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Effects of simulated climate change on the structure and functioning of *Sphagnum* peatlands – an experimental and modeling study

Thesis Advisor: Edward MITCHELL

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Jury :

Pr. Edward MITCHELL	-	University of Neuchâtel
Dr. Richard PAYNE*	-	University of York
Dr. Vincent JASSEY*	-	EPFL, Lausanne
Dr. Enrique LARA*	-	University of Neuchâtel
Dr. Colomban DE VARGAS	-	Station biologique de Roscoff, UMPC
Pr. Daniel GILBERT	-	Univ. Franche–Comté, Besançon

* rapporteur

UNIVERSITÉ DE NEUCHÂTEL

CLIM PEAT

UNIVERSITÉ DE NEUCHÂTEL

Faculté des sciences
Secrétariat-décanat de Faculté
Rue Emile-Argand 11
2000 Neuchâtel - Suisse
Tél: + 41 (0)32 718 2100
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

Monsieur Matthieu MULOT

Titre:

"Effects of simulated climate change on the structure and functioning of *Sphagnum* peatlands – an experimental and modeling study"

sur le rapport des membres du jury composé comme suit:

- Prof. Edward Mitchell, directeur de thèse, Université de Neuchâtel, Suisse
- Dr Enrique Lara, Université de Neuchâtel, Suisse
- Dr Vincent Jassey, EPF Lausanne, Suisse
- Dr Richard Payne, University of York, United Kingdom
- Dr Colomban de Vargas, CNRS, Station Biologique de Roscoff, France
- Prof. Daniel Gilbert, Université de Franche-Comté, France

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Abstract

Effects of simulated climate change on the structure and functioning of *Sphagnum* peatlands – an experimental and modeling study

Abstract: The main objective of this thesis was to investigate the response of Sphagnum dominated peatland to water table manipulations in an integrative approach. We conceived a mesocosms experiment that simulates artificial peatlands at different water regimes.

We first show that our mesocosms behave according to the literature, with respiration and decomposition rates enhanced by the combined effects of water and temperature. We hypothesize that this C cycle dynamic is regulated by microbial activity. To address this question, we investigate the topological change of micro eukaryotic interaction network along the gradient and show that the structure of the network evolves along the gradient, showing a change point around -10 cm.

Then we study the functional response of one functional group of micro eukaryotes, the testate amoebae. We show that environmental filtering constrained by manipulated water level promotes traits selection, and that this traits response can be used to mathematically model Hutchinson niche concept. Finally, we demonstrated the phenotypic plasticity on *Hyalosphenia papilio*, showing that traits selection acts both between species by community replacement, and within species by phenotypic plasticity.

We propose a framework to model environmental filtering, integrating all components of this study.

Keywords: Peatland, carbon, protist, climate change, testate amoebae, modeling.

Résumé

Effets de changements climatiques simulés sur la structure et le fonctionnement des tourbières à sphaignes – Expériences et modélisation

Résumé : L'objectif principal de cette thèse était d'investiguer la réponse des tourbières à sphaigne à des manipulations de niveau de nappe dans une approche intégrative. Nous avons conçu une expérience en mésocosmes qui simule des tourbières artificielles à différents niveau de nappe.

Nous montrons dans un premier temps que les mésocosmes ont un comportement cohérent avec la littérature, avec une respiration et une décomposition amplifiées par les effets combinés du niveau d'eau et de la température. Nous posons l'hypothèse que cette dynamique du cycle du carbone est régulée par l'activité microbienne. Pour corroborer cette hypothèse, nous analysons les changements topologiques des réseaux d'interaction des micro–eucaryotes le long du gradient de nappe et montrons que la structure du réseau évolue le long du gradient, montrons un point d'inflexion autour de -10 cm.

Ensuite, nous étudions la réponse fonctionnelle d'un groupe de micro–eucaryotes, les amibes à thèque. Nous montrons que le filtre environnemental contraint par les niveaux d'eau artificiels promeut la sélection de traits fonctionnels, et que cette réponse des traits peut être utilisée pour modéliser mathématiquement le concept de niche tel que décrit par Hutchinson. Enfin, nous démontrons la plasticité phénotypique de *Hyalosphenia papilio*, montrant que la sélection de traits a lieu à la fois par remplacement des communautés et par plasticité phénotypique au sein d'une espèce.

Nous proposons un cadre théorique pour modéliser le filtre environnemental, intégrant tous les composants de cette étude.

Mots–clés: Tourbière, carbone, protiste, changements climatiques, amibes à thèque, modélisation

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I have to express my gratitude to Emily. Maybe you read the thesis until this point and were courageous enough to dig a bit between the french words of this section. In any case, you're probably partly responsible for this document. You thoroughly proofread my application letter for this position and came full circle (not sure I'm using this expression the right way) by proofreading my introduction. Thank you so much for the push you gave me to start my PhD journey.

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Everything not saved
will be lost.

NINTENDO
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CHAPTER 1

Introduction

Although it might seem simple to present peatlands, the complexity of such systems requires entire books to draw a near exhaustive picture of them. We chose clarity over completeness to introduce this thesis, as this introduction is not intended to be an extensive review of *Sphagnum* peatland functioning. Nevertheless, we provide all the information necessary to fully apprehend this thesis. The reader wishing to learn more about a specific part of this introduction may refer to existinf complete works [Gopal 1990, Rydin 2006, Martini 2007, Reddy 2008]. As this thesis focuses principally on peatland microbial response to water level manipulation, we chose to outline the introduction from a global peatland picture to a microbe orientated view. We will first present the general characteristics of peatlands and their potential vulnerability to climate changes, and will then detail the microbial processes involved in the peatland functioning. Finally we will present the structure of the thesis.

1.1 Peatlands: complex systems for which hydrology is crucial

We begin with a concise overview of the studied system. As our study focuses primarily on microbial interactions within the bryosphere (subsection 1.3.1), we will not thoroughly describe the vegetation, but rather emphasize the importance of hydrology for the system functioning.

Peatlands are wetlands, that is, lands where the water level is near the ground surface, in some cases inducing anoxic conditions in the substrate. Hydrology is such that it induces specialized fauna and flora. According to [Warner 1997], a wetland is defined as a land that is saturated with water long enough to promote wetland or aquatic processes as indicated by poorly drained soils, hydrophytic vegetation and various kinds of biological activities which are adapted to a wet environment. Peatlands are a particular type of wetlands characterized by a more or less thick layer of peat, which is formed by the accumulation, degradation and compaction of organic matter (mainly plant rhizosphere remains and dead bryophytes) under anoxic condition driven by water-logged soil.

Depending on environmental conditions driving their evolution, peatlands present subcategories : marshes, swamps, bogs, and fens. Our focus is on bogs and fens, which present an abundance of bryophytes of the *Sphagnum* genus. Bogs and fen are classified along a bog – poor fen – rich fen series. Bogs are ombrotrophic, meaning that water and nutrient input is supplied by precipitations, and are thus characterized by the absence of plants indicators of minerotrophy (*i.e.* dissolved minerals as main ressource). Conversely, fens are more minerotrophic, receiving water and nutrients from streams or springs. Rich fens are less acidic (pH 5–8) than poor fens (pH 4–5.5) and presents a higher

plant diversity. Although bogs are always oligotrophic, fens (rich or poor) range from oligotrophic to eutrophic. In fens, richness refers to floristic richness rather than nutrient richness [Rydin 2006]. Based on site physico-chemical properties and vegetation [Bridgham 1996], several classification systems have been proposed. The most widely adopted in the Northern Hemisphere is the one drawn by [Racey 1996] and summarized in [Rydin 2006], reported in Figure 1.1.

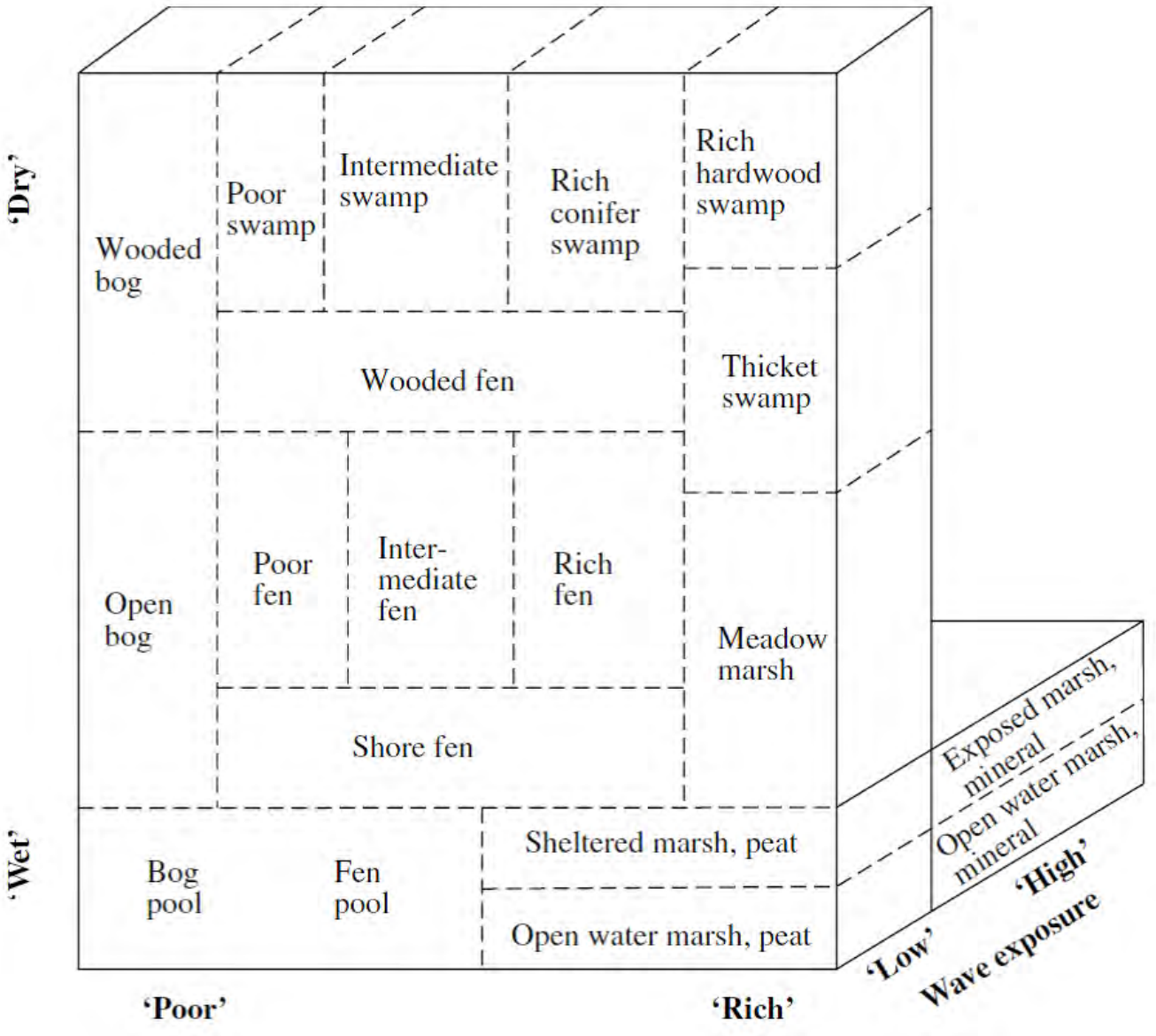

Figure 1.1: Classification of peatlands, from [Racey 1996, Rydin 2006]. Our studied stystem belong to the bogs–fens series, characterized by high water level.

The vascular plants diversity of *Sphagnum*-dominated peatlands is quite low, and *Eriophorum vaginatum* is often the most abundant vascular plant in the Northern hemisphere. The abundance and diversity of vascular plants increases as water level decreases. This vegetation change is quite well marked along a microtopographic gradient [Payette 2001]. Indeed peatlands are not flat, and microtopography plays an important role in system functioning. The topographic structure of peatlands can

be divided into three mains habitats; pools, hummocks and lawns (Figure 1.2). These microhabitats differ in wetness (because of different relative elevations above water table) resulting in plant diversity and microbial diversity [Jassey 2011b, Marcisz 2014a]. Indeed, this succssion of microhabitats offer the possibility for different microbes and plant to coexist wuthin the same peatland, while inhabiting distinct niches.

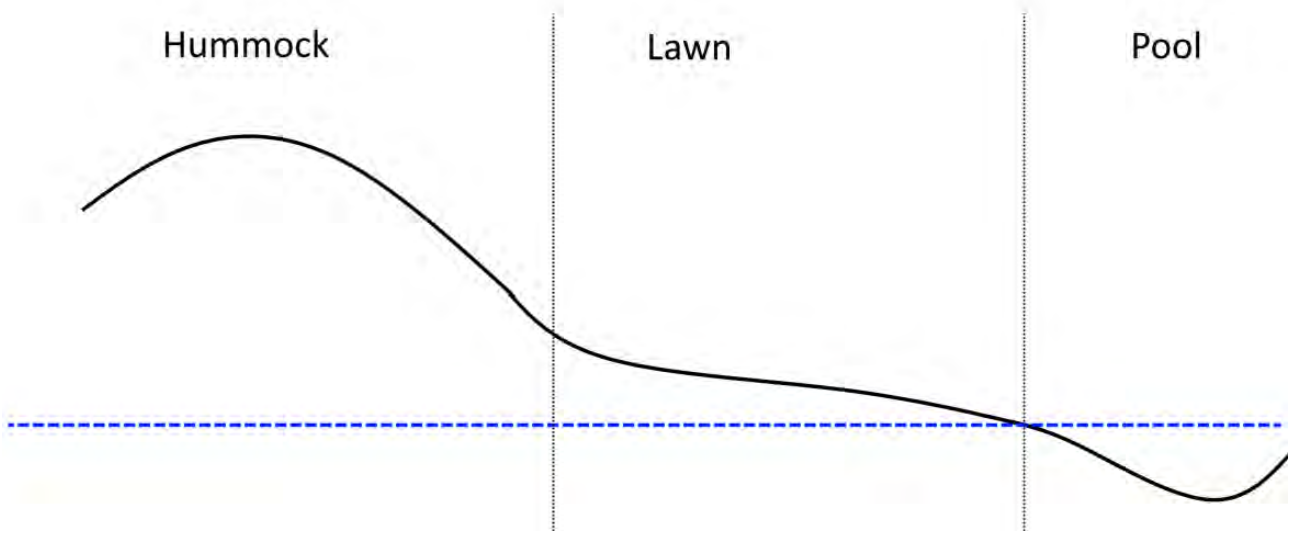

Figure 1.2: Microtopography in peatlands. The blue dashed line indicates the water level. The plain black line indicate the soil surface. The microtopography induces contrasted habitats due to the hydrological gradient it creates. Hummocks rise well above the water table, and are relatively dry microhabitat, while pools are permanently flooded

Hummocks are the driest microhabitat in *Sphagnum*-dominated peatlands. Raised 20 to 40 cm above water level, they express the highest diversity of vascular plants and presents dwarf shrubs. Lawns are mid-wet micro-habitats, situated 5–20 cm above the water level, and present a high diversity of graminoids. They also present the highest diversity of mosses (*Sphagnum fuscum*, *S. rubellum*, *S. balticum*, and *S. cuspidatum*) [Rydin 2006]. Pools are permanently water-filled basins, often with some vegetation at their edges. Of course, micro-habitats are not as clearly segregated as represented in the figure, and thus a wide variety of intermediate states exists from pool (wet) to hummocks (dry).

1.2 Peatland, C cycling and Global Environmental Changes (GEC)

1.2.1 C cycling

Because of their hydrology, peatlands are characterized by anoxia, inducing a low decomposition rate, which in turns induces carbon to be stored within the peat layer (carbon sink function). The upper part of the peatland, aerated, is called the acrotelm, while the lower part, water saturated, is called the catotelm. The mosses of the acrotelm accumulate C from atmospheric CO_2 via the process of photosynthesis. *Sphagnum* stems grow continuously, the upper living part growing on top of the decaying dead yellow part. Consequently, the dead parts compact and accumulate within the peat layer, resulting in organic matter accretion (Figure 1.3). The key to C accumulation in peatlands is not high net primary production (217 – 3194 $g_C.m^2.year^1$ [Gopal 1990]) (NPP) but low decomposition rate [Ito 2004]: as the accumulation to the catotelm is made under anoxic conditions, only a small part of this organic matter is decomposed. This leads the *Sphagnum* peatlands behaving as a C sink. Hence, peatlands are valuable pools of sequestered C and understanding their response to predict potential feedbacks on the global C cycle is crucial [Belyea 2004, Rydin 2006, Yu 2006].

1.2.2 Global Environmental Changes

1.2.2.1 Global context

As described previously, the peatland C sink function is mainly due to low decomposition rate induced by high water level. One can expect this C accumulation to be altered by ongoing Global Environmental Changes (GEC. The three major factors are climate change, atomspheric N deposition, and land use changes) [Pachauri 2015]. One scenario is the accelerated decomposition of organic matter and the resulting increase of greenhouse gases (GHG, mainly CO_2 and CH_4) in the atmosphere.

The vast majority of peatlands are situated in the Northern hemisphere, and contain about 1/3 of the world's soil C stock in an area accounting for only 3–5 % of the global land surface [Turunen 2002, Mitra 2005, Frokling 2007](Figure 1.4). *Sphagnum*-dominated peatlands situated in Boreal and Subarctic areas and expected to experience large climate changes in the coming century. As a result, identifying and quantifying the potential feedbacks from these high-latitude ecosystems is essential for future climate projections.

In Europe, peatlands have been much affected by peat harvesting or drainage for agriculture and forestry [Chapman 2003], and the preservation of the remaining natural sites and the restoration of damaged peatlands is now a EU priority [Raeymaekers 1999]. In addition to direct human impacts, peatlands are currently exposed to indirect human impacts such as climate change and atmospheric deposition of nitrogen, which will affect their structure (e.g. vegetation and soil communities) and functioning (e.g. C balance) and possibly invalidate previous findings or projection

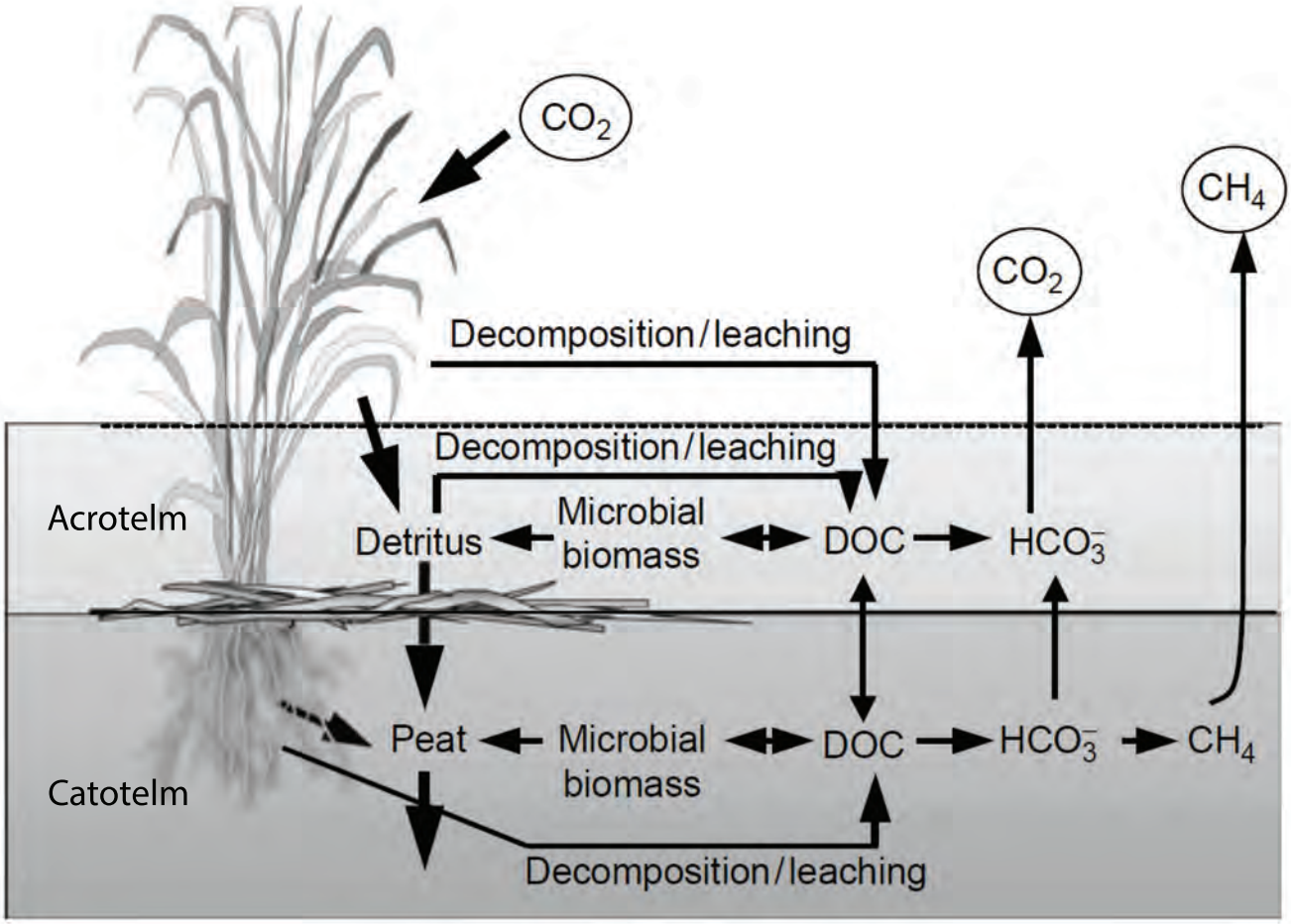

Figure 1.3: Carbon sequestration in peatland. Atmospheric carbon is used by plants to produce biomass. Part of this biomass will be degraded by the detrital foodweb, and some carbon will be returned to the atmosphere as CO_2 or CH_4. Part of this biomass will remain in the peat, consequently storing carbon from the atmoshpere to the peat. This ability to store carbon is responsible for the peatland carbon sink function. from [Reddy 2008]

based on steady state climate setting. Indeed, peatlands that are currently recovering from past damage (e.g. through spontaneous regeneration or as a result of restoration) may fail to recover fully [Glatzel 2004, Samaritani 2011]. As shifts in vegetation are slow, and even the presence of keystone species such as *Sphagnum sp.* do not necessarily indicate the full recovery of a C-sequestering function [Francez 2000], other indicators such as testate amoebae (Protists) are being considered as early indicators of ecosystem dynamics and functioning [Buttler 1996, Laggoun-Défarge 2008].

It is still not clear how fast peatlands respond to changes in temperature and droughts in continental climate (relatively fra from the sea shore) settings [Booth 2010a] and, since continental regions account for a significant proportion of all northern hemisphere peatlands (e.g. much of Russia and North America), little is known on how this will affect the global C cycling. These questions can therefore be considered as research priorities to better understand peatland ecosystem/biodiversity responses to climate warming.

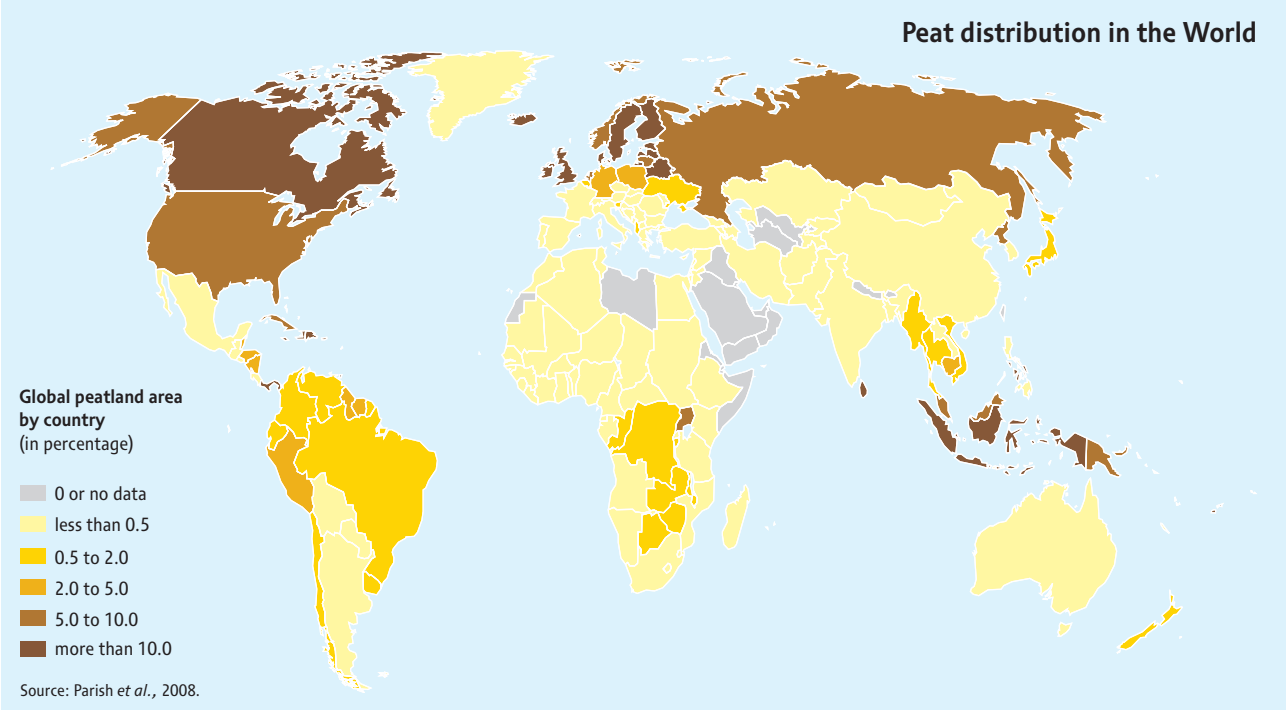

Figure 1.4: Peat distribution in the World. Most of the european peatlands have been damaged by anthropic activities. The nothern hemisphere shows a dense distribution of peatlands, especially in boreal zones [Parish 2008].

1.2.2.2 Climate previsions and their potential impacts on peatland functinning

A previously detailed, peatlands functioning is caused by the particular hydrology occurring in these ecosystems. The key to optimum peatland functioning is water-logged soil, and thus a higher water table. This water level depends greatly on climatic conditions, especially temperature (related to evaporation rate) and precipitations. Modifications of any of those two variables can alter the peatland functioning and thus their C balance. The following projections are based on Coupled Model Intercomparison Project Phase 5 (CMIP5) multi-model mean projections (i.e., the average of the model projections available) for the 2081–2100 period under the RCP2.6 and RCP8.5.

In the next century, precipitations are predicted to increase up to 30% in the northern hemisphere, with emphasis in the boreal region. Meanwhile, the Mediterranean area is expected to undergo slightly drier condition (-10–20% precipitations). This is conjugated with an estimated reduction of mean relative humidity of -1 to -10% and an associated increase of mean evaporation up to 0.6 $mm.day^{-1}$. Finally, average temperatures are expected to increase from 1.75 to 2 °C in the northern hemisphere. These climatic changes are likely to modify the hydrology of peatlands by modifying their water table level. While increasing precipitations may temporarily increase the water level, the combined effect of temperature elevation and evaporation increase would potentially lower the water level [Collins 2013].

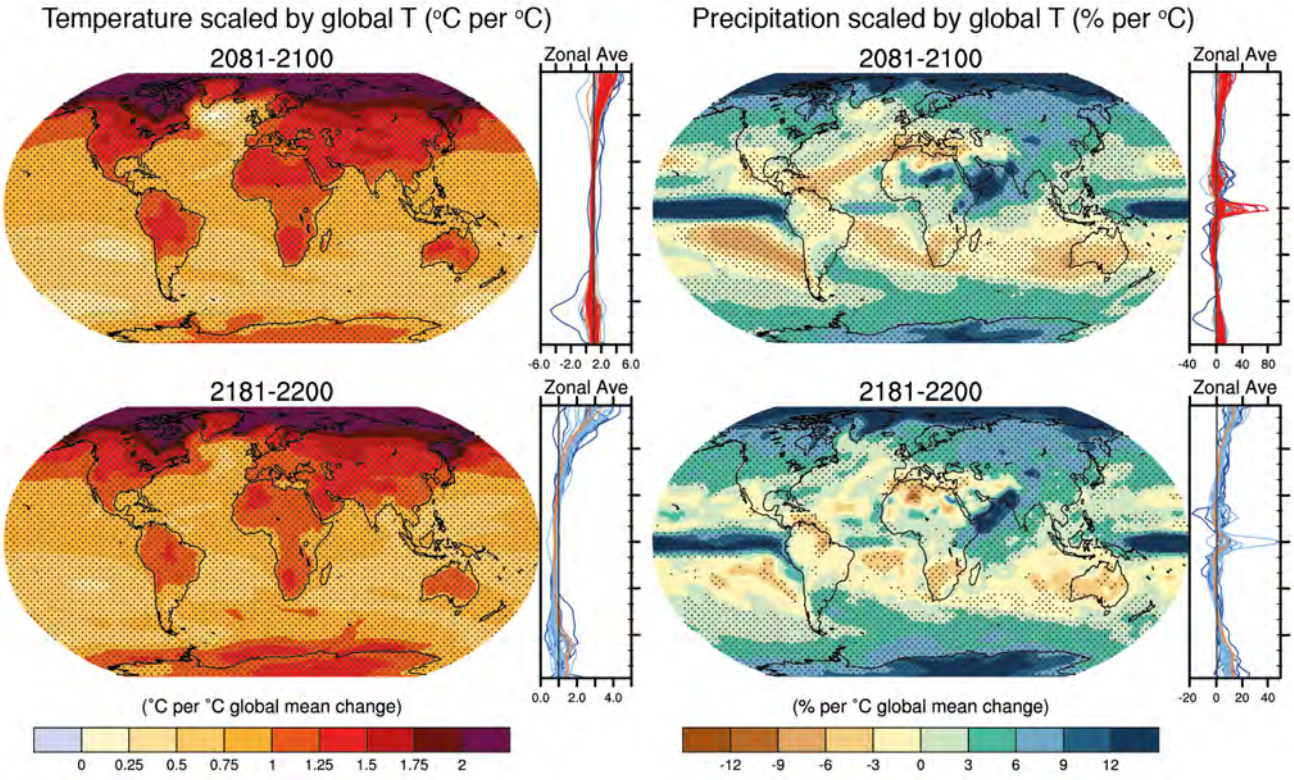

Figure 1.5: Temperature (left) and precipitation (right) change patterns derived from transient simulations from the CMIP5 ensembles, scaled to 1 °C of global mean surface temperature change. The patterns have been calculated by computing 20-year averages at the end of the 21st (top) and 22nd (bottom) centuries and over the period 1986–2005 for the available simulations under all RCPs, taking their difference (percentage difference in the case of precipitation) and normalizing it, grid-point by grid-point, by the corresponding value of global average temperature change for each model and scenario. The normalized patterns have then been averaged across models and scenarios. The colour scale represents degrees Celsius (in the case of temperature) and percent (in the case of precipitation) per 1 °C of global average temperature change. Stippling indicates where the mean change averaged over all realizations is larger than the 95% percentile of the distribution of models. Zonal means of the geographical patterns are shown for each individual model for RCP2.6 (blue), 4.5 (light blue), 6.0 (orange) and 8.5 (red). RCP8.5 is excluded from the stabilization figures. [Collins 2013]

These extended periods of drought can temporarily turn the peatland in to C source, especially during the growing season [Pullens 2016]. Generally, elevated temperature and low precipitations reduce carbon accumulation, notably by reducing mosss productivity [Bragazza 2016]. Temperature alone can promote C output by a 2–3 folds range [Silvola 1996]. Temperature increase, added to water level drawdown has both short term and long term effect on peatland functioning. Effect of WTD is important, as lowering of the water table by 1 cm can increase CO_2 fluxes by 7.1 $mg_{CO_2}.m^{-2}.h^{-1}$ at 12°C and 9.5 $g_{CO_2}.m^{-2}.yr^{-1}$ [Silvola 1996]. Consequently, peatland drainage can double CO_2 fluxes [Silvola 1996]. This effect is rapid (1-2 day after water table drawdown) [Dinsmore 2008]. Low water table also alters peatland C sink function by lowering photosynthesis by 24 to 42% [Blodau 2004].

Blodau estimates that overall, the change in C mineralization induced by low water table is the main contributor to CO_2 exchange rate.

Lowered water level would modify the peatlands functioning in different ways. Firstly, before system functioning, drier peatlands are more likely to experience fires [Kettridge 2015]. This higher probability for peatlands to undergo fire under drier conditions is the most direct way for C export from a peatland. For instance, in 1997, wildfires in drained Indonesian peatlands led to the release of 0.95–2.57 Gt of carbon into the atmosphere [van der Werf 2010, Page 2002]. This elevated risk of fire is not only due to drier soil condition but also because of the vegetation shift occurring on drained *Sphagnum* peatland.

Water level drawdown promotes the development of vascular plants, especially graminoids [Breeuwer 2009]. This change in plant composition can be explained by a N enrichment of pore water in drier conditions [Robroek 2014], thus promoting the growth of N dependent plants. Interestingly, this nutrient dynamics is driven by plant community as well as microtopography [Macrae 2012]. This change in plant community composition and nutrient cycling also modify the microbial communities (see following section) by promoting the develpoment of saprotrophs and other heterotrophic organisms (mainy fungi) and bacteria [Mariotte 2015, Robroek 2014].

Ongoing climate changes scenarios suggest that northern hemisphere peatlands could experience water level (WTD) drawdown. This drawdown would thus have a cascading effect on the peatland functionning, by modifying plant community composition along with microbial communities, promoting larger heterotrophic microbial activities and higher decomposition rate as well as lower photosynthesis activity. This higher decomposition rate in drier conditions is also explained by the fact that vascular plant litter decomposes faster than *Sphagnum* litter [Bragazza 2009]. Consequently, water level decrease induced by climate change could alter peatland C cycling and potentially turn them into C sources, temporarily or permanently.

1.2.3 Role of the microbial communities

As explained previously, the key to C accumulation is low decomposition rate, driven by the anoxic conditions in the peatland water-saturated soil [Yu 2006]. The C sink function of peatlands is expected to be sensitive to variations in soil water content induced by climate change [Davidson 2006, Bragazza 2009, Briones 2014]. Micro-eukaryotic organisms have been shown to drive key ecosystems functions, especially soil C cycling [Fitter 2005, Nielsen 2011]. Our knowledge of the food webs in peatlands is still poor [Gilbert 1998, Mieczan 2013, Gilbert 2015, Mieczan 2015], but it is clear that microbial activities drive C cycling at least partly [Jaatinen 2008, Briones 2014, Lin 2014, Jassey 2015]. Meanwhile, it appears that water level has an impact on those microbial communities, and hence on C cycling [Choi 2007, Faubert 2010, Dinsmore 2013, Gažovič 2013]. Consequently, there is a need to understand the response of peatland microbial community structure to GEC, and especially with focus on changes water level.

1.3 Microbial communities in peatland, and the concept of the bryosphere

1.3.1 Concepts of bryosphere and sphagnosphere

Recently, bryophytes were modelled as a complete earth compartment, the bryosphere [Lindo 2010, Jonsson 2014]. The concept of the bryosphere is defined as, the combined complex of living and dead moss tissue and associated organisms, representing a tightly coupled system of both above and belowground processes. It covers a large proportion of the terrestrial Earth's surface and exists at the interface of the lithosphere, pedosphere, atmosphere, and hydrosphere [Lindo 2010]. These microorganisms inhabiting the bryosphere are small metazoa, bacteria, protists, algae and fungi, constituting the "bryobiota". Among these organisms, some spend their entire lifecycle in the bryosphere and are therefore called byophiles, while others, called bryoxenes, only spend a fraction of their lifecycle in the bryosphere. Together, these organisms form the detrital foodweb of the bryosphere [Lindo 2010, Smith 1982, Döbbeler 1997, Anderson 2006, Kauserud 2008]. Recently, this concept was scaled down to the "Sphagnosphere" [Chiapusio 2013] and [Jassey 2011a]. Chiapusio and Jassey define the Sphagnosphere as: "a specific microecosystem between living *Sphagnum* and its associated microorganisms". The Sphagnosphere represents, according to the authors, the first scale in peatlands allowing determining any relation with its surrounding environment [Chiapusio 2013].

1.3.2 bryobiota

The bryobiota in peatlands encompasses a broad range of organisms from bacteria to micro-metazoa (<2mm). In a C cycling perspective, it is useful to divide those organisms into trophic groups. It is especially important to discriminate autotrophs, mixotrophs, and heterotrophs. Autotrophs are key players in the peatland microbial foodweb, as they constitute the basal food resource on which grazers and small predators will feed. They are principally green algae and cyanobacteria. The bacteria of the Sphagnosphere are not well known, with the exception of N-fixing cyanobacteria which form symbiotic associations with mosses [DeLuca 2002, Solheim 2002, Adams 2008]. Peatlands are rich in microalgae, and their diversity seems to be related to the rich–poor gradient in much the same way as that of vascular plants. Species of *Euglena* and *Chlamydomonas* are very numerous, occurring in tens of thousands per cm^2 in *Sphagnum* carpets [Hingley 1993]. Desmidiaceae are diverse and abundant in peatlands: a boreal rich fens may contain about 100 species, a poor fen about 35, and a bog area about 15 species [Flensburg 1965]. In the same habitat diatoms may be more numerous, but there are fewer species [Hingley 1993], especially in silica-deficient bogs. Most microalgae have a wide geographical distribution, and largely the same species are found in Europe and North America. One interesting alga is *Chlorella*, which constitute the symbiont of several mixotrophic testate amoebae [Gomaa 2014].

Mixotrophy in peatlands is poorly documented, with the exception of the mixotrophy occurring in testate amoebae and some ciliates and chrysophytes [Mieczan 2013]. Testate amoebae (TA) are a well-studied functional group of protists ranging mostly from 5 to more than 200 μm. These unicellular organisms live in a shell (test) whose shape varies between species. As this shell is resistant to decay, it remains in the peat when the cell dies. Furthermore, it has been shown [Charman 1992, Booth 2008, Jassey 2011c, Marcisz 2014a], that the distribution of testate amoebae is strongly correlated to Water Table Depth and/or pH, even at a fine scale [Mitchell 2000]. Therefore, TA are excellent bioindicators and are especially useful to infer past hydrological change [Booth 2002, Booth 2008, Charman 2007, Payne 2007, Lamarre 2013]. Testate amoebae occur with high diversity in *Sphagnum*, and there may be over 2000 individuals per cm^3 [Tolonen 1992], many of which have very precise habitat requirements. Testate amoebae can be heterotrophic, eating notably small ciliates and whatever microbial ressource available [Jassey 2013b], or mixotrophic; the latest playing a significant role in the C sink function in peatland [Jassey 2015], and representing often more than 70 % of the peatland microbial biomass [Jassey 2013b, Marcisz 2014a].

Finally, a broad range of heterotrophic microorganisms occur in the Sphagnosphere. Bacteria and fungi are the main heterotrophic microorganisms in peatlands [Gilbert 2015, Thormann 2006]. Bacteria are more abundant deep within the peat [Waksman 1929], while fungi are abundant in the aerated surface layers of the peat [Rydin 2006]. Fungi characteristic of ombrotrophic peat bogs are of the genera *Penicillium*, *Cladosporum*, *Trichoderma*, *Mucor*, *Mortierella*, *Cephalosporium*, and *Geotrichum*. Some heterotrophs will feed on *Sphagnum* litter (saprotrophs), for instance fungi or bacteria. Motile microorganisms will exhibit more predatory feeding habits, such as the ciliates and testate amoebae.

1.4 From peatland to mesocosm... to peatlands

1.4.1 From peatland...

As discussed, peatlands are complex ecosystems with a diverse detrital food-web that is sensitive to environmental gradient, specifically to moisture, and thus water level. In a perspective of GEC, it becomes necessary to understand the response of *Sphagnum* peatlands to change in their hydrology. However, it is quite challenging to investigate the whole system functioning at once in a natural peatland. Luckily, the physical properties of *Sphagnum* make them a good candidates for Natural Model System (NMS) [Srivastava 2004, Rosen 2005]. The framework proposed by Rosen (2005) and applied to the bryosphere by [Lindo 2010] requires mosses-based (sphagnosphere) experiments conducted within the laboratory or field in order to compare experimental findings with theoretical models and to scale the improved models up to the bryosphere.

In the bryosphere NMS framework, it appears critical to investigate the effect of water level on *Sphagnum*-dominated peatland functioning. Indeed, the main effect of water table depth is to deter-

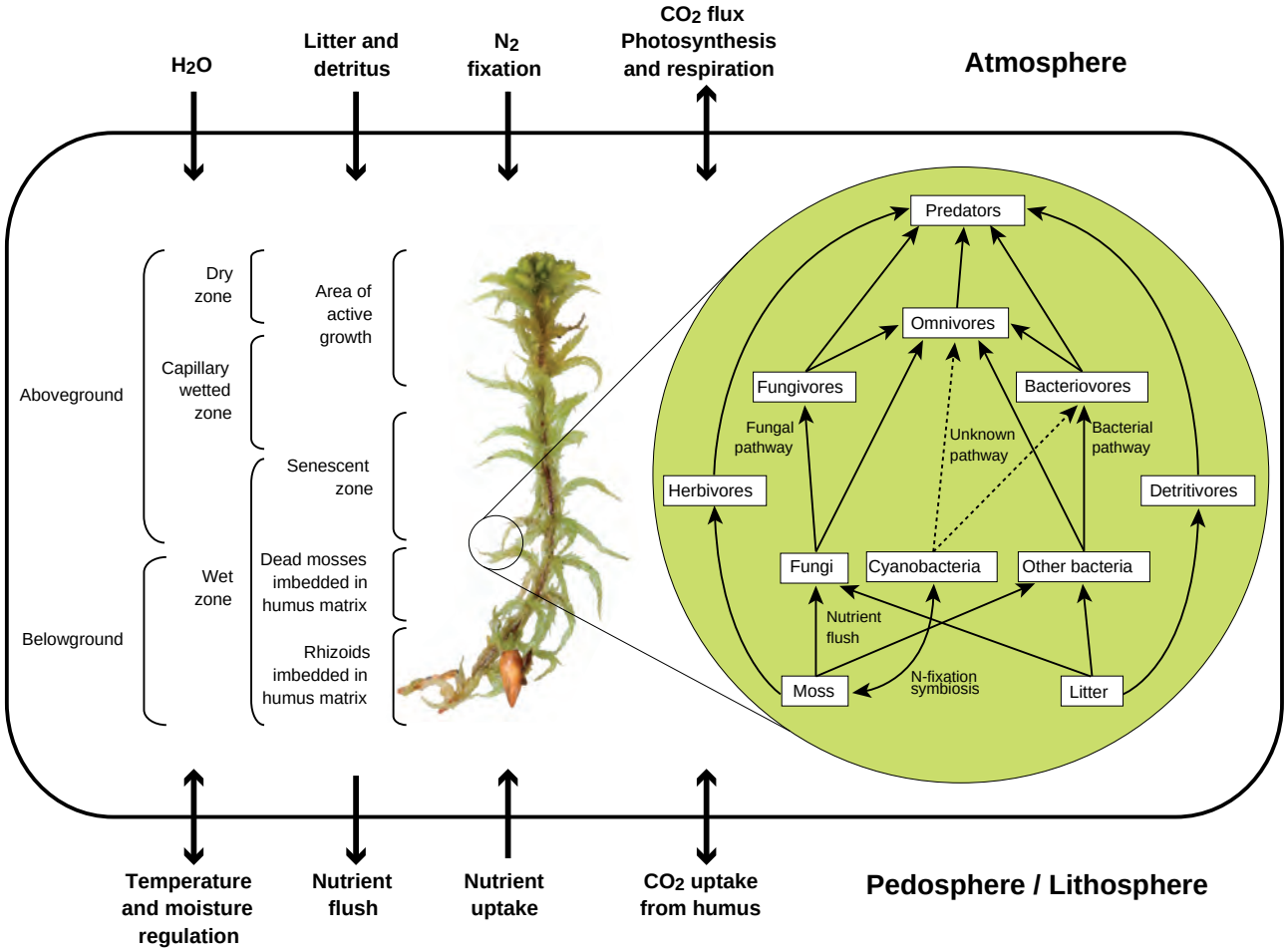

Figure 1.6: The Sphagnosphere detrital foodweb. A wide variety of microorganisms coexist in the water film surrounding *Sphagnum* shoots. *Sphagnum* and phototrophic organisms (mainy algae and cyanobacteria) are the primary food source. Living moss is consummed by herbivorous microorganisms, while litter it consumed by detritivores. Algae and bacteria are grazed upon by predators.[Lindo 2010], modified

mine the anaerobic zone, in which litter decomposition is lower than in aerobic zones [Laiho 2006]. According to [Bragazza 2009] the percentage of mass loss is on average about 2.3 times higher in the aerobic layer for both vascular plant and *Sphagnum* litter. This loss of mass is the consequence of modification occurring in the bryobiota, the detrital food-web playing an important role in the C cycling.

The NMS framework implies modeling. The purpose of modeling is to mathematically express the laws and rules constraining Nature (the existence of things insofar as it is governed by universal – causal – laws [Kant 2001]). The idea of using mathematics to describe Nature, or ecosystems, seems quite recent in ecology; numerical ecology formally emerged in the early 1960's with the works of Serge Frontier. However, in actuality, it is an ancient idea that appeared alongside philosophy, and that was already present withinin Aristotelian School, in IVth century BC. More recently, Galilei wrote in the Assayer (1623) that the book of Nature "cannot be understood unless one first learns to

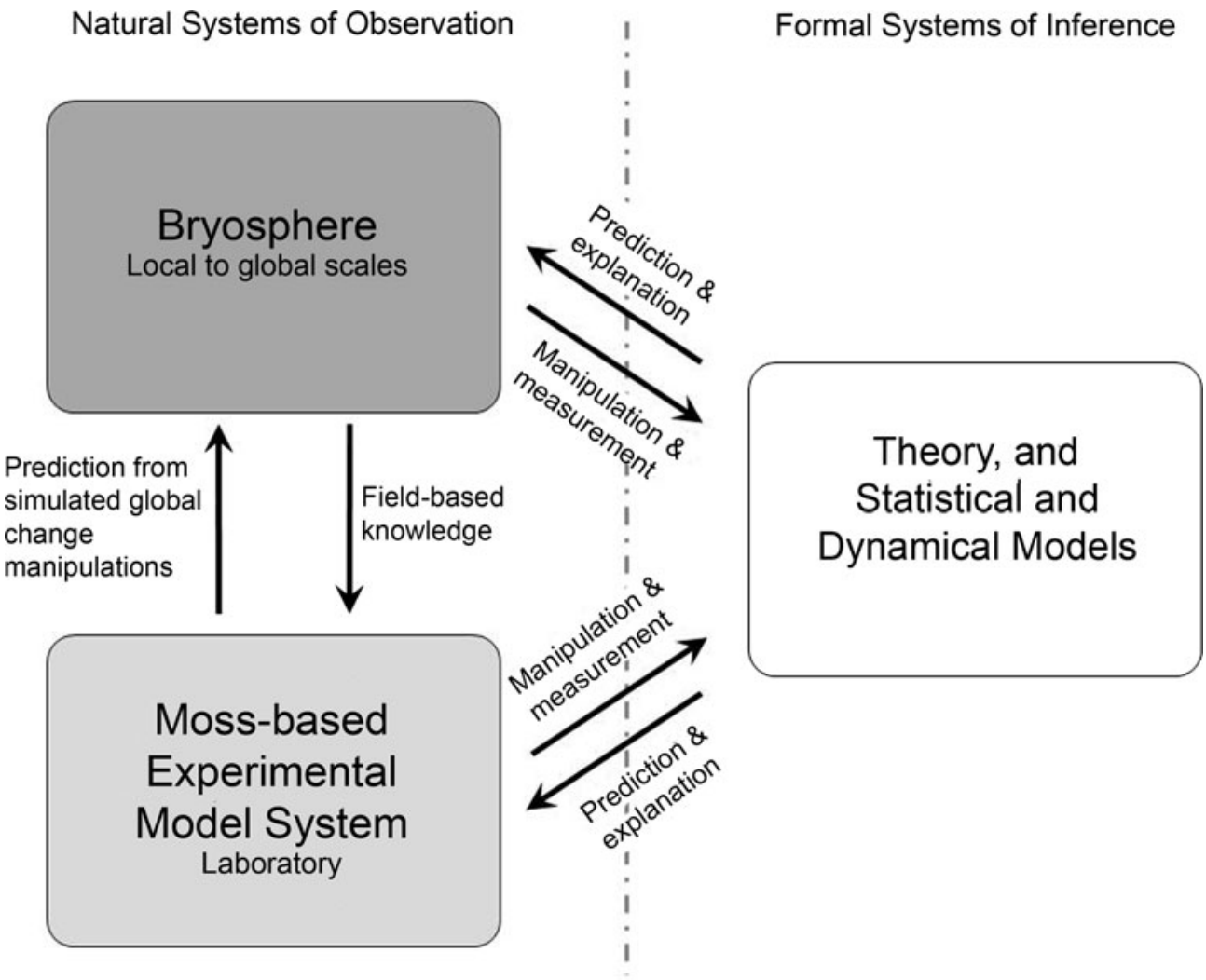

Figure 1.7: Natural Model System proposed in [Lindo 2010] and [Rosen 2005]. This framework requires mosses-based (sphagnosphere) experiments conducted within the laboratory or field in order to compare experimental findings with theoretical models and to scale the improved models up to the bryosphere.

comprehend the language and read the letters in which it is composed. It is written in the language of mathematics, and its characters are triangles, circles, and other geometric figures without which it is humanly impossible to understand a single word of it; without these, one wanders about in a dark labyrinth." [Stillman Drake 1957]. With these words, Galilei drew two hypotheses:

1. Mathematics is the language of Nature, and scientists need to learn this language to communicate with Nature. An experiment is therefore only a recording of a message already existing in a natural mathematical form.

2. Mathematics is a human language, and is the most precise language. To understand Nature, scientists must constrain Nature to speak this language, and an experiment is therefore a coercive operation. The experimenter forces Nature to express in a language not its own.

In any case, understanding Nature requires that experiments be conducted. Kant, in the preface to the second edition (1787) of the *Critique of Pure Reason*, agrees with the second hypothesis. He also strongly emphasizes the necessity of conducting experiments in order to understand Nature. He wrote:

> When Galilei experimented with balls of a definite weight on the inclined plane, when Torricelli caused the air to sustain a weight which he had calculated beforehand to be equal to that of a definite column of water, or when Stahl, at a later period, converted metals into lime, and reconverted lime into metal, by the addition and subtraction of certain elements; a light broke upon all natural philosophers[1]. They learned that *reason only perceives that which it produces after its own design*; that it must not be content to follow, as it were, in the leading-strings of nature, but must proceed in advance with principles of judgement according to unvarying laws, and compel nature to reply its questions. For accidental observations, made according to no preconceived plan, cannot be united under a necessary law. But it is this that reason seeks for and requires. It is only the principles of reason which can give to concordant phenomena the validity of laws, and *it is only when experiment is directed by these rational principles that it can have any real utility. Reason must approach nature with the view, indeed, of receiving information from it, not, however, in the character of a pupil, who listens to all that his master chooses to tell him, but in that of a judge, who compels the witnesses to reply to those questions which he himself thinks fit to propose*. To this single idea must the revolution be ascribed, by which, after groping in the dark for so many centuries, natural science was at length conducted into the path of certain progress. [Kant 1999]

Therefore, Kant tells us that understanding Nature requires designing experiments in which we constrain a parameter. In our case, we designed a mesocosm experiment in which we manipulate the water level of peat monolith topped by *Sphagnum* layers. The mesocosm approach seemed extremely fitted for a study within the framework proposed by [Singh 2010] (Figure 1.8).

1.4.2 ... to mesocosms

Our study aims at investigating the response of microbial communities to water table depth and, thus to evaporation and precipitation, in relation to broader climatic changes. It also covers to some extent the effect of temperature, as the experiment ran during few years with contrasted summer/winter cycles. We also investigated the response of the system (peatland) by measuring decomposition and respiration. Now, a controversy had existed with the use of microcosms for ecology modeling, with Carpenter notably stating:

[1] Actually, Kant uses *Naturforscher*, which translates by *naturalist*. In the french edition, this word is translated by *physicist*.

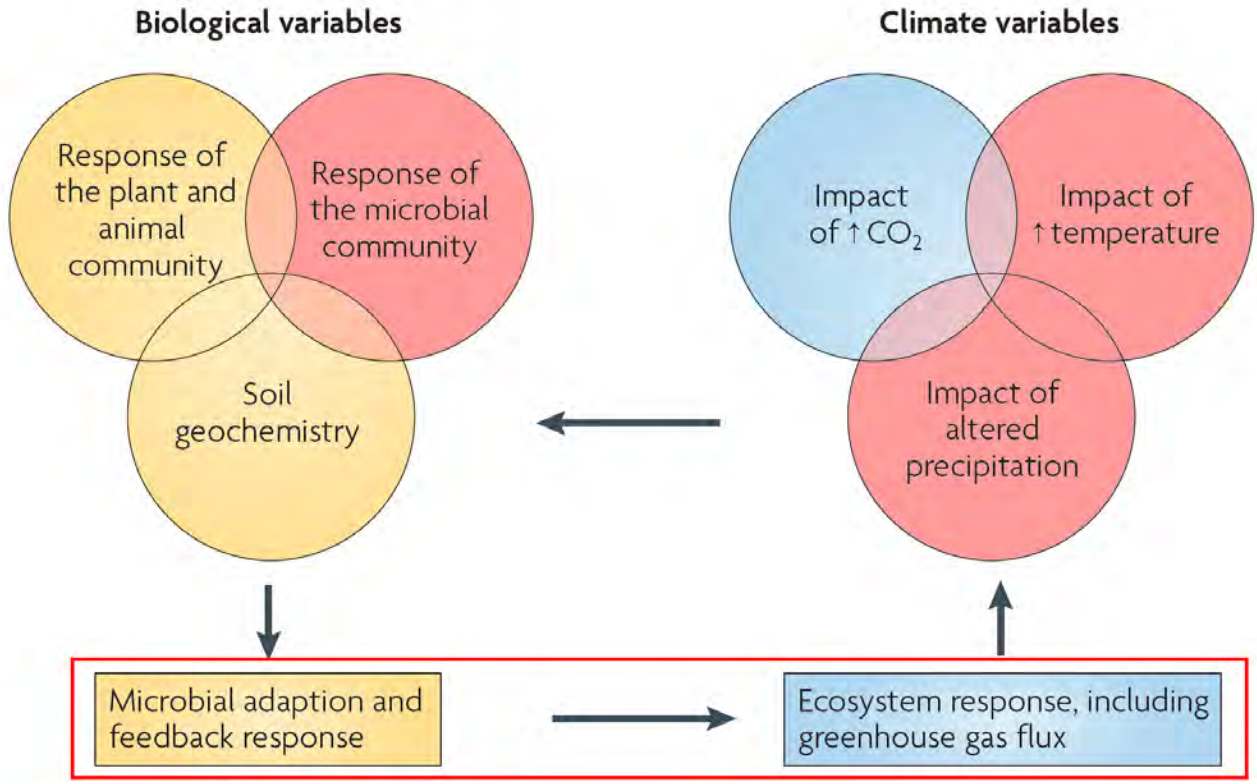

Figure 1.8: Framework for future research on climate change and ecological responses, proposed by [Singh 2010] (modified). Aspects covered by our study are colored in red. Our study aims at investigating the response of the microbial community to water table depth and, thus to precipitations. It also covers to some extent the effect of temperature, as the experiment ran during several years with contrasted summerwinter cycles. We also investigated the response of the system (peatland) by measuring decomposition and respiration

> It is irresponsible for academic ecology to produce larval microcosmologists by canalizing graduate students into careers of small-scale experimentation. There is cognitive danger that the microcosm (rather than the ecological system) will become the object of study, leading to needless confusion as results are overinterpreted and overextended. As ecology becomes more and more a science done indoors by urbanites, there is significant risk of losing our sense of context. Already there is a shortage of students and postdoctoral students who have the practical knowledge and natural-history background to function outdoors. Graduate curricula in ecology could fill gaps in practical knowledge through courses in hardware, lumberyards, construction, boat and motor maintenance, field methods, and so forth. But graduates who lack a deep appreciation of natural history and real ecosystems, which can come from extensive field experience but not from the campus, have deficient educations. [Carpenter 1996]

Besides, quite similar criticisms were made towards community ecology, stating that it was not scalable to ecosystem ecology [Loreau 2010].

Luckily, several studies have shown microcosm experiments to be useful to validate empirical models built from field observation or unveil mechanisms scalable to field [Daehler 1996, Drake 1996,

Jaffee 1996, Verhoef 1996, Srivastava 2004, Benton 2007, Jiang 2008]. A few mesocosms experiments were even built in order to investigate peatlands, but they only investigated a single response of the peatland, not their functioning as a whole. For instance, studies either focused on decomposition [Thormann 2004], GHG exchange —including in some cases the role of microbial communities in these exchanges [Morris 2002, Rinnan 2005, Haapala 2011, Rahman 2011, van Winden 2012]—, DOC [Preston 2011], or plant communities [Dieleman 2015]. Few mesocosm studies assessed the effect of water table depth manipulation on *Sphagnum* peatlands, and existing studies focused only on one response variable, such as GHG [Blodau 2003, Tiiva 2009, Faubert 2010]. Most mesocosm studies investigated only one explanatory variable. A notable exception, however, was the mesocosms built by Bridgham and colleagues [Bridgham 1999, Bridgham 2008] where both water level and temperature were manipulated.

Our mesocosm experiment was designed in the context of the Climpeat project, to study the response of *Sphagnum* peatlands to hydrological changes. The aim of this design was to allow a whole ecological study, to understand the effects of simulated climate change on the structure and functioning of *Sphagnum* peatlands. Our climate change scenario is based on water table variation, accordingly to IPCC previsions, expecting of increased precipitation a more marked contrast between summer and winter precipitation patterns, with more frequent drought or flooding events. The findings from the mesocosms experiment will then be compared to the result from the field experiment taking place on the polish site, from were some studies were already published [Slowinska 2010, Marcisz 2014b].

1.5 Organization of the thesis

This present work follows a nesting doll structure, describing the experiment and the findings from a global perspective (the system respiration and decomposition rate) to a local (the response of one single species) perspective (Figure 1.9).

1.5.1 Mesocosms

Our mesocosm experiment allowed us to perform a multiscale study on the response of *Sphagnum* peatland to water table manipulations. Each of the studied scales corresponds to a distinct chapter.

In chapter 2, we will describe the mesocosm setup, that has already been published [Mulot 2015]. This chapter presents the building process of the mesocosms, the issues we had to cope with and the solution we found to overcome them. It describes the cost of the experiment and the strategy used to handle missing data. Appendices associated to this chapter present a setup guide for the mesocosms, the source code and schematics of the two devices we designed and built to control and monitor the water level in each mesocosm, and the source code of a program we wrote to monitor weather forecast in order to protect the experiment from inclement weather, such as hails or frost.

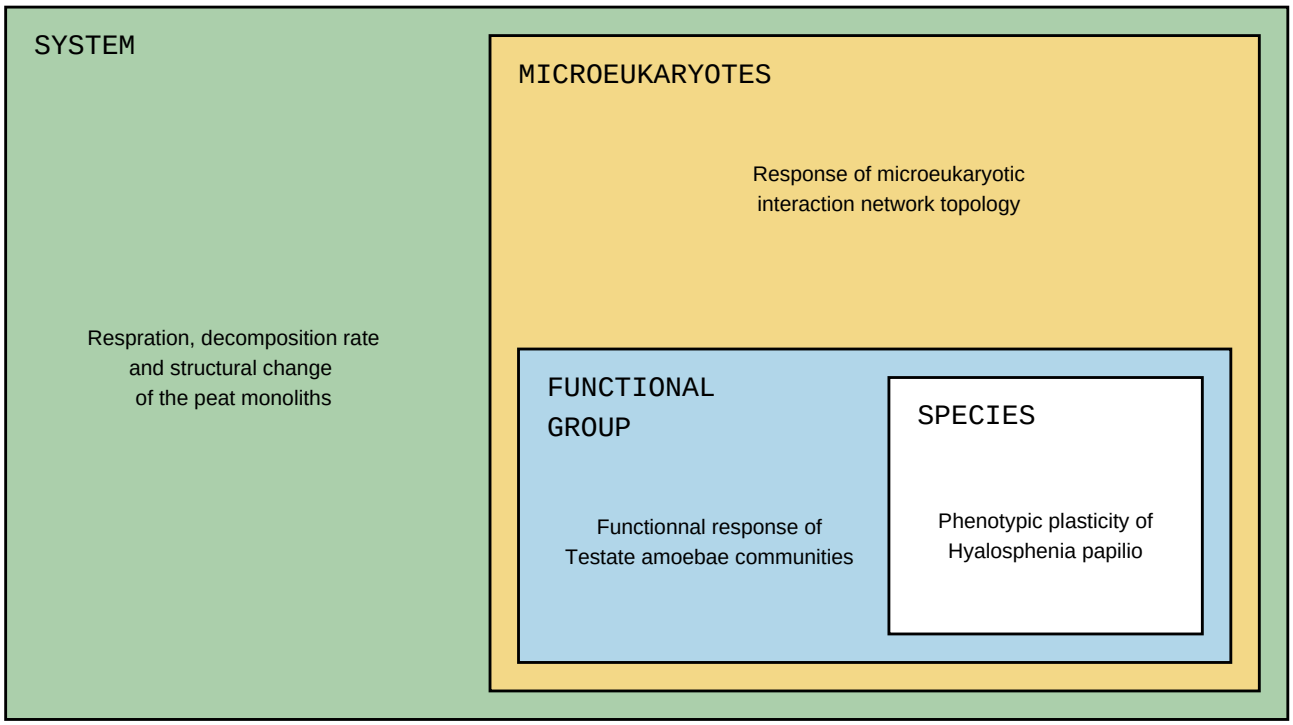

Figure 1.9: Structure of the thesis. The chapter 2 (not shown on the diagram) describes the mesocosms experiment. Chapter three describes the system response to the constrained water level. Chapter four presents the response of microeukaryotic interaction network structure. Chapter five focuses on one functional group of protists, the testate amoebae. Finally, chapter six investigates the response of one single species within this functional group, *Hyalosphenia papilio*.

1.5.2 System scale

At the global scale, we studied the respiration and litter decomposition of the peat monoliths along a water level gradient at different temperatures. This chapter is not intended to be published separately, but to be joined with chapter 4. Within the present work, it aims to validate the geochemical behaviour of our mesocosm experiment. We first made a brief review of the expected responses to WTD and temperature, and then compared it to the actual results obtained in the mesocosm experiment.

We used litter bags for litter decomposition and direct CO_2 measurements using a Licor–8100. The objective of this part is to quantify the effect of the WTD treatment on the system functioning. It allows data comparison against the literature to ensure that our mesocosm design behaves accordingly to available data. Secondly, it allows linking microbial responses to system functional responses. We present a short review of the existing models for CO_2 estimation, and point out the need to integrate microbial interactions to these models.

1.5.3 Response of the microbial communities

In the chapter 4, we will study the changing structure of the bryobiota by High Throughput Sequencing (HTS) using co-occurence network to model the interaction between the microeukaryotes. The co-occurrence network (also called interaction network) is an elegant way to model microbial interaction as graphs. It relies on non-random associations between pairs of species or Operational Taxonomic Units (OTUs). Although the nature of the interactions behind non-random association is

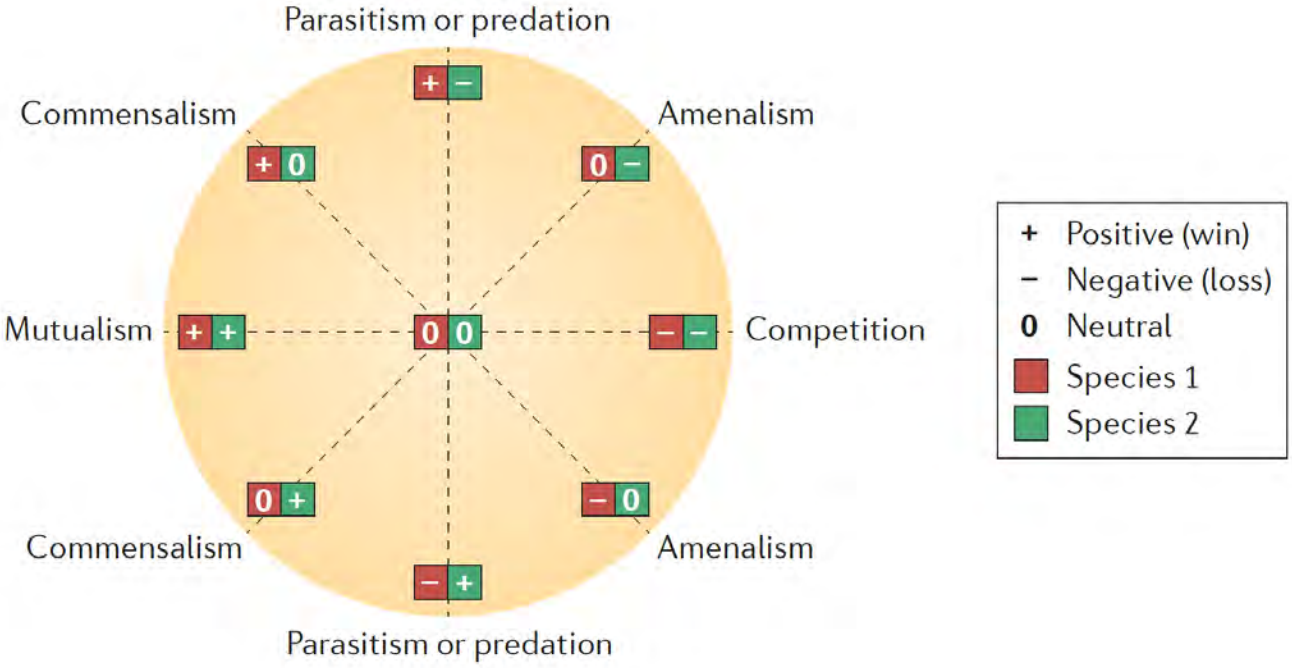

Figure 1.10: Summary of ecological interactions between members of different species. The wheel display introduced by [Lidicker 1979] has been adapted to summarize all possible pairwise interactions. For each interaction partner, there are three possible outcomes: positive (+), negative (–) and neutral (0). For instance, in parasitism, the parasite benefits from the relationship (+), whereas the host is harmed (–); this relationship is thus represented by the symbol pair + –. From [Faust 2012]

unknown, such networks were shown to correlate with the C cycle in marine systems [Guidi 2016], which makes them good candidate for NMS in our context of peatland C cycling. In our case, the interaction networks were built from Operational Taxonomic Unit (OTU) matrices revealed by High Throughput Sequencing of the V9 region of the 18S SSU rDNA. This chapter intends to map WTD to network topology, allowing to map network topology to C cycling using data obtained in chapter 4. To achieve this study, we conceived a R package, gradnet, that will be published along with the paper. The source and documentation of the package are available in the appendices of this thesis.

Here, our hypothesis was that a strong environmental gradient would modify the nature of the interaction between organisms composing the microbial foodweb. Such change in the microbial interaction would be reflected on the network topology. As networks are mathematical objects with mathematical properties, it should be possible to correlate these properties to our investigated wa-

ter table gradient. Changes in the interactions would most likely indicate that changes occur at the functional level of the microbial food web.

1.5.4 Functionnal response of TA communities

In chapter 5, we will present the shift in the testate amoeba (TA) community along the artificial water level gradient with an emphasis on functional diversity. The aim of this chapter is to understand how changes in system functioning reflect in traits selection through environmental filtering processes. We used light microscopy to study the shift of TA communities through time according to the treatments. Then, we used the fourth corner approach to compute the functional response of the TA communities [Dray 2014].

TA are a well-studied and documented functional group of protists that are abundant and frequent in peatlands. This available literature makes them suited for functional diversity analysis [Fournier 2015a]. We assume that environment filtering select functions through microbial community turnover. Functions ecological abilities that can be related to traits, *i.e.* species or individual characteristics, related to environments. For instance, feeding habits, morphology, etc. are functions. The functional approach may be more ecologically informative than the classical community approach. Indeed, functions can be identical among two different communities while identical communities can potentially exhibit different functions through specific phenotypic plasticity. The phenotypic plasticity, that is the ability or organisms to change its phenotype depending on the environment, is studied in the chapter six.

1.5.5 Response of *Hyalosphenia papilio*

Chapter six focuses on one mixotrophic species of TA, *Hyalosphenia papilio*. The choice of *Hyalosphenia papilio* as a model species is due to its ease of identification, its abundance, its documented response to WTD [Booth 2010b] and its trophic position. Indeed, mixotrophic species have been shown to be crucial in C cycling and in the microbial loop [Jassey 2013b, Jassey 2015]. We will study its morphological response to environmental gradient and its possible implication for protist taxonomy.

In this chapter, we study the morphological response of *H. papilio* in 1) the mesocosm experiment 2) a natural peatland in Linje (Poland) and 3) 37 peatlands over Europe. We investigate the environmental factors influencing the morphology of *H. papilio* (size, shape, and pore numbers). We focus on climatic variables and water level. Finally, we used single cell sequencing technique to assess whether this morphological variation was due to phenotypic plasticity or if it was genetically determined.

CHAPTER 2

A mesocosm approach to study the response of *Sphagnum* peatlands to hydrological changes: setup, optimization and performances.

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as published in Mires and peat [Mulot 2015].

2.1 Summary

Sphagnum-dominated peatlands are major C pools and sinks but these functions are threatened by climate change. There is therefore a need to better understand how microclimatic changes (soil temperature, moisture and water table depth) are affecting their functioning. Experimental studies on *Sphagnum* peatlands using precise controlled conditions are relatively rare, especially those aiming to understand the system as a whole. Furthermore, the existing mesocosm designs are generally only briefly described in the literature. In this paper, we provide a comprehensive description of a mesocosm experiment designed to study the response of *Sphagnum* peatlands to water table manipulation. We define our experimental setup (3 water levels x 3 amplitude of fluctuations x 5 replicates), explain how we built the mesocosms, the issues we faced and the solutions we chose to solve them. We provide a detailed description of the devices we conceived to manipulate the water level, including software codes and electronic diagram, and explain how to face data loss in such experimental design. We show that it is possible to build a reliable and powerful experimental setup at a moderate cost using standard technology. The aim of this paper is to provide a useful resource for future researchers willing to design similar experiments.

2.2 Introduction

Northern *Sphagnum* peatlands are a significant yet threatened carbon (C) pool. Although they represent 3-5 % of the worldwide land area [Frokling 2007] they hold 1/3 of the soil C pool [Turunen 2002] , representing 500 ($\pm$ 100) gigatons of C (GtC) in northern peatlands [Yu 2012] which is comparable

to the atmospheric C pool (around 750 GtC). In peatlands, the key to C accumulation is low decomposition rate, which is in great part driven by the anaerobic conditions in the water-saturated soil. Therefore the C sink function of peatlands is likely to be very sensitive to climate-induced variations in soil water content [Davidson 2006, Bragazza 2009]. Studying how on-going climate change and associated changes in soil water content affect the functioning of peatlands and especially their C-sequestration is thus a research priority.

Sphagnum-dominated peatlands are primarily situated in the Boreal and Subarctic areas, which are experiencing the largest climate changes, making the identification and quantification of potential feedbacks from these high-latitude ecosystems essential for future climate projections [Pachauri 2015]. One scenario is that rising temperature and drought would increase the decomposition rate in *Sphagnum* peatlands and thus cause a release of C to the atmosphere, inducing a positive feedback to global warming [Belyea 2004, Rydin 2006].

Although *Sphagnum* peatlands have been widely studied in the last decades, only relatively few experimental studies were performed. Moreover, they were usually carried out on field, and the majority of the few existing mesocosms experiments only investigated a single response of the peatland, and not its functioning as a whole. For instance, studies either focused on decomposition, [Thormann 2004], GHG exchange including in some cases the role of microbial communities in these exchanges [Morris 2002, Rinnan 2005, Haapala 2011, Rahman 2011, van Winden 2012], DOC [Preston 2011], or plant communities [Dieleman 2015]. Few mesocosm studies assessed the effect of water table depth manipulation on *Sphagnum* peatlands, and existing studies focused only on one response variable, such as GHG [Blodau 2003, Tiiva 2009, Bridgham 2008, Faubert 2010]. Most of the mesocosms studies investigate only one explanatory variable. A notable exception, however, was the mesocosms built by Bridgham and colleagues [Bridgham 1999, Bridgham 2008] where both water level and temperature effects were manipulated. Nevertheless, here again, the mesocosms were only briefly described, making it difficult for other researchers to reproduce or evaluate exactly the setup.

We designed a mesocosm experiment to study the response of *Sphagnum* peatlands to hydrological changes. The aim of this design is to allow a whole ecological study, to understand the effects of simulated climate change on the structure and functioning of *Sphagnum* peatlands, at different levels: microbial communities, water chemistry, decomposition, respiration, etc. Our climate change scenario is based on water table variation, accordingly to IPCC previsions, expecting increasing precipitation and a more marked contrast between summer and winter precipitation pattern, with more frequent drought or flooding events. The scope of this paper is to provide a comprehensive description of our experimental setup along with the issues we had to cope with and the solutions chosen to overcome these issues, to help researchers designing or planning their future experiments. As is typically the case, the experimental setup presented here is the result of time and budget constraints and iterative improvements. We discuss the strengths and weaknesses of this setup as well as possible improvements.

2.3 Methods

2.3.1 Experimental design

Mesocosms were built to assess the effects of water table variations on the ecological functioning of a pure *Sphagnum fallax* layer through time. We defined three average water table depths (AWTD: 4 cm, 15 cm, and 25 cm below the top of the moss carpet), for each we determined three amplitudes of fluctuation (± 2 cm, ± 7.5 cm, ± 12.5 cm). Each treatment was replicated five times. The 45 mesocosms were installed outdoors along in two rows (22 and 23 mesocosms), in the botanical garden of Neuchâtel, Switzerland (46°59'59.95"N; 6°56'0.33"E; Alt: 554 m), in late July 2012. The treatments were randomly placed along the two rows.

Table 2.1: Parameters of the nine treatments. Water table depth (WTD) is defined in cm from the top of the *Sphagnum fallax* layer at the onset of the experiment. Fluctuation Amplitude (FA) designates in cm the potential variation of WTD around the average WTD

Treatment code	HL	HM	HH	ML	MM	MH	LL	LM	LH
Average WTD	High (4 cm)			Medium (15 cm)			Low (25 cm)		
FA(code)	Low	Medium	High	Low	Medium	High	Low	Medium	High
FA [cm]	4	10	25	4	10	25	4	10	25
Max WTD [cm]	2	-1	-8.5	13	10	2.5	23	20	13
Min WTD [cm]	6	9	17	17	20	28	27	30	38

2.3.2 Mesocosm functionning

2.3.2.1 Hardware

Each mesocosm is made of a polypropylene tank (type WIVA VAT4, 60 L) containing a perforated PVC tube. In this PVC tube is a peat core on top of which lies a *Sphagnum fallax* layer. The PVC tube is 45 cm long, has a diameter of 12 cm and is perforated with 5 mm holes spaced ca. 5 cm apart both horizontally and vertically, all around the tube. The *Sphagnum* layer is placed 5 cm below the upper edge of the tube at the beginning of the experiment. The tank is filled with water, and the water level is set and regulated according to one of the treatments described above. Each mesocosm is defined by three conditions: Average Water Table Depth (AWTD), Maximum Water Table Depth (MaxWTD) and Minimum Water Table Depth (MinWTD), as shown in Figure 2.1. The MaxWTD is constrained by a 3.5 mm drainage hole drilled in the side of the tank.

The minimum level is controlled by a mechanical automatic system (toilet flush device, Geberit Impuls380): when the water table depth drops below the minimum level, the valve of the device opens, allowing water to flow into the tank until the water level reaches the minimum level. When MinWTD is reached, it lifts the float of the device, switching the valve off (Figure 2.1). The water for MinWTD was initially supplied by a Millipore filtration device (Elix 10 model), providing MilliQ

water. However, this device was too sensitive to frost and was then replaced by a simpler filtration cartridge composed of three layers of sand, polyester wadding and activated charcoal. The cartridge is filled with deionised water and exits through a UV lamp for sterilisation. The cartridge with its deionised water supply is located in a small garden shelter situated 4 m above the mesocosms, and thus the water is supplied by gravity. The rationale behind using deionised water is that the amount of supplied water necessary to maintain a minimum water level was very small, and it therefore impacted very little the water chemical composition (less than 1% of the mesocosm water was supplied by this system, the vast majority being meteoric water). The great benefit of using deionised and sterile water is that it prevented the blooming of algae into the pipes, which would lead to clogging. Similarly, deionised water was easily stored in tanks exposed to temperature variations, without being colonized by bacteria or algae.

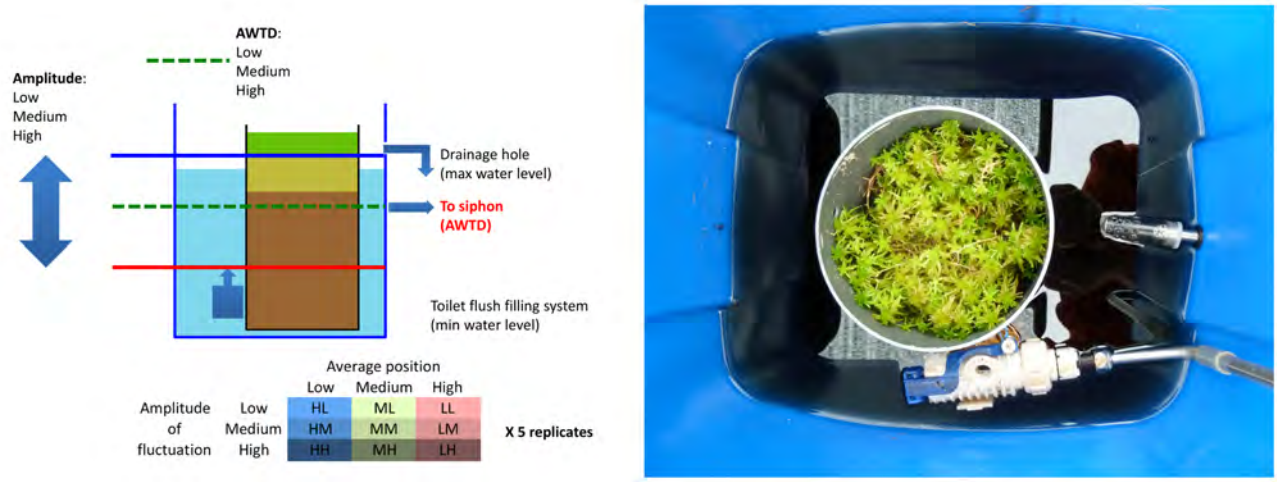


Figure 2.1: Diagram and picture of one mesocosm taken from above. The minimum water level is set by the toilet flush device (white device on the picture), which is mounted on a rail fixed on the PVC tube. Water is supplied through the stainless steel pipe. The lower stainless steel pipe is connected to the siphon. A filter was added to avoid large particles entering and clogging the system.

To ensure WTD homogeneity, all five replicated mesocosms of each intermediate and high amplitude treatments were connected together via silicone pipes plugged into a siphon. The replicated mesocosms of the stable treatment (with low amplitude of fluctuation) did not require to be connected together. The connection to the tank (mesocosm or siphon) was made of a 10 cm stainless steel pipe (0.5 cm diameter) plugged into the tank and sealed by a rubber O-ring. Silicone pipes were plugged onto the stainless steel pipes. To simulate horizontal water transfers, a system of pumps dynamically regulated by a microcontroller controled the water level decrease from MaxWTD to AWTD.

The mesocosms were shaded underneath a net filtering 50 % of solar radiation, to simulate the shading effect of vascular plants growing on natural conditions. The net also prevented damages caused by animals, mainly birds. To simulate the higher amount of rainfall occurring at the altitude from which the *Sphagnum* and peat was sampled (ca. 1000 m a.s.l.), we added the lid of the tanks as

a gutter, tilted at 45° and with the lower edge placed over the top of the tank, to increase rain water input by ca 70 % estimated as follows:

$$S_{tot} = S_{tank} + S_{tank} \times \cos(45) \approx 1.71 \times S_{tank} \quad (2.1)$$

where Stot is the total rainfall input surface and Stank the tank rainfall input surface. The equation is dimensionless. Finally, a Styrofoam cover was placed around the border of each PVC tube, to avoid direct insolation and unwanted heating on the side of the peat core.

Environmental variables were monitored hourly, with the exception of WTD which is monitored every twelve hours. The site was equipped with a Decagon PYR sensor placed under the net, along with high-resolution rain gauge (Decagon ECRN-100) and air moisture and temperature sensor (Decagon VP-3). In one replicate of each treatment, a Decagon E5-TM soil moisture sensor was inserted 5 cm under the top of the *Sphagnum* layer. The E5-TM sensors were calibrated with the default Decagon calibration equation (Topp equation) at the time of the installation, but the raw data can be corrected a posteriori using a *Sphagnum*-specific calibration equation that was calculated from the *Sphagnum* mosses we used in the project. A HOBO Logger 8K - UA-001-08 temperature sensor (+/- 0.53C accuracy) was placed in every mesocosm between the old and the new peat (i.e. base of the *Sphagnum* carpet, 15 cm deep).

2.3.2.2 Biological material

Peat cores were extracted from a cutover bog in the Vallée des Ponts-de-Martel, Canton Neuchâtel (Marais rouge sud: 47°00'00.22"N; 006°44'57.01"E; Alt: 999 m) using a peat corer (Buttler et al. 1998). The peat cores were immediately inserted into the PVC tubes while still on the sampling site, as to preserve the vertical structure of the peat. *Sphagnum fallax* was sampled in the Creux de l'Épral peatland, Canton Jura (47°12'18.31"N; 006°56'05.83"E; Alt: 990 m), using a homemade *Sphagnum* corer, which consists of a cylindrical steel blade of the same diameter as the PVC tubes. The corer was 25 cm deep and allowed sampling the intact *Sphagnum* layer. All mesocosms were seeded at the beginning of the experiment with an extract of microorganisms from pool, hummock and lawn from the Cachot peatland (47° 0'17.15"N; 6°39'52.21"E; Alt: 1052 m) so as to provide the full range of soil organisms potentially adapted to the different water table regimes. It would have been preferable to use full peat monoliths, instead of harvesting peat and *Sphagnum* separately, but it was not possible in our case. This issue is detailed in the discussion section.

2.3.2.3 WTD monitoring and WTD control

The key variable to monitor in our design is WTD, which is recorded every 12 hours by a piezometer data logger. One water level sensor is installed in one mesocosm of each treatment. A water level sensor consists of a 600 mm eTape™ Continuous Fluid Level Sensor produced by Milone Technolo-

gies. It is a solid-state sensor with a resistive output that varies with the level of the fluid. The nine water level sensors are connected to an Arduino Uno R3 microcontroller board through a 16-Channel Analog/Digital Multiplexer/Demultiplexer CD74HC4067 breakout board manufactured by Sparkfun. The WTD is recorded into a SD card in csv (comma-separated values) format, through Adafruit Data logging shield, equipped with a SD card reader and a Real Time Clock (RTC) module. Originally, each water level sensor required individual calibration to transform input voltage into WTD. MiloneTM now produces a 0-5V Linear Output Module that facilitates calibration. The electronic diagram and microcontroller code are provided in supplementary material.

The water table decrease, to simulate horizontal water drainage through the acrotelm, is also controlled by an Arduino Uno R3 microcontroller. In each siphon is a self-priming pump (DC pump by Trossen Robotics) and a water level float switch, which is used to inform the microcontroller whether the WTD is above or below AWTD. Every hour, the microcontroller will check the water level for each treatment, and will run the appropriate pump for a given time if the water level is above the AWTD. The pumps runtimes have been calibrated so that WTD can decrease from max WTD to AWTD in one week, assuming no precipitations. Lowering of the water table below the AWTD level then only depends on evaporation. The three stable treatments (with low amplitude of fluctuation) are not connected to a siphon, as stated above, and have no actively regulated water table decrease. The electronic diagram and microcontroller code are provided in supplementary material.

For our design, we chose to install electricity on the experimental site, rather than having autonomous devices. However, it is reasonably easy to build similar devices, solar powered. To achieve that, we can use a LiPo Rider electronic card, whose purpose is to deliver a stable 5V by pairing a solar panel and a battery. If the experiment is close to a water stream, we can add power by adding one or several 3.6V Micro Hydro Generator, which are low cost turbines converting water flow to electricity, requiring only a flow as little as 3 L/min. Both devices (LiPo Rider and Micro Hydro Generator) cost less than 50 USD. The frost-sensitive components of the system (toilet flush device, filtration device and pumps) are removed during the winter months.

2.4 Results

2.4.1 Cost

The whole design has a moderate cost, especially because it was handcrafted. The overall cost is less than 10,000 USD, including sensors (Table 2.2). Comparatively, WTD monitoring solely was estimated at 34,500 CHF (ca. 38,300 USD) by a local company. We were able to build our data logger and water regulating device because of the tremendous rise of the DIY trend in the USA, which brought to the market cheap yet efficient electronic components, along with a huge documentation. Especially, the Arduino microcontroller board came out in 2005 and quickly became a reference. Because of its open-source nature and its highly competitive price (ca. 30 USD), many compatible

boards and shields were developed, allowing performing various tasks that only skilled engineers could previously do, such as reading sensors input, controlling motors, valves, and so on. Its programming simplicity makes it extremely handy to use. The microcontroller is programmed in C++, which makes it accessible even for beginner programmers. The Arduino board comes with a dedicated IDE (Integrated Development Environment) and only requires a USB port to be configured. The IDE is written in Java and is therefore compatible with any operating system. Some recent applications henceforth allow controlling the board with a smartphone (iOS or Android). A more complete case study of the use of Arduino board for environmental monitoring has been described in [Fisher 2012].

Table 2.2: Cost of the hardware (in Swiss francs, approximately = USD) used for setting up the mesocosm experiment. The net cost, i.e. the cost of the hardware specifically bought and non-reusable represents ca. 30% of the total cost of the experiment.

Item	Cost (CHF)	Specifically bought	Reusable
Tanks (WIVA VAT4, 60 L)	540	yes	barely
Pipes	200	yes	no
PVC tubes	200	yes	barely
WTD sensors (see sup. mat.)	450	yes	yes
WTD data logger (see sup. mat.)	100	yes	yes
WTD controller (see sup. mat.)	300	yes	yes
Toilet flush devices (Geberit)	1,800	yes	barely
Soil moisture sensors (9 x Decagon E5-TM @ 172.-)	1,548	no	yes
PYR sensor (Decagon)	238	no	yes
Rain gauge (Decagon ECRN-100)	307	no	yes
Decagon data loggers (3 x EM50 @ 458.-)	1,375	no	yes
HOBO data loggers (8K - UA-001-08)	1,800	no	yes
Various components (cables, power plugs, etc.)	200	yes	no
Filtration cartridge	150	yes	yes
Garden shelter	300	yes	yes
Total cost of all items	9,508		
Total items specifically bought		4,240	
Total reusable items			6,568

The time spent learning designing the electronic part should also be taken into account for the cost estimation. As our devices are very simple and the online documentation extremely abundant, it took approximately two full weeks to learn the Arduino and electronic basics and two full weeks to build and install the devices, for a total of 160 hours. It is largely compensated by the fact that we did not have to pay anyone any longer to measure the water table depth daily once the water level sensors were in place. At the beginning of the experiment, we had to hire a technician to measure the water level daily using a lasermeter. The workload was ca. five hours a week, corresponding to 260 hours annually. Therefore, the technician cost was compensated in ca. eight months. These eight months

are largely compensated by saving the cost of ca. 38,300 USD estimated by the local company for the sole water level monitoring.

2.4.2 Environmental data

2.4.2.1 Dealing with missing data

Overall, the system was fairly reliable. Nevertheless, several problems were encountered. First, having several sensors implies a higher probability of a technical issue occurring in at least one of them. However, this quantity of sensors and loggers prevents a global data shutdown, which would be dramatic from an experimental point of view. This problem aside, the sensors were reliable: over two years, only 4.19% of the 1,239,540 expected data points were missing or erroneous. Problems occurred among others in the HOBO Logger 8K - UA-001-08 temperature sensor, whose batteries sometimes failed due to the severe frost of winter 2012-2013. Similarly, a damaged cable on one E5-TM sensor induced power drainage in one Decagon datalogger, leading the batteries to empty too rapidly, and the data recorded by this sensor were not reliable. Finally, the air moisture and temperature sensor did not handle the high temperature of summer 2013, and yielded erroneous output data during this period.

Table 2.3: Missing data and solutions used to cope with data gaps.

Sensor	Data loss [%]	Impacted sensors /total	Data gap >10 days	Solution
Soil moisture	3.65%	2/9	yes	Generalized additive model based on the seven remaining sensors and valid data + rainfall, pyranometer, piezometer, and air temperature data
Soil temperature	3.65%	2/9	yes	*idem*
Peat temperature	4.11%	30/45	yes (3 sensors)	Amelia II
Air temperature	13.70%	1/1	yes	Generalized additive model based on valid data and nearest national weather station temperature data
Air relative humidity	13.70%	1/1	yes	Generalized additive model based on valid data + rainfall, pyranometer, and air temperature data
Rain gauge	2.74%	1/1	yes	Linear model based on valid data and nearest national weather station precipitation data
Pyranometer	0.00%	0/1	/	/
Piezometer	4.11%	45/45	no	Linear interpolation
Overall	4.19%	81/112		

To overcome the data gap induced by defective sensors or data loggers, several strategies were used, and are summarized in Table 2.3. The Amelia II R package [Honaker 2011] is well suited

for small data gaps and time series, and was used to computationally infer scarce missing data entries, using the built-in modified expectation maximization importance sampling (EMis) algorithm, called EMB. For longer data gap (greater than 10 days), depending on the variable to reconstruct, several tools are available, including least square linear regression (using lm function from R package, [Chambers 1991], autoregressive moving average (ARMA) [Gurland 1954], autoregressive integrated moving average (ARIMA) models or their seasonal equivalent SARMA and SARIMA. However, these classical models might not be the most accurate nor the best suited, regarding the nature of our dataset. For instance, to reconstruct missing air temperature data, we fitted a linear model expressing recorded air temperature as a response variable to the air temperature recorded at the nearest national weather station. We then computed the missing values based on this model. Similarly, we reconstructed missing soil moisture data using a generalized additive model (GAM, a generalization of the GLM) expressing soil moisture as the result of air temperature, water table depth, soil temperature, solar radiation, and precipitations variables. The model performed well and gave a Root Mean Square Error (RMSE) of 0.076. Consequently, our setup allowed us to monitor finely the environmental data, as shown on Figure 2.2. The model fitting highly depends on local climatic conditions specific to where the experiment takes place. The aim of such models are not to draw general equations for understanding climatic mechanisms, but rather to rely on correlation between climatic variables to infer missing data points. Therefore, no universal model could be developed that would be suitable for any research location around the world. An increasingly used and efficient method to infer missing data is artificial neural network (ANN) [Kuligowski 1998, Kuligowski 1998]. The main strength of ANNs is that they can infer any mathematical function (universal approximation theorem, [Hornik 1991]. The drawback is that they require a large input dataset, and the structure of the network has to be chosen carefully to avoid overfitting.

2.4.2.2 Homogeneity assessment and trials

One concern about our design was the potential presence of a row effect. Indeed, our 45 mesocosms were installed in two rows, one oriented SSE, the second oriented NNW. We analysed the temperature data recorded by the HOBO data loggers, inserted in each mesocosm at the catotelm/acrotelm interface. Over the first nine months, there was no significant temperature difference between rows as determined by one-way ANOVA (nine months mean temperature for each mesocosm, $p = 0.475$), confirming that the location of each mesocosm had no impact on its average temperature. It is however likely that the daily temperature range or other variables differed between the rows but our goal here was not to analyse this in detail. Further analyses of the temperature dynamic among water level treatments are yet to be performed.

The WTD control required several trials and prototypes before being effective. Consequently, the three amplitudes of fluctuation were operational after one year only. Our first idea was to use medical drips to induce a slow leakage as horizontal water transfer simulation. It was unsuccessful due to peat

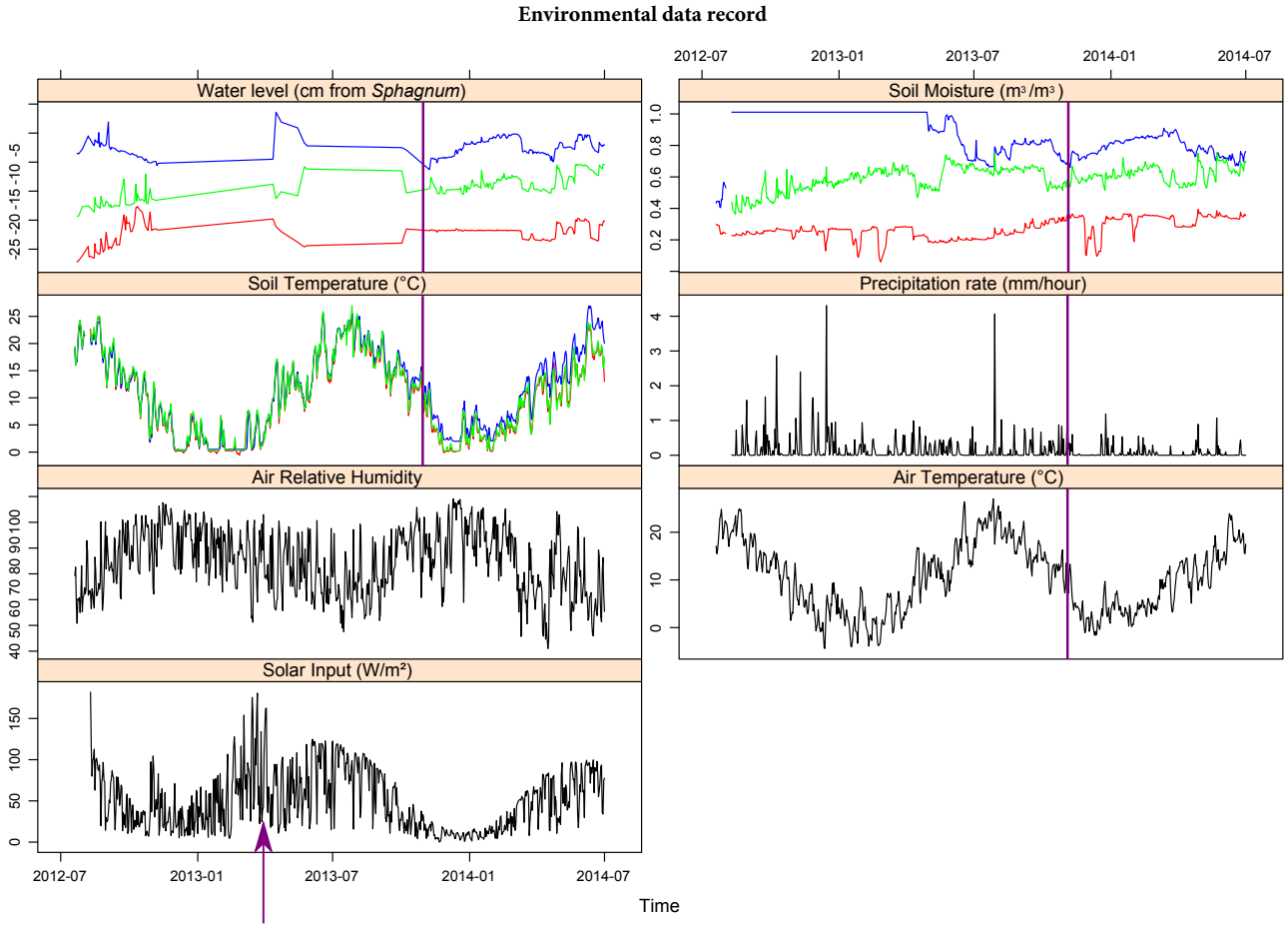


Figure 2.2: Environmental data recorded at the experimental site (black) and in three mesocosms: LM (red), MM (green), and HM (blue). The WTD dynamic regulation was effective from November 2013 onwards. I was removed during the freezing period and reinstalled after the thaw. The significant reduction of solar input occurring in spring 2013 (purple arrow) is due to the re-installation of the net, which was removed during the previous winter to avoid it being damaged by the heavy snowfalls. The net filters ca. 50% of the solar input. It was not removed in the following winter as there was virtually no snowfall.

particles released by the peat core, clogging the drip. We then attempted to build peat-made slow leaks (water flowing from the pipes into pierced bottles filled with peat, as an attempt to simulate natural infiltration), but the water flow through those devices was too high, and the water level could not rise above AWTD. The final design, using dynamically regulated pumps, appeared to perfectly overcome these constrains. Connecting replicates of each treatment to each other allowed us to maintain homogeneity between the replicates even in case of unpredicted events (leaks, clogs, etc.). We recommend using pipes with a strict minimum diameter of 5mm to avoid clogging, with ideally a larger diameter to lower the resistivity of the pipes connecting replicates, that can induces a small WTD differences among the replicates of a same treatment. However, pipes with a diameter greater than 3 cm should be used with care, because it increases the risk of leak at the O-ring level. From July 2012 to September 2013, we observed a significant difference between the three AWTD treatments

(ANOVA, $p < 0.01$), for which the water level remained approximately constant over this period. The sparse clogging induced some punctual disturbance that had overall no effect on the AWTD over time. From November 2013 to present (April 2015) the nine treatments were well contrasted (shown in Figure 2.2: November 2013 to August 2014), although we had to fix some leakage issues. Additionally, it seems that water level, controlled by the float/pump/regulator system, decreased slightly slower than initially planned and calibrated. Although it had no impact for our setup, this could be an issue for experiments where the water level drop has to be finely controlled ($\pm 1mm.h^{-1}$ for instance). However, this can easily be fixed by modifying the microcontroller program to precisely adjust the settings.

2.5 Discussion

As understanding the responses of peatlands to climate change is crucial in the global warming context, it is important to develop protocols and methods allowing manipulating micro-climatic conditions to fill existing knowledge gaps and improve peatland-climate feedback models. Experimental field studies are essential to assess true ecosystem functioning. However field manipulations are difficult and expensive to develop and typically only allow testing for small climatic changes. For instance, field Open Top Chambers (OTC) design allows a temperature increase ranging from 1 to 4°C only [Huguet 2013]. There is a need of mesocosms studies, in which many parameter can be controlled, and if necessary highly contrasted. Although our system relied only on WTD control and monitoring, we could easily control other parameters such as rainfall, solar input, wind, and so forth, using similar devices than those used for WTD regulation, but installed in a growth chamber. The water level regulation device could easily be modified to control fans or heating resistors and the pumps could be set up as to simulate rainfalls by pumping rainwater from a tank onto the mesocosms.

We showed that it is possible and relatively simple (with some practical skills) to build an efficient mesocosm experiment to assess the effect of water table depth on *Sphagnum* peatlands. Although efficient, this system is not perfect and could be improved in several ways, as we made several mistakes and learned a lot during the construction and maintenance of our experiment. Perhaps the main limitation is the rather small diameter of the peat core, which induces some edge effect. The advantage of this choice is that it requires less peat and is therefore less damaging to natural ecosystems (a critical issue in countries like Switzerland where there are no vast expanses of peatlands and most of the remaining peatlands are protected). The solution would be to fill the entire tank with peat and cover it with *Sphagnum*, and to use a small tube (e.g. 5-10 cm diameter) for WTD monitoring and manipulation. The counterpart of this design is that it requires much larger amounts of peat and *Sphagnum*, consequently damaging the original harvest site. For instance, using the whole surface of the 60L tanks would have required more than $7m^2$ of *Sphagnum*, instead of the $0.6m^2$ necessary for our design. Given that peatlands and *Sphagnum* are protected in Switzerland, we would not have been allowed to harvest such a surface of *Sphagnum*. The weight of the peat to carry also has to be taken

into account: considering a peat density of ca. 1.1 (wet peat) we had to transport ca. 230 kg of peat to build the mesocosms. If we had to use the whole 60L tanks filled up to 5/6, we would have had to transport 2,245 kg of peat. The final choice should therefore be a compromise.

Secondly, harvesting peat and *Sphagnum* separately rather than as single monoliths as it is commonly done was not the best theoretical scenario but still was the best practical choice. We needed peat of a specific chemical composition, similar to what can be found in the natural peatland where the field experiment part of the project was held (i.e. Linje mire in Poland). We found this peat in a cutover peatland where only few mosses were growing. On the second hand, we needed pure *Sphagnum fallax* carpets. We obtained authorization for *Sphagnum* sampling in a peatland covered with patches of pure *Sphagnum fallax* carpet, but unfortunately the peat on this site was not of the desired nature (too minerotrophic). As our study focused primarily on the relatively short-term changes in microbial communities from the top three centimetres of the *Sphagnum* layer with three much contrasted treatments (water level), the effect of this artificial setup was probably lower than the effect of the water level itself. Moreover, the *Sphagnum* layers were 20 cm thick, allowing a proper hydrological behaviour in this part. For accurate estimations of respiration and decomposition, it would be preferable to use larger, single peat monoliths. Similarly, the fact that the peat cores were not insulated did not allow simulating the naturally occurring temperature gradient in the cores, thus also affecting respiration processes. However, the impact of this isothermality of the peat core on the microbial communities inhabiting the top three centimetres of the *Sphagnum* layer is likely to be less critical, as the temperature of the moss surface is mostly driven by atmospheric and water temperature.

Thirdly, another limitation is that the contrasted treatments we used caused major changes in the system. Under wetter conditions the high growth rate of *Sphagnum* and limited soil respiration caused the mosses to grow ca. 5cm above the limit of the tube while in the low water table treatments *Sphagnum* growth was minimal, soil respiration was high and the surface of the peat subsided by ca. 10cm. Thus after nine months there was a ca. 20cm height difference between the two most contrasted treatments. The first year, we decided to level the *Sphagnum* layers back to their original position by adding or removing peat from underneath the *Sphagnum* layer. However, this manipulation created disturbance in the mesocosm, especially in the *Sphagnum* structure, and should be avoided if the study aims primarily at assessing peat decomposition. Again, this was less critical for our surface microbial studies. Nevertheless we decided not to reproduce this manipulation after the second growing season. This issue could be solved by installing the peat cores on a jack, to maintain the top of the *Sphagnum* carpet at the same level in all treatments. If the entire tank had been filled with peat, then the water level could be adapted to the *Sphagnum* layer level by levelling the siphon connecting the replicates up or down.

Fourthly, the cost and design of the monitoring setup could be greatly improved. Up to now, we had to go to the experimental site to retrieve the data from the different data loggers. This was not an issue because our experimental site is easily and quickly accessible. Nevertheless, for less accessible experiment, or for maintenance issue, we would rather setup data logging on a webserver, through

GPRS connection. It is possible to do so using commercial data loggers (ex. Decagon), which are expensive, or to use any microcontroller board (as Arduino, Tiva, or Parallax propeller) associated with a GPRS shield. Although it is possible to setup our own webserver from scratch, various platforms already exists, such as Nimbits (http://www.nimbits.com) and Xively (https://xively.com/) and can be easily used along with homemade data loggers. The GPRS shield associated to an Arduino board can for instance be used with the Pushingbox service (http://www.pushingbox.com/) to send data to a Google spreadsheet, via simple POST requests.

At last, building and maintaining such a system requires permanent weather condition awareness. Every systems dealing with water is extremely sensitive to frost and the sensitive parts have to be easily removable during winter. In our setup, the pumps are installed on a rack that can be disassembled and stored during winter. Indeed, the frost would permanently damage the pumps. Similarly, we had to disassemble the toilet flush devices. For this reason, it is crucial to label every piece carefully accordingly to the mesocosm to which it belongs, so it is not necessary to recalibrate the whole system in spring. To prevent sudden frost, or potentially harmful events such as hail, we developed a Python script parsing online weather forecast daily, using the wundergound website API. This script sends us emails if it detects frost, storm, hail, or any given keyword. It is light and can for instance be installed on a university server or a RaspberryPi, and launched periodically using Linux cron command scheduler. The code is provided in supplementary material.

To conclude, mesocosm approaches are useful to study global change impacts on ecosystems. Although at first glance often simple in their conception, they easily become a challenge to optimise and maintain. We hope the description given here will be useful to other researchers planning comparable experiments. It may indeed be interesting to develop a standard mesocosm system that could then be used in multisite studies. In such a context cost and robustness will be critical as well as optimised and automated data transfer to allow project coordinators to follow in real time the progress of the whole experiment.

CHAPTER 3

Respiration – decomposition: validating mesocosms response to Water Table Depth

3.1 Introduction

As described in the general introduction and in chapter 2, northern *Sphagnum* peatlands are now recognized as an important, threatened carbon (C) pool. As the key to C accumulation is low decomposition rate induced by high water level, studying how on-going climate change and associated changes in soil water content affect the functioning of peatlands and especially their C-sequestration is a research priority. The scientific community became aware of the importance of understanding such system during the early 2000's, when the number of papers published on this topic (keywords: peatldand, climate, carbon) increased tremendously, by more than 500 % between 2000 and 2014 (Figure 3.1, ISI database).

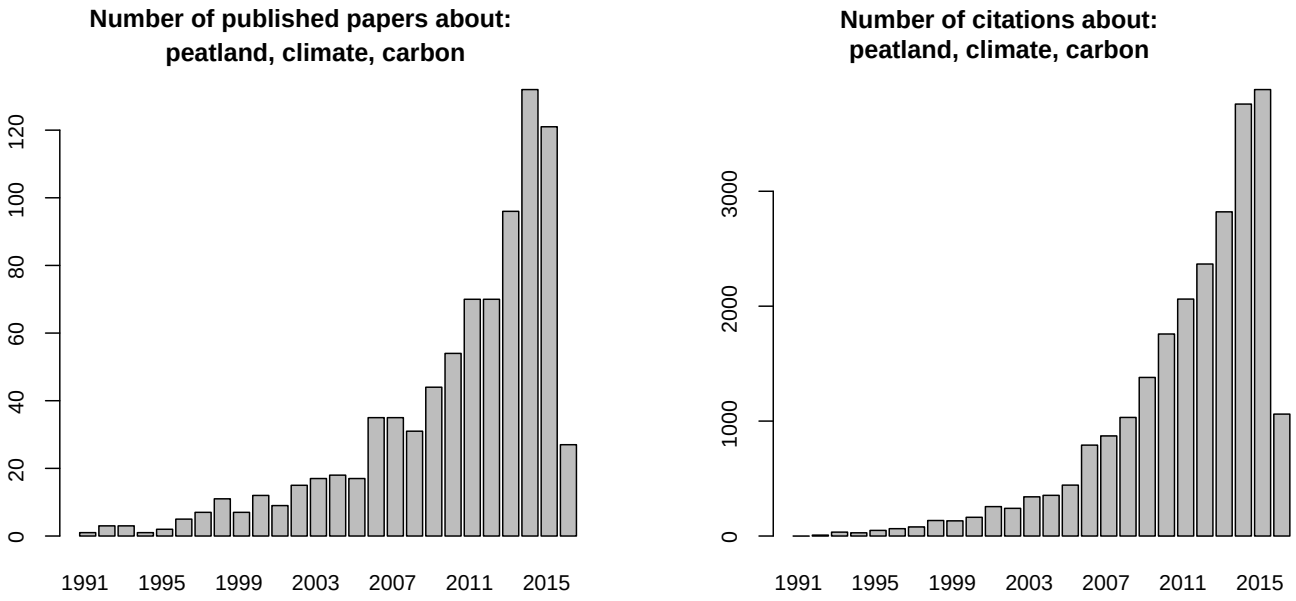


Figure 3.1: Number of published and cited papers since 1900 about peatland, climate and carbon. Data were retrieved from ISI web of knowledge. No publication was found before 1990.

Historically Bridgham (1992) and Gorham (1991) were amongst the firsts to point out the importance of understanding the relationships between peatland respiration and ongoing climate change.

They showed that respiration in peatlands varies annually depending on climate and warned about peatland vulnerability to climate change [Gorham 1991, Bridgham 1992]. Moore, in 1998, concluded that the storage of C in peatlands is sensitive to all C cycle components (biotic and biotic factors) and is therefore difficult to predict. To him, the challenge was to develop quantitative models capable of predicting each peatland evolution [Moore 1998], considering that peatlands have local behaviours [Sulman 2010, Adkinson 2011]. More than a decade later, although a significant number of studies were published, the problem remains the same. In a review, Limpens (2008) concluded that "key uncertainties remain concerning the existence of perturbation thresholds, the relative strengths of the CO_2 and CH_4 feedbacks, the links among peatland surface climate, hydrology, ecosystem structure and function, and trace gas biogeochemistry as well as the similarity of process rates across peatland types and climatic zones. Progress on these research areas can only be realized by stronger co-operation between disciplines that address different spatial and temporal scales." [Limpens 2008]. In the literature, three major factors appear to be good candidate to predict C fluxes in peatland [Grant 2012]:

1. Temperature
2. Water table depth (WTD)
3. Vegetation cover

3.1.1 Temperature

Temperature has been shown to be a major C cycling driver. Liu et al (2016), in a study conducted in Zoige Peatland in China, reported that an increase from 8 °C to 18 °C (which is well above IPCC previsions, around 2–3 °C) associated with oxidization increased CO_2 output by 176 %. Warming alone caused respiration to increase by 73% and oxidization alone by 40.7 % [Liu 2016]. Even only 1°C warming can accelerate total ecosystem respiration rates more than 50 % during the growing season, leading to a possible global increase in peatland heterotrophic respiration of 38–100 megatonnes of C per year [Dorrepaal 2009]. This enhanced effect of temperature increase during the growing season has been observed in several studies, and peatlands can turn into carbon source during summer [Pullens 2016]. Aboveground soil temperature is more important to net CO_2 exchange than deep soil warming and is more important than WTD to predict CO_2 on a daily basis, but average WTD position is well correlated to global CO_2 emissions [Bubier 1998]. The effect of temperature is therefore rapid, if not immediate, and respiration varies during the day. The effect of WTD, however, is more constant through time, as WTD fluctuates less rapidly than temperature, and the responses of the peatland to WTD changes take longer time to occur.

Interestingly, the effect of temperature on CO_2 fluxes depends on the WTD. Q_{10} temperature coefficient (a measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10 °C) has been shown to vary depending on the WTD, from 2.9 with

water tables of 0-20 cm and 2.0 with water tables below 20 cm in natural peatlands. [Silvola 1996]. Additionally, temperature will impact C cycle on a long term by altering vegetation cover.

3.1.2 Water Table Depth

Carbon output (as CO_2 or CH_4) is highly correlated to Water Table Depth [Gorham 1991], whose fluctuation can cause large interannual respiration variability. For instance, Aslan-Sungur et al. (2016) reported respiration rates (NEE measured with eddy-covariance at Yenicaga temperate peatland, Turkey) with a 3 folds variation range between 3 consecutive years (246, 244 and 663 $g_C.m^{-2}.yr^{-1}$ for 2011, 2012, and 2013 respectively). In their experiment, the peak of CO_2 emissions occurred in the dry summer of 2013 when WTD was below a threshold value of -60 cm and soil water content below 70% by volume. Water availability index (an index to estimate the balance between forecast streamflow and current reservoir storage, in hydrology) was found to have the strongest explanatory power for variations in monthly ecosystem respiration [Aslan-Sungur 2016], which naturally lead to consider its relationship with the vegetation cover.

Effect of WTD is important, as lowering of the water table by 1 cm can increase CO_2 fluxes by 7.1 $mg_{CO_2}.m^{-2}.h^{-1}$ at 12°C and 9.5 $g_{CO_2}.m^{-2}.yr^{-1}$ in ombrotophic peatlands [Silvola 1996]. Consequently, peatland drainage can double CO_2 fluxes [Silvola 1996]. Low water table also alters peatland the C sink function by lowering photosynthesis. A mesocosm experiment using peat cores placed under controlled WTD showed photosyntesys activity reduced by 24 to 42% as WTD was lowered of 30 cm [Blodau 2004]. Nevertheless Blodau estimates that overall, the change in C mineralization induced by low water table is the main contributor to CO_2 exchange rate.

As previously expressed, the effect of temperature is dependent on the WTD, and reciprocally. Interannual carbon exchange variability was shown to be linked primarily to changes in WTD ($R^2 = 0.89$) but also constrained by temperature (with an improved R^2 of 0.96 with WTD and T combined) [Strachan 2016]. In fact, Bubier et al. (2003) showed that WTD was the strongest control on respiration in the drier summer, whereas surface peat temperature explained most of the variability in the wetter summer [Bubier 2003]. Therefore, C exchange in peatlands is a result of temperature and WTD, the two combined modifying the functioning of the peatland. As water availability, directly related to WTD and temperature, is the main C cycle driver during dry periods, we can understand that the vegetation cover and associated exudates, impacted by water availability, has a role in peatlands C dynamics. Actually, Helfter (2015) concludes that peatland C exchange is modulated by two dominant factors:

- phenology of the plant community, driven by winter air temperature and impacting photosynthetic potential and net CO_2 uptake during the growing season (colder winters are linked to lower summer NEE),
- WTD, which enhanced soil respiration and decreased Gross Primary Production during droughts. WTD could be offset by higher precipitation [Helfter 2015].

3.1.3 Importance of vegetation

Studies conducted on peatlands with different vegetation covers supported Helfter's conclusions, which were anticipated by Bubier et al (2003). According to Bubier, our ability to predict ecosystem responses to ongoing climate change depends on a more complete understanding of the factors that control NEE across a range of peatland plant communities [Bubier 2003].

It was shown that warming of approximately 1 °C promotes respiration of ancient peatland carbon (up to 2100 years old) when dwarf-shrubs or graminoids are present, while it is not observed when only bryophytes are present [Walker 2016]. Walker et (2016) suggest that plant-induced peat respiration could contribute up to 40 % of ecosystem CO_2 emissions. A similar study showed similar results, with the lowest CO_2 fluxes measured at ombrotrophic sites dominated by *Sphagnum fuscum* (78-127 $mg_{CO_2}.m^{-2}.h^{-1}$ at 12 °C, 60-200 $g_{CO_2}.m^{-2}.yr^{-1}$) and the highest CO_2 fluxes measured at ombrotrophic sites with abundant understorey vegetation (183-259 $mg_{CO_2}.m^{-2}.h^{-1}$ at 12 °C, 290-340 $g_{CO_2}.m^{-2}.yr^{-1}$) [Silvola 1996]. This difference can be partly explained by the fact that *Sphagnum* species are usually difficult to decompose compared with most co-inhabiting vascular plants [Limpens 2003, Dorrepaal 2005, Verhoeven 1995]. Thus, a greater proportion of vascular plants tends to increase the decomposition rate.

Carbon exchange in peatlands is thus the consequence of the interaction between biotic and abiotic factors, with possible feedbacks from one factor to another. Temperature and WTD induce different soil moisture which in turn drive peat structure and plant communities. Ongoing climate change (with extended periods of drought) can for instance restructure the plant community composition, in turn increasing porewater phenolic concentrations, which results in decreased microbial carbon use efficiency and enhanced carbon release. [Dieleman 2016]. As a consequence, studies of C cycling need to capture the spatial and temporal variance of biotic and abiotic factors and their interactions to project the likely impacts of environmental change [Armstrong 2015] (Figure 3.2).

For our project, we designed a mesocosm experiment that aims to simulate *Sphagnum* peatland at different WTD regimes. Before drawing ecological conclusions from this experiment, we must ensure that the peat monoliths exhibit behaviour comparable with what is observed in natural settings. Using litter bags and direct CO_2 measurements, we here aim to compare the C cycle dynamics (actually only respiration and decomposition, which account only for a small part of the C cycle) of our experimental system with literature data.

3.2 Methods

3.2.1 Experimental design

We designed a mesocosms experiment [Mulot 2015] that aims to simulate *Sphagnum* peatland at different WTD. A mesocosm is a tank filled with water, in which we placed peat monoliths topped

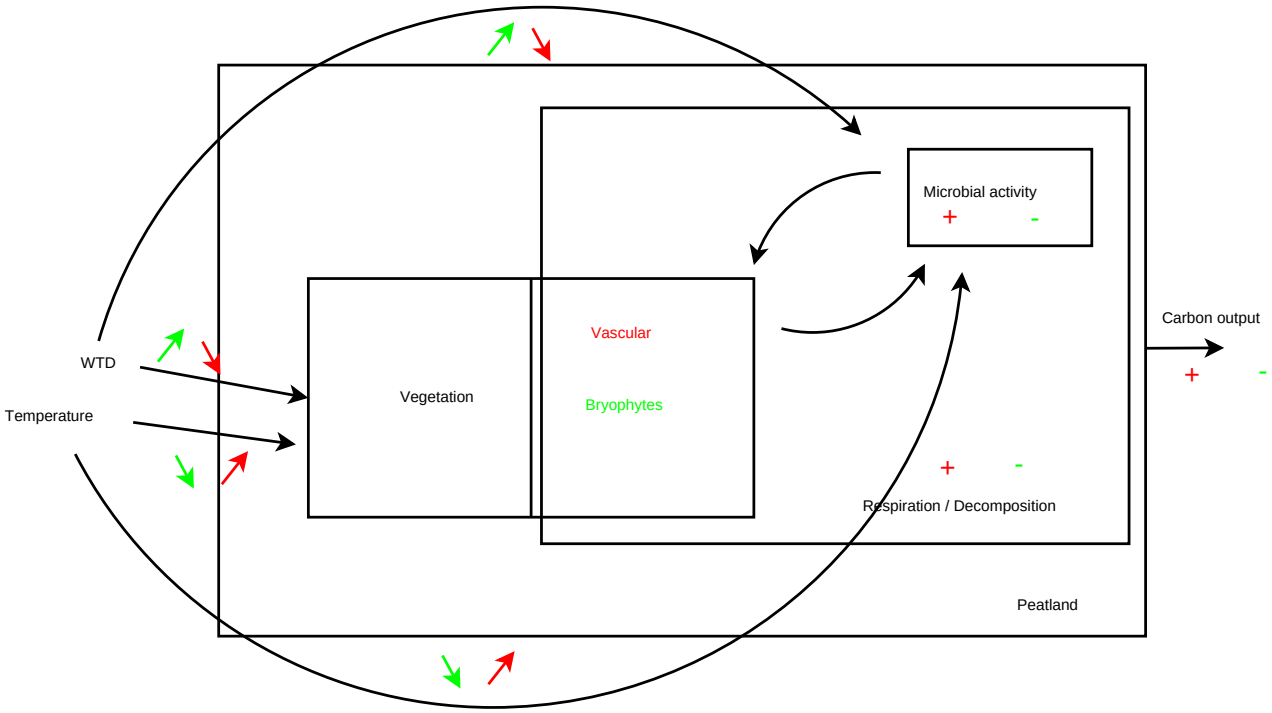


Figure 3.2: Effects of WTD, temperature and vegetation cover on C cycling in peatlands. Low water table added to elevated temperatures promote the growth of vascular plants associated with enhanced microbial activities. The two combined induce higher decomposition and respiration rates, and thus increased carbon export. Feebacks exist between vegetation cover and microbial activities

with a pure *Sphagnum fallax* layer. We defined three average water table depths (AWTD: 4 cm, 15 cm, and 25 cm below the top of the moss carpet, thereafter named High, Intermediate, and Low), for each we determined three amplitudes of fluctuation ($\pm$ 2 cm, $\pm$ 7.5 cm, $\pm$ 12.5 cm) [Mulot 2015]. Each treatment (3AWTD $\times$3 amplitudes = 9 subtreatments) was replicated five times. The experiment started in August 2012.

Environmental variables were monitored hourly, with the exception of WTD which was monitored every twelve hours. The site was equipped with a Decagon PYR sensor, along with high-resolution rain gauge (Decagon ECRN-100) and air moisture and temperature sensor (Decagon VP-3). In one replicate of each treatment, a Decagon E5-TM soil moisture sensor was inserted 5 cm under the top of the *Sphagnum* layer.

3.2.2 Litter decomposition

We measured litter decomposition using litter bags [Singh 1977]. Each litter bags was 3.5 $\times$ 7 cm and made of PET net (Sefar 07-500/39, mesh size $500\mu m$). Litter bags were filled with 0.3 g $\pm$ 0.01 of either dead *Sphagnum fallax* or dead *Eriophorum vaginatum* litter, and then sealed with a sewing machine. We ran three litter decomposition campaigns (03/11/2012–30/04/2013, 30/04/2013–16/12/2013 and 16/12/2013–23/10/2014, hereafter named run1, run2 and run3, respectively). Each

time, we inserted two litter bags (one with *Sphagnum fallax*, one with *Eriophorum vaginatum*) in each mesocosm. The litterbags were inserted horizontally 10 cm below the *Sphagnum* surface. Litterbags were then collected at the end of the campaign. Litter decomposition was measured as follow: litter was weighted before being inserted in the litterbags. Then, the sealed litterbags were weighted before installation and after collection, and mass loss was computed. Before being weighted, litterbags were dried for 24h at 40 °C and cooled in a dryer with Silica-gel. As the three litterbags campaign had different duration, we standardized the mass loss as:

$$D = \frac{\frac{B_{Tf} - B_{T0}}{L}}{d} \tag{3.1}$$

With D, the mass loss per day, expressed in percentages, B_{Tf} the mass of the sealed litterbag after collection in grams, B_{T0} the mass of the sealed litterbag before installation, L the initial mass of litter, in grams, and d, the number of days of the sampling campaign.

For each campaign (run) we computed the average Growing-Degree-Day (measure of heat accumulation) as

$$G\bar{D}D = \frac{1}{n}\sum_{i=1}^{n} GDD_i \tag{3.2}$$

with

$$GDD_i = \frac{T_{\max_i} + T_{\min_i}}{2} - T_{\text{base}} \tag{3.3}$$

with n the number of day in the run, $T_{\max_i}$ the maximum temperature of day i, $T_{\min_i}$ the minimum temperature of day i and $T_{\text{base}} = 10$. When $T_{\min_i} < T_{\text{base}}$, we considered $GDD_i = 0$

3.2.3 Respiration

We measured CO_2 flux using a Licor-8100. We designed an adapter to fit the chamber of the device onto the mesocosms. The adapter is equiped with a tight sheath that surrounds the peat monolith to avoid lateral gas exchange. Measurements were performed from 11/03/2014 to 28/08/2014 to cover a range of temperature from 10.5 °C to 30.5 °C. For each measurement, WTD was measured precisely relatively to the top of the *Sphagnum* layer. We covered a WTD gradient ranging from 23 cm to -1 cm. Each measurement consisted of 30 sec of pre-purge, 4 minutes recording, followed by 30 sec of post purge. CO_2 flux in $\mu mol.m^{-2}.s^{-1}$ was computed by exponential regression (Equation 3.4) using a 1min dead band. Temperature at 5 cm above the *Sphagnum* was recorded for each measurement. CO_2 flux is estimated by

$$F_c = \frac{10VP_0\left(1 - \frac{W_0}{1000}\right)}{RS(T_0 + 273.15)} . \frac{\partial C'}{\partial t} \tag{3.4}$$

where F_c is the soil CO_2 flux rate ($\mu mol.m^{-2}.s^{-1}$), V is volume (cm^3, chamber + pipes), P_0 is the initial pressure (kPa), W_0 is the initial water vapor mole fraction ($mol.mol^{-1}$), S is the soil surface

area (cm^2), T_0 is initial air temperature (°C), and $\frac{\partial C'}{\partial t}$ is the initial rate of change in water-corrected CO_2 mole fraction ($\mu mol.mol^{-1}.s^{-1}$).

We modelled respiration response with a General Additive Model (GAM). GAM is a generalized linear model in which the linear predictor depends linearly on unknown smooth functions of some predictor variables. It is written under the form

$$g(\mathrm{E}(Y)) = \varepsilon + f_1(x_1) + f_2(x_2) + \cdots + f_m(x_m) \tag{3.5}$$

Where g is the link function and $\mathrm{E}(Y)$ the exponential family function applied to the response variable and ε is the error therm. f_i are functions applied to explanatory variable x_i.

3.2.4 *Sphagnum* layer structure

Sphagnum layers topping the peat monolith originated from the same *Sphagnum fallax* lawn and had exactly the same initial structure. Living *Sphagnum* and yellow peat were measured before the lawns being placed onto the peat monoliths. On 30/04/2013, *ie* 9 months after the beginnning of the experiment, we temporarily removed the *Sphagnum* layers from the peat monolith and we measured the thickness of the yellow mosses and green mosses. We then computed the structural (thickness) evolution of the *Sphagnum* layers relatively to their original dimensions. Although this evolution is difficult to relate to available literature, it is useful to see if the structural response follow the respiration and decomposition patterns.

3.3 Results

3.3.1 Litter decomposition

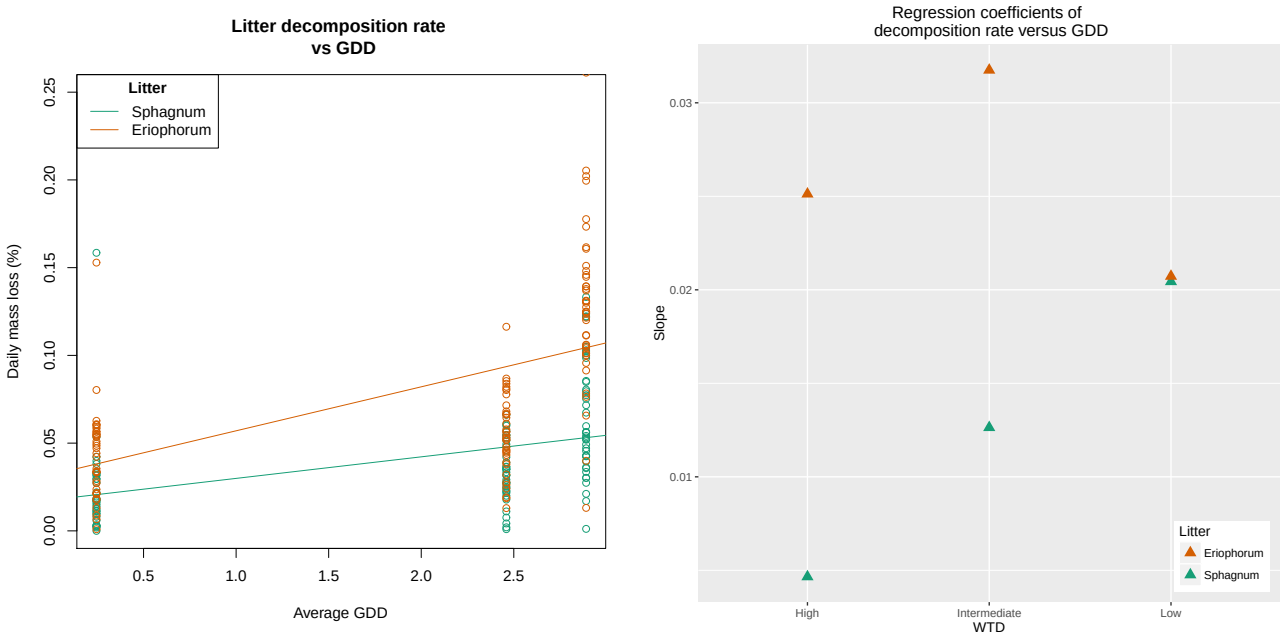
Litter decomposition differed depending on the sampling campaign and the type of litter. The three sampling campaigns (hereafter named *runs*) differed mainly by their temperature. Winter 2013 was harsh, and the mean GDD computed during the first campaign was 0.24. Average temperatures recorded during the second and third campaigns were significantly higher, with GDD of 2.89 and 2.46 respectively. Overall, *Eriophorum* decomposition rate was higher than *Sphagnum* litter decomposition rate (+100 %, +116 % and +100 % for Run 1, 2 and 3 respectively).

The decomposition rate increased significantly with GDD (linear regression, $p < 0.05$), with *Eriophorum* litter responding twice as fast to GDD as *Sphagnum* litter (Figure 3.3).

Decomposition rates increased with water table depth. At low temperature (run 1, $G\bar{D}D = 0.24$), the effect of WTD was greater than at more elevated temperature (runs 2 and 3, both were >10 °C, with both $G\bar{D}D > 2$), were there is almost no difference between the intermediate and high treatment (**??**). We tested the combined effect of WTD, temperature and litter type with an analysis of variance, the litter type and WTD being nested in temperature. The three terms were significant (see Table 3.2).

Table 3.1: Decomposition (in percentage per day) per litter type and per run. Overall, *Eriophorum* decomposition rate was higher than *Sphagnum* litter decomposition rate (+100 %, +116 % and +100 % for Run 1, 2 and 3 respectively)

Run	Litter	mean	sd	min	max
run1	*Sphagnum*	0.02	0.03	0.00	0.16
run2		0.06	0.03	0.00	0.13
run3		0.03	0.02	0.00	0.07
run1	*Eriophorum*	0.04	0.03	0.00	0.15
run2		0.13	0.04	0.01	0.26
run3		0.06	0.05	0.01	0.36



(a) (b)

Figure 3.3: **(a)** Decomposition rate (in percentage per day) versus GDD. *Eriophorum* litter decomposition rate reponded twice as fast to GDD as *Sphagnum* litter. The low R^2 is explained by the 3 treatments being combined altogether in this plot. **(b)** Slopes of the regression between daily decomposition rate and GDD, per litter type and WTD. The response to GDD is highest for *Eriophorum* in Intermediate WTD. The response of *Sphagnum* decomposition to GDD increases as the water level decreases. However, *Eriophorum* litter decomposition is less impacted by increasing GDD in dry environment.

3.3.2 Respiration

We modelled respiration response with a General Additive Model (GAM) under the form

$$g(\mathrm{E}(CO_2)) = \varepsilon + f_1(WTD) + f_2(Temp) \tag{3.6}$$

Table 3.2: ANOVA of decomposition rate response versus GDD, litter type, and WTD. At low temperature (run 1, $G\bar{D}D = 0.24$), the effect of WTD is more important than at more elevated temperature (runs 2 and 3, both were >10 °C, with both $G\bar{D}D > 2$), where there is almost no difference between the intermediate and high treatment.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
GDD	1	0.11	0.11	70.78	0.0000
GDD:litter	1	0.10	0.10	63.37	0.0000
GDD:WTD	9	0.03	0.00	2.32	0.0166
Residuals	220	0.34	0.00		

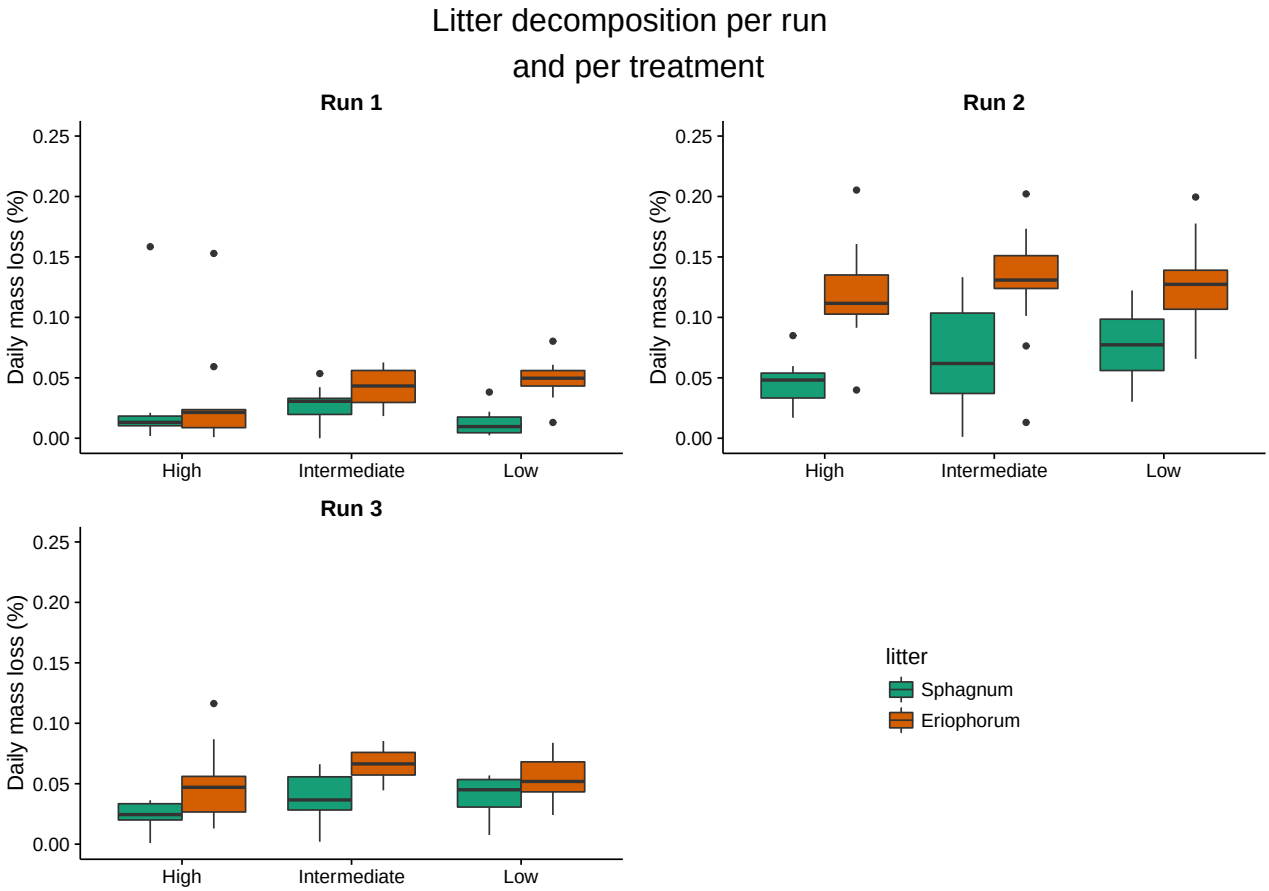


Figure 3.4: Decomposition rate per litter type and per run. Overall, decomposition rate were twice higher for vascular plant than that for *Sphagnum* litter. The decomposition was enhanced by low WTD. The differences observed between the three runs were likely to temperature differences between the three runs. The decomposition rate increased with the temperature.

f_1 and f_2 are here thin plate regression spline smooth functions [Wood 2003]. g is a gaussian function and E is an identity function.The model was built using 123 CO_2 measurements. The model performed well, with 86.2% of the variance explained by the parameters (Table 3.3).

The confidence interval for each component is small along the whole gradient. Interestingly the response of the respiration to DWT is not linear but show an inflexion around -10cm. The respiration

Table 3.3: Summary of the GAM function. Overall, the model explains 86.2% of the deviance. both smooth terms are significant

Family: gaussian
Link function: identity
Formula: $g(\mathrm{E}(CO_2)) = \varepsilon + f_1(WTD) + f_2(Temp)$

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.0370	0.0256	40.51	<2e-16	***

Approximate significance of smooth terms:

	edf	Ref.df	F	p.value	
s(WTD)	2.734	3.395	58.77	<2e-16	***
s(temp)	3.828	4.684	90.70	<2e-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 . 0.1 ' ' 1

R-sq.(adj) = 0.854, Deviance explained = 86.2%
GCV = 0.085873, Scale est. = 0.080594, n = 123

response is more rapid when water level is high, and mild when the water table is below 10 cm (Figure 3.5). On the other hand, respiration response to temperature is almost linear. This is in line with litter decomposition data, where decomposition rate is higher in drier, warmer conditions (Figure 3.3 and Figure 3.4).

3.3.3 *Sphagnum* layer structure

On 30/04/2013, *ie* 9 months after the beginnning of the experiment, we measured the structure of the *Sphagnum* layers. Up to ths date, the water fluctuation system was not active, and we consequently had three average water table depth and 15 replicates each. We observed a clear difference along the three AWTD treatments. The thickness of both living *Sphagnum* (green part) and yellow peat were positively correlated with water level, as shown by figure 3.6.

To assess whether this difference was due to differential *Sphagnum* growth or to shrinking, we computed the *Sphagnum* and yellow peat growth relatively to their original structure. It appears that this difference was actually due to a reduction of both living mosses and yeallow peat thickness, as shown in table 3.4.

3.4 Discussion

In a context of ongoing climate changes, we designed and built a mesocosm experiment to assess the effect of hydrological changes on the functioning of *Sphagnum* dominated peatland. We tested the C

Table 3.4: *Sphagnum* and yellow peat growth. Both living *Sphagnum* and yellow peat exhibit a negative growth in some treatments, caused either by increased decomposition or by shrinking.

	Treatment	mean	sd
Sphagnum growth (cm)	High	-0.07	1.81
	Intermediate	0.18	1.73
	Low	-1.37	1.39
Yellow peat growth (cm)	High	0.00	1.32
	Intermediate	-1.57	0.96
	Low	-3.11	1.52

cycle dynamics of our mesocosms to ensure that their functioning is in line with field observations. This chapter is not intended to be published as is and will likely be merged with the chapter 4, to link peatland C sink function to micro-eukaryotic interaction networks structure.

3.4.1 General behavior of the mesocosms

Overall, our system behaved accordingly to the literature. Respiration increased with temperature, and with WTD. WTD and temperature (and GDD) have a cross effect both on respiration and decomposition rate [Strachan 2016]. This effect might or might not be the cause of the structural change of the *Sphagnum* layer during the first nine months. Indeed, it is difficult to determine if the observed thickness reduction is due to higher decomposition rate or to shrinkage. One idea was to perform X-ray computed tomography (CT) on the *Sphagnum* layers to evaluate the bulk density in all the mesocosms (Figure 3.7). A trial was successfully conducted in Lausanne hospital, and CT scan seems to be a promising tool to better understand structural change in peatland. However, due to time constraints, we decided not to pursue with this analysis.

Results from our litterbag experiments were also in line with the literature [Bragazza 2009], but the design did not allow us to compute a time dependant decomposition model as described in [Olson 1963]. Such models require the same bags to be weigted at different time points. However, the decomposition rate was shown to be strongly correlated to climate, especially temperature (Figure 3.3) [Meentemeyer 1978, Aerts 1997, Gholz 2000, Liski 2003]. The differentiation by litter type was also similar to that reported in the literature, where *Sphagnum* litter decomposes slower than vascular plant litter [Bragazza 2009]. Our data showed that *Eriophorum* litter decomposition response to temperature was greater than that of *Sphagnum*. This result is also corroborated by the literature [Hobbie 1996, Thormann 2004, Moore 2005, Breeuwer 2008]. Finally, the higher decomposition rate observed in the drier mesocosms (more than 2 times higher than in the high water level mesocosms) is consistent with the literature reporting litter decomposition rate increase by 2.3 in aerobic vs anaerobic layer [Bragazza 2009, Laine 2009]. Our litterbag experiments lack litter chemistry analysis, but they nevertheless show that the results from our mesocosm experiment exhibit similar behavior as natural settings and other experiments.

3.4.2 Is a GAM good enough to model respiration? A short review of the literature

GAM modeling is probably not good enough for mechanistic modeling, but is useful to grasp a picture of the system response. GAM models are useful to predict a local behaviour, but poor to model the general response of a wide variety of peatlands. Our design does not really allow proper respiration modeling, as the dimensions of our peat cores probably limit the inertia we would observe in natural peatlands, where thermodynamic processes are buffered by the peat pile. Nevertheless, the direction of the mesocosm response is comparable to existing models found in the literature.

Mechanistics models for peatland respiration exist, but they often make poor use of WTD. For instance, one model ([Kandel 2013]) estimates ecosystem respiration R_E as a temperature dependent function

$$R_E = (R_{base} + R_{fb}) = R_0 e^{bT} + (\beta \times RVI) e^{bT} \tag{3.7}$$

where R_{base} represents the basal soil respiration which is independent of foliar biomass, R_{fb} includes all direct and indirect contribution of foliar biomass in ecosystem respiration, (i.e.,both aboveground and belowground respiration), R_0 is the basal soil respiration at 0°C (independent of foliar biomass), β is a scaling parameter between RVI and R_{fb}, b is the temperature sensitivity of respiration and T is the temperature (°C). RVI is the ratio vegetation index, variable related to metabolically active plant material [Kuzyakov 2010, Wohlfahrt 2010].

Although this model is very interesting from a mechanistic point of view, it is complicated to use for predictions, and does not take WTD into account. It does not consider inner processes occuring in the peatland either. The McGill Wetland Model (MWM) [St-Hilaire 2010] based on the general structure of the Peatland Carbon Simulator (PCARS) [Frolking 2002], is more sophisticated. It considers WTD as a continuous variable and tries to model each inner process as a distinct compartments. For instance, the MWM models photosynthesis (using Rubisco carboxylation rate), canopy conductance, mosses conductance, autotrophic respiration, and decomposition. Canopy conductance uses volumetric soil water content as parameter, which is dependent on WTD. Volumetric soil-water content is evaluated at two depth (d) corresponding to the centre of the fibric and hemic peat layers:

$$\theta_l = \theta_P \left[\frac{W - d}{\Psi_{sat}} \right]^{\frac{-1}{b}} \tag{3.8}$$

where W is the water table depth, Ψ_{sat} is the soil matric potential at saturation and b is the soil texture parameter of the peat layer [Letts 2000]. θ_l is the volumetric soil water content and θ_P is the soil porosity

This model, which performs relatively well, does not however take into account the effects of WTD variability on CO_2 exchange. Mezbahuddin et al. (2014) attempted to tackle this issue by developing a model based on three assumptions (quoted from [Mezbahuddin 2014]):

1. Shallow WTD in the rainy season (November–April) causes lower net ecosystem productivity (NEP) mainly through slower CO_2 fixation due to reduced nutrient availability and uptake caused by slower nutrient transformation and root growth and uptake resulting from slower O_2 diffusion through wet soils.

2. When WTD increases during the early dry season (May–July), more rapid O_2 transport into larger unsaturated soil zones enables faster root growth and microbial nutrient transformations that in turns results in more rapid root nutrient uptake and CO_2 fixation which contributes to a higher NEP. Increased O_2 availability in this hydroperiod may, however, result in more rapid aerobic decomposition in deeper peat layers. Drying of surface residues and near surface peat layers at the same time can reduce surface and near surface soil respiration thereby offsetting the increase in deeper peat respiration, resulting in no net increase of R_E

3. Deeper WTD during the late dry season (August–October), causes greater desiccation of near surface peat which forces declines in root and canopy water potentials, and consequently in canopy conductance and CO_2 fixation, thereby reducing NEP. Further deepening of the aerobic peat zone during this hydroperiod may lead to an increase in deeper peat respiration which exceeds reduction in near surface peat respiration through desiccation, raising Re and further lowering NEP

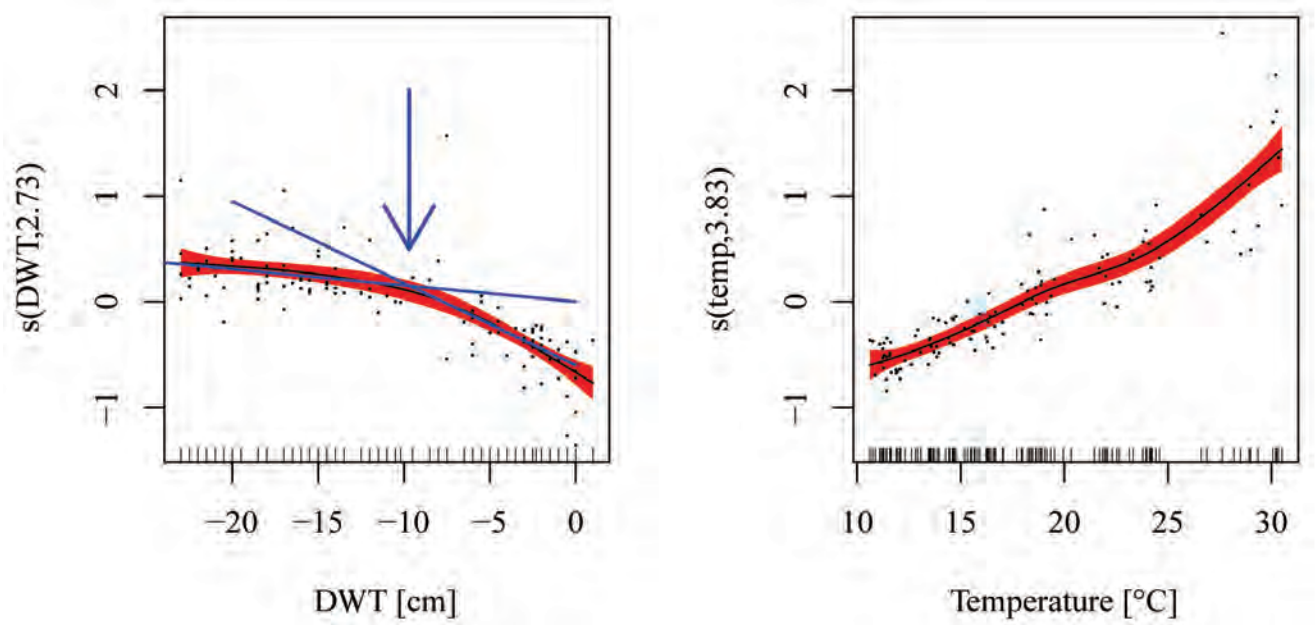
The great advantages of this model (*ecosys*) developped by Mezbahuddin et al. (2014) is that it takes into account the processes by which WTD affects CO_2 [Mezbahuddin 2014]. Grant (2012), describes that

> the modeling of WTD effects on peatland CO_2 exchange in *ecosys* is based on the explicit coupling of oxidation–reduction reactions which drive C and N transformations in soil, roots and mycorrhizae with gaseous and aqueous transfers of the substrates and products of these reactions through soil and root profiles with dynamic WTD. This coupling allowed the model to simulate complex responses of CO_2 exchange to changes in WTD. [Grant 2012]

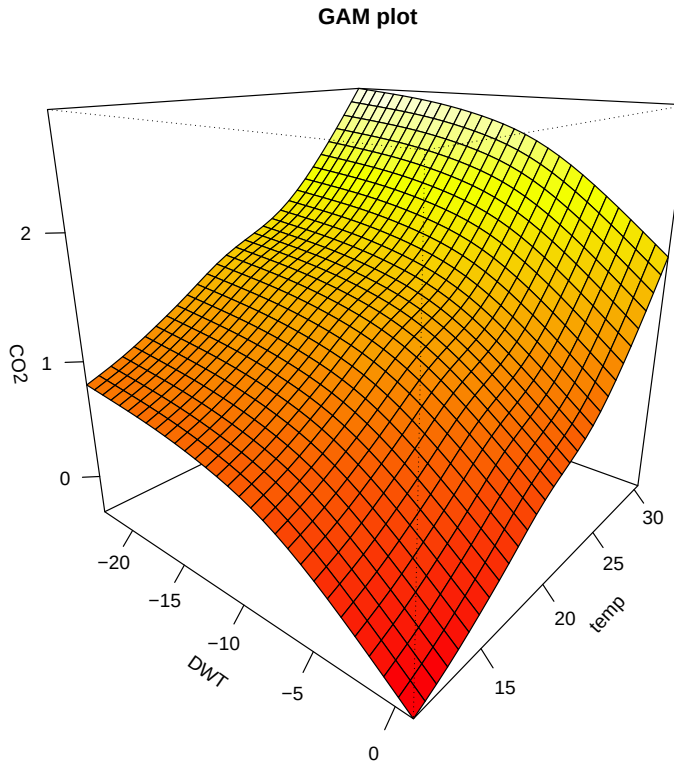
3.4.3 What about microbial activity ?

It appears that modeling and understanding peatland respiration is an arduous task. Many of the existing models rely on compartments that are mechanistic black boxes, and they work with different level of abstraction. It is certain that the GAM fitted in this chapter is very poor from a mechanistic point of view. It however explains 86 % of the mesocosm respiration response, by taking into account only WTD and temperature. Yet, this GAM is a black box with the highest level of abstraction. Indeed, it does not take into account the underlying processes driving C cycle at all. Kandel's model (2013) is one layer deeper in the mechanistic peatland modeling, but fails to integrate inner processes occurring

in peatland. Grant (2012) and Mezbahuddin (2014) are a layer deeper in the mechanistic modeling, but they barely consider microbial activity, which is altered by WTD. However, their models aknowledge that microbial activity induces differences in the soil biogeochemisty. We can assume that, in our case too, the respiration response to WTD is induced by microbial communities whose structure and interactions are impacted by WTD. Therefore, understanding the structuring and functioning of micro-eukaryotic communities along a WTD gradient is one key to understanding peatland C cycling.

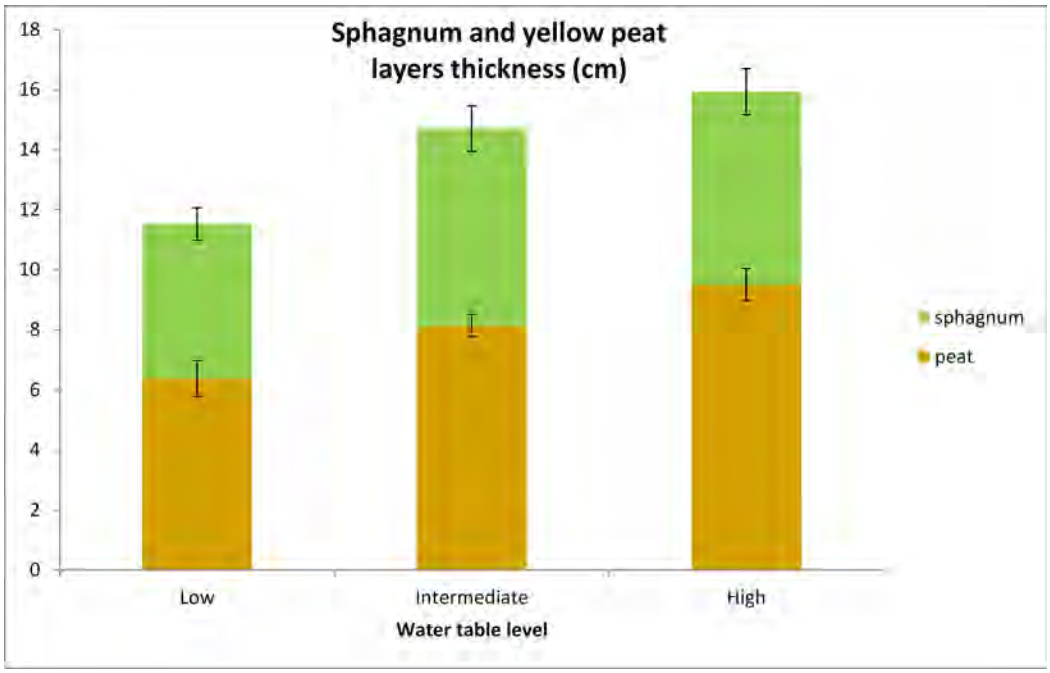


(a) Partial contribution of the smoothed explanatory variables. The left panel presents respiration response curve against WTD. Right panel presents respiration response curve aginst temperature. On the left panel, blue lines are piecewise regressions. the blue arrow shows the intersection between these two piecewise regressions.



(b) Visualisation of the GAM model. Formula: $g(\mathrm{E}(CO_2)) = \varepsilon + f_1(WTD) + f_2(Temp)$. DWT is expressed in cm from the top of *Sphagnum*, temperature in °C, and CO_2 in $\mu mol.m^{-2}.s^{-1}$.

Figure 3.5: Partial contributions **(a)** and 3D plot **(b)** of the GAM espressing CO_2 as the response of WTD and Temperature



(a) Differences in *Sphagnum fallax* layer structure after 9 months on 3 different WTD (-4 cm, -15 cm, and -25 cm below the top of the moss carpet: High, Intermediate and Low). The differences are due to a reduction of the layers thickness in dry condition, rather than *Sphagnum* growth in wet conditions

(b) Picture of a *Sphagnum* layer from a wet mesocosm (High treatment, left) and from a dry mesocosm (Low treatment, right), after nine months. The *Sphagnum* layers of every plot were structurally identical at T0

Figure 3.6: Effect of experimental water table manipulation on the thickness of the *Sphagnum* carpet and the underlying young peat

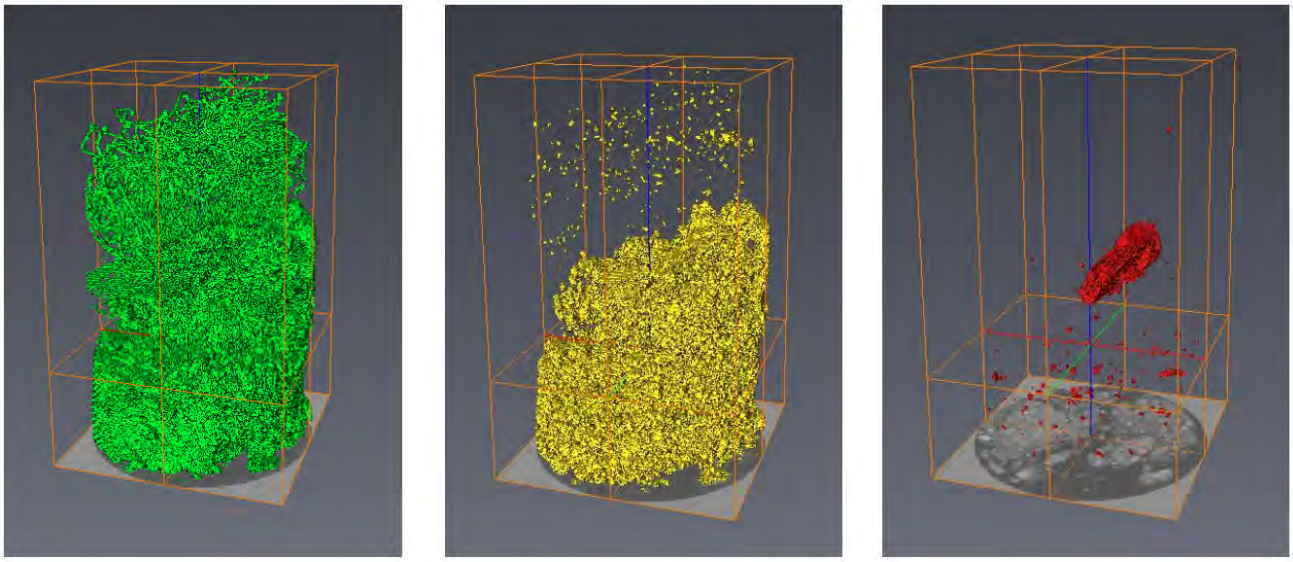


Figure 3.7: Test of X-ray computed tomography on *Sphagnum* layer. From left to right: partition of the particles from smaller to larger. The green CT (computerized tomography) represents living *Sphagnum* and associated thin dead shoots, the yeallow CT represents the top of the peat monolith. the red CT is the HOBO temperature sensor placed between the peat monolith and the *Sphagnum* layer.

CHAPTER 4

Detecting changepoints in micro–eukaryotic community structure and keystone species along ecological gradients using co–occurrence network metrics

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4.1 Introduction

4.1.1 Co-occurrence networks

Understanding the metacommunity dynamics in relation to biotic and abiotic perturbations is key to forecast ecosystems evolution in the actual context of Global Environmental Changes (GEC) [Leibold 2002, Suttle 2007, Gilman 2010]. Such studies are routinely conducted using classical diversity indices and species distribution, but are seldom performed with the aim to investigate the dynamics of species interactions in response to perturbation [Tylianakis 2008], likely because biodiversity based studies are more easily conducted [McCann 2007].

Co-occurrence networks are rapidly becoming a pivotal tool to model species interactions. The underlying idea is that co-occurrence, on the basis of correlation (i.e. non-random association or dissociation), represents the whole range of biotic and ecologic interactions (direct and indirect interactions) driving community structure [Fuhrman 2009, Faust 2012, HilleRisLambers 2012]. One of the main strength of co-occurrence networks is their ability to describe the state of a system regardless of the trophic position of the involved taxa. The drawback is that it is difficult to derive ecological indicators from blind network computation. Research on co-occurrence networks is now rapidly increasing with the recent development of high throughput sequencing (HTS). Indeed, it is now possible to investigate distribution patterns of microbial communities, *i.e.* organisms smaller than 100 μm, with a decent taxonomic resolution and an almost complete screening on the diversity. Micro-organisms drive key ecosystems functions, especially in soil C cycling [Fitter 2005, Nielsen 2011]. However, often, the Operational Taxonomic Units (OTUs) unveiled by HTS have so far rarely been adequately documented in term of taxonomy and thus interpreted in any depth with respect to ecology. Hence co-occurrence networks allow inferring the functional role of those poorly known taxa without prior knowledge about their ecology, for instance by computing their centrality in the interaction network.

Co-occurrence networks are too complex to be directly understood by human mind, but their representation, a graph, is a mathematical object whose properties can be used to numerically characterize the networks. Initially developed in the fields of social sciences and graph theory, many tools were adapted by biologists to describe the topology of biological networks and the characteristics (centrality) of their nodes. Networks are for instance used in physiology, to investigate metabolic pathways [del Rio 2009] or understand protein folding [Atilgan 2004]. Regardless of the framework in which networks are analyzed, centrality measures are always a prime choice of descriptors, either at the local/mesoscale (scale of a node or subgraph) or the global scale (scale of the network). Centrality measures describe the "importance" of a node in the network. Various indices exist, but the most commonly used are betweenness centrality (BC: the fraction of shortest paths going through a node. High BC indicates a that a node is an important crossroad in the network), eigenvector centrality (EC, the principal eigenvector of the network adjacency matrix.), degree centrality (DC, the number of edges of a node. High DC indicates a highly connected node) and closeness centrality (CC, the sum of distances of a node from all other nodes composing the graph. High CC indicated a node locally highly connected) [Freeman 1977]. An illustration of these different metrics is given in Figure 4.1.

4.1.2 Topological keystone species

Besides network description, these topology and centrality metrics are used to infer topological "keystone" species, i.e. species whose removal would have a major impact on the network topology. Several approaches have been developed to identify such nodes within a network. However, the major bias with the available concepts developed to identify "keystone" species is that they are mostly based on food webs (e.g. [Vasas 2006]), or energy directed networks, with an *a priori* bias that prevents scaling the concepts up to the pure co-occurrence network, which involves both direct (trophic) and indirect (competition, niche construction [F. John Odling-Smee 2013] etc.) interactions. Existing keystone indices heavily rely on trophic interaction [Jordan 1999, Libralato 2006], biomass and/or functions [Power 1996, Ortiz 2013a, Ortiz 2013b], and are therefore not suited for co-occurrence networks. For instance, a recent study promotes he use of DC and BC as key characteristics to identify keystone nodes in metabolic pathways [Roume 2015]. The rationale is that nodes that are very *between* (and thus central) but poorly connected are critical nodes in the metabolic pathway. Yet, here again, this approach assumes flows in the network.

The search for keystoneness indicators has also been performed empirically, for instance by generating artificial networks from generalized Lotka–Volterra equations (dynamic trophic networks), iteratively removing nodes, and measuring the network stability after each removal [Berry 2014], but the methodology used in this case implies that selected centrality descriptors are suited for Lotka–Volterra based networks, and thus cannot be blindly generalized to other networks. In the latter study, Berry and Widder (2014) proposed four criteria to identify keystone species: high DC, high CC, low

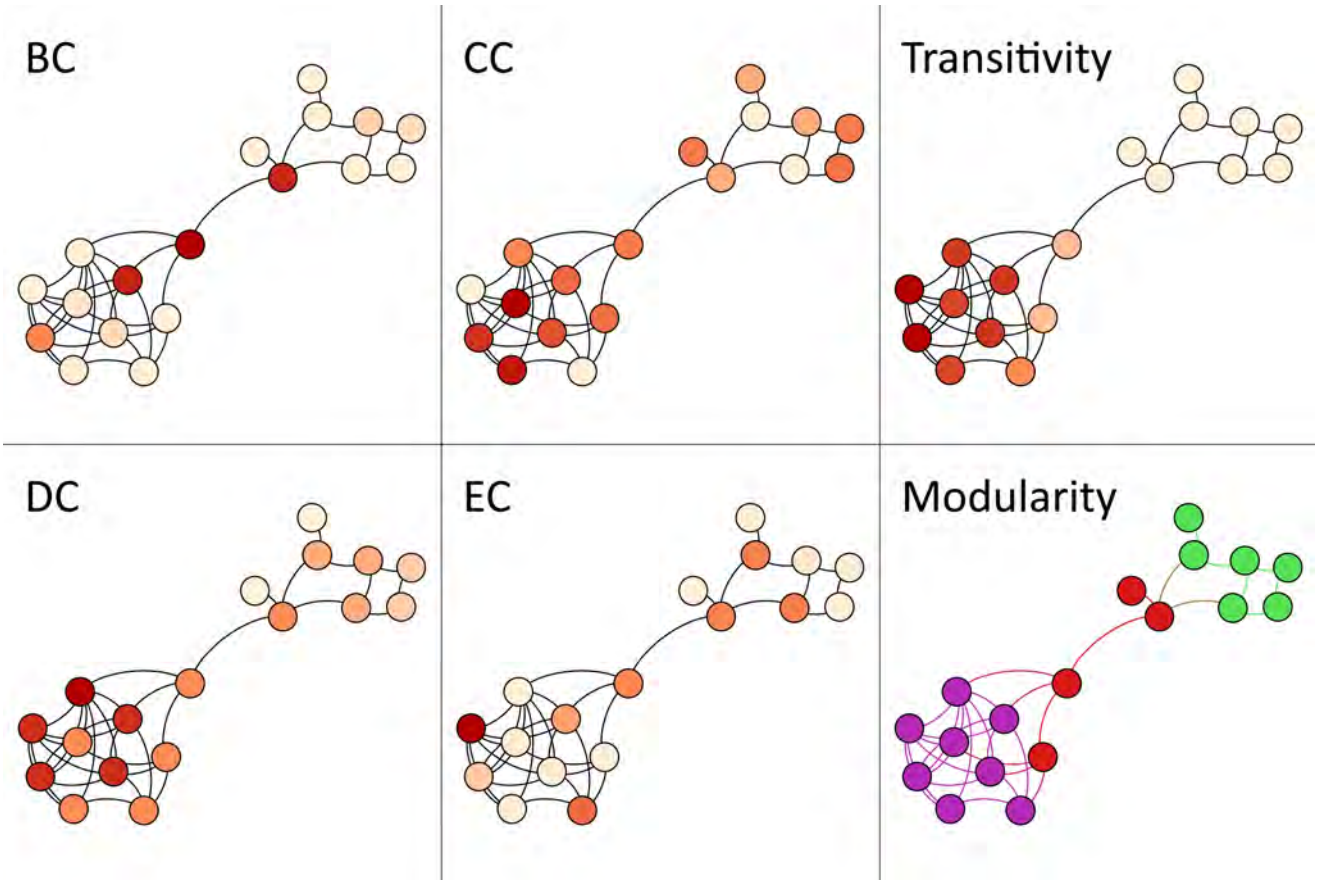

Figure 4.1: Common centrality and topology measures. BC (betweenness centrality) is the fraction of shortest paths going through a node. EC (eigenvector centrality) is the node eigenvalue of the principal eigenvector of the network adjacency matrix. DC is the number of edges connected to a node. CC (closeness centrality) is the sum of distances of a node from all other nodes composing the graph. Transitivity represents how close a node is to form a complete graph with its neighbors. For all indices, values are proportional to color intensity. Modularity is the partitioning of a network into subnetworks. In this case, colors indicate possible subnetworks.

BC, and high transitivity. The transitivity of a node quantifies how close its neighbors are to being a complete graph, i.e. a graph in which all nodes are connected to each other [Watts 1998].

In order to overcome current limitations in identifying keystone species it is useful to look at similar concepts developed in other (unrelated but with comparable complexity) fields. In graph theory attempts to solve the so called "Critical Node Detection Problem" (CNDP) [Arulselvan 2009, Ventresca 2015] are based on network topology analysis. For instance, critical nodes in metabolic pathway were identified by defining the concepts of choke point (critical points in metabolic networks, which when inactivated may lead to an organism's failure to produce or consume particular metabolites) and load point (ratio of BC and DC, which assume that a node with high betweenness and few edges is critical for the network) [Rahman 2006]. In the case of co-occurrence networks, given the complexity of the underlying interactions involved in the network, a good approach could be to use general graph theory theorems and metrics, designed for a broad range of networks (power

grids, roads, disease spread, Internet, etc.). A recent study [Allesina 2009] showed the efficiency of using a modified version of the Pagerank algorithm, which was developed by Google to rank the web pages by their importance within the internet network. Although the modified Pagerank algorithm developed by Allesina and Pascual was conceived to solve the CNDP in food-web networks, it can be adapted to interaction networks. Recent attempts to solve the CNDP were based on the analysis of networks as electrical circuits [Zhu 2014] and hence developing current flow–based centrality index [Brandes 2005].

A common feature of these approaches is that they assume flows in the network, which is not necessarily the case in co-occurrence networks. Recently, a publication developed the idea of building a generalized keystone index (GKI) using linear combination of several centrality indices (DC, BC, CC, EC, information centrality (IC), and subgraph centrality (SC) [Estrada 2005]) by factor analysis [Estrada 2007], which has the advantage of taking into account the centrality of the nodes at all the levels of the network. IC and SC are described in the methods section. This indice, called Generalized Keystone Index (GKI) has been shown to be valid for ecological network, but has not been tested on co-occurrence networks. This indice is purely topological and can be used in a context where flows are unknown or absent.

4.1.3 Evolution of network structure along a gradient – topological change-points

Up to now, ecological studies on interaction networks and associated centrality metrics have mainly focused on the temporal or spatial distribution of communities [Barberán 2012], and, to our knowledge, none have aimed to investigate the response of communities along ecological gradients. In the context of GEC induced by global warming, environmental factor driving microbial communities are predicted to shift from their actual value [Pachauri 2015], thus creating a need to understand the stability of interaction networks [Tylianakis 2010]. Networks metrics could potentially be used to infer ecological thresholds and thus be relevant to investigate the sensitivity of ecosystem functions to GEC. Here we explore this question using the micro-eukaryotic communities living in *Sphagnum* peatlands.

Northern *Sphagnum* peatlands are amongst the most threatened ecosystems by GEC. Although they represent 3–5 % of the worldwide land area [Frokling 2007] they hold 1/3 of the soil C pool, which is comparable to the atmospheric C pool (around 750 GtC). C cycling in peatlands thus plays an important role in global C sequestration [Bragazza 2006]. The key to C accumulation is low decomposition rate, which is primarily driven by the anaerobic conditions in the water-saturated peatland soil [Yu 2006]. The C sink function of peatlands is expected to be sensitive to variations in soil water content induced by GEC [Davidson 2006, Bragazza 2009, Briones 2014]. Studying how on-going climate change and associated changes in soil water content affect the functioning of peatlands and especially their C-sequestration is thus a research priority. Knowledge of the food webs in peatlands

is still limited [Gilbert 1998, Mieczan 2013, Gilbert 2015, Mieczan 2015], but it is clear that microbial communities are key actors in the C cycle of peatlands [Jaatinen 2008, Briones 2014, Lin 2014, Jassey 2015]. Water level is a critical factor driving these microbial communities and hence C cycling [Choi 2007, Faubert 2010, Dinsmore 2013, Gažovič 2013]. Consequently, understanding the response of peatland microbial community structure to GEC, and especially water level is a research priority.

For this study, we computed interaction networks along a water level gradient using a sliding window. Generally, networks are computed using sample from different ecosystems, and the topologies of the inferred networks are then compared between these distinct ecosystems. In the case of a strongly influential gradient, samples collected at the extremes of this gradient belong to different ecosystems. Ecological changepoints are the value of the gradient for which a system transitions from one state to another (Figure 4.2). We assumed that:

1. Community structure is modified by an environmental gradient, and thus by WTD in peatlands [Marcisz 2014a, Fournier 2015b]

2. The interactions between conserved species are modified along the gradient [Ikeda 1992, Suttle 2007, Juliano 2009, Gilman 2010, Hillyer 2010]

3. The structure of the networks reflects these changes and will vary dynamically along the investigated environmental gradient, that is here water level.

4. The structure of the network can show a topological changepoint along the investigated gradient

4.2 Methods

4.2.1 Experimental design, DNA collection and sequencing

We designed and built a mesocosm experiment to assess the effect of experimental water table depth manipulation on the functioning of *Sphagnum* peatlands [Mulot 2015]. Each mesocosm is made of a tank containing a pierced PCV tube in which a peat core topped by a *Sphagnum fallax* layer is placed. The tank is filled with water according to the treatment. We defined three average water levels (-4 cm, -15 cm and -25 cm from the top of the *Sphagnum* layer). For each of these three average water levels, we set three theoretical amplitudes of fluctuation ($\pm$ 2 cm, $\pm$ 5 cm or $\pm$ 12.5 cm around the average water level). These nine treatments (three average water levels $\times$ three amplitudes) were replicated five times for a total of 45 mesocosms. The experiment started in August 2012. All *Sphagnum* layers originated from the same peatland and were seeded at the beginning of the experiment with an extract of pool, hummock and lawn to provide a full community potential.

The three theoretical amplitudes of fluctuations were in practice not as contrasted as expected as these depended on extended drought periods, which did not occur during the course of the experiment.

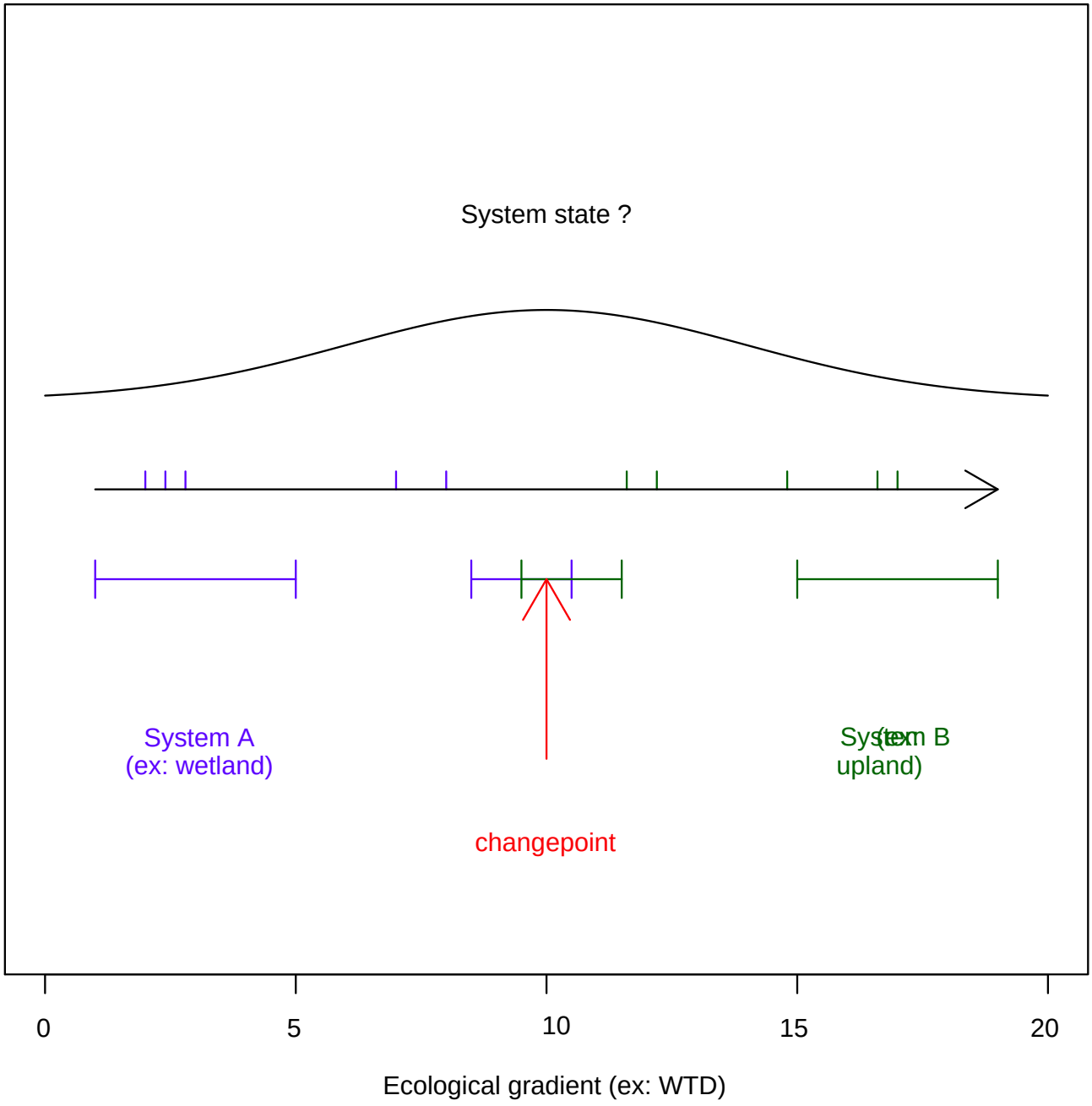

Figure 4.2: Transition state between two systems along a continuous environmental gradient: theoretical framework for computing networks along a gradient. A and B are two distinct ecosystems, segregated by a specific environmental factor. Computing networks using a sliding window along the investigated gradient allows measuring the topological response of the network, and could help identify ecological changepoints. The assumption behind this framework is that the structure of the network reflects the state of the system.

Thus we ended up with nine treatments with rather stable water levels (max SD = 4.6 cm). However, due to differential *Sphagnum* growth or peat compaction and decomposition in the three different treatments the actual water levels measured in the experiment since August 2013 corresponded to a

continuum of 45 water levels, ranging from -23.7 cm to +12.4 cm (standing water) relative to the top of the *Sphagnum* layer.

Samples were collected in September 2014, after two years of experimental water table manipulation. We sampled three *Sphagnum* capitula (top 1cm) in each mesocosm and transferred them in 2 ml tubes with 1 ml of Lifeguard™ soil DNA preservation solution. DNA was extracted with a Mobio Powersoil® DNA Isolation Kit following the manufacturer's recommendations [Lara 2009]. The SSU rRNA V9 region was amplified following the procedure described in [Amaral-Zettler 2009], using Promega's GoTaq polymerase (without proofreading activity). Sequencing was performed with Illumina's Hi Seq technology, using V3 chemistry (Fasteris, Geneva, Switzerland).

The resulting sequences were processed following the pipeline described in [Vargas 2015]. Paired-end sequences were first demultiplexed using SABRE software, and then merged using FLASH [Magoc 2011] with default settings. Tags and primers regions of the $44,191,424$ resulting sequences were then trimmed. Sequences with at least one mis-sequenced nucleotide in the primers regions were discarded, for a total of $15,750,875$ conserved sequences. Quality filtering was performed by evaluating the expected error in a 50 bp sliding window and removing sequences with more than 1% of error in the worst quality window. The $14,254,591$ remaining sequences ($44,415$ unique sequences) were clustered into $6,191$ OTUs using SWARM [Mahé 2014], with a 1 bp threshold. OTUs taxonomic assignation was performed using ggsearch [Pearson 1988, Pearson 2014] against a curated version of the PR^2 database [Guillou 2013].

4.2.2 Numerical analysis

Numerical analyses were carried out in R statistical software [R Core Team 2014], with the exception of modularity [Blondel 2008] that was computed using Gephi [Bastian 2009] for visualization purpose. Prior to any analysis, OTUs belonging to Embryophyceaea were discarded, as well as those accounting for less than 0.1% of the total remaining sequences. The rationale is to discard over abundant *Sphagnum* sequences and to trim the tail of the reads distribution. The analyses further described were performed at the OTU level. An R package to perform the network analysis over the gradient, gradnet, is currently under development, and the documentation can be found in the appendices.

4.2.2.1 Community changes

To compare the network topology response to classical diversity analysis, we first computed diversity indices for the 45 samples. We first applied a Hellinger transformation on the community matrix, to normalize the abundance distribution. Hellinger transformation compensates the semi-quantitative nature of the read counts. Based on this matrix we then calculated species richness, Shannon diversity, Simpson diversity, Pielou evenness and Hill evenness. We then detected threshold in the communities structural response to water level gradient by piecewise regression and TITAN analysis [Baker 2010]. TITAN aims at detecting changes in taxa distributions along an environmental gradient, by using

indicator species scores to integrate occurrence, abundance and directionality of taxa responses. The TITAN analysis was performed with 500 permutations and 500 bootstraps.

4.2.2.2 Networks changes

Co-occurrence networks were computed based on Spearman correlation coefficient. As this coefficient (ρ) is a ranked correlation, it is independent from abundances distribution. Unlike Pearson's correlation coefficient, which assumes linear relationship between variables, Spearman's correlation only tests for a monotonic relationship between the two variables. This avoids spurious correlations, which may artificially inflate networks and reduce specificity [Friedman 2012]. Besides, correlation coefficient has been shown to outperform other metrics like dissimilarity matrices in terms of sensitivity and specificity [Berry 2014]. As ρ is orientated (ρ ranges from -1 to 1), we computed separately positive and negative correlations, representing respectively positive and negative interactions. We produced adjacency matrices by retaining correlation for which the absolute value of ρ was greater than 0.8 and the associate p-value lower than 0.05. The ρ threshold is defined according to the Maximum Relatedness Subnetwork principle, which conserves the overall topology of the network while removing noise [Lee 2010]. Correlations were computed using Hmisc R package [Jr 2015]. To compute p-values, we first generated permutations and bootstrap distributions, with 1000 iterations each, by shuffling taxon abundances and resampling from samples with replacement, respectively. The measure-specific p-value was then obtained as the probability of the null value (represented by the mean of the permutation distribution) under a Gauss curve fitted to the mean and standard deviation of the bootstrap distribution. Measure-specific p-values were multiple-testing-corrected with the Benjamini-Hochberg False Discovery Rate algorithm. The network objects were then constructed using statnet [Handcock 2008] and igraph [Csardi 2006] R packages, and exported as gexf files for gephi, via the rgexf R package [Yon 2015].

Networks were computed by sliding window along the water level gradient. We set a window of 10 samples width and a moving step of one. As a consequence, we computed 35 positive interaction networks, 35 negative interaction networks, and 35 global interaction networks, for a total of 105 networks. We chose a window of 10 samples to linearize the WTD gradient and to perform efficient jackknifing. Along with networks generation, we computed topology descriptors for each graph, using an iterative leave one out jackknife procedure (iteratively removing each site and computing the metrics of the network generated from the truncated matrix) [Zappelini 2015]. For each produced network, we computed the average DC, maximum DC (number of edges of the most connected node), average BC, maximum BC, connectedness (probability that at least one chain of links connects all the nodes), connectance (ratio on links and all possible links) [Dunne 2002, Heleno 2012], number of nodes, number of connected and unconnected nodes, transitivity, modularity, and number of edges. These metrics are topological descriptors of the networks [Csardi 2006]. From these metrics, we

computed a generalized topological index (GTI) built similarly to the GKI described in the following section.

We used piecewise regression to detect changepoints in the networks topological descriptors along the water level gradient, with the R package segmented [Muggeo 2003]. We then compared topological change points and community change points obtained by TITAN.

4.2.2.3 Keystone species detection

We searched for keystone species using the generalized keystone index (GKI) [Estrada 2007]. The GKI is the scores of the nodes computed from the linear combination of BC, IC, EC, SC, DC, and EC. This linear combination is obtained by Principal Component Analysis (PCA), which permits to transform the multidimensional space of centrality measures into a reduced space of principal components. The GKI is the scores of the nodes along the first component of the PCA.

OTUs (nodes) were classified into feeding habits group (plant feeding, bacterivorous, predators, plant parasites, ectomycorrhizes, mixotrophs, phototrophs, predators of bacteria small protists, parasites and saprotrophs, alguivores, phagotrophs, omnivores) and broader trophic categories (autotrophs, mixotrophs, heterotrophs), and the average GKI of each feeding group and each trophic level was computed along the water level gradient. The underlying idea is to understand the topologic position of each feeding group and trophic category at different water table depths. We used piecewise regression to detect changepoints in the average keystoneness of the trophic groups along the water level gradient.

4.3 Results

4.3.1 α and β diversity analysis

Read abundance in the mesocosms was mainly dominated by Metazoa, whose number remained however constant throughout the WTD gradient (Figure 4.3). These metazoa were small arthropods and nematods, mostly. Chlorophyta, Streptophyta, Dinophyta and Discoba all decreased in abundance from -220 mm to ca -90 mm and then increased continuously toward the wet end of the gradient. Conversely, Cryptophyta, Ciliophora and Stramenopiles exhibit the exact inverse response pattern, decreasing from ca -110 mm. This indicates a community shift from this tipping point.

Overall, we observed a near linear negative correlation between β-diversity indices and water level (Figure 4.4). OTU richness expressed a more complex pattern, with an increase from -236 mm to -172 mm (80 to 90 OTUs), then a decrease until -64 mm (62 OTUs). Finally OTU richness increased continuously from -64 mm to +124 mm (83 OTUs). Hill's evenness linearly decreased, but showed a small inflexion around -64 mm, concomitantly with the richness drop. Interestingly, this inflexion was not shown by Shannon's or Simpson's index, as the diversity loss buffered the evenness of the sample. Piecewise regressions on each index showed a mean breakpoint at -117.9 mm (sd: 61.4).

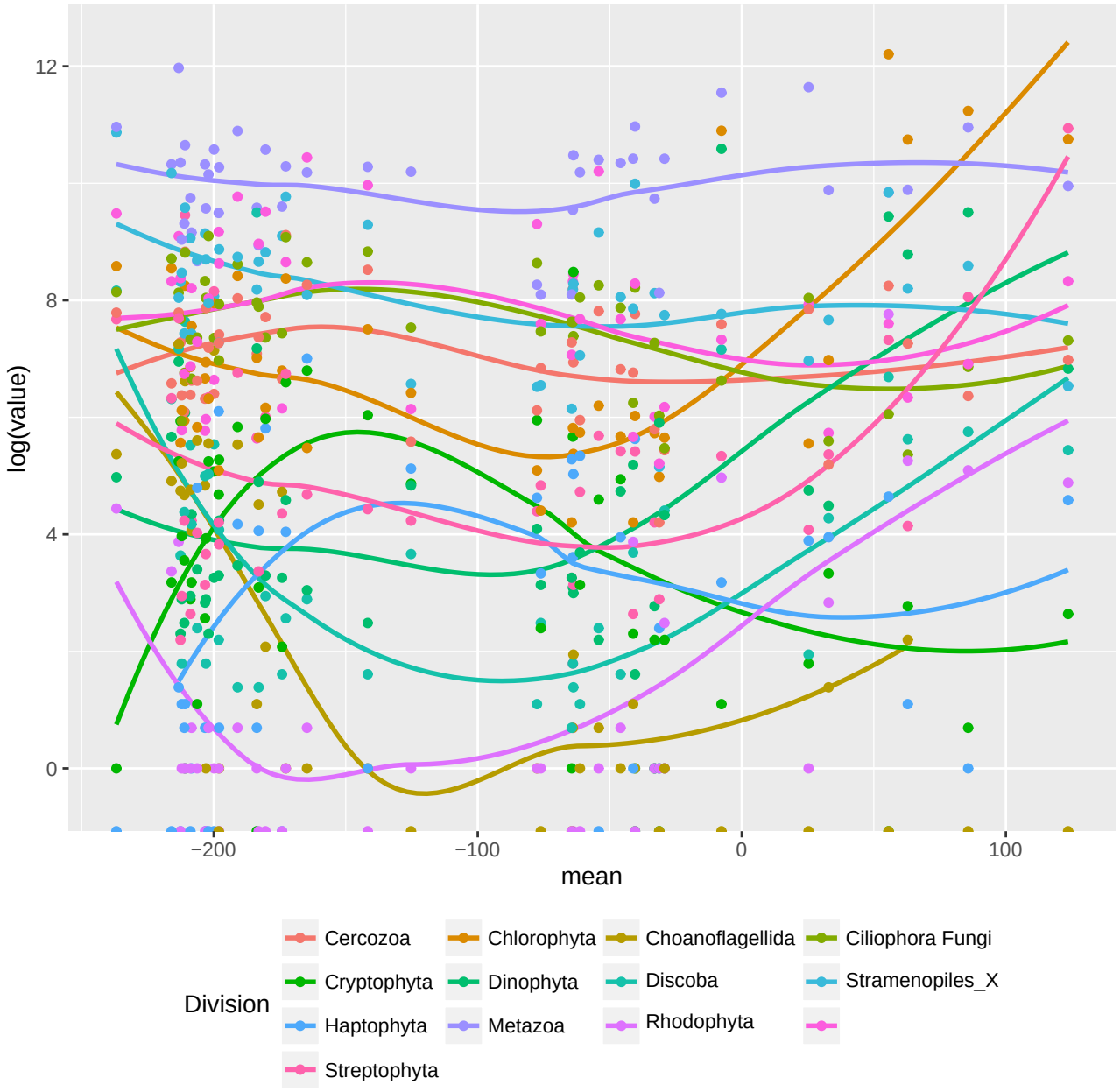

Figure 4.3: Abundance (log transformed) of reads per division. Diversity is mainly dominated by Metazoa (arthropods and nematodes). The read abundace distribution shows a community shift between -50 mm and -110 mm, where Cryptophyta, Ciliophora and Stramenopiles are replace by Chlorophyta, Streptophyta, Dinophyta and Discoba. Curves are cubic spline regressions

Although it was difficult to delimitate ecological changepoints from univariate diversity indices, the TITAN analysis showed a clear pattern. A community based threshold is shown at -101.4 mm, with a cumulated positive z score greater than 100. Iterative computation by keeping more OTUs than those occurring more than 0.1% did not improve the results of TITAN (up to 3777 OTUs meeting the

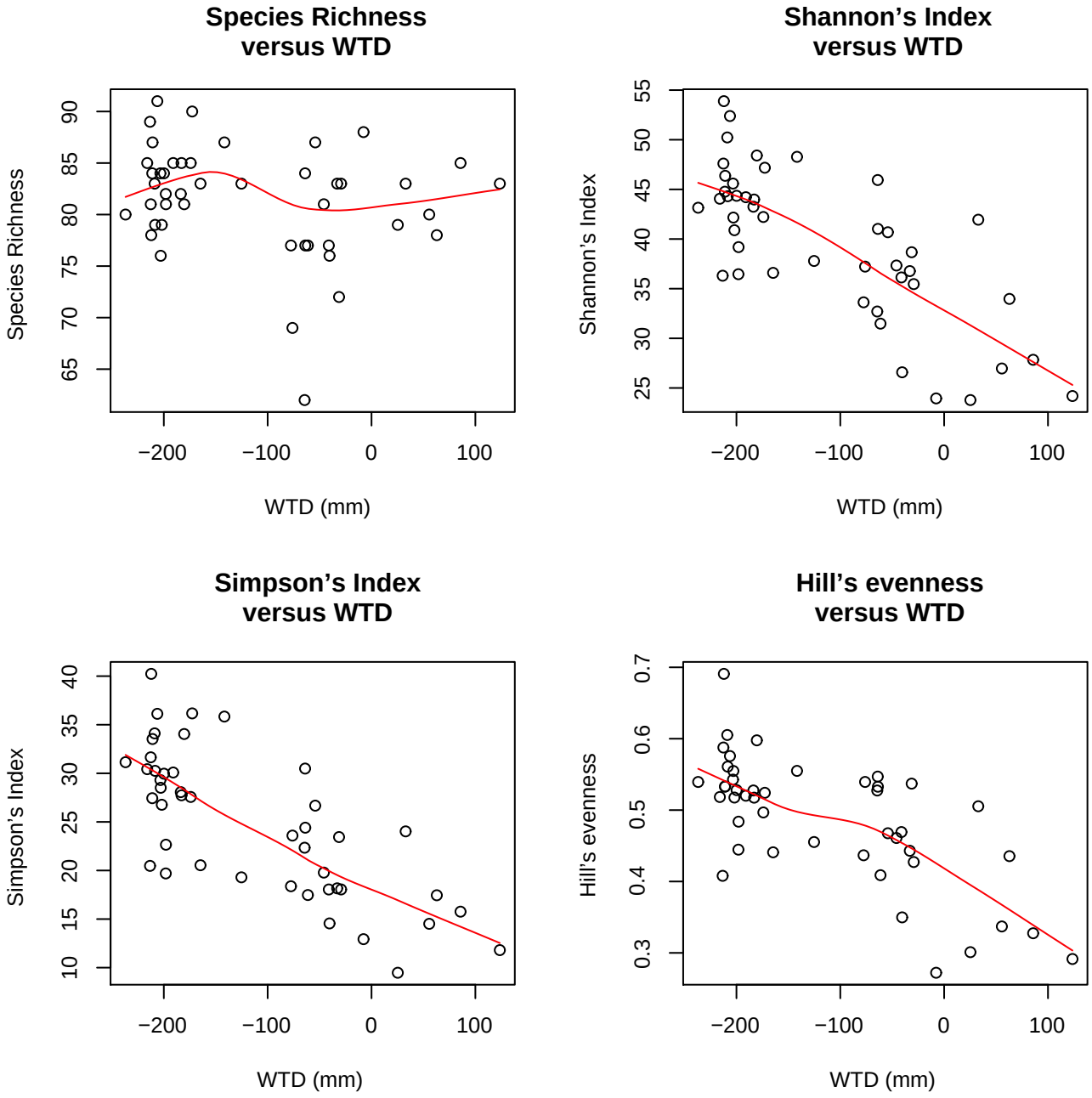

Figure 4.4: Diversity indices along WTD gradient. Red lines are local polynomial regression (loess). OTU richness remained somewhat constant throughout the whole WTD gradient. Shannon, Simpson, and Hill's indices decreased continuously toward the wet end of the gradient. This indicates a turnover in the community composition

criterion for TITAN, *i.e.* occurring more than three times per sample on average). This result confirms the mean changepoint computed from α and β diversity indices.

4.3.2 Network analysis

First, the network changed along the WTD gradient by its structure. Different edges were called depending on the WTD. The phase plot (Figure 4.5) illustrates these topological changes. A great number of edges are spelled[1] between -130 mm and -90 mm, resulting in a denser network. Some edges are preserved along the whole gradient, but on average edges are conserved on only 23 % of the gradient (sd = 42) (Figure 4.5).

We computed general topology metrics (connected nodes, connectance, connectedness, clusters, etc, cf Figure 4.6) as well as average centrality indices (BC, DC, Transitivity, etc.). All these metrics expressed a complex pattern that is similar between the three types of interaction networks (positive, negative, and global interaction networks). Generally, the metrics response along the water level was a curve expressing one changepoint. We identified the position of these changepoints by piecewise regression and found a mean changepoints around -121 mm (sd: 49.3). Interestingly, the pattern expressed by the connectivity indicators (links, connectance, connectedness and degree) showed an inverse response in negative and positive interaction network. While the number of positive interactions increased until the changepoints (-121 mm), the number of negative interaction decreased. This indicates most likely less competition.

We computed a generalized topological index (GTI), defined as the score of the network along the first component of a PCA computed from topology metrics. The GTI shows a changepoint at -109 mm on the global interaction network, which is consistent with the changepoints computed from individual topological indices. We retrieved similar results from positive and negative interaction networks.

4.3.3 Keystone species and average keystoneness per feeding group and trophic level

We used GKI to identify keystone species along the water level gradient (Table 4.1). The GKI express the overall centrality of a node at all the levels of the network (local, meso, or global). We then computed the average keystoneness of feeding group or trophic level (Figure 4.7). Changepoints detected via piecewise regression are consistent with those inferred by TITAN, topology indices, and diversity indices. Indeed, the regression showed average changepoints at -100.3 (sd: 42.4) or -113.8 (sd: 13.6) with respectively feeding groups and trophic levels. The graph of the feeding group keystoneness along the WTD gradient is not shown in this chapter.

Four of the 24 identified keystone organisms are ectomycorrhizes or fungi, but do not belong to the same taxa depending on the WTD. The sliding window procedure along with spatially separated mesocosms ensure that the presence of ectomycorrhize as keystone species is not due to mesocosms or sample contamination.

[1] In a dynamic network, an edge spell is the apparition of an edge in the network.

Table 4.1: Best keystone organisms indeintified per network along the WTD. Four of the 24 identified keystone organisms are ectomycorrhizes or fungi, but do not belong to the same taxa depending on the WTD.

WTD	OTU	GKI	Closest assignation (BLAST)	Trophic mode
-213.6	80_226_3295	6.9	*Lactarius* sp	ectomycorrhize
-210.3	80_226_3295	6.7	*Lactarius* sp	ectomycorrhize
-209.0	142_359_1538	6.9	*Clavulina*	ectomycorrhize
-207.8	142_359_1538	7.7	*Clavulina*	ectomycorrhize
-206.6	93_258_2785	8.8	*Chrysophyceae*	bacterivorous
-205.2	38_90_12085	7.5	*Monhysteridae* (nematode)	predator
-203.9	60_178_4858	9.5	*Prismatolaimus* (nematode)	feed on protists and bacteria
-201.9	20_26_22768	8.1	*Plectus* sp. (nematode)	bacterivorous
-199.3	31_56_15507	7.4	*Chrysophyceae*	bacterivorous
-196.8	56_158_5654	10.2	*Chrysophyceae*	mixotrophic or bacterivorous (?)
-194.2	56_158_5654	7.6	*Chrysophyceae*	mixotrophic or bacterivorous (?)
-191.3	56_158_5654	9.2	*Chrysophyceae*	mixotrophic or bacterivorous (?)
-188.3	56_158_5654	9.5	*Chrysophyceae*	mixotrophic or bacterivorous (?)
-184.5	77_219_3404	7.7	Glissomonad	bacterivorous
-178.7	77_219_3404	10.1	Glissomonad	bacterivorous
-171.4	56_158_5654	7.4	*Chrysophyceae*	mixotrophic or bacterivorous (?)
-159.4	71_209_3773	8.5	*Trebouxiophyceae*	phototrophic
-147.9	47_127_7943	6.0	*Cladosporium*	saprotrophic or parasitic (plants)
-136.0	52_145_6374	6.7	*Knufia*	saprotrophic and parasitic (plants)
-124.1	71_209_3773	6.5	*Trebouxiophyceae*	phototrophic
-112.4	66_191_4266	6.6	**unidentified**	**unknown**
-101.1	31_56_15507	6.0	*Chrysophyceae*	bacterivorous
-89.3	85_240_3145	8.5	*Galerina*	ectomycorrhize
-77.4	85_240_3145	8.0	*Galerina*	ectomycorrhize
-67.4	85_240_3145	6.0	*Galerina*	ectomycorrhize
-58.9	85_240_3145	5.9	*Galerina*	ectomycorrhize
-54.4	142_359_1538	5.8	*Clavulina*	ectomycorrhize
-49.9	64_185_4684	5.6	*Arcellinida*	feed on bacteria + small protists
-46.4	14_16_34490	5.9	*Geocentrophora*	predator
-40.8	46_123_8102	6.1	*Lepidocyrtus*	bacteria + fungi + algae
-31.9	82_231_3258	8.0	*Apatococcus*	phototrophic
-22.5	142_359_1538	6.6	*Clavulina*	ectomycorrhize
-11.5	142_359_1538	8.1	*Clavulina*	ectomycorrhize
-0.6	142_359_1538	8.1	*Clavulina*	ectomycorrhize
12.1	64_185_4684	8.1	*Arcellinida*	feed on bacteria + small protists

Between -150 mm and -100 mm, the keystoneness of the phagotrophs and of the saprotrophs decreased (graph not shown). At the trophic level, this is illustrated by a decrease of the keystoneness of primary producers (autotrophs) associated with an decrease of the keystoneness of the mixotrophs and heterotrophs Figure 4.7. The decrease of the mixotrophs keystoneness precedes the decrease of predators (heterotrophs) keystoneness. Interestingly, the keystoneness of bacterivorous organisms is

relatively stable and neutral accross the water level gradient. In wet condition, with standing water, keystoneness of plant feeding and omnivorous organism increased, supported by an increase of the keystoneness of level 3 and 4 organisms. At -100 mm, the keystoneness of the heterotrophs becomes higher than the keystoneness of the autotrophs (Figure 4.7), showing that the food resource for grazing and predation is probably higher when the water level is high.

4.3.4 Changepoints — synthesis

To estimate the variance explained by water level on network properties, we used 3rd order polynomial models ($F = \varepsilon + ax + bx^2 + cx^3$, where x is the water level and F the response matrix or variable). We used a PERMANOVA (adonis function of vegan package) [Oksanen 2015] for diversity indices, topological metrics, trophic level and feeding group keystoneness, and linear regression followed by ANOVA for GTI. The results are given in Table 4.2, along with changepoints. Overall, water level explains 42% of the diversity changes, but only 8 to 13% of the network topology, hence suggesting more complex interaction patterns, with potential feedback and indirect niche modifications.

Table 4.2: Detected changepoints by different indices and variance explained by water level on treatment (diversity or network properties, or node properties). NS indicate that the coefficient is not significant. Overall, water level explains 42% of the diversity changes, but only 8 to 13% of the network topology, hence suggesting more complex interaction patterns, with potential feedback and indirect niche modifications

				R^2 ($ax + bx^2 + cx^3$)		
	mean changepoint (mm)	SD	total R^2	a	b	c
Communities (TITAN)	-101.4	-	-	-	-	-
diversity indices	-117.9	61.4	0.44	0.44	NS	NS
topological indices	-121.6	49.3	0.132	0.078	0.017	0.037
GTI (polynomial regression + varpart)	-109.6	-	0.049	0.035	0.014	NS
feeding group keystoneness	-101.0	42.4	0.048	0.039	0.003	0.006
trophic level keystoneness	-114.4	13.6	0.084	0.069	NS	0.015

We chose the 21st generated global network (Figure 4.8), which correspond to -112.4 mm, as a network representative of a changepoint. The most keystone organism of this graph was an unidentified fungi, with 11 edges (8 interactions with heterotrophic organisms, 2 with autotrophs, and 1 with a mixotroph) and a GKI of 5.62.

4.3.5 Type of interactions

We characterized the type of interactions along the gradient and built a global network covering the whole WTD gradient. In this network, the weight of the edges are their conservation ratio throughout the gradient. Therefore, the centrality indices for the nodes composing this network take the weight

of the links into account. The most keystone organism of this network is *Apatococcus sp.*, a green phototrophic alga. This results suggest that this alga is basal and crucial in the community stability. Among the ten most keystone organisms of this network, three are autotrophic (*Apatococcus*, and two unidentified *Trebouxiophyceae*). The seven others are six fungi and one nematode.

The type of the links does not vary along the gradient (Figure 4.9). Nevertheless, high water level seems to promote heterotroph–heterotroph interaction and limit autotroph–heterotroph and mixotroph–heterotroph interaction. Autotroph–mixotroph interaction reaches an optimum when WTD is around -100 mm and at the dry end of the gradient.

4.4 Discussion

In this chapter, we used co-occurrence networks to investigate microeukaryotic community structure evolution along an environmental gradient. We tried to detect ecological threshold along this gradient by investigating changepoints of network topological indicators and nodes centrality indices.

Our understanding of the ecological informations carried by co-occurence network is still poor, although a sound framework emerged recently [Faust 2012] and the structure of microbial communities co-occurence networks has been shown to be correlated to ecosystem functioning, especially C cycling [Guidi 2016]. In our study, we aimed to study the topological changes of microbial communities co-occurence network along an ecological gradient. investigated the response of network structure along a WTD gradient in en experimental setting designed to study the reponse of *Sphagnum* peatland to climate change.

Our study brought to light a new methodological approach for co-occurence network use in ecology, that is to compute networks with a sliding window accross ane environmental gradient. We showed that it is possible to detect ecological changepoint — *i.e.* the value of an environmental variable (gradient) from which an ecosystem transitions from one state to another — using topology descriptors of a network. We used a recently created but yet still poorly used centrality index, the GKI [Estrada 2007], to infer topological keystone species. Indeed, unlike food webs, co-occurence networks do not represent direct trophic interaction but a broader set of interaction whose type is unknown. Hence we have to rely solely on the network topology to assess the relative importance of a species within this network. The GKI allows estimating the relative importance of a species at all the scale of the network (from its centrality amongst its closest neighbors to its centrality within the whole network).

4.4.1 Detecting changepoint using co-occurrence network

Overall, water level had a strong effect on the interaction network topology, with a GTI showing a changepoint around -10 cm. Especially, the effect of water level of trophic group centrality was nonlinear and showed an optimum around -10 cm, marked by an increase of the keystoneness of

autotrophs. This confirms Mitchell and al. (2003) hypothesis stating that water level is a key factor for autotrophic microbial assimilation in *Sphagnum* by defining the thickness of the water film on the *Sphagnum* mosses [Mitchell 2003]. It appears that this changepoint, -10 cm, is also the point from which the community starts to shift.

Detected changepoints are remarkably consistent, regardless of the approach used to infer them. Overall, the mean changepoints computed from trophic level keystoneness showed the lowest standard deviation, confirming the importance of trophic interaction in peatland functioning. Compared to classical threshold detection methods [Andersen 2009, Francesco Ficetola 2009], threshold inference using trophic groups gives an insightful information about the ecological dynamic of the studied gradient.

For instance, at the trophic level, changepoint is illustrated by an increase of the keystoneness of the primary producers associated with a decrease of the keystoneness of the mixotrophs and top predators. However, some of the most involved primary producers, *Chlorella*, are the photosynthetic symbiont of several mixotrophic testate amoebae [Gomaa 2014], which were not detected by our bioinformatics pipeline, and are nonetheless known to play a significant role in C cycling in peatland [Jassey 2015].

This emphasizes the necessity of improving the sequencing and barcoding effort of microeucaryotes [Adl 2007, Guillou 2013]. Indeed, many species known for inhabiting peatlands and found in our samples by optical microscopy do not have matching data base entries. Ongoing projects are aiming to overcome this challenge, such as Eukref (http://eukref.org/) and soon to be UniEuk, that was notably presented in ECOP Sevilla (September 2015). This effort is necessary to unveil the exhaustive picture of the microbial interactions occurring in a given habitat, which is made possible by HTS technology.

4.4.2 Challenges

This study points out a need for a unified framework to investigate and understand co-occurrence networks in ecology. Even if a sound background has recently been drawn [Faust 2012, Berry 2014, Williams 2014], rigorous pipelines allowing comparisons among studies have yet to be developed. Computers power allows going beyond classical diversity and community structure indices, and understanding not only community composition but also community interaction dynamic along environmental gradient. However, we still lack a unified framework to routinely conduct these studies, similarly to what is done with α and β diversity analyses. Besides, although it is clear that the structure of the network relates the ecosystem state, the way the signal is embedded into the network topology is not clear [Gray 2014]. It is necessary to understand how the network topology reflect underlying ecological processes.

Tools to investigate topological changes of a network along a gradient are also lacking. Although intuitively a network evolving along a gradient is mathematically very similar to a network evolving

through time, this is not the case. In a time evolving network (a dynamic network) the state of a network at a time t is dependent of the state of the network at $t-1$, and the dynamics of such networks can thus be modelled by differentials equations. This is not the case in gradient dependent network where the state of a network on each state of the gradient is independent. As the topological change of a network due to an environmental change is time dependent, modeling this evolution in respect to both time and environmental parameters is a key to understanding environmental filtering. We proposed here to classify the OTU according to their trophic position, but one further step would be to classify the OTU according to their function. Indeed, structural changes in the interaction network are likely to be a consequence of a functional response of the community.

4.4.3 Deciphering trophic group contributions

Although this study is well-advanced in its methodology, more work need to be achieved to decipher the role and contribution of each trophic group. We worked with the 97 most abundant OTU to validate the pipeline, but we aim to expand our study by taking into acount more than 1000 OTU. We estimated that computing the network on a regular office computer would take approximatively two to three weeks, if possible at all, given the tremendous amount of memory required to perform the millions of permutations to generate the data. Nevertheless, including more OTU is required to get a finer signal on the contribution of each trophic group within the microbial food web. This ongoing work is arduous as all the OTU have to be manually assigned to a trophic group, and the broad range of organisms covers by HTS requires experts of differents taxa to be consulted. Here again, integrative databases like UniEuk seem to be the key to bridge ecology and bioinformatics.

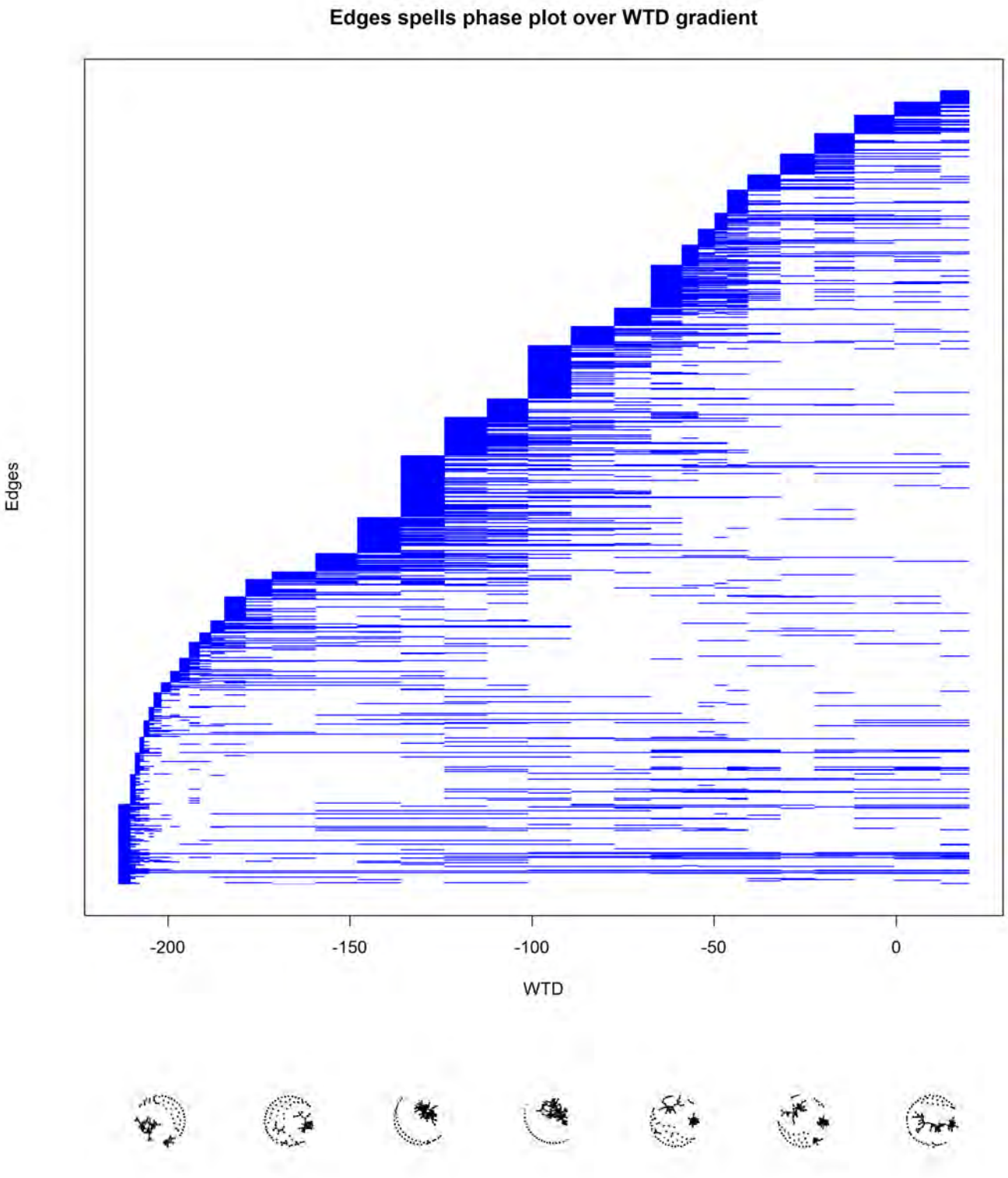

Figure 4.5: Network structure and edge spells. **top:** Phase plot of the network. Each line corresponds to a different edge spell along the WTD gradient. Many edges are spelled between -130 mm and -90 mm, resulting in a dense network around -100 mm. The total number of edges along the WTD gradient is expressed as the variable *"Link"* in Figure 4.6. **bottom:** general structure of the network along the WTD

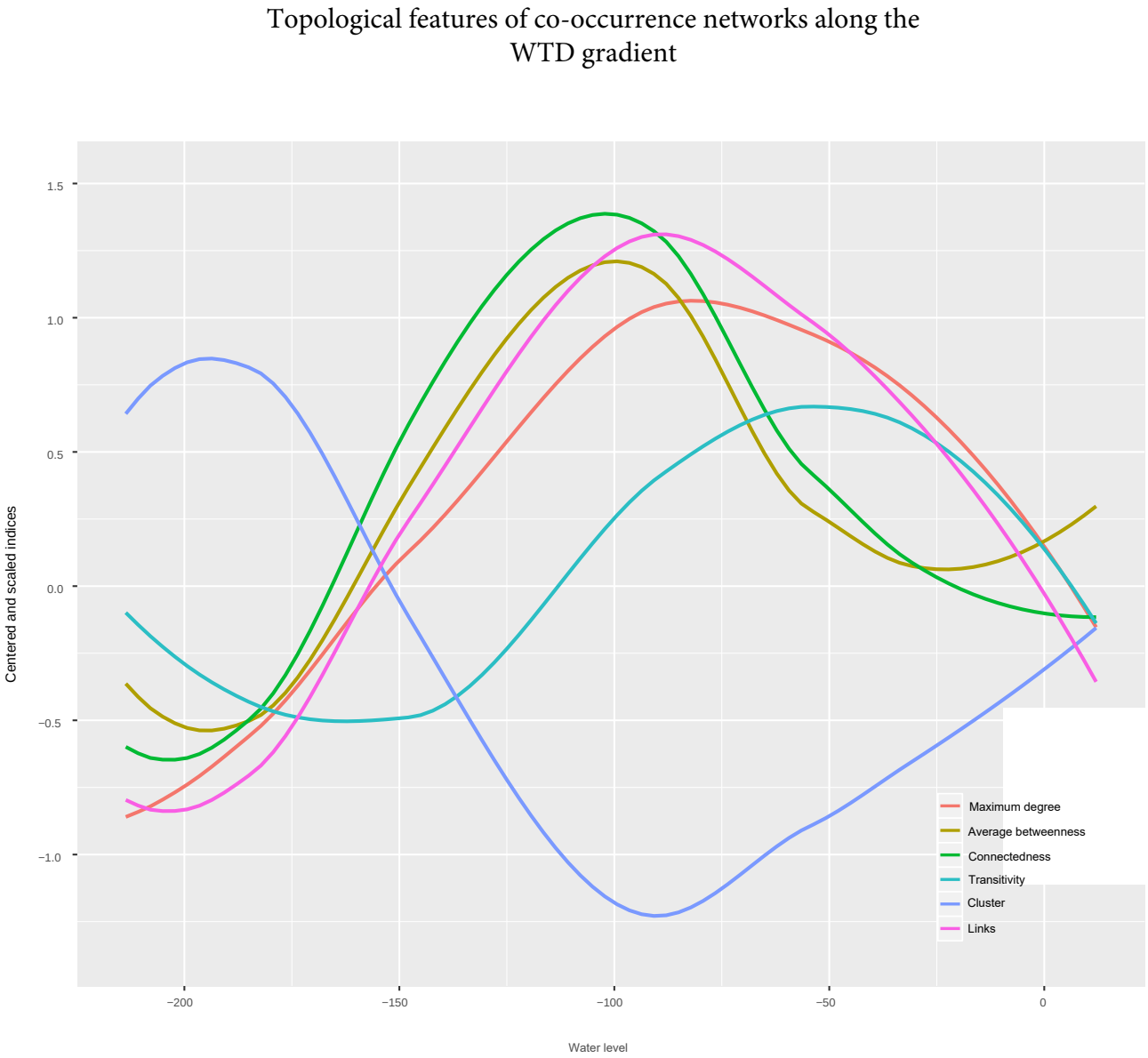

Figure 4.6: Co-occurrence network topology evolution along WTD gradient. A clear changepoint can be observed around -100 mm, marked overall by a higher connectivity. Maximum degree is the number of links of the most connected node. Betweeness is the overall centrality of each node. Connectedness is the ration btween realized links and possible links. Transitivity indicates how much the graph is to being complete. Cluster indicate the number of topological modules in the network. Link indicates the number of links in the network.

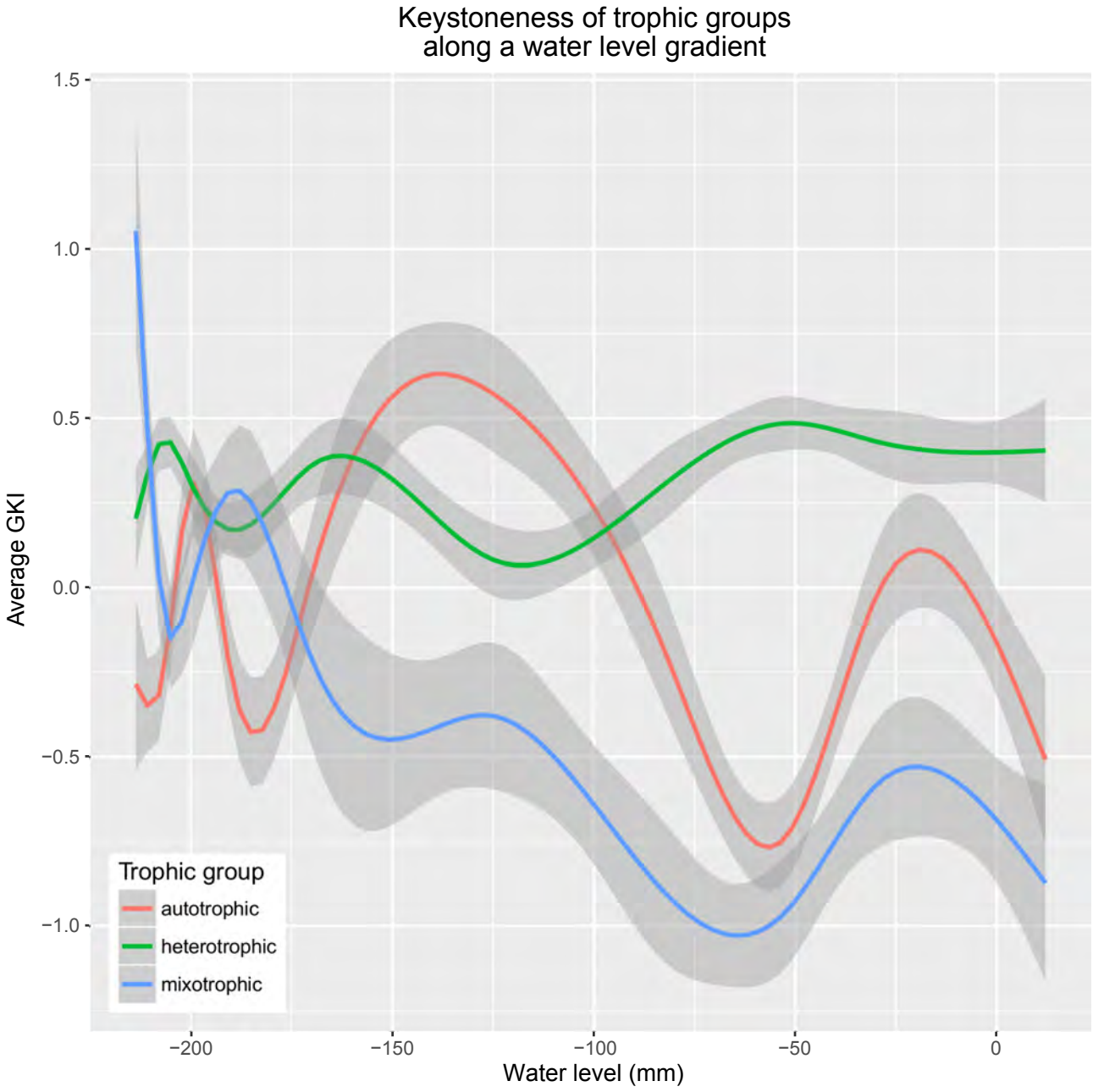

Figure 4.7: Average keystoneness of trophic groups along WTD. Between -150 mm and -100 mm, the keystoneness of the phagotrophs and of the saprotrophs decreased (graph not shown). At the trophic level, this is illustrated by a decrease of the keystoneness of primary producers (autotrophs) associated with an decrease of the keystoneness of the mixotrophs and heterotrophs Figure 4.7. The decrease of the mixotrophs keystoneness precedes the decrease of predators (heterotrophs) keystoneness. Interestingly, the keystoneness of bacterivorous organisms is relatively stable and neutral accross the water level gradient

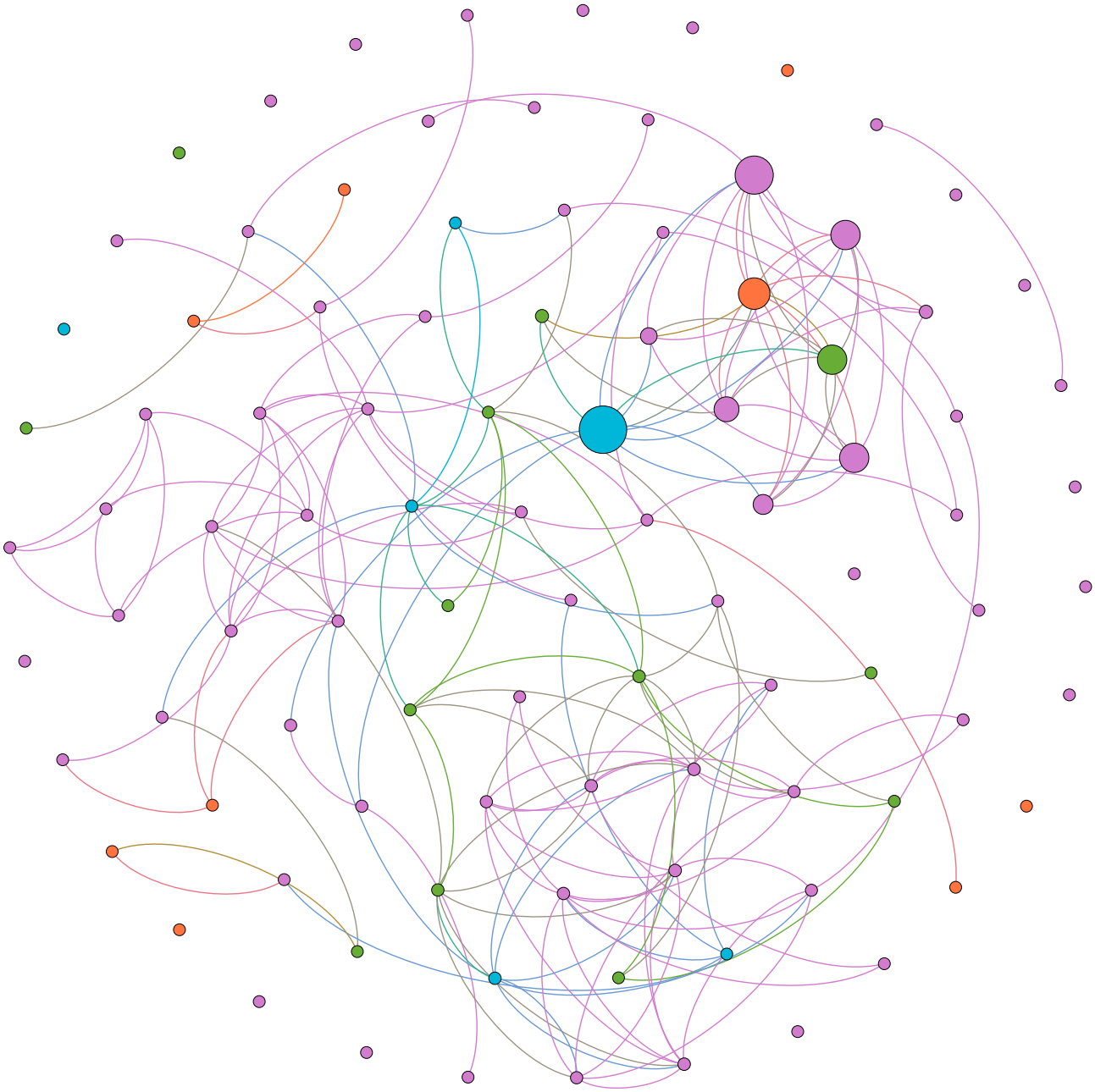

Figure 4.8: Global network at the tipping point. Labels are masked on purpose. Colors represent the trophic group, with blue = unkown, green = autotrophy, orange = mixotrophy, purple = heterotrophy. Size is proportionnal to the keystoneness.

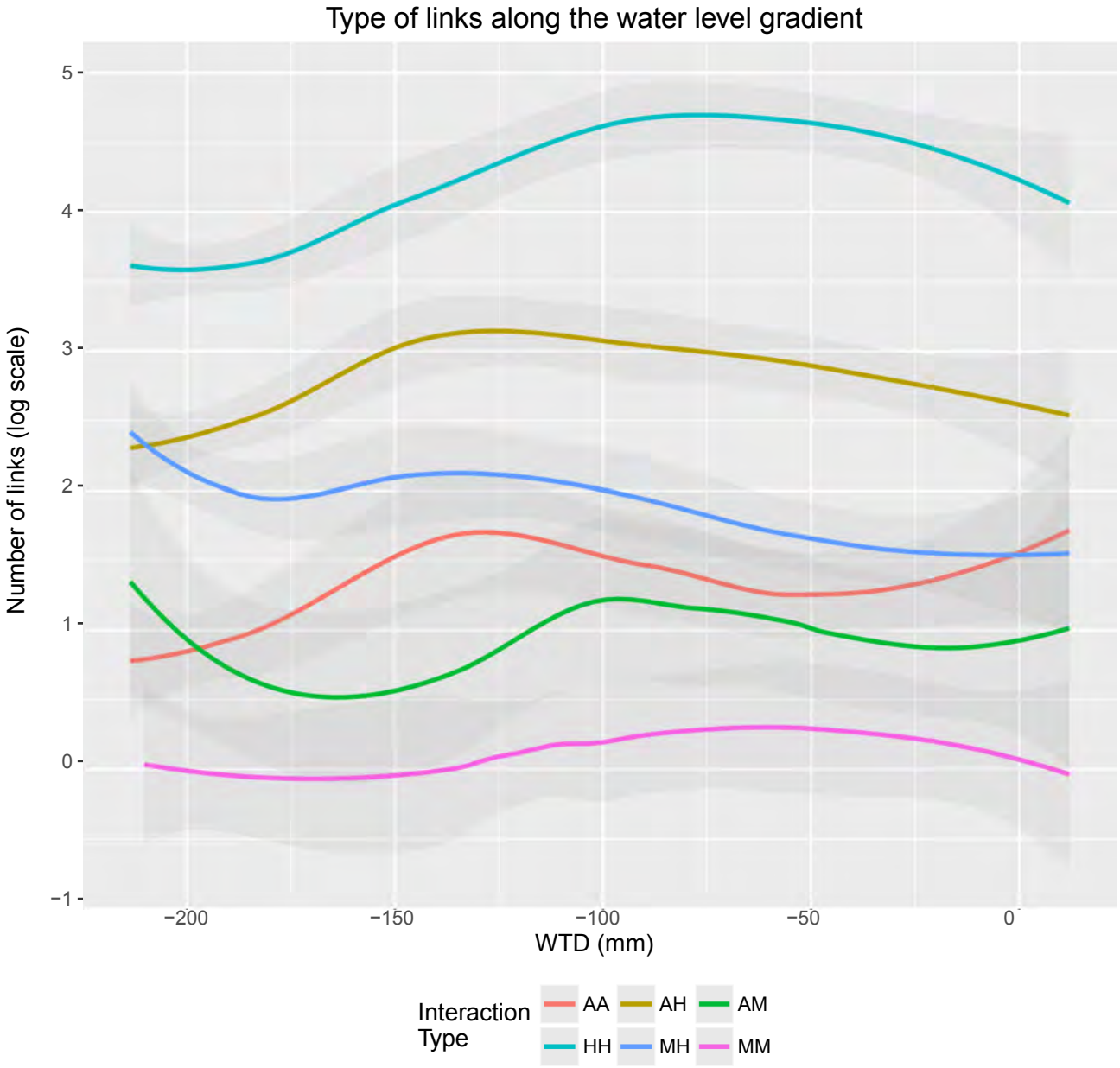

Figure 4.9: Typology of the interaction along the WTD gradient. **A**: autotroph, **H**: heterotroph, **M**: mixotroph (thus **AA** = autotroph – autotroph, and so on...). Curves are local polynomial regressions

CHAPTER 5

Functional diversity of peatland testate amoebae: finding relevant traits to assess the response of communities to ecological stress

5.1 Introduction

Ecosystems are at the crossroads of various interests, either political, ecological, or economical. With climate changes, population growth, resources availability, and economic development, their resistance or resilience to stresses and disturbances are of importance for human sustainability. Interest in measuring their responses to environmental changes started to be taken into account at the political level in the 1990's, by calculating a monetary value of the services they provide to human welfare [Costanza 1997]. The perfect example is the recent political awareness about bee loss. While ecologist are focused on how it will impact the ecosystems, politics understand more easily that "if bees and other pollinators were to disappear completely, the cost to the UK economy could be up to £440 million per year"[1].

If the first step was to forge a broad consensus on preserving ecosystems and bringing to light the importance of biodiversity, finding and testing relevant proxies to monitor the evolution of ecosystems still need further researches [Bartkowski 2015]. In their review, Bartkowski et al. (2015) defined the characteristics of good ecosystem indicators. First, they should take into account several aspects of biodiversity further than species richness solely (e.g. functional diversity), and describe the structure and assemblage of this diversity. Second, the relation between biodiversity proxies and human wellbeing (provided through ecosystem services) should be clear. One way to satisfy both criteria is to use a multiple-proxy approach to estimate the complexity of biodiversity structure and related ecosystemic services, by combining habitat measurement, species based indices (Shannon, Simpson), functional diversity, etc. [Bartkowski 2015]

Functional diversity (FD) emerged at the very beginning of the 21st century as a tool to model ecosystem functioning. It is based on the relationship between environmental constraints and the community structure, through functional traits [Keddy 1992, Woodward 1991]. It is designed to assess the impact of global changes (biotic - effect traits - and abiotic- response traits) on both community structure and ecosystem functioning [Chapin 2000]. Lavorel and Garnier conceptualized a global

[1] BBC: *Loss of bees could be 'a blow to UK economy'*, http://www.bbc.com/news/10371300

framework with these approaches by integrating analyses of response traits (relating to environmental constraint) and analyses of functional effects of species on ecosystem functioning [Lavorel 2002]. The authors highlighted that species traits could simultaneously explain the effects of changes on community structure and the feedback from species presence on ecosystem functioning, by differentiating effect traits (traits that structure the niche) and response traits (traits that are a response to environmental conditions).

To be relevant for functional diversity, traits selected for FD analysis should be chosen in order to be related to niche characteristics, either as response traits or as effect traits [Mlambo 2014, Violle 2007]. According to Mlambo (2014), selected traits for functional diversity have to be related directly or indirectly to ecosystem processes. Up to now, the FD approach has been used to study plant or fish communities assemblages [Gravel 2016], and the traits used and validated in the literature are specific to plant ecology [Cornwell 2008, Lavorel 2002] or fishes [Villéger 2010, Toussaint 2016]. There is thus a need to develop trait-based approaches suited for our system, that is micro-eukaryotic communities assemblage in *Sphagnum*-dominated peatland.

Sphagnum-dominated peatlands are now well recognized in terms of ecosystem services [Bonn 2016]. In these systems, the low decomposition rate driven by water-logged soils induces a carbon (C) sink function. Henceforth, peatlands are valuable pools of sequestered C accumulated over the last millenniums, and understanding their response to predicting potential feedbacks on the global C cycle becomes crucial [Belyea 2004, Rydin 2006, Yu 2006]. Indeed, northern hemisphere peatlands contain about 1/3 of the world's soil C stock in an area accounting for only 3–5 % (around 400 million ha) of the global land surface [Frokling 2007, Turunen 2002]. The ecosystem services provided by peatlands is thus estimated around 20,000 USD.ha^{-1}.year^{-1}, *i.e.* 12 trillion USD.year^{-1} for the worldwide peatland [Costanza 1997, Batzer 2014]. In addition to direct human impacts (harvesting, drainage, etc.), peatlands are exposed to indirect human impact such as climate change, which will affect their functioning, especially the C sink function. *Sphagnum*-dominated peatlands, primarily situated in Boreal and Subarctic regions, are expected to experience large climate changes in the coming century, making the identification and quantification of potential feedbacks from these high-latitude ecosystems essential for future climate projections [Pachauri 2015]. As shifts in vegetation are slow, other taxa such as testate amoebae are being considered as early indicators of ecosystem dynamics and functioning [Buttler 1996, Laggoun-Défarge 2008]. Furthermore, it has been shown that TA are pontentially better indicators of micro-environmental gradient than vegetation [Koenig 2015].

Testate amoebae (TA) are a polyphyletic group of protists building a shell (called test). Their decay-resistant shell, well preserved in soil and aquatic sediments after the organisms death, allows inferring past TA communities for paleoenvironmental reconstruction [Chambers 2012, Talbot 2010]. Moreover, a recent study showed that TA traits can also be used instead of TA community composition for paleoenvironmental reconstruction [Fournier 2015a]. TA are known to be well correlated with some environmental gradients like soil moisture, water table depth, pH, or nutrients content [Booth 2005, Jassey 2011b, Swindles 2009]. Nonetheless, recent studies suggest that even the

well-known species are in fact complex of different species with different ecological preferences [Kosakyan 2013, Singer 2015].

Although testate amoebae are a well-studied group in peatlands, and seem to be good proxies for environmental disturbances (especially WTD and pH), they show some limitations. The informative power of TA relies heavily (on theory) on the accuracy of their identification. Given that identification is often made by graduate or undergraduate students who are still learning, we can assume that a significant yet unknown fraction of the TA used in ecological studies are misidentified. Although it seems that this taxonomic inconsistency has few effect on our perception of the ecological signal of the communities [Payne 2011], recent findings have shown that wrongly identified specimen could be ecologically misleading, especially if morphologically similar species have different ecological preferences [Singer 2015]. Indeed, it has been demonstrated that TA exhibit pseudo-cryptic diversity, that is species that differ only by small morphological details but are genetically distinct and have different ecological preferences [Singer 2015]. It is therefore necessary to develop tools conserving the ecological informative power of TA, while setting free from potential identification biases. Ideally, those tools should be more ecologically relevant than TA α and β diversity indices to relate to ecosystem functioning.

The main driver of peatland functioning is the high water level that induces anoxic conditions and thus low decomposition rates, leading to the peatland C sink function [Belyea 2004, Rydin 2006]. Micro-eukaryotic communities are one of the major contributor to this peatland C sink function, especially mixotrophic TA that often account for $> 70\%$of the peatland microbial biomass [Jassey 2013b, Jassey 2015]. These organisms have been shown to be sensitive to water table variation [Amesbury 2012, Jassey 2011b, Marcisz 2014a, Booth 2005, Swindles 2009, Mieczan 2009]. However, the determinants of TA community assemblage through environmental filtering are still unclear. The mechanistic approach to community assemblage states that community membership is constrained to those species with the appropriate functional traits to reach a site (*i.e.*, overcome a dispersal filter) and establish under the circumstances set by the environment and other organisms (i.e., the abiotic and biotic filters) [Belyea 1999, Lebrija-Trejos 2010]. Therefore, there is a need to understand the functional responses of TA to water level induced environmental filtering to better assess the effect of water level on peatland functioning, in a modeling perspective. It is especially important to understand the dynamic processes involved in this environmental filtering.

To assess the response of microbial communities to changes in water level through time, we designed a mesocosm experiment [Mulot 2015]. We artificially manipulated the water table and we monitored the response of TA communities with a dual approach. We monitored the changes in community structure both through the succession of species and through the variation in trait community structure [Ackerly 2007]. Our hypothesis was that community weighted mean of traits (CWM: the abundance-weighted mean of the species trait values within a community) and TA community structure would exhibit a comparable signal to water level manipulation, while the trend shown by

CWM would be more ecologically informative than community structure to understand ecosystem functioning.

5.2 Material and methods

5.2.1 Experimental design

We designed a mesocosm experiment to assess the effects of water table depth on the TA communities of *Sphagnum fallax* through time. A mesocosm was made of a tank filled with water. In each tank was a pierced PVC tube (45 cm high, 12 cm of diameter) containing a peat core on top of which lied a *Sphagnum fallax* layer. The water level in each mesocosm was adjusted to maintain three average water table depths (AWTD: -4 cm, -15 cm, and -25 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 simultaneously on the same *Sphagnum* patch, in the Creux de l'Épral peatland, Canton Jura (47°12'18.31"N; 006°56'05.83"E; Alt: 990 m), and were seeded with a water extract from pool, hummock, and lawn to provide the full community potential [Mulot 2015]. Therefore, the communities were identical at T0 for all the mesocosms. The top three centimeters 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), 27/03/2014 (T6). Several environmental variables related to peatland ecology were recorded. Soil and air moisture, soil and air temperature, rainfall, solar radiation were measured hourly, using decagon devices (E5-TM, ECRN-100, PYR, and VP-3 respectively). Water level was recorded twice a day using automatic custom-made piezometers as described in (Mulot et al., 2015).

5.2.2 TA isolation and characterization

Testate amoebae were extracted by sieving and backsieving through mesh filters [Jassey 2011b]. We counted a minimum of 100 individuals per samples, with the exception of some late dry samples where TA density was very low and a total of only 50 individuals could be reached [Payne 2009]. As the diversity in these samples was low, and given that we had five replicates per treatment, having only 50 specimens in a few samples was less critical. Identification was made at the species level, but species were gathered into broad morphological groups to overcome possible identification biases [Heger 2009]. Especially, specimen form the *Nebela tincta* group were gathered as *Nebela tincta s.l.*. *Euglypha* and *Cyclopyxis* were also gathered in a broad group each. Seven samples were discarded because of disturbances in the mesocosm or thechnical problems during sampling or preparation, resulting in 98 final samples.

The selected traits were chosen in order to be related either to a response to environmental changes or to niche characteristics, as recommended in the literature [Lavorel 2002, Mlambo 2014,

Messier 2010]. As little work has previously been undertaken regarding traits on TA, some of them were selected based on expert opinion or on previous ecological studies [Arrieira 2015, Fournier 2012, Fournier 2015a]. We selected the following traits for functional diversity analysis:

1. Phylogenetic group (Amphitremidae, Arcellida, Euglyphida): different phylogenetic groups are related to different stable strategy and different function in the niche [Wanner 2008], especially their contribution in the food web.

2. Mixotrophy (binary: mixotrophic = 1, other =0). Mixotrophy is a key trait in oligotrophic conditions and plays a role in peatland C cycling [Jassey 2015] and was shown to be correlated to water level [Fournier 2015a].

3. Shell composition (protein, silica or agglutinated), which may reflect the availability of different substrates (e.g. mineral particles of adequate size, silica) or the relative cost of building a self-secreted shell by comparison with an agglutinated one [Fournier 2012]

4. Shell shape (cylindrical, discoid, hemispheric, ovoid or pyriform), which gives an information about the TA resistance to drought and thus the ability to remain active in thinner water films and contribute to the food web in dry conditions

5. Aperture position (terminal, ventral, ventral-central), which represents the ability to survive in thin water film , and thus the ability to remain active in thinner water films and contribute to the food web in dry conditions

6. Test compression (binary: 1 = compressed), which also provides information related to survival potential in drier situations and thus potential contribution to the food web in dry conditions

7. Biovolume [Loeuille 2005, Makarieva 2008], which was shown to be related to DWT and bio-climatic variables [Bobrov 1995, Jassey 2013a, Laggoun-Défarge 2008]. They were calculated following Fournier et al. (2012b) in relation with the shape of the shell. The biovolume is related to the metabolic rate and the capacity of the food web to process energy.

8. Shell length of the shell and aperture width (related to prey size and food web functioning, [Gomaa 2014, Jassey 2013a]) .

The morphological dimensions were measured directly at 400× magnification using an inverted IX-81 Olympus microscope, and the Olympus Cell sens dimension software.

5.2.3 Numerical analysis

To compare TA community response to TA trait responses we calculated community based indices and traits based diversity indices . Community based indices were computed using the R package

vegan [Oksanen 2015], while functional diversity indices were calculated using the R package FD [Laliberté 2010, Laliberté 2014].

Community based indices were species richness (number of species per sample), Shannon entropy (uncertainty to predict the species identity of an individual randomly sampled in the sample), community evenness and Fisher's alpha. Fisher's alpha is useful to compare diversity among communities varying in number of individuals because it is independent of sample size. The functional diversity indices were functional richness, functional divergence, functional evenness, and Rao entropy. The Functional richness (FRich) represents the volume of functional space filled by the species community [Mason 2005, Mouillot 2013, Villéger 2008]. It expresses the proportion of available functional space occupied by species. Functional evenness (FEve) represents the distribution of species abundance in niche space, define by the selected traits [Mason 2005, Villéger 2008]. Mason (2005) defined FEve so as to be orthogonal with FRich even if they are both related to the same entities. Functional divergence (FDiv) calculates the degree to which abundance distribution in niche space maximizes divergence in functional characters within the community [Mason 2005, Villéger 2008, Ricotta 2011]. A high degree of FDiv is correlated to high degree of niche differentiation among species in the community [Mouchet 2010]. Rao's quadratic entropy index (RaoQ) incorporate both the relative abundance of species and a measure of pairwise functional differences between species [Botta-Dukát 2005]. It represents the dispersion of the species in the space of selected traits [Laliberté 2010]. This index is not affected by the splitting of a species into two functionally identical species with the same abundance [Gerisch 2012, Mason 2005].

We computed the Community Weighted Mean (CWM) in each samples. The CWM describes changes in traits composition of a community [Ricotta 2011]. To compare the response of community traits through and community composition to water level through time, we computed principal response curve (PRC) analysis based on time and water level [Van den Brink 1999]. PRC is a multivariate tool based on eigenvector ordination that has been shown to be useful for the analysis of time series obtained from biomonitoring programs, detecting trends at a single sampling site, and comparing sampling sites with a reference site [van den Brink 2009].

Analysis were carried out on R statistical software [R Core Team 2014].

5.3 Results

We compared approaches based on species community composition and composition of functional traits. Even if these two approaches are not directly comparable (Mlambo, 2014), we can evaluate the magnitude and the ecological meaning of the signal given by each family of indexes (specific or functional). The communities were similar if not identical between all the plots at T0 (mean Bray-Curtis distance of 0.1, SD=0.05). Overall, we identified 13,959 TA in 98 samples over seven sampling dates spanning from 02/08/2012 (T0), to 27/03/2014 (T6). On average, 142 individuals were identified for each sample. The total diversity was 17 morpho-species. The richest samples

were the dry plots at T4-T5 with a species richness of 10-11. The poorest sample (Intermediate, T5) showed only two species. Overall, *Hyalosphenia papilio* was the most abundant, representing 79% of the total count. The second most abundant group was *Nebela tincta s.l.*, representing only 5.3% of the diversity Table 5.1. Consequently, our study does not cover the full TA diversity but only the most dominant taxa.

Table 5.1: Absolute distribution of morphospecies in the dataset. Communities are dominated by *Hyalosphenia papilio* in wet conditions throughout the whole experiment and during the first months in the dry mesocosms. Overall, *Hyalosphenia papilio* was the most abundant, representing 79% of the total count. To compare, the second most abundant group was *Nebela tincta s.l.*, representing only 5.3% of the diversity

Morpho-species	mean	sd	median	min	max
Amphitrema wrightianum	0.10	0.51	0.00	0.00	4.00
Arcella catinus	5.92	7.83	3.00	0.00	58.00
Archerella flavum	0.81	2.97	0.00	0.00	19.00
Argynnia dentistoma	0.19	0.78	0.00	0.00	6.00
Assulina muscorum	1.31	2.57	0.00	0.00	21.00
Assulina seminulum	0.10	0.39	0.00	0.00	2.00
Centropyxis aculeata	2.05	5.06	1.00	0.00	36.00
Corythion dubium	4.56	21.37	0.00	0.00	146.00
Cyclopyxis eurystomakahli	0.17	0.56	0.00	0.00	4.00
Euglypha compressaciliata	1.80	2.96	1.00	0.00	17.00
Heleopera rosea	2.03	5.74	0.00	0.00	39.00
Hyalosphenia elegans	1.21	2.27	0.00	0.00	13.00
Hyalosphenia papilio	112.39	73.77	101.50	1.00	360.00
Nebela tincta s.l.	7.50	15.30	3.00	0.00	125.00
Phryganella acropodia	1.43	4.60	0.00	0.00	30.00
Physochila griseola	0.62	3.29	0.00	0.00	27.00
Trinema lineare	0.24	1.06	0.00	0.00	8.00

5.3.1 Community structure

Overall, species richness was higher in dry plots than in wet plots, mainly because wet plots were dominated by one species, *Hyalosphenia papilio*, which represented around 80% of the diversity. The high abundance of *Hyalosphenia papilio* caused the communities to be odd in wet plot, as shown by the evenness plot. The evenness was indeed lower in wet mesocosms Figure 5.1. The dry mesocosms exhibited the most drastic change in community structure with an increase of the species richness (from 6 to 9 species) and to an increase in evenness. This increase in evenness was due to the sharp decrease (*ca.* 80%) of *Hyalosphenia papilio* relative abundance from the dry mesocosms (from ca. 90% at T0 to ca. 20% at T6). This effect is also visible in the Fisher's alpha, whose log properties linearize the signal. All the diversity indices showed small variability due to seasonality.

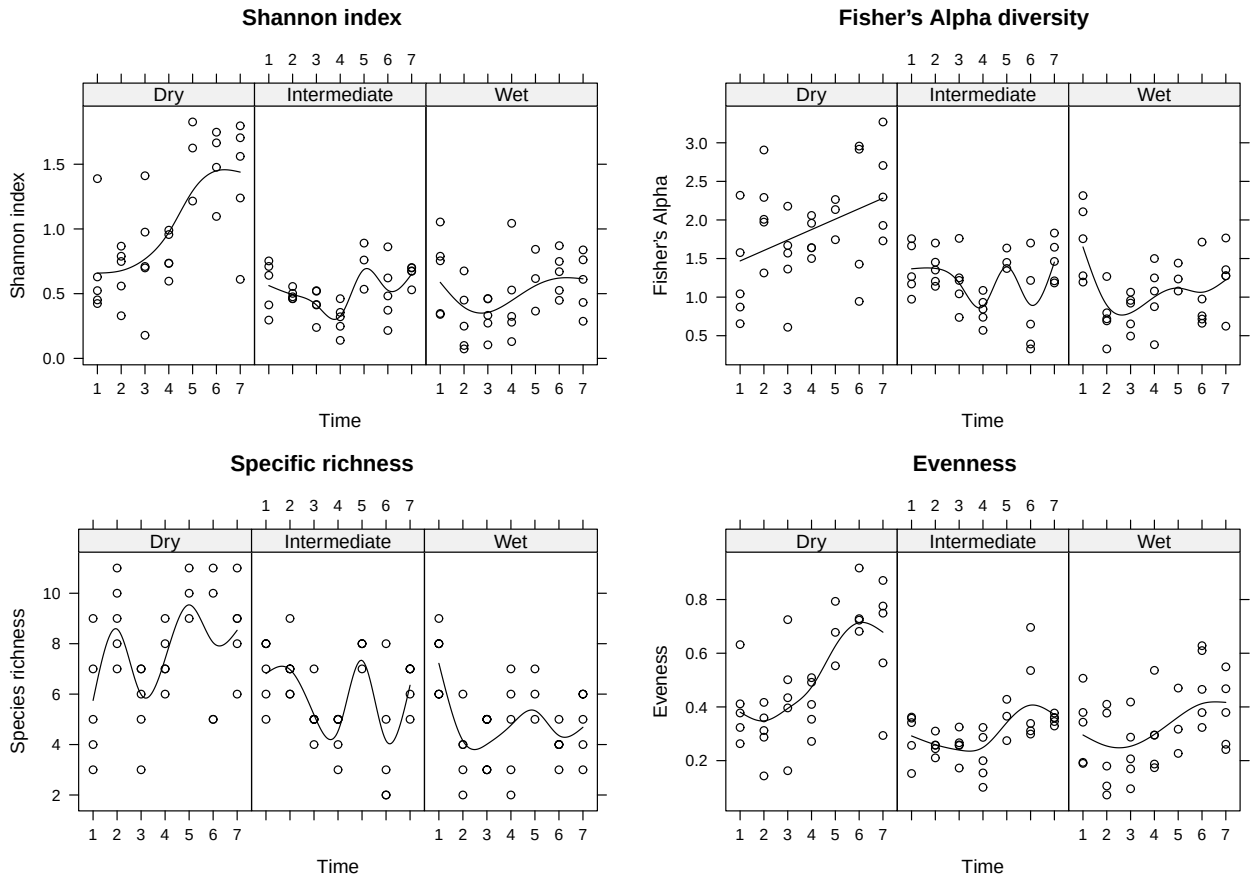

Figure 5.1: Taxonomic diversity indices of TA community in an experimental setup. Wet, Intermediate and Dry refers to water level, respectively -4 cm, -15 cm, and -25 cm from the top of *Sphagnum*. Lines are cubic spline regressions. The dry mesocosms exhibited the most drastic change in community structure with an increase of the species richness (from 6 to 9 species) and to an increase in evenness. This increase in evenness was due to the sharp decrease (*ca.* 80%) of *Hyalosphenia papilio* relative abundance from the dry mesocosms.

5.3.2 Community composition of traits

In our experiment, FRich (Figure 5.2) increased continually in dry plots showing a better use of the available resources by the species Figure 5.2. In intermediate and wet plots the FRich decreased first then increased during the two years of experiment. It could indicate that in winter months the species community did not fill the whole range of niche resources. In Figure 5.2, FEve decreased only slowly in each treatment, showing no clear changes in how regularly species filled the functional space. In contrast the FDiv (Figure 5.2) increased rapidly in dry plot indicating that the niche differentiation had clearly augmented. In intermediate and wet plots, the FDiv varied following seasonal changes in the niche. Interestingly, FDiv was quite stable throughout the duration of the experiment in the Intermediate plot, seasonality aside, showing that the system was overall stable. Generally, dry mesocosms were experiencing the most abrupt shift in the functional response, whatever the index. This denotes a clear and fast modification of the niche induced by the treatment.

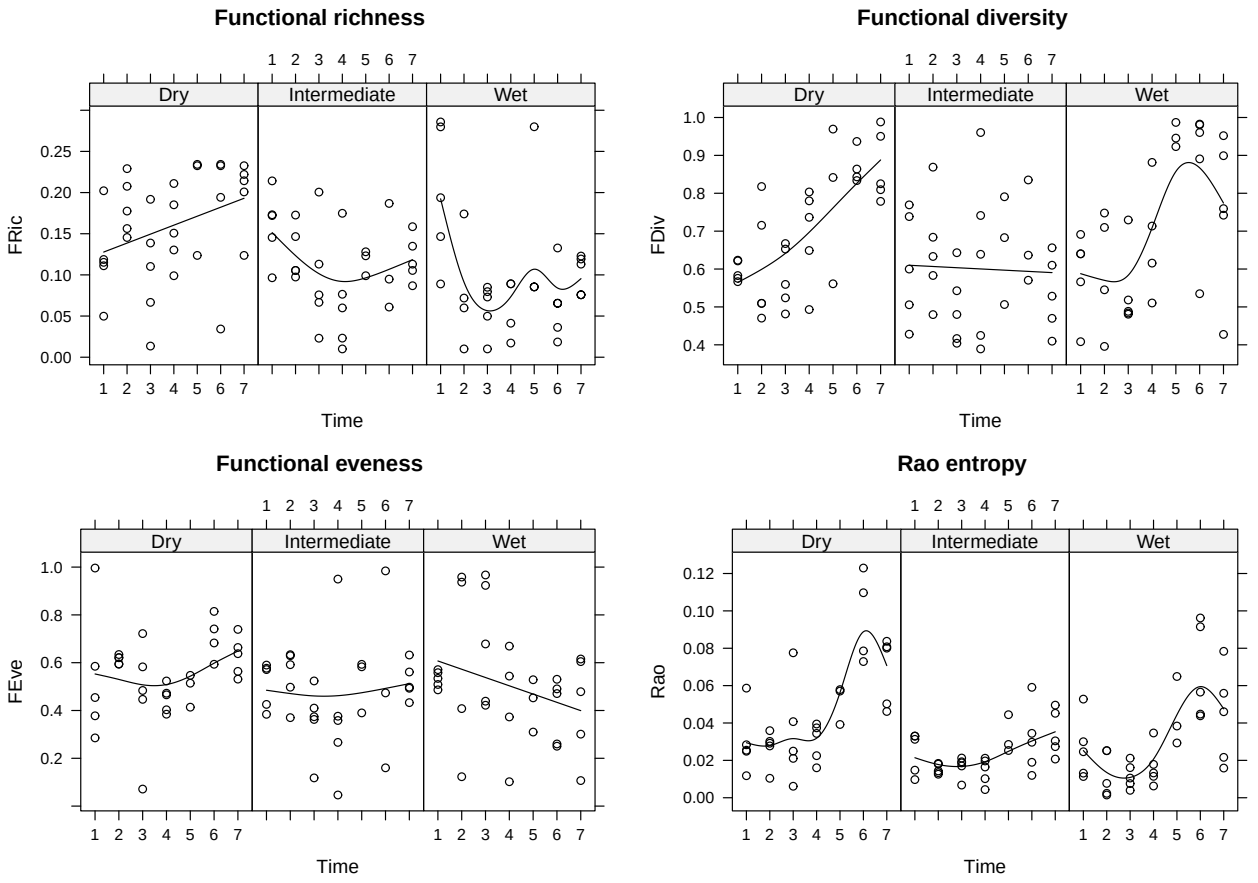

Figure 5.2: Functional diversity indexes per sampling time and treatment. Wet, Intermediate and Dry refers to water level, respectively -4 cm, -15 cm, and -25 cm from the top of *Sphagnum*. Lines are cubic spline regressions. Functional diversity increased rapidly in dry plot indicating that the niche differentiation had clearly augmented. In intermediate and wet plots, the functional diversity varied following seasonal changes in the niche.

5.3.3 Principal response curves

We performed principal response curves (PRC) analyse with either species matrix (Hellinger transformed) or CWM matrix (Gower distance and standardized) as response, and treatment and time as explanatory variables. The two PRCs (on species and CWM) were significant (anova.cca method from Oksanen et al., 2015, $p < 0.01$) and explained approximatively the same amount of variance (ca. 60%).

Both PRCs show that the communities, that were actually similar at the beginning ogf the experiment, started to diverge in response to the new constrained water levels. More specifically, the two PRC show the same pattern, with communities from the dry treatment transitioning immediately, whereas communities from the wet mesocosms oscillated mildly depending on the season, alternatively diverging and converging from and to the intermediate communities. In fact, the wet mesocosms showed a rather constant hydrology, as they were almost saturated permanently, the soil moisture

Table 5.2: Variance explained by Principal Response Curves species matrix (Hellinger transformed) or CWM matrix (Gower distance and standardized) as response and treatment and time as explanatory variables. The two PRCs were significant and explained 61.9% and 60% of the variance, respectively

	variance explained (%)	
	CWM	species
Conditional	18.6	14.2
Constrained	41.4	47.8
Unconstrained	40.0	38.1
Total	100	100

varying from 90% to 100%. The Intermediate mesocosms, however, saw their water content varying depending on the season, under the influence of temperature, solar radiation, and precipitation. Thus, it was actually the Intermediate communities that oscillated. Nevertheless, here, Intermediate treatment was used as a base level to which dry and wet treatment were compared.

The communities' PRC first showed that TA response was in line with the literature. Wet and Intermediate mesocosms were dominated by *Hyalosphenia papilio*, and were later colonized by *Phryganella acropodia*, whose ecological optimum for water level is around -3 cm [Payne 2007]. Conversely, dry plots were rapidly colonized by typically smaller species such as *Nebela tincta s.l.* and *Corythion sp.*, as well as some *Euglypha* species. The functional response showed that dry conditions selected ventral pseudostome, along with small, ovoid, agglutinated or silica tests. These characters were long believed to provide an advantage to cope with hydrological stress. Wet conditions selected larger species, with a protein-built test. More importantly, mixotrophy was strongly selected by the wet treatment.

5.4 Discussion

Our study is the first, to our knowledge, to investigate the dynamics of environmental filtering through time on TA assemblages. In this study we investigated the response of TA communities to constrained 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.

5.4.1 Effect of the treatment

The effect of water level on communities assemblages was stronger and more rapid in the dry plots. The water level constrained in the dry plots, ca. -25 cm, is indeed known to strongly disturb peatland functioning and efforts in peatlands restoration are made to raise the water table above this level [Quinty 2003]. In our system the response to water level was immediate because the structure of the mesocosms didn't buffer water table drawdown as would happen in natural peatland. As a con-

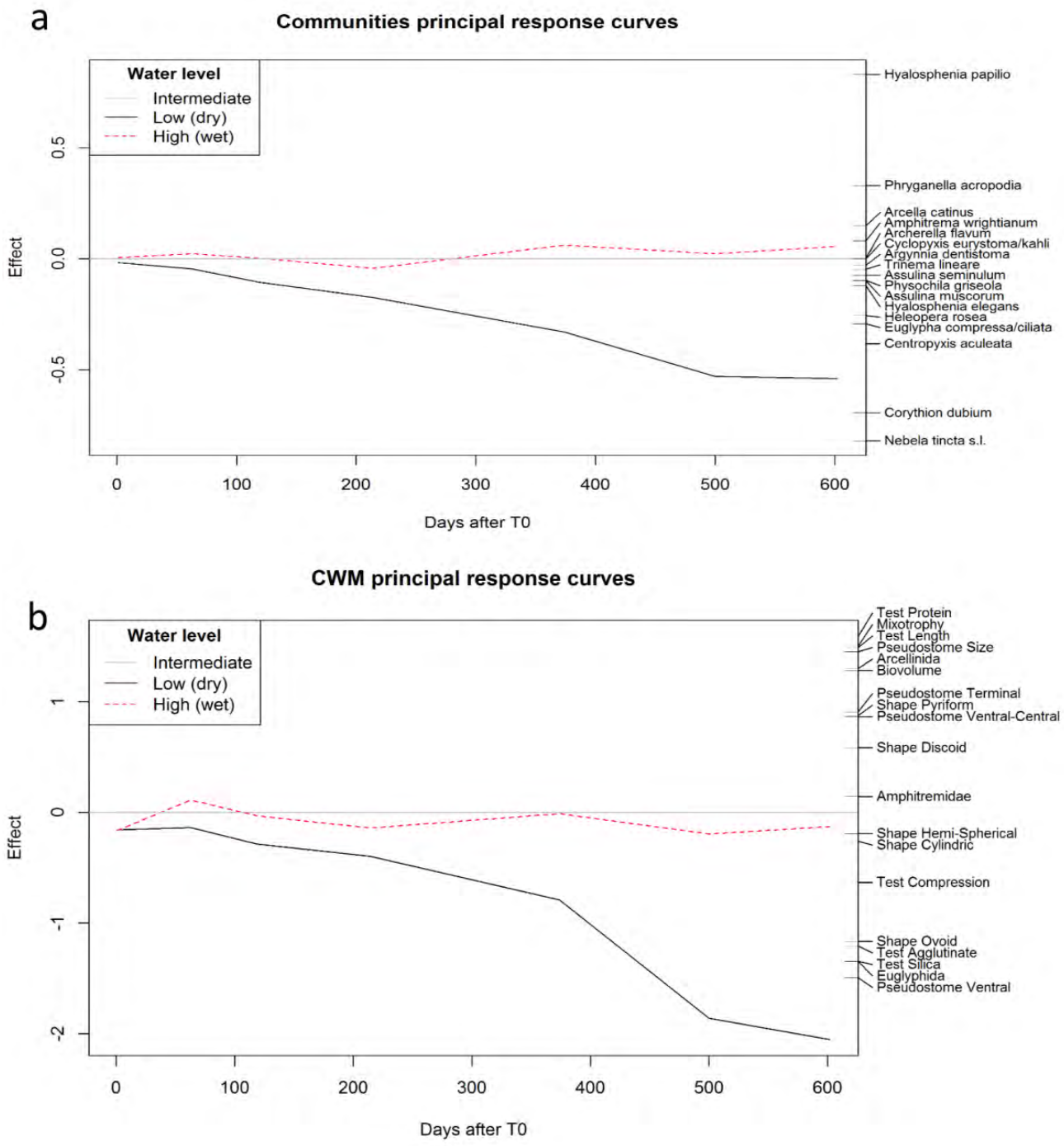

Figure 5.3: Principal Response Curves (PRC) for **(a)** the species matrix (Hellinger transformed), or **(b)** the CWM matrix (Gower distance and standardized) as response, and the treatment (WTD) and time as explanatory variables. Wet, Intermediate and Dry refers to water level, respectively -4 cm, -15 cm, and -25 cm from the top of *Sphagnum*

sequence, microbial communities responded immediately to the water level decrease, but the effect of a similar drainage in natural peatland would probably be slower. PRCs suggest that the effect of the dry treatment reached a plateau after one year and a half, and thus that by then the communities

had adapted to their new constrained environment. This 1.5 year period covers the whole range of extreme climatic events that the peatland is susceptible to undergo, especially summer drought and winter frost that frame the niche in the temperature and moisture dimensions [Aguilar 2012]. As our TA communities are originated from the top 3cm of the *Sphagnum* carpet, they are subjected to climate variability (Figure 5.4).

The shift occurring in the wet and intermediate plots are less marked. The TA communities of these two treatments exhibit a clear oscillating pattern, alternatively converging and diverging from each other. This oscillation can be explained by seasonality. In summer, the elevated temperature, low precipitations, and important solar radiation will significantly impact the *Sphagnum* moisture in the intermediate plots. Such climate would however have significantly weaker effect on the wetness of the wet plots, which are almost permanently flooded. This variability of the intermediate plot wetness can actually occur at high frequency, for instance when a hot sunny day is followed by a stormy night. The *Sphagnum* moisture would in this case range from ca. 20% to ca. 100% in less than 24 hours. Such climatic events would have much less effect on the dry or the wet plots, thus leading to more stable communities. Consequently, species from the intermediate plots have to be able to sustain rapid microclimatic changes. Climatic variability has been shown to be determinant for niche structuring for a broad range of organisms, including vertebrates [Quintero 2013] and some protists [Aguilar 2012], and has been determined crucial for niche structure forecasting in a context of global warming [Jackson 2009]. However, these models have been built for broad climatic variation, taking into account big organisms, such as plant or mammals. In our case, we can probably transpose this model to a smaller scale, as protists are small organisms with shorter life cycles.

5.4.2 Environmental filtering

Our experimental design allowed us to investigate the environmental filtering effect of water table depth on TA assemblages in peatland. As expected, high water level selecte larger species, and to favor mixotrophy. This is not surprising, as mixotrophic TA represent up to 70% of the microbial biomass in peatlands. The mixotrophy is a feeding strategy that allows to overcome oligotrophic conditions that characterize peatlands [Gomaa 2014, Jassey 2013b]. Moreover, mixotrophy in TA has been shown to shape C cycling dynamics in peatlands [Jassey 2015], and was thus expected to be highly favored by high water level.

This mixotrophy selection is associated with protein or secreted tests. Proteinaceous tests are translucent, allowing sunlight to reach photosymbionts. Test secretion is still a poorly known process [Netzel 1983, Nomura 2014] but it is likely that this requires more energy and thus a higher metabolic activity that building agglutinated test. The correlation between mixotrophy and size is hence logical, as biovolume is correlated with metabolism [Makarieva 2008] and the number of mitochondria [Kosakyan 2015]. Biovolume is then positively correlated with water level, leading in finding larger preys, that would explain the selection of TA species with larger pseudosome. The drawback is that

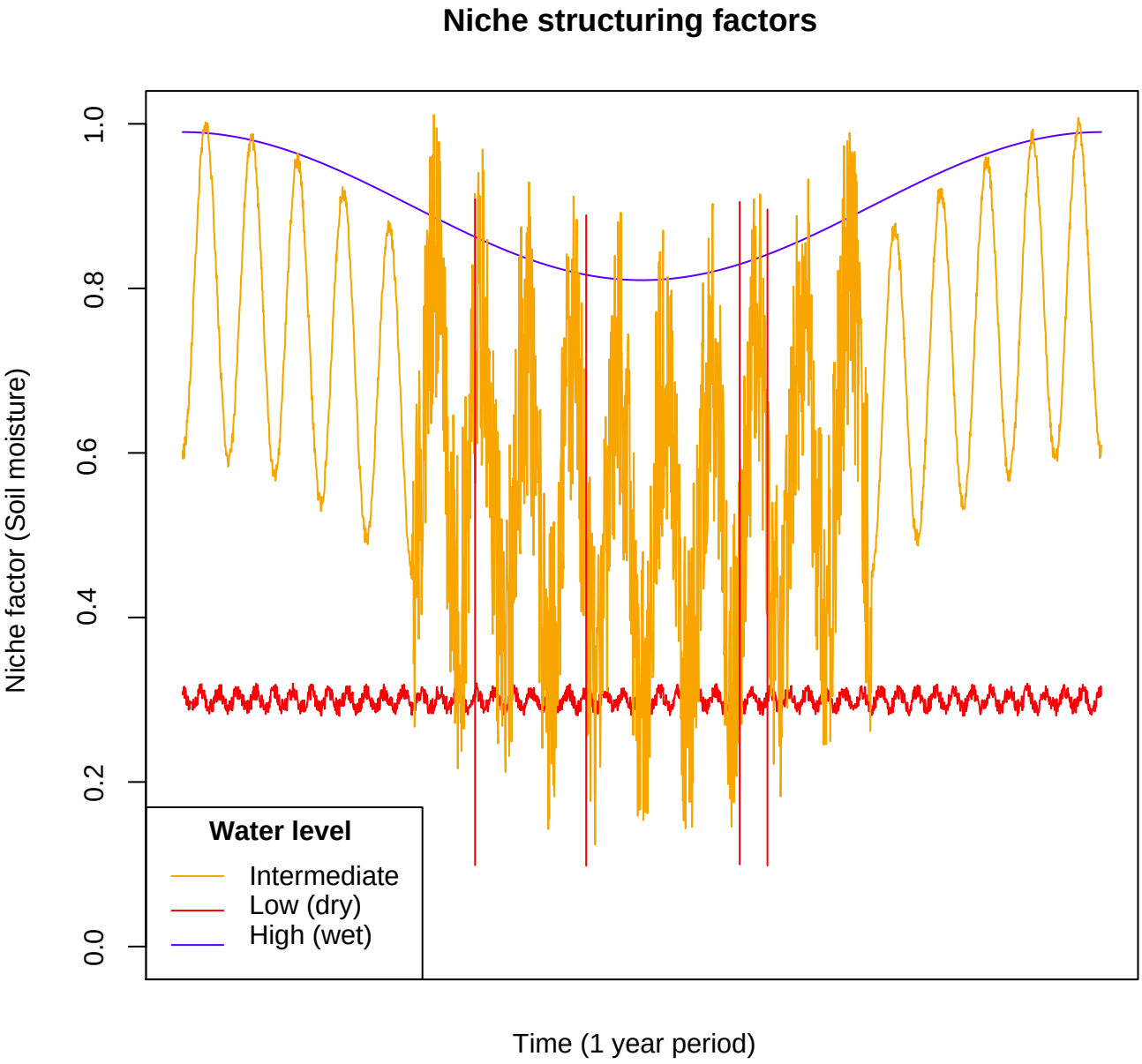

Figure 5.4: Theoretical niche factor. This factor here is moisture supposedly undergone by the top 3 cm of the *Shagnum* stems. High WTD plot are almost permanently flooded and therefore show low variability, except for summer when water level decrease moderately. Dry mesocosms also experience small changes, and rainfal input are rapidly drained, and thus show punctual moist event. Intermediate plot, however, exhibit the highest variability and moisture range. Thus WTD is not the sole factor driving communities, but moisture variability plays a part too.

larger species are more vulnerable to drought and are highly dependent on moisture to survive. For instance, *Hyalosphenia papilio* has pores in its shell, whose number increases with the water level [Booth 2010b]. Although this is clearly an advantage in wet environments for water exchange, these pores are a structural weakness when it comes to cope with drought.

Ventral aperture (plagiostome), and even more a hidden aperture (cryptostome) are good strategies to cope with drought, while cylindric and hemi-spherical thecae seem to be advantageous to handle frequent moisture variations. Dry mesocosms selected Euglyphid TA, which are very common in uplands (in contrast with wetlands to which peatlands belong)[Seppey 2015, Szelecz 2014, Tsyganov 2012]. Beside, TA communities from the dry mesocosms are somewhat similar to communities occurring in upland soil. *Euglypha s.l.*, notably, are commonly found in uplands soil. the genus *Euglypha* provides a good example of traits selection. *Euglypha rotunda* is frequent in upland soil and dry mosses and is small (ca. $60\mu m$), while *Euglypha compressa* is common in *Sphagnum* peatlands and larger (up to $120\mu m$). In general, traits selected by environmental filtering in the dry plots are traits associated with soil TA, showing that a drained peatland is slowly transitioning from a wetland system to an upland system.

5.4.3 Implications for future ecological surveys

This study shows that functional traits are intrinsically more ecologically informative than the identity of the corresponding species to understand the ecology of an ecosystem. 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. For instance, peatlands along a geographical gradient can select different communities while conserving the same ecology, and thus showing similar functional response. Especially, TA species distribution is highly related to the hemisphere where the peatland is located, although two different peatlands from each hemisphere can behave identically. It is impossible to compare Northern and Southern hemisphere peatlands based on TA communities, as all distance-based algorithms would segregate them firstly by the geographical gradient, potentially masking weaker ecological signal. However, such algorithm would behave adequately on a functional response matrix.

Our aim was to assess the relevance of functional diversity for understanding environmental filtering. It is clear that environmental filtering selects traits through community replacement, but it is possible that morphological traits are also selected by phenotypic plasticity. TA species have size variation ranges [Bobrov 1995] that are potentially ecologically meaningful, even if phenotypic plasticity in TA has never been formally proven. One way to take this signal into account is to measure every TA individually along an environmental gradient, which would however be an immense work. Nevertheless, these morphological characters make TA relevant even without prior knowledge of their taxonomy or their ecology. An ecologist non-specialist of TA could use the functional response based on morphologic characterization of the observed specimen. It would be quite straightforward (yet time consuming) for test shape, pseudostome position, size, etc. The mixotrophy is less obvious to identify if the observed specimen is dead or has lost its green photosymbionts. Nonetheless, functional diversity analysis is both practical and informative.

5.4.4 Perspective for mathematical niche modeling

Niche shift and system dynamics lack mechanistic solution in ecology, especially for microbial populations. Most existing models have a too broad scale and consider geographical gradient as a primary niche factor, based on presence–absence data [Ladau 2013, Hirzel 2002]. Several approaches attempt to predict population dynamics, notably by the use of Lotka-Volterra equations. This becomes difficult when metabolic and reproduction rates are unknown. Models for small scale ecosystems (typically the soil medium or the sphagnoshere) exist, but they assume a prior knowledge of the ecological optimum and/or the physiological characteristics of the targeted organisms [Larsen 2012, Lennon 2012, Fournier 2016]. Consequently, the existing niche modeling algorithms are not well suited for microbial niche definition, especially in the meaning of Hutchinson who defined the niche as a n-dimensional hypervolume, where the dimensions are environmental conditions and resources, that define the requirements of an individual or a species to live and for its population to persist [Hutchinson 1957].

Looking at the functional response could address ecological questions while sparing the need to predict population dynamics, and provide an elegant way to model niches. Mathematically, the niche realized (ReN) of a community can be defined by the linear combination of each species functional vector and can be represented as the vector $\vec{ReN} \in \mathbb{R}^n$, such as

$$\vec{ReN} = \sum_{i=1}^{n} c_i \vec{v_i} \tag{5.1}$$

where $\vec{v_i} \in \mathbb{R}^n$ is the functional vector of species i, whose dimensions are functions and components the weight of the functions, and c_i is the relative abundance of species i. Interestingly, the CWM is a linearized equivalent of $\vec{ReN}$. The niche space filled by the community can thus be modelled as

$$V = \left| \vec{ReN} \times I \right| \tag{5.2}$$

where I is the Identity matrix of the dimension of $\vec{ReN}$. For instance, let us consider that a community occupies a 3 dimensional niche, and that this niche is represented as $\vec{ReN} = \begin{pmatrix} 1 \\ 2 \\ 1 \end{pmatrix}$, thus:

$$V = \left| \vec{ReN} \times I \right| = \left| \begin{pmatrix} 1 \\ 2 \\ 1 \end{pmatrix} \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \right| = \begin{vmatrix} 1 & 0 & 0 \\ 0 & 2 & 0 \\ 0 & 0 & 1 \end{vmatrix} = 2 \tag{5.3}$$

as illustrated by Figure 5.5. Therefore, comparing the niches of two communities is equivalent to comparing their respective vectors, for instance looking for their colinearity.

We showed in this study that the volume derived from $\vec{ReN}$ is deformed by an environmental gradient and that this deformation is time-dependant. Consequently, the effect of an environmental

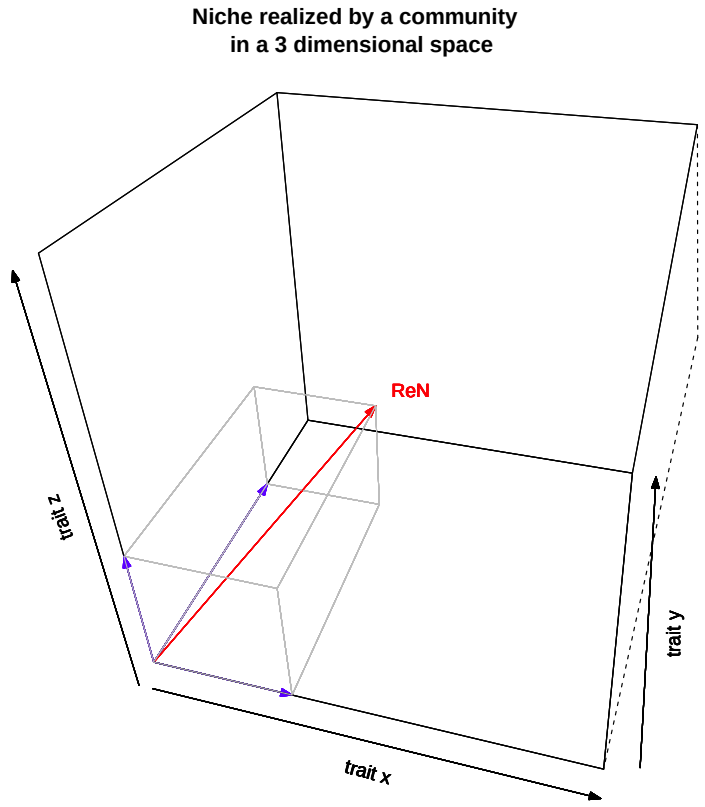

Figure 5.5: Representation of the niche realized by a community. $\vec{ReN}$ is the vector of the realized niche. Blue arrows are the vectors associated with each traits. The box is the niche space occupied by the community

variable on the realised niche $\vec{ReN}$ could be represented as a strain tensor G. The characterisation of this tensor would then be a promising way to model the structuring effect of selected environmental factors on niche topology. Especially, in this context, environmental filtering could be represented as $\frac{\partial G}{\partial t}$.

CHAPTER 6

Genetic determinism vs. phenotypic plasticity in protist morphology

6.1 Introduction

Untangling the relationships between morphology and phylogeny is key to building a reliable taxonomy, but remains a challenge even for well–known organisms [Grbic 2015]. Finding reliable discriminant traits to separate closely–related taxa is especially difficult for microscopic organisms such as nematodes, rotifers and unicellular protists that often lack clear diagnostic traits. Molecular studies have repeatedly revealed extensive evidence for "pseudocryptic" diversity; genetically distinct species that differ by only very subtle morphological details, typically only revealed by detailed morphometric analyses or electron–microscopy. The criteria to distinguish these species were often previously considered to be invalid (e.g. [Hebert 2004]). The existence of extensive pseudocryptic diversity suggests that global biodiversity is considerably underestimated [Šlapeta 2005] and has implications for applications such as biomonitoring, for which accurate taxonomy is essential. Cryptic diversity has been documented in numerous groups of protists including diatoms [Kooistra 2010, Poulíčková 2010, Mann 2010], haptophytes [Medlin 2007, Sáez 2003], foraminiferans [Morard 2009], euglyphid testate amoebae [Chatelain 2013] and hyalospheniid testate amoebae [Corno 2006, Kosakyan 2013, Singer 2015]. However, phenotypic plasticity has also been documented in several protist groups including diatoms and ciliates. Evidence exists for both subtle phenotypic effects, with no implication for taxonomy [Trobajo 2011], and stronger phenotypic effects, which, if not understood as such, would possibly lead to erroneous taxonomic splitting [Tuffrau 2000, Bartual 2008]. In some cases (e.g. planktonic foraminifera), detailed analysis has shown that what was previously interpreted as phenotypic plasticity, is in fact cryptic diversity [Ujiié 2014].

Testate amoebae are a polyphyletic group of primarily terrestrial and freshwater free–living shelled protozoa that clearly illustrate the challenges that come with defining species in micro–eukaryotes [Kosakyan 2013, Singer 2015, Bobrov 2004, Oliverio 2014, Schlegel 2003]. Testate amoebae show evidence for both clear correlation between morphological and genetic diversity [Gomaa 2012, Heger 2011, Kosakyan 2012, Wylezich 2002], and phenotypic plasticity, in response to food sources and temperature [Medioli 1987, Wanner 1999]. Discriminating taxonomically relevant traits from phenotypic plasticity is therefore challenging. Beyond simply causing taxonomic confu-

sion, this issue potentially undermines their reliability as bioindicators [Mitchell 2008, Payne 2011, Payne 2013] and their value as model a group in microbial biogeography [Heger 2009].

Here we address the questions of cryptic diversity and phenotypic plasticity using *Hyalosphenia papilio* [Leidy 1879] as a model. *H. papilio* is one of the most widely distributed, abundant, and well–studied testate amoeba species. It is a mixotrophic (i.e. it contains endosymbiotic algae) testate amoeba with a smooth proteinaceous shell. *H. papilio* has a circumboreal distribution and is abundant in northern *Sphagnum* peatlands [Gilbert 2015, Swindles 2009, Woodland 1998]. Because of its decay–resistant shell, ease of identification, and sensitivity to variations in peatland water table depth, *H. papilio* is a key bioindicator in peatland ecology [Charman 1992, Marcisz 2014b, Marcisz 2014a, Mitchell 1999] and palaeoecology, where sub–fossil testate amoeba communities are used to infer past hydrological conditions [Booth 2002, Booth 2008, Charman 2001, Lamarre 2013, Qin 2013]. *H. papilio* thus makes an excellent model to consider questions of phenotypic plasticity and genetic diversity. Considerable morphological variability of *H. papilio* has been reported with respect to shell size (90–175 μmin length) and the number of pores of *H. papilio* (2–10) [Bobrov 2004, Bobrov 1995] has recently been shown to correlate with water table depth [Booth 2010b], which suggests that this morphological trait might represent an example of environmentally–driven phenotypic plasticity. Alternatively, pore number and shell size could be inherited, and forms with high numbers of pores selected in moist environments. Indeed, in some members of the family (i.e. *Nebela collaris* group in *Hyalospheniidae*), a correlation between subtle variations in shell morphology and Cytochrome oxidase I gene (COI) sequence variation has been found [Kosakyan 2013, Singer 2015]. Two recent studies have shown that *H. papilio* is genetically highly diverse, and constitutes a species complex [Oliverio 2014, Heger 2013]; however, no morphological data support the separation of these genetically–distinct forms. Thus, it may well be that individuals with different pore numbers are genetically distinct. Shells with varying numbers of pores have been observed in another hyalosphenid testate amoebae: *Nebela gimlii* [Singer 2015]. This morphological character is therefore relevant for other Hyalospheniidae species. It follows that the observed morphological variation in *H. papilio* could be either due to genetic determinism or phenotypic plasticity. Our aim was to determine the relative influence of both factors using a combination of experimental and observational approaches. To our knowledge, the relative contribution of genetic and ecophysiological factors in determining morphology has never been addressed in protists. This knowledge–gap illustrates the considerable challenges in protist taxonomy.

6.2 Methods

6.2.1 Morphological analyses

We studied the morphological variability of *Hyalosphenia papilio* using three complementary approaches:

1. a manipulative experiment with controlled conditions,
2. an observational study of a within–site natural ecological gradient,
3. an observational study across 37 European peatlands .

The first two components of our study focussed on *H.papilio* pore number and water table, as wetness is known to be an important control on testate amoeba ecology and previous research suggests a link with pore numbers [Booth 2010b]. The third component takes a broader perspective and focuses on biovolume and climate, based on evidence for temperature–size relationships in other protists [Bobrov 1995]. In our experimental study we also considered a suite of other morphological traits to investigate whether they differed systematically in respose to manipulated water table depth.

The manipulative experiment was designed to assess the effects of water table variation on the microbial communities of *Sphagnum fallax* mesocosms through time. We constructed mesocosms that consisted of a peat monolith topped by a *Sphagnum fallax* layer [Mulot 2015]. The water level in each mesocosm was adjusted to maintain three water table depths (AWTD: -4 cm, -15 cm, and -25 cm, hereafter referred to as "high", "intermediate" and "low" treatments). There were five replicates per treatment with a total of 15 mesocosms. From all mesocosms, we collected 3–5 shoots of the upper 3 cm (living part) of *Sphagnum* mosses. This was done seven times ($08/08/2012$ (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)) across the experimental period. Amoebae were extracted from *Sphagnum* shoots by filtering and back–filtering using mesh sizes of 250 and 20 μm following the procedures described in [Jassey 2011c, Nguyen-Viet 2008]. The number of pores of *H. papilio* specimen was counted for all the dates under 400$\times$ magnification. Ten specimens from the five mesocosms of respectively low and high water level treatment from T0 and T4 (i.e. ca. 1 year after T0) were randomly selected, observed and photographed at 400$\times$ magnification. The following morphological traits were measured using imageJ [50] (Figure 6.1): Maximum length (a), maximum width (b), shell depth (Dp), pseudostome (aperture) width (c), orthogonal distance from the pseudostome to the maximum width axis (d), orthogonal distance from the pseudostome to the position of the top pore (i.e. the pore most distant from the aboral end of the shell) (e), linear distance from the pseudostome to the top pore (f), neck angle (α), pore number (p).

The natural gradient study was designed to investigate the response of testate amoeba communities to a water table gradient in a natural setting. We investigated the water table gradient in Linje mire (53° 11'15"N, 18° 18'34"E), a *Sphagnum fallax*–dominated peatland in Poland [Marcisz 2014b, Slowinska 2010]. In each of five sites within the peatland, five permanently marked plots were selected in hummocks or lawns. *Sphagnum fallax* mosses were collected five times in the year 2013 ($20/04$, $07/06$, $20/07$, $01/09$ and $25/10$), covering a full growing season. Samples were prepared as above and *H. papilio* pore number counted.

Finally, in the European–scale study we aimed to identify links between shell size and climate. We sampled mosses in 37 peatlands distributed across Europe (including 10 specimens from the ma-

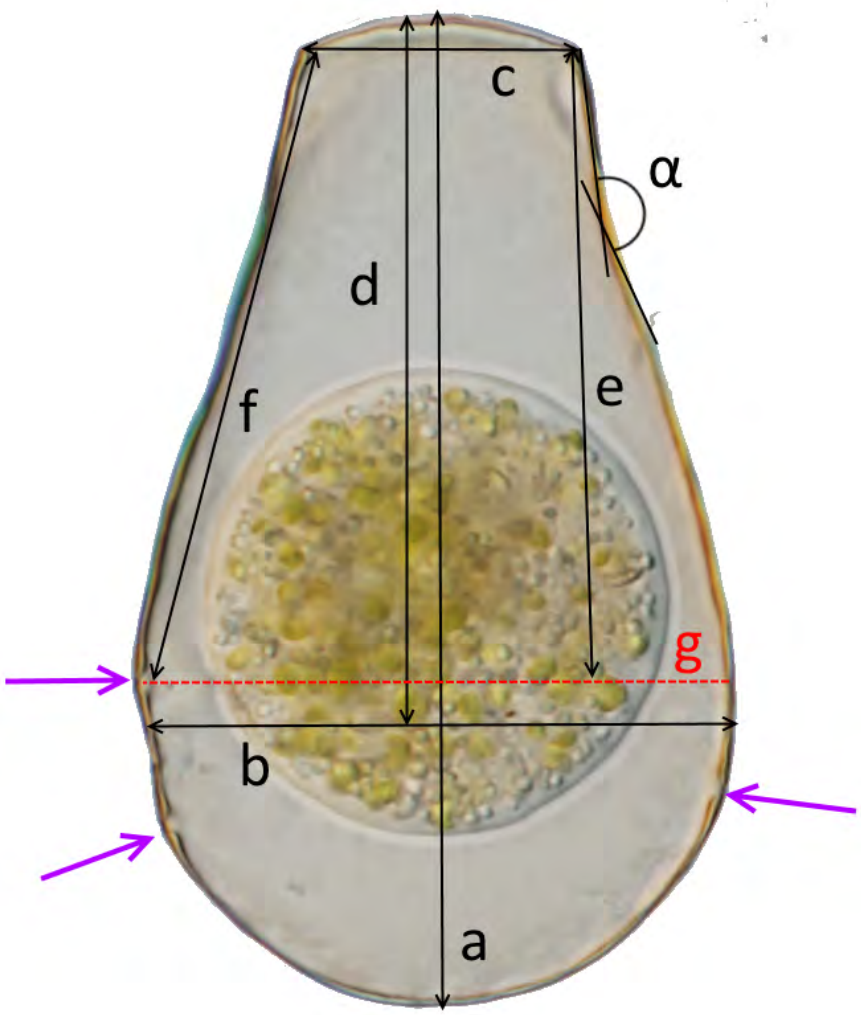

Figure 6.1: Morphological measurements of *Hyalosphenia papilio* specimens. (a) maximum length, (b) maximum width, (c) pseudostome width, (d) orthogonal distance from the pseudostome to the maximum width axis, (e) orthogonal distance from the pseudostome side to height of the highest pore, (f) linear distance from the pseudostome side to the first encountered pore, (α) angle of the neck, (g) not measured, axis passing by the highest pores, orthogonal to (a) axis, to measure (e). The number of pores was also counted (purple arrows).

nipulative experiment T0 samples) (Table X). We extracted testate amoebae, as above, and calculated biovolume using the formula of Charrière et al. [Charrière 2006]. The dataset yielded measurements for 318 specimens which were related to bioclimatic data for these sites.

6.2.2 Data analysis

The morphological variability of *H. papilio* in relation to water table depth in the mesocosm experiment was tested using multivariate ratio analysis (MRA) [Baur 2011]. Morphological trait measure-

ments were centred and scaled prior to analysis. We first computed a shape PCA to investigate how the morphology of the specimens was correlated to the treatments and time (explicitly T0 + High water level, T0 + Low water level, T4 + High water level, T4 + Low water level). We then calculated hierarchical clusters [Murtagh 1985] based on morphological trait ratios using Ward's method on a Euclidean dissimilarity matrix to understand the relationship between morphologically consistent groups and treatments. Finally, we performed a Fisher's linear discriminant analysis (LDA) to extract the best segregating ratios for clusters.

For the pore number data from both the manipulative experiment and the within—peatland natural gradient study we retained all samples with a minimum of 20 *H. papilio* specimens and tested the changes in morphotype abundance (Hellinger transformed matrix) through time using Principal Response Curves (PRC; [Van den Brink 1999]) and ANOVA [Chambers 1992]. For the European–scale study, we used bioclimatic data (BioClim [Hijmans 2005]) plus altitude [Jarvis 2008], and spatial structure (PCNM eigenvectors [Borcard 2002]) as explanatory variables for biovolume. For each of these two subsets (bioclimatic and spatial) the variables which significantly ($p < 0.05$) explained biovolume were selected using glmulti [Calcagno 2010]. The retained spatial eigenvectors were then divided into three spatial matrices: macroscale, mesoscale and microscale. The relative contribution of the selected bioclimatic variables was tested using the Relaimpo package [Grömping 2006].

6.2.3 Phylogenetic analysis

For the phylogenetic analysis, *H. papilio* specimens with contrasting morphologies (number of pores, size) were isolated from the manipulative experiment. Prior to isolation, each individual was photographed, and fixed in a guanidine thiocyanate buffer [Chomczynski 1987]. Mitochondrial COI sequences were obtained using a nested PCR protocol [Gomaa 2014] using a Promega GoTaq G2 kit. Sequences are deposited in GenBank with the following accession numbers XX–XX (nb. accession numbers will be added after paper acceptance).

The sequences were aligned and controlled manually using MUSCLE [Edgar 2004], and BioEdit [Hall 1999]. A maximum likelihood tree was built in MEGA6 [Tamura 2013] using the Tamura–Nei model with 16 gamma–distributed rate variation across sites and a proportion of invariant sites. Node robustness was tested using 500 bootstraps. We performed a Bayesian analysis in order to confirm the topology observed with Maximum likelihood using MrBayes v3.2 [Ronquist 2003] with a general time reversible model of sequence evolution with 6 gamma–distributed rate variation across sites and a proportion of invariable sites. Bayesian MCMC analyses were carried out with two simultaneous chains, adding generations until the standard deviation of split frequencies fell below 0.01. We used sequences from *Nebela ansata* (GenBank number JN849055.1, JN849054.1) and *N. galeata* (Genbank number JN849060.1, JN849059.1, JN849058.1) to root all trees, as these species were shown to be closely related to *H. papilio* in a COI gene–based phylogeny of Hyalospheniidae [Kosakyan 2012]. We then assigned our sequences to the different lineages described in [Heger 2013].

6.3 Results

6.3.1 Morphometric patterns in relation to water table depth

The shape PCA of morphometric data for the manipulative experiment showed that the morphology of *Hyalosphenia papilio* was different between the two sampling occasions but not between water

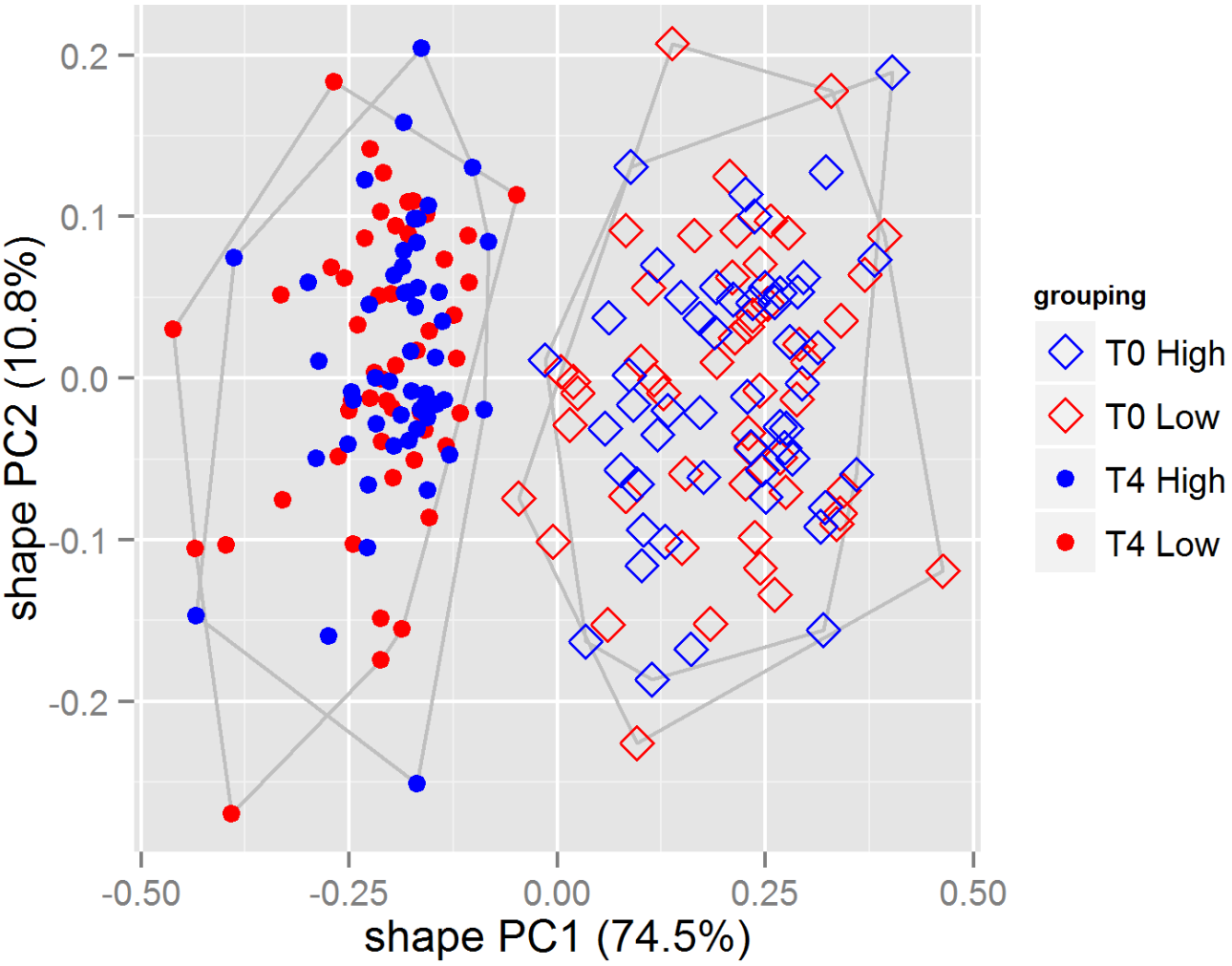

Figure 6.2: Scatterplot of first against second shape Principal Component of morphometric data of 200 *Hyalosphenia papilio* individuals, from High (-4 cm) and Low (-25 cm) water table treatment at T0 and T4. The variance explained by each PC is given in parentheses. The treatments are morphologically segregated along the first axis of the PCA.

The LDA ratio extractor showed that the best segregating ratios for the two groups (T0 vs. T4) were angle/length and width/pores (Table 6.1).

Table 6.1: Best discriminating ratios obtained by Linear Discriminant Analysis. The best discrimating ratio between specimen of T0 and T4 are angle/L and l/pores. As angle and pores number are fairly constant between the two sampling dates, specimens are in fact discriminated by an isometric size reduction.

Groups	best ratios	range T4	range T0	SD	delta
r1 T0-T4	angle/L	0.81-1.30	1.22-1.66	4.89	0.51
r2 T0-T4	l/pores	26.38-46.01	18.09-29.46	2.13	0.71

Neither pore number nor the width/length ratio differed significantly between T0 and T4 (Wilcoxon test, $p > 0.05$). The morphological segregation between T0 and T4 was thus due to a simple isometric size reduction as also attested by the Pearson product moment correlation between length and isometric size of 0.99 (i.e. geometric means of morphological traits [Baur 2014]). Thus the biovolume distribution did not overlap between T0 and T4. Two exceptions to this general pattern were however, the angle of the neck, which was more pronounced at T4 than T0 (mean = 119.5 °, SD = 5.60 vs. $171.97° \pm 13.49$; $p - -value < 0.05$, test = ANOVA) and the shell depth, which was normally distributed around a mean of 19.54 μm regardless of the overall size of the shell or of any other measured morphological feature. Although the PCA showed considerable overlap in the two treatments at both time points, the conditional inference tree of biovolume distribution against treatment (High/Low) and time (T0/T4) revealed a significant reduction in shell length at T + 1 year in the low water table treatment (Figure 6.3).

In the manipulative experiment, the mean pore number increased from 2.02 to 2.18 pores per specimens in the high water level treatment from T0 to T6, while it decreased from 2.04 to 2.01 in the low water level treatment (t–test, $p < 0.05$; Figure 6.4). In the field observational study mean pore number was also on average slightly higher in wet conditions (lawn), but this difference was not statistically significant (t–test, p=0.7; Fig. 4).

The two PRC analyses showed a similar response, although only the PRC performed on the manipulative experiment samples was significant (table supponline). The PRC of pore number in the mesocosm study (Figure 6.5) showed that specimens with more than two pores tend to become more abundant in the wet plots throughout the year, whereas dry plots were consistently dominated by two pore specimens. This response became apparent one year after the beginning of the experiment (August 2013).

6.3.2 Morphometric patterns in relation to climate

In our analysis of links between bioclimate and morphology, 13 variables were significant: altitude, annual precipitation, mean diurnal temperature range, isothermality, mean temperature of the coldest month, precipitation of the driest month, precipitation of the wettest month, precipitation of the coldest quarter, precipitation of the driest and wettest quarters, annual temperature range, precipitation of

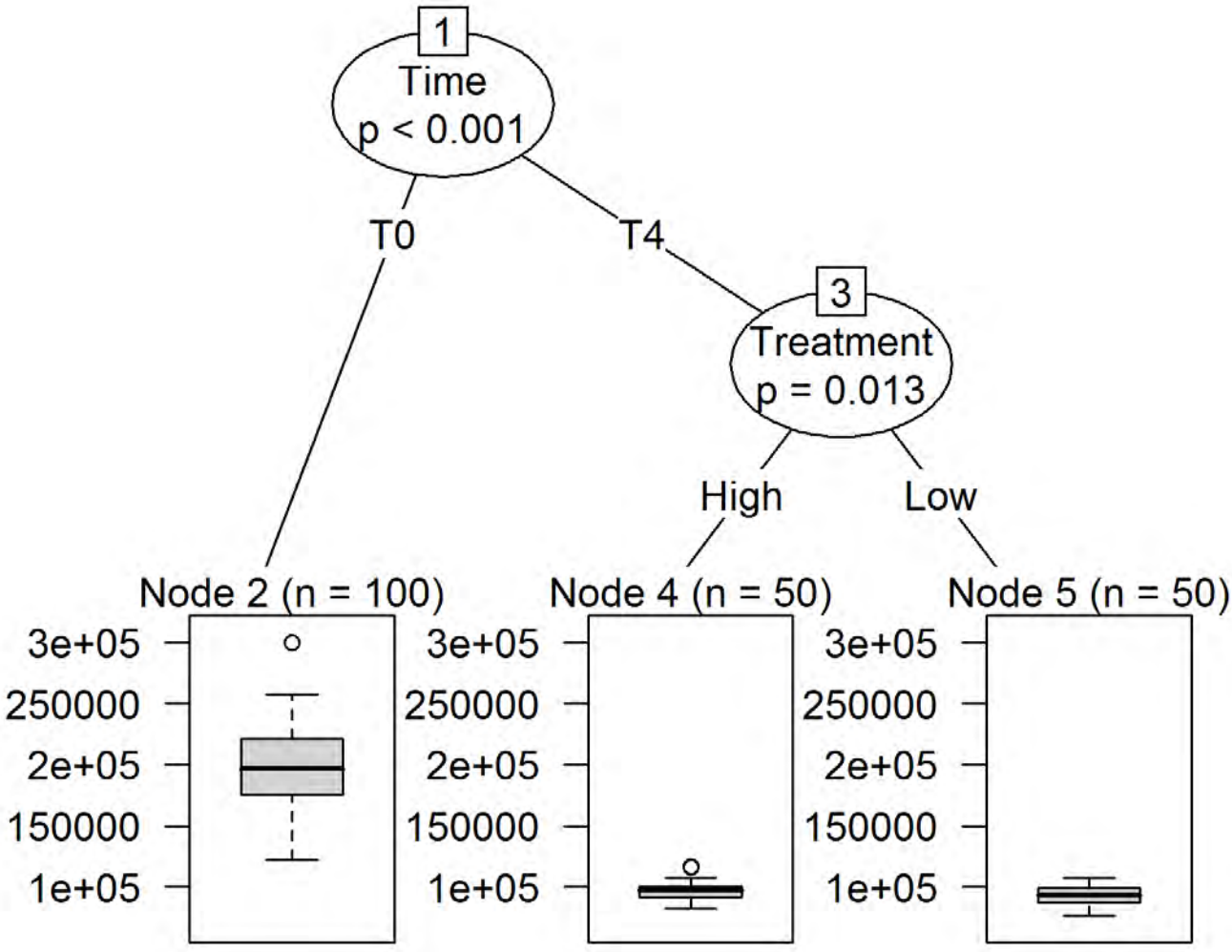

Figure 6.3: Conditional inference tree (CIT) of *Hyalosphenia papilio* specimen biovolume. The tree shows a reduction in shell length at T4 in the low water table treatment.

warmest quarter and precipitation seasonality (see sup n for more details). For the spatial data, we retained PCNM 1–5 (macroscale), 7–9 (mesoscale), 12–16 (microscale).

Variance partitioning analysis showed that the four groups of variables jointly explained 48% of the variance in biovolume. The bioclimatic data explained the highest proportion of variance (11% alone, 44% associated with spatial data). Geographical location explained less than 7% of the variance, equally shared between mesoscale and microscale structure (Figure 6.6). The four explanatory matrices were significant (ANOVA, $p < 0.05$). The size variation over this dataset is considerable with an almost three fold range in length (70.3 μm in Store Mosse, Sweden and 200.9 μm in Cena, Latvia). Size also varied considerably in relation to microhabitat (ANOVA, $p < 0.05$), with larger specimen occurring in lawns (mean length: 129.4 μm, standard deviation: 16.98), as opposed to hummocks (mean: 104.56 $\mu m \pm 13.03$).

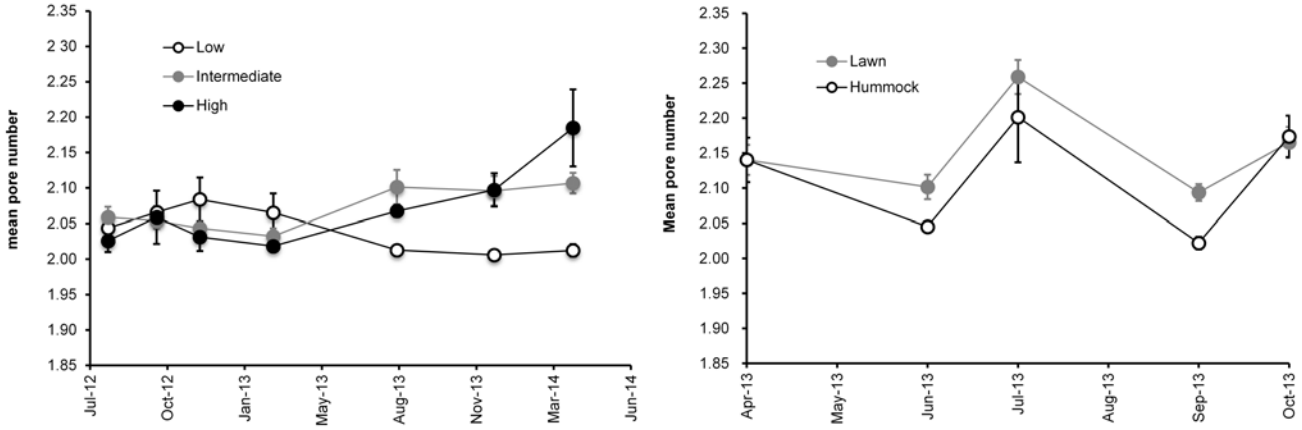

Figure 6.4: Mean variation in pore number over time in the mesocosm experiment (left) and field study (right), respectively per habitat and treatment. Shading of points illustrates the degree of wetness (the darker the wetter).

6.3.3 Phylogenetic analysis

We added our 14 new COI sequences from individual *H. papilio* cells to the 301 sequences of Heger et al. (2013). The topologies of both the strict consensus ML and Bayesian trees were identical and showed that these sequences could all be attributed to two of the 12 lineages of Heger et al. (2013): Six sequences from cells with three or four pores were grouped into haplotype A and eight sequences from cells with two to five pores belonged to haplotype J in Heger et al. (2013); and being thus relatively distantly related. Cluster analyses of the morphometric measurements of these 14 shells using the same approach as described above did not reveal any pattern congruent with the phylogenetic tree. Therefore our phylogenetic reconstructions showed no correlation between taxonomic positions and pore numbers or other morphological traits (Figure 6.7).

6.4 Discussion

6.4.1 Pore number in *Hyalosphenia papilio* shells

The mesocosm study showed that the average number of pores of *Hyalosphenia papilio* increased in wetter conditions, whilst specimens with more than two pores tended to disappear from the driest plots. This shift occurred abruptly and may have been initiated by warm and dry conditions during the first summer. However, it was impossible to detect a significant response of pore number to water table in the field observational study, regardless of the studied period (annual or by sampling date).

The precise function of pores in *H. papilio* is not known. Pores possibly allow water flow between the TA shell interior and the external environment [Leidy 1879]. A reduced number of pores would limit such flow. This may be advantageous under drier conditions when the thickness of the water film surrounding the TA shell is reduced. As such, reduced pore number might reduce the risk of

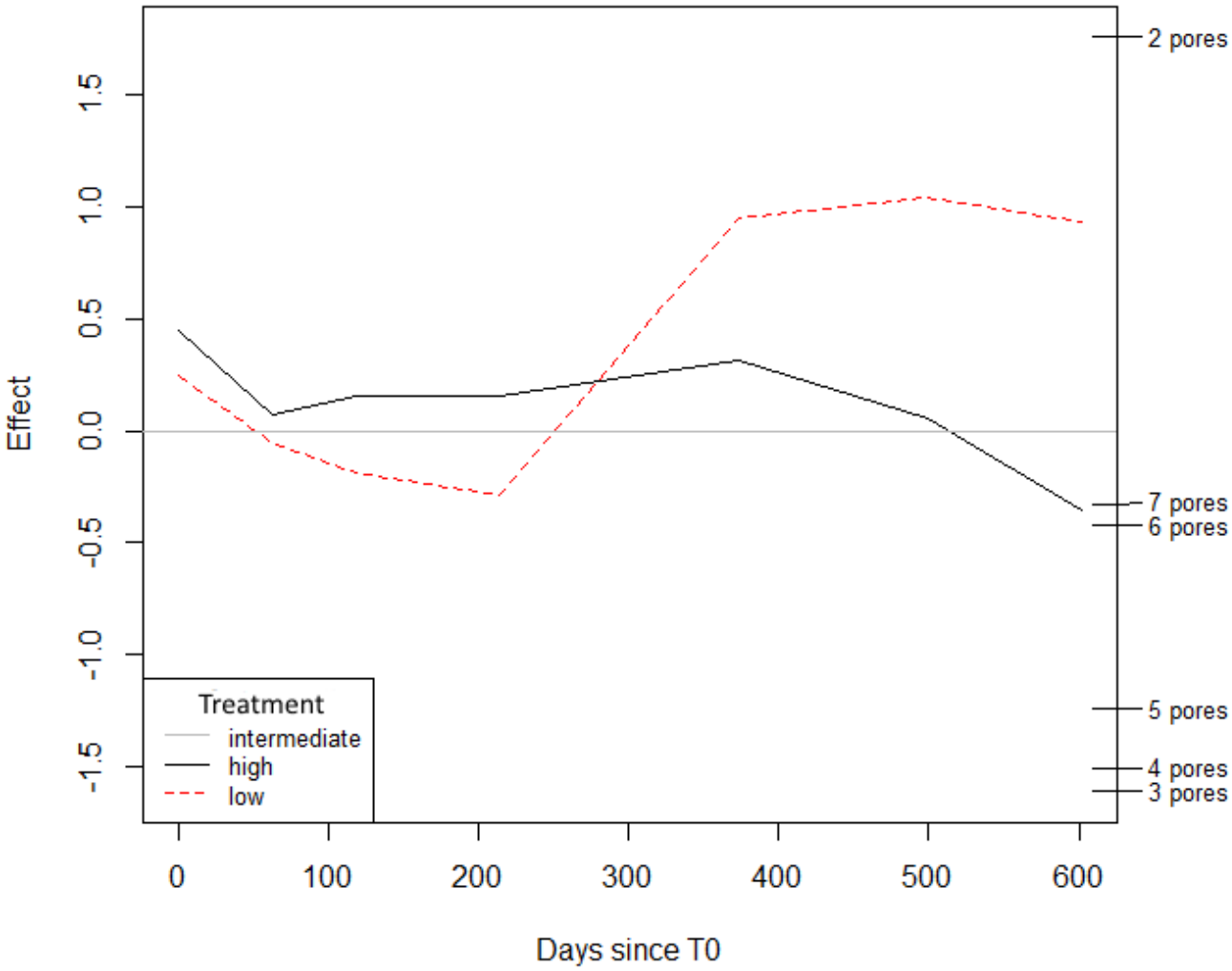

Figure 6.5: Principal Response Curve of Hellinger transformed morphotypes (pores number) abundance distribution in the mesocosm experiment by treatment (water level).

desiccation, and could be interpreted as an adaptive response of TA to drought. In a microsite where the water film thickness changes fast, a reduced number of pores could be especially advantageous by representing an adaptation to the driest conditions regularly experienced by the amoebae. This may explain why, in the within–peatland natural gradient study Linje mire, we observed fewer pores in the sites where oscillations of water table depth were greatest. This suggests that pore number and possibly other morphological traits are determined not only by the average values of ecological factors but also by their range of variation.

The change in pore number we observed in the mesocosm experiment could be seen as evidence for phenotypic plasticity. However it remained possible that different genetic species with different

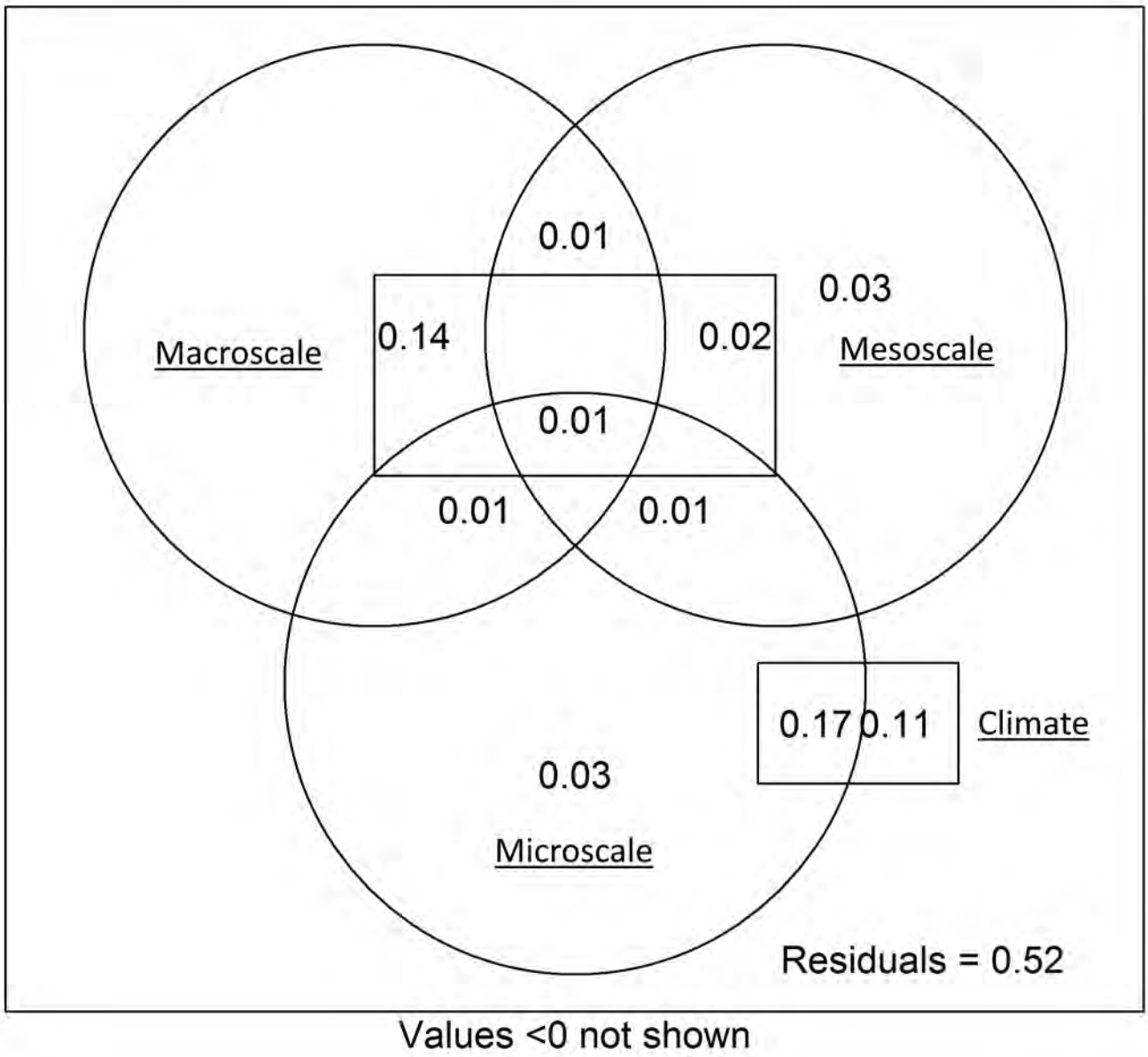

Figure 6.6: Variance partitioning of *Hyalosphenia papilio* biovolume in 37 *Sphagnum* peatlands across Europe. Macroscale variables were PCNM axis 1-5, mesoscale variables were PCNM axis 7-9 and microscale variables were PCNM axis 12–16. Bioclimatic variables were altitude, annual precipitation (BIO12), mean diurnal temperature range (BIO2), isothermality (BIO3), mean temperature of the coldest month (BIO6), precipitation of the driest month (BIO14), precipitation of the wettest month (BIO13), precipitation of the coldest quarter (BIO19), precipitation of the driest and wettest quarters (BIO17 and BIO16) and annual temperature range (BIO1). Bioclimatic variables are represented by the rectangles

pore numbers co-occurred in our initial community and that the shift we observed was due to a shift in community composition among closely related but morphologically distinct pseudo-cryptic taxa.

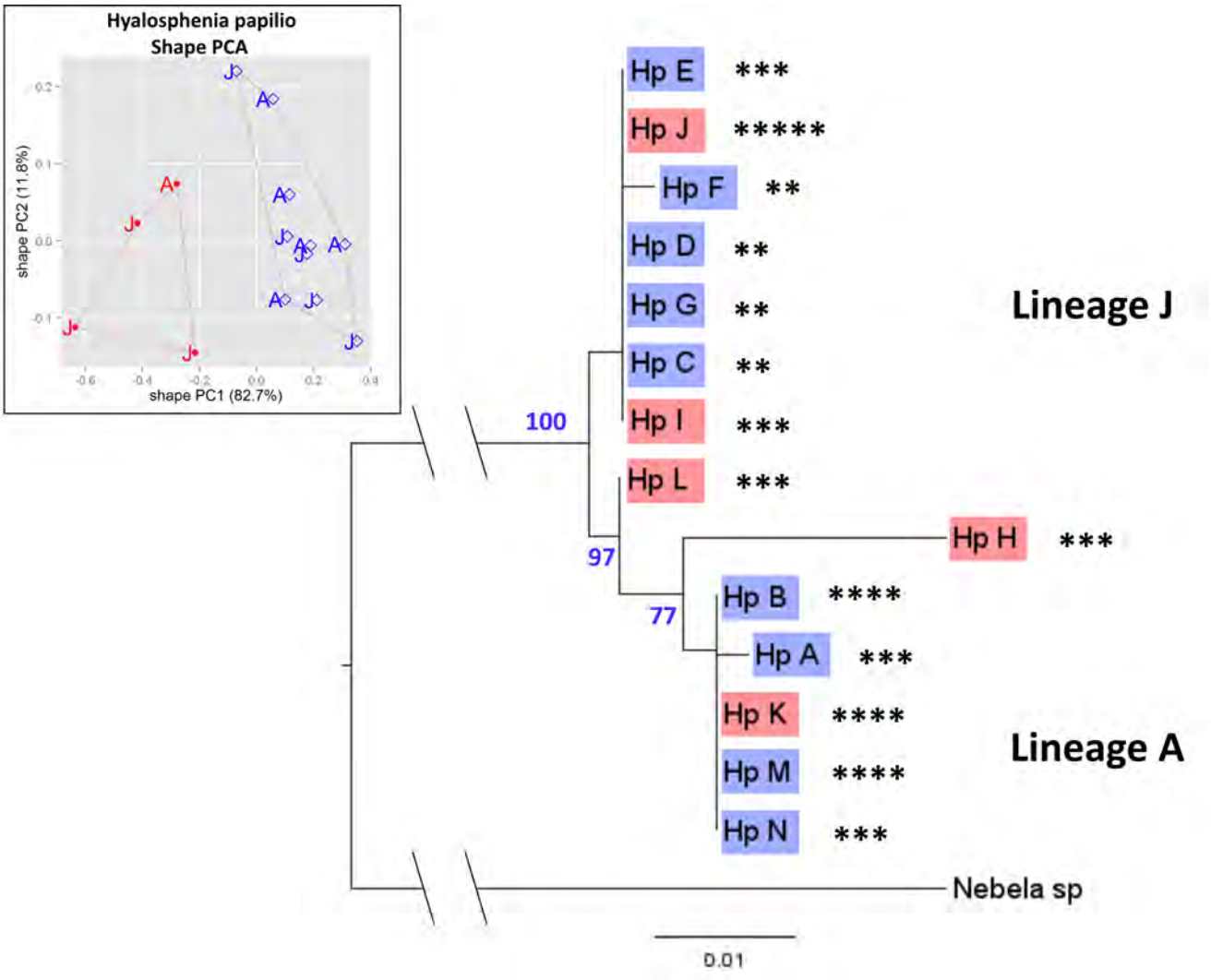

Figure 6.7: Molecular phylogenetic analysis by Maximum Likelihood method based on the Tamura-Nei model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Blue numbers indicate the posterior probability of the bipartition, computed by Bayesian inference using MrBayes. Blue and red highlighting indicates the morphological cluster to which the cell belongs, computed using the MRA approach. The groups were computed based on morphological trait ratios using the Ward method on a previously obtained Euclidean dissimilarity matrix. Stars indicate the number of pores. **Top left corner**: Shape PCA of the ratios, with hull drawn over morphological clusters

To clarify this point, a genetic study was thus necessary. Our barcoded specimens fell within the two clades observed in Western Europe by Heger et al. (2013), clades A and J. Whilst the two-pored and five-pored types were represented only in lineage J, shells with three and four pores were found in both lineages. Shell morphology did not correlate either with affiliation to clade A or J. These results suggest that neither shell dimensions and shape, nor pore number are genetically determined. This is perhaps surprising given the fact that clades A and J are distantly related within the *H. papilio* species

complex and would therefore be expected to exhibit more morphological differences than two more closely-related clades.

Booth & Meyers (2010) recorded a wider range of pore numbers but their study included the broadest range of natural conditions where *H. papilio* can be found. Taken together, the results from both studies would suggest the existence of both phenotypic plasticity and genetic determinism. It is indeed likely that more clades - some of which possibly have a genetically determined higher number of pores - were included in the broader range of conditions sampled by Booth & Meyers (2010). Alternatively the morphotypes with higher pore numbers may only occur in very specific conditions (bog pools) that do not correspond to the wettest treatment in our manipulative experiment.

6.4.2 *Hyalosphenia papilio* shell size

Over a one year period, we observed a clear reduction in TA shell size in the mesocosm experiment, while the effect of water table depth, although significant, was comparatively weaker. Specimens originated from a wet *Sphagnum* carpet sampled at ca. 1000m a.s.l. At the time of sampling the amoebae had spent one year in the mesocosms under very different climatic conditions at ca. 500 m a.s.l. (i.e. ca. 28% less rain – 1800 mm vs. 1500 mm – and ca. $1.5°C$ warmer on average). The observed size shift therefore suggests a response to climate, and specifically the warmer conditions at the experimental site. This result disagrees with observations made on freshwater "'naked amoebae"' taken as a whole, for which no clear correlation between temperature and biovolume was identified [Atkinson 2003]. However, "'naked amoebae"' are a polyphyletic assemblage of a large diversity of organisms which do not share common ecology [Pawlowski 2009] and can be expected to respond differently to temperature changes. Atkinson and colleagues [Atkinson 2003] proposed that higher temperatures increase metabolism in protists. Reduced size increases the contact surface with the environment relative to the biovolume. Consequently, a reduction in size as a response to increased temperature, may therefore be a mechanism for TA to deal with temperature induced metabolism. This prediction has been confirmed in cells from phytoplankton blooms, where increased temperature reduced the cell size [Sommer 2008]. In the case of the mixotrophic *H. papilio*, CO_2 input is not directly beneficial to host cells but to the *Chlorella* symbionts that are enclosed within. A reduction in amoeba size should increase symbionts' access to CO_2 and enhance their photosynthesis. As symbionts are responsible for a substantial part of *H. papilio* carbon input [Jassey 2012, Jassey 2015] a reduction in amoeba size under warmer conditions may be indirectly beneficial. This pattern is in agreement with the temperature-organism size rules, which have been widely documented in ectothermic animals [Atkinson 1994]. Besides temperature, cells were significantly smaller in the wetter treatment (Figure 6.4). Again this may be an adaptative response to a thinner water film.

Our study in mesocosms covered only a limited period in time, and spanned a limited temperature gradient, and results can be expected to vary in a larger, *in situ* setting. As in the mesocosm study, in the survey at European scale, smaller specimens were observed in drier microhabitats (i.e.

hummocks; ANOVA, microhabitat nested in site,$p < 0.05$), in agreement with the reduction in shell length at T4 in the low water table treatment. However biovolume was correlated positively to the temperature of the coldest quarter and isothermality (accounting respectively for 12% and 11% of explained variance), and negatively with variables representing instability (mean diurnal temperature range, annual temperature range). As for pores, it seems that the mean environmental condition is less critical than its magnitude of fluctuation for *H. papilio* biovolume (supp3). The contrasting correlation with temperature between the study at the European scale and the mesocosms suggests that other factors such as food quality and availability, selective pressure from predators etc., likely influence the biovolume of *H. papilio*. Such effects would be in line with modeling studies showing that an organism's size is also constrained by its position in the food web [Loeuille 2005]. To summarize, although our data show diverse and complex morphological variability, it does not allow the derivation of general temperature–size rules. More experimental data would be necessary to achieve this.

6.4.3 James's rule and beyond?

These results demonstrate that the morphology of *H. papilio* can vary over space and time in response to changes in its habitat. Such patterns could be due to within population changes (population size-shift hypothesis – i.e. James' rule) or to community changes (species shift hypothesis – i.e. Bergman's rule) [Daufresne 2009] . Our results clearly show that the variability in pore numbers as well as size is driven by phenotypic plasticity, and not pseudo-cryptic genetic diversity. This supports the population size–shift hypothesis despite the fact that the *H. papilio* populations of the mesocosms included two genetically divergent (i.e. cryptic) species. If the population size-shift hypothesis (James' rule) is accepted two further hypotheses could explain the changes: 1) an increase in the proportion of juvenile stages (i.e. population age-structure shift hypothesis) and 2) a shift in size at a given age (size-at-age shift hypothesis) [Daufresne 2009]. These latter two hypotheses can only be tested in organisms that show developmental stages or that grow as they age, which is probably not the case in TA. Unlike many protists, size is supposed to be constant in individuals of protist species with a shell, except those that include a swarmer flagellate cell ("gamete") in their life cycles like foraminiferans and radiolarians. Shifts in shell size can thus be hypothesised to occur during cell formation, and our data suggest that such shifts could be driven by environmental conditions.

6.4.4 Implications for protist taxonomy, biodiversity assessment and biogeography

Our data for *Hyalosphenia papilio* show one example where unicellular organisms display morphological variation that is not correlated to genetic diversity, or at least where morphology is more influenced by external factors than by genetic inheritance *per se*. Parameters such as temperature and water table depth (i.e. water film thickness around the cells) are correlated with morphological

traits, which suggests that variation in morphology does not occur at random but is an adaptation to environmental conditions. Similar adaptations have been shown to arise very quickly in the ciliate *Cyclidium glaucoma*, in just a single generation [Forster 2013]. Other examples of rapid morphological changes that are the product of phenotypic plasticity have been documented in different species of unicellular eukaryotes including other testate amoebae [Medioli 1987, Wanner 1999]. This contrast with observations that small morphological differences can be used to separate species, a situation called "'pseudo-cryptic diversity"', which prevails among many species of protozoa and microalgae [Sáez 2003, Morard 2009], including arcellinid testate amoebae [Gomaa 2015] and even close relatives of *Hyalosphenia papilio* [Kosakyan 2013]. Therefore, the relative contribution of genetic inheritance and phenotypic plasticity in shaping morphology varies depending on the taxonomic group and the trait under consideration. Thus, our data call for a reassessment of taxonomy based on morphology alone. TA substantial increase in taxonomic research on these globally still under-studied organisms is clearly beeded. This will quite likely lead to a reassessment of estimates of global microbial eukaryotic diversity.

CHAPTER 7

General discussion

7.1 Background

In this work, we investigated peatland response to water level changes using a mesocosm approach. This fulfills a need to understand, preserve and/or restore the peatlands C sink function. Although peatlands represent only ca. 4 % of the continent area, they hold 1/3 of the soil C pool, corresponding approximatively to the atmospheric C pool [Turunen 2002, Mitra 2005, Frokling 2007]. The C sink function is the process by which peatlands store atmospheric C within the peat . This process is achieved by unbalanced ratio between carbon input by photosynthesis (Net Primary Production, NPP) and carbon output by respiration and decomposition [Gopal 1990]. In peatlands, the key to the C sink function is not NPP but low decomposition rate induced by anoxic conditions. These anoxic conditions are the consequence of a high water level that characterizes peatland.

Over the past century, peatlands have sustained a large amount of damages due to anthropic activities. Agricultural drainage probably impacted peatlands the most, by lowering the water table. Moreover, peat has been harvested for horticulture, and to produce heat or electricity. Indeed, peat is an accumulation of organic matter and is therefore an efficient fuel. These anthropic damages are tremedous in certain countries, like in Switzerland, where 90 % of the peatlands areas have been destroyed over the last century [Grapf 2007]. In the context of ongoing climate change, peatlands are threatened by possible extensive periods of drought, which could alter peatlands in two ways. First, dry peat in hot summers may burn, which releases large amounts of C in the atmosphere. For instance, peatland fires that occurred in Indonesia in 2015 released an estimated 1.75 billion metric ton of CO_2 equivalent, which is more than the annual GHG emissions of Germany (http://www.globalfiredata.org/updates.html). Secondly, extensive period of drought can alter the peatland C sink function by causing a decrease in the water table, then inducing aerobic conditions in the surface of the peatland, consequently unbalancing the input/output ratio toward more C emission due to higher decomposition rate. This C release feed back positively to global warming, by increasing greenhouse effect. In order to reduce or possibly reverse the current trend of C loss from peatlands, a need to preserve peatlands [Raeymaekers 1999], and to restore damaged peatlands.

Now, ecology is not (yet) the prime concern of government leaders and administrators, and peatland conservation has to put forward ecosystemic services provided by the peatland. Ecosystemic services rely on the monetary values of an ecosystem, *i.e.* how much it would cost to a country if the said ecosystem were lost. This monetary value is difficult to estimate (although a C trade market

exists), but peatlands have other assets. First, they host a specialized flora and fauna, often endemic or with limited geographical distribution. A wide variety of bryophytes of the *Sphagnum* genera are found in peatlands, along with carnivorous plants such as *Drosera sp* and *Sarracenia sp*. Secondly, due to low decomposition rate, peat is an archive within which organic remains are preserved. This archive function is important for archeology (as bodies and tools are mummified in the peat [Painter 1991]) and also for paleoenvironmental reconstruction, as it is possible to infer past climatic conditions (up to thousands years) by analyzing organic matter (plants and microorganisms) remains in peat cores.

There is thus a need to understand peatland functioning in order to preserve the remaining sites and restore the already damaged sites [Raeymaekers 1999]. The C sink function, and more generally the overall biogeochemical functioning of peatlands is driven by microbial communities inhabiting the peatland, within both the oxic and anoxic layers of the peat. To understand peatland functioning, we need a framework linking the ecosystem function to the microbial activity. As the main factor driving peatland C cycle is the water level, this framework should take the water level into account.

7.2 Achievements and advances

In this thesis, we investigated the response of *Sphagnum* peatland with an integrative approach using a mesocosm experiment. The two principal challenges lied in the mesocosm design on one hand, and the complexity of the nesting doll approach on the other hand. To make this study consistent, we structured it as a nested doll, from a global perspective to a local perspective. We first presented the mesocosm design. Then we investigated the C cycle dynamic at the system scale, to validate the mesocosm response to WTD. The following chapter addressed the response of microeukaryotic communities inhabiting the upper *Sphagnum* layer, and we then investigated the functional response of a functional group of microeukaryotes: the testate amoebae. Finally, we investigated the morphological response of one testate amoebae species, *Hyalosphenia papilio*, to understand the effect of WTD at the species level.

7.2.1 Mesocosm design and construction

First, we designed and built a mesocosm experiment to assess the effect of water table manipulation on *Sphagnum* peatlands. Initially, it was planned to have the mesocosms ready by July 2012, but the experiment started only mid-August. The dynamic water table regulation system was effective only in autumn 2013. The mesocosms did not fully work as expected, as the water table fluctuations were not very effective. Nevertheless, the treatments were contrasted enough to successfully conduct the ecological study. We had to cope with many issues, including data loss. We had to design electronic devices to monitor the experiment. As the challenges we faced were numerous, we decided to publish the experimental design along with a setup guide, electronic diagrams, and the source of

the microcodes and scripts we developed for this experiment [Mulot 2015]. We hope that it could be useful for researchers aiming to conduct similar research.

7.2.2 Response of the system – respiration and decomposition

Then, we investigated the response of the peat monoliths to different water levels. We measured the respiration and the decomposition rate, and we looked at the structural change occurring in the *Sphagnum fallax* layer topping the peat monoliths. The aim of this part was first to validate the system response of the mesocosm by comparing our own measurement with the literature. The second aim was to characterize the C cycle dynamic in order to associate it with the microeukaryotic response. Our mesocosms behaved according to the literature, with a higher decomposition rate for vascular plant litter than for bryophyte litter. The sensitivity of the decomposition rate to temperature was also higher for vascular plant litter than for bryophyte litter. We modelled the respiration in response to water level and temperature using a GAM. The models showed that respiration is positively correlated to water level and temperature, the response to the water level showing a small inflexion point around -10 cm, indicating a likely change in the microbial activity below and above this threshold. This model explained 86 % of the respiration response but lacks mechanistic solutions. We discussed the existing models taking into account water level and temperature, and none of these models takes microbial activity into account in their respective set of equations, although the most sophisticated models acknowledge the role of the microbial activity.

7.2.3 Response of the microeukaryotic communities – topological evolution on interation networks

In the third chapter, we analyzed the topological evolution of microeukaryotic interaction network along the water level gradient. Interaction network are based on non-random association or dissociation and represent the whole range of direct (predation, etc.) and indirect (niche construction) interactions between species composing a community. We hypothesized that the nature of the interaction would be altered by a strong environmental filter, as is water level in peatlands, and that these changes would be reflected on the topology of the inferred networks. These topological changes, reflecting the state of the ecosystem should therefore allow us detecting changepoints, that is a critical value of an environmental factor (here water level) from which an ecosystem transition from one state to another. Furthermore, we hypothesized that the structure of the network should allow us to detect keystone species, ie species whose removal would alter the network integrity.

To our knowledge, interaction network inference along an environmental gradient has never been performed before, and we developed a R package to perform this analysis. We found topological changepoints between -12 cm and -10 cm regardless of the indicators used to infer them. We also found that the most important contributor to the network (keystone species) changed along the wa-

ter level gradient and that the changepoint inferred by the topological descriptors of the network corresponded to the point where heterotrophs become more keystone than autotrophs, showing that the food resource for grazing and predation is probably higher when the water level increases. This also corresponded to the maximal number of autotroph–mixotroph interactions within the network. Finally, we found that the most keystone organism across the whole gradient was *Apatococcus sp*, suggesting that this alga, or similar alga, is basal and crucial in peatland functioning.

7.2.4 Response of testate amoebae – comparison between communities response and functional response

In the fifth chapter, we focused on a functional group of protists, the testate amoebae (TA). Testate amoebae are unicellular organisms living in a shell whose morphology varies between species, making them relatively easy to identify. They are a well studied group in peatland and their ecology is generally known, numerous paper having been published on this topic. We compared the response of the TA communities (species composition divergence) with TA functional response (traits selected by the environment). We analyzed samples between 3 different water levels along a time series, allowing us to characterize the temporal divergence of the TA communities. Overall, the signals obtained by TA community or by CWM (community weighted mean, the traits matrix) were very similar, although the CWM response was less linear than the community response. High water level promotes mixotrophy, which is a key feature in peatlands, as it allows overcoming oligotrophic conditions. This rise of mixotrophy is due to the domination of *H. papilio* in the wet samples. The intermediate samples showed an oscillating seasonal pattern corresponding to changes in the *Sphagnum* layer moisture throughout the years.

The communities were stable after one year, ie after they underwent climatic extrema (summer and winter), which acted as high and low filters. It is very likely that traits selection is related to the changes in the topology of the interaction networks, as water level promotes different trophic group depending on the water level. Therefore, it seems that environmental filtering acts by selecting traits, which leads to community shift and caused the ecosystem functional response. This functional response seems to be more ecologically informative that sole communities composition analysis, and allows mathematically comparing each communities realized niches in the meaning of Hutchinson [Hutchinson 1957].

7.2.5 Response of *Hyalosphenia papilio* – Genetic determinism vs. phenotypic plasticity in protist morphology

Finally, we targeted one species amongst the TA, *Hyalosphenia papilio*. *Hyalosphenia papilio* is a common, abundant TA in the *Sphagnum* dominated peatland of the northern hemisphere. *H. papilio* has been shown to express great morphological variability (size and pored number), and to be in

fact a complex of species. The relationship between morphology and phylogeny was yet unknown and it was unclear whether morphological divergences were due to phenotypic plasticity or genetic determinism. We analyzed the morphology of thousands of *H. papilio* specimens in relation with the environmental factor in our mesocosm experiment, in a field experimental setting and in a European survey. We sequenced some specimens for which we analyzed the morphology and found that the morphology of *H. papilio* is not related to the lineage to which it belongs but is explained both by environmental and geographic factors. This suggests that environmental filtering acts by selecting traits both by community renewal and phenotypic plasticity.

7.3 Limitations and perspectives of the present thesis

In this thesis, we showed that investigating one system by different yet simultaneous approaches and perspective gives a more complete understanding of the said system. Although it is clear that mesocosms designs show some limits, and that conclusions drawn from such experiments are not all scalable to natural ecosystems, they allow conducting integrative studies. Such integrative approach has a great potential to bridge microbial communities and ecosystem function, and have many possible applications, from theoretical ecology to landscape management [Song 2014].

This work, although performed rigorously, showed some limitations. Firstly, the size of the mesocosms does not really allow deriving general models. Because of the small diameter of the peat cores (12 cm), they underwent rapid thermal exchange that does not occur in natural peatlands, where heat transfer is almost entirely taking place at the interface between the soil and the atmosphere. Consequently, the respiration data we obtained are fitted for our experimental design but cannot be scaled up to natural settings. Nevertheless, this thermodynamic limitation had little if no effect at all on the microbial activity of the top 3 cm of the *Sphagnum fallax* mosses, whose behaviour is likely to be identical or similar both in the mesocosms and in natural settings. One solution would have had been to fill the whole mesocosm with peat and not have used peat cores in PVC tubes, but it was impossible, as detailed in chapter 2.

Secondly, despite of the nested doll structure, this work is not entirely integrative. We tried to investigate the mesocosms response at different scales, from the broadest (system) to the smallest (species) and we can legitimately estimate that we achieved a a vertically integrative study. However, we failed to be integrative on a horizontal perspective. For instance, we did not investigate the water chemistry. We had no information about the DOC, pH, C/N, etc. Similarly, we did not consider the effect of atmospheric deposition, and assumed that this deposition was identical amongst all the mesocosms. Finally, we didt not study at all the vegetation shift along WTD. We probably could have investigated the mesocosms response(s) with a greater depth, but we actually were limited by the amount of work to perform.

Finally, the experiment was probably slightly ambitious for a single PhD thesis, given the number of topic it covers. We had to learn peatland ecology, C cycle analysis, TA ecology and identification,

functional ecology. This alone would be sufficient to conduct a PhD study. Yet, we went further and developped a pipeline to process High Throughput Sequencing data, and extended this research by developping a framework to compute interaction network along ecological gradients. This PhD work probably would have been more horizontally integrative if we were two PhD students working on the same experiment.

This study clearly showed some limitations. However, it allowed us to better understand the inner processes driving peatland evolution under constrained WTD. From the data we collected and analysed, we tried to draw an environmental filtering modeling framework, that would combine all the effects observed at all the scale of the mesocosms response.

7.4 Proposition of environmental filtering modeling framework

To preserve and restore peatlands, we need to understand the underlying ecological processes driving peatland evolution. In this thesis, we brought to light the cascading effect of environmental filtering. Environmental filtering is often not considered for itself and is just a black box concept, although it is one of the most enduring concepts in the study of community assembly and dynamics [Kraft 2014]. Initially, the concept of environmental filter(ing) was created to describe traits selection in plants community assembly constrained by abiotic conditions [van der Valk 1981, Bazzaz 1991]. The concept broadened with time and is increasingly used in many studies of community assembly, succession, invasion biology and biogeography [Weiher 1998, Richardson 2000, Cornwell 2006, Whitfeld 2012] We propose here a framework to model environmental filtering (Figure 7.1). The idea is to consider environmental filtering as a hub to which existing models focusing on specific ecological aspects can be plugged.

As we saw in chapter 5, environmental factors promote traits selection. This traits selection is caused by both communities' replacement and by phenotypic plasticity (chapter 6). This induces a community functional response. The selected traits modify the nature of the interactions between organisms composing the meta-community (chapter 4), resulting in species' niche modifications. Indeed, the traits selected within a community of co-occurring species are expected to reflect the processes that have lead to community assembly [Kraft 2007], such as competitive interactions and environmental filtering [Ackerly 2003, Stubbs 2004]. There are therefore two components to the environmental filtering: abiotic (effect of the environmental variable) and biotic (feedback of the interactions between organisms, notably by niche modification). The environmental filtering is a dynamic process that becomes stable after all time-dependent ecological extrema have been reached by the system. In our case, this required one year and a half, when the system sustained both cold winter and dry summer.

The environmental filtering process is thus oscillating at the beginning. Once the system has reached equilibrium, the function of the investigated ecosystem is modified and can be modeled. In this work, we investigated the response of the C cycle dynamic (chapter 3). This modification of the

system functioning might or might not generate feedbacks that will alter environmental conditions of the investigated system. In our case, a modification of the water level will alter the C sink function of the peatland, which will result in higher atmospheric CO_2 concentrations, which would increase global warming and impact the hydrologic regime of the same peatland.

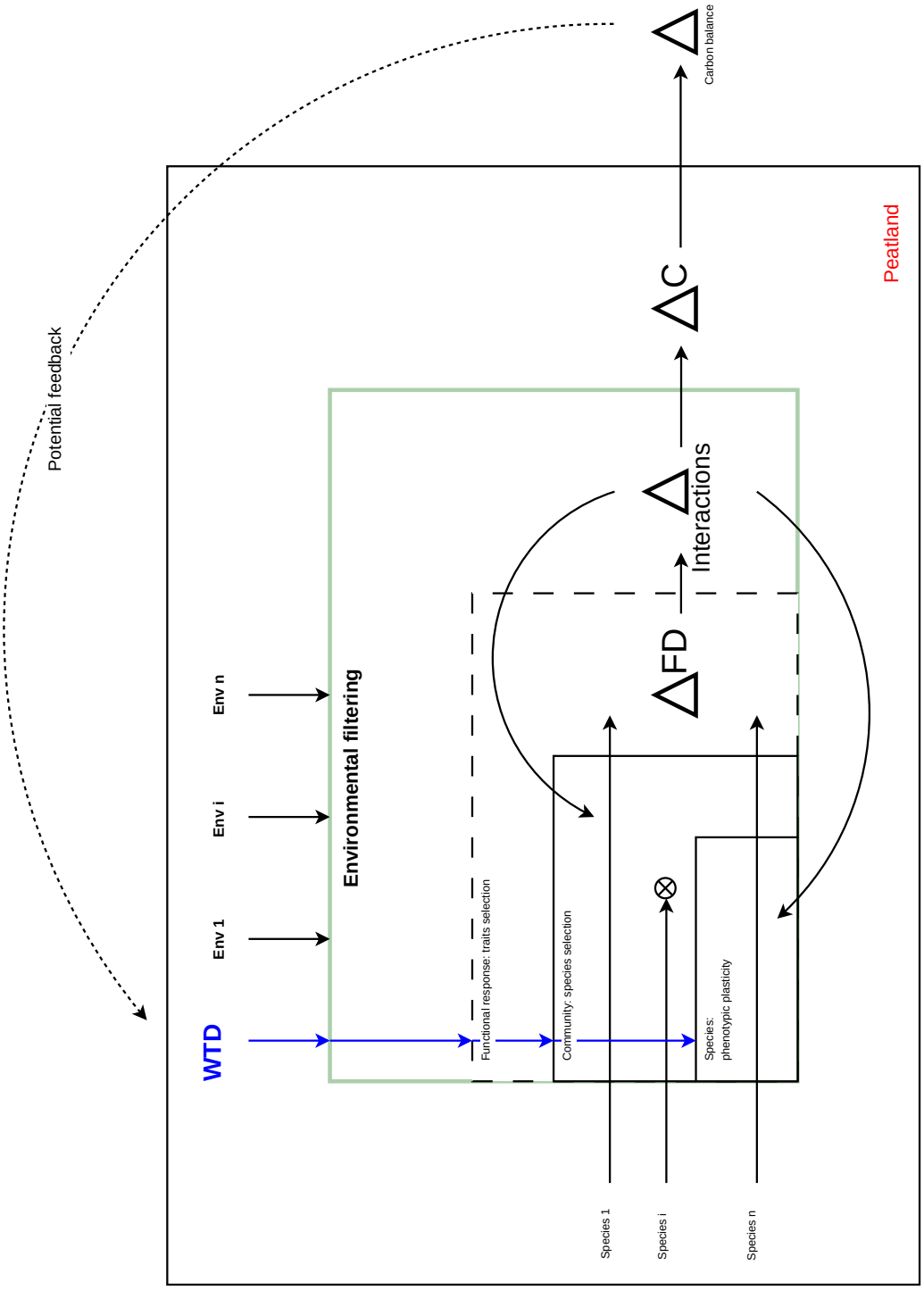

Figure 7.1: Proposition of environmental filtering framework. Environmental factors promote traits selection both by communities' replacement and by phenotypic plasticity (chapter 6). This induces a community functional response, in turn modifying the nature of the interactions between organisms composing the meta-community (chapter 4), resulting in species' niche modifications. These modifications of the microbial loop alters the system functionning.

APPENDIX A

Appendices

To save trees, and because not all the appendices are easily printable, we made a supponline containing everything necessary:

`https://goo.gl/SplZgv`

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