean**	**se**	**n**	**mean**	**se**	**n**	**mean**	**se**
Amphitrema wrightianum	9.4	4.1	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	3.1	0.4
Archerella flavum	10.9	8.5	Wet	5	4.6	2.2	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
Cyclopyxis eurystoma/kahli	11.1	9.2	Wet	5	0.5	0.3	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.4	0.4	5	0.0	0.0	5	0.0	0.4
Physochila griseola	15.2	7.1	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.5	0.5	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
Hyalosphenia papilio	16.1	10.1	Wet	5	84.4	3.9	5	92.4	3.2	5	92.6	1.9	5	89.1	4.3	3	84.9	4.5	5	78.4	4.1	5	84.4	0.3
Centropyxis aculeata	17.0	7.0	Wet	5	0.8	0.5	5	0.0	0.0	5	0.2	0.2	5	2.7	1.0	3	1.5	0.3	5	0.0	0.0	5	1.7	0.0
Hyalosphenia elegans	19.2	9.9	Wet	5	0.3	0.2	5	0.4	0.4	5	0.3	0.2	5	3.0	1.5	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
Phryganella acropodia	21.6	11.5	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	6.9	2.6	5	15.2	3.8	5	7.4	0.0
Heleopera rosea	22.3	6.9	Wet	5	1.2	0.8	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	2.7	1.0	5	0.3	0.2	5	1.0	0.0
Argynnia dentistoma	23.7	7.0	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.2	0.2	3	0.0	0.0	5	0.0	0.0	5	0.0	3.5
Corythion dubium	23.7	11.0	Wet	5	0.0	0.0	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	3	0.2	0.2	5	0.0	0.0	5	0.0	0.7
Nebela tincta s.l.	23.8	12.6	Wet	5	1.8	0.6	5	2.7	1.1	5	2.5	0.8	5	1.5	1.0	3	0.2	0.2	5	1.0	0.7	5	0.3	0.4
Trinema lineare	24.2	11.3	Wet	5	0.4	0.3	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	3.0
Arcella catinus	24.7	8.7	Wet	5	3.5	1.4	5	3.9	2.2	5	2.3	1.4	5	1.8	0.6	3	3.2	1.2	5	4.3	1.8	5	1.1	0.0
Euglypha compressa/ciliata	25.5	9.6	Wet	5	1.7	0.5	5	0.4	0.3	5	1.0	0.6	5	1.2	0.8	3	0.0	0.0	5	0.0	0.0	5	0.4	0.6
Assulina muscorum	25.5	11.4	Wet	5	0.6	0.5	5	0.0	0.0	5	1.2	1.0	5	0.0	0.0	3	0.0	0.0	5	0.7	0.7	5	0.6	1.5
Assulina seminulum	29.7	12.7	Wet	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
Amphitrema wrightianum	9.4	4.1	Inter	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
Archerella flavum	10.9	8.5	Inter	5	0.0	0.0	5	0.3	0.2	5	0.0	0.0	5	0.0	0.0	3	0.2	0.1	5	0.6	0.4	5	0.0	0.2
Cyclopyxis eurystoma/kahli	11.1	9.2	Inter	5	0.2	0.1	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.1	0.1	5	0.0	0.0
Physochila griseola	15.2	7.1	Inter	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.2	0.1	5	0.0	0.0	5	0.2	1.6
Hyalosphenia papilio	16.1	10.1	Inter	5	86.4	2.7	5	89.5	0.6	5	90.5	1.3	5	92.9	1.6	3	80.6	3.3	5	81.6	6.5	5	83.9	0.6
Centropyxis aculeata	17.0	7.0	Inter	5	0.8	0.2	5	0.1	0.1	5	0.3	0.3	5	0.2	0.2	3	0.5	0.4	5	0.0	0.0	5	0.5	0.0
Hyalosphenia elegans	19.2	9.9	Inter	5	0.7	0.3	5	1.9	0.6	5	0.5	0.2	5	0.0	0.0	3	1.7	1.5	5	0.4	0.3	5	0.4	0.2
Phryganella acropodia	21.6	11.5	Inter	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	2.6	0.7
Heleopera rosea	22.3	6.9	Inter	5	0.5	0.5	5	0.2	0.1	5	1.4	0.8	5	0.0	0.0	3	1.5	1.3	5	0.2	0.2	5	1.1	0.0
Argynnia dentistoma	23.7	7.0	Inter	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.3	0.2	3	0.0	0.0	5	0.1	0.1	5	0.0	1.1
Corythion dubium	23.7	11.0	Inter	5	0.2	0.2	5	0.1	0.1	5	0.2	0.2	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	0.7
Nebela tincta s.l.	23.8	12.6	Inter	5	3.6	0.6	5	2.5	0.8	5	1.3	0.4	5	0.8	0.3	3	1.6	0.9	5	0.1	0.1	5	2.0	0.2
Trinema lineare	24.2	11.3	Inter	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	5	0.2	0.2	3	0.0	0.0	5	0.0	0.0	5	0.0	0.0
Arcella catinus	24.7	8.7	Inter	5	6.5	2.0	5	2.7	0.8	5	4.2	1.3	5	4.9	1.5	3	10.5	2.8	5	15.7	6.2	5	7.1	0.0
Euglypha compressa/ciliata	25.5	9.6	Inter	5	0.1	0.1	5	0.7	0.2	5	1.0	0.8	5	0.1	0.1	3	0.6	0.3	5	0.0	0.0	5	0.5	0.0
Assulina muscorum	25.5	11.4	Inter	5	0.8	0.2	5	1.8	0.7	5	0.5	0.3	5	0.6	0.4	3	2.4	1.8	5	1.2	0.9	5	1.8	0.2
Assulina seminulum	29.7	12.7	Inter	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	5	0.0	0.0	5	0.0	1.1
Amphitrema wrightianum	9.4	4.1	Dry	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	4	0.0	0.0	5	0.0	0.0
Archerella flavum	10.9	8.5	Dry	5	4.5	3.1	5	1.3	1.3	5	0.3	0.3	5	0.1	0.1	3	0.1	0.1	4	0.0	0.0	5	0.0	0.0
Cyclopyxis eurystoma/kahli	11.1	9.2	Dry	5	0.4	0.4	5	0.4	0.2	5	1.5	1.5	5	0.0	0.0	3	0.0	0.0	4	0.0	0.0	5	0.0	0.0
Physochila griseola	15.2	7.1	Dry	5	0.0	0.0	5	0.1	0.1	5	0.0	0.0	5	0.0	0.0	3	5.5	2.8	4	0.0	0.0	5	1.1	0.9
Hyalosphenia papilio	16.1	10.1	Dry	5	81.3	5.9	5	85.7	2.6	5	77.7	6.9	5	77.6	3.9	3	42.5	12.9	4	25.2	5.4	5	16.9	6.1
Centropyxis aculeata	17.0	7.0	Dry	5	0.5	0.4	5	1.2	0.4	5	1.0	0.7	5	2.4	1.0	3	9.0	3.3	4	10.6	8.1	5	7.6	5.1
Hyalosphenia elegans	19.2	9.9	Dry	5	0.5	0.4	5	0.8	0.5	5	1.3	0.9	5	0.2	0.2	3	2.1	0.2	4	0.7	0.4	5	1.1	0.5
Phryganella acropodia	21.6	11.5	Dry	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	4	0.0	0.0	5	0.0	0.0
Heleopera rosea	22.3	6.9	Dry	5	0.7	0.3	5	0.3	0.2	5	0.9	0.6	5	0.5	0.3	3	8.8	1.9	4	2.7	2.0	5	9.0	5.1
Argynnia dentistoma	23.7	7.0	Dry	5	0.0	0.0	5	0.1	0.1	5	0.0	0.0	5	1.7	0.6	3	0.0	0.0	4	0.0	0.0	5	0.1	0.1
Corythion dubium	23.7	11.0	Dry	5	0.7	0.7	5	0.7	0.5	5	0.0	0.0	5	0.4	0.3	3	0.8	0.5	4	24.3	9.8	5	33.7	16.4
Nebela tincta s.l.	23.8	12.6	Dry	5	4.3	1.9	5	3.1	1.0	5	9.6	2.9	5	12.4	3.9	3	23.2	10.5	4	25.9	7.0	5	21.8	5.7
Trinema lineare	24.2	11.3	Dry	5	0.0	0.0	5	0.3	0.3	5	0.9	0.9	5	0.6	0.4	3	0.9	0.9	4	0.2	0.2	5	0.0	0.0
Arcella catinus	24.7	8.7	Dry	5	5.3	1.9	5	2.8	0.9	5	3.2	1.4	5	1.6	0.8	3	2.5	1.4	4	4.0	1.9	5	1.8	0.8
Euglypha compressa/ciliata	25.5	9.6	Dry	5	1.8	0.8	5	1.8	0.5	5	3.0	1.0	5	2.3	0.8	3	3.7	2.0	4	3.3	2.0	5	4.4	2.1
Assulina muscorum	25.5	11.4	Dry	5	0.0	0.0	5	1.4	0.3	5	0.4	0.4	5	0.1	0.1	3	0.7	0.3	4	2.2	1.3	5	2.3	0.7
Assulina seminulum	29.7	12.7	Dry	5	0.0	0.0	5	0.2	0.2	5	0.0	0.0	5	0.1	0.1	3	0.3	0.3	4	1.0	0.6	5	0.1	0.1

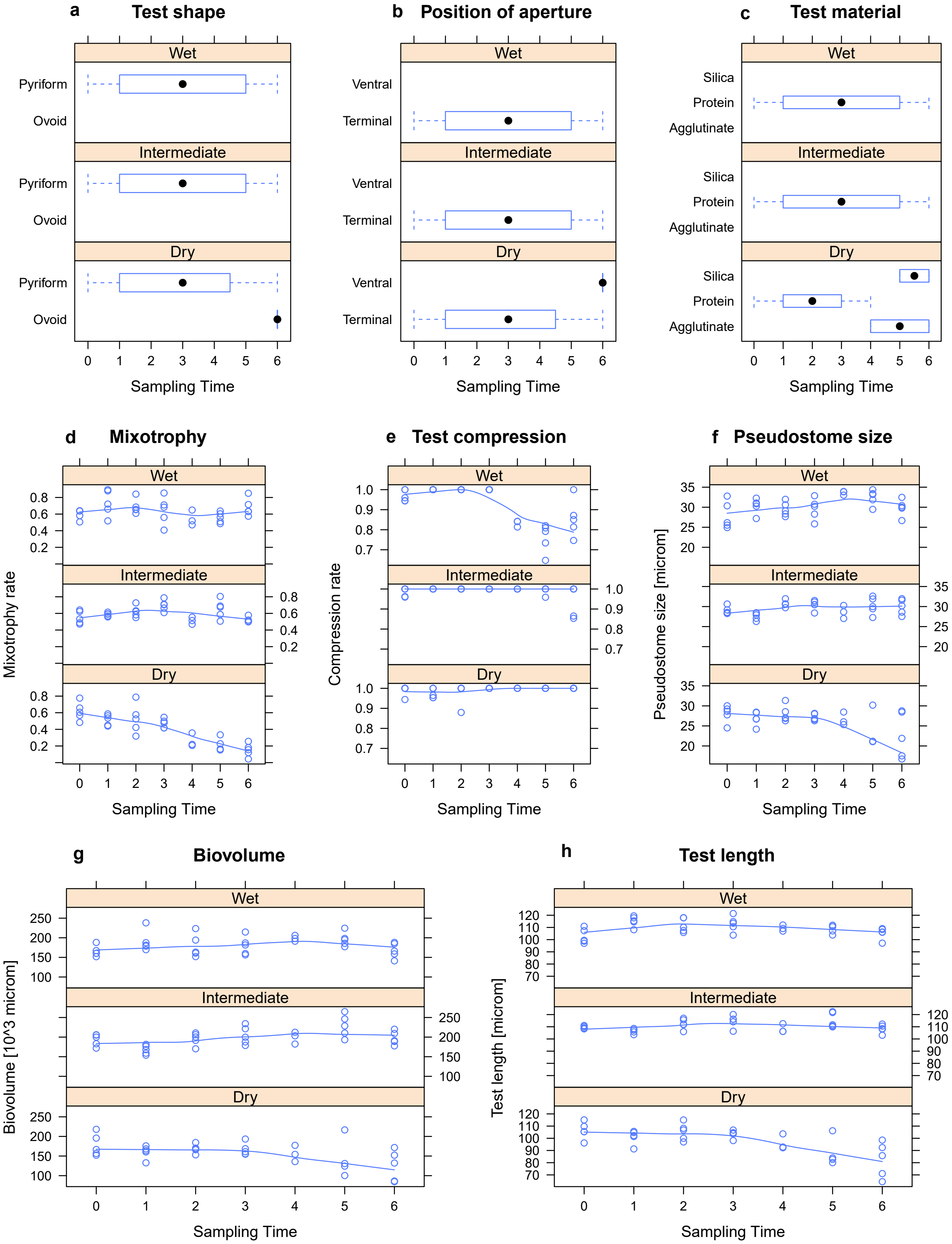


Figure 4.2 – Temporal changes in community weighted mean of traits (CWM) from testate amoeba communities in *Sphagnum fallax* mesocosms with controlled water table depth: a-c dominant traits for each treatment, d-h temporal patterns of individual traits with fitted smooth regression lines.

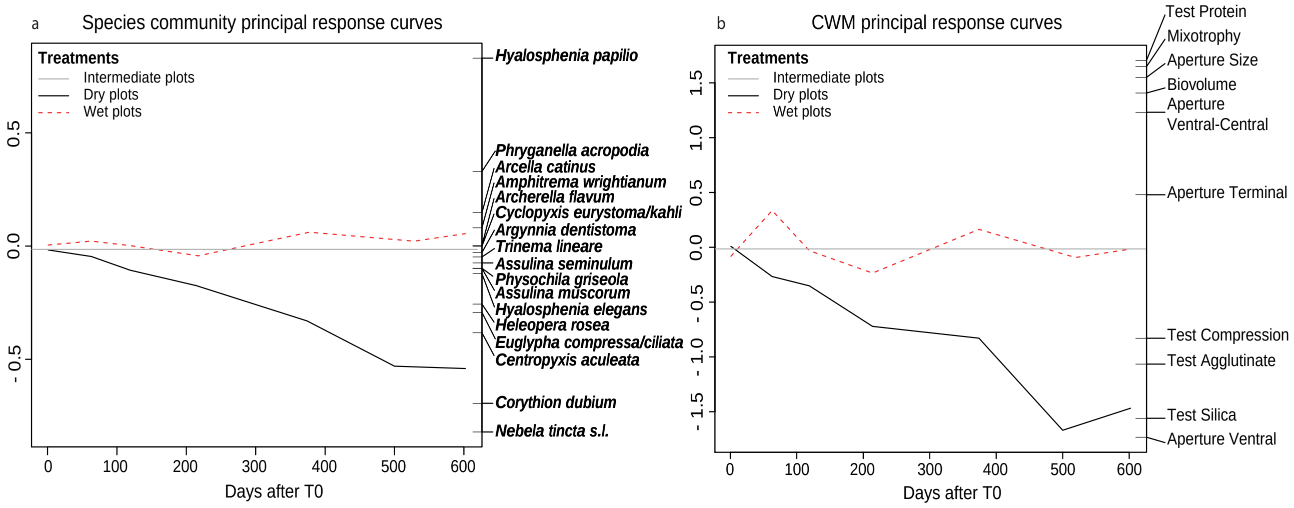


Figure 4.3 – Principal Response Curves (PRC) on testate amoeba community data from *Sphagnum fallax* mesocosms with controlled water table depth for (a) the species (Hellinger transformed), and (b) the CWM of traits (Gower distance and standardized) as response, and the treatment (WTD) and time as explanatory variables. Intermediate water level plots were defined as "control" treatment, the two other lines represented the variation of communities in comparison with intermediate plots. The measured water table depth (WTD) from the top of the *Sphagnum* layer in the wet, intermediate and dry treatments were respectively -4cm, -15cm, and -25cm.

cies PRC was 14.2, 47.8 and 38.1, respectively, while these fractions in the CWM PRC were 17.8, 43.2 and 38.9 (Figure 4.3). Testate amoeba communities do not diverge significantly between the intermediate and wet plots (Figure 4.3a), both being dominated by *Hyalosphenia papilio*. By contrast, the communities in dry plots gradually diverged over time mainly due to the replacement of *H. papilio* species from the *Nebela tincta* complex and *Corythion dubium*.

The PRC based on community weighted mean of traits (Figure 4.3b), shows a very similar pattern, but with more fluctuation. No clear difference is observed between the wet and intermediate plots. The dry plots diverged rapidly from the other two treatments. The traits positively correlated to the change were ventral aperture, more compressed agglutinate or silica made test, small shell and aperture sizes. By contrast, mixotrophy, test made of protein, and larger species characterized the wet and intermediate plots.

4.4 Discussion

We investigated the response of testate amoeba communities to experimentally-manipulated water level by comparing two approaches: community composition and functional composition responses. The two approaches showed similar response patterns, with water level acting as a strong environmental filter.

4.4.1 Effect of the treatment on community composition and inferred water table depth

The transfer function allowed us to assess how well the theoretical water table depth inferred from the testate amoeba community structure in the mesocosms matched the observed water table. The principal response curves showed the general patterns of response and allowed identifying the most responsive taxa.

At the beginning of the experiment, communities were largely dominated by *Hyalosphenia papilio*. The optimal water level for *H. papilio* as calculated in the transfer function was $-16\text{cm} \pm 10\text{cm}$ suggesting that intermediate plots (-15cm) were best suitable for this species and that dry and wet plots would be less – but both equally so – favourable to this species. This is in line with previous studies that have regularly identified *H. papilio* as an indicator of moderately moist conditions (Booth and Meyers, 2010; Lamentowicz and Mitchell, 2005). However, the theoretical optimum water table for this species may be lower than the true optimum because calculations were based on one-off water table measurements taken in summer (Mitchell et al., 1999), as in most transfer function studies (but see Sullivan and Booth, 2011), while the average water table over the growing season is most likely higher due to higher rainfall and/or lower evaporation in spring and autumn. In support to this view, the proportion of *H. papilio* found at the beginning and at the end of the experiment was exactly the same in wet plots (84.4%) and only slightly lower (but not significantly so) in intermediate plots (T0: 86.4%, T6: 83.9%). This indeed suggests that the optimum average water level for this species is between -4 and -15 cm.

The lack of contrast between the intermediate and wet treatments as shown in the PRC suggests that wet indicators failed to become established in the wet plots. This could be due to several causes. As mesocosms were isolated from natural peatlands, colonisation did not compensate for local species extinction and potentially more competitive species absent at the beginning of the experiment could not reach the plots. Aiming to overcoming this bias, we seeded all plots with "bog microbial cocktail" (i.e. a mixture of water extracted from pools, lawns and hummocks), with the hope that both dry and wet indicator taxa would develop in the corresponding treatments. However, despite adding this "bog microbial cocktail", wet indicator species were either too rare to be detected at the beginning of the experiment (e.g. *Amphitrema wrightianum*, *Physochila griseola*), or were observed in low density and then rapidly declined (e.g. *Archerella flavum*, *Cyclopyxis eurystoma/kahli*). Therefore, well-established species such as *H. papilio* could persist over time even in potentially suboptimal conditions (Fournier et al., 2016; Holyoak et al., 2005; Leibold et al., 2004). Obviously, this bog microbial cocktail addition did not suffice to provide viable populations of species from the full range of micro-environmental conditions. The reason may be that bog pool taxa are especially sensitive to disturbance and did not survive long-enough to build large populations, especially given the strong dominance of *H. papilio.* This should be further tested but if true would suggest that these wet indicators are indeed of especially high bioindication value for peatland conservation.

By contrast to wet indicators, all dry indicator taxa were detectable at the beginning of the experiment, although at very low abundance. It is therefore not surprising that the effect of water level depth on testate amoeba communities, as reflected by the inferred water table as well as the PRC, was stronger and more rapid in dry plots. The mean inferred water level in dry plots decreased progressively and reached the experimental water table depth at T5. This shift was primarily due to a clear decrease of *H. papilio* and an increase in abundance of species characteristic for drier situations such as *Nebela tincta* s.l., *Corythion dubium* and *Euglypha compressa/ciliata* (Bobrov et al., 2002; Booth, 2001; Lamentowicz and Mitchell, 2005; Mieczan, 2009). With a water level of $-25\mathrm{cm}$ still within its theoretical range of tolerance ($16\pm10\mathrm{cm}$), *H. papilio* maintained its population until T3, before decreasing abruptly in favour of *Nebela tincta* first and then (after T4) *Corythion dubium.* These two species together accounted for more than half of the testate amoeba community at T5 and T6. Therefore, even in suboptimal conditions, *H. papilio* dominated the community structure during eight months (including one winter) before better-adapted taxa were able to take over at the beginning of the second growing season.

In our experiment, the response to the low water level treatment was fast: a clear response was already visible at T1, two months after T0. It is possible that microbial communities responded more rapidly to the lowered water level than in a natural site because the structure of the mesocosms did not buffer water table drawdown as would happen in natural peatland. If true, the effect of a similar drainage in natural peatland would be slower. Further experiments *in situ* would be needed to test this. Nevertheless, the dataset used to calibrate the transfer function was taken in the Jura Mountains, including the two peatlands where the *Sphagnum* carpets (Creux de l'Epral, Jura, 990 m a.s.l.) and the "bog microbial cocktail" (Le Cachot, Neuchâtel, 1050 m a.s.l.) were collected (Mitchell et al., 1999; Mulot et al., 2015). Accordingly, the shift in the community structure observed in the dry treatment is coherent with natural communities observed in drier microsites in *Sphagnum* peatland of the region (Mitchell et al., 1999).

The observed change in testate amoeba community structure could be related to the growth of both bacteria and small micro eukaryotes stimulated by oxygenation of the peat (Bragazza et al., 2016; Jassey et al., 2013b). This could explain the shift in testate amoeba community towards species belonging to another food web level (i.e. bacterivores). Such a community shift has been interpreted as being indicative for a change in C cycling at the surface of *Sphagnum* peatlands leading to the release of C to the atmosphere and faster nutrient cycling (Gilbert et al., 2003; Jassey et al., 2013a, 2012). The water level in the dry plots, ca. $-25\mathrm{cm}$, is indeed known to strongly disturb peatland functioning (Dieleman et al., 2014; Jassey et al., 2013a; Mitsch and Gosselink, 2007) and peatland restoration generally aims to raise the water table above this level (Quinty and Rochefort, 2003).

4.4.2 Environmental filtering and functional traits

Our experimental design allowed us to investigate the environmental filtering effect of water table on testate amoeba functional traits. High water level selected for larger species, and favoured mixotrophs (Figure 4.3), as expected (Jassey et al., 2012, 2015). Mixotrophic testate amoeba represent up to 70% of the microbial biomass in *Sphagnum* peatlands (Jassey et al., 2013b) and were shown to contribute to C sequestration in peatlands (Jassey et al., 2015). Mixotrophy is a feeding strategy well adapted for oligotrophic aquatic conditions that characterize the pools of *Sphagnum* peatlands (Gomaa et al., 2014; Jassey et al., 2013a). Mixotrophy was thus expected to be favoured by high water level.

In our study, mixotrophy was associated with test made of protein (i.e. *H. papilio*, *Archerella flavum*). Proteinaceous tests are translucent, allowing sunlight to reach photosymbionts. Test secretion is still a poorly known process (Netzel, 1983; Nomura et al., 2014) but it is likely that this requires more energy and thus a higher metabolic activity than building an agglutinated test using material from the environment. The relationship between mixotrophy and size is hence logical, as biovolume is correlated with metabolism (Makarieva et al., 2008) and the number of mitochondria (Kosakyan et al., 2015). Biovolume is positively correlated with water level and thus prey size can be expected to increase under wetter conditions. This in turn would explain the selection in wet mesocosms of testate amoeba species with larger pseudostome which were shown to occupy higher tropic levels as inferred from carbon and nitrogen stable isotope analyses (Jassey et al., 2013b, 2012). The drawback is that larger species are dependent on constant moisture to survive, be it due to their dependency on specific prey or to their likely lower physiological tolerance to drought. It should however be noted that not all peatland mixotrophic testate amoebae build proteinaceous shells (e.g. *Amphitrema* ssp., *Heleopera sphagni*, *Placocista spinosa*) and *Amphitrematidae* have small apertures.

Ventral (plagiostome) or hidden (cryptostome) apertures as well as test compression, are considered as adaptations to drought, allowing testate amoebae to remain active even in a thinner water film (Fournier et al., 2015; Gilbert et al., 2003; Lamentowicz et al., 2015). In support for this view, in dry plots ventral aperture emerged as a significant trait at T6. Furthermore, community structure shifted towards the dominance of silica and agglutinate test composition, a common trait in mineral soils (Seppey et al., 2016; Szelecz et al., 2014; Tsyganov et al., 2012). On the contrary, in wet plots, where a compressed test does not represent a specific advantage, the proportion of species with a compressed test declined.

4.4.3 Future use of functional traits in ecological studies of testate amoebae

As highlighted by the PRC, functional traits are intrinsically more ecologically informative than the identity of the corresponding species to interpret the functioning and the evolution of our system. Taxonomy-based analyses could be comparatively more difficult to interpret in relation to ecological processes. Indeed, selected traits relate directly to the ecology and the abilities of the investigated species, and provide a finer and more consistent signal than the community composition. As misidentification currently represents a limitation of ecological and palaeoecological studies of testate amoebae (Heger et al., 2009; Mitchell et al., 2014; Payne et al., 2011), although some information is lost using functional traits, this approach can inform more directly about the status of an ecosystem (Fournier et al., 2015, 2012).

An additional advantage of the traits approach is that it allowed overcoming biogeographical limitations. Indeed, as testate amoebae are increasingly being shown to have limited geographical distribution (Smith et al., 2008; Swindles et al., 2014), a strict taxonomical approach would lead to high community turnover along geographical gradients that may not correspond to changes in functioning. Using a functional traits approach allows focusing specifically on the functionally relevant signal in the community data. Our aim was to assess the relevance of functional diversity for understanding environmental filtering. An advantage of the trait approach is that non-specialists of testate amoeba taxonomy may use the functional response based on morphologic characterization of the observed specimen or a relatively crude morphotype approach. Functional diversity analysis is therefore both practical and informative.

4.5 Conclusion

We studied the response of testate amoeba communities to experimentally manipulated water table, with the aim to better understand the impact of climatic changes on *Sphagnum* peatlands. The response of testate amoeba communities to experimentally manipulated water table depth was in line with the known ecological preferences of species documented in observational studies and coherent with the impacts on vegetation and decomposition.

The shift in selected traits also reflect the change in humidity content of the top-layer *Sphagnum* (not quantified but clear from visual observations). The reduced dominance of mixotrophic species and the shift to smaller taxa suggests functional changes in communities with an alteration of the microbial food web (i.e. increase in bacterivory vs. eukaryvory) and impact on C cycling (i.e. faster C turnover and reduced C fixation by mixotrophs). This is in line with recent evidence for a key role played by mixotrophic testate amoebae in the functioning of *Sphagnum* peatlands (Jassey et al., 2015). As these changes are relatively easy to measure, using well selected functional traits on testate amoeba community could give peatlands managers practical and resource-efficient tools to monitor the evolution or recovery of protected peatlands. The next steps will be to further experiment the effects of water table changes (e.g. drought followed by a recovery), combining mesocosms experiments and field studies and to further assess how selected response and effect traits can inform on the evolution of natural or semi-natural ecosystems facing environmental changes.

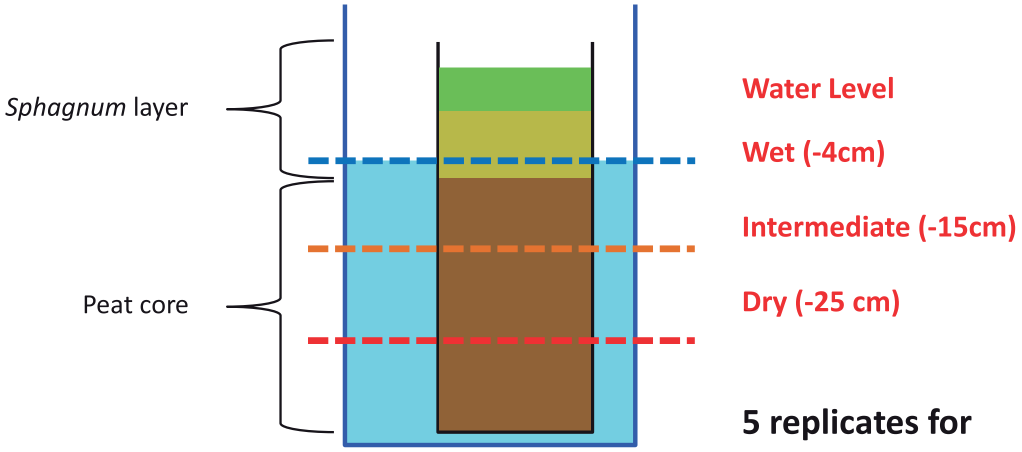
4.6 Acknowledgements

This project was supported by grant N° PSPB-013/2010 from Switzerland through the Swiss Contribution to the enlarged European Union and additional funds from the University of Neuchâtel.

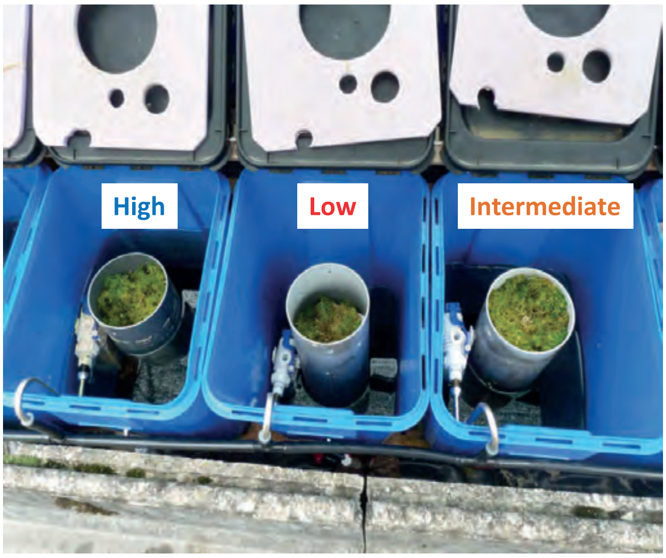
Appendix

Experimental design

Outline of one mesocosm :



5 replicates for each treatment



Change in *Sphagnum* carpet after one year of experiment

Chapter 5

Can *Sphagnum* testate amoeba communities recover from drainage? Results from a three year mesocosm experiment.

Isabelle Koenig[a], Florence Schwendener[a], Matthieu Mulot[a,c], Edward A. D. Mitchell[a,b]

Abstract

Sphagnum peatlands represent a globally significant pool and sink of carbon but these functions are threatened by ongoing climate change. Testate amoebae are useful bioindicators of hydrological changes. Existing inference models are based only on observational studies and very little experimental work has been done on the impact of water table changes on communities.

We used a mesocosm experimental setting to assess first during a "disturbance phase" the impact of three contrasted water table position simulating wet (−4cm), intermediate (−15cm) and dry (−25cm "stress") conditions and subsequently during a "recovery phase" at −10cm for all plots. The experiment lasted a total of three years. We assessed the response of testate amoeba communities using taxonomic and functional trait approaches (community weighted means of traits – CWM). The selected functional traits were hypothesised to be correlated to moisture content (response traits: shell size, aperture position) or trophic role (effect traits: mixotrophy, aperture size controlling prey range).

The mixotrophic species *Hyalosphenia papilio* dominated the wet and intermediate plots during the disturbance phase. In dry plots, the community shifted to a dominance of "dry indicators" (*Corythion dubium*, *Nebela tincta*, *Cryptodifflugia oviformis*) and with a CWM of traits corresponding to adaptation to thin water films (loss of mixotrophy, and dominance of smaller taxa with ventral or ventral-central aperture). During the recovery phase, communities in some wet and intermediate plots shifted either towards a more balanced community with some indicators – and associated traits – of drier conditions while other wet and intermediate plots retained similar communities, the difference between these two groups being reflected by the macroscopic characteristics of the *Sphagnum* carpet (better preserved in the second group). In the former dry plots, indicators and traits of wet conditions increased by the end of the experiment.

This is the first experiment simulating a disturbance and subsequent recovery in *Sphagnum* peatlands and focusing on the response of testate amoebae. The results generally confirmed that testate amoebae respond within a few months to hydrological changes and thus represent useful biomonitors for assessing current and past hydrological changes in *Sphagnum* peatlands.

Keywords: Mesocosm experiment, testate amoebae, functional traits, recovery, disturbance, *Sphagnum* peatlands, bio-indicators, water table depth.

[a] Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland

[b] Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

[c] Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 7144, Adaptation et Diversité en Milieu Marin, Equipe EPEP, Station Biologique de Roscoff, F-29680 Roscoff, France

Acta Protozoologica, accepted

5.1 Introduction

The increasing influence of human activities on the biosphere makes it necessary to assess how direct and indirect impacts are affecting ecosystems (MEA, 2005). Biomonitoring is an efficient approach for evaluating environmental health in general and specifically the impact of perturbation but also ecosystem restoration (MEA, 2005; Bonnett et al., 2011; Bonn et al., 2016; Norris et al., 2011; Church et al., 2014). Bioindicator taxa must be sensitive enough to rapidly react to environmental changes, and have measurable characteristics (e.g. community composition, morphology, or function) that are correlated to relevant processes (e.g. C sequestration Niemi and McDonald, 2004; Payne, 2013). Protists play essential functional roles and are useful bioindicators in aquatic as well as terrestrial ecosystems (Caron et al., 2009; Wilkinson and Mitchell, 2010; Norris et al., 2011; Pinto et al., 2014). Our focus here is on the use of testate amoebae living in *Sphagnum* peatlands as indicators of hydrological changes.

Testate amoebae are a polyphyletic group of unicellular protists building a shell, named "test". Although they are generally studied as a homogeneous ecological and functional group, they belong to three phylogenetic groups, each of which has specific morphological traits: i) Arcellinida (Kosakyan et al., 2016), with "lobose" (wide) pseudopods, ii) Euglyphida, (Cavalier-Smith and Chao, 2003) with "filose" (narrow) pseudopods and (for most group) a test produced with self-secreted silica scales (idiosomes), both groups with a single pseudostome (aperture) to the test and iii) Amphitremidae (Gomaa et al., 2013) with "filose" pseudopods and two pseudostomes. Some taxa (in all three groups) possess photosynthetic endosymbionts (i.e. they are mixotrophic). Identification is based on test characteristics, thus allowing the use of this group in palaeoecological studies (Charman, 2001). Tests are made of self-secreted protein, self-secreted silica plates (idiosomes) or of agglutination (xenosomes) of either prey [diatom frustules, euglyphid plates, i.e. kleptoplastidy –][] (Lahr et al., 2015) or mineral particles present locally (Ogden and Hedley, 1980; Delaine et al., 2017). Testate amoebae are a dominant group of micro-organisms in wet and humid environments (Gilbert et al., 1998; Gilbert and Mitchell, 2006) and play a central important role in soil microbial food webs and C cycling in *Sphagnum* peatlands (Wilkinson and Mitchell, 2010; Jassey et al., 2015). Their community structure is well correlated with key environmental gradients such as humidity (generally measured as water table depth), pH, or nutrient richness (Mitchell et al., 2008b).

Recently, the functional diversity framework has been applied to testate amoebae and results are promising for an application in biomonitoring and paleoenvironmental reconstruction (Fournier et al., 2012, 2015; Galka et al., 2013; Kajukało et al., 2016; van Bellen et al., 2016). Selected morphological and physiological traits were shown to be linked to processes, such as ecosystem recovery following perturbation (wetlands, floodplain, Fournier et al., 2012). As functional traits are directly linked to ongoing processes, they could be used to monitor ecosystem functioning and give information of ecological niche dimensions (Mouillot et al., 2007; Holt, 2009; Kearney et al., 2010). This approach has been developed initially for plants, macro-invertebrates and fishes and was more recently applied to micro-eukaryotes and especially testate amoebae (Fournier et al., 2012, 2015; Arrieira et al., 2015; Kajukało et al., 2016; Marcisz et al., 2016).

To be relevant, functional traits must be related to known ecological processes and must be measurable at the species level (Violle et al., 2007; Mlambo, 2014). In practice, traits are most often selected based on correlations revealed in observational ecological studies. Although such studies are quite convincing, they should ideally be tested further using controlled experiments to link traits to a specific environmental change. Mesocosm experiments provide the necessary level of control to assess how selected traits respond to manipulation, while other variables are kept (approximately) constant in the different treatments (Benton et al., 2007).

We set up a mesocosm experiment to simulate the effect of water table changes on *Sphagnum*-peatlands (Mulot et al., 2015). Over twenty months (August 2012 to March 2014), we followed the response of testate amoeba community structure to three water levels: dry (−25cm from the moss surface = D), intermediate (−15cm = I) and wet (−4cm = W). We assessed the changes in taxonomic and functional diversities (Koenig et al. submitted). At the end of the experiment, the mesocosms were kept in operation, providing the opportunity for further experimentation. In March 2015, all water levels were set at −10cm to monitor the resilience of testate amoeba communities. Samples were collected and analysed in May and September 2015 to assess: 1) How the testate amoeba communities will recover poststress and 2) How similar the taxonomic and functional trait responses will be.

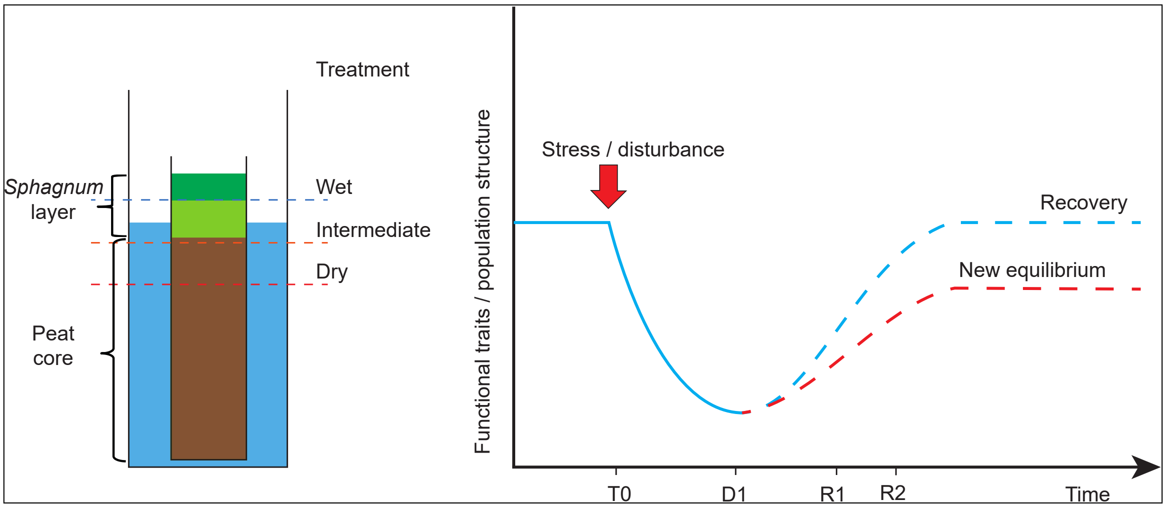


Figure 5.1 – Left: schematic cross-section through a mesocosm showing the peat and *Sphagnum* layer. Right: expected evolution of community structure or traits over the stress and recovery phases. At the onset of the experiment (T0) the community included the whole range of species living in pool, lawn and hummock, taken in a natural *Sphagnum* peatland. D1 is the point of maximum disturbance effect and R1 and R2 are sampling points during the recovery phase. Dotted lines represented the possible evolution of both species community structure and community weighted mean of functional traits in response to disturbance. Full recovery depended on the survival potential of species. The new equilibrium represents the situation when the local conditions or present species pool do not allow a full recovery of the original state. In our case this is due to the fact that some species are likely to be lost during the disturbance phase.

5.2 Material and Methods

5.2.1 Experimental design

Mesocosms were constituted of tanks filled with water (Figure 5.1). In each tank a pierced PVC tube (45cm high, 12cm diameter) was filled with a peat core topped with a carpet of *Sphagnum fallax* (Mulot et al., 2015). The system allowed manipulating the water level independently in each mesocosm. The *Sphagnum* carpets were collected simultaneously on the same *Sphagnum* patch, in the Creux de l'Epral peatland, Canton du Jura (47°12′18.3″N; 6°56′5.83″E; elevation: 990m a.s.l.), and were seeded with a water extract from pool, hummock and lawn to provide the full community potential at the beginning of experiment, 02/08/2012 (T0) (Mulot et al., 2015). The water table was set at −4cm (wet treatment), −15cm (intermediate treatment) and −25cm (dry treatment), with five replicates of each treatment. The disturbance effect was recorded at 27/03/2014 (D1). One year later (27/03/2015) water level was set at −10cm in all mesocosms (i.e. beginning of recovery phase) and we monitored the changes in the testate amoeba community twice during the growing season. Samplings were made on 18/05/2015 (R1) and 24/09/2015 (R2, Figure 5.1). At each sampling date, we collected the top three centimetres of *Sphagnum* stems. Sample codes indicate treatment, replicate and sampling date, e.g. D4_R2 corresponds dry treatment, replicate 4 sampled on 24/09/2015.

5.2.2 Testate amoeba isolation and characterization

Testate amoebae were extracted by sieving and back-sieving through mesh filters (15 - 200μm, Booth et al., 2010; Jassey et al., 2011). We aimed for minimum counts of 100 individuals per sample alive and dead pooled together. In some plots, testate amoeba density was very low and less than 50 individuals were counted (D2_D1: 47; D4_D1: 29; W2_D1: 28; W2_R1: 17). Identification was made following Charman et al. (2004) and Mitchell (2003)'s keys at the species or species complex level. Some taxa were grouped into broader morphological groups, in particular taxa from the *Nebela tincta* group (Kosakyan et al., 2013). *Cyclopyxis arcelloides* includes *Cyclopyxis kahli* (found only in one sample at R2) and *C. eurystoma*. *Heleopera sylvatica* includes *H. petricola*. To reduce the impact of rare species, taxa that do not exceed a mean of 3% in one treatment at one sampling time (n=5) were discarded (*Argynnia dentistoma*, *Assulina seminulum*, *Bullinularia indica*, *Cryptodifflugia sacculus*, *Heleopera sphagni*, *Hyalosphenia elegans*, *Plagiopyxis labiata*, *Sphenoderia lenta*, *Trigonopyxis arcula*, *Trinema complanatum*, *Wailesella eboracencis*) before carrying out any further analyses.

Table 5.1 – Description, ecological meaning and references for the six functional traits selected for testate amoeba taxa.

	Unit	Description	Type of trait	Ecological meaning	References
Biovolume	μm^3	Volume of shell (90% occupied by the living amoeba)	Response	Related to the metabolic rate and the capacity of the food web to process energy.	Fournier et al. (2012) Laggoun-Defarge et al. (2008) Makarieva et al. (2008)
Aperture position	Factor	From a terminal aperture to the completely central one.	Response	Represents the ability to survive in thin water films and thus the ability to remain active and contribute to the food web in dry conditions.	Fournier et al. (2012) Lamentowicz et al. (2015)
Test material	Factor	Protein made, Idiosomes (secreted bio-siliceous plates), Xenosomes (built with particles collected in environment: organic debris, diatom frustules, mineral particles)	Response	Availability of material and / or prey to construct the test. Source of material appear to be a major regulator of abundance and repartition of testate amoeba along Sphagnum parts.	Gilbert and Mitchell (2006)
Test compression	Binary	0: not compressed 1: compressed	Response	Survival potential in drier situations and thus potential contribution to the food web in dry conditions.	Fournier et al. (2012)
Mixotrophy	Binary	Presence (1) or not (0) of photosynthetic endosymbionts	Effect	Mixotrophy is a key factor in oligotrophic conditions and plays a role in peatland C cycling.	Fournier et al. (2015) Jassey et al. (2015, 2011)
Aperture size	µm	Width of the shell aperture	Effect	Related to prey size and food web functioning	Jassey et al. (2013a)

5.2.3 Functional traits

Traits chosen to assess the impact of water level on community structure followed the requirements of functional diversity (Table 5.1, Lavorel and Garnier, 2002; Messier et al., 2010; Mlambo, 2014). Test compression, an aperture in ventral position and small biovolume allow amoebae to stay active in thin water film (Laggoun-Defarge et al., 2008; Fournier et al., 2012; Tsyganov et al., 2012). Mixotrophy is believed to be an adaptation to low nutrient availability (Fournier et al., 2012; Jassey et al., 2013a) while test material informs on the abundance of mineral particles or prey incorporated in test construction (Gilbert et al., 2003; Gilbert and Mitchell, 2006; Jassey et al., 2011; Schwind et al., 2016). Finally, aperture size was demonstrated to be correlated to prey size and thus the trophic level, small apertures corresponding to microbial feeder and large apertures to predator of micro-eukaryotes and micro-metazoan (Jassey et al., 2013a; Gomaa et al., 2014). The morphological dimensions were measured directly at 400x magnification using an inverted IX-81 Olympus microscope, and the *Olympus cellSens dimension* software. Biovolume was calculated according to the general shape of the shell, applying the formulas of Fournier et al. (2015).

5.2.4 Numerical analyses

Gradient analyses

The structure and temporal changes of the testate amoeba communities in the three treatments was first assessed based on the relative abundance of species over sampling time. Species were classified according to their optimal water table depth calculated with a transfer function (weighted averaging regression) based on an independent dataset (Mitchell et al., 1999).

We then computed the community weighted mean (CWM) of selected traits (biovolume, aperture position, test compression, test composition, mixotrophy and aperture size) in each sample. CWM represented the average of each trait value weighted by the relative abundance of each species (Dray and Legendre, 2008; Ricotta and Moretti, 2011).

We analysed the temporal patterns of community structure and community weighted mean of functional traits using principal component analyses (PCA) in which we projected passively treatment and time. Prior to PCA, the species dataset was Hellinger transformed according to Legendre and Gallagher (2001) and the CWM of traits dataset was standardised. Permutational multivariate analysis of variance was used to assess the difference in testate amoeba community structure between sampling times for each treatment separately. This analysis partitions dissimilarities between groups and tests the significance of those partitioning (Anderson, 2001; Oksanen, 2015). We used a Bray-Curtis distance matrix of relative abundance of species with 999 permutations and analysed each treatment separately. The significance between sampling time for community weighted mean of traits was tested by analyses of variance with random effect and Tukey multiple comparisons of mean (Bonferroni correction), for each treatment separately.

Null models for functional diversity

To disentangle habitat selection and biotic interaction, recent studies suggested the use of null models to test the assembly processes between species community structure, phylogenetical dataset and functional traits (Webb et al., 2006; Cavender-Bares et al., 2009; Mason et al., 2008; Chase et al., 2011). These methods are applicable also with species abundance instead of phylogenetical communities. The standardized effect size of mean pairwise distance (ses.mpd) compare the observed distance separating samples based on species community structure in relation with the pool of functional traits, and the same distance based on a random community matrix with respect to the specific richness of the original community and the original traits matrix (Kembel, 2010). The standardized effect size is calculated by:

$$\text{ses.mpd} = \frac{\text{mean}(M_{\text{null}}) - M_{\text{observed}}}{\text{sd}(M_{\text{null}})} \tag{5.1}$$

where M_{null} = randomized mean pairwise distance, M_{observed} = observed pairwise distance, $\text{sd}(M_{\text{null}})$ = standard deviation of randomized pairwise distance (Kembel, 2010). When ses.mpd is significantly lower than expected by chance, the main drivers of community structure are related to habitat filtering; when it is significantly higher, biotic factors are more likely to be the driver. As the traits matrix included binary variables, semi-quantitative factors and quantitative traits, the Gower distance was applied, using the method “Podani” for ordinal variables (Gower, 1971; Podani, 1999).

All analyses were carried out with the R statistical software (R Core Team, 2016). Ordinations were computed using the package vegan (Oksanen, 2015). The community weighted mean of functional traits was calculated using the package FD (Laliberté et al., 2014). Standardized effect size and null model were computed with package Picante (Kembel et al., 2010) and optima and tolerance of species were calculated with the package rioja (Juggins, 2015).

5.3 Results

5.3.1 Testate amoeba community composition and treatment effects on community structure

Hyalosphenia papilio was the only taxon found in all samples and the most abundant overall, accounting for over half for the community on average and a median abundance of 72.9%. *Corythion dubium*, *Nebela tincta* s.l., *Assulina muscorum*, *Phryganella acropodia*, *Centropyxis aculeata*, *Arcella catinus*, *Heleopera rosea*, and *Euglypha ciliata* each contributed $>$ 2% of the overall community and occurred in $>$ 45% of all samples (Table 5.2). Eighteen other morphotaxa were less abundant and frequent (Table 5.2).

The three treatments had contrasted effects on the relative abundance of individual taxa (Table 5.3). In wet and intermediate treatments, the abundance of *H. papilio* decreased while *A. muscorum* and *P. acropodia* increased. Specific richness did not change significantly in these two treatments. In dry plots, the situation was different (Table 5.3). By R1, *H. papilio* declined sharply (to 6.7% on average) and the community was dominated by *C. dubium*, *N. tincta*, and *Cryptodifflugia oviformis*. By R2, *H. papilio* began to recover regaining dominance (23.6%) and the community was co-dominated by *N. tincta* (15.2%), *H. rosea* (12.2%), *Physochila griseola*, *Cyclopyxis arcelloides*, *C. aculeata* and *A. muscorum* ($7.8 - 8.6$%). This was the most balanced community in the entire experiment. The specific richness in dry plots increased significantly from 5.2 taxa at T0 to 10.2 taxa at R1 (ANOVA p-adjusted T0-R1: 0.002) and 10.6 taxa at R2 (ANOVA p-adjusted

Table 5.2 – List of testate amoeba taxa, number and relative frequency of occurrence, mean, standard error (se), median, minimum and maximum relative abundance. Taxa are ordered by mean relative abundance over the four sampling times and within all treatments ($n = 60$). Rare taxa (i.e. maximum abundance in any single treatment and time <3%) are indicated in grey and were excluded from numerical analyses.

Morphotaxa	N	n/N	mean	se	median	min	max
Hyalosphenia papilio Leidy, 1874	60	100.0%	56.6%	4.1%	72.9%	0.6%	94.7%
Corythion dubium Taranek, 1871	29	48.3%	7.1%	2.1%	0.0%	0.0%	83.4%
Nebela tincta s.l. (Leidy) sensu Kosadyan and Lara, 2012	41	68.3%	7.0%	1.3%	2.2%	0.0%	41.7%
Assulina muscorum Greeff, 1888	39	65.0%	6.7%	1.7%	1.2%	0.0%	70.0%
Phryganella acropodia (Hertwig & Lesser, 1874) Hopkinson, 1909	28	46.7%	4.1%	1.0%	0.0%	0.0%	33.3%
Centropyxis aculeata (Ehrenberg, 1838)	36	60.0%	3.0%	0.8%	0.6%	0.0%	30.8%
Arcella catinus Penard, 1890	44	73.3%	2.9%	0.4%	1.7%	0.0%	10.9%
Heleopera rosea Penard, 1890	32	53.3%	2.7%	0.7%	0.6%	0.0%	25.2%
Euglypha ciliata type	37	61.7%	2.0%	0.4%	0.7%	0.0%	13.7%
Cryptodifflugia oviformis Penard, 1902	5	8.3%	1.4%	0.7%	0.0%	0.0%	31.6%
Physochila griseola (Wailes & Penard, 1911)	13	21.7%	1.2%	0.4%	0.0%	0.0%	17.6%
Cyclopyxis arcelloides (Deflandre, 1929)	13	21.7%	1.0%	0.6%	0.0%	0.0%	37.7%
Archerella flavum (Archer, 1877) Loeblich and Tappan, 1961	10	16.7%	0.9%	0.4%	0.0%	0.0%	16.4%
Amphitrema wrightianum Archer, 1869	11	18.3%	0.8%	0.3%	0.0%	0.0%	11.1%
Heleopera sylvatica Penard, 1890	14	23.3%	0.6%	0.2%	0.0%	0.0%	8.9%
Pseudodifflugia gracilis Schlumberger, 1845	12	20.0%	0.5%	0.2%	0.0%	0.0%	5.9%
Hyalosphenia elegans Leidy, 1874	22	36.7%	0.5%	0.1%	0.0%	0.0%	5.1%
Heleopera sphagni Leidy, 1874	11	18.3%	0.5%	0.2%	0.0%	0.0%	9.6%
Trinema complanatum Penard, 1890	5	8.3%	0.2%	0.1%	0.0%	0.0%	7.4%
Assulina seminulum (Ehrenberg, 1848)	8	13.3%	0.1%	0.0%	0.0%	0.0%	0.9%
Sphenoderia lenta Schlumberger, 1845	3	5.0%	0.1%	0.0%	0.0%	0.0%	1.8%
Bullinularia indica (Penard, 1907)	3	5.0%	0.1%	0.0%	0.0%	0.0%	2.1%
Plagiopyxis labiata Penard, 1910	2	3.3%	0.0%	0.0%	0.0%	0.0%	1.6%
Wailesella eboracencis (Wailes & Penard, 1911)	1	1.7%	0.0%	0.0%	0.0%	0.0%	2.4%
Cryptodifflugia sacculus Penard, 1902	1	1.7%	0.0%	0.0%	0.0%	0.0%	1.3%
Argynnia dentistoma (Penard, 1890)	1	1.7%	0.0%	0.0%	0.0%	0.0%	0.6%

T0-R2: 0.002). Within treatment, the community structure changed significantly over the duration of the whole experiment (multivariate analyse of variance, dry plots R2: 58.5%, p-value 0.001; intermediate plots R2: 40.1%, p-value 0.001; wet plots R2: 36.8%, p-value 0.016, Table 5.4).

The principal component analysis (PCA) based on species data (Figure 5.2a) further illustrated these patterns: At T0 communities were strongly dominated by *H. papilio*, and not significantly different among treatments. Communities in the intermediate and wet treatments shifted from T0 to D1 with increasing representation of *P. acropodia* and *A. muscorum* (bottom right quadrat of the ordination diagram). At the start of the recovery period, the community structure diverged between two groups of samples. The communities of six samples (I1, I2, I4, I5, W1, W3) was still dominated by *H. papilio*, but to a lesser extent, together with *A. muscorum* and *P. acropodia*, during the whole recovery period. The last four samples (I3, W2, W4, W5, labelled on the PCA) diverged towards a community composition dominated by *A. muscorum* and *C. dubium* and with the quasi disappearance of *H. papilio* (centre of the diagram); the communities in these plots were more similar to those of dry plots at D1 and R1. W2 should be interpreted very carefully at R1 as only 18 individuals were counted, but the overall community structure recovered in density at R2 (117 individuals counted). In dry plots, the community structure diverged from the wet and intermediate plots (left of the diagram). At D1, it was dominated by *N. tincta*, *H. rosea*, and *C. dubium* and had further diverged by R1 with a highest relative abundance of *C. dubium*. By R2, community structure was again similar to that of D1.

5.3.2 Treatment effects on testate amoeba functional traits

The first two axes of the PCA based on community weighted mean (CWM) of functional traits (Figure 5.2b) explained over 80% of the variance in the data (axis 1 = 59.6%, axis 2 = 20.7%). As axis 2 was significant based on the Kaiser-Guttmann rule (Kaiser, 1991) but just not significant according to the broken stick model (MacArthur, 1957), we decided to interpret both axes, as broken stick models often underestimates the significance (Cangelosi and Goriely, 2007). The three treatments showed contrasting temporal patterns in the CWM ordination space. At T0 all treatments were correlated to a high proportion of mixotrophic species (left of the diagram), a compressed, proteinaceous test, with a terminal aperture and a large biovolume, reflecting the dominance of *H. papilio*. In the Intermediate treatment, no strong change was observed. In the wet plots, a shift was observed towards taxa with smaller biovolume and aperture size, test built with xenosomes and idiosomes and a ventral position of the

Table 5.3 – Mean relative abundance and standard error (se, $n = 5$) and specific richness of testate amoeba taxa in *Sphagnum fallax* from a mesocosm experiment on four sampling times, details for the three treatments (dry, intermediate, and wet). T0: onset of the experiment, D1: after eight months of treatment, R1 and R2: three, and six months after maintaining the water level −10cm. Taxa are listed in increasing water table depth optimum (Opt). Grey bars in cells are proportional to the mean relative abundance of each taxa in plots. The difference in mean specific richness was only significant in the dry treatment between T0-R1 and T0-R2 (ANOVA and Tukey HSD test, in bold).

				T0		D1		R1		R2	
Morphotaxa	Opt.	Tol.	Treat.	Mean	se	Mean	se	Mean	se	Mean	se
Amphitrema wrightianum	9.4	4.1	Wet	0.0%	0.0%	3.1%	0.4%	6.1%	2.4%	0.5%	0.5%
Pseudodifflugia gracilis	9.4	7.5	Wet	0.0%	0.0%	0.0%	0.0%	0.4%	0.4%	1.0%	0.7%
Archerella flavum	10.9	8.5	Wet	4.7%	2.2%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%
Cyclopyxis arcelloides	11.1	9.2	Wet	0.6%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Physochila griseola	15.2	7.1	Wet	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.2%	0.9%
Hyalosphenia papilio	16.1	10.1	Wet	85.0%	3.9%	84.4%	3.5%	55.5%	11.2%	36.1%	14.6%
Centropyxis aculeata	17.0	7.0	Wet	0.8%	0.5%	1.7%	1.5%	1.3%	1.2%	7.1%	6.4%
Cryptodifflugia oviformis	19.5	13.2	Wet	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Euglypha ciliata	19.6	11.8	Wet	1.7%	0.5%	0.4%	0.4%	0.5%	0.4%	5.7%	2.5%
Phryganella acropodia	21.6	11.5	Wet	0.0%	0.0%	7.4%	3.0%	17.0%	5.2%	11.1%	6.0%
Heleopera rosea	22.3	6.9	Wet	1.2%	0.8%	1.0%	0.6%	0.0%	0.0%	1.9%	1.3%
Heleopera sylvatica	23.0	11.9	Wet	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.0%	0.7%
Corythion dubium	23.7	11.0	Wet	0.0%	0.0%	0.0%	0.0%	6.4%	1.8%	4.2%	2.4%
Nebela tincta s.l.	23.8	12.7	Wet	1.8%	0.6%	0.3%	0.3%	1.2%	1.2%	4.4%	3.0%
Arcella catinus	24.7	8.7	Wet	3.5%	1.4%	1.1%	0.7%	1.4%	1.1%	1.8%	0.8%
Assulina muscorum	25.5	11.4	Wet	0.7%	0.5%	0.6%	0.4%	10.2%	7.3%	22.9%	14.3%
Specific richness				6.6	0.7	4.8	0.6	5.8	0.6	8.2	1.5
Amphitrema wrightianum	9.4	4.1	Inter	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%
Pseudodifflugia gracilis	9.4	7.5	Inter	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.7%
Archerella flavum	10.9	8.5	Inter	0.0%	0.0%	0.0%	0.0%	0.3%	0.3%	1.8%	1.2%
Cyclopyxis arcelloides	11.1	9.2	Inter	0.2%	0.1%	0.0%	0.0%	0.2%	0.2%	1.4%	1.2%
Physochila griseola	15.2	7.1	Inter	0.0%	0.0%	0.2%	0.2%	0.0%	0.0%	0.9%	0.9%
Hyalosphenia papilio	16.1	10.1	Inter	87.2%	2.7%	84.2%	1.1%	68.2%	4.8%	56.2%	8.0%
Centropyxis aculeata	17.0	7.0	Inter	0.9%	0.2%	0.5%	0.2%	0.6%	0.5%	4.3%	4.3%
Cryptodifflugia oviformis	19.5	13.2	Inter	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Euglypha ciliata	19.6	11.8	Inter	0.1%	0.1%	0.5%	0.2%	0.3%	0.2%	0.4%	0.3%
Phryganella acropodia	21.6	11.5	Inter	0.0%	0.0%	2.6%	1.6%	5.3%	3.1%	6.1%	3.7%
Heleopera rosea	22.3	6.9	Inter	0.5%	0.5%	1.1%	0.6%	0.3%	0.3%	1.3%	1.2%
Heleopera sylvatica	23.0	11.9	Inter	0.0%	0.0%	0.0%	0.0%	0.2%	0.2%	0.9%	0.5%
Corythion dubium	23.7	11.0	Inter	0.2%	0.2%	0.0%	0.0%	6.6%	6.0%	1.3%	0.8%
Nebela tincta s.l.	23.8	12.7	Inter	3.6%	0.6%	2.0%	0.7%	1.4%	1.4%	4.0%	3.5%
Arcella catinus	24.7	8.7	Inter	6.6%	2.0%	7.1%	1.1%	1.4%	0.5%	3.0%	0.8%
Assulina muscorum	25.5	11.4	Inter	0.8%	0.2%	1.8%	0.7%	15.2%	3.3%	17.4%	4.9%
Specific richness				5.8	0.5	6.0	0.3	6.6	0.5	7.6	1.5
Amphitrema wrightianum	9.4	4.1	Dry	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Pseudodifflugia gracilis	9.4	7.5	Dry	0.0%	0.0%	0.0%	0.0%	1.1%	0.6%	3.4%	0.8%
Archerella flavum	10.9	8.5	Dry	4.6%	3.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Cyclopyxis arcelloides	11.1	9.2	Dry	0.4%	0.4%	0.0%	0.0%	1.1%	1.1%	8.4%	7.6%
Physochila griseola	15.2	7.1	Dry	0.0%	0.0%	1.1%	0.9%	2.9%	1.7%	8.6%	3.5%
Hyalosphenia papilio	16.1	10.1	Dry	81.7%	5.7%	17.1%	6.1%	6.7%	2.6%	23.6%	6.6%
Centropyxis aculeata	17.0	7.0	Dry	0.5%	0.4%	7.7%	5.1%	2.8%	1.1%	8.2%	1.9%
Cryptodifflugia oviformis	19.5	13.2	Dry	0.0%	0.0%	0.0%	0.0%	18.3%	5.9%	0.0%	0.0%
Euglypha ciliata	19.6	11.8	Dry	1.8%	0.8%	4.5%	2.1%	4.2%	1.7%	5.0%	1.2%
Phryganella acropodia	21.6	11.5	Dry	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.3%
Heleopera rosea	22.3	6.9	Dry	0.7%	0.3%	9.2%	5.3%	3.7%	0.8%	12.2%	2.6%
Heleopera sylvatica	23.0	11.9	Dry	0.0%	0.0%	0.0%	0.0%	1.3%	1.0%	3.1%	1.7%
Corythion dubium	23.7	11.0	Dry	0.7%	0.7%	33.9%	16.5%	30.1%	9.4%	3.5%	1.7%
Nebela tincta s.l.	23.8	12.7	Dry	4.3%	1.9%	22.2%	5.9%	24.8%	6.6%	15.2%	1.8%
Arcella catinus	24.7	8.7	Dry	5.3%	2.0%	1.9%	0.8%	1.2%	0.8%	0.4%	0.2%
Assulina muscorum	25.5	11.4	Dry	0.0%	0.0%	2.3%	0.7%	1.9%	0.9%	7.8%	5.3%
Specific richness				5.2	0.9	7.4	0.4	**10.2**	0.7	**10.6**	0.5

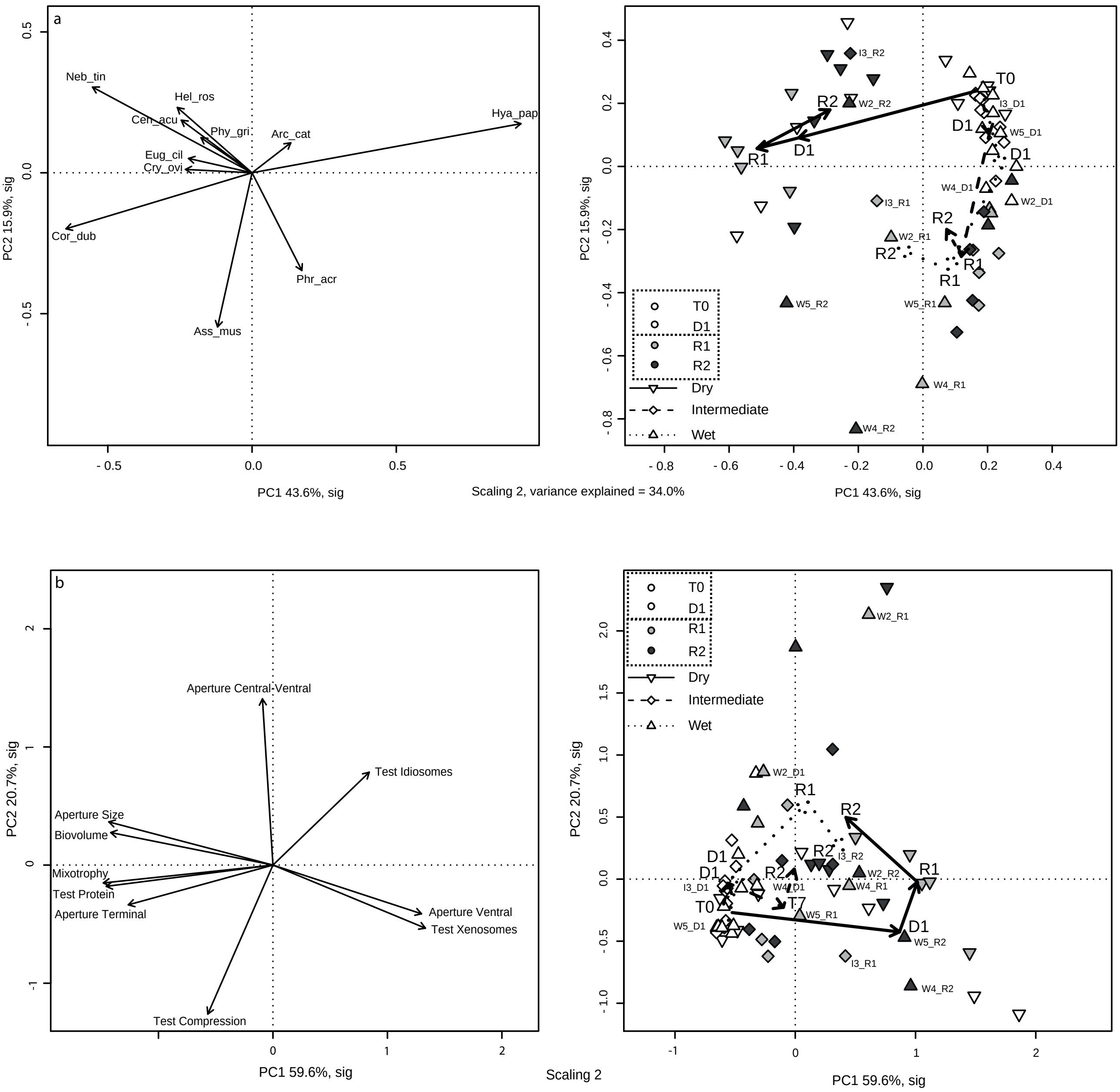


Figure 5.2 – Principal component analyses (PCA) of a) testate amoeba species and b) community weighted mean (CWM) of functional traits in *Sphagnum* fallax from a mesocosm experiment simulating water table changes. The species dataset was Hellinger transformed and the CWM data were scaled. Projection of descriptors (left) and samples (right), scaling 2. On the right plots, arrows represent the time line for each treatment (mean coordinates of the five sampling plots of each treatment and time). In both PCAs, axes 1 and 2 were the only significant axes and accounted respectively for 60.4% (species based) and 80.2% (CWM based) of the variance. Characteristic plots for wet and intermediate treatments were labelled. Taxa abbreviations: Amp_wri: *Amphitrema wrightianum*, Pse_gra: *Pseudodifflugia gracilis*, Arc_fla: *Archerella flavum*, Cyc_arc: *Cyclopyxis arcelloides*, Phy_gri: *Physochila griseola*, Hya_pap: *Hyalosphenia papilio*, Cen_acu: *Centropyxis aculeata*, Hel_syl: *Heleopera sylvatica*, Cry_ovi: *Cryptodifflugia oviformis*, Eug_cil: *Euglypha ciliata*, Phr_acr: *Phryganella acropodia*, Hel_ros: *Heleopera rosea*, Cor_dub: *Corythion dubium*, Neb_tin: *Nebela tincta* s.l., Arc_cat: *Arcella catinus*, Ass_mus: *Assulina muscorum*.

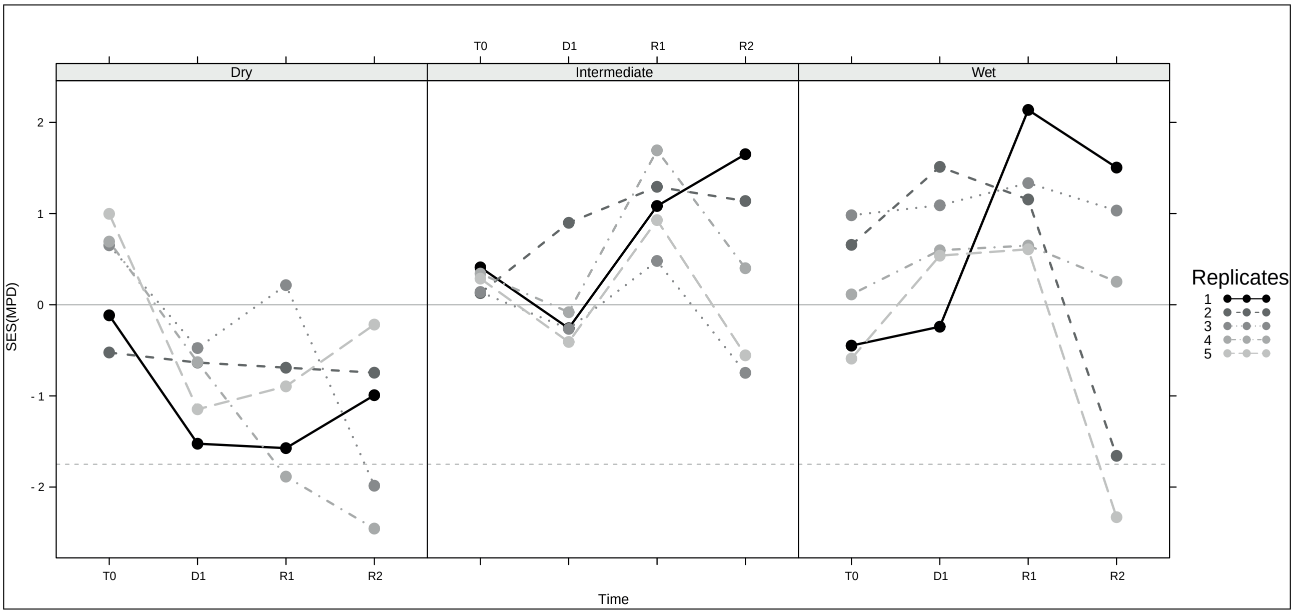


Figure 5.3 – Evolution of standardized effect size of mean pairwise distance between sampling plots relatively to a null model (random species matrix with respect to observed specific richness, see text for details) of testate amoeba community weighted mean (CWM) of functional traits data from a *Sphagnum fallax* mesocosm experiment simulating water table changes. The mean pairwise distance represents the distance separating communities based on the pool of functional traits. Horizontal grey dotted line represents the limit of p-value of 0.05, points below the line are significantly different from the null model. Each replicate was represented separately, with a grey scale.

aperture (axis 2). As for the community biplot, the dispersion of samples from the wet plots was wide, for the recovery phase (R1 and R2): W2 and W3 are correlated to a ventral-central aperture, with non-compressed, idiosomes tests, while W4 and W5 are correlated to smallest taxa with a small ventral aperture and a test built from xenosomes. This composition of traits is similar to that observed for dry plots at maximum disturbance (D1-R1). In dry plots, the CWM of traits evolved towards small species, with small, ventral pseudostome and compressed test made with xenosomes at D1, corresponding to the maximal effect of the treatment. After restoring the hydrological conditions, the community mix of traits returned towards larger species, with a central-ventral aperture and idiosomes tests.

Figure 5.3 shows the evolution of standardised effect size of mean pairwise distance (ses.mpd) based on species traits for each plot. In dry plots, the trend was negative since D1, all treatments and sampling dates but one showing negative values as compared to the null model, two of these plots being significantly different from the null model. In wet plots, the trend was towards a positive distance from the null model but two samples (W2 and W5) showed the opposite at R2, again illustrating the high dispersion of responses in this treatment. In intermediate plots, no clear trend was visible.

5.4 Discussion

Our aim was to assess the resilience capacity the *Sphagnum* testate amoeba community following an experimental hydrological perturbation which included three treatments: 1) wet (mild perturbation), 2) dry (severe perturbation) and 3) intermediate (“no perturbation”, considered here as control). The duration of the first experiment was long enough (almost three years) to induce a deep change in mesocosm functioning. In dry samples, the mineralisation of peat was obvious and *Sphagnum fallax* was replaced by other bryophytes and ferns, like in drained peatlands (Grosvernier et al., 1997; Graf et al., 2010).

Our results showed that the duration of the perturbation (i.e. thirty-two months) was clearly sufficient to allow both the moss (i.e. amoeba habitat) *Sphagnum fallax* and their associated microbial communities (among which testate amoebae) to respond first to the experimental hydrological perturbation and second to the experimental “restoration”. Both the testate amoeba community structure and community weighted mean of functional traits reacted within one growing season to the restored conditions. The response patterns we observed are generally in line with known ecological preferences of species derived from observational studies and the response of testate amoeba communities to a manipulative field experiment simulating a hydrological

Table 5.4 – Permutational multivariate analysis of variance of testate amoeba community dataset from Sphagnum fallax mesocosm experiment simulating water table level changes by treatment and between sampling time. Sampling time was significantly different in all treatments.

Treatment		Df	SumsOfSqs	MeanSqs	F-value	R^2	p-value
Dry	Time	3	2.34	0.78	7.52	0.58	0.00
	Residuals	16	1.66	0.10		0.41	
	Total	19	4.00			1.00	
Intermediate	Time	3	0.37	0.12	3.56	0.40	0.00
	Residuals	16	0.55	0.03		0.60	
	Total	19	0.92			1.00	
Wet	Time	3	0.88	0.29	3.11	0.37	0.02
	Residuals	16	1.51	0.09		0.63	
	Total	19	2.39			1.00	

Df: degree of freedom, SumOfSqs: sequencial sum of square, MeanSqs: mean square.

gradient (Marcisz et al., 2014).

5.4.1 Community structure and response to water table changes

At the beginning of the experimentation, the community was dominated by *Hyalosphenia papilio*, a frequently dominant taxon in oligotrophic *Sphagnum* peatlands and especially *Sphagnum fallax* poor-fens (Lamentowicz and Mitchell, 2005; Jassey et al., 2013b).

In the dry treatment, the community structure shifted by D1, as expected, towards a dominance of taxa characteristic for drier conditions: *Nebela tincta* s.l., *Corythion dubium*, *Assulina seminulum*, *Centropyxis aculeata* (Bobrov et al., 1999; Mitchell et al., 1999; Lamentowicz and Mitchell, 2005), which is similar to the community structure observed on bare peat or early stages of spontaneous cutover bog regeneration (Buttler et al., 1996; Laggoun-Defarge et al., 2008). This effect remained until R1 and a recovery became clear by R2 (Mitchell et al., 1999; Mazei et al., 2009).

Present at the beginning of the experiment, *Archerella flavum*, a mixotrophic species well adapted to situations where nutrient availability is low (Jassey et al., 2013b, 2015) and characteristic for moderately wet micro-habitats (Mazei et al., 2009), disappeared completely in the dry plots. The increase in species richness at R1 and R2 suggests that some taxa were not detected at T0, possibly due to the strong dominance of *H. papilio*, but were recorded at R1 and R2 when this species decreased.

Unlike the evolution in dry plots, neither the intermediate nor the wet treatment showed a clear change in community composition during the disturbance period (T0-D1). It seems that the water level contrast between the intermediate and wet treatments was not high enough to affect the population dynamics of *H. papilio* and the community structure thus remained similar in both treatments until D1. This lack of contrasted response of *H. papilio* between these two treatments is in line with its relatively wide tolerance to water level (Table 5.3 Booth and Meyers, 2010).

During the recovery phase, with a mean water table position at −10cm, the relative abundance of *H. papilio* declined and that of several taxa related to drier conditions increased (i.e. *Assulina muscorum*, *Phryganella acropodia*, Figure 5.4 Bobrov et al., 1999; Mitchell et al., 1999). However, two patterns were observed (Fig 2a): the community structure of some wet and intermediate plots changed and became similar to that of the dry treatment, while the other wet and intermediate plots remained relatively stable during this phase, despite a decrease of *H. papilio*. This unexpected trend is however coherent with the contrasted evolution of the mesocosms (Suppl. Figure 5.1). Indeed, when the water level was moved to −10cm in all plots, we observed that the structure and vitality of the moss carpet had considerably changed in some mesocosms. This was most clear in dry treatments where the S*phagnum fallax* carpet was quite completely replaced by other bryophytes and ferns (Suppl. Figure 5.1), but also in some wet and intermediate plots.

5.4.2 Treatment effects on testate amoeba functional traits

This clear community shift in the dry treatment corresponded to a shift in traits with an increased representation of smaller taxa, of shells with small ventral pseudostomes, a loss of mixotrophy, and an increase in idiosomes and xenosomes tests. These changes are in line with the drier conditions and thinner water film (Fournier et al., 2015; Marcisz et al., 2016; Payne et al., 2016b). The clear decrease in mixotrophy (related to the diminution of *H. papilio* which was not replaced by another mixotrophic taxa) indicates that mixotrophy does not represent an advantage in drier conditions, even when light availability remains constant. Mixotrophy was shown to be related to nutrient content and C cycling: a higher proportion of mixotrophs in the community being associated with higher C sequestration (Jassey et al., 2013b, 2015). The reduction of mixotrophy thus indicates an important functional change in the testate amoeba community in the dry plots during the disturbance phase. The shift in traits also indicates a lowering of the trophic level the community (i.e. more bacterivory as opposed to eukaryvory), suggesting a faster turnover of carbon and nutrients (Jassey et al., 2013a, 2015; Fournier et al., 2015).

During the recovery phase (R1-R2) in the dry treatment, the shifts of both community structure and traits towards the situation of T0 are in line with the increased humidity. This suggests that the functional niche and the food web structure responded the manipulated water table in the dry treatment (Kearney et al., 2010; Jassey et al., 2013a). Moreover, the trend towards the recovery of the original niche indicates that mesocosms are adequate tools to model the evolution of natural ecosystem (Benton et al., 2007).

The CWM did not differ significantly between the wet and intermediate treatments. In these two treatments, changes occurred only after D1, with a slow transition towards traits indicating drier conditions, at least in some plots. CWM of traits further suggests that the state of *Sphagnum* layer was the main driver in wet & intermediate plots (Suppl. Figure 5.1) while water table depth impacts more strongly the testate amoeba community structure in the dry plots. This is in line with previous observations on the stronger role of moisture conditions in the moss carpet in shaping testate amoeba communities rather than a possible direct impact of water table position (Buttler et al., 1996; Mitchell et al., 1999; Booth, 2001, 2002; Jassey et al., 2011; Payne et al., 2016a).

5.4.3 Community assembly: habitat filtering vs. biotic factors

We used the null model approach to disentangle biotic from abiotic drivers of community assembly. Changes in ecological niche should impact on testate amoeba community structure and this will be reflected by the CWM of traits. The distance between sampling plots (mean pairwise distance) should be significantly lower than the distance calculated by chance in plots where abiotic drivers are more important than competition (Chase et al., 2011; Fournier et al., 2012; Arrieira et al., 2015). In our experiment, the trend in dry plots was negative (significantly in two replicates, Figure 5.3) and, indicated that the community of testate amoeba was mainly drifted by environmental filters (Villéger et al., 2008). The same pattern occurred in two replicates of the wet treatment (W2 and W5), consistently with the state of *Sphagnum* stems in these plots. The shift towards less anoxic conditions (related to dryness) imply an important constraint for the communities living in *Sphagnum* and, as one of the most important groups, testate amoebae respond strongly to the change. By highlighting directly the loss of mixotrophy and the increase of smaller taxa, mainly bacterivorous, CWM of traits showed that dryness deeply affects ecosytem functioning and impact on nutrient and carbon cycles (Jassey et al., 2013a,b, 2015).

5.5 Conclusion and suggestion for future work

Our goal was to monitor the recovery of testate amoeba community to a water level comparable to natural *Sphagnum* peatlands using a mesocosms experiment. Due to the relatively small diameter of the moss carpets (12cm), survival of *Sphagnum* mosses cannot be guaranteed. Larger mesocosms would be useful to conduct longer-term experiments, but at a higher cost. The choice of the size is clearly a tradeoff. Furthermore, over the course of our experiment, the differential growth of the mosses and mineralization of the underlying peat caused the surface of the moss carpet to strongly diverge among treatments (ca. 20cm height difference by the end of the experiment), thus creating additional microclimatic effect (shading). Nevertheless, the survival of testate amoeba communities throughout the experiment, allowed us to assess for the first time experimentally community recovery in response to a raise in water level comparable to what is aimed for in peatland restoration projects. The results show the influence of both the water table and the structure of the moss carpet and confirm the usefulness of testate amoebae as biomonitors in peatland.

The response patterns of the community weighted mean of functional traits were similar to those of testate amoeba community structure and both were well correlated to ecological constraints. The CWM approach however presents the

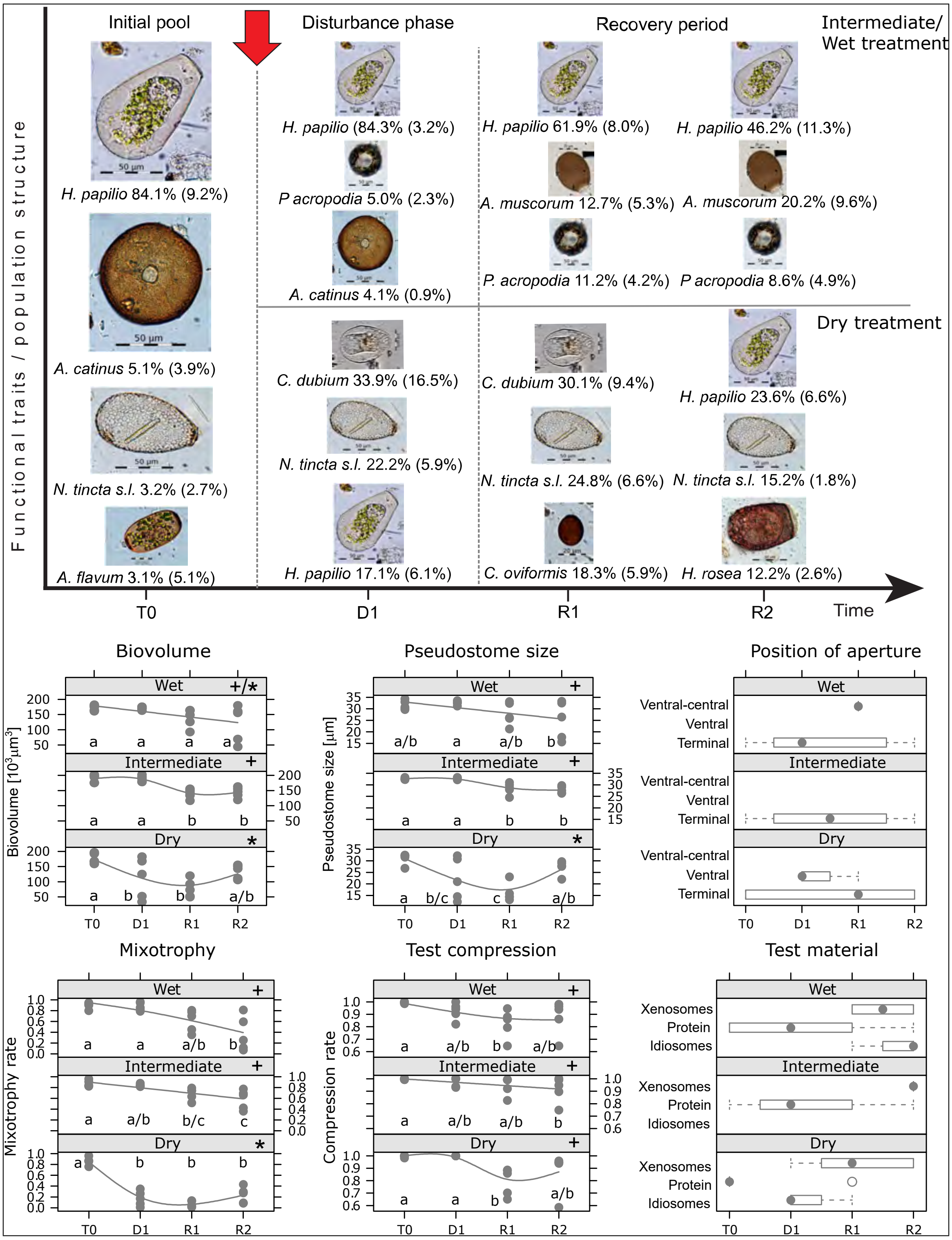


Figure 5.4 – Top: summary representation of the evolution of testate amoeba communities from a *Sphagnum fallax* mesocosm experiment simulating water table changes. The size of illustrated species is approximately propotional to their relative body size. As communities in the intermediate and wet treatments were not significantly different, they are pooled together for the species based evolution (upper part). The three most abundant taxa (mean abundance) at each sampling time are represented (four at T0). Present at the beginning of the experiment, *A. flavum* recovered only slightly in intermediate plots at R2 (not shown). Small taxa (*P. acropodia*, *A. muscorum*, *C. oviformis*, *C. dubium*) increased after the disturbance (arrow) and in dry plots *H. papilio* almost disappeared at R1 and recovered at R2. Bottom: Evolution of testate amoeba functional traits. Different signs or letters indicate significant differences. The lines show the cubic spline interpolation of the CWM of trait at each sampling point ($n = 5$). As for species-based evolution, in the wet and intermediate treatments the disturbance effect remained in the recovery phase in some plots, as shown by the increasing spread of points and constantly decreasing spline line. By contrast, in dry plots, the maximum stress response was observed at D1 and R1 followed by a partial recovery at R2. In the dry treatment, the dominant positions of the pseudostome was ventral at D1 and terminal at R1.

advantage of revealing more directly the impact of a stress, as noted for the diverging response of some wet/intermediate plots in the recovery phase. In addition, the functional diversity approach circumvents to some extent the need for high taxonomic resolution, which may not be easily achieved for non-specialists and can lead to erroneous interpretation in some cases (Heger et al., 2009; Mitchell et al., 2014). Testate amoeba taxonomy is constantly changing and molecular based studies are revealing cryptic diversity (Lara et al., 2007; Heger et al., 2009; Kosakyan et al., 2013, 2016; Singer et al., 2015; Oliverio et al., 2014). This will require a renewed effort to characterise the ecological information that can be inferred from this larger number of taxa. Until a better taxonomy is available, the functional diversity approach, based on easily-measurable morphological traits represents a powerful approach to infer ecological processes (Fournier et al., 2012, 2015; de Bello et al., 2010; Mitchell et al., 2014). Measuring the proportion of mixotrophs, the size and position of the pseudostome or the size category of testate amoebae is thus a promising approach to monitor the impact of perturbations or restoration on Sphagnum peatlands (Fournier et al., 2015; Marcisz et al., 2016; Payne et al., 2016a). To our knowledge these tools have not yet been used in environmental consulting or by managers, but we feel that the potential is clearly there.

5.6 Acknowledgements

This experiment was the follow up of a project supported by grant N° PSPB-013/2010 from Switzerland through the Swiss Contribution to the enlarged European Union and additional funds from the University of Neuchâtel.

Appendix

Supplementary material

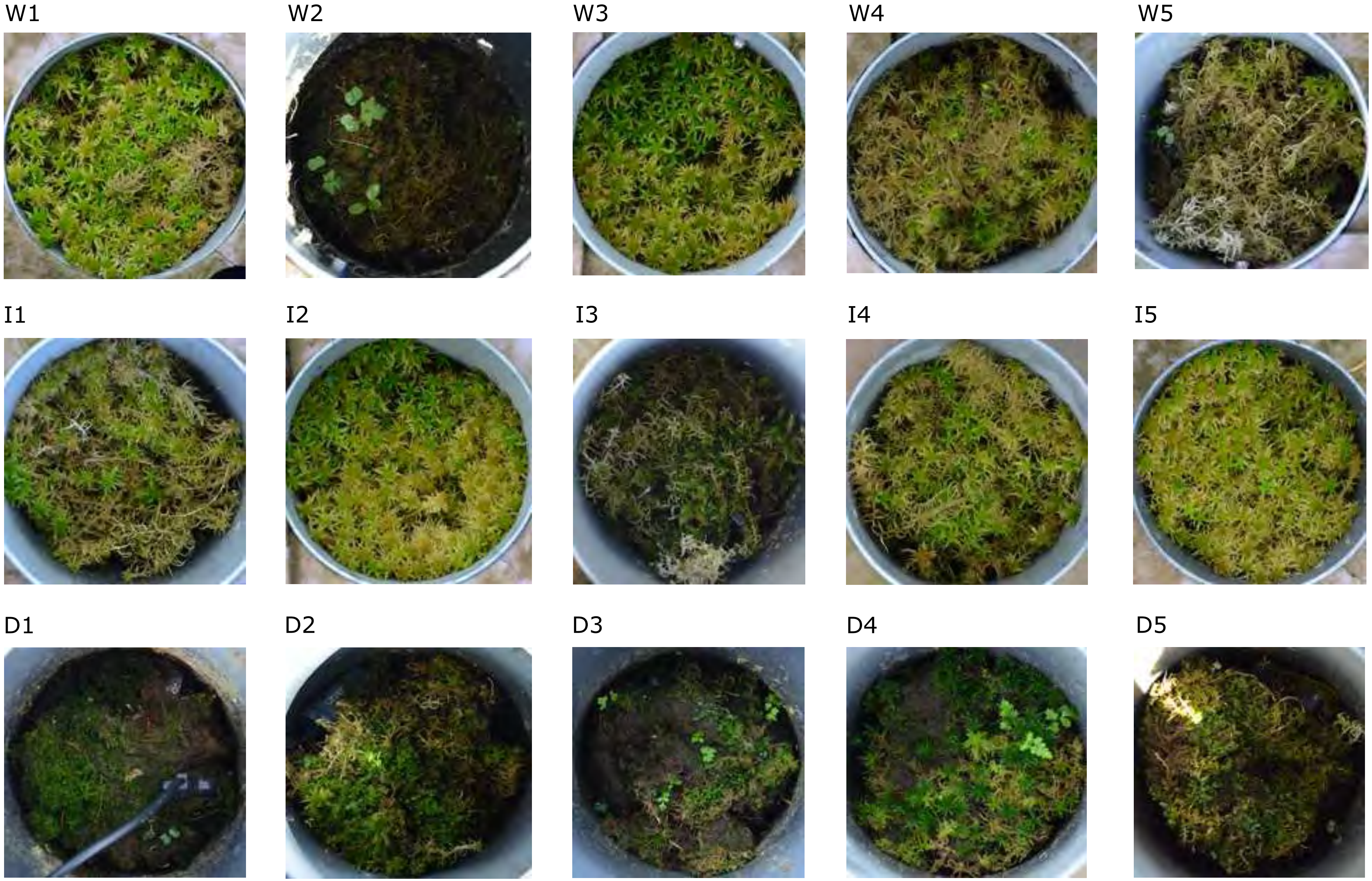
Table 5.A1 – Correspondence of sampling codes and date between the first experiment (Koenig et al., accepted) and this experiment.

	Treatment			Sampling dates				Replicates
Treatment/Date	Dry	Intermediate	Wet	2012-08-02	2014-03-27	2015-05-18	2015-09-24	
Koenig et al. (accepted)	BM	IM	HM	T0	T6	na	na	1-5
Recovery experiment	D	I	W	T0	D1	R1	R2	1-5

Table 5.A2 – Whole dataset of testate amoebae, the last column (Total) giving the total number of individuals counted per sample.

Site	Date	Time	Treatment	Amp_wri	Arc_cat	Arc_fla	Ass_mus	Cen_acu	Cor_dub	Cry_ovi	Cyc_eur	Eug_cil	Hel_ros	Hel_sph	Hya_pap	Neb_tin	Phr_acr	Phy_gri	Total
D1	02.08.2012	T0	Dry	0.0%	8.4%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	2.3%	0.8%	0.0%	85.5%	2.3%	0.0%	0.0%	131
D2	02.08.2012	T0	Dry	0.0%	0.0%	5.6%	0.0%	0.8%	0.0%	0.0%	0.0%	0.0%	1.6%	0.0%	90.4%	1.6%	0.0%	0.0%	125
D3	02.08.2012	T0	Dry	0.0%	7.4%	16.7%	0.0%	1.9%	0.0%	0.0%	1.9%	3.7%	0.9%	0.0%	59.3%	8.3%	0.0%	0.0%	108
D4	02.08.2012	T0	Dry	0.0%	9.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.2%	0.0%	0.0%	87.3%	0.0%	0.0%	0.0%	63
D5	02.08.2012	T0	Dry	0.0%	1.2%	0.0%	0.0%	0.0%	3.5%	0.0%	0.0%	0.0%	0.0%	0.0%	85.9%	9.4%	0.0%	0.0%	85
I1	02.08.2012	T0	Intermediate	0.0%	9.8%	0.0%	0.0%	0.5%	1.0%	0.0%	0.0%	0.0%	0.0%	0.0%	84.0%	4.6%	0.0%	0.0%	194
I2	02.08.2012	T0	Intermediate	0.0%	1.6%	0.0%	1.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	95.9%	1.3%	0.0%	0.0%	315
I3	02.08.2012	T0	Intermediate	0.0%	1.8%	0.0%	1.2%	1.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	90.9%	4.8%	0.0%	0.0%	165
I4	02.08.2012	T0	Intermediate	0.0%	11.1%	0.0%	0.5%	1.0%	0.0%	0.0%	0.5%	0.5%	0.0%	0.0%	82.9%	3.5%	0.0%	0.0%	199
I5	02.08.2012	T0	Intermediate	0.0%	8.5%	0.0%	1.2%	1.2%	0.0%	0.0%	0.6%	0.0%	2.4%	0.0%	82.3%	3.7%	0.0%	0.0%	164
W1	02.08.2012	T0	Wet	0.0%	2.7%	6.1%	2.7%	0.7%	0.0%	0.0%	0.0%	2.0%	0.7%	0.0%	83.8%	1.4%	0.0%	0.0%	148
W2	02.08.2012	T0	Wet	0.0%	3.6%	11.5%	0.0%	0.6%	0.0%	0.0%	0.6%	2.4%	0.6%	0.0%	79.4%	1.2%	0.0%	0.0%	165
W3	02.08.2012	T0	Wet	0.0%	8.6%	5.7%	0.0%	2.9%	0.0%	0.0%	1.4%	2.9%	0.0%	0.0%	74.3%	4.3%	0.0%	0.0%	70
W4	02.08.2012	T0	Wet	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%	4.4%	0.0%	94.2%	0.7%	0.0%	0.0%	137
W5	02.08.2012	T0	Wet	0.0%	2.8%	0.0%	0.6%	0.0%	0.0%	0.0%	0.0%	1.1%	0.6%	0.0%	93.3%	1.7%	0.0%	0.0%	180
D1	27.03.2014	D1	Dry	0.0%	3.4%	0.0%	1.3%	7.4%	0.0%	0.0%	0.0%	2.7%	26.2%	0.0%	26.2%	28.2%	0.0%	4.7%	149
D2	27.03.2014	D1	Dry	0.0%	4.3%	0.0%	4.3%	2.1%	17.0%	0.0%	0.0%	10.6%	2.1%	0.0%	17.0%	42.6%	0.0%	0.0%	47
D3	27.03.2014	D1	Dry	0.0%	1.1%	0.0%	0.6%	0.0%	83.9%	0.0%	0.0%	0.6%	0.6%	0.0%	0.6%	12.6%	0.0%	0.0%	174
D4	27.03.2014	D1	Dry	0.0%	0.0%	0.0%	3.4%	27.6%	6.9%	0.0%	0.0%	0.0%	17.2%	0.0%	34.5%	10.3%	0.0%	0.0%	29
D5	27.03.2014	D1	Dry	0.0%	0.5%	0.0%	2.0%	1.5%	61.9%	0.0%	0.0%	8.4%	0.0%	0.0%	7.4%	17.3%	0.0%	1.0%	202
I1	27.03.2014	D1	Intermediate	0.0%	5.3%	0.0%	2.6%	0.0%	0.0%	0.0%	0.0%	0.9%	0.0%	0.0%	83.3%	0.9%	6.1%	0.9%	114
I2	27.03.2014	D1	Intermediate	0.0%	8.7%	0.0%	0.0%	0.6%	0.0%	0.0%	0.0%	0.6%	1.2%	0.0%	82.1%	0.0%	6.9%	0.0%	173
I3	27.03.2014	D1	Intermediate	0.0%	10.2%	0.0%	1.1%	0.5%	0.0%	0.0%	0.0%	0.0%	3.2%	0.0%	82.4%	2.7%	0.0%	0.0%	187
I4	27.03.2014	D1	Intermediate	0.0%	4.1%	0.0%	4.1%	0.0%	0.0%	0.0%	0.0%	0.0%	1.4%	0.0%	87.8%	2.7%	0.0%	0.0%	74
I5	27.03.2014	D1	Intermediate	0.0%	7.4%	0.0%	1.2%	1.2%	0.0%	0.0%	0.0%	1.2%	0.0%	0.0%	85.2%	3.7%	0.0%	0.0%	81
W1	27.03.2014	D1	Wet	1.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.2%	0.0%	84.1%	1.6%	9.5%	0.0%	63
W2	27.03.2014	D1	Wet	3.6%	3.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	75.0%	0.0%	17.9%	0.0%	28
W3	27.03.2014	D1	Wet	2.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	93.4%	0.0%	3.9%	0.0%	76
W4	27.03.2014	D1	Wet	3.9%	0.0%	0.0%	2.0%	7.8%	0.0%	0.0%	0.0%	2.0%	0.0%	0.0%	78.4%	0.0%	5.9%	0.0%	51
W5	27.03.2014	D1	Wet	3.6%	1.8%	0.0%	0.9%	0.9%	0.0%	0.0%	0.0%	0.0%	1.8%	0.0%	91.1%	0.0%	0.0%	0.0%	112
D1	18.05.2015	R1	Dry	0.0%	0.9%	0.0%	1.8%	1.8%	32.9%	34.7%	0.0%	5.0%	1.8%	0.9%	12.6%	2.3%	0.0%	5.4%	222
D2	18.05.2015	R1	Dry	0.0%	4.6%	0.0%	1.3%	6.6%	34.9%	11.8%	0.0%	2.0%	2.0%	0.0%	1.3%	34.9%	0.0%	0.7%	152
D3	18.05.2015	R1	Dry	0.0%	0.6%	0.0%	1.3%	0.0%	23.7%	29.5%	0.0%	2.6%	5.1%	0.0%	1.9%	35.3%	0.0%	0.0%	156
D4	18.05.2015	R1	Dry	0.0%	0.0%	0.0%	5.7%	4.1%	0.8%	4.1%	5.7%	11.5%	6.6%	0.0%	13.9%	38.5%	0.0%	9.0%	122
D5	18.05.2015	R1	Dry	0.0%	0.0%	0.0%	0.0%	1.8%	59.6%	11.9%	0.0%	0.9%	3.7%	0.0%	4.6%	17.4%	0.0%	0.0%	109
I1	18.05.2015	R1	Intermediate	0.0%	1.3%	0.0%	11.1%	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%	0.7%	78.4%	0.0%	7.8%	0.0%	153
I2	18.05.2015	R1	Intermediate	0.0%	1.1%	0.0%	16.6%	0.0%	0.5%	0.0%	1.1%	0.0%	0.0%	0.0%	64.2%	0.0%	16.6%	0.0%	187
I3	18.05.2015	R1	Intermediate	0.0%	0.8%	0.0%	5.0%	2.5%	30.3%	0.0%	0.0%	0.0%	1.7%	0.8%	51.3%	6.7%	0.8%	0.0%	119
I4	18.05.2015	R1	Intermediate	0.0%	0.7%	1.3%	24.2%	0.0%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	72.5%	0.0%	0.7%	0.0%	153
I5	18.05.2015	R1	Intermediate	0.0%	3.2%	0.0%	19.2%	0.6%	1.3%	0.0%	0.0%	0.6%	0.0%	0.0%	74.4%	0.0%	0.6%	0.0%	156
W1	18.05.2015	R1	Wet	0.4%	1.3%	0.0%	0.0%	0.0%	4.4%	0.0%	0.0%	0.0%	0.0%	1.3%	78.8%	0.0%	13.7%	0.0%	226
W2	18.05.2015	R1	Wet	11.1%	5.6%	0.0%	0.0%	5.6%	11.1%	0.0%	0.0%	0.0%	0.0%	5.6%	22.2%	5.6%	33.3%	0.0%	18
W3	18.05.2015	R1	Wet	0.0%	0.0%	0.0%	0.0%	0.5%	2.0%	0.0%	0.0%	0.0%	0.0%	0.0%	77.1%	0.0%	20.5%	0.0%	205
W4	18.05.2015	R1	Wet	9.6%	0.0%	0.0%	38.4%	0.0%	4.8%	0.0%	0.0%	0.7%	0.0%	0.0%	36.3%	0.0%	10.3%	0.0%	146
W5	18.05.2015	R1	Wet	8.7%	0.0%	0.0%	13.3%	0.0%	9.3%	0.0%	0.0%	2.0%	0.0%	0.0%	61.3%	0.0%	5.3%	0.0%	150
D1	24.09.2015	R2	Dry	0.0%	0.0%	0.0%	0.8%	11.8%	1.7%	0.0%	2.5%	4.2%	8.4%	1.7%	42.9%	9.2%	1.7%	15.1%	119
D2	24.09.2015	R2	Dry	0.0%	0.0%	0.0%	0.0%	10.5%	9.6%	0.0%	0.0%	0.9%	21.1%	0.0%	30.7%	21.1%	0.9%	5.3%	114
D3	24.09.2015	R2	Dry	0.0%	1.1%	0.0%	2.3%	3.4%	1.1%	0.0%	0.0%	6.8%	18.2%	0.0%	30.7%	15.9%	0.0%	20.5%	88
D4	24.09.2015	R2	Dry	0.0%	0.0%	0.0%	8.1%	4.4%	0.0%	0.0%	40.7%	6.7%	8.9%	0.0%	8.9%	17.8%	0.0%	4.4%	135
D5	24.09.2015	R2	Dry	0.0%	0.9%	0.0%	31.9%	12.9%	6.0%	0.0%	0.9%	8.6%	8.6%	1.7%	10.3%	17.2%	0.9%	0.0%	116
I1	24.09.2015	R2	Intermediate	0.0%	2.9%	0.0%	17.6%	0.0%	2.9%	0.0%	0.0%	0.0%	0.0%	0.0%	66.2%	0.0%	10.3%	0.0%	136
I2	24.09.2015	R2	Intermediate	0.0%	2.1%	2.8%	26.2%	0.0%	0.0%	0.0%	5.5%	0.0%	0.7%	9.7%	35.9%	0.0%	17.2%	0.0%	145
I3	24.09.2015	R2	Intermediate	0.8%	0.8%	0.0%	0.0%	23.0%	4.0%	0.0%	0.8%	1.6%	6.3%	0.0%	38.1%	19.0%	0.8%	4.8%	126
I4	24.09.2015	R2	Intermediate	0.0%	3.6%	5.8%	23.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	61.6%	1.4%	0.7%	0.0%	138
I5	24.09.2015	R2	Intermediate	0.0%	5.6%	0.0%	16.2%	0.0%	0.0%	0.0%	0.0%	0.6%	0.0%	0.0%	77.1%	0.6%	0.0%	0.0%	179
W1	24.09.2015	R2	Wet	0.0%	4.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%	2.8%	78.9%	0.0%	13.4%	0.0%	142
W2	24.09.2015	R2	Wet	2.8%	2.8%	0.0%	1.9%	33.6%	0.9%	0.0%	0.0%	15.0%	6.5%	0.0%	23.4%	6.5%	1.9%	4.7%	107
W3	24.09.2015	R2	Wet	0.0%	1.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.8%	0.0%	0.9%	60.2%	0.0%	34.3%	0.0%	108
W4	24.09.2015	R2	Wet	0.0%	0.0%	0.7%	71.5%	0.7%	9.5%	0.0%	0.0%	2.9%	0.0%	0.0%	10.9%	0.0%	3.6%	0.0%	137
W5	24.09.2015	R2	Wet	0.0%	0.0%	0.0%	46.0%	2.2%	11.7%	0.0%	0.0%	8.0%	3.6%	0.0%	7.3%	16.8%	2.9%	1.5%	137

Figure 5.A1 – Picture of the mesocosms on 2015, the 23rd of September (R2) with *Sphagnum fallax* carpets. Mesocosms are ordered according to code W: Wet, I: Intermediate, D: Dry treatment.



Chapter 6

Discussion and Conclusion to: Using testate amoeba community structure and functional diversity to assess the impact of climate changes on ecosystem functioning: finding relevant and convenient tools for bio-monitoring of *Sphagnum* peatlands evolution or recovery.

6.1 Context

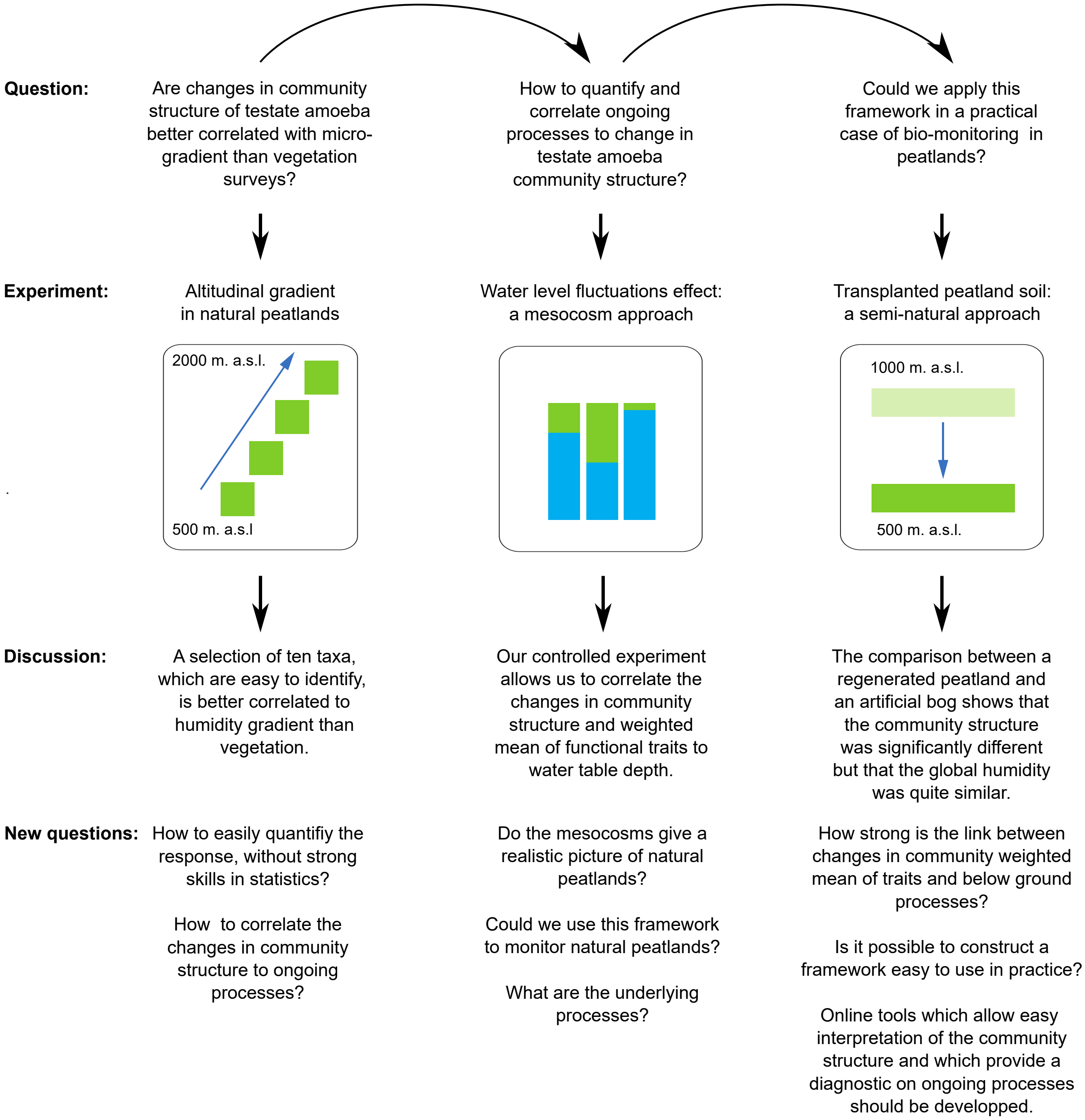
With ongoing climate changes and human pressure on ecosystems worldwide, the understanding and quantifying of services provided by ecosystems has become highly important (IPCC, 2014; MEA, 2005). Ecosystem services include all the functions contributing to human well-being and the monetary values of these services can be estimated (Costanza et al., 1998; MEA, 2005). Ecosystem services provided by peatlands include climate regulation, either by increasing carbon fixation or by reducing carbon emissions. The carbon pool and sink function of peatlands can however easily be lost when these ecosystems are damaged (Belyea and Malmer, 2004; Fleischer et al., 2016; Mitsch and Gosselink, 2007).

Sphagnum peatlands, mainly found in boreal regions (but not exclusively), are characterised by waterlogged soils with anoxic conditions, high acidity and low nutrient content (Gobat et al., 2010; Mitsch and Gosselink, 2007). Due to the anoxic conditions, mineralisation rates are low which leads to the accumulation of carbon in the soil (Bridgham et al., 2006; Maltby and Immirzi, 1993). While peatlands represent only 3% of the Earth's land surface, they contain ca. 500±100 giga–tons of carbon (Gt C), which represents the equivalent of 1/3 to 1/2 of all atmospheric carbon (Gorham, 1991; Ramsar, 2013; Yu, 2012).

Peatlands have suffered from direct (peat harvesting, drainage, conversion to agricultural land, afforestation) and indirect (pollution by nutrients, climate change, alteration of regional hydrology) impacts. Since the end of the 20^{th} century, an increasing interest emerged to protect and restore the reminding peatlands and, in Western Europe, a substantial amount of money was spent in revitalisation projects (Andersen et al., 2017). However, in their review, Andersen et al. (2017) highlighted that in most cases, little if nothing is invested in monitoring restoration success, and they suggested the implementation of long-term monitoring and research programmes to assess the success of conducted measurements. In this context, the development of bio-monitoring tools that are easy to handle and to implement will greatly improve the follow-up of peatland restoration and will allow the comparison between sites. Until now, peatlands monitoring was mainly conducted using vegetation surveys (Bonnett et al., 2011; Graf et al., 2010). These require profound knowledge in botany, are time consuming (quantitative survey) or are based on estimation (semi-quantitative survey). In addition, they depend on the season and need to be conducted by the same botanist to avoid a bias in estimation (Vittoz and Guisan, 2007). Moreover, the impact of perturbation or regeneration measures will be reflected by a change in vegetation many years later. In this context and as most of the functions of peatlands are related to waterlogged soils (i.e. carbon fixation, preservation of specialised plants, water filtration, buffer effect against flood, etc. Haapalehto et al., 2011; MEA, 2005), finding organisms that respond more rapidly to changes in the water content is of primary importance.

6.2 Biomonitoring of *Sphagnum* peatlands through changes in testate amoeba community structure and weighted mean of functional traits

The central point of this thesis was to test the use of testate amoeba community in the context of *Sphagnum* peatlands monitoring and to address some theoretical issues related to the selection of functional traits. As the carbon fixation function in *Sphagnum* peatlands is mainly related to the level of water table, monitoring tools giving direct or indirect information on humidity and/or water level depth are essential. Such tools have to be well correlated to ecosystem functioning and thus to ecosystem services and must be easy to use also for non-specialists (e.g. natural park managers and their staff, environmental consultants). To achieve this, we followed a three-step approach (Figure 6.1) by: 1) comparing changes in testate amoeba and vegetation community structures along an altitudinal gradient of *Sphagnum* peatlands in Switzerland; 2) defining a framework allowing to monitor change in testate amoeba community structure and selecting functional traits indicating a water stress in a controlled experiment; 3) applying this framework in the follow-up of a transplanted bog and comparing the functioning of the bog to a reference site. Each step was planned to answer questions emerging from the previous one and aims to refine the use of testate amoeba community structure and functional traits in bio-monitoring of *Sphagnum* peatlands.



Question:

Are changes in community structure of testate amoeba better correlated with micro-gradient than vegetation surveys?

How to quantify and correlate ongoing processes to change in testate amoeba community structure?

Could we apply this framework in a practical case of bio-monitoring in peatlands?

Experiment:

Altitudinal gradient in natural peatlands

Water level fluctuations effect: a mesocosm approach

Transplanted peatland soil: a semi-natural approach

Discussion:

A selection of ten taxa, which are easy to identify, is better correlated to humidity gradient than vegetation.

Our controlled experiment allows us to correlate the changes in community structure and weighted mean of functional traits to water table depth.

The comparison between a regenerated peatland and an artificial bog shows that the community structure was significantly different but that the global humidity was quite similar.

New questions:

How to easily quantifiy the response, without strong skills in statistics?

How to correlate the changes in community structure to ongoing processes?

Do the mesocosms give a realistic picture of natural peatlands?

Could we use this framework to monitor natural peatlands?

What are the underlying processes?

How strong is the link between changes in community weighted mean of traits and below ground processes?

Is it possible to construct a framework easy to use in practice?

Online tools which allow easy interpretation of the community structure and which provide a diagnostic on ongoing processes should be developped.

Figure 6.1 – Global overview of the thesis with each step as described in text. The whole project was constructed to test the possible use of testate amoeba community structure and community weighted mean of functional traits in practical peatlands management.

6.2.1 Testate amoeba community structure as a biomonitoring tool in *Sphagnum* peatlands

Using taxonomic groups to follow changes in ecosystems requires some prerequisites (Bonnett et al., 2011; Noss, 1990; Trepel, 2007). According to Noss (1990), a bioindicator should be (amongst others): 1) sufficiently sensitive to provide early warning of change; 2) distributed over a broad geographical area; 3) able to give continuous assessment over a wide range of stress; 4) easy and cost-effective to measure, collect, assay and calculate; 5) relevant to ecologically significant phenomena. Furthermore, in ecosystems particularly sensible to stress and disturbances, the impact of sampling should be as little as possible. Testate amoebae fulfil several (if not all) above assumptions and could be used in bio-monitoring programmes (Payne, 2013). As a dominant taxonomic and functional group in *Sphagnum* peatlands, they play a central role in carbon and nutrient cycles and respond rapidly to changes in their environment (Gilbert and Mitchell, 2006; Jassey et al., 2013a, 2015; Marcisz et al., 2014; Wilkinson, 2008; Wilkinson and Mitchell, 2010). Moreover, changes in testate amoeba community structure are expected to reflect ongoing processes, particularly related to humidity, acidity and nutrient cycles (Bobrov et al., 2002; Gilbert and Mitchell, 2006; Qin et al., 2013; Wilkinson, 2008; Wilkinson and Mitchell, 2010).

The use of testate amoeba community structure in peatlands and wetlands is now well established but requires profound knowledge in taxonomy and ecological interpretation (Heger et al., 2009; Mitchell et al., 2008b; Payne, 2013). Moreover, as community structure might per se be different at reference sites, it could be difficult to interpret ongoing processes only based on the comparison of community structures between different sites. The functional trait based approach allows to overcome some of these limitations in bio-monitoring (de Bello et al., 2010; Fournier et al., 2015). Our work gave new evidence to the relationship between functional traits and niche constraints. Establishing a framework using easy measurements with an optical microscope and the addition of manipulations e.g. water stress should help to implement the use of testate amoebae in bio-monitoring of *Sphagnum* peatlands.

In chapter 2, we assessed that a selection of ten testate amoeba morphotaxa was better correlated to the humidity gradient and to moss carbon and nitrogen contents along the altitudinal gradient than vegetation (vascular plants and bryophytes). By reflecting humidity and nutrient content of *Sphagnum* patches, these ten morphotaxa could thus be used to follow the impact of revitalisation measures and to assess the impact of climate change on peatlands. Moreover, the identification of these morphotaxa is relatively easy using an optical microscope and sampling could be done without prior knowledge other than choosing the patches according to the aim of the study. In addition, as the switch between carbon sink and source in *Sphagnum* peatlands is directly linked to the water table depth (Bragazza et al., 2009; Fleischer et al., 2016; Gorham, 1991), these taxa provide indirect information on organic matter mineralisation (Bragazza et al., 2016; Jassey et al., 2013a).

This first experiment was limited by the lack of reference studies using these taxa as a biomonitoring tool. Here, we used indirect and direct ordinations but these approaches require expertise in multivariate analyses and may have a deterrent effect on the staff of natural ecosystem managements. By analysing a broader range of samples, from different macro-climatic zones and micro-environmental conditions, we should be able to calibrate the ratio of each morphotaxa in the community structure and to relate it to the water level and carbon balance. Comparison tables, both visual and quantitative, are required, allowing interpreting the community structure in terms of the proportion of each taxon. Moreover, controlled experiments and additional proxies could add value and thus support the implementation of testate amoeba as a robust biomonitoring tool.

6.2.2 Assessing the impact of water table depth on testate amoeba community structure and weighted mean of functional traits

In the mesocosm study (chapters 4 & 5), we followed the changes in testate amoeba community structure and functional traits in a controlled experiment of manipulated water table. Our experiment covered the whole process from the start of the disturbance to the recovery after the rewetting process. By isolating one variable, the other variables staying comparable between treatments, we could assess the change in community structure related to the variation in humidity content. The functional trait approach brought an additional dimension to the experiment and allowed us to highlight some mechanisms hidden behind the shift in community structure and particularly the niche filtering process (Maire et al., 2012; Mouillot et al., 2007).

Recent studies correlated testate amoeba functional traits to local conditions and particularly humidity (Fournier et al., 2012, 2015; Kajukało et al., 2016; Marcisz et al., 2016; Payne et al., 2016b; Schwind et al., 2016), food web functioning (Jassey et al., 2012, 2013b), nutrients and availability of micro-particles for shell building (Jassey et al., 2015; Schönborn, 1963; Wanner and Meisterfeld, 1994). In the first part of the experiment (chapter 4), we correlated the lowering of the water table with a shift in community weighted mean of traits towards: 1) an increased proportion of small taxa; 2) a hidden pseudostome (ventral or ventral-central); 3) a diminution of the pseudostome size; and 4) the loss of mixotrophy. This set of traits reflects a drastic change in ecosystem functioning, related to a thickening of the acrotelm (i.e. the oxic layer in *Sphagnum* peatlands) which stimulates the development of primary decomposers, bacteria and fungi (Andersen et al., 2013; Bragazza et al., 2016). In the recovery experiment (chapter 5), we simulated a regeneration process by increasing the water table in the replicates. Testate amoeba community responded to changes in less than six months and the response was in line with previous studies showing that the structure of the community was correlated to micro-environmental gradients such as the position along the stem or humidity content (Jassey et al., 2011; Mieczan, 2010; Mitchell et al., 1999). The evolution of dry plots suggested that the use of mesocosms, as ecosystem modelling, to study restoration ecology in *Sphagnum* peatland is realistic. The change in testate amoeba community structure went towards the recovery of taxa that are correlated to medium-wet conditions like *Hyalosphenia papilio* and *Heleopera rosea*, and the decline of taxa adapted to dryness like *Nebela tincta* s.l. and *Corythion dubium*. The community weighted mean of traits showed a recovery of mixotrophy, an increase in test length, variation of the pseudostome size and terminal pseudostome position, indicating that local conditions were less drastic and allowed testate amoeba community to recover. The C fixation possibly resumed (or at least the rate of C loss declined – inferred from the increase in proportion of mixotrophs), and the trophic level of testate amoeba community increased, indicating a change in food web functioning (Gravel et al., 2016; Jassey et al., 2013b; Wilkinson and Mitchell, 2010).

6.2.3 Practical application of testate amoeba in bio-monitoring

The third step was to apply our framework in practise. The hot and dry summer of 2015 gave us the opportunity to make use of a real-life situation with the follow-up of an artificial bog experiment in the Botanical Garden of Neuchâtel (Chapter 3). We decided to monitor the community of testate amoebae based on taxonomic and functional approaches and to compare it to a reference site, located in Bois des Lattes (canton of Neuchâtel.

Our results indicated that the community structures in the Botanical Garden were significantly different than those found in Bois des Lattes on bare peat. Moreover, in the Botanical Garden in 2015, the shift in community structure between living testate amoebae and empty amoeba tests indicated that most of the original population died out, probably due to the dryness. The empty tests community structure in the Botanical Garden in 2015 indicated more humid conditions than the one found in Bois des Lattes. In the Botanical Garden, *Sphagnum* patches from various sites from the Jura were transplanted into the Botanical Garden and some of them were maintained in wet conditions for at least two years until the bog was built. The community has survived in most patches until the summer of 2015.

After the dry and hot summer, in the Botanical Garden (both sampling dates), the community structure was dominated by *Corythion dubium*, a pioneer taxon related to dry conditions (Payne et al., 2015; Smith, 1996). We assumed that a transitional phase was ongoing in 2015 between the transplantation of *Sphagnum* patches into the Botanical Garden and the community found in 2016. Based on community weighted mean of traits, both sites were significantly different but to a lower extent. In Bois des Lattes, the broad presence of taxa with a test made of xenosomes (mainly *Difflugia* ssp.), linked to the local conditions, i.e. the presence of mineral particles, explained most of the difference. A focus on traits related to humidity constraints, revealed that, in the Botanical Garden, the humidity rate was comparable to the humidity conditions found in *Sphagnum* patches laying on bare peat, in Bois des Lattes. We concluded that after the hot and dry summer of 2015, the niche constraints in the Botanical Garden were similar to one found in the reference site. The community weighted mean of traits confirmed the transitional status of the 2015 sampling in the Botanical Garden and the difference between the community weighted mean of traits in the living population and empty tests clearly indicated a shift towards drier conditions.

The shift in community structure is difficult to interpret as the ecological preference of *Corythion dubium* is not clearly established yet. Most of studies linked *C. dubium* to the margin of ombrotrophic peatlands (Mitchell et al., 1999; Opravilova and Hajek, 2006). Often related to the driest part of acid peatbogs, this taxon could be also found in forest humus, in Arctic or Antarctic areas (Beyens et al., 1990; Payne et al., 2015; Royles et al., 2013; Smith, 1996). Thus, interpreting that the bog is shifting towards meadow, forest or other kind of terrestrial ecosystems, only based on the wide presence of *C. dubium*, is hasty. The trend in the Botanical Garden evolved from a situation with low niche filtering and high food web level for testate amoeba community – reflected by CWM of traits on empty tests in 2015 – towards more stressed conditions with high niche filtering – related to a thinner water film – in 2016. This indicated that testate amoeba community had a lower level in food web functioning, this being related to the loss of larger taxa, often predators of smaller ones (Fournier et al., 2012; Jassey et al., 2012, 2013b; Mouillot et al., 2007). Compared with Bois des Lattes, where we sampled on bare peat, the functioning of the artificial bog is rather similar in 2016, confirming that the water table never reached the ground level over the last few months.

6.3 Linking community structure, functional traits and ecosystem functioning

6.3.1 Taxonomic community structure

The shift in community structure is summarised in Figure 6.2, including the main taxa found during this thesis and ordered regarding their tolerance to water stress. *Amphitrema wrightianum* and to a lesser extend *Archerella flavum*, the two taxa found in waterlogged conditions, may be described as flagship taxa for a high water level, as they will be quickly impacted by dryness. For instance, they were never found alive neither in the Botanical Garden nor in Bois des Lattes, but were very abundant in pools along the altitudinal gradient and they reappeared in some plots after rewetting of the mesocosms during the recovery period. On the other end of the humidity gradient are testate amoebae living in dryer conditions and some of them could be defined as flagship taxa of dryness or perturbation, particularly *Assulina muscorum*, *Corythion dubium*, some big *Euglypha* ssp., *Nebela militaris* and small taxa of *N. tincta* group. By comparing the proportion of these taxa in the testate amoeba community along a sequence of sampling (e.g. according to a natural gradient, the reference site, along a time series, etc.) a first diagnosis can be made and allows an insight in ongoing processes. But using only taxonomic dynamics in monitoring may hide the signal because some taxa (for example, H*yalosphenia papilio* or *H. elegans*) can widely dominate the community, as it was the case in the mesocosm experiment. Moreover, as some groups were understudied, inferring below ground processes only based on community structure is slightly hazardous.

6.3.2 Community weighted mean of functional traits

The functional traits approach allows to interpret ongoing processes and to link them to global functioning of the peatland. In waterlogged conditions, the environmental constraints on testate amoeba community are less drastic and testate amoebae are represented at several levels of food web structure from predatory to bacterivorous (Alley, 1982; Jassey et al., 2011; Tilman, 1982). When water stress increases, adaptations which allow the individual to stay active in a thin water film like hidden pseudostome (ventral or ventral-central), small and compressed test are better adapted and these traits dominated the community weighted mean (Figure 6.3). Testate amoeba predators are often large taxa with a wide terminal pseudostome (Fournier et al., 2015; Gilbert et al., 2003; Jassey et al., 2012; Lamentowicz et al., 2013). These traits represent a disadvantage when the water film is thin and lead to the diminution of predatory testate amoeba taxa. The predation pressure on small, bacterivorous taxa decreased, explaining the shift, but little is really known about these relationships (Geisen et al., 2017; Jassey et al., 2012; Wilkinson, 2008). Our results in mesocosms are in line with some pioneer's works made in projects of floodplain restoration, peatlands monitoring, palaeo-ecology reconstruction, etc. (Fournier et al., 2015; Jassey et al., 2013a; Kajukało et al., 2016; Marcisz et al., 2016). These studies suggested that biovolume, pseudostome size and position, and test compression reflect changes in humidity level and thus give insight in belowground processes. But further works are needed to really understand the complexity of the relationships within the testate amoeba community, and to link it to niche filtering and to select meaningful functional traits.

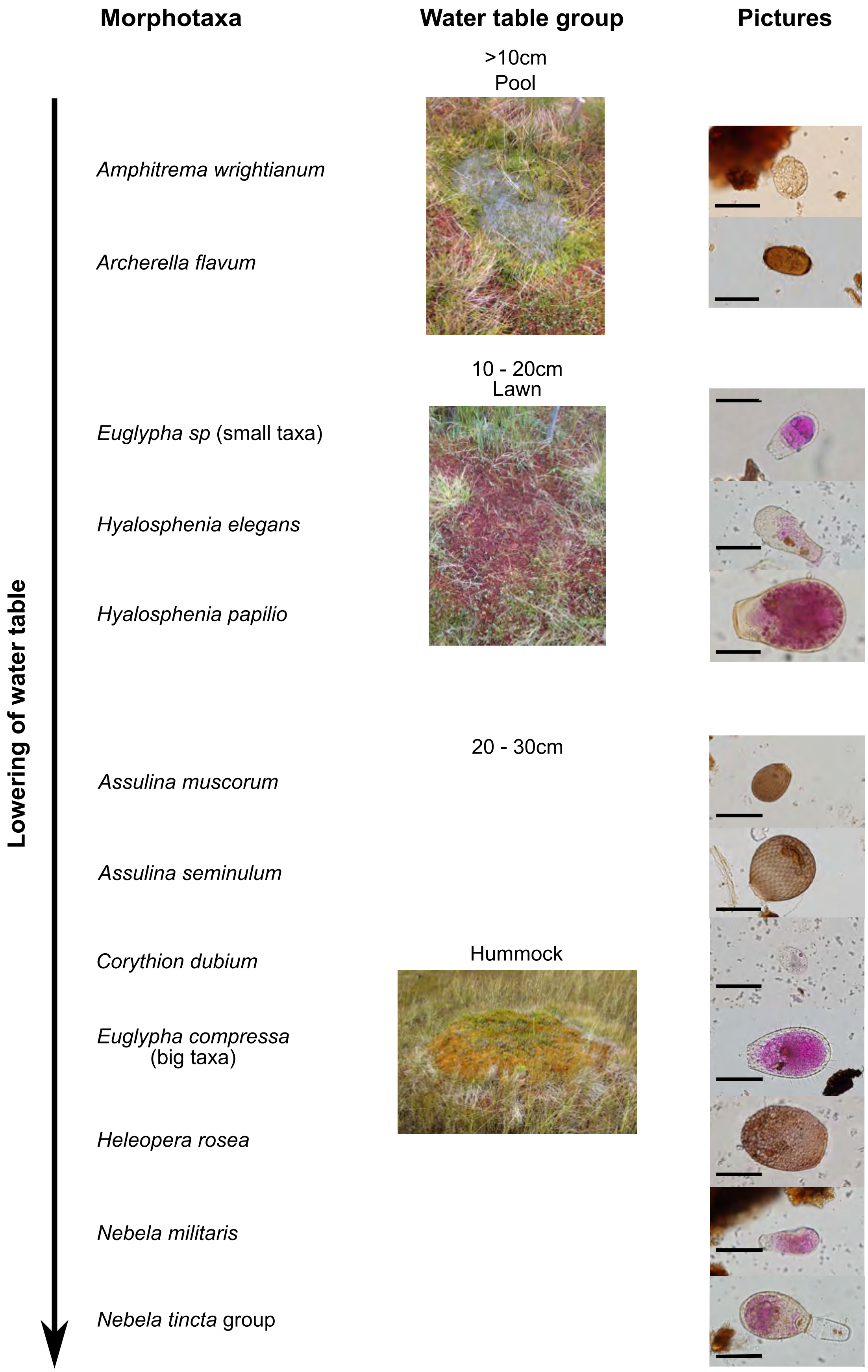


Figure 6.2 – Summary of testate amoeba community structure. The arrow indicated the direction of water stress, corresponding to the lowering of water table. Morphotaxa are grouped in three categories corresponding to water content: high humidity content (water table depth – WTD: $< 10\mathrm{cm}$, corresponding to pools), medium humidity content (WTD: 10 to $20\mathrm{cm}$, corresponding to lawns) and dry conditions (WTD: 20 to $30\mathrm{cm}$ corresponding to hummocks). Pictures respect the proportion between individuals and black line measure $50\mu\mathrm{m}$. NB: only morphotaxa related to this work are included but they do not represent the whole community that could be found in each micro-habitat.

Mixotrophy is both an effect trait reflecting the impact of the taxa on niche dynamic and a response trait to local conditions (Lavorel and Garnier, 2002; Stoecker, 1998; Violle et al., 2007). Mixotrophy gives an advantage when nutrient availability is low or variable by giving an alternative source of energy (Jassey et al., 2015; Stoecker, 1998). It was assessed to enhance carbon fixation in peatlands but this function is lost with rising temperatures or decreasing humidity content, either by the shift towards more heterotrophy (in mixotrophic taxa) or by the loss of mixotrophic taxa themselves (Jassey et al., 2015; Wilken et al., 2013). Thus, including the mixotrophy in bio-monitoring should reflect carbon fixation function together with humidity content (Jassey et al., 2015). In our experiments (Figure 6.3), mixotrophy was the trait which was the best in relation with dryness. In these studies, mixotrophy was mainly depending on the wide presence of *Hyalosphenia papilio*. This nutrition mode is often related to humidity content but as *H. papilio* lives in the very upper part of *Sphagnum* stems, where the light availability is maximal (Marcisz et al., 2014; Mitchell, 2004; Schönborn, 1963), the relationship is not so obvious. Mixotrophy is an important feature in carbon fixation, but it remains largely understudied and available studies were mainly conducted with planktonic or bacterial communities (Matantseva and Skarlato, 2013) or are related to phylogeny (Gomaa et al., 2014). In the context of climate changes, carbon storage and carbon exchange, a deeper understanding of the link between mixotrophy and carbon cycle, also in terrestrial ecosystems, is essential and could help to reduce the pressure on peatlands and wetlands worldwide.

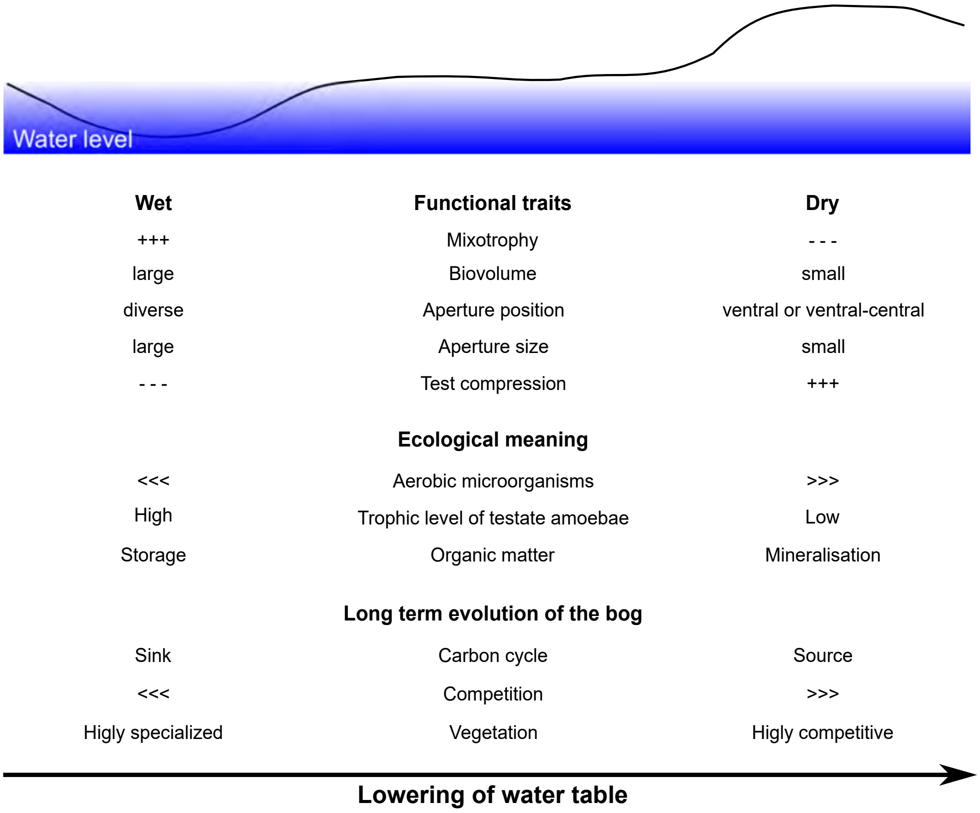
6.3.3 Below-ground – above-ground relationships

If the lowering of the water table impacts the testate amoeba community as an environmental filter, the opposite effect occurs at the upper scale (Figure 6.3). Waterlogged and oligotrophic soils act as a strong driver on plant communities and only taxa with adaptations to the very drastic conditions found in peatlands could settle in (Manneville et al., 2006; Mitsch and Gosselink, 2007). Specialised plants have often low growing rates and are less competitive (K-strategists) than generalist and they can difficultly compete against taxa with high growing rates and high competition qualities (r-strategists). The drainage of peatlands leads to successional changes towards a new equilibrium, often meadows followed by forests (Jukaine et al., 1995; Punttila et al., 2016). Revitalisation measures usually include rewetting by rising water level, removal of trees and introduction of peatlands vegetation (Quinty and Rochefort, 2003; Schumann and Joosten, 2008). Nevertheless, even after rewetting measures, vegetation needs time to recover. In Finland, Haapalehto et al. (2011) found, that if rewetting measures in degraded peatlands reverses the vegetation succession with recovering of bog taxa like *Sphagnum* ssp, and E*riophorum vaginatum*, the process is slow, even if some studies highlighted that recovery could occur in less than eight years (Poulin et al., 2013). Haapalehto et al. (2011) also showed that some vascular plants common in wet hollows (*Scheuchzeria palustris*, *Carex limosa*) were still absent after ten years. Assessing the changes in humidity sooner than by monitoring vegetation successions will improve the follow-up of revitalisation measure in peatlands, at least by indicating if measures for rewetting were sufficient or not.

6.4 Perspectives and follow up

When up-scaled to the entire ecosystem, our results suggested that the regeneration process of damaged peatlands, by rising the ground water level, impact sufficiently, and rapidly, the abiotic conditions in the soil to induce major changes in biotic composition and associated functions. The growing body of evidence demonstrates the clear and rapid response of testate amoebae to hydrological changes in *Sphagnum* peatlands and how these communities relate to essential functions such as C sequestration (Bragazza et al., 2016; Buttler et al., 1998; Laggoun-Defarge et al., 2008). Given the global concern about the effects of ongoing climate change on biodiversity, ecosystem functioning and ultimately ecosystem function relevant to human well-being, potentially simple and informative monitoring tools are precious. The analysis of testate amoebae to assess spatial patterns (e.g. along natural or human-impacted ecological gradients) and temporal (e.g. following ecosystem restoration) changes in the carbon sink function is such a tool. From a management perspective, testate amoebae offer the advantage of providing insights on below-ground processes that are directly relevant for a key function and that change faster than vegetation.

Our framework allowed us to highlight ongoing processes in mesocosms and in the artificial peatbog in the Botanical Garden, but more work should be done to refine the tool. For example,



Wet	Functional traits	Dry
+++	Mixotrophy	- - -
large	Biovolume	small
diverse	Aperture position	ventral or ventral-central
large	Aperture size	small
- - -	Test compression	+++
	Ecological meaning	
<<<	Aerobic microorganisms	>>>
High	Trophic level of testate amoebae	Low
Storage	Organic matter	Mineralisation
	Long term evolution of the bog	
Sink	Carbon cycle	Source
<<<	Competition	>>>
Higly specialized	Vegetation	Higly competitive

Lowering of water table

Figure 6.3 – Evolution of functional traits of testate amoebae along the stress gradient and impact of change in water content at the microbial and ecosystem scales. The shift in traits indicated that environmental filtering increased on the community of testate amoebae, but at the ecosystem scale, the lowering of water table represented a decrease of niche constraints which could favour the arrival of more competitive and fast-growing plants coming from the surroundings.

we do not know the long-term evolution of testate amoeba communities in recovery areas like in Bois des Lattes. The sampling we conducted gave us a snapshot of the situation. Our results indicate that the situation should be stable (little variation between living population and empty tests), but we need to test it all along the year as seasonality is known to affect testate amoeba population (Lamentowicz et al., 2013; Lara et al., 2011; Warner et al., 2007). Permanent plots in regeneration areas and in natural peatlands, with seasonal or annual sampling could improve our knowledge on variation of testate amoeba community structure and community weighted mean of traits in response to rewetting measures. Even if discussed today, testate amoebae are quite cosmopolite and identical morphotaxa are found overall in comparable ecosystems, at least in the northern hemisphere (Finlay and Clarke, 1999; Mitchell et al., 2008b). Therefore, creating a monitoring tool based on testate amoebae is not an utopia. Recently, a transfer function was made with a pan-European dataset of testate amoebae with the aim to calibrate the reconstruction of palaeohydrology across Europe (Amesbury et al., 2016). To achieve that, they defined morphotaxa (related as "type") to overcome taxonomic inconstancy, and provided a calibration framework that should allow the reconstruction of past climates by inferring water table fluctuation in European peatlands. The same work should be done to construct and calibrate identification keys for bio-monitoring. Moreover, as most relevant traits in testate amoebae are related to specific characteristics (mixotrophy, pseudostome position, test compression, test material, even class size, etc.), a reference guide comparable to the databases for life-history traits of European flora (Kleyer et al., 2008) and for micro-invertebrate (BETSI, 2012; Moretti et al., 2017), should be set up as an interpretation key for ongoing processes. Large existing datasets could then be used to calibrate this tool, applicable in peatlands monitoring at a large scale, at least in Europe. As highlighted by Andersen et al. (2017) and Bonnett et al. (2011), following revitalisation measures with easy-to-handle, inexpensive tools is primordial, both to assess the success of revitalisation, particularly in terms of carbon and nutrient cycles, and also for biodiversity preservation, and to provide a feedback to taxpayers.

Furthermore, few researches, if any (but see Meyer et al., 2013), were conducted on testate amoeba functional traits responding to variation in nitrogen and carbon dioxide content or impact of pollutants on *Sphagnum* peatlands. These factors were known to deeply impact peatlands evolution and induced massive changes in the community structure of testate amoebae (Bragazza et al., 2006, 2012; Breeuwer et al., 2008; Meyer et al., 2012; Mitchell, 2004; Nguyen-Viet et al., 2008). The definition of functional traits well correlated to changes in nutrient content of peatlands will add value to support the introduction of testate amoebae in practical bio-monitoring. Even if peatlands restoration is mainly related to the water table depth, atmospheric deposition of fertilisers could shift a bog to a fen or allow fast-growing plants to settle even in humid conditions and switch a peatland from carbon sink to carbon source (Bragazza et al., 2006; Freeman et al., 2004; Turunen et al., 2004).

6.5 Conclusion

At each step of this work, we aimed to provide conditions which could be applied in practice. Testate amoeba communities react quickly to changes in humidity and a selection of ten easy-to-determine taxa together with community weighted mean of functional traits on the whole community made it possible to analyse ongoing processes. With only two hours per sample on well-selected samples (i.e. along a humidity gradient, with respect of patch size, along a regeneration gradient, etc.) the ongoing processes could be assessed with small means and no prior taxonomic training. The use of testate amoeba functional traits as a tool to follow changes has only recently started to be explored and is not yet used in applied research (Marcisz et al., 2014, 2016; Payne et al., 2016a). With further development and optimisation, this kind of tool could be very useful for peatland ecosystem management, and could be used by non-specialists, in complement to routine approaches such as vegetation surveys or water table depth measurements. User-friendly illustrated identification keys with ecological information for each morphotaxon could help the implementation of testate amoeba community and functional traits analyses in *Sphagnum* peatlands bio-monitoring. An additional step could be made with the set-up of a web platform, allowing to upload datasets of testate amoeba communities and community weighted mean of traits, based only on taxonomic inventory. A full analysis of these communities should then help to widely use testate amoebae in bio-monitoring. Such platforms already exist, for example, the NINJA platform (Nematode INdicator Joint Analysis) that gives a diagnosis of soil quality based on nematodes community structure (Sieriebriennikov et al., 2014).

Peatlands are now rare in Switzerland, they have suffered a loss of 90% of surfaces together with an alteration of hydrology and/or nutrient pollution in most of the remaining surfaces (Graf et al., 2010; Grünig, 1994). Conserving the remaining bogs and restoring the damaged ones is therefore a major challenge (Graf et al., 2010). Peatlands host glacial relic communities of plants, animals and other organisms, most of which are locally threatened and further endangered by climate changes and human pressure. Additional tools to assess their health and recent evolution based not only on vegetation surveys but also focusing on belowground organisms and processes would provide useful information to help to support conservation and restoration efforts. This ultimately improves the capacity of peatlands to provide ecosystem services in Switzerland.

Bibliography

Ackerly, D. D. and Cornwell, W. K. A trait-based approach to community assembly: partitioning of species trait values into within-and among-community components. *Ecology letters*, 10(2):135–145, 2007. 00464. Page 4, Page 62

Alley, T. R. Competition theory, evolution, and the concept of an ecological niche. *Acta Biotheor*, 31(3): 165–179, September 1982. ISSN 0001-5342, 1572-8358. doi:10.1007/BF01857239. 00101. Page 101

Amesbury, M. J., Mallon, G., Charman, D. J., Hughes, P. D. M., Booth, R. K., Daley, T. J., and Garneau, M. Statistical testing of a new testate amoeba-based transfer function for water-table depth reconstruction on ombrotrophic peatlands in north-eastern Canada and Maine, United States. *Journal of Quaternary Science*, 28(1):27–39, January 2013. ISSN 0267-8179. doi:10.1002/jqs.2584. Page 6, Page 61

Amesbury, M. J., Swindles, G. T., Bobrov, A., Charman, D. J., Holden, J., Lamentowicz, M., Mallon, G., Mazei, Y., Mitchell, E. A., Payne, R. J., Roland, T. P., Turner, T. E., and Warner, B. G. Development of a new pan-European testate amoeba transfer function for reconstructing peatland palaeohydrology. *Quaternary Science Reviews*, 152:132–151, November 2016. ISSN 02773791. doi:10.1016/j.quascirev.2016.09.024. 00005. Page 63, Page 104

Andersen, R., Chapman, S., and Artz, R. Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry*, 57:979–994, 2013. ISSN 00380717. doi:10.1016/j.soilbio.2012.10.003. 00059. Page 33, Page 100

Andersen, R., Farrell, C., Graf, M., Muller, F., Calvar, E., Frankard, P., Caporn, S., and Anderson, P. An overview of the progress and challenges of peatland restoration in Western Europe. *Restor Ecol*, 25(2):271–282, March 2017. ISSN 1526-100X. doi:10.1111/rec.12415. 00003. Page 97, Page 104

Anderson, M. J. A new method for non-parametric multivariate analysis of variance: NON-PARAMETRIC MANOVA FOR ECOLOGY. *Austral Ecology*, 26(1):32–46, February 2001. ISSN 14429985. doi:10.1111/j.1442-9993.2001.01070.pp.x. 00000. Page 82

Anderson, O. R. The density and diversity of gymnamoebae associated with terrestrial moss communities (Bryophyta : Bryopsida) in a northeastern US forest. *Journal of Eukaryotic Microbiology*, 53(4):275–279, July 2006. ISSN 1066-5234. doi:10.1111/j.1550-7408.2006.00103.x. Page 6

Arneth, A., Harrison, S. P., Zaehle, S., Tsigaridis, K., Menon, S., Bartlein, P. J., Feichter, J., Korhola, A., Kulmala, M., O'Donnell, D., Schurgers, G., Sorvari, S., and Vesala, T. Terrestrial biogeochemical feedbacks in the climate system. *Nature Geosci*, 3(8):525–532, 2010. ISSN 1752-0894. doi:http://www.nature.com/ngeo/journal/v3/n8/suppinfo/ngeo905$_S1.html$. Page 5, Page 15

Arrieira, R. L., Fatoreto Schwind, L. T., Bonecker, C. C., and Lansac-Toha, F. A. Use of functional diversity to assess determinant assembly processes of testate amoebae community. *Aquatic Ecology*, 49(4):561–571, 2015. doi:10.1007/s10452-015-9546-z. 00000 WOS:000363963300011. Page 4, Page 6, Page 61, Page 79, Page 88

Artz, R. R. E., Chapman, S. J., Siegenthaler, A., Mitchell, E. A. D., Buttler, A., Bortoluzzi, E., Gilbert, D., Yli-Petays, M., Vasander, H., and Francez, A.-J. Functional microbial diversity in regenerating cutover peatlands responds to vegetation succession. *Journal of Applied Ecology*, 45(6):1799–1809, December 2008. ISSN 00218901, 13652664. doi:10.1111/j.1365-2664.2008.01573.x. 00025. Page 49

Barber, K. E. Peatlands as scientific archives of past biodiversity. *Biodivers Conserv*, 2(5):474–489, 1993. ISSN 0960-3115, 1572-9710. doi:10.1007/BF00056743. 00106. Page 5

Barnett, A. J., Finlay, K., and Beisner, B. E. Functional diversity of crustacean zooplankton communities: towards a trait-based classification. *Freshwater Biology*, 58(8):1755–1765, 2013. doi:10.1111/j.1365-2427.2007.01733.x. 00113. Page 61

Bartkowski, B., Lienhoop, N., and Hansjürgens, B. Capturing the complexity of biodiversity: A critical review of economic valuation studies of biological diversity. *Ecological Economics*, 113:1–14, 2015. ISSN 0921-8009. Page 61

Baselga, A. and Orme, C. D. L. betapart : an R package for the study of beta diversity: *Betapart package. Methods in Ecology and Evolution*, 3(5):808–812, October 2012. ISSN 2041210X. doi:10.1111/j.2041-210X.2012.00224.x. 00000. Page 37

Belyea, L. R. and Lancaster, J. Assembly rules within a contingent ecology. *Oikos*, pages 402–416, 1999. 00469. Page 62

Belyea, L. and Malmer, N. Carbon sequestration in peatland: patterns and mechanisms of response to climate change. *Global Change Biology*, 10(7):1043–1052, 2004. ISSN 1365-2486. doi:10.1111/j.1529-8817.2003.00783.x. 00000. Page 5, Page 6, Page 61, Page 97

Benton, T. G., Solan, M., Travis, J. M. J., and Sait, S. M. Microcosm experiments can inform global ecological problems. *Trends in Ecology & Evolution*, 22(10):516–521, October 2007. ISSN 0169-5347. doi:10.1016/j.tree.2007.08.003. 00143. Page 10, Page 79, Page 88

BETSI, A. Database for Functional Traits of Soil Invertebrates. *French Foundation for Biodiversity Research*, 2012. 00002. Page 4, Page 104

Beyens, L. and Chardez, D. An annotated list of testate amoebae observed in the Arctic between the longitudes 27 degrees E and 168 degrees W. *Arch Protistenkd*, 146(2):219–233, November 1995. ISSN 0003-9365. Page 27

Beyens, L., Chardez, D., Delandtsheer, R., Debock, P., and Jacques, E. Testate Amebas Populations from Moss and Lichen Habitats in the Arctic. *Polar Biol*, 5(3):165–173, 1986. ISSN 0722-4060. doi:10.1007/Bf00441696. 00000. Page 44

Beyens, L., Chardez, D., Debaere, D., Debock, P., and Jacques, E. Ecology of Terrestrial Testate Amebas Assemblages from Coastal Lowlands on Devon Island (Nwt, Canadian Arctic). *Polar Biol*, 10(6):431–440, June 1990. ISSN 0722-4060. Page 101

Blaum, N., Mosner, E., Schwager, M., and Jeltsch, F. How functional is functional? Ecological groupings in terrestrial animal ecology: towards an animal functional type approach. *Biodivers Conserv*, 20(11):2333–2345, 2011. ISSN 0960-3115. doi:10.1007/s10531-011-9995-1. Page 4

Bobrov, A. A., Charman, D. J., and Warner, B. G. Ecology of testate amoebae (Protozoa: Rhizopoda) on peatlands in western Russia with special attention to niche separation in closely related taxa. *Protist*, 150 (2):125–36, August 1999. ISSN 1434-4610 (Print) 1434-4610 (Linking). Page 6, Page 87

Bobrov, A., Charman, D., and Warner, B. Ecology of testate amoebae from oligotrophic peatlands: specific features of polytypic and polymorphic species. *Biology Bulletin*, 29(6):605–617, 2002. ISSN 1062-3590. Page 44, Page 69, Page 99

Bockstael, N. E., Freeman, A. M., Kopp, R. J., Portney, P. R., and Smith, V. K. On Measuring Economic Values for Nature. *Environmental Science & Technology*, 34(8):1384–1389, 2000. ISSN 0013-936X. doi:10.1021/es990673l. 00428. Page 61

Bonn, A., Allott, T., Evans, M., Joosten, H., and Stoneman, R. *Peatland Restoration and Ecosystem Services: Science, Policy and Practice.* Cambridge University Press, 2016. 00002. Page 61, Page 79

Bonnet, L. Le peuplement thécamobien des sols. *Revue d'Ecologie et de Biologie du Sol*, 1:123–408, 1964. 00000. Page 16, Page 37

Bonnet, L. Ecologie de quelques euglyphidae (thécamoebiens, filosea) des milieux édaphiques et para édaphiques (première partie: genres corythion et trinema). *Bulletin de la Société d'Histoire Naturelle de Toulouse*, 127:7–13, 1991. Page 44

Bonnett, S. A., Ross, S., Linstead, C., and Maltby, E. A review of techniques for monitoring the success of peatland restoration. Technical Report, Natural England, 2009. 00004. Page 6

Bonnett, S. A., Linsted, R., Ross, S., and Maltby, E. Guidelines for monitoring the success of peatland restoration. Technical Report, Natural England, 2011. 00002. Page 6, Page 79, Page 97, Page 99, Page 104

Booth, R. K. Ecology of testate amoebae (Protozoa) in two Lake Superior coastal wetlands: Implications for paleoecology and environmental monitoring. *Wetlands*, 21(4):564–576, December 2001. ISSN 0277-5212. doi:10.1672/0277-5212(2001)021[0564:eotapi]2.0.co;2. Page 6, Page 44, Page 48, Page 69, Page 88

Booth, R. K. Testate amoebae as paleoindicators of surface-moisture changes on Michigan peatlands: modern ecology and hydrological calibration. *J Paleolimnol*, 28(3):329–348, September 2002. ISSN 0921-2728. doi:10.1023/A:1021675225099. Page 34, Page 44, Page 88

Booth, R. K., Hotchkiss, S. C., and Wilcox, D. A. Discoloration of polyvinyl chloride (PVC) tape as a proxy for water-table depth in peatlands: validation and assessment of seasonal variability. *Funct Ecol*, 19(6): 1040–1047, December 2005. ISSN 0269-8463. doi:10.1111/j.1365-2435.2005.01048.x. 00019. Page 18

Booth, R. K., Sullivan, M. E., and Sousa, V. A. Ecology of testate amoebae in a North Carolina pocosin and their potential use as environmental and paleoenvironmental indicators. *Ecoscience*, 15(2):277–289, 2008. ISSN 1195-6860. doi:10.2980/15-2-3111. Page 6, Page 26

Booth, R., Lamentowicz, M., and Charman, D. Preparation and analysis of testate amoebae in peatland paleoenvironmental studies. *Mires and Peat*, 7(02):1–7, 2010. Page 18, Page 36, Page 62, Page 80

Booth, R. K. Testate amoebae as proxies for mean annual water-table depth in Sphagnum-dominated peatlands of North America. *Journal of Quaternary Science*, 23(1):43–57, January 2008. ISSN 0267-8179. doi:10.1002/jqs.1114. Page 34, Page 61

Booth, R. K. and Meyers, B. Environmental Controls on Pore Number in Hyalosphenia papilio: Implications for Paleoenvironmental Reconstruction. *Acta Protozoologica*, 49(1):29–35, 2010. ISSN 0065-1583. Page 68, Page 87

Booth, R. K. and Zygmunt, J. R. Biogeography and comparative ecology of testate amoebae inhabiting Sphagnum-dominated peatlands in the Great Lakes and Rocky Mountain regions of North America. *Diversity and Distributions*, 11(6):577–590, 2005. Page 15, Page 61

Boyd, J. and Banzhaf, S. What are ecosystem services? The need for standardized environmental accounting units. *Ecological Economics*, 63(2–3):616–626, 2007. ISSN 0921-8009. doi:10.1016/j.ecolecon.2007.01.002. 01302. Page 3

Bragazza, L., Buttler, A., Habermacher, J., Brancaleoni, L., Gerdol, R., Fritze, H., Hanajik, P., Laiho, R., and Johnson, D. High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. *Global Change Biol*, 18(3):1163–1172, March 2012. ISSN 1354-1013. doi:10.1111/j.1365-2486.2011.02585.x. Page 105

Bragazza, L. Sphagnum Niche diversification in Two Oligotrophic Mires in the Southern Alps of Italy. *Bryologist*, 100(4):507–515, 1997. ISSN 0007-2745. 00000. Page 25

Bragazza, L. and Gerdol, R. Are nutrient availability and acidity-alkalinity gradients related in Sphagnum-dominated peatlands? *Journal of Vegetation Science*, 13:473–482, 2002. Page 25

Bragazza, L., Freeman, C., Jones, T., Rydin, H., Limpens, J., Fenner, N., Ellis, T., Gerdol, R., Hajek, M., Hajek, T., Lacumin, P., Kutnar, L., Tahvanainen, T., and Toberman, H. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences of the United States of America*, 103(51):19386–19389, December 2006. ISSN 0027-8424. doi:10.1073/pnas.0606629104. Page 105

Bragazza, L., Buttler, A., Siegentaller, A., and Mitchell, E. A. D. Plant Litter Decomposition and Nutrient Release in Peatlands. In *Carbon Cycling in Northern Peatlands*. American Geophysical Union, 2009. Page 99

Bragazza, L., Buttler, A., Robroek, B. J. M., Albrecht, R., Zaccone, C., Jassey, V. E. J., and Signarbieux, C. Persistent high temperature and low precipitation reduce peat carbon accumulation. *Glob Chang Biol*, 2016. ISSN 1365-2486. doi:10.1111/gcb.13319. 00000. Page 5, Page 47, Page 48, Page 69, Page 99, Page 100, Page 103

Breeuwer, A., Heijmans, M., Robroek, B. J. M., Limpens, J., and Berendse, F. The effect of increased temperature and nitrogen deposition on decomposition in bogs. *Oikos*, 117(8):1258–1268, August 2008. ISSN 00301299, 16000706. doi:10.1111/j.0030-1299.2008.16518.x. 00043. Page 105

Bridgham, S. D., Megonigal, J. P., Keller, J. K., Bliss, N. B., and Trettin, C. The carbon balance of North American wetlands. *Wetlands*, 26(4):889–916, 2006. 00452. Page 5, Page 6, Page 97

Briones, M. J. I., McNamara, N. P., Poskitt, J., Crow, S. E., and Ostle, N. J. Interactive biotic and abiotic regulators of soil carbon cycling: evidence from controlled climate experiments on peatland and boreal soils. *Global change biology*, 20(9):2971–2982, 2014. 00012. Page 61

Buckland, P. C. Peatland archaeology: a conservation resource on the edge of extinction. *Biodiversity & Conservation*, 2(5):513–527, 1993. 00063. Page 33

Buttler, A., Grosvernier, P., and Matthey, Y. Development of Sphagnum fallax diaspores on bare peat with implications for the restoration of cut-over bogs. *J Appl Ecol*, 35(5):800–810, October 1998. ISSN 0021-8901. Page 25, Page 103

Buttler, A., Warner, B. G., Grosvernier, P., and Matthey, Y. Vertical Patterns of Testate Amoebae (Protozoa: Rhizopoda) and Peat-Forming Vegetation on Cutover Bogs in the Jura, Switzerland. *New Phytol*, 134(2): 371–382, October 1996. ISSN 0028646X. Page 18, Page 26, Page 34, Page 61, Page 87, Page 88

Cadotte, M. W., Cavender-Bares, J., Tilman, D., and Oakley, T. H. Using Phylogenetic, Functional and Trait Diversity to Understand Patterns of Plant Community Productivity. *PLoS ONE*, 4(5):e5695, 2009. doi:10.1371/journal.pone.0005695. Page 3

Cadotte, M. W., Carscadden, K., and Mirotchnick, N. Beyond species: functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology*, 48(5):1079–1087, 2011. ISSN 1365-2664. doi:10.1111/j.1365-2664.2011.02048.x. Page 3, Page 4, Page 10, Page 61

Cangelosi, R. and Goriely, A. Component retention in principal component analysis with application to cDNA microarray data. *Biology direct*, 2(1):2, 2007. 00108. Page 83

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., and Naeem, S. Biodiversity loss and its impact on humanity. *Nature*, 486(7401):59–67, 2012. ISSN 0028-0836. doi:10.1038/nature11148. 01363. Page 3

Carlson, M. L., Flagstad, L. A., Gillet, F., and Mitchell, E. A. D. Community development along a proglacial chronosequence: are above-ground and below-ground community structure controlled more by biotic than abiotic factors? *Journal of Ecology*, 98(5):1084–1095, 2010. ISSN 1365-2745. doi:10.1111/j.1365-2745.2010.01699.x. Page 15

Caron, D. A., Worden, A. Z., Countway, P. D., Demir, E., and Heidelberg, K. B. Protists are microbes too: a perspective. *The ISME journal*, 3(1):4–12, January 2009. ISSN 1751-7370 (Electronic) 1751-7362 (Linking). doi:10.1038/ismej.2008.101. Page 79

Cavalier-Smith, T. and Chao, E. E. Y. Phylogeny and Classification of Phylum Cercozoa (Protozoa). *Protist*, 154(3–4):341–358, October 2003. ISSN 1434-4610. doi:10.1078/143446103322454112. Page 79

Cavender-Bares, J., Kozak, K. H., Fine, P. V. A., and Kembel, S. W. The merging of community ecology and phylogenetic biology. *Ecol Lett*, 12(7):693–715, 2009. ISSN 1461-023X. doi:10.1111/j.1461-0248.2009.01314.x. Page 82

Chambers, F. M., Booth, R. K., De Vleeschouwer, F., Lamentowicz, M., Le Roux, G., Mauquoy, D., Nichols, J. E., and Van Geel, B. Development and refinement of proxy-climate indicators from peats. *Quaternary International*, 268:21–33, 2012. 00062. Page 15, Page 61

Chapin III, F. S., Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M., Reynolds, H. L., Hooper, D. U., Lavorel, S., Sala, O. E., and Hobbie, S. E. Consequences of changing biodiversity. *Nature*, 405(6783):234–242, 2000. Page 3, Page 4

Chapman, S., Buttler, A., Francez, A., Laggoun-Defarge, F., Vasander, H., Schloter, M., Combe, J., Grosvernier, P., Harms, H., Epron, D., Gilbert, D., and Mitchell, E. Exploitation of northern peatlands and biodiversity maintenance: a conflict between economy and ecology. *Front. Ecol. Environ.*, 1(10):525–532, 2003. ISSN 1540-9295. doi:10.2307/3868163. 00000 WOS:000221791700013. Page 5, Page 33

Charman, D. J. Biostratigraphic and palaeoenvironmental applications of testate amoebae. *Quaternary Sci Rev*, 20(16-17):1753–1764, 2001. ISSN 0277-3791. Page 6, Page 15, Page 18, Page 36, Page 79

Charman, D. J. and Warner, B. G. Relationship between Testate Amebas (Protozoa, Rhizopoda) and Microenvironmental Parameters on a Forested Peatland in Northeastern Ontario. *Can J Zool*, 70(12):2474–2482, December 1992. ISSN 0008-4301. doi:10.1139/z92-331. Page 6

Charman, D., Hendon, D., and Woodland, W. The identification of testate amoebae (Protozoa: Rhizopoda) in peats, Technical Guide No. 9. In Association, Q. R., editor, *Quaternary Science Reviews*, volume 23, page 1869. Paul, Hughes, 2004. ISBN 0-907780-48-2. Page 18, Page 62, Page 80

Chase, J. M., Kraft, N. J., Smith, K. G., Vellend, M., and Inouye, B. D. Using null models to disentangle variation in community dissimilarity from variation in -diversity. *Ecosphere*, 2(2):1–11, 2011. 00213. Page 82, Page 88

Church, A., Fish, R., Haines-Young, R., Mourato, S., Tratalos, J., Stapleton, L., and Willis, C. *UK National Ecosystem Assessment Follow-on. Work Package Report 5: Cultural ecosystem services and indicators.* UK national ecosystem assessment follow-on: cultural ecosystem services and indicators, 2014. 00035. Page 79

Clarke, K. Non-parametric multivariate analyses of changes in community structure. *Australian journal of ecology*, 18(1):117–143, 1993. 09001. Page 64

Colwell, R. K. Biodiversity: concepts, patterns, and measurement. *The Princeton guide to ecology*, pages 257–263, 2009. 00091. Page 3

Cornwell, W.K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E, and Eviner, V. T. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters*, pages 1065–1071, 2008. Page 4

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'neill, R. V., Paruelo, J., and others. The value of the world's ecosystem services and natural capital. *Ecological economics*, 25(1):3–16, 1998. 00729. Page 3, Page 97

Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S. J., Kubiszewski, I., Farber, S., and Turner, R. K. Changes in the global value of ecosystem services. *Global Environmental Change*, 26:152–158, 2014. ISSN 0959-3780. doi:10.1016/j.gloenvcha.2014.04.002. 00849. Page 3

Coûteaux, M.-M. and Darbyshire, J. F. Functional diversity amongst soil protozoa. *Applied Soil Ecology*, 10(3): 229–237, 1998. Page 6

Daniels, R. E. and Eddy, A. Handbook of European 'Sphagna'. *National environmental research council*, 1990. ISSN 0-904282-82-1. Page 17

Davidson, E. A. and Janssens, I. A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440(7081):165–73, March 2006. ISSN 1476-4687 (Electronic) 0028-0836 (Linking). doi:10.1038/nature04514. Page 5, Page 15, Page 33

Davis, S. R. and Wilkinson, D. M. The conservation management value of testate amoebae as 'restoration' indicators: speculations based on two damaged raised mires in northwest England. *Holocene*, 14(1):135–143, January 2004. ISSN 0959-6836. doi:10.1191/0959683604h1696rp. 00000. Page 15, Page 34

Díaz, S., Lavorel, S., Chapin, F. S., Tecco, P. A., Gurvich, D. E., and Grigulis, K. Functional diversity—at the crossroads between ecosystem functioning and environmental filters. *Terrestrial ecosystems in a changing world*, pages 81–91, 2007. Page 3, Page 4

Díaz, S., Purvis, A., Cornelissen, J. H. C., Mace, G. M., Donoghue, M. J., Ewers, R. M., Jordano, P., and Pearse, W. D. Functional traits, the phylogeny of function, and ecosystem service vulnerability. *Ecol Evol*, 3(9): 2958–2975, September 2013. ISSN 2045-7758. doi:10.1002/ece3.601. 00075. Page 4

Daza Secco, E., Haapalehto, T., Haimi, J., Meissner, K., and Tahvanainen, T. Do testate amoebae communities recover in concordance with vegetation after restoration of drained peatlands? *Mires and Peat*, 18(18):1–14, 2016. doi:10.19189/MaP.2016.OMB.231. 00000. Page 34, Page 49

de Bello, F., Lavorel, S., Díaz, S., Harrington, R., Cornelissen, J. H., Bardgett, R. D., Berg, M. P., Cipriotti, P., Feld, C. K., Hering, D., and others. Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation*, 19(10):2873–2893, 2010. 00375. Page 90, Page 99

Delaine, M., Bernard, N., Gilbert, D., Recourt, P., and du Châtelet, E. A. Origin and diversity of testate amoebae shell composition: Example of Bullinularia indica living in Sphagnum capillifolium. *European Journal of Protistology*, 59:14–25, 2017. 00000. Page 79

Delarze, R., Gonseth, Y., and Galland, P., editors. *Guide des milieux naturels de Suisse : écologie, menaces, espèces caractéristiques*. Rossolis, Bussigny, 2e éd., entièrement revue et corrigée edition, 2008. ISBN 978-2-940365-16-6. Page 21

Diaz, S., Lavorel, S., de Bello, F., Quetier, F., Grigulis, K., and Robson, T. M. Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences of the United States of America*, 104(52):20684–9, 2007. ISSN 1091-6490 (Electronic) 0027-8424 (Linking). doi:10.1073/pnas.0704716104. Page 4

Dieleman, C. M., Branfireun, B. A., McLaughlin, J. W., and Lindo, Z. Climate change drives a shift in peatland ecosystem plant community: Implications for ecosystem function and stability. *Global Change Biology*, 21 (1):388–395, 2014. ISSN 13541013. doi:10.1111/gcb.12643. 00021. Page 69

Dolédec, S. and Chessel, D. Rythmes saisonniers et composantes stationnelles en milieu aquatique I- Description d'un plan d'observations complet par projection de variables. *Acta Oecologica, Oecologia Generalis*, 8 (3):403–426, 1987. Page 19

Dray, S. and Legendre, P. Testing the species traits-environment relationships: the fourth-corner problem revisited. *Ecology*, 89(12):3400–3412, 2008. ISSN 0012-9658. 00231. Page 36, Page 64, Page 82

Dunger, W., Wanner, M., Hauser, H., Hohberg, K., Schulz, H. J., Schwalbe, T., Seifert, B., Vogel, J., Voigtlander, K., Zimdars, B., and Zulka, K. P. Development of soil fauna at mine sites during 46 years after afforestation. *Pedobiologia*, 45(3):243–271, May 2001. ISSN 0031-4056. doi:10.1078/0031-4056-00083. Page 62

Ecker, K., Kuchler, M., Feldmeyer-Christe, E., Graf, U., and Waser, L. T. Predictive mapping of floristic site conditions across mire habitats: Evaluating data requirements. *Community Ecol*, 9(2):133–146, December 2008. ISSN 1585-8553. doi:10.1556/Comec.9.2008.2.2. 00000. Page 8, Page 17, Page 49

Euliss Jr, N. H., Mushet, D. M., Smith, L. M., Conner, W. H., Burkett, V. R., Wilcox, D. A., Hester, M. W., and Zheng, H. Ecosystem Services: Developing Sustainable Management Paradigms Based on Wetland Functions and Processes. In *Wetland Techniques*, pages 181–227. Springer, 2013. 00000. Page 4

European Commission. Directions towards sustainable agriculture, 1999. 00000. Page 61

Finlay, B. J. and Clarke, K. J. Ubiquitous dispersal of microbial species. *Nature*, 400(6747):828–828, 1999. ISSN 0028-0836. Page 6, Page 104

Finlayson, C. M. *Monitoring ecological change in wetlands.* na, 1994. 00019. Page 61

Fisher, B., Turner, K., Zylstra, M., Brouwer, R., de Groot, R., Farber, S., Ferraro, P., Green, R., Hadley, D., Harlow, J., Jefferiss, P., Kirkby, C., Morling, P., Mowatt, S., Naidoo, R., Paavola, J., Strassburg, B., Yu, D., and Balmford, A. Ecosystem Services and Economic Theory: Integration for Policy-Relevant Research. *Ecol Appl*, 18(8):2050–2067, 2008. ISSN 1051-0761. doi:10.1890/07-1537.1. 00000. Page 3

Fleischer, E., Khashimov, I., Hölzel, N., and Klemm, O. Carbon exchange fluxes over peatlands in Western Siberia: Possible feedback between land-use change and climate change. *Sci. Total Environ.*, 545-546:424–433, 2016. ISSN 1879-1026. doi:10.1016/j.scitotenv.2015.12.073. 00000. Page 33, Page 97, Page 99

Foissner, W. Biogeography and dispersal of micro-organisms: A review emphasizing protists. *Acta Protozool*, 45(2):111–136, 2006. ISSN 0065-1583. 00409. Page 6

Fournier, B., Malysheva, E., Mazei, Y., Moretti, M., and Mitchell, E. A. Toward the use of testate amoeba functional traits as indicator of floodplain restoration success. *European Journal of Soil Biology*, 49:85–91, March 2012. ISSN 11645563. doi:10.1016/j.ejsobi.2011.05.008. 00023. Page 4, Page 6, Page 34, Page 47, Page 61, Page 62, Page 63, Page 70, Page 79, Page 81, Page 88, Page 90, Page 100, Page 101

Fournier, B., Lara, E., Jassey, V. E., and Mitchell, E. A. Functional traits as a new approach for interpreting testate amoeba palaeo-records in peatlands and assessing the causes and consequences of past changes in species composition. *The Holocene*, 25(9):1375–1383, 2015. Page 6, Page 34, Page 47, Page 48, Page 61, Page 63, Page 70, Page 79, Page 81, Page 87, Page 88, Page 90, Page 99, Page 100, Page 101

Fournier, B., Coffey, E. E. D., van der Knaap, W. O., Fernandez, L. D., Bobrov, A., and Mitchell, E. A. D. A legacy of human-induced ecosystem changes: spatial processes drive the taxonomic and functional diversities of testate amoebae in Sphagnum peatlands of the Galapagos. *Journal of Biogeography*, 43(3):533–543, 2016. doi:10.1111/jbi.12655. 00000 WOS:000370450900010. Page 6, Page 34, Page 47, Page 61, Page 69

Fraser, L. H. and Keddy, P. The role of experimental microcosms in ecological research. *Trends in Ecology & Evolution*, 12(12):478–481, 1997. ISSN 0169-5347. doi:10.1016/S0169-5347(97)01220-2. 00000. Page 10

Freeman, C., Fenner, N., Ostle, N. J., Kang, H., Dowrick, D. J., Reynolds, B., Lock, M. A., Sleep, D., Hughes, S., and Hudson, J. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature*, 430(6996):195–198, 2004. 00518. Page 105

Frolking, S. and Roulet, N. T. Holocene radiative forcing impact of northern peatland carbon accumulation and methane emissions. *Global Change Biology*, 13(5):1079–1088, 2007. 00193. Page 61

Frontier, S., Pichod-Viale, D., Leprêtre, A., Davoult, D., and Luczak, C. *Ecosystèmes : structure, fonctionnement, évolution : [Master, CAPES, agrégation].* Sciences SUP. Sciences de la vie. Dunod, Paris, 4e éd. edition, 2008. ISBN 978-2-10-051935-4. 00000. Page 4

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., Slade, E. M., Steffan-Dewenter, I., Emmerson, M., Potts, S. G., Tscharntke, T., Weisser, W., and Bommarco, R. Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proceedings. Biological sciences / The Royal Society*, 282(1801):20142620, 2015. ISSN 1471-2954 (Electronic) 0962-8452 (Linking). doi:10.1098/rspb.2014.2620. Page 4

Galka, M., Miotk-Szpiganowicz, G., Goslar, T., Jesko, M., van der Knaap, W. O., and Lamentowicz, M. Palaeohydrology, fires and vegetation succession in the southern Baltic during the last 7500 years reconstructed from a raised bog based on multi-proxy data. *Palaeogeography Palaeoclimatology Palaeoecology*, 370:209–221, January 2013. ISSN 0031-0182. doi:10.1016/j.palaeo.2012.12.011. Page 79

Gavazov, K., Ingrisch, J., Hasibeder, R., Mills, R. T., Buttler, A., Gleixner, G., Pumpanen, J., and Bahn, M. Winter ecology of a subalpine grassland: Effects of snow removal on soil respiration, microbial structure and function. *Science of the Total Environment*, 590:316–324, 2017. Page 47

Geisen, S., Rosengarten, J., Koller, R., Mulder, C., Urich, T., and Bonkowski, M. Pack hunting by a common soil amoeba on nematodes: Pack-hunting protists attacking nematodes. *Environmental Microbiology*, 17 (11):4538–4546, 2015. ISSN 14622912. doi:10.1111/1462-2920.12949. 00000. Page 47

Geisen, S., Mitchell, E. A. D., Wilkinson, D. M., Adl, S., Bonkowski, M., Brown, M. W., Fiore-Donno, A. M., Heger, T. J., Jassey, V. E. J., Krashevska, V., Lahr, D. J. G., Marcisz, K., Mulot, M., Payne, R., Singer, D., Anderson, O. R., Charman, D. J., Ekelund, F., Griffiths, B. S., Rønn, R., Smirnov, A., Bass, D., Belbahri, L., Berney, C., Blandenier, Q., Chatzinotas, A., Clarholm, M., Dunthorn, M., Feest, A., Fernández, L. D., Foissner, W., Fournier, B., Gentekaki, E., Hájek, M., Helder, J., Jousset, A., Koller, R., Kumar, S., La Terza, A., Lamentowicz, M., Mazei, Y., Santos, S. S., Seppey, C. V. W., Spiegel, F. W., Walochnik, J., Winding, A., and Lara, E. Soil protistology rebooted: 30 fundamental questions to start with. *Soil Biology and Biochemistry*, 111:94–103, 2017. ISSN 0038-0717. doi:10.1016/j.soilbio.2017.04.001. 00000. Page 101

Gerisch, M., Agostinelli, V., Henle, K., and Dziock, F. More species, but all do the same: contrasting effects of flood disturbance on ground beetle functional and species diversity. *Oikos*, 121(4):508–515, 2012. ISSN 1600-0706. doi:10.1111/j.1600-0706.2011.19749.x. Page 4

Gilbert, D. and Mitchell, E. Microbial diversity in Sphagnum peatlands. *Developments in Earth Surface Processes*, 9:287–318, 2006. ISSN 0928-2025. Page 6, Page 15, Page 34, Page 37, Page 44, Page 47, Page 63, Page 79, Page 81, Page 99

Gilbert, D., Amblard, C., Bourdier, G., and Francez, A. J. The Microbial Loop at the Surface of a Peatland:Structure, Function, and Impact of Nutrient Input. *Microbial Ecol*, 35(1):83–93, January 1998. ISSN 0095-3628. doi:10.1007/s002489900062. 00000. Page 6, Page 61, Page 79

Gilbert, D., Mitchell, E. A. D., Amblard, C., Bourdier, G., and Francez, A. J. Population dynamics and food preferences of the testate amoeba Nebela tincta major-bohemica-collaris complex (Protozoa) in a Sphagnum peatland. *Acta Protozool*, 42(2):99–104, May 2003. ISSN 0065-1583. 00054. Page 6, Page 69, Page 70, Page 81, Page 101

Gómez-Baggethun, E., de Groot, R., Lomas, P. L., and Montes, C. The history of ecosystem services in economic theory and practice: From early notions to markets and payment schemes. *Ecological Economics*, 69(6): 1209–1218, 2010. ISSN 0921-8009. doi:10.1016/j.ecolecon.2009.11.007. 00636. Page 3

Gobat, J.-M., Aragno, M., and Matthey, W. *Le sol vivant.* Number 14 in Science et ingénierie de l'environnement. Presses Polytechniques et Universitaires Romandes, Lausanne, 3ème édition revue et augmentée edition, 2010. 00000. Page 5, Page 97

Gomaa, F., Mitchell, E. A. D., and Lara, E. Amphitremida (Poche, 1913) Is a New Major, Ubiquitous Labyrinthulomycete Clade. *PLoS ONE*, 8(1):e53046, January 2013. doi:10.1371/journal.pone.0053046. 00020. Page 79

Gomaa, F., Kosakyan, A., Heger, T. J., Corsaro, D., Mitchell, E. A., and Lara, E. One Alga to Rule them all: Unrelated Mixotrophic Testate Amoebae (Amoebozoa, Rhizaria and Stramenopiles) Share the Same Symbiont (Trebouxiophyceae). *Protist*, 165(2):161–176, March 2014. ISSN 14344610. doi:10.1016/j.protis.2014.01.002. 00007. Page 70, Page 81, Page 103

Goodall, D. W. *Mires: Swamp, Bog, Fen and Moor.* Elsevier Scientific Publishing Compagny, Amsterdam, 1983. Page 5

Gorham, E., Janssens, J. A., and Glaser, P. H. Rates of peat accumulation during the postglacial period in 32 sites from Alaska to Newfoundland, with special emphasis on northern Minnesota. *Can J Bot*, 81(5): 429–438, May 2003. ISSN 0008-4026. doi:10.1139/B03-036. Page 15

Gorham, E. Northern Peatlands: Role in the Carbon Cycle and Probable Responses to Climatic Warming. *Ecol Appl*, 1(2):182–195, May 1991. ISSN 10510761. 00000. Page 33, Page 97, Page 99

Gower, J. C. A general coefficient of similarity and some of its properties. *Biometrics*, pages 857–871, 1971. 02821. Page 82

Graf, U., Wildi, O., Kuchler, M., and Ecker, K. Five-year changes in Swiss mire vegetation. *Bot Helv*, 120(1): 15–27, 2010. ISSN 0253-1453. doi:10.1007/s00035-010-0071-3. Page 6, Page 26, Page 49, Page 86, Page 97, Page 105

Gravel, D., Albouy, C., and Thuiller, W. The meaning of functional trait composition of food webs for ecosystem functioning. *Phil. Trans. R. Soc. B*, 371(1694):20150268, 2016. 00001. Page 4, Page 100

Grime, J. P. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American naturalist*, pages 1169–1194, 1977. ISSN 0003-0147. Page 4

Grünig, A. *Mires and man: mire conservation in a densely populated country-the Swiss experience: excursion guide and symposium proceedings of the 5th Field Symposium of the International Mire Conservation Group (IMCG) to Switzerland 1992.* Swiss Federal Institute for Forest, Snow and Landscape Research, 1994. 00028. Page 34, Page 105

Grosvernier, P., Matthey, Y., and Buttler, A. Growth potential of three Sphagnum species in relation to water table level and peat properties with implications for their restoration in cut-over bogs. *J Appl Ecol*, 34(2): 471–483, April 1997. ISSN 0021-8901. doi:10.2307/2404891. Page 21, Page 86

Haapalehto, T. O., Vasander, H., Jauhiainen, S., Tahvanainen, T., and Kotiaho, J. S. The Effects of Peatland Restoration on Water-Table Depth, Elemental Concentrations, and Vegetation: 10 Years of Changes. *Restoration Ecology*, 19(5):587–598, September 2011. ISSN 1526-100X. doi:10.1111/j.1526-100X.2010.00704.x. 00059. Page 97, Page 103

Hajek, M., Rolecek, J., Cottenie, K., Kintrova, K., Horsak, M., Poulickova, A., Hajkova, P., Frankova, M., and Dite, D. Environmental and spatial controls of biotic assemblages in a discrete semi-terrestrial habitat: comparison of organisms with different dispersal abilities sampled in the same plots. *J Biogeogr*, 38(9): 1683–1693, September 2011. ISSN 0305-0270. doi:10.1111/j.1365-2699.2011.02503.x. 00000. Page 15

Hajkova, P., Bojkova, J., Frankova, M., Opravilova, V., Hajek, M., Kintrova, K., and Horsak, M. Disentangling the effects of water chemistry and substratum structure on moss-dwelling unicellular and multicellular micro-organisms in spring-fens. *Journal of Limnology*, 70:54–64, 2011. ISSN 1129-5767. doi:10.3274/jl11-70-s1-04. 00000. Page 6, Page 15

Hassol, S. *Impacts of a warming Arctic-Arctic climate impact assessment.* Cambridge University Press, 2004. 00406. Page 61

Hausmann, K., Hülsmann, N., and Radek, R. Protistology. 3rd completely revised edition. *Berlin, Schweitzerbart*, 2003. 00014. Page 6

Hedberg, P., Saetre, P., Sundberg, S., Rydin, H., and Kotowski, W. A functional trait approach to fen restoration analysis. *Appl Veg Sci*, 16(4):658–666, October 2013. ISSN 1654-109X. doi:10.1111/avsc.12042. 00008. Page 4, Page 9

Heger, T., Mitchell, E., Ledeganck, P., Vincke, S., Van de Vijver, B., and Beyens, L. The curse of taxonomic uncertainty in biogeographical studies of free-living terrestrial protists: a case study of testate amoebae from Amsterdam Island. *Journal of biogeography*, 36(8):1551–1560, 2009. ISSN 1365-2699. doi:10.1111/j.1365-2699.2009.02094.x. 00036. Page 36, Page 70, Page 90, Page 99

Holt, R. D. Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proc Natl Acad Sci U S A*, 106(Suppl 2):19659–19665, 2009. ISSN 0027-8424. doi:10.1073/pnas.0905137106. 00258. Page 79

Holyoak, M., Leibold, M. A., and Holt, R. D. *Metacommunities: spatial dynamics and ecological communities.* University of Chicago Press, 2005. ISBN 0-226-35064-9. Page 47, Page 69

Honaker, J. and King, G. What to do About Missing Values in Time Series Cross-Section Data. *American Journal of Political Science*, 54(3):561–581, 2010. Page 19

Honaker, J., King, G., and Blackwell, M. AMELIA II, A Program for Missing Data, 2011. 01013. Page 19

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A. J., Vandermeer, J., and Wardle, D. A. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecol Monogr*, 75(1):3–35, 2005. ISSN 0012-9615. doi:10.1890/04-0922. Page 3, Page 4

IPCC. Climate Change 2013 The Physical Science Basis. Technical report, OMM, Geneva, 2013. Page 61

IPCC. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Technical report, United Nations Environmeant Programme (UNEP) and World Meteorological Organization (WMO), Cambridge, United Kingdom & New York, NY, USA, 2014. Page 3, Page 6, Page 97

Jahns, H. M., editor. *Guide des fougères, mousses et lichens d'Europe : plus de 650 espèces photographiées.* Les guides du naturaliste. Delachaux et Niestlé, Paris, réimpr. edition, 2007. ISBN 978-2-603-00684-9. Page 17

Jari Oksanen, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs, and Helene Wagner. vegan: Community Ecology Package, 2016. 00129. Page 37

Jassey, V. E. J., Chiapusio, G., Mitchell, E. A. D., Binet, P., Toussaint, M.-L., and Gilbert, D. Fine-Scale Horizontal and Vertical Micro-distribution Patterns of Testate Amoebae Along a Narrow Fen/Bog Gradient. *Microbial Ecology*, 61(2):374–385, February 2011. ISSN 0095-3628. doi:10.1007/s00248-010-9756-9. 00000. Page 44, Page 61, Page 62, Page 63, Page 80, Page 81, Page 88, Page 100, Page 101

Jassey, V. E. J., Shimano, S., Dupuy, C., Toussaint, M.-L., and Gilbert, D. Characterizing the feeding habits of the testate amoebae Hyalosphenia papilio and Nebela tincta along a narrow "fen-bog" gradient using digestive vacuole content and 13 C and 15 N isotopic analyses. *Protist*, 163(3):451–464, 2012. Page 47, Page 69, Page 70, Page 100, Page 101

Jassey, V. E. J., Chiapusio, G., Binet, P., Buttler, A., Laggoun-Defarge, F., Delarue, F., Bernard, N., Mitchell, E. A. D., Toussaint, M. L., Francez, A. J., and Gilbert, D. Above- and belowground linkages in Sphagnum peatland: climate warming affects plant-microbial interactions. *Global Change Biol*, 19(3):811–823, 2013a. ISSN 1354-1013. doi:10.1111/Gcb.12075. Page 6, Page 47, Page 48, Page 62, Page 63, Page 69, Page 70, Page 81, Page 88, Page 99, Page 101

Jassey, V. E. J., Meyer, C., Dupuy, C., Bernard, N., Mitchell, E. A. D., Toussaint, M.-L., Metian, M., Chatelain, A. P., and Gilbert, D. To What Extent Do Food Preferences Explain the Trophic Position of Heterotrophic and Mixotrophic Microbial Consumers in a Sphagnum Peatland? *Microbial Ecology*, 66(3):571–580, October 2013b. ISSN 0095-3628, 1432-184X. doi:10.1007/s00248-013-0262-8. 00018. Page 37, Page 47, Page 48, Page 69, Page 70, Page 87, Page 88, Page 100, Page 101

Jassey, V. E. J., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F., Fournier, B., Gilbert, D., Laggoun-Défarge, F., Lara, E., T. E. Mills, R., Mitchell, E. A. D., Payne, R. J., and Robroek, B. J. M. An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. *Scientific Reports*, 5:16931, 2015. ISSN 2045-2322. doi:10.1038/srep16931. 00004. Page 6, Page 36, Page 47, Page 48, Page 62, Page 63, Page 70, Page 71, Page 79, Page 81, Page 87, Page 88, Page 99, Page 100, Page 103

Jassey, V. E. J., Lamentowicz, M., Bragazza, L., Hofsommer, M. L., Mills, R. T., Buttler, A., Signarbieux, C., and Robroek, B. J. Loss of testate amoeba functional diversity with increasing frost intensity across a continental gradient reduces microbial activity in peatlands. *European Journal of Protistology*, 2016. ISSN 09324739. doi:10.1016/j.ejop.2016.04.007. 00000. Page 6, Page 61

Jong, R. d., Blaauw, M., Chambers, F. M., Christensen, T. R., Vleeschouwer, F. d., Finsinger, W., Fronzek, S., Johansson, M., Kokfelt, U., Lamentowicz, M., Roux, G. L., Mauquoy, D., Mitchell, E. A. D., Nichols, J. E., Samaritani, E., and Geel, B. v. Climate and Peatlands. In Dodson, J., editor, *Changing Climates, Earth Systems and Society*, International Year of Planet Earth, pages 85–121. Springer Netherlands, 2010. ISBN 978-90-481-8715-7 978-90-481-8716-4. 00027 DOI: 10.1007/978-90-481-8716-4_5. Page 15

Juggins, S. rioja: Analysis of Quaternary Science Data, 2012. 00102. Page 20

Juggins, S. rioja: Analysis of Quaternary Science Data, 2015. Page 64, Page 82

Jukaine, Laine, J., Vasander, H., and Laiho, R. Long-Term Effects of Water Level Drawdown on the Vegetation of Drained Pine Mires in Southern Finland. *Journal of Applied Ecology*, 32(4):785–802, 1995. ISSN 0021-8901. doi:10.2307/2404818. 00244. Page 103

Junk, W. J., An, S., Finlayson, C. M., Gopal, B., Květ, J., Mitchell, S. A., Mitsch, W. J., and Robarts, R. D. Current state of knowledge regarding the world's wetlands and their future under global climate change: a synthesis. *Aquat Sci*, 75(1):151–167, 2012. ISSN 1015-1621, 1420-9055. doi:10.1007/s00027-012-0278-z. 00061. Page 5

Kaiser, H. F. Coefficient Alpha for a Principal Component and the Kaiser-Guttman Rule. *Psychological Reports*, 68(3):855–858, 1991. ISSN 0033-2941. doi:10.2466/pr0.1991.68.3.855. 00071. Page 83

Kajukało, K., Fiałkiewicz-Kozieł, B., Gałka, M., Kołaczek, P., and Lamentowicz, M. Abrupt ecological changes in the last 800 years inferred from a mountainous bog using testate amoebae traits and multi-proxy data. *European Journal of Protistology*, 2016. ISSN 0932-4739. doi:10.1016/j.ejop.2016.04.003. 00000. Page 61, Page 79, Page 100, Page 101

Kattge, J., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Bönisch, G., Garnier, E., Westoby, M., Reich, P. B., Wright, I. J., Cornelissen, J. H. C., Violle, C., Harrison, S. P., Van BODEGOM, P. M., Reichstein, M., Enquist, B. J., Soudzilovskaia, N. A., Ackerly, D. D., Anand, M., Atkin, O., Bahn, M., Baker, T. R., Baldocchi, D., Bekker, R., Blanco, C. C., Blonder, B., Bond, W. J., Bradstock, R., Bunker, D. E., Casanoves, F., Cavender-Bares, J., Chambers, J. Q., Chapin Iii, F. S., Chave, J., Coomes, D., Cornwell, W. K., Craine, J. M., Dobrin, B. H., Duarte, L., Durka, W., Elser, J., Esser, G., Estiarte, M., Fagan, W. F., Fang, J., Fernández-Méndez, F., Fidelis, A., Finegan, B., Flores, O., Ford, H., Frank, D., Freschet, G. T., Fyllas, N. M., Gallagher, R. V., Green, W. A., Gutierrez, A. G., Hickler, T., Higgins, S. I., Hodgson, J. G., Jalili, A., Jansen, S., Joly, C. A., Kerkhoff, A. J., Kirkup, D., Kitajima, K., Kleyer, M., Klotz, S., Knops, J. M. H., Kramer, K., Kühn, I., Kurokawa, H., Laughlin, D., Lee, T. D., Leishman, M., Lens, F., Lenz, T., Lewis, S. L., Lloyd, J., Llusià, J., Louault, F., Ma, S., Mahecha, M. D., Manning, P., Massad, T., Medlyn, B. E., Messier, J., Moles, A. T., Müller, S. C., Nadrowski, K., Naeem, S., Niinemets, ., Nöllert, S., Nüske, A., Ogaya, R., Oleksyn, J., Onipchenko, V. G., Onoda, Y., Ordoñez, J., Overbeck, G., Ozinga, W. A., Patiño, S., Paula, S., Pausas, J. G., Peñuelas, J., Phillips, O. L., Pillar, V., Poorter, H., Poorter, L., Poschlod, P., Prinzing, A., Proulx, R., Rammig, A., Reinsch, S., Reu, B., Sack, L., Salgado-Negret, B., Sardans, J., Shiodera, S., Shipley, B., Siefert, A., Sosinski, E., Soussana, J.-F., Swaine, E., Swenson, N., Thompson, K., Thornton, P., Waldram, M., Weiher, E., White, M., White, S., Wright, S. J., Yguel, B., Zaehle, S., Zanne, A. E., and Wirth, C. TRY – a global database of plant traits. *Global Change Biology*, 17(9):2905–2935, 2011. ISSN 1365-2486. doi:10.1111/j.1365-2486.2011.02451.x. 00585. Page 4

Kearney, M., Simpson, S. J., Raubenheimer, D., and Helmuth, B. Modelling the ecological niche from functional traits. *Philos Trans R Soc Lond B Biol Sci*, 365(1557):3469–3483, 2010. ISSN 0962-8436. doi:10.1098/rstb.2010.0034. 00133. Page 34, Page 47, Page 79, Page 88

Keddy, P. A. Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science*, 3(2):157–164, 1992. Page 3, Page 10, Page 61

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., and Webb, C. O. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, 26(11):1463–1464, June 2010. ISSN 1367-4803. doi:10.1093/bioinformatics/btq166. 00000. Page 82

Kembel, S. An introduction to the picante package, 2010. 00004. Page 82

Kleyer, M., Bekker, R., Knevel, I., Bakker, J., Thompson, K., Sonnenschein, M., Poschlod, P., van Groenendael, J., Klimeš, L., Klimešová, J., Klotz, S., Rusch, G., Hermy, M., Adriaens, D., Boedeltje, G., Bossuyt, B., Dannemann, A., Endels, P., Götzenberger, L., Hodgson, J., Jackel, A.-K., Kühn, I., Kunzmann, D., Ozinga, W., Römermann, C., Stadler, M., Schlegelmilch, J., Steendam, H., Tackenberg, O., Wilmann, B., Cornelissen, J., Eriksson, O., Garnier, E., and Peco, B. The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology*, 96(6):1266–1274, November 2008. ISSN 00220477, 13652745. doi:10.1111/j.1365-2745.2008.01430.x. 00661. Page 104

Koenig, I., Feldmeyer-Christe, E., and Mitchell, E. A. D. Comparative ecology of vascular plant, bryophyte and testate amoeba communities in four Sphagnum peatlands along an altitudinal gradient in Switzerland. *Ecological Indicators*, 54:48–59, 2015. doi:10.1016/j.ecolind.2015.01.043. 00002 WOS:000355365500005. Page 34, Page 61

Kosakyan, A., Heger, T. J., Leander, B. S., Todorov, M., Mitchell, E. A., and Lara, E. COI barcoding of Nebelid testate amoebae (Amoebozoa: Arcellinida): extensive cryptic diversity and redefinition of the Hyalospheniidae Schultze. *Protist*, 163(3):415–34, May 2012. ISSN 1618-0941 (Electronic) 1434-4610 (Linking). doi:10.1016/j.protis.2011.10.003. Page 36, Page 62

Kosakyan, A., Gomaa, F., Mitchell, E. A. D., Heger, T. J., and Lara, E. Using DNA-barcoding for sorting out protist species complexes: A case study of the Nebela tincta–collaris–bohemica group (Amoebozoa; Arcellinida, Hyalospheniidae). *European journal of protistology*, 49(0):222–37, May 2013. ISSN 0932-4739. doi:10.1016/j.ejop.2012.08.006. 00016. Page 80, Page 90

Kosakyan, A., Mulot, M., Mitchell, E. A., and Lara, E. Environmental DNA COI barcoding for quantitative analysis of protists communities: A test using the Nebela collaris complex (Amoebozoa; Arcellinida; Hyalospheniidae). *European Journal of Protistology*, 51(4):311–320, August 2015. ISSN 09324739. doi:10.1016/j.ejop.2015.06.005. 00002. Page 70

Kosakyan, A., Lahr, D. J. G., Mulot, M., Meisterfeld, R., Mitchell, E. A. D., and Lara, E. Phylogenetic reconstruction based on *COI* reshuffles the taxonomy of hyalosphenid shelled (testate) amoebae and reveals the convoluted evolution of shell plate shapes. *Cladistics*, 2016. ISSN 07483007. doi:10.1111/cla.12167. 00000. Page 79, Page 90

Krashevska, V., Bonkowski, M., Maraun, M., and Scheu, S. Testate amoebae (protista) of an elevational gradient in the tropical mountain rain forest of Ecuador. *Pedobiologia*, 51(4):319–331, 2007. ISSN 00314056. doi:10.1016/j.pedobi.2007.05.005. Page 15

Kremen, C. and Ostfeld, R. S. A Call to Ecologists: Measuring, Analyzing, and Managing Ecosystem Services. *Frontiers in Ecology and the Environment*, 3(10):540–548, 2005. ISSN 15409295. doi:10.2307/3868610. 00299. Page 3

Körner, C. The use of ‘altitude’in ecological research. *Trends Ecol Evol*, 22(11):569–574, 2007. ISSN 0169-5347. 00000. Page 25

Lê, S., Josse, J., and Husson, F. FactoMineR: An R package for multivariate analysis. *Journal of statistical software*, 25(1):1–18, 2008. Page 20

Laggoun-Defarge, F., Mitchell, E., Gilbert, D., Disnar, J.-R., Comont, L., Warner, B. G., and Buttler, A. Cutover peatland regeneration assessment using organic matter and microbial indicators (bacteria and testate amoebae). *Journal of Applied Ecology*, 45(2):716–727, April 2008. ISSN 0021-8901. doi:10.1111/j.1365-2664.2007.01436.x. Page 6, Page 26, Page 34, Page 61, Page 63, Page 81, Page 87, Page 103

Lahr, D. J., Bosak, T., Lara, E., and Mitchell, E. A. The Phanerozoic diversification of silica-cycling testate amoebae and its possible links to changes in terrestrial ecosystems. *PeerJ*, 3:e1234, 2015. 00008. Page 79

Laliberté, E., Legendre, P., and Shipley, B. FD: measuring functional diversity from multiple traits, and other tools for functional ecology, 2014. Page 34, Page 38, Page 64, Page 82

Lamentowicz, L., Gabka, M., Rusinska, A., Sobczynski, T., Owsianny, P. M., and Lamentowicz, M. Testate Amoeba (Arcellinida, Euglyphida) Ecology along a Poor-Rich Gradient in Fens of Western Poland. *International Review of Hydrobiology*, 96(4):356–380, September 2011. ISSN 1434-2944. doi:10.1002/iroh.201111357. 00014. Page 44

Lamentowicz, M., Lamentowicz, ., van der Knaap, W., Gąbka, M., and Mitchell, E. Contrasting Species—Environment Relationships in Communities of Testate Amoebae, Bryophytes and Vascular Plants Along the Fen–Bog Gradient. *Microbial ecology*, 59(3):499–510, 2010. ISSN 0095-3628. doi:10.1007/s00248-009-9617-6. Page 6, Page 15, Page 26

Lamentowicz, M., Bragazza, L., Buttler, A., Jassey, V. E. J., and Mitchell, E. A. D. Seasonal patterns of testate amoeba diversity, community structure and species–environment relationships in four Sphagnum-dominated peatlands along a 1300 m altitudinal gradient in Switzerland. *Soil Biology and Biochemistry*, 67(0):1–11, 2013. ISSN 0038-0717. doi:10.1016/j.soilbio.2013.08.002. Page 6, Page 26, Page 27, Page 34, Page 44, Page 48, Page 101, Page 104

Lamentowicz, M., Gałka, M., Lamentowicz, ., Obremska, M., Kühl, N., Lücke, A., and Jassey, V. Reconstructing climate change and ombrotrophic bog development during the last 4000years in northern Poland using biotic proxies, stable isotopes and trait-based approach. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 418:261–277, 2015. ISSN 00310182. doi:10.1016/j.palaeo.2014.11.015. 00007. Page 6, Page 34, Page 61, Page 63, Page 70, Page 81

Lamentowicz, M. and Mitchell, E. A. D. The Ecology of Testate Amoebae (Protists) in Sphagnum in North-western Poland in Relation to Peatland Ecology. *Microbial Ecology*, 50(1):48–63, 2005. ISSN 1432-184X. doi:10.1007/s00248-004-0105-8. Page 36, Page 40, Page 48, Page 68, Page 69, Page 87

Lara, E., Heger, T. J., Mitchell, E. A. D., Meisterfeld, R., and Ekelund, F. SSU rRNA reveals a sequential increase in shell complexity among the euglyphid testate amoebae (Rhizaria : Euglyphida). *Protist*, 158(2):229–237, April 2007. ISSN 1434-4610. doi:10.1016/j.protis.2006.11.006. 00029. Page 90

Lara, E., Mitchell, E. A. D., Moreira, D., and López García, P. Highly Diverse and Seasonally Dynamic Protist Community in a Pristine Peat Bog. *Protist*, 162(1):14–32, 2011. ISSN 1434-4610. doi:10.1016/j.protis.2010.05.003. Page 104

Lauber, K., Wagner, G., Gfeller, E., and Gygax, A., editors. *Flora Helvetica : flore illustrée de Suisse*. P. Haupt, Bern, 3e éd. entièrement rev. / avec la collab. de andreas gygax edition, 2007. ISBN 978-3-258-07206-7. Page 26

Laughlin, D. C. Applying trait-based models to achieve functional targets for theory-driven ecological restoration. *Ecol Lett*, 17(7):771–784, 2014. ISSN 1461-0248. doi:10.1111/ele.12288. 00044. Page 9

Laurila-Pant, M., Lehikoinen, A., Uusitalo, L., and Venesjärvi, R. How to value biodiversity in environmental management? *Ecological Indicators*, 55:1–11, August 2015. ISSN 1470160X. doi:10.1016/j.ecolind.2015.02.034. 00025. Page 61

Lavorel, S. and Garnier, E. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional ecology*, 16(5):545–556, 2002. Page 3, Page 4, Page 34, Page 36, Page 61, Page 63, Page 81, Page 103

Lebrija-Trejos, E., Pérez-García, E. A., Meave, J. A., Bongers, F., and Poorter, L. Functional traits and environmental filtering drive community assembly in a species-rich tropical system. *Ecology*, 91(2):386–398, 2010. 00219. Page 62

Ledeganck, P., Nijs, I., and Beyens, L. Plant functional group diversity promotes soil protist diversity. *Protist*, 154(2):239–249, July 2003. ISSN 1434-4610. doi:10.1078/143446103322166536. Page 15

Legendre, P. and Gallagher, E. D. Ecologically meaningful transformations for ordination of species data. *Oecologia*, 129(2):271–280, 2001. ISSN 0029-8549, 1432-1939. doi:10.1007/s004420100716. 01802. Page 37, Page 82

Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., Holt, R. D., Shurin, J. B., Law, R., Tilman, D., Loreau, M., and Gonzalez, A. The metacommunity concept: a framework for multi-scale community ecology. *Ecol Lett*, 7(7):601–613, July 2004. ISSN 1461-023X. doi:10.1111/j.1461-0248.2004.00608.x. Page 69

Lemonis, N.) palécoécologie d'une tourbière de russie du nord par une approche multi-indicateurs (thécamibes, macrorestes de plantes, pollens et étude de la tourbe). Master's thesis, University of Neuchâtel, 2012. Page 36, Page 40

Litchman, E., Klausmeier, C. A., Schofield, O. M., and Falkowski, P. G. The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. *Ecology letters*, 10 (12):1170–1181, 2007. 00277. Page 4

Littlewood, N., Anderson, P., Artz, R., Bragg, O., Lunt, P., and Marrs, R. Peatland biodiversity. *IUCN UK Peatland Programme, Edinburgh*, 2010. 00011. Page 5

Londo, G. Decimal Scale for Releves of Permanent Quadrats. *Vegetatio*, 33(1):61–64, 1976. ISSN 0042-3106. Page 17, Page 35

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J., Hector, A., Hooper, D., Huston, M., Raffaelli, D., and Schmid, B. Biodiversity and ecosystem functioning: current knowledge and future challenges. *science*, 294(5543):804–808, 2001. ISSN 0036-8075. Page 3, Page 4

MacArthur, R. and Wilson, E. *The Theory of Island Biogeography*. Princeton University Press, Princeton, 1967. Page 33

Macarthur, R. and Levins, R. The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The American Naturalist*, 101(921):377–385, 1967. ISSN 0003-0147. 02441. Page 10

MacArthur, R. H. On the relative abundance of bird species. *Proceedings of the National Academy of Sciences*, 43(3):293–295, 1957. 01075. Page 83

Maire, V., Gross, N., Börger, L., Proulx, R., Wirth, C., Pontes, L. d. S., Soussana, J.-F., and Louault, F. Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. *New Phytologist*, 196(2):497–509, 2012. ISSN 0028646X. doi:10.1111/j.1469-8137.2012.04287.x. 00052. Page 99

Makarieva, A. M., Gorshkov, V. G., Li, B.-L., Chown, S. L., Reich, P. B., and Gavrilov, V. M. Mean mass-specific metabolic rates are strikingly similar across life's major domains: evidence for life's metabolic optimum. *Proceedings of the National Academy of Sciences*, 105(44):16994–16999, 2008. 00144. Page 63, Page 70, Page 81

Malmer, N., Horton, D. G., and Vitt, D. H. Element Concentrations in Mosses and Surface Waters of Western Canadian Mires Relative to Precipitation Chemistry and Hydrology. *Ecography*, 15(1):114–128, January 1992. ISSN 09067590. Page 25

Maltby, E. and Immirzi, P. Carbon dynamics in peatlands and other wetland soils regional and global perspectives. *Chemosphere*, 27(6):999–1023, 1993. ISSN 0045-6535. doi:10.1016/0045-6535(93)90065-D. 00000. Page 5, Page 97

Manneville, O., Vergne, V., Villepoux, O., and Matthey, W. *Le monde des tourbières et des marais: France, Suisse, Belgique et Luxembourg*. Les références du naturaliste. Delachaux et Niestlé, Paris, nouvelle édition, septembre 2006 edition, 2006. ISBN 978-2-603-01390-8. 00004 OCLC: 949664796. Page 103

Marcisz, K., Fournier, B., Gilbert, D., Lamentowicz, M., and Mitchell, E. A. Response of Sphagnum Peatland Testate Amoebae to a 1-Year Transplantation Experiment Along an Artificial Hydrological Gradient. *Microbial Ecology*, 67(4):810–818, 2014. ISSN 0095-3628. Page 6, Page 27, Page 47, Page 61, Page 62, Page 87, Page 99, Page 103, Page 105

Marcisz, K., Colombaroli, D., Jassey, V. E. J., Tinner, W., Kołaczek, P., Gałka, M., Karpińska-Kołaczek, M., Słowiński, M., and Lamentowicz, M. A novel testate amoebae trait-based approach to infer environmental disturbance in Sphagnum peatlands. *Sci Rep*, 6, September 2016. ISSN 2045-2322. doi:10.1038/srep33907. 00005. Page 34, Page 47, Page 48, Page 61, Page 79, Page 87, Page 90, Page 100, Page 101, Page 105

Mason, N. W., Lanoiselée, C., Mouillot, D., Wilson, J. B., and Argillier, C. Does niche overlap control relative abundance in French lacustrine fish communities? A new method incorporating functional traits. *Journal of Animal Ecology*, 77(4):661–669, 2008. 00053. Page 82

Matantseva, O. V. and Skarlato, S. O. Mixotrophy in microorganisms: Ecological and cytophysiological aspects. *Journal of Evolutionary Biochemistry and Physiology*, 49(4):377–388, July 2013. ISSN 0022-0930, 1608-3202. doi:10.1134/S0022093013040014. 00011. Page 103

Mazei, Y. A., Tsyganov, A. N., and Bubnova, O. A. Structure of a community of testate amoebae in a sphagnum dominated bog in upper sura flow (Middle Volga Territory). *Biology Bulletin*, 34(4):382–394, August 2007. ISSN 1062-3590. doi:10.1134/s1062359007040115. Page 61

Mazei, Y. A., Tsyganov, A. N., and Bubnova, O. A. The Species Composition And Community Structure Of Testate Amoebae In Sphagnum Bogs Of Northern Karelia (The White Sea Lowland). *Zoologichesky Zhurnal*, 88(7):771–782, July 2009. ISSN 0044-5134. 00000. Page 62, Page 87

Mazei, Y. A., Blinokhvatova, Y. V., and Embulaeva, E. A. Specific features of the microspatial distribution of soil testate amoebae in the forests of the Middle Volga Region. *Arid Ecosystems*, 1(1):46–52, 2011. 00006. Page 62

Mazei, Y. Biodiversity patterns in protozoan communities: linking processes and scales. *Protistology*, 5(4), 2008. 00005. Page 62

McGill, B. J., Enquist, B. J., Weiher, E., and Westoby, M. Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, 21(4):178–185, 2006. ISSN 0169-5347. 01550. Page 4

McMullen, J. A., Barber, K. E., and Johnson, B. A paleoecological perspective of vegetation succession on raised bog microforms. *Ecological Monographs*, 74(1):45–77, February 2004. ISSN 0012-9615. doi:10.1890/02-4046. Page 15

MEA. *Ecosystems and human well-being*. Number vol 1-5 in The Millennium Ecosystem Assessment series. The Island Press, Washington D.C., 2005. ISBN 1559632283 (pbk. alk. paper vol. 1) 1559633913 (pbk. alk. paper vol. 2) 1559632704 (pbk. alk. paper vol. 3) 1559631864 (pbk. alk. paper vol. 4) 1559633875 (pbk. alk. paper vol. 5). Page 3, Page 4, Page 5, Page 61, Page 79, Page 97

Meisterfeld, R. Order arcellinida kent, 1880. *The illustrated guide to the Protozoa*, 2:827–860, 2002a. 00160. Page 6

Meisterfeld, R. Testate amoebae with filopodia. *The illustrated guide to the protozoa*, 2:1054–1084, 2002b. 00124. Page 6

Messier, J., McGill, B. J., and Lechowicz, M. J. How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters*, 13(7):838–848, 2010. ISSN 1461-0248. doi:10.1111/j.1461-0248.2010.01476.x. Page 63, Page 81

Meyer, C., Gilbert, D., Gillet, F., Moskura, M., Franchi, M., and Bernard, N. Using "bryophytes and their associated testate amoeba" microsystems as indicators of atmospheric pollution. *Ecological Indicators*, 13 (1):144–151, February 2012. ISSN 1470-160X. doi:10.1016/j.ecolind.2011.05.020. Page 105

Meyer, C., Desalme, D., Bernard, N., Binet, P., Toussaint, M.-L., and Gilbert, D. Using testate amoeba as potential biointegrators of atmospheric deposition of phenanthrene (polycyclic aromatic hydrocarbon) on "moss/soil interface-testate amoeba community" microecosystems. *Ecotoxicology*, 22(2):287–294, March 2013. ISSN 0963-9292, 1573-3017. doi:10.1007/s10646-012-1025-x. 00005. Page 104

Mieczan, T. Ecology of testate amoebae (Protists) in *Sphagnum* peatlands of eastern Poland: Vertical microdistribution and species assemblages in relation to environmental parameters. *Annales de Limnologie - International Journal of Limnology*, 45(1):41–49, 2009. ISSN 0003-4088, 2100-000X. doi:10.1051/limn/09003. Page 15, Page 61, Page 69

Mieczan, T. Vertical Microzonation Of Testate Amoebae And Ciliates In Peatbog Waters In Relation To Physical And Chemical Parameters. *Polish Journal of Ecology*, 58(4):729–739, 2010. ISSN 1505-2249. Page 100

Mitchell, E. A. D. Response of testate amoebae (protozoa) to N and P fertilization in an arctic wet sedge tundra. *Arct Antarct Alp Res*, 36(1):78–83, February 2004. ISSN 1523-0430. doi:10.1657/1523-0430(2004)036[0078:Rotapt]2.0.Co;2. 00000. Page 27, Page 103, Page 105

Mitchell, E. A. D., Buttler, A. J., Warner, B. G., and Gobat, J. M. Ecology of testate amoebae (Protozoa : Rhizopoda) in Sphagnum peatlands in the Jura mountains, Switzerland and France. *Ecoscience*, 6(4):565–576, 1999. ISSN 1195-6860. doi:10.1080/11956860.1999.11682555. Page 36, Page 40, Page 44, Page 63, Page 66, Page 68, Page 69, Page 81, Page 87, Page 88, Page 100, Page 101

Mitchell, E. A. D., Buttler, A., Grosvernier, P., Rydin, H., Albinsson, C., Greenup, A. L., Heijmans, M., Hoosbeek, M. R., and Saarinen, T. Relationships among testate amoebae (Protozoa), vegetation and water chemistry in five Sphagnum-dominated peatlands in Europe. *New Phytologist*, 145(1):95–106, January 2000. ISSN 0028-646X. doi:10.1046/j.1469-8137.2000.00550.x. Page 25, Page 49

Mitchell, E. A. D., Bragazza, L., and Gerdol, R. Testate amoebae (Protista) communities in Hylocomium splendens (Hedw.) BSG (Bryophyta): Relationships with altitude, and moss elemental chemistry. *Protist*, 155(4):423–436, December 2004. ISSN 1434-4610. Page 15, Page 16

Mitchell, E. A. D. Pack hunting by minute soil testate amoebae: nematode hell is a naturalist's paradise. *Environ Microbiol*, 17(11):4145–4147, 2015. ISSN 1462-2920. doi:10.1111/1462-2920.13025. 00000. Page 6

Mitchell, E. A. D. and Gilbert, D. Vertical Micro-Distribution and Response to Nitrogen Deposition of Testate Amoebae in Sphagnum. *The Journal of eukaryotic microbiology*, 51(4):480–490, 2004. ISSN 1066-5234. doi:10.1111/j.1550-7408.2004.tb00400.x. Page 18, Page 35, Page 44

Mitchell, E. A. D., Charman, D. J. G., and Warner, B. G. Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodiversity and Conservation*, 17(9):2115–2137, 2008a. 00215. Page 6

Mitchell, E. A. D., Payne, R. J., and Lamentowicz, M. Potential implications of differential preservation of testate amoeba shells for paleoenvironmental reconstruction in peatlands. *Journal of Paleolimnology*, 40(2): 603–618, August 2008b. ISSN 0921-2728. doi:10.1007/s10933-007-9185-z. 00000. Page 6, Page 15, Page 34, Page 48, Page 61, Page 79, Page 99, Page 104

Mitchell, E. A. D., Payne, R. J., van der Knaap, W. O., Lamentowicz, L., Gabka, M., and Lamentowicz, M. The performance of single- and multi-proxy transfer functions (testate amoebae, bryophytes, vascular plants) for reconstructing mire surface wetness and pH. *Quaternary Research*, 79(1):6–13, January 2013. ISSN 0033-5894. doi:10.1016/j.yqres.2012.08.004. Page 15, Page 61

Mitchell, E. A. D., Lamentowicz, M., Payne, R. J., and Mazei, Y. Effect of taxonomic resolution on ecological and palaeoecological inference – a test using testate amoeba water table depth transfer functions. *Quaternary Science Reviews*, 91(0):62–69, 2014. ISSN 0277-3791. doi:10.1016/j.quascirev.2014.03.006. Page 15, Page 19, Page 62, Page 63, Page 70, Page 90

Mitchell, E. A. The identification of Nebela and similar species with indications on their ecology and distribution. *University of Anchorage, Alaska*, 2003. 00005. Page 18, Page 62, Page 80

Mitsch, W. J. and Gosselink, J. G. *Wetlands*. John Wiley & Sons, New York, 3ème édition edition, 2000. 00000. Page 3, Page 5, Page 6, Page 49

Mitsch, W. J. and Gosselink, J. G. *Wetlands*. John Wiley & Sons, john wiley & sons edition, 2007. 00118. Page 33, Page 69, Page 97, Page 103

Mlambo, M. C. Not all traits are 'functional': insights from taxonomy and biodiversity-ecosystem functioning research. *Biodiversity and conservation*, 23(3):781–790, 2014. Page 4, Page 34, Page 61, Page 63, Page 79, Page 81

Moore, P. D. and Bellamy, D. J. *Peatlands*. Springer-Verlag, 1974. ISBN 978-0-387-91112-0. 00635. Page 5

Moore, T. R. and Knowles, R. The Influence of Water-Table Levels on Methane and Carbon-Dioxide Emissions from Peatland Soils. *Can J Soil Sci*, 69(1):33–38, 1989. ISSN 0008-4271. doi:10.4141/cjss89-004. Page 15

Moreno-Mateos, D., Power, M. E., Comín, F. A., and Yockteng, R. Structural and Functional Loss in Restored Wetland Ecosystems. *PLOS Biol*, 10(1):e1001247, 2012. ISSN 1545-7885. doi:10.1371/journal.pbio.1001247. Page 6

Moretti, M. and Legg, C. Combining plant and animal traits to assess community functional responses to disturbance. *Ecography*, 32(2):299–309, April 2009. ISSN 09067590, 16000587. doi:10.1111/j.1600-0587.2008.05524.x. 00075. Page 34

Moretti, M., Dias, A. T. C., de Bello, F., Altermatt, F., Chown, S. L., Azcárate, F. M., Bell, J. R., Fournier, B., Hedde, M., Hortal, J., Ibanez, S., Öckinger, E., Sousa, J. P., Ellers, J., and Berg, M. P. Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. *Functional Ecology*, 31(3):558–567, March 2017. ISSN 02698463. doi:10.1111/1365-2435.12776. 00006. Page 104

Mouillot, D., Dumay, O., and Tomasini, J. A. Limiting similarity, niche filtering and functional diversity in coastal lagoon fish communities. *Estuarine, Coastal and Shelf Science*, 71(3–4):443–456, 2007. ISSN 0272-7714. doi:10.1016/j.ecss.2006.08.022. 00085. Page 4, Page 34, Page 79, Page 99, Page 101

Mouillot, D., Graham, N. A., Villéger, S., Mason, N. W., and Bellwood, D. R. A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution*, 28(3):167–177, 2013. ISSN 0169-5347. Page 34

MétéoSuisse. Bulletin climatologique année 2015, 2016. 00000. Page 33, Page 36, Page 38

MétéoSuisse. Bulletin climatologique année 2016, 2017. 00000. Page 33, Page 36, Page 38

Mulhauser, B., Mulot, M., Tritz, J., Gueniat, S., Koenig, I., D'Inverno, M., and Mitchell, E. A. D. Creation of a raised bog in the botanical garden of Neuchâtel: a tool for research, collections and public information. In *Eurogard VII*, Paris, accepted. 00000. Page 8, Page 35

Mulot, M., Varidel, D., and Mitchell, E.A.D. A mesocosm approach to study the response of Sphagnum peatlands to hydrological changes: setup, optimisation and performance. *Mires and Peat*, 16(09):1–12, 2015. ISSN 1819-754X. 00000. Page 10, Page 62, Page 69, Page 79, Page 80

Mulot, M., Marcisz, K., Grandgirard, L., Lara, E., Kosakyan, A., Robroek, B. J., Lamentowicz, M., Payne, R. J., and Mitchell, E. A. Genetic determinism vs. phenotypic plasticity in protist morphology. *Journal of Eukaryotic Microbiology*, 2017. Page 47

Netzel, H. Formation Of The Thecal Wall By Polyphase Secretion In The Testate Ameba Netzelia-Oviformis (Rhizopodea, Lobosia). *ARCHIV FUR PROTISTENKUNDE*, 127(4):351–381, 1983. 00001. Page 70

Nguyen-Viet, H., Bernard, N., Mitchell, E. A. D., Badot, P. M., and Gilbert, D. Effect of lead pollution on testate amoebae communities living in Sphagnum fallax: An experimental study. *Ecotoxicology and Environmental Safety*, 69(1):130–138, 2008. ISSN 0147-6513. doi:10.1016/j.ecoenv.2007.02.007. Page 105

Niemi, G. J. and McDonald, M. E. Application of Ecological Indicators. *Annual Review of Ecology, Evolution, and Systematics*, 35(1):89–111, December 2004. ISSN 1543-592X, 1545-2069. doi:10.1146/annurev.ecolsys.35.112202.130132. 00655. Page 79

Nomura, M., Nakayama, T., and Ishida, K.-i. Detailed process of shell construction in the photosynthetic testate amoeba Paulinella chromatophora (Euglyphid, Rhizaria). *Journal of Eukaryotic Microbiology*, 61(3): 317–321, 2014. 00006. Page 70

Norris, K., Bailey, M., Keith, A., Maskell, L., Reading, C., Turner, S., Vanbergen, A., Watt, A., and others. Biodiversity in the context of ecosystem services [chapter 4]. Technical report, UK National Ecsystem Assessment, 2011. 00023. Page 79

Noss, R. F. Indicators for Monitoring Biodiversity: A Hierarchical Approach. *Conservation Biology*, 4(4): 355–364, 1990. ISSN 0888-8892. 03279. Page 99

Ogden, G. G. and Hedley, R. H. An Atlas of Freshwater Testate Amoebae. *Soil Science*, 130(3):176, 1980. ISSN 0038-075X. Page 79

Oksanen, J. Vegan: an introduction to ordination. *URL http://cran. r-project. org/web/packages/vegan/vignettes/introvegan. pdf*, 2015. 00008. Page 19, Page 64, Page 82

Oliverio, A. M., Lahr, D. J., Nguyen, T., and Katz, L. A. Cryptic diversity within morphospecies of testate amoebae (Amoebozoa: Arcellinida) in New England bogs and fens. *Protist*, 165(2):196–207, 2014. 00021. Page 90

Opravilova, V. and Hajek, M. The variation of testacean assemblages (Rhizopoda) along the complete base-richness gradient in fens: A case study from the Western Carpathians. *Acta Protozool*, 45(2):191–204, May 2006. ISSN 0065-1583. 00000. Page 18, Page 44, Page 101

Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R., Church, J. A., Clarke, L., Dahe, Q., Dasgupta, P., and others. *Climate change 2014: synthesis Report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change.* IPCC, 2014. 00393. Page 15, Page 61

Parsons, P. A. Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. *Biological Reviews*, 80(04):589–610, 2005. ISSN 1469-185X. Page 4

Pavoine, S., Vela, E., Gachet, S., De Bélair, G., and Bonsall, M. B. Linking patterns in phylogeny, traits, abiotic variables and space: a novel approach to linking environmental filtering and plant community assembly. *Journal of Ecology*, 99(1):165–175, 2011. ISSN 1365-2745. Page 4

Payne, R. J., Kishaba, K., Blackford, J. J., and Mitchell, E. A. D. Ecology of testate amoebae (Protista) in south-central Alaska peatlands: building transfer-function models for palaeoenvironmental studies. *Holocene*, 16 (3):403–414, April 2006. ISSN 0959-6836. doi:10.1191/0959683606hl936rp. Page 27

Payne, R. J. Seven reasons why protists make useful bioindicators. *Acta Protozoologica*, 52(3):105, 2013. 00000. Page 79, Page 99

Payne, R. J. and Mitchell, E. A. D. How many is enough? Determining optimal count totals for ecological and palaeoecological studies of testate amoebae. *J. Paleolimn.*, 42(4):483–495, 2008. ISSN 0921-2728 1573-0417. doi:10.1007/s10933-008-9299-y. Page 36

Payne, R. J. and Pates, J. M. Vertical stratification of testate amoebae in the Elatia Mires, northern Greece: palaeoecological evidence for a wetland response to recent climatic change, or autogenic processes? *Wetlands Ecology and Management*, 17(4):355–364, August 2009. ISSN 0923-4861. doi:10.1007/s11273-008-9112-8. Page 62

Payne, R. J., Charman, D. J., Matthews, S., and Eastwood, W. J. Testate amoebae as palaeohydrological proxies in Surmene Agacbasi Yaylasi peatland (Northeast Turkey). *Wetlands*, 28(2):311–323, June 2008. ISSN 0277-5212. doi:10.1672/07-42.1. Page 34

Payne, R. J., Lamentowicz, M., and Mitchell, E. A. D. The perils of taxonomic inconsistency in quantitative palaeoecology: experiments with testate amoeba data: The perils of taxonomic inconsistency in quantitative palaeoecology. *Boreas*, 40(1):15–27, January 2011. ISSN 03009483. doi:10.1111/j.1502-3885.2010.00174.x. 00017. Page 36, Page 61, Page 70

Payne, R. J., Mitchell, E. A. D., Hung, N.-V., and Gilbert, D. Can pollution bias peatland paleoclimate reconstruction? *Quaternary Research*, 78(2):170–173, September 2012. ISSN 0033-5894. doi:10.1016/j.yqres.2012.05.004. Page 61

Payne, R. J., Belyakova, O., and Mazei, Y. Diversity and community ecology of forest epiphyte testate amoebae from European Russia. *European Journal of Protistology*, 51(5):450–459, October 2015. ISSN 0932-4739. doi:10.1016/j.ejop.2015.02.006. 00004. Page 100, Page 101

Payne, R. J., Babeshko, K. V., van Bellen, S., Blackford, J. J., Booth, R. K., Charman, D. J., Ellershaw, M. R., Gilbert, D., Hughes, P. D. M., Jassey, V. E. J., Lamentowicz, u., Lamentowicz, M., Malysheva, E. A., Mauquoy, D., Mazei, Y., Mitchell, E. A. D., Swindles, G. T., Tsyganov, A. N., Turner, T. E., and Telford, R. J. Significance testing testate amoeba water table reconstructions. *Quaternary Science Reviews*, 138:131–135, 2016a. ISSN 0277-3791. doi:10.1016/j.quascirev.2016.01.030. 00005. Page 88, Page 90, Page 105

Payne, R. J., Creevy, A., Malysheva, E., Ratcliffe, J., Andersen, R., Tsyganov, A. N., Rowson, J. G., Marcisz, K., Zielińska, M., Lamentowicz, M., Lapshina, E. D., and Mazei, Y. Tree encroachment may lead to functionally-significant changes in peatland testate amoeba communities. *Soil Biology and Biochemistry*, 98:18–21, 2016b. ISSN 00380717. 00000. Page 47, Page 87, Page 100

Petchey, O. L. and Gaston, K. J. Functional diversity (FD), species richness and community composition. *Ecology Letters*, 5(3):402–411, 2002. ISSN 1461-0248. Page 3

Petchey, O. L. and Gaston, K. J. Functional diversity: back to basics and looking forward. *Ecology Letters*, 9 (6):741–758, 2006. ISSN 1461-0248. 00876. Page 34

Pinto, R., de Jonge, V. N., and Marques, J. C. Linking biodiversity indicators, ecosystem functioning, provision of services and human well-being in estuarine systems: Application of a conceptual framework. *Ecological Indicators*, 36(0):644–655, 2014. ISSN 1470-160X. doi:10.1016/j.ecolind.2013.09.015. 00025. Page 79

Podani, J. Extending Gower's general coefficient of similarity to ordinal characters. *Taxon*, pages 331–340, 1999. 00186. Page 82

Poulin, M., Andersen, R., and Rochefort, L. A New Approach for Tracking Vegetation Change after Restoration: A Case Study with Peatlands. *Restoration Ecology*, 21(3):363–371, 2013. ISSN 1526-100X. doi:10.1111/j.1526-100X.2012.00889.x. 00038. Page 103

Punttila, P., Autio, O., Kotiaho, J., Kotze, J., Loukola, O., Noreika, N., Vuori, A., and Vepsäläinen, K. The effects of drainage and restoration of pine mires on habitat structure, vegetation and ants. *Silva Fennica*, 50(2), 2016. ISSN 22424075. doi:10.14214/sf.1462. 00004. Page 103

Qin, Y., Mitchell, E. A. D., Lamentowicz, M., Payne, R. J., Lara, E., Gu, Y., Huang, X., and Wang, H. Ecology of testate amoebae in peatlands of central China and development of a transfer function for paleohydrological reconstruction. *Journal of Paleolimnology*, 50(3):319–330, October 2013. doi:10.1007/s10933-013-9726-6. 00015 WOS:000324068900005. Page 6, Page 34, Page 99

Quinty, F. and Rochefort, L. *Peatland restoration guide.* Canadian Sphagnum Peat Moss Association, 2003. 00081. Page 6, Page 69, Page 103

R Core Team. *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2016.* ISBN 3-900051-07-0, 2016. 00000. Page 19, Page 37, Page 64, Page 82

Ramsar. *The Ramsar Convention Manual: a guide to the Convention on Wetlands (Ramsar, Iran, 1971).* Ramsar Convention Secretariat, Gland, Switzerland, 6th ed. edition, 2013. 00000. Page 3, Page 5, Page 33, Page 97

Ricotta, C. and Moretti, M. CWM and Rao's quadratic diversity: a unified framework for functional ecology. *Oecologia*, 167(1):181–188, 2011. 00000. Page 34, Page 82

Rosenfeld, J. S. Functional redundancy in ecology and conservation. *Oikos*, 98(1):156–162, 2002. ISSN 0030-1299, 1600-0706. doi:10.1034/j.1600-0706.2002.980116.x. 00263. Page 4

Royles, J., Amesbury, M. J., Convey, P., Griffiths, H., Hodgson, D. A., Leng, M. J., and Charman, D. J. Plants and Soil Microbes Respond to Recent Warming on the Antarctic Peninsula. *Current Biology*, 23(17):1702–1706, September 2013. ISSN 0960-9822. doi:10.1016/j.cub.2013.07.011. 00026. Page 101

Ruiz-Jaen, M. C. and Mitchell Aide, T. Restoration success: how is it being measured? *Restoration ecology*, 13 (3):569–577, 2005. Page 9

Rydin, H. a., Jeglum, J. K., and Jeglum, J. K. *The biology of peatlands, 2e.* Oxford university press, 2013. 00758. Page 61

Rykiel, E. J. Towards a definition of ecological disturbance. *Australian Journal of Ecology*, 10(3):361–365, 1985. ISSN 1442-9993. Page 4

Samaritani, E., Siegenthaler, A., Yli-Petays, M., Buttler, A., Christin, P. A., and Mitchell, E. A. D. Seasonal Net Ecosystem Carbon Exchange of a Regenerating Cutaway Bog: How Long Does it Take to Restore the C-Sequestration Function? *Restor Ecol*, 19(4):480–489, July 2011. ISSN 1061-2971. doi:10.1111/j.1526-100X.2010.00662.x. 00000. Page 15

Schönborn, W. Die Stratigraphie lebender Testaceen im Sphagnetum der Hochmoore. *Limnologica*, 1(4): 315–321, 1963. 00040. Page 100, Page 103

Schönborn, W. Untersuchungen über die Zoochlorellen-Symbiose der Hochmoor-Testacean. *Limnologica*, pages 173–176, 1965. 00000. Page 48

Schönborn, W. Population dynamics and production biology of testate amoebae (Rhizopoda, Testacea) in raw humus of two coniferous forest soils. *Archiv für protistenkunde*, 132(4):325–342, 1986. ISSN 0003-9365. 00000. Page 27

Schnoor, T., Bruun, H. H., and Olsson, P. A. Soil Disturbance as a Grassland Restoration Measure—Effects on Plant Species Composition and Plant Functional Traits. *PLoS ONE*, 10(4):e0123698, 2015. ISSN 1932-6203. doi:10.1371/journal.pone.0123698. 00000. Page 4

Schröter, D., Wolters, V., and De Ruiter, P. C. C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos*, 102(2):294–308, 2003. ISSN 1600-0706. doi:10.1034/j.1600-0579.2003.12064.x. Page 6

Schumann, M. and Joosten, H. Global peatland restoration manual. *Institute of Botany and Landscape Ecology, Greifswald University, Germany*, 2008. 00039. Page 103

Schwind, L. T. F., Arrieira, R. L., Bonecker, C. C., Lansa-Tôha, F. A., and others. Chlorophyll-a and Suspended Inorganic Material Affecting the Shell Traits of Testate Amoebae Community. *Acta Protozoologica*, 2016(3): 145–154, 2016. 00000. Page 37, Page 81, Page 100

Seppey, C. V. W., Fournier, B., Szelecz, I., Singer, D., Mitchell, E. A. D., and Lara, E. Response of forest soil euglyphid testate amoebae (Rhizaria: Cercozoa) to pig cadavers assessed by high-throughput sequencing. *International Journal of Legal Medicine*, 130(2):551–562, March 2016. ISSN 0937-9827, 1437-1596. doi:10.1007/s00414-015-1149-7. 00003. Page 70

Sieriebriennikov, B., Ferris, H., and de Goede, R. G. M. NINJA: An automated calculation system for nematode-based biological monitoring. *European Journal of Soil Biology*, 61(0):90–93, 2014. ISSN 1164-5563. doi:10.1016/j.ejsobi.2014.02.004. Page 105

Silva, D. M., Batalha, M. A., and Cianciaruso, M. V. Influence of fire history and soil properties on plant species richness and functional diversity in a neotropical savanna. *Acta Botanica Brasilica*, 27(3):490–497, 2013. ISSN 0102-3306. doi:10.1590/S0102-33062013000300005. 00004. Page 4

Singer, D., Kosakyan, A., Pillonel, A., Mitchell, E. A. D., and Lara, E. Eight species in the Nebela collaris complex: Nebela gimlii (Arcellinida, Hyalospheniidae), a new species described from a Swiss raised bog. *European Journal of Protistology*, 51(1):79–85, 2015. doi:10.1016/j.ejop.2014.11.004. 00008 WOS:000351031600007. Page 90

Smith, H. G. Diversity of Antarctic terrestrial protozoa. *Biodiversity and Conservation*, 5(11):1379–1394, November 1996. ISSN 0960-3115. doi:10.1007/bf00051984. Page 100, Page 101

Smith, H. G., Bobrov, A., and Lara, E. Diversity and biogeography of testate amoebae. *Biodivers Conserv*, 17 (2):329–343, February 2008. ISSN 0960-3115. doi:10.1007/s10531-007-9260-9. Page 6, Page 70

Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K., Tignor, M., and Miller, H. *Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. O.F. Canziani M.L. Parry J.P. Palut, cambridge university press edition, 2007. 01796. Page 5

Sousa, W. P. The role of disturbance in natural communities. *Annual review of ecology and systematics*, 15: 353–391, 1984. ISSN 0066-4162. Page 4

Stockmarr, J. Tablets with spores used in absolute pollen analysis. *Pollen et Spores*, 13:615–621, 1971. Page 18, Page 36

Stoecker, D. K. Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *European Journal of Protistology*, 34(3):281–290, 1998. 00187. Page 47, Page 103

Suding, K. N., Lavorel, S., Chapin, F. S., Cornelissen, J. H. C., Díaz, S., Garnier, E., Goldberg, D., Hooper, D. U., Jackson, S. T., and Navas, M.-L. Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology*, 14(5):1125–1140, 2008. ISSN 1365-2486. doi:10.1111/j.1365-2486.2008.01557.x. 00413. Page 36

Sullivan, M. E. and Booth, R. K. The Potential Influence of Short-term Environmental Variability on the Composition of Testate Amoeba Communities in Sphagnum Peatlands. *Microbial Ecology*, 62(1):80–93, July 2011. ISSN 0095-3628. doi:10.1007/s00248-011-9875-y. Page 26, Page 27, Page 34, Page 68

Swindles, G. T., Charman, D. J., Roe, H. M., and Sansum, P. A. Environmental controls on peatland testate amoebae (Protozoa: Rhizopoda) in the North of Ireland: Implications for Holocene palaeoclimate studies. *Journal of Paleolimnology*, 42(1):123–140, July 2009. ISSN 0921-2728. doi:10.1007/s10933-008-9266-7. Page 15, Page 34, Page 61

Swindles, G. T., Reczuga, M., Lamentowicz, M., Raby, C. L., Turner, T. E., Charman, D. J., Gallego-Sala, A., Valderrama, E., Williams, C., Draper, F., Honorio Coronado, E. N., Roucoux, K. H., Baker, T., and Mullan, D. J. Ecology of Testate Amoebae in an Amazonian Peatland and Development of a Transfer Function for Palaeohydrological Reconstruction. *Microbial Ecology*, 68(2):284–298, August 2014. ISSN 0095-3628, 1432-184X. doi:10.1007/s00248-014-0378-5. 00016. Page 70

Szelecz, I., Fournier, B., Seppey, C., Amendt, J., and Mitchell, E. Can soil testate amoebae be used for estimating the time since death? A field experiment in a deciduous forest. *Forensic science international*, 236:90–8, March 2014. ISSN 1872-6283 (Electronic) 0379-0738 (Linking). doi:10.1016/j.forsciint.2013.12.030. 00000. Page 70

Talbot, J., Richard, P. J. H., Roulet, N. T., and Booth, R. K. Assessing long-term hydrological and ecological responses to drainage in a raised bog using paleoecology and a hydrosequence. *Journal of Vegetation Science*, 21(1):143–156, 2010. Page 15, Page 61

Tilman, D. *Resource competition and community structure*, volume 17. Princeton University Press, 1982. ISBN 0-691-08302-9. Page 47, Page 101

Todorov, M. Observation on the soil and moss testate amoebae (Protozoa: Rhizopoda) from Pirin Mountain (Bulgaria). *Acta Zool. Bulg.*, 50:19–29, 1998. 00000. Page 15

Tolonen, K., Warner, B. G., and Vasander, H. Ecology of Testaceans (Protozoa, Rhizopoda) in Mires in Southern Finland .1. Autecology. *Arch Protistenkd*, 142(3-4):119–138, December 1992. ISSN 0003-9365. Page 6

Trepel, M. Evaluation of the implementation of a goal-oriented peatland rehabilitation plan. *Ecological Engineering*, 30(2):167–175, 2007. ISSN 0925-8574. doi:10.1016/j.ecoleng.2006.11.003. 00023. Page 6, Page 99

Tsyganov, A. N., Aerts, R., Nijs, I., Cornelissen, J. H. C., and Beyens, L. Sphagnum-dwelling Testate Amoebae in Subarctic Bogs are More Sensitive to Soil Warming in the Growing Season than in Winter: the Results of Eight-year Field Climate Manipulations. *Protist*, 163(3):400–414, May 2012. ISSN 1434-4610. doi:10.1016/j.protis.2011.07.005. 00023. Page 63, Page 70, Page 81

Tsyganov, A. N., Komarov, A. A., Mitchell, E. A. D., Shimano, S., Smirnove, O. V., Aleynikov, A. A., and Mazei, Y. A. Additive partitioning of testate amoeba species diversity across habitat hierarchy within the pristine southern taiga landscape (Pechora-Ilych Biosphere Reserve, Russia). *European Journal of Protistology*, 51 (1):42–54, 2015. doi:10.1016/j.ejop.2014.11.003. 00003 WOS:000351031600004. Page 62

Turner, T. E. and Swindles, G. T. Ecology of Testate Amoebae in Moorland with a Complex Fire History: Implications for Ecosystem Monitoring and Sustainable Land Management. *Protist*, 163(6):844–855, 2012. ISSN 1434-4610. doi:10.1016/j.protis.2012.02.001. 00000. Page 15

Turunen, J., Tomppo, E., Tolonen, K., and Reinikainen, A. Estimating carbon accumulation rates of undrained mires in Finland–application to boreal and subarctic regions. *The Holocene*, 12(1):69–80, 2002. 00509. Page 5, Page 61

Turunen, J., Roulet, N. T., Moore, T. R., and Richard, P. J. Nitrogen deposition and increased carbon accumulation in ombrotrophic peatlands in eastern Canada. *Global Biogeochemical Cycles*, 18(3), 2004. 00132. Page 105

Vačkář, D., ten Brink, B., Loh, J., Baillie, J. E. M., and Reyers, B. Review of multispecies indices for monitoring human impacts on biodiversity. *Ecological Indicators*, 17(0):58–67, 2012. ISSN 1470-160X. doi:10.1016/j.ecolind.2011.04.024. Page 3, Page 6

van Bellen, S., Mauquoy, D., Payne, R. J., Roland, T. P., Hughes, P. D., Daley, T. J., Loader, N. J., Alayne Street-Perrott, F., Rice, E. M., and Pancotto, V. A. An alternative approach to transfer functions? Testing the performance of a functional trait-based model for testate amoebae. *Palaeogeography, Palaeoclimatology, Palaeoecology*, December 2016. ISSN 00310182. doi:10.1016/j.palaeo.2016.12.005. 00000. Page 34, Page 48, Page 61, Page 79

Van den Brink, P. J. and Ter Braak, C. J. Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. *Environmental Toxicology and Chemistry*, 18(2):138–148, 1999. 00539. Page 64

Vandermeer, J. H. Niche Theory. *Annual Review of Ecology and Systematics*, 3(1):107–132, 1972. doi:10.1146/annurev.es.03.110172.000543. 00000. Page 47

Villéger, S., Mason, N. W., and Mouillot, D. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, 89(8):2290–2301, 2008. ISSN 0012-9658. Page 88

Villéger, S., Novack-Gottshall, P. M., and Mouillot, D. The multidimensionality of the niche reveals functional diversity changes in benthic marine biotas across geological time: Long-term functional diversity changes. *Ecology Letters*, 14(6):561–568, June 2011. ISSN 1461023X. doi:10.1111/j.1461-0248.2011.01618.x. 00000. Page 34, Page 36

Vincke, S., Van de Vijver, B., Nijs, I., and Beyens, L. Changes in the testacean community structure along small soil profiles. *Acta protozoologica*, 45(4):395–406, 2006. Page 6

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E. Let the concept of trait be functional! *Oikos*, 116(5):882–892, 2007. ISSN 1600-0706. doi:10.1111/j.0030-1299.2007.15559.x. Page 4, Page 61, Page 79, Page 103

Violle, C., Nemergut, D. R., Pu, Z., and Jiang, L. Phylogenetic limiting similarity and competitive exclusion. *Ecology letters*, 14(8):782–787, 2011. 00187. Page 61

Vitousek, P. M. Human Domination of Earth's Ecosystems. *Science*, 277(5325):494–499, 1997. ISSN 00368075, 10959203. doi:10.1126/science.277.5325.494. 07202. Page 3

Vittoz, P. and Guisan, A. How reliable is the monitoring of permanent vegetation plots? A test with multiple observers. *Journal of Vegetation Science*, 18(3):413–422, 2007. ISSN 1654-1103. Page 6, Page 97

Wallace, K. J. Classification of ecosystem services: Problems and solutions. *Biological Conservation*, 139(3–4): 235–246, 2007. ISSN 0006-3207. doi:10.1016/j.biocon.2007.07.015. 00000. Page 3

Wanner, M. and Meisterfeld, R. Effects of some environmental factors on the shell morphology of testate amoebae (Rhizopoda, Protozoa). *European Journal of Protistology*, 30(2):191–195, 1994. ISSN 0932-4739. doi:10.1016/S0932-4739(11)80029-5. Page 100

Wanner, M., Elmer, M., Kazda, M., and Xylander, W. E. Community assembly of terrestrial testate amoebae: how is the very first beginning characterized? *Microbial ecology*, 56(1):43–54, 2008. ISSN 1432-184X. doi:10.1007/s00248-007-9322-2. Page 6

Warner, B. G. and Charman, D. J. Holocene Changes On A Peatland In Northwestern Ontario Interpreted From Testate Amebas (Protozoa) Analysis. *Boreas*, 23(3):270–279, September 1994. ISSN 0300-9483. 00000. Page 6

Warner, B. G. *Methods in quaternary ecology.* Geoscience Canada. Reprint series 5. Geological Association of Canada, St. John's Newfoundland, 1990. ISBN 0-919216-42-0. Page 6

Warner, B., Asada, T., and Quinn, N. Seasonal influences on the ecology of testate amoebae (Protozoa) in a small Sphagnum peatland in Southern Ontario, Canada. *Microbial ecology*, 54(1):91–100, July 2007. ISSN 0095-3628. doi:10.1007/s00248-006-9176-z. Page 27, Page 104

Waughman, G. J. Chemical Aspects of the Ecology of Some South German Peatlands. *J Ecol*, 68(3):1025–1046, 1980. ISSN 0022-0477. 00140. Page 25

Webb, C. O., Losos, J. B., and Agrawal, A. A. Integrating phylogenies into community ecology. *Ecology*, 87(7): S1–S2, July 2006. ISSN 0012-9658. doi:10.1890/0012-9658(2006)87[1:Ipice]2.0.Co;2. Page 82

White, P. S. and Pickett, S. T. A. Natural disturbance and patch dynamics: an introduction. *Academic Press*, pages 3–13, 1985. 00009. Page 4

Wilken, S., Huisman, J., Naus-Wiezer, S., and Donk, E. Mixotrophic organisms become more heterotrophic with rising temperature. *Ecology letters*, 16(2):225–233, 2013. 00041. Page 61, Page 103

Wilkinson, D. M. What is the upper size limit for cosmopolitan distribution in free-living microorganisms? *J. Biogeogr.*, 28(3):285–291, 2001. ISSN 0305-0270. doi:10.1046/j.1365-2699.2001.00518.x. 00103 WOS:000169689800001. Page 6

Wilkinson, D. M. Testate amoebae and nutrient cycling: peering into the black box of soil ecology. *Trends Ecol. Evol. (Amst.)*, 23(11):596–599, 2008. ISSN 0169-5347. doi:10.1016/j.tree.2008.07.006. 00037. Page 6, Page 99, Page 101

Wilkinson, D. M. and Mitchell, E. A. D. Testate Amoebae and Nutrient Cycling with Particular Reference to Soils. *Geomicrobiol J*, 27(6-7):520–533, September 2010. ISSN 0149-0451. doi:10.1080/01490451003702925. Page 79, Page 99, Page 100

Wilkinson, D. and Davis, S. Rapid assessments of microbial biodiversity using relationships between Genus and Species richness. Studies on Testate Amoebae. *order*, 2000. Page 19

Woodland, W. A., Charman, D. J., and Sims, P. C. Quantitative estimates of water tables and soil moisture in Holocene peatlands from testate amoebae. *Holocene*, 8(3):261–273, 1998. ISSN 0959-6836. doi:10.1191/095968398667004497. Page 6

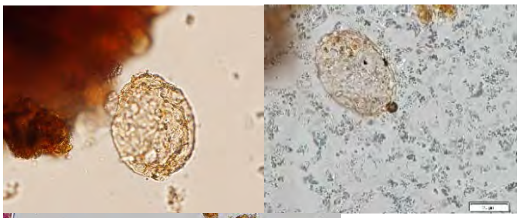
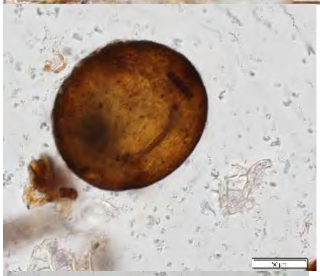
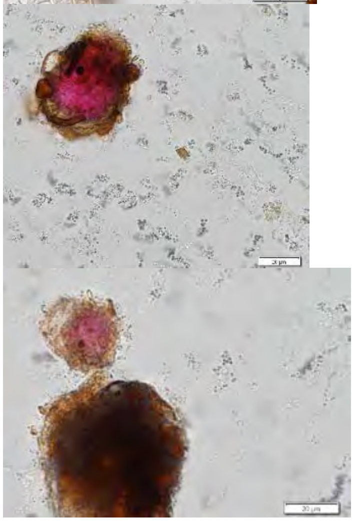
Woodward, F. I. and Diament, A. D. Functional Approaches to Predicting the Ecological Effects of Global Change. *Functional Ecology*, 5(2):202–212, 1991. ISSN 0269-8463. doi:10.2307/2389258. 00090. Page 3, Page 61

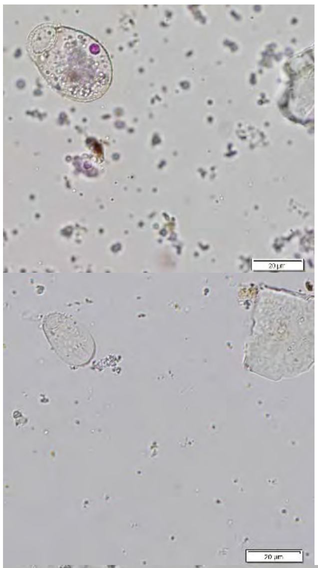
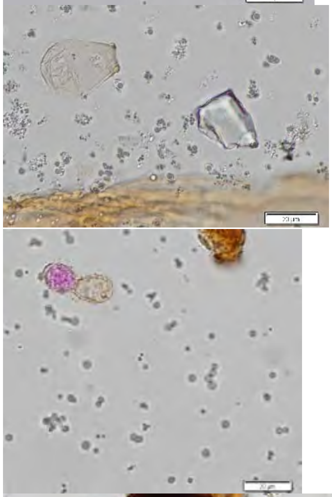
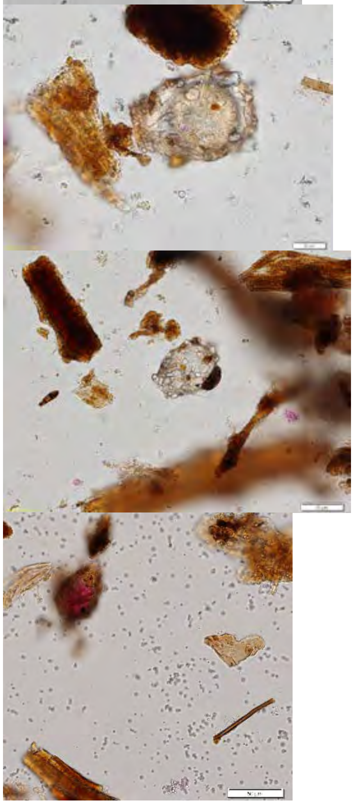
Yu, Z. C. Northern peatland carbon stocks and dynamics: a review. *Biogeosciences*, 9(10):4071–4085, October 2012. ISSN 1726-4189. doi:10.5194/bg-9-4071-2012. 00111. Page 5, Page 33, Page 97

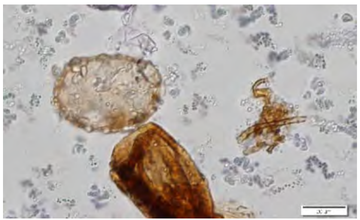
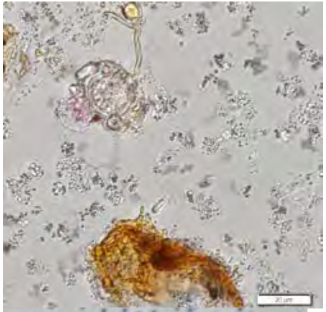
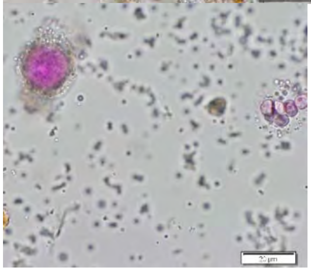
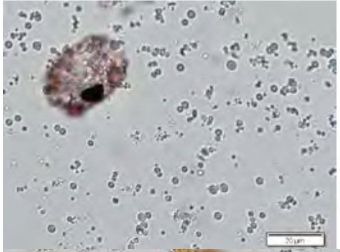
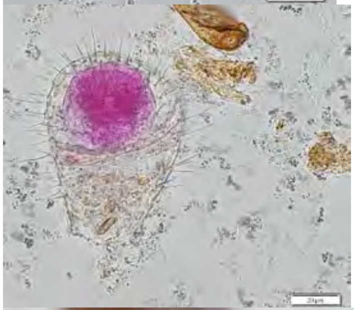
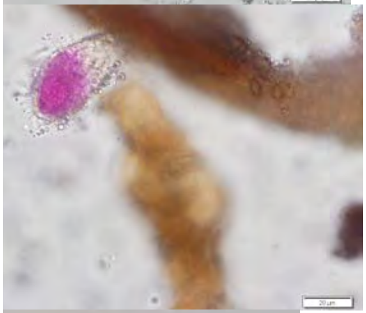
Zerbe, S., Steffenhagen, P., Parakenings, K., Timmermann, T., Frick, A., Gelbrecht, J., and Zak, D. Ecosystem Service Restoration after 10 Years of Rewetting Peatlands in NE Germany. *Environmental Management*, 51 (6):1194–1209, 2013. ISSN 0364-152X, 1432-1009. doi:10.1007/s00267-013-0048-2. 00011. Page 6

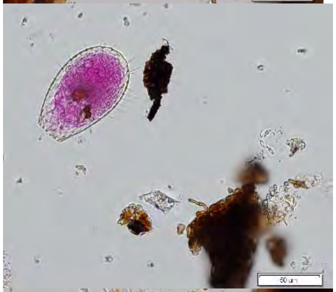
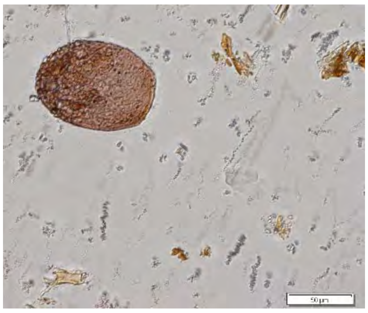
Appendix A

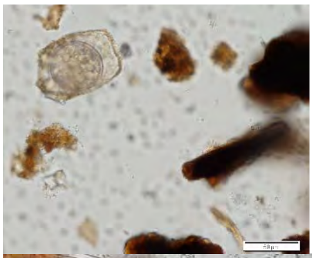
Portfolio

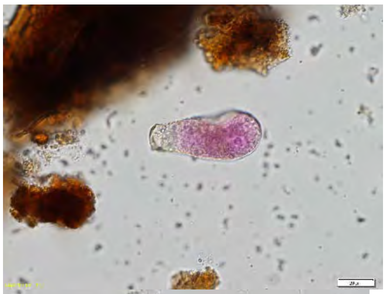
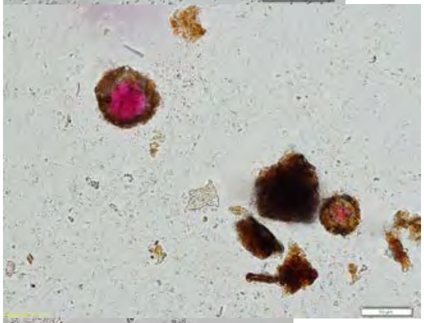
Morphotaxa	**Taxa included**	**Pictures**
Amphitrema wrightianum	*Amphitrema wrightianum* *Amphitrema stenostoma*	
Archerella flavum	*Archerella flavum*	
Assulina muscorum	*Assulina muscorum*	
Assulina seminulum	*Assulina seminulum*	
Bullinularia indica	*Bullinularia indica*	
Centropyxis aerophila	*Centropyxis aerophila* *Centropyxis cassis* *Centrophyxis platystoma* *Cyclopyxis kahli*	

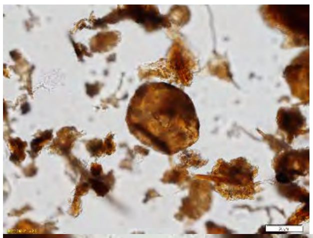
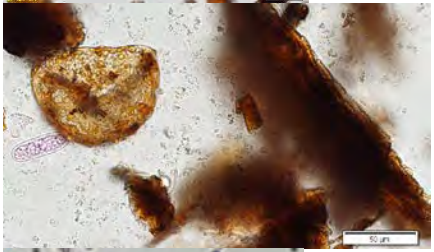
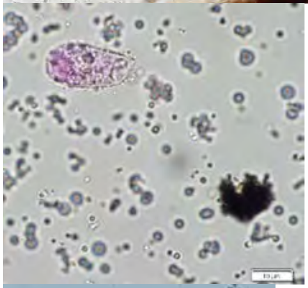
Morphotaxa	**Taxa included**	**Pictures**
Corythion dubium	*Corythion dubium* *Corythion delamarei*	
Cryptodifflugia sp	*Cryptodifflugia minuta* *Cryptodifflugia oviformis*	
Difflugia lucida	*Difflugia brevicola* *Difflugia lucida* *Difflugia pyriformis* *Difflugia pristis*	

Morphotaxa	**Taxa included**	**Pictures**
Difflugia pulex	*Difflugia pulex* *Difflugia humilis* *Cryptodifflugia sacculus* *Schoenbornia humicola* *Pseudodifflugia sp*	   
Euglypha “small species”	*Euglypha ciliata* *Euglypha rotunda* *Euglypha laevis*	  

Morphotaxa	**Taxa included**	**Pictures**
Euglypha "large species"	*Euglypha compressa* *Euglypha penardi* *Euglypha strigosa* *Euglypha tuberculata*	   
Heleopera rosea	*Heleopera rosea*	

Morphotaxa	**Taxa included**	**Pictures**
Heleopera sylvatica	*Heleopera sylvatica*	 
Hyalosphenia elegans	*Hyalosphenia elegans*	
Hyalosphenia minuta	*Hyalosphenia minuta*	
Hyalosphenia papilio	*Hyalosphenia papilio* *Hyalosphenia ovalis*	
Nebela collaris	*Nebela collaris* *Nebela bohemica*	 

Morphotaxa	Taxa included	Pictures
Nebela militaris	*Nebela militaris*	
Nebela tincta	*Nebela tincta* *Nebela guttata* *Nebela gimlii* *Nebela rotunda*	  
Phryganella acropodia	*Phryganella acropodia* *Cyclopyxis eurystoma*	 

Morphotaxa	**Taxa included**	**Pictures**
Trigonopyxis arcula	*Trigonopyxis arcula*	 
Trinema lineare	*Trinema lineare*	 

Appendix B

Isabelle Koenig

Curriculum Vitae

Rue des Perrières 25
CH-2340 Le Noirmont
📱 *+41 (0)78 621 32 22*
☎ *+41 (0)32 422 89 01*
✉ *ikoenig@bluewin.ch*
in *isabellekoenig*

I am a dynamic scientist interested in ecosystems functioning and in the impact of climate changes on ecosystems. I use descriptive, experimental and theoretical approaches to handle this complex study field.

Education

2012–2017	**Ph.D in biology**, *University of Neuchâtel*, Switzerland. Testate amoeba community structure and functional diversity to assess climate changes on ecosystem functioning.
2009–2011	**M.Sc in biogeosciences**, *Universities of Neuchâtel and Lausanne*, Switzerland. Comparative ecology of vascular plant, bryophyte and testate amoeba communities in four *Sphagnum* peatlands along an altitudinal gradient in Switzerland
2006–2009	**B.Sc in biology**, *University of Neuchâtel*, Switzerland.
2000–2002	**Certificate in Prevention and Control of Diseases**, *IRSP*, Lausanne, Switzerland.
1984–1989	**Graduation in nursing**, *Ecole de soins infirmiers Bois-Cerf*, Lausanne, Switzerland.

Ph.D thesis

Title	*Using testate amoeba community structure and functional diversity to assess climate changes on ecosystem functioning: finding relevant and convenient tools for bio-monitoring of Sphagnum peatlands evolution or recovery.*
Supervisors	Prof. E.A.D. Mitchell Dr. R.-C. Le Bayon
Description	This thesis was conducted at the crossroad between theoretical and applied ecology. The central point was to test the use of testate amoeba community structure in the context of *Sphagnum* peatlands monitoring, and to address some theoretical issues related to the selection of functional traits. This work was structured along a gradient of perturbation, from natural peatlands along an altitudinal gradient, to a mesocosm study with manipulated water level. The main aim was to assess the potential of testate amoeba in ecological and functional monitoring of *Sphagnum* peatlands, together with the understanding of ongoing processes.

Professional situation

2013–today	**Ph.D Assistant**, *University*, Neuchâtel. Teaching (practical works, statistical support) and mentoring undergraduate and postgraduate thesis. Organising and managing two scientific symposiums.

2014–2017 **Project Manager**, *Nomad – Home care organisation*, La Chaux-de-Fonds.
Situational analysis, implementating corrective measures, creating perennial structure for prevention and control of diseases.
Staff training and supervising in hospital hygiene, establishing a professional network in prevention and control of diseases.

01.2012–12.2012 **Scientific Collaborator**, *University*, Neuchâtel.
Scientific writing.

Academic achievements

Detailed achievements:

- Ecological design, sampling and statistical interpretation of data;
- Microscopic identification of testate amoeba and pictures;
- Scientific writing and effective public presentation;
- Organising of a scientific symposium:
  - Leadership of the whole organisation;
  - Planning organisation, work delegation and supervision;
  - Contingency management;
- Teaching:
  - Practical work: protistes and invertebrates, Bachelor of Science;
  - Practical work: field works in peatlands;
  - Statistical teaching: descriptive statistics, multivariate analyses, transfer functions, graphical data representation;
  - Assistance and support for Master of Science students.

Miscellaneous

1999–2001 **Travelling Sale Representative in hospital hygiene**, *Henkel-Ecolab*, Basel.
Counselling and sales. Staff training in hospital hygiene, in nursing homes and senior's facilities.

1989–1999 **Registered Nurse**, *Public hospitals*, Geneva and Delémont.
Nursing and teaching. Nursing student's supervision.

Languages

French	Native	
English	B2 level	*Conference presentations, reading & writing scientific articles, online courses, etc.*
German	B1 level	*By Henkel-Ecolab, most of the teaching and working meetings in German.*

IT skills

Microsoft	Word, Excel, PowerPoint
Statistics	R, RStudio
Adobe	Illustrator, Acrobat Pro, InDesign
Open source	Libre Office, Inkscape, LaTeX
OS	Windows, Linux Mint

Oral presentations

October 2016	TEBIS V, Traits Ecologiques et Biologiques des organIsmes des Sols, Toulouse, France
September 2015	VII European Congress of Protistology, Sevilla, Spain
May 2015	End meeting of the Polish-Swiss project "Climpeat", Torun, Poland
September 2014	7th International Symposium On Testate Amoeba, Poznan, Poland

May 2014	Progress meeting of the Polish-Swiss project “Climpeat”, Frasne, France
October 2013	Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland
October 2013	Colloque franco-suisse “Zones humides: fonctionnement, indicateurs, gestion et restauration”, Frasne, France

Posters

February 2017	Biology17, annual Swiss conference on ecology, evolution, systematics and conservation, Bern, Switzerland
February 2014	Annual meeting of the Swiss Soil Science Society (SSSS), Nyon, Switzerland
February 2013	32nd meeting of the German society for protozoology (DGP), Kartaise Ittingen, Switzerland
January 2013	Peatland ecology and management, Neuchâtel, Switzerland

Articles

Isabelle Koenig, Elizabeth Feldmeyer-Christe, and Edward A. D. Mitchell. Comparative ecology of vascular plant, bryophyte and testate amoeba communities in four Sphagnum peatlands along an altitudinal gradient in Switzerland. *Ecological Indicators*, 54:48–59, 2015. 00002 WOS:000355365500005.

Interests

Natural science	Ornithology, botany, protists, soil biology
Membership	Société des Naturalistes Franc-Montagnards, Swiss Botany Society, International Society of Protistologists
Sports	Mountain biking, cross-country skiing, walking