Peatland ecological engineering: testing an approach to strengthen enzymic latch mechanism and impede carbon emissions in post-extracted unrestored and Sphagnum farming system

Thèse

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Résumé

Contexte et objectif Les tourbières agissent comme de vastes réservoirs de carbone (C) en raison d'une production plus élevée de matière organique par rapport à sa décomposition. Malheureusement, les tourbières ont subi des transformations significatives par le passé, liées notamment à des activités humaines comme le drainage et l'extraction de tourbe. Ces perturbations ont augmenté la décomposition de la matière organique, d'où la transformation de puits de C à long terme en sources de C atmosphérique. Le remouillage est utilisé comme outil d'ingénierie écologique pour améliorer les propriétés hydrophysiques des tourbières perturbées, permettant de limiter les émissions de C et de fournir un substrat approprié pour la restauration des tourbières et la culture de la sphaigne. Un autre outil, soit l'addition de composés phénoliques, a été testé en laboratoire dans le passé pour évaluer si le mécanisme de verrou enzymatique (MVE) peut réduire la décomposition de la matière organique, mais plusieurs résultats rapportés semblent contradictoires. Par conséquent, cette thèse vise à évaluer l'effet du remouillage et l'ajout de produits phénoliques sur le renforcement du MVE, et à évaluer comment ce mécanisme peut limiter la décomposition de la matière organique dans deux modèles expérimentaux, soient des tourbières qui n'ont pas été restaurées après l'extraction de la tourbe et un site de culture de sphaigne au Québec (Canada). Cette thèse se divise en trois sous-objectifs : le premier se concentre sur les tourbières non restaurées après extraction de la tourbe et les deux autres sur un système de culture de sphaigne.

Matériel et méthodes Pour le 1er sous-objectif concernant la gestion des planches d'exploitation, une expérience impliquant le remouillage et trois traitements phénoliques a été menée sur deux secteurs de tourbières non restaurées à la suite à l'extraction horticole de la tourbe (NR) qui différaient selon le nombre d'années depuis la fin d'extraction (NR-1 an et NR-41 ans). Pour le 2^{e} sous-objectif concernant la gestion de cultures de sphaignes, une expérience avec trois traitements phénoliques a été établie dans deux bassins de culture de sphaigne dominés par les sous-genres *Acutifolia* ou *Sphagnum*. Pour le 3^{e} sous-objectif toujours en relation avec la gestion de cultures de sphaignes, une expérience avec trois traitements phénoliques a été menée dans des bassins de cultures ayant deux stades de développement de tapis de sphaignes du sous-genre *Acutifolia* : tapis établis d'un an et de neuf ans en âge. Des copeaux broyés de biomasse aérienne fraîche de *Picea mariana* (testés

uniquement dans le 1er sous-objectif), des granules de bois, de vieilles racines récoltées lors du hersage de la tourbe et un traitement témoin sans ajout représentaient les traitements phénoliques. L'efficacité des traitements a été mesurée par les échanges de dioxyde de carbone (CO_2), la concentration de composés phénoliques solubles dans la tourbe et l'activité des enzymes extracellulaires. La productivité (pour le 2e sous-objectif) et l'accumulation de la biomasse de sphaignes (pour les 2e et 3e sous-objectifs) ont également été prises en compte.

Résultats Les résultats du 1er sous-objectif ont montré que le niveau de la nappe phréatique des deux secteurs de tourbières non restaurées n'a que légèrement augmenté à la suite du remouillage. Les émissions de CO_2 et l'activité des enzymes de polyphénol oxydase et des hydrolases n'ont pas été affectées par le remouillage et l'ajout de produits phénoliques. Dans le 2e sous-objectif, l'ajout de produits phénoliques a mené à des émissions de CO_2 plus élevées par rapport au témoin pour les deux sous-genres, *Acutifolia* et *Sphagnum*. L'addition de composés phénoliques n'a pas augmenté la concentration en composés phénoliques solubles dans la tourbe, ni la productivité et l'accumulation de biomasse de sphaigne pour les deux sous-genres. L'activité des différentes enzymes étudiées n'a pas été limitée en réponse aux additions phénoliques. Les résultats du 3e sous-objectif ont révélé que les ajouts phénoliques étaient incapables de limiter les émissions de CO_2 et l'activité enzymatique aux deux stades de développement des tapis de sphaignes du sous-genre *Acutifolia*.

Conclusion Cette étude n'a pas réussi à démontrer le renforcement du MVE en réponse au remouillage et à l'ajout de composés phénoliques. Pour le 1er sous-objectif, les conditions oxiques dues à un niveau bas de la nappe phréatique (< -45 cm) n'auraient pas déclenché le verrou enzymatique. Pour tous les sous-objectifs, le non-renforcement du MVE pourrait être l'absence d'effets inhibiteurs des composés phénoliques testés. Nous pensons également que les émissions élevées de CO_2 détectées dans cette étude pourraient être liées à la décomposition des produits phénoliques. Bien que le MVE n'ait pas été validé dans ces expériences de terrain à court terme, cette étude a contribué à la compréhension du MVE en réponse à des applications de produits phénoliques. Des recherches supplémentaires sont nécessaires pour identifier des produits phénoliques susceptibles de limiter la décomposition de la tourbe. Également, la comparaison des variables environnementales entre les couches superficielles de la tourbe avec les couches plus profondes dans le profil de tourbe pourrait

aider à améliorer notre compréhension du MVE et par conséquent des processus de décomposition en tourbière.

Abstract

Background and aim Peatlands act as vast carbon (C) reservoirs due to an imbalance between higher organic matter production compared to its decomposition. Unfortunately, in the recent past, considerable changes such as drainage and peat extraction due to anthropogenic activities have been impacting peatlands. These land conversions disturbed the functioning of peatlands by increasing organic matter decomposition: hence converting long-term C sink to atmospheric C source. Previously, ecological engineering tools such as rewetting was used to reverse hydro-physical properties of peatlands that can limit C emissions and provides better substrate conditions conducive to Sphagnum farming. Also, another tool could be phenolic addition as trialed at lab-based level to test the enzymic latch mechanism (ELM) but reports from the literature reveal contradictory results. Therefore, this dissertation aimed to assess the role of rewetting (blocking former ditches with dams) or enhanced rewetting (by an irrigation system through channels) with phenolic additions on strengthening of the ELM. More specifically, to test how ELM can limit peat decomposition in two experimental models such as post-extracted unrestored peatlands and Sphagnum farming system in Québec, Canada. This thesis is therefore divided into three sub-objectives: the first one focused on post-extracted unrestored peatlands and the other two sub-objectives were focused on Sphagnum farming system.

Material and methods For the first sub-objective related to management of post-extracted peat fields, an experiment involving rewetting along with three distinct phenolic treatments was conducted on two post-extracted unrestored (UNR) sectors (young and old) that differed in age (UNR-1 yr and UNR-41 yr) since the horticultural peat extracting activities ceased. For the second sub-objective aiming to improve *Sphagnum* culture, an experiment with the addition of three phenolic treatments was established in two cultivation basins dominated by the *Acutifolia* or *Sphagnum* subgenus species in a Sphagnum farming system. For the third sub-objective, an experiment with three phenolic treatments was conducted on two developmental stages — 1 yr-established carpet and 9 yr-established carpet — with *Sphagnum* species of the *Acutifolia* subgenus. The developmental stages refer to the cultivation age of carpets composed of *Acutifolia* subgenus species in a Sphagnum farming system. The phenolic treatments were: *Picea mariana* aboveground fresh wood chips (used

only in the first sub-objective) or wood pellets (wood), old roots from peat harrowing (root), and a subplot with no additions (control). The effectiveness of treatments was assessed mainly by carbon dioxide exchange, peat soluble phenolics and extracellular enzyme activities. *Sphagnum* productivity (for the second sub-objective) and biomass accumulation (for the second and third sub-objectives) were also accounted.

Results In the case of first sub-objective, water table level at UNR-1 yr sector was slightly increased as a result of rewetting (-48 cm) compared to non-rewetted (-57 cm) plots. Largely, carbon dioxide (CO_2) emissions, phenol oxidase, and hydrolase enzyme activities at both UNR sectors were not inhibited in response to rewetting and phenolic additions. As for second sub-objective, phenolic additions showed higher CO_2 values (more CO_2 release compared to CO_2 uptake) as net ecosystem exchange compared to control for both *Acutifolia* and *Sphagnum* subgenus carpets. Phenolic additions were unable to induce positive effect on peat soluble phenolics, productivity, and biomass accumulation within both subgenera *Sphagnum* carpets. Enzyme activities were not limited in response to phenolic additions also for both subgenera *Sphagnum* carpets. For the third sub-objective, phenolic additions were unable to limit CO_2 emissions and enzyme activities at both developmental stages of *Acutifolia* subgenus *Sphagnum* carpets.

Conclusion The results of the three experimental approaches showed little support in the strengthening of ELM in response to rewetting and phenolic treatments. Specifically for the first sub-objective, we assumed that oxic conditions due to still low water table level (< -45 cm) did not create enzymic latch, which is required for strengthening of ELM. Overall, for all sub-objectives, another reason for failure in detecting ELM could be absence of inhibitory effects of phenolic additions on enzyme activities. We also assumed that higher CO_2 emissions detected in this study might be linked with the decomposition of phenolic products themselves. Though ELM in response to rewetting and phenolic additions on these short-term field experiments is not validated, this study has contributed to greater understanding of the ELM in response to field phenolic additions. Still, further research is warranted to compare environmental variables between the top surface and vertical peat profile depth to better understand rewetting and phenolic addition effects on peat decomposition processes.

Also, additional investigations are required to identify phenolic products that could limit peat decomposition.

Table of contents

List of tables

List of figures

List of appendices

Tables

Figures

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Foreword

This doctoral thesis is written as an article-based thesis where three chapters also serving as three articles are sandwiched between a general introduction and conclusion. All the chapters were prepared by me as first author with suitable corrections and suggestions from my director, Line Rochefort and co-director Chris Freeman. All the field work, data collection and statistical analysis required for this thesis was done by me.

Brief description of the thesis sections is explained below:

1. **Abstract** covers a brief background related to the subject of this thesis followed by overall objective, methods, results, and conclusion.

2. A general **introduction** outlines the status, problem and possible solution around the idea of this thesis, which is "to combine rewetting (blocking former ditches with dams) or enhanced rewetting (by irrigation system through channels) with phenolic addition at field based level to strengthen enzymic latch and to test how it can suppress enzymes activities, reduce *Sphagnum* decomposition, promotes *Sphagnum* productivity, and limit greenhouse gas (GHG) emissions in two experimental models such as post-extracted unrestored peatlands and Sphagnum farming system in Québec, Canada."

3. **Chapter 1** will be submitted in a SCI journal. Talal Asif, Line Rochefort, Chris Freeman, Christian Dunn and Mélina Guêné-Nanchen (2024). Peatland ecological engineering: testing an approach to limit carbon dioxide emissions under enzymic latch mechanism in post-extracted unrestored peatlands. TA developed and implemented the research design and wrote manuscript. LR and CF provided suggestions on experimental design. LR, CF, CD, and MGN reviewed the manuscript. LR received the financial support for this study from National Sciences and Engineering Research Council of Canada (NSERC) through a Collaborative Research and Development Grant supported by the Canadian Sphagnum Peat Moss Association (CSPMA) and its members (grant no. CRDPJ 769 517951 – 17).

4. **Chapter 2** was submitted on 02 January 2025 in the Frontiers in Earth Science journal. Talal Asif, Line Rochefort, Chris Freeman, Christian Dunn, Hojeong Kang, and Mélina Guêné-Nanchen (2024). Phenolic supplements: testing an approach to limit *Sphagnum*

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5. **Chapter 3** was submitted on 19 October 2024 in the Heliyon journal and is currently under review. Talal Asif, Line Rochefort, Chris Freeman, Christian Dunn, Hojeong Kang, and Mélina Guêné-Nanchen (2024). Testing phenolic enrichment effect on enzymic latch mechanism at two stages of *Acutifolia* establishment in a Sphagnum farming system. TA developed and implemented the research design and wrote manuscript. LR and CF provided suggestions on experimental design. LR, CF, CD, HK, and MGN reviewed the manuscript. LR received the financial support for this study from National Sciences and Engineering Research Council of Canada (NSERC) through a Collaborative Research and Development Grant supported by the Canadian Sphagnum Peat Moss Association (CSPMA) and its members (grant no. CRDPJ 769 517951 – 17).

6. A general **conclusion** illustrates the concise results obtained from the three chapters and provides suitable suggestions to be considered for future research.

The results of this thesis have been presented in the following symposiums or conferences:

Science Symposium PERG & SER-EC, 6-7 April 2022, oral presentation, Université Laval, Québec, QC, Canada.

28th PERG Symposium & 2023 McGill Carbon team Annual Meeting, 1 March 2023, oral presentation, McGill University, Montreal, QC, Canada.

Centre for Northern Studies (CEN) annual conference, 16-17 February, poster presentation, Université du Québec à Rimouski, QC, Canada.

I International Symposium on Growing Media, Compost Utilization and Substrate Analysis for Soilless Cultivation, RE3 conference, 13 June 2023, oral presentation, Québec City convention center, Québec, QC, Canada.

RE3 conference, 6 June 2023, oral presentation, Québec City convention center, Québec, QC, Canada.

Introduction

Natural peatlands are unique ecosystems where net primary production is greater than organic matter decomposition, leading to organic matter accumulation over time (Gorham, 1991). Worldwide, such an imbalance between productivity and decomposition results in 450 to 650 gigatons (Gt) of carbon storage in peatlands (FAO, 2020). The largest contribution of carbon storage in peatlands comes from northern peatlands with a range of 400 to 550 Gt of carbon (UNEP, 2022). Global peatlands cover an area of 500 million hectares (Mha), of which Asia (33%) and North America (32%) comprise more peatland area than the other regions, for example Oceania, Europe, Latin America, and the Caribbean. Peatlands are threatened by various anthropogenic activities such as drainage for agriculture, afforestation, peat extraction, oil sand mining, reservoir construction, and road construction. Peatland degradation due to anthropogenic activities can destabilize the natural carbon cycle (Fig. 0.1) or sink function of peatlands by enhancing organic matter decomposition; thus, transforming peatlands from sinks to the source of carbon (Waddington & Price, 2000; Wang et al., 2015). Rewetting which raises the water table level, can reverse the drainage impact to some extent and assist in limiting carbon dioxide (CO_2) emissions from peatlands. Rewetting could also provide a new suitable peat substrate for Sphagnum farming, which is a form of paludiculture that aims to cultivate and produce *Sphagnum* biomass on a renewable and cyclical basis. In the recent past, experiments using ecological engineering tools (rewetting and phenolic addition) for limiting peat decomposition, measured through inhibited enzyme and microbial activities, have been done in lab-based and greenhouse experiments (Fenner & Freeman, 2011; Bonnett et al., 2017; Fenner & Freeman, 2020; Alshehri et al., 2020). To date, no general paradigm has been formulated that ecological engineering tools inhibit the enzyme and microbial activities in peatlands based on the enzymic latch mechanism (ELM) theory presented by Freeman et al. (2001). To uncover, how rewetting along with phenolic additions can limit peat decomposition in two different experimental models (post-extracted unrestored peatlands and Sphagnum farming sites) under the mechanism of the ELM is the basis of this study.

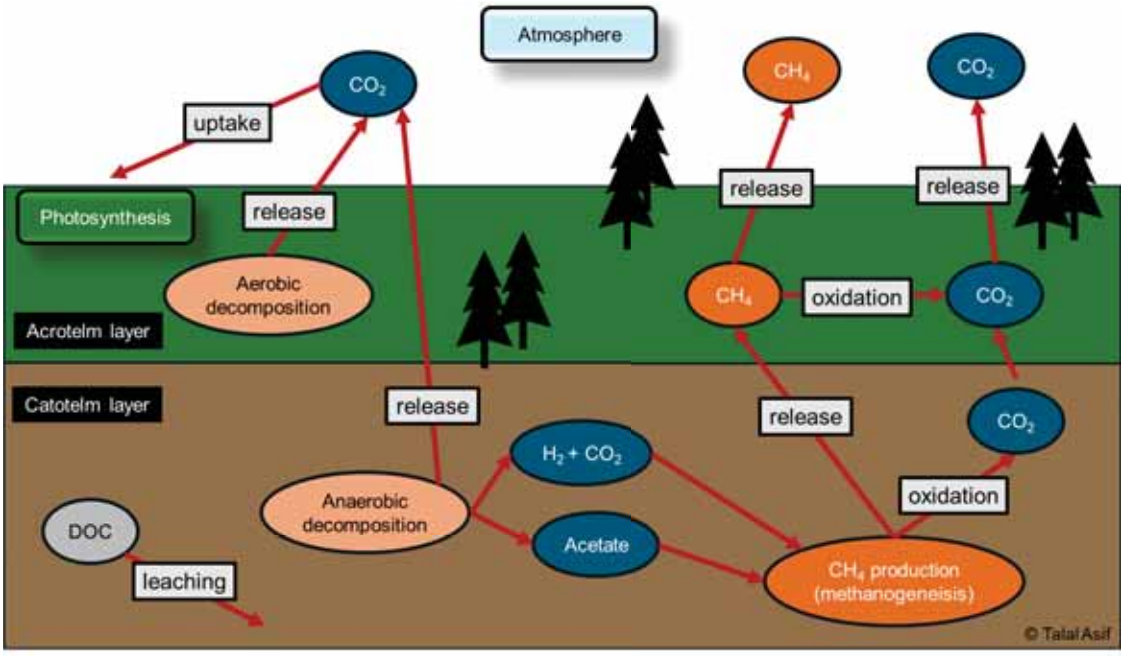

Fig. 0.1. Conceptual framework of the peatlands carbon cycle. The balance between carbon dioxide (CO_2) absorption via photosynthesis and release via respiration between the atmosphere and the ecosystem is termed NEE. Where NEE is the sum of gross ecosystem productivity (GEP) and ecosystem respiration (ER). CO_2 uptake by plants via photosynthesis from the atmosphere is called gross ecosystem productivity (GEP). Whereas the release of CO_2 via aerobic and anaerobic respiration from the ecosystem to the atmosphere is known as ecosystem respiration (ER). Decomposition results in CH_4 production via methanogenesis which is released directly or oxidized by methanotrophs in the form of CO_2 from the ecosystem to the atmosphere. Dissolved organic carbon (DOC) represents an important component of the carbon cycle and is lost through leaching.

Decomposition dynamics

Decomposition is a comminution process that involves complete or partial disintegration of large organic matter into simple organic and inorganic substances like CO_2, CH_4, and dissolved organic carbon (DOC) (Moore & Basiliko, 2006; Rydin & Jeglum, 2013). Organic matter decomposition is controlled by biotic (microbial community) and abiotic (oxygen availability, pH, temperature, nutrient status, substrate quality, moisture content) factors (Aerts, 1997; Gartner & Cardon, 2004; Laiho, 2006). In peatlands, a slow rate of decomposition is ascribed to anoxia, low pH, nutrient immobilization, low temperature, limited microbial activity, and *Sphagnum* acid (van Breeman, 1995; Freeman et al., 2001a). Among these, primarily oxygen availability in response to a lowered water table level within the peat profile has a critical role in peatland decomposition (Freeman et al., 2001a). Johnson & Damman (1991) observed a decline in *S. fuscum* decomposition within the vertical positions of hummock (oxic/anoxic layers). After 22 months, Johnson & Damman (1991) found higher mass loss in the oxic layer (12.8%) than intermediate anoxic (10.6%) and anoxic layer (9.5%). Similarly, a negative correlation was found between wetness level and *Sphagnum* decomposition in a mire complex composed of a raised bog and wet fen located in central southern Sweden (Bengtsson et al., 2016). Recently, researchers conducted a litter bag decomposition experiment and noticed a positive correlation between *Sphagnum* mass loss and oxygen (oxic and anoxic layers) availability. For example, after two years of decomposition, Mäkilä et al. (2018) observed higher mass loss at -10 cm hummock depth — for *S. fuscum* (18.3%) and *S. magellanicum* (18.8%) — than at -30 cm hummock depth — for *S. fuscum* (13.6%) and *S. magellanicum* (15.1%) — and at -50 cm hummock depth — for *S. fuscum* (13.3%) and *S. magellanicum* (11.3%). Overall, *Sphagnum* mass loss on an annual basis has been reported at a different vertical position of the hummock layer by different studies, for instance, Johnson & Damman (1993) (8- 25 %), Moore & Basiliko (2006) (5-30 %), Asif et al. (14-17 % unpublished data).

Traditionally peatlands are water-saturated habitats with a slow decomposition rate that allows organic matter accumulation in peatlands. Decomposition constraints will be affected by drainage and continuous global climate warming. The model projection for 21st century global warming estimated a potential rise of 1.1-6.4 °C in surface temperature in relation to enhanced greenhouse gas (GHG) emissions (IPCC, 2007). Peatland drainage in response to

peat extraction and global warming declines the water table level and eventually, waterlogged peatlands are exposed to the oxic environment, and as described above resulting in increased CO_2 emissions. In this paradigm together with IPCC (2014) and the Paris Agreement (UNFCCC, 2015), "Carbon lock" in peatlands can be partially secured through ecological engineering options that will ultimately help in impeding peatland decomposition.

Enzymic latch mechanism: A general paradigm for carbon sequestration

The decomposition process is strongly affected by the activities of microorganisms. Peatland organic matter accumulation is attributed to the limited microbial activities on account of anoxia (Belyea, 1996; Freeman et al., 1996; Moore & Basiliko, 2006). Partial decay due to anoxia results in the accumulation of phenolic compounds that further allow organic matter accumulation. Phenolic compounds are potent inhibitors of microorganisms and in particular extracellular enzymes (phenol oxidase and hydrolase enzymes) produced by fungi and bacteria (Wetzel, 1992; Freeman et al., 2001a; Fenner et al., 2005). In the last few decades, a wide array of research proved that oxygen constraint on phenol oxidase is the primary latch for "Carbon-lock" and its prevention from being re-released as CO_2 into the atmosphere (Freeman et al., 2001a; Fenner & Freeman, 2011). Phenol oxidase is the suit of enzymes, which in the presence of oxygen can completely oxidize phenolic compounds (McLatchey & Reddy, 1998). In addition to a lack of oxygen, low pH and low temperature can also limit phenol oxidase activity (Williams et al., 2000; Freeman et al., 2001b). It has been shown that phenol oxidase limited activity indirectly affects another group of enzymes (hydrolase enzymes) through inhibitory effects imposed by an accumulation of phenolic compounds (Wetzel, 1992; Freeman et al., 2001b). Therefore, phenol oxidase activity is also an important factor in the whole decomposition process. Freeman et al. (2001a) combined all the above-mentioned constraints in the process of peatland decomposition into the phrase 'enzymic latch'. In natural peatlands, an enzymic latch exists with varying strength between oxic and anoxic layers. However, lowered water table level due to drainage and peat extraction can allow oxygen to ingress deeper layers resulting in the weakening of enzymic latch (enhanced phenol oxidase activity), which will malfunction peatland carbon sequestration through peat oxidation.

Conceptual model of the enzymic latch mechanism

Natural and drained (degraded, peat extraction, and post-extracted unrestored) peatlands can have different enzymic latch mechanism (ELM) strengths (strong or weak) depending on water and oxygen availability. Essentially, the ELM is a group of constraints that exist in a cyclical process. Briefly, oxygen limitation impedes phenol oxidase activity which favours phenolic accumulation, which are potent inhibitor of hydrolase enzymes responsible for organic matter decomposition. To better understand ELM, please read Fig. 0.2 caption.

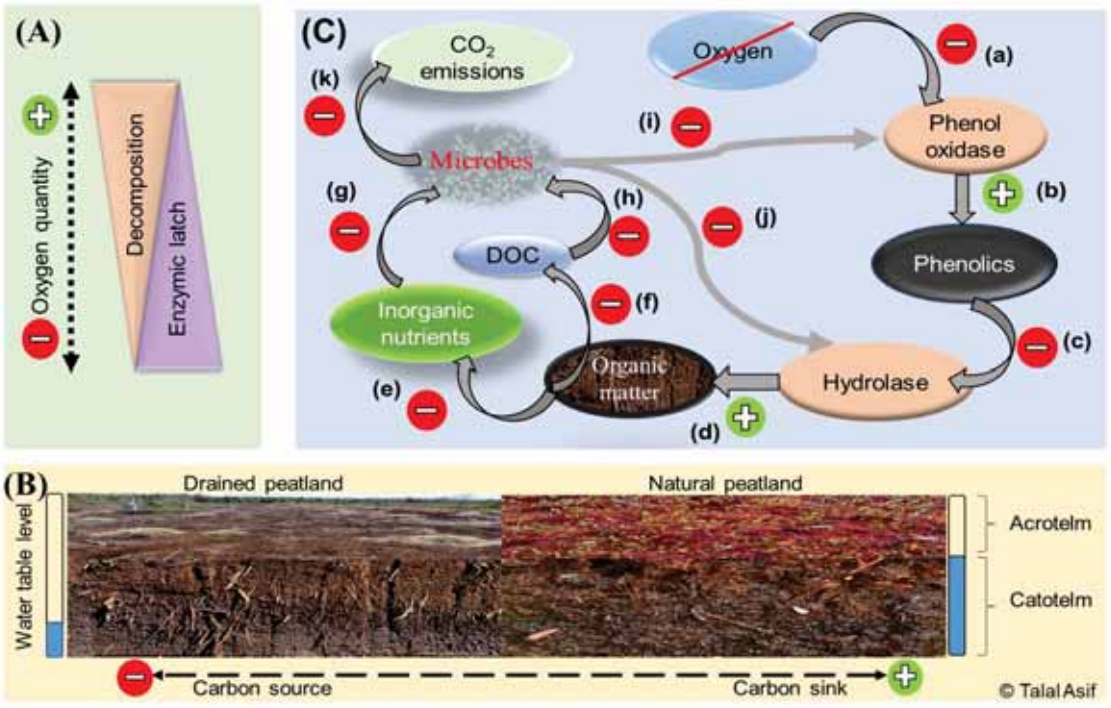

Fig. 0.2. Conceptual model of the enzymic latch mechanism (ELM). Adapted from the conceptual model of the ELM as proposed by Freeman et al. (2001 & 2012). In this regard, the ELM model is presented with three concepts such as 1) decomposition and ELM relationship in response to oxygen availability (Fig. 0.2A), 2) natural and drained peatland with an illustration of two proxies: water table level and carbon sink (Fig. 0.2B) and, 3) cyclical process of the ELM with the assumption of zero oxygen level (Fig. 0.2C). Positive (+) and negative (-) signs show an increase and decrease in quantity (Fig 0.2A & B), whereas positive (+) signs indicate an accumulation, and negative (-) signs indicate a limited response of variables caused by the former response variable (Fig. 0.2C). In Fig. 0.2C, at first, the absence of oxygen (anaerobic conditions) impedes phenol oxidase activity (a), inactive phenol oxidase and continuous anoxia cause accumulation of inhibitory phenolic compounds (b), higher phenolic concentration in turn limit hydrolase enzyme activities, the primary enzyme that causes organic matter to decay (c), limited activity of hydrolase enzymes favors organic matter accumulation (d), which results in immobilization of inorganic nutrients (e),

and dissolved organic carbon, DOC, (f), nutrient and DOC mobilization is important for microbes; however, their immobilization leads to reduced microbial activity (g & h), limited microbial activity will result in reduced de novo synthesis of phenol oxidase enzymes (i), hydrolase enzymes (j) and ultimately results in lower emissions (k).

Ecological engineering of decomposition: A solution for impeding enzymes

Rewetting – A building agent for phenolics

Rewetting, by raising the water table level, can reverse the drainage impact to some extent and assist in limiting CO_2 emissions from peatlands. Rewetting could be achieved by blocking ditches either by constructing dams or filling the ditches with peat (Fig.0.3 & 0.4).

Fig. 0.3. Drainage ditch blocking with the aid of excavator during winter of 2020. Photo credit: Kathy Pouliot, Peatland Ecology Research Group.

A shallower water table level will limit oxygen infiltration into peat layers resulting in reduced edaphic enzyme activities and de novo enzyme synthesis from fungi and bacteria. Based on ELM, phenolics in the peat profile will be accumulated and the enzymic latch will start to work cyclically if constraints continue to exist (Figure 0.2C). Rewetting post-extracted unrestored peatlands could restore ecological functioning, but it depends on the time since rewetting and site conditions (remaining peat depth, oxic layer depth above water table level, and peat quality). The lower water table level due to drainage or climate warming can cause peat subsidence and rewetting post-extracted unrestored peatlands can reverse this effect depending on the site conditions. Subsidence in peatland due to shrinkage and

compression are reversible. On the other hand, subsidence caused by peat oxidation is irreversible. For instance, extreme droughts cause a permanent change in peat structure that

Fig. 0.4. Stagnant water observed during summer of 2021in the ditch in response to dam construction.

fails to recover peat volume even after rewetting or site saturation (Price, 2003; Kennedy & Price, 2005). But, in case of shrinkage and compression along with low oxidation level continued water table level rise can lead to the accumulation of organic matter with limited GHG emissions (Price, 2003; Wilson et al., 2016). Rewetting effects can be different depending on the time scale and can improve the hydrological, biogeochemical, and ecological functioning of the peatlands. In the long term, it improves water table level, increases soil moisture content, and favours natural succession with the establishment of native species. Once anaerobic conditions are achieved, it further limits microbial activity

like natural peatlands (Freeman et al., 2001a; Urbanová & Bárta, 2020). In the short term, water fluctuation can disturb peatland functioning; for example, Kim et al. (2021) did a mesocosm experiment and discovered that rapid fluctuation of water table level inhibited *Sphagnum* growth and enhanced decomposition, which ultimately destabilized the carbon sink function of peatlands. In another study, relatively stable water table level fluctuation of less than 15 cm was found to accelerate CO_2 uptake and *Sphagnum* production in comparison to unstable water table fluctuation greater than 15 cm (Brown et al., 2017). It is, therefore, important to study the interaction of rewetting with phenolic additions in in-situ conditions for a better understanding of ELM.

Phenolics - A tool for strengthening enzymic latch mechanism

Phenolic compounds are a diverse group of secondary metabolites produced by plant metabolism. They are classified by the presence of hydroxyl group and derivatives attached to the aromatic ring. Based on chemical structure, they are categorized as phenolic acid, flavonoids, stilbenes, and lignans (Min et al., 2015; Dunn & Freeman, 2018). *Sphagnum* lacks lignin which is usually recalcitrant in the process of decomposition however they are rich in *Sphagnum* acid which is a phenol derivative. It is well reported that phenolics act as potent inhibition compounds against enzymes (van Breeman, 1995; Wetzel, 1992; Freeman et al., 2001). However, increased oxygen availability in peat profile due to anthropogenic activities has threatened the fate of phenolic amounts by increased extracellular enzyme activities. Based on ELM, it is assumed that the decomposition rate and carbon emissions in peatlands could be reduced by strengthening ELM with the aid of rewetting and phenolic addition. However, some of the studies contradict this theory as they showed no regulation of enzyme activities in response to soluble phenolics and speculated that changes in enzyme activities could be due to soil conditions (Sun et al., 2010; Romanowicz et al., 2015). Therefore, there is no general agreement that which *Sphagnum* compounds inhibit decomposition and specifically phenolics would have a key role in strengthening ELM. So, there is a need to revisit the role of phenolics in terms of the ELM on a large scale.

Phenolic supplements

In addition to available phenolics in peatland, an external phenolic supplement could help in regulating the decomposition process. The addition of external phenolics could add

constraints on microbial metabolism and eventually limit the de novo synthesis of extracellular enzymes from fungi and bacteria. At last, phenolic external additions could also slow down edaphic enzyme activities. Phenolic addition can be achieved either from natural products, commercially available phenols, or industrial waste (Fig. 0.5). For example, natural products include wood chips (Alshehri et al., 2020; Fenner & Freeman, 2020), wood by-products (sawdust, bark), and biochar from wood. Commercially available phenols include gallic acid, cinnamic acid, tannic acid, sodium lignosulphonate acid, calcium lignosulphonate acid, and others (Dunn & Freeman, 2018). Industrial waste is enriched with phenolics such as mixed sludge which is a by-product of the pulp and mill industry. In recent decades, studies have shown that added phenolics played a crucial role in limiting enzyme activities (Fenner & Freeman, 2011; Alshehri et al., 2020), whereas other studies found the opposite or no effect of external phenolic addition on enzyme activities (Sun et al., 2010, Urbanová & Hájek, 2021, Hájek & Urbanová, 2024). Though former studies provide a baseline for understanding enzyme response to externally added phenolic in laboratory-based experiments, however more evidence is required for testing and validating ELM at a large scale in natural conditions.

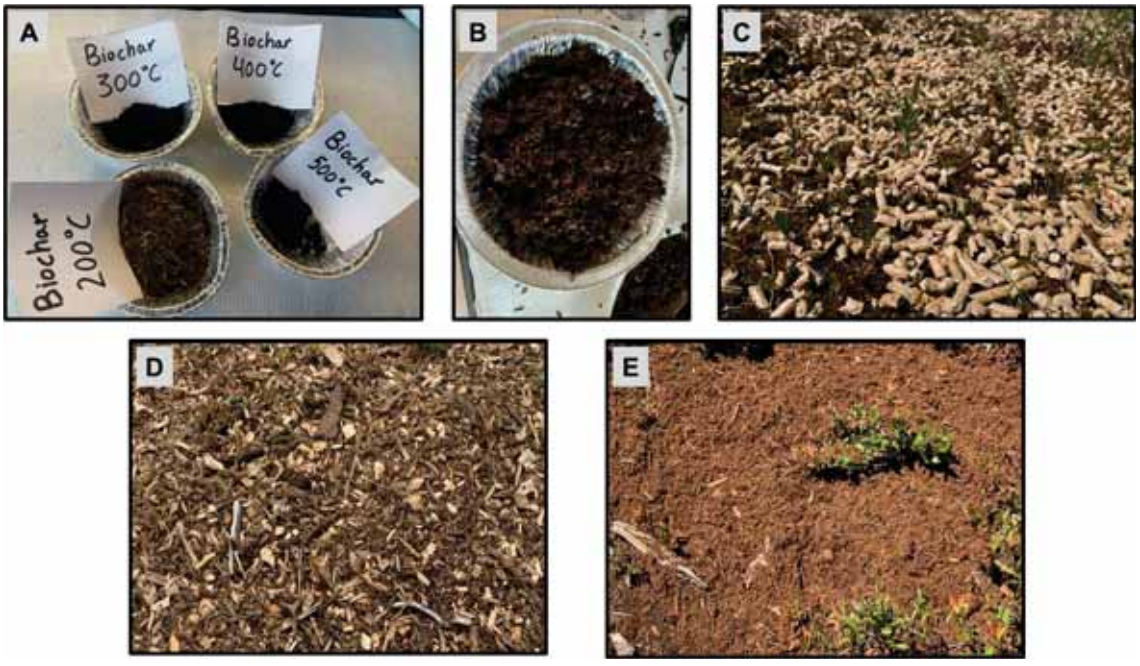

Fig. 0.5. Examples of phenolic products, wood biochar (A), cranberry (B), commercial wood pellets (C), aboveground fresh wood chips (D), and old ground roots from peat harrowing (E).

Drained peatlands

Status

Globally, 12% of the total peatland area (500 Mha) is degraded by anthropogenic activities to the extent that peat is no longer accumulated, and the remaining peat is continuously being oxidized thereby transforming peatlands from CO_2 sink to a source (Fig. 0.6). Peatland drainage and degradation, excluding fires, is responsible for approximately 2 Gt of CO_2 equivalent per year of GHG emissions, roughly representing about 4% of total global anthropogenic emissions. In Canada, 1.1% of the peatland area (119 Mha) has been disturbed by various anthropogenic activities such as agriculture, resource extraction (including horticulture peat extraction and oil mining), hydro dam construction, and forestry operations. Within the drained peatland area of Canada, horticultural peat extraction represents only 0.025 Mha, of which only 60% area is under active peat extraction and the rest is restored, reclaimed, or managed for other use (UNEP, 2022).

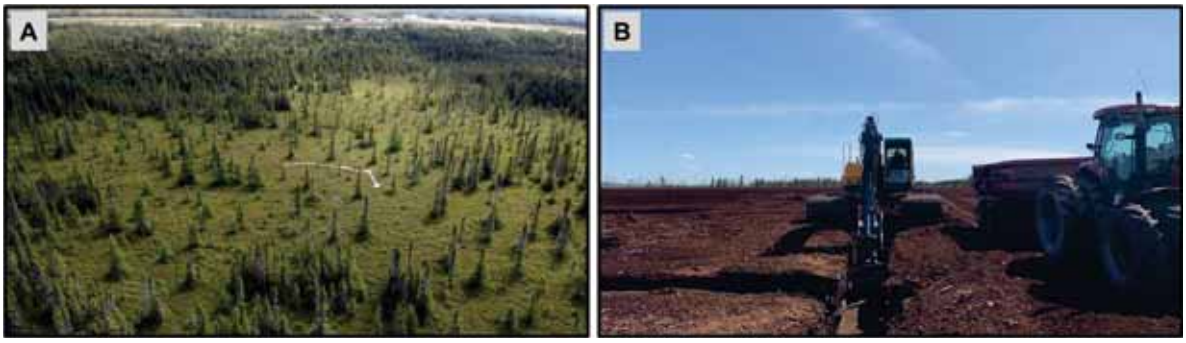

Fig. 0.6. Illustration of natural peatland (A) versus horticultural extracted peatland (B) where excavator is operating for cleaning drainage ditches.

Prior to peat extraction, peatlands are drained by constructing ditches followed by vegetation removal. Peatland drainage changes peat hydro-physical properties such as lowering water level, decreasing soil moisture content, and peat subsidence. For example, a lower water table level causes a reduction in peat volume, which is generally linked to shrinkage, compression, and oxidation of peat (Price, 1996, 1997, & 2003). Increased water table level fluctuation leads to the contraction in peat above the water table level and is termed as shrinkage, whereas compression refers to the stress change on peat below the water table level (Kennedy & Price, 2005; Kettridge et al., 2013; Liu et al., 2020). Peatland drainage and

extraction favour peat oxidation (aerobic decomposition) and so CO_2 release into the atmosphere. Consequently, peat extraction followed by industrial abandonment is a persistent source of CO_2 (Waddington et al., 2002).

Time lapse in post-extracted unrestored peatlands

Peatland extraction over a specific location usually lasts 20-50 years. But throughout the commercial life of a site, there can be some years where a particular deposit is not needed depending on economic demands (Fig. 0.7). Generally, unrestored peatlands result in higher CO_2 emissions and lower methane (CH_4) efflux (Strack et al., 2016). It is evident in the literature that after industrial abandonment GHG emissions differ between the ages of unrestored peatlands as there is a lot of yearly variation due to climatic conditions of a particular year. For instance, Waddington et al. (2002) estimated CO_2 emissions over two years from two post-extracted peatlands that differed in age, old site (7-8 years) and young site (2-3 years), since cessation of industrial activities. The first year was a dry year and had higher CO_2 emissions but still old site emitted higher CO_2 emissions for both years such as 399 g C m^{-1} $season^{-1}$ (1998), 112 g C m^{-1} $season^{-1}$ (1999) compared to young site 363 g C m^{-1} $season^{-1}$ (1998), 88 g C m^{-1} $season^{-1}$ (1999) However, on the other hand, sites following a degraded period of ~15-16 years depicted a significant differences in CO_2 emissions between the years due to seasonal climatic variation, for example an average CO_2 emissions of 173 g C m^{-2} y^{-1} (estimated in 2014) and 259 g C m^{-2} y^{-1} (estimated in 2015) to the atmosphere (Rankin et al., 2018). Logically, a lower water table level creates the oxic environment and stimulates enzymes or microbial activities which ultimately leads to higher CO_2 and lower CH_4 emissions (Freeman et al., 2001; Waddington & McNeil, 2002; Wilson et al., 2013). Conversely, other studies reported that long-term drainage suppressed enzymes and microbial activities of aged peat under the oxic environment which can diminish emissions of CO_2 (Croft et al., 2001; Urbanová & Bárta, 2016). Besides this conflict, it is suggested that CO_2 emissions from unrestored peatlands can be reduced if hydrological conditions return to a point that favours carbon accumulation following sustainable production of *Sphagnum* biomass (Joosten et al., 2012; Gaudig et al., 2018).

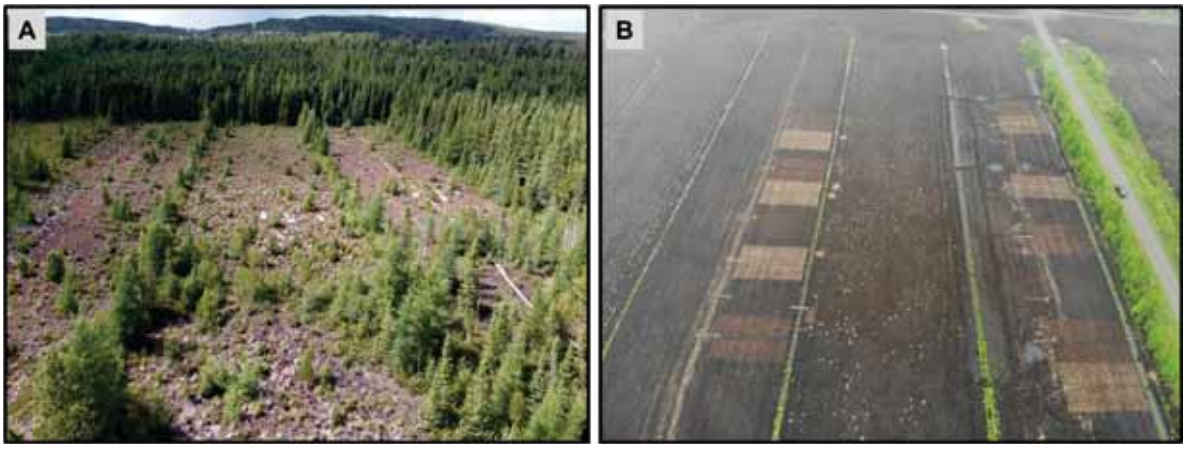

Fig. 0.7. Aerial view of two post-extracted unrestored peatland sectors in the Rivière-du-Loup peatland complex, where sectors represent 41 years (A) and less than 1 year (B) of cessation of industrial activities.

Sphagnum farming – the concept for sustainable use of post-extracted peatlands

Sphagnum farming is proposed to be a suitable alternative for rewetted post-extracted peatlands by which ecosystem services could be partially restored. For example, *Sphagnum* biomass accumulation can restore peat accumulation activity with limited decomposition and with time it can shift from a source of carbon to a sink, it can also help in water and nutrient regulation, and it can offer habitat for various biodiversity. Non-decomposed *Sphagnum* biomass is a valuable raw material for many purposes such as donor material for restoration, organic potting, floral moss (orchid propagation), and roofing (reeds). In addition, Sphagnum farming can also provide sustainable biomass as an alternate product that can replace extracted peat, perlite in horticulture substrates (Emmel, 2008; Reinikainen et al., 2012; Jobin et al., 2014; Müller & Glatzel, 2021) and ultimately can reduce pressure on peat extraction.

Global picture of Sphagnum farming

Currently, *Sphagnum* cultivation trials are in progress on degraded and cutover peatlands in Canada, Chile, Denmark, Finland, Germany, Ireland, Japan, Latvia, Lithuania, New Zealand, South Korea, and The Netherlands. In the coming decades, demand for non-decomposed *Sphagnum* biomass will continue to rise (Caron et al., 2015), therefore, research on optimizing *Sphagnum* farm productivity is gaining priority. A recent study on axenic in vitro propagation from a variety of *Sphagnum* species proposed that a sustainable supply of donor material in response to high demand is only possible through axenic propagations in

bioreactors (Heck et al., 2021). However, most studies are focused on the sustainable production of non-decomposed *Sphagnum* biomass in degraded or cutover peatlands (Landry & Rochefort, 2009; Pouliot et al., 2015; Gaudig et al., 2018). In this context, non-decomposed *Sphagnum* biomass collected from Sphagnum farming sites and natural peatlands resulted in similar *Sphagnum* establishment on rewetted peatland (Hugron & Rochefort, 2018).

In Canada, *Sphagnum* farms are constructed using the Moss Layer Transfer Technique with slight modification (Fig. 0.8, Rochefort et al., 2003; Quinty & Rochefort, 2003). Briefly, the site is prepared (including refreshing and reprofiling), basins, irrigation canals, and irrigation systems are constructed based on specific site characteristics. Afterward, donor material is spread with a straw mulch cover to improve microclimatic conditions to favour *Sphagnum* establishment. Phosphorous fertilization is used for large-scale peatland restoration where it helps the growth of *Polytrichum* species that limits frost heaving and stabilizes peat surface for *Sphagnum* growth. However, in Sphagnum farming system, phosphorous fertilization is not used as it could provide favourable growth conditions for plant species other than *Sphagnum*. Later, dams are constructed, and an automatic irrigation system supplies water from a nearby water source into the irrigation canals. It is important not to flood the basins as it can promote fungus growth and displace the *Sphagnum* and straw cover. Preferably, the water table level in the irrigation canal is maintained between 0 to -5 cm from the surface for optimum *Sphagnum* growth. For an overview of site and Sphagnum farming in Canada, see Gutierrez Pacheco et al. (2021) and Guêné-Nanchen & St-Hilaire (2022).

Fig. 0.8. Aerial view (A) and ground level view (B) of Sphagnum farming (A) where mosses of *Sphagnum* were cultivated in 2013.

***Sphagnum* biology**

The *Sphagnum* genus plays a vital role in the peatland ecosystem through its slow decomposition and higher productivity, leading to carbon storage as long-term peat deposits. Moss species of *Sphagnum* creates their specific habitat through acidification and waterlogging that does not permit most of the vascular plants and microorganisms to grow in their habitat (Rydin et al., 2006). However, *Sphagnum* growth and decay vary among species and have been widely studied either in-situ or ex-situ environments (Johnson and Damman, 1993; Gunnarsson, 2005; Bengtsson et al., 2016). In general, biotic (microorganisms) and abiotic (mean annual temperature, precipitation/water table, and intrinsic properties of the species) factors influence *Sphagnum* growth and decay dynamics (Coulson & Butterfield, 1978; Moore, 1989; Asada et al., 2003; Rydin & Jeglum, 2013). Among these, the water table level within the peatland holds a key role in controlling *Sphagnum* productivity and decomposition. For example, Weltzin et al. (2000) found that *Sphagnum* (*S. capillifolium*, *S. magellanicum,* and *S. fuscum* combined) productivity in wet treatment (311 g m^{-2} y^{-1}) was higher in comparison to intermediate (162 g m^{-2} y^{-1}) and dry (236 g m^{-2} y^{-1}) water treatments. Several studies have suggested that a stable water table level promotes *Sphagnum* growth and concurrently CO_2 sequestration (Silvola et al., 1996; Tuittila et al., 2004; Brown et al., 2017; Kim et al., 2021).

Furthermore, the three taxonomic groups of *Sphagnum* moss (Fig. 0.9, *Acutifolia*, *Sphagnum*, and *Cuspidata*) are spatially distributed on different topographic levels (hummocks, hollows, and lawns) based on biotic and abiotic factors. Mosses of *Sphagnum* subgenus can have different decay, productivity, and stem density patterns. Overall, moss species of *Acutifolia* subgenus are found in hummocks and lawns of peatlands where they form dense moss carpets and are tolerant to lower water table levels. Moss species of *Acutifolia* subgenus are characterized by lower decomposition, lower productivity, and high stem densities. Mosses from *Sphagnum* subgenus inhabit the lawns of peatlands where they have less dense moss carpet compared to the *Acutifolia* subgenus but tolerate variations in water table levels. *Sphagnum* subgenus also has a low decay rate compared to the *Cuspidata* group, but biomass production is greater than the *Acutifolia* subgenus. Conversely, mosses from *Cuspidata* subgenus reside in hollows where they have loose moss carpets that do not hold water close to the surface. Species of *Cuspidata* subgenus have in general higher

Fig. 0.9. Three taxonomic groups of *Sphagnum* moss: *Acutifolia* subgenus (A), *Sphagnum* subgenus (B-C), and *Cuspidata* subgenus (D). Photo credit: Gilles Ayotte, Université Laval, FSAA, Phytologie.

growth rate (stem elongation) productivity but the absence of water close to the surface results in frequent desiccation during the growing season and forms loose carpet mats, less resistant to desiccation. The absence of water close to the surface results in frequent desiccation events and consequently impacts the biomass accumulation potential (Rochefort et al., 1990; Johnson & Damman, 1991; Bengtsson et al., 2016). In a review, Moore et al. (1998) found a productivity and growth difference between the microtopographic position of the species. As, productivity (29-142 g m^{-2}) and growth (4-24 mm) — for lawn species: *S. angustifolium*, *S. warnstorfii*, *S. angustifolium* — and productivity (70-84 g m^{-2}) and growth (6-9 mm) for hummock species (*S. fuscum* and *S. capillifolium*). Asada et al. (2003) reported high productivity with low growth values of *Sphagnum* for hummock species in comparison to hollow and lawn species. For instance, Asada et al. (2003) measured production (mean

240 g m^{-2}) and growth (mean 15.5 mm) — for hummock species: *S. austinii*, *S. fuscum*, *S. rubellum*, and *S. papillosum* — and production (mean 200 g m^{-2}) and growth (mean 37 mm) for hollow and lawn species (*S. tenellum*, *S. pacificum,* and *S. lindbergii*).

In the Sphagnum farming system, studies have reported different *Sphagnum* moss productivity results that can be attributed to vast scale factors, such as climatic conditions, water table level and nutrient status. Therefore, it is important to choose the right species for cultivation based on specific objectives.

Sphagnum subgenus selection for cultivation

The selection of *Sphagnum* subgenus for cultivation in the Sphagnum farming system varies depending on the intended use of the biomass. Broadly, mosses from *Acutifolia* and *Sphagnum* subgenus are preferred over moss species of *Cuspidata* subgenus due to better quality biomass and lower decomposition rate. For instance, if the aim is to generate raw material for horticultural growing substrates and for peatland restoration, then moss species of *Acutifolia* and *Sphagnum* subgenus would be more appropriate as they are more tolerant to desiccation and produce better quality products. Hugron & Rochefort (2018) conducted a *Sphagnum* reintroduction experiment on a vacuum extracted peatland where they found that cultivated *Sphagnum* had similar regeneration capacity compared to the moss fragments from natural peatlands. Thus, the study suggested that cultivated *Sphagnum* could be used for peatland restoration. Numerous plant cultivation experiments have indicated that several moss species of *Acutifolia* and *Sphagnum* subgenus are suitable components for growing substrates. However, it's worth noting that the growth rate of plants may not be consistent when using substrates containing moss species from the *Cuspidata* subgenus (Gaudig et al., 2018).

To date, in Sphagnum farming system limited *Sphagnum* moss species have been cultivated on degraded and drained peatlands namely, *S. rubellum*, *S. papillosum*, *S. magellanicum*, *S. flavicomans*, *S. fuscum*, *S. fallax*, *S. fimbriatum*, *and S. palustre* (Krebs et al., 2012; Gaudig et al., 2014; Pouliot et al., 2015; Gaudig et al., 2017). Thus, there is sufficient room for conducting research on *Sphagnum* farms such as estimation of productivity and

decomposition along with GHG emissions under ELM at different stages of *Sphagnum* establishment.

Significance and implication of the study

Peat extraction operation on drained peatlands is usually active for 20 years on average. It ceases based on multiple reasons, such as when the remaining peat deposit is less than 40 cm, when the peat quality is not of adequate quality, when there is low economic demand or shortage of industrial resources. Such drained peatlands could be left unrestored while awaiting reactivation of the site with no management of protecting exposed peat from oxidation, leading to continuous CO_2 emissions. Rewetting creates anoxic conditions that inhibit extracellular enzymes and ultimately accumulate phenolic compounds that are potent inhibitors in the decomposition process. Although rewetting is a well-known approach to slow down decomposition, some outcomes of recent studies did not support the inhibitory roles of phenolic in peat decomposition (Sun et al., 2010; Urbanová & Hájek, 2021) as explained in ELM by Freeman et al. (2012). Therefore, we need to revisit the phenomena of rewetting along with phenolic addition to better understand the ELM in order to reduce exposed peat oxidation from peatlands which could be left unused for different period of time.

Sphagnum peat is a favoured component in growing media due to its unique qualities such as low bulk density, high water retention capacity but along with porous aeration, low pH, and slow rate of decomposition. In the last few decades, these unique properties along with low production costs created a high demand for *Sphagnum* peat growing media. Therefore, the high demand for peat resulted in a large volume of peat extraction from natural peatlands by the horticulture industry. To limit pressure on natural peatlands like peat harvesting and donor material collection, an alternate approach of cyclic and renewable *Sphagnum* biomass having high production and slow rate of decomposition is needed. In this context, there is a need to understand ELM's role through phenolic additions in limiting *Sphagnum* decomposition, leading to enhanced productivity and biomass accumulation in a Sphagnum farming system.

Research objectives

The overall objective of this thesis is to evaluate the effectiveness of rewetting (by dams) or enhanced rewetting (by irrigation system) along with phenolic additions on the strengthening of the enzymic latch mechanism (ELM) in limiting peat decomposition in two experimental models such as post-extracted unrestored peatlands and Sphagnum farming system in Québec, Canada (Fig. 0.10). Increasing the abundance of peat phenolics by rewetting and phenolic additions is a prerequisite for effectively testing ELM. To fill above-mentioned knowledge gap, experiments were conducted based on three sub-objectives to better understand ELM's role in limiting decomposition measured through proxies such as CO_2 flux and extracellular enzyme activities.

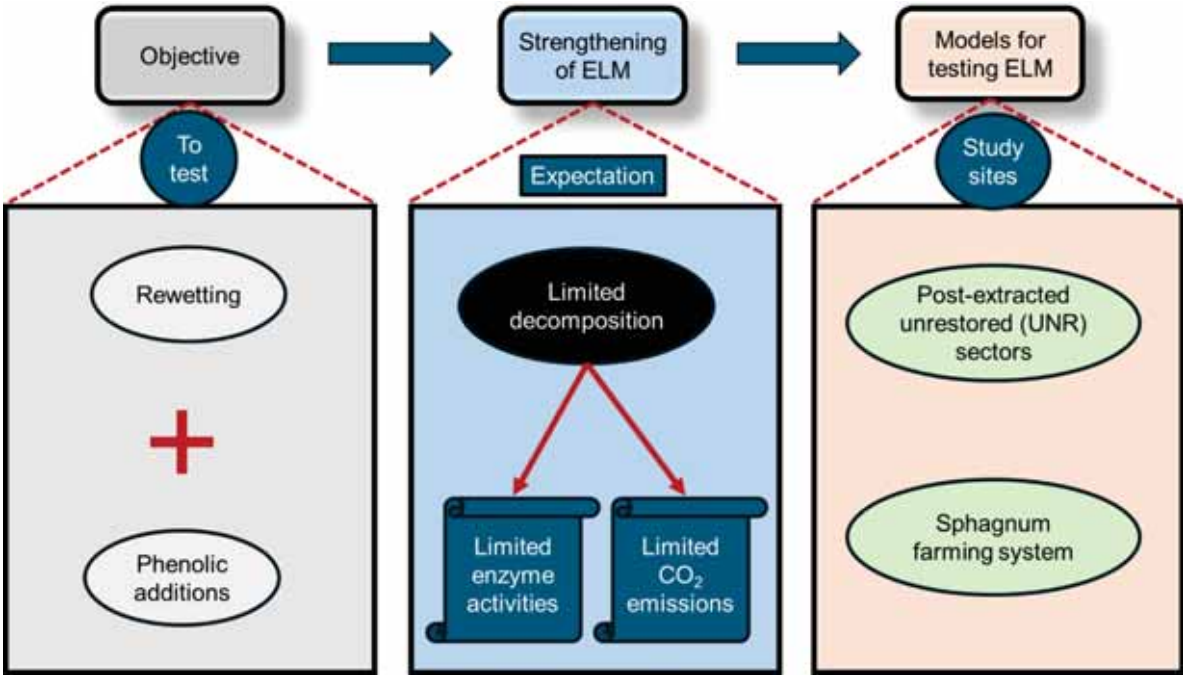

Fig. 0.10. Conceptual framework of the overall objective of the thesis concentrated on testing rewetting or enhanced rewetting with phenolic addition on a large scale to strengthen enzymic latch mechanism (ELM) and to test 1) how ELM can suppress enzyme activities and limit CO_2 emissions at post-extracted unrestored (UNR) peatlands that differed in age since cessation of industrial activities, 2) how ELM can reduce enzyme activities, limit CO_2 emissions, and promotes *Sphagnum* biomass accumulation in a Sphagnum farming system with different age (corresponding to the number of years of *Sphagnum* growth) of *Sphagnum* establishment.

The first sub-objective, which is addressed in Chapter 1, aimed to evaluate the impact of large-scale rewetting associated with phenolic addition on the strengthening of the ELM.

More specifically on the suppression of enzyme activities and decrease of CO_2 emissions at two post-extracted unrestored sectors (old and young), differing in age (UNR-1 yr and UNR-41 yr) since extraction activities ceased. Rewetting and phenolic addition effects were assessed mainly by peat respiration, peat soluble phenolics, and extracellular enzyme activities (Fig. 0.11).

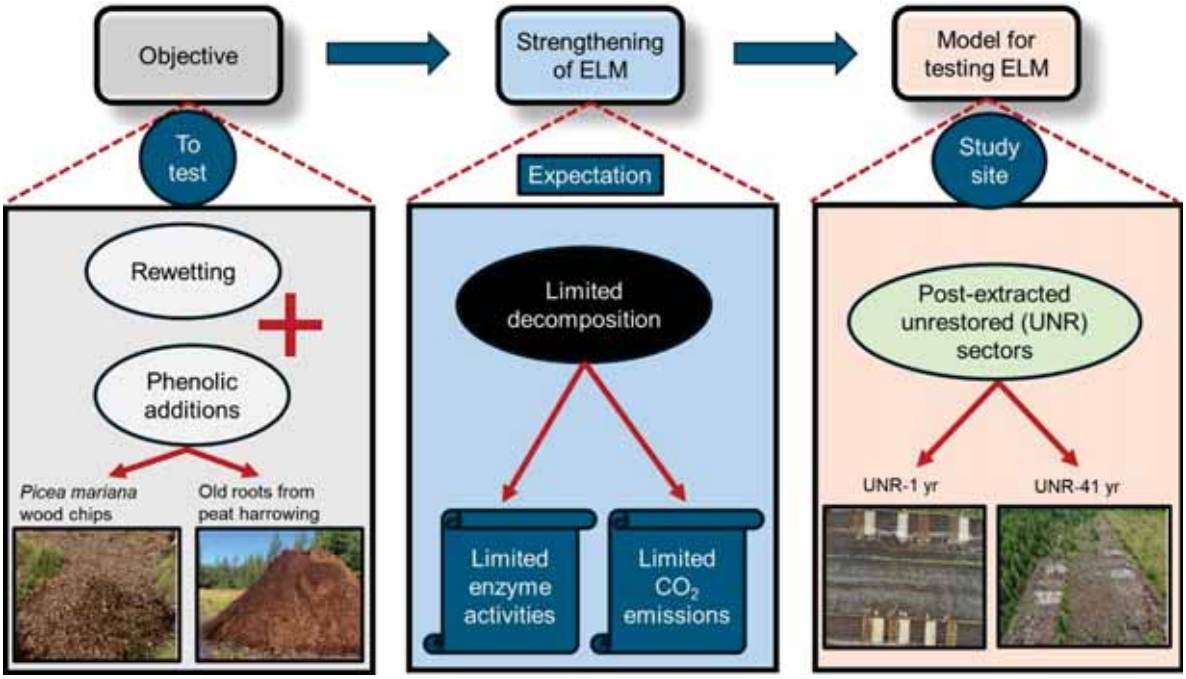

Fig. 0.11. Conceptual framework of the first sub-objective focused on testing rewetting with phenolic addition (*Picea mariana* aboveground fresh wood chips and old roots from peat harrowing) on a large scale to strengthen enzymic latch mechanism (ELM) and test how ELM can suppress enzyme activities and limit CO_2 emissions at two post-extracted unrestored (UNR) sectors (old and young), differing in age (UNR-1 yr and UNR-41 yr) since extraction activities ceased.

The second sub-objective, which is addressed in Chapter 2, aimed to evaluate the impact of phenolic additions to strengthen ELM, decrease *Sphagnum* decomposition, and therefore increase *Sphagnum* productivity and CO_2 uptake in the context of the two *Sphagnum* subgenus differing in their morphology (with the species tested) in a Sphagnum farming system. Phenolic addition effects were analyzed mainly by assessing net ecosystem exchange, peat soluble phenolics, and extracellular enzyme activities (Fig. 0.12).

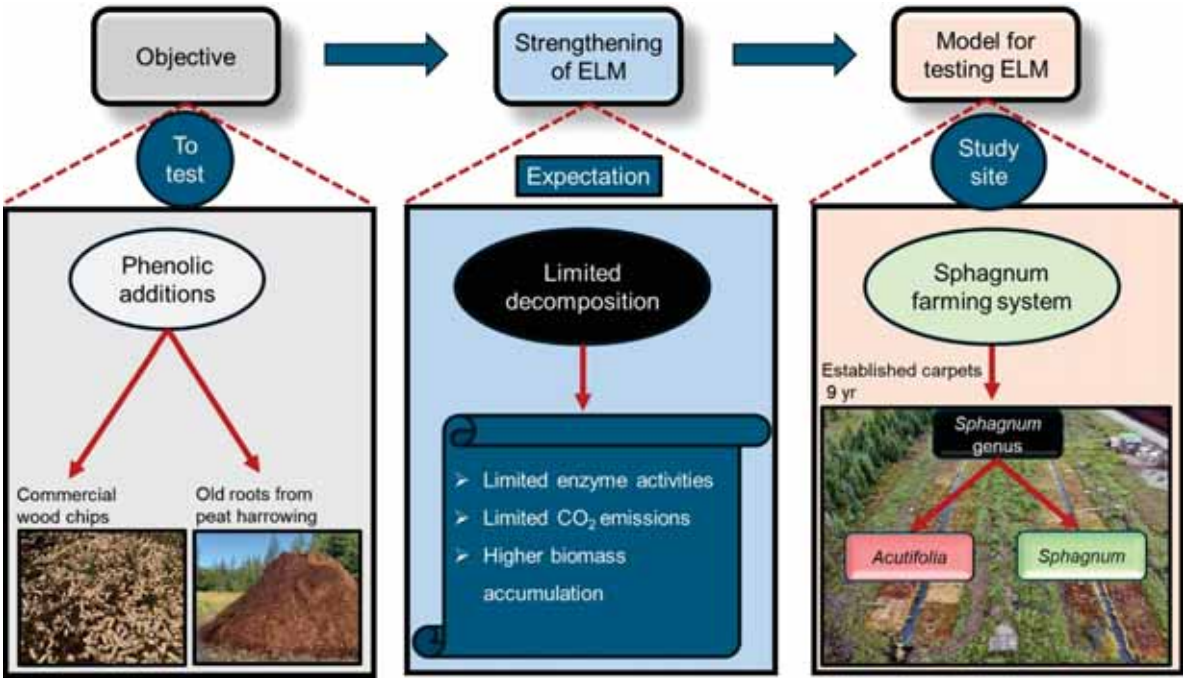

Fig. 0.12. Conceptual framework of the second sub-objective, which focused on testing phenolic addition (commercial wood pellets and old roots from peat harrowing) to strengthen enzymic latch mechanism (ELM) and test how ELM can reduce enzyme activities, limit CO_2 emissions, and promote *Sphagnum* biomass accumulation, thereby increasing *Sphagnum* productivity and CO_2 uptake in the context of the two widely structured *Sphagnum* subgenus (*Acutifolia* and *Sphagnum*) established in 2013 in a Sphagnum farming system.

The third sub-objective, which is addressed in Chapter 3, aimed to evaluate the role of phenolic additions on ELM's effectiveness in limiting *Acutifolia* subgenus decomposition and thereby enhancing biomass accumulation at two developmental stages — 1 yr-established carpet and 9 yr-established carpet — corresponding to the creation of the basins in a Sphagnum farming system. Like the second sub-objective, treatment effects were assessed mainly by net ecosystem exchange, peat soluble phenolics, and extracellular enzyme activities (Fig. 0.13).

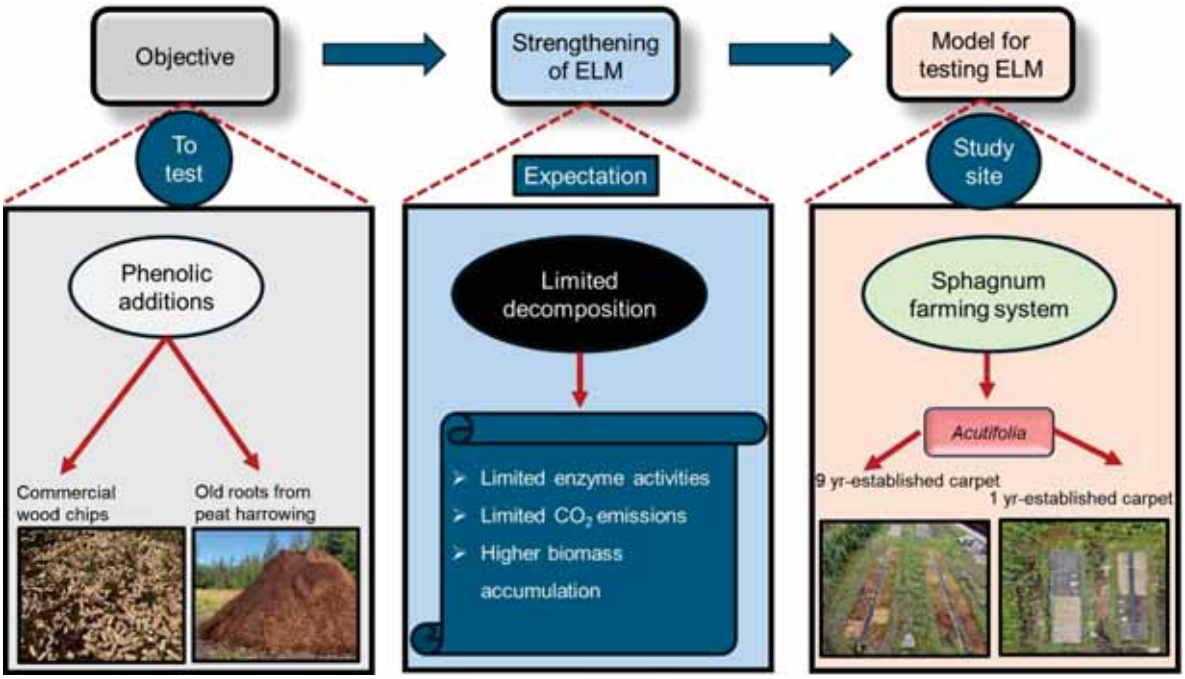

Fig. 0.13. Conceptual framework of the third sub-objective addresses to test strengthening of enzymic latch mechanism (ELM) via phenolic additions (commercial wood pellets and old roots from peat harrowing) and to see how ELM can reduce enzyme activities, limit CO_2 emissions, and promote *Sphagnum* biomass accumulation at two developmental stages — 1 yr-established carpet and 9 yr-established carpet — corresponding to the number of years of *Sphagnum* growth in a Sphagnum farming system.

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Chapter 1 Peatland ecological engineering: testing an approach to limit carbon dioxide emissions under enzymic latch mechanism in post-extracted unrestored peatlands

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1.1 Résumé

Cette étude a testé le remouillage de tourbières non restaurées après extraction de tourbe et l'ajout de composés phénoliques pour renforcer le mécanisme de verrou enzymatique (MVE) afin de protéger les dépôts de tourbe. Trois traitements phénoliques ont été évalués sur des secteurs non restaurés depuis 1 ou 41 ans: copeaux de *Picea mariana*, vieilles racines et témoin. Les performances ont été mesurées par le dioxyde de carbone (CO_2), les composés phénoliques et l'activité enzymatique. Le remouillage (-48 cm) a légèrement augmenté le niveau de la nappe phréatique mais a conduit à des émissions de CO_2 plus élevées, surtout dans le secteur non remouillé (-57 cm) d'un an. Contrairement aux attentes, les composés phénoliques n'ont ni augmenté la concentration en composés phénoliques solubles ni limité l'activité enzymatique, sauf pour l'arylsulphatase dans le secteur de 41 ans, sans réduire les émissions de CO_2. Le manque de conditions anoxiques pourrait expliquer l'échec du MVE.

1.2 Abstract

Enzyme activities and phenolics play a vital role in limiting organic matter decomposition in peatlands according to the enzymic latch mechanism (ELM). In this study, rewetting post-extracted peatlands along with phenolic additions was tested to strengthen ELM in order to protect exposed peat deposits awaiting restoration from further oxidation.

A rewetting experiment with the addition of three phenolic treatments was tested on two post-extracted unrestored (UNR) sectors (young and old) that differed in age (UNR-1 yr and UNR-41 yr) since the cessation of commercial extracting activities in Québec, Canada. Phenolic treatments included i) *Picea mariana* aboveground fresh wood chips (wood); ii) old roots from peat harrowing (root); and iii) no addition (control). The performance of the treatments was assessed mainly by measuring carbon dioxide (CO_2) flux, peat soluble phenolics, and extracellular enzyme activities.

Rewetting (-48 cm) compared to non-rewetted (-57 cm) plots slightly increased the water table level but showed higher CO_2 emissions at UNR-1 yr sector. Contrary to ELM, rewetting and phenolic addition did not enhance soluble phenolics, nor did they inhibit phenol oxidase, and hydrolase enzyme activities at both UNR sectors. An exception is for wood and root additions at the rewetted UNR-41 yr sector that limited arylsulphatase activity, but CO_2 emissions were not reduced. In the current study, we assume that phenolic product decomposition might have contributed to greater CO_2 emissions. Overall, failure in ELM detection close to the peat surface could be linked to the absence of anoxic conditions responsible for inhibiting phenol oxidase activity.

1.3 Introduction

Peatlands are unique ecosystems where net primary production exceeds decomposition over time, leading to the accumulation of organic matter (Gorham, 1991). Such an imbalance accounts for carbon (C) storage in peatlands in the range of 450 to 650 gigatons (Gt), with a major contribution of 400 to 550 Gt C from northern peatlands (FAO, 2020; UNEP, 2022). Globally, peatlands are drained for agriculture, forestry, resource exploration, and exploitation (mining, petrol or peat extraction) or lost through flooding by hydro dams and degraded by overgrazing. At present, 12% of global peatlands have been degraded and serve as carbon dioxide (CO_2) sources. Drained peatlands are responsible for about 4% (2 Gt CO_2e per year) of total global greenhouse gas (GHG) emissions (UNEP, 2022). Over the past few decades, on a small scale, ecological engineering tools such as rewetting, which raises water table (WT) level, and phenolic additions have been tested to evaluate the response of enzymes and microbes in limiting CO_2 emissions. However, contradictory results such as positive, negative, and neutral effects of peatland ecological engineering on enzymic latch mechanism (ELM) were presented from these experiments (Freeman et al., 2001; Fenner & Freeman, 2011; Wang et al., 2015; Fenner & Freeman, 2020; Alshehri et al., 2020; Urbanová & Hájek, 2021). Yet in the literature, it is unclear how ecological engineering tools can manipulate enzyme and microbial activities to limit CO_2 emissions from drained or unrestored peatlands.

In peatlands, a slow rate of decomposition is ascribed to abiotic factors like anoxia, low pH, nutrient immobilization, low temperature, high concentration of phenolics, and biotic factors such as limited microbial and extracellular enzyme (phenol oxidases and hydrolases) activities (Aerts, 1997; Freeman et al., 2001; Gartner & Cardon, 2004; Laiho, 2006). Among these factors, oxygen constraint on phenol oxidase enzyme was proposed to be an initial step for such a lower decomposition rate (Freeman et al., 2001). Phenol oxidase is an oxygen-dependent enzyme that can degrade phenolic compounds; however, anoxia limits its activities and helps to build up inhibitory phenolic compounds. Accumulation of phenolic compounds restricts the hydrolase enzyme, responsible for polysaccharide depolymerization and nutrient cycling (Freeman et al., 2001a; Dunn et al., 2014). All the constraints identified above were termed as the ‘enzymic latch’ mechanism (Fig. 1.1). However, drainage can reverse the enzymic latch process leading to enhanced peat decomposition and CO_2 emissions (Fenner & Freeman, 2011).

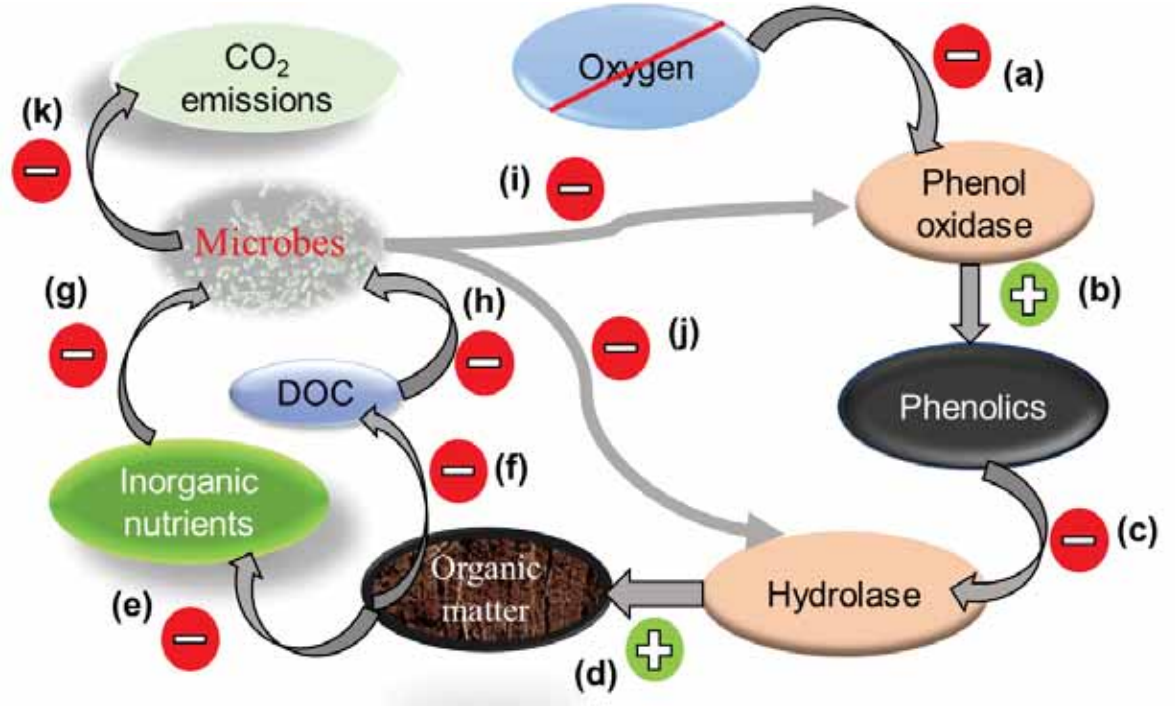

Fig. 1.1. Conceptual figure of the enzymic latch mechanism (ELM). The "–" sign indicates a constraint (negative effect) and "+" sign indicates an accumulation (positive effect). Briefly, steps involved in the ELM, oxygen absence (anaerobic conditions) impedes phenol oxidase (a) activity (an extracellular enzyme that breaks down phenolics). Inactive or limited phenol oxidase enzyme activities coupled with anoxia allow further accumulation of phenolics (b, inhibitory compounds). Phenolic accumulation in turn limits hydrolase enzyme activity (c, a primary enzyme that causes organic matter to decay). Limited activity of hydrolase enzyme favors organic matter accumulation (d) and nutrients (e) and DOC (f) immobilization. Nutrient and DOC release is important for microbes; however, their limited amount leads to reduced microbial activity (g & h). Further, reduced microbial activity will not be able to add up more enzymes (i & j Phenol oxidase & hydrolase) and ultimately results in lower carbon dioxide (CO_2) emissions (k). All the constraints identified above make up ELM.

In Canada, peatlands cover approximately 119 million hectares (ha), of which 35,314 ha have been extracted for horticulture purposes (Rochefort et al., 2022). Peatlands drained for horticultural peat extraction are closed when the residual peat deposit is less than 1 m in depth or when the peat quality declines below a certain level, for other technical reasons such as low economic demand for peat, lack of industrial resources (machinery, labor), and long-term planning (Premier Tech, Consumers and Growers, personal communication). These peat fields that are left unrestored for a moment (hereafter referred to as post-extracted unrestored) result in continuous CO_2 emissions (Strack et al., 2016). Oxygen ingresses into

drained peat layers and creates an oxic environment that stimulates enzyme and microbial activities, which in turn accelerates aerobic decomposition leading to higher CO_2 emissions (Freeman et al., 2001; Waddington & McNeil, 2002; Wilson et al., 2013). Furthermore, it is well documented that the amount of GHG emitted varies between post-extracted peatlands depending on their age (time since peat extracting activity ceased) due to climatic variations and hydro-physical properties of peat. For example, Waddington et al. (2002) reported CO_2 emissions over two years between two post-extracted peatlands that differed in age, young site (2-3 years) and old site (7-8 years). During the dry year, seasonal emissions were higher at the old site (399 g C m^{-2}) compared to the young site (363 g C m^{-2}). In the wet year, seasonal emissions were lower compared to the dry year, but still, seasonal emissions were greater at the old site (112 g C m^{-2}) compared to the young site (88 g C m^{-2}). On the other hand, Rankin et al. (2018) studied CO_2 emissions for two consecutive years from post-extracted peatlands that were subjected to 15-16 years of cessation in industrial activities. The authors reported CO_2 emissions of 173 g C m^{-2} y^{-1} and 259 g C m^{-2} y^{-1} in 2014 and 2015, respectively, differences most likely due to different seasonal climates.

The draining of peatlands affects their hydro-physical properties such as lowering WT level, decreasing peat moisture content, causing peat oxidation, subsidence, and compression, all effects leading to CO_2 release back into the atmosphere (Price, 1996; Price, 1997; Price, 2003; Waddington et al., 2002). However, rewetting can reverse the drainage impacts to some extent except for oxidative changes. Rewetting will limit oxygen infiltration into peat layers resulting in reduced edaphic enzyme activities and de novo enzyme synthesis (Freeman et al., 2012). Rewetting is a well-known approach to slow down decomposition, but its effects on CO_2 emissions depend on multiple factors, mainly the time since rewetting and site conditions (remaining peat depth, oxic layer depth above WT level, and peat quality). Phenolics act as potent inhibition compounds against enzymes and will be accumulated in response to rewetting as explained in ELM (van Breeman, 1995; Wetzel, 1992; Freeman et al., 2001). In addition to available phenolics in peatland, an external phenolic supplement could help in limiting de novo synthesis of extracellular enzymes. Sourcing of phenolic additions can be from natural products (above and belowground tree biomass), commercially available phenols, or industrial waste (Dunn et al., 2014; Alshehri et al., 2020; Fenner & Freeman, 2020; Urbanová & Hájek, 2021). Recently, a study found no effect of low and high

molecular weight humic substances, added as a phenolics supplement in peat samples, on enzyme activities (e.g. Urbanová & Hájek, 2021) while others found a significant effect of added phenolics on enzyme activities, where added phenolics represented wood chips of *Picea mariana*, *Thuja occidentalis*, and *Larix laricina* (e.g. Alshehri et al., 2020). A better understanding of ELM in response to ecological engineering tools is needed to formulate strategies for reducing CO_2 emissions from post-extracted unrestored peatlands.

The overall objective of the current study was to evaluate the impact of rewetting associated with phenolic addition on the strengthening of the ELM, and more specifically on the suppression of enzyme activities and decrease of CO_2 emissions at two post-extracted unrestored peatlands of different ages since extraction activities ceased. Precisely, we aim to answer the following questions: 1) What are the mean CO_2 fluxes, enzyme activities, and peat soluble phenolics at both post-extracted unrestored sectors? 2) Do CO_2 fluxes, enzyme activities, peat soluble phenolics, and WT levels differ between rewetted and non-rewetted plots or among phenolic treatments? 3) What are the driving factors of ELM, and do they vary between post-extracted unrestored sectors, rewetted and non-rewetted plots? We hypothesized that: 1) The young unrestored sector would have higher CO_2 emissions compared to the old unrestored sector. 2) Rewetting would limit CO_2 emissions and enzyme activities compared to non-rewetting. 3) Rewetting along with phenolic addition would strengthen the enzymic latch mechanism compared to non-rewetting.

1.4 Methods

1.4.1 Study sites

In 2021, two post-extracted unrestored (UNR) peatland sectors in the Rivière-du-Loup peatland complex (Québec, Canada) were selected based on the number of years since the cessation of peat extraction activity (hereafter referred to as age). The first UNR sector representing the young UNR sector within the Verbois peatland was selected where horticultural peat extraction was carried out from 1979 to 2013 and later had been left unrestored. However, industrial harrowing started again in Verbois during the spring of 2021 for reprofiling but immediately stopped for our study purposes. Now, the young UNR sector represents less than 1 year of cessation of industrial activities (later referred to as "UNR-1 yr"; 47°50′ N, 69°26′ W). The UNR-1 yr sector had only bare peat. The second UNR sector

within the Bois-des-Bel (BDB) peatland was chosen to serve as an old UNR sector where horticultural peat was extracted from 1972 to 1980. It has since remained unrestored for 41 years following the cessation of peat extraction activities (hereafter referred to as "UNR-41 yr"; 47°58′ N, 69°25′ W). With time, spontaneous vegetation had colonized the UNR-41 yr sector with dominant species being *Kalmia angustifolia*, *Rhododendron groenlandicum*, *Vaccinium angustifolium*, *Eriophorum vaginatum*, *Betula populifolia*, *Picea mariana*, *Larix laricina* and no recovery of the *Sphagnum* moss carpet. In the current study, three peat fields were chosen at both UNR sectors for the implementation of the experimental design. The peat depth was on average 2.5 m and 0.9 m at the UNR-1 yr and UNR-41 yr sectors. Differences in peat thickness at both UNR sectors are due to peat extraction and subsidence.

1.4.2 Experimental design

The experiment was implemented to represent a split-split plot design at both UNR sectors, with age and rewetting treatment as the main plot factor, replications as the subplot factor, and phenolic addition as the sub-subplot factor. For the rewetting treatment, at both UNR sectors, dams were constructed by blocking ditches in the fall of 2020 at only one peat field (hereafter referred to as rewetted plots). Dams were constructed after every -20 cm decline in altitude from a reference point. At both UNR sectors, ditches were voluntarily not blocked at one of the three peat fields (hereafter called as non-rewetted plots). Rewetted plots and non-rewetted plots (with active ditches) were separated by a buffer peat field. To be clear, rewetting should not be confused with flooding. In the North American context, rewetting refers to the raising of the WT level as much as possible close to the surface without flooding as often seen in peatland rewetting projects in Europe. For the phenolic treatments, two products were chosen that were locally available and had different concentrations of soluble phenolics: *Picea mariana* aboveground fresh wood chips (wood; 1.245 mg g^{-1} phenolics) and *Picea mariana* old roots from peat harrowing (root; 0.159 mg g^{-1} phenolics) extracted from peat fields. Both these products were chipped using a commercial chipping machine. The wood chips measured approximately 2.5 cm × 2.5 cm ± 0.2, while the root chips were fibrous, ranging from 0.3 mm × 0.1 mm ± 0.05. Based on previous greenhouse experiments and personal field experience, a 2 kg m^{-2} fresh weight dosage of phenolic products was opted. On both UNR sectors (except buffer peat field), phenolic treatments were applied on 18 m × 10 m bare peat plots (vegetation was excluded in UNR-41 yr sector) with a fresh weight dosage

of 2 kg m^{-2} in June of 2021. A control plot with no phenolic addition was also included. In total, this study included 36 experimental units: 2 (age) × 2 (rewetting treatments) × 3 (phenolic treatments) × 3 (phenolic treatment replications). In each experimental unit, all measurements and sampling were done at approximately 15 m distance perpendicular to the ditches (Fig. A1.1)

1.4.3 Soil respiration

Soil respiration (SR; g CO_2 $m^{-2}d^{-1}$) from bare peat plots was measured bi-monthly during the growing season of 2022 (May to August/September). The opaque closed chamber method was used to determine SR. Stainless steel collars, 60 cm × 60 cm × 20 cm (height), with grooves on top were inserted in bare peat at a 15 m distance perpendicular to the ditch. The opaque chamber (60 cm × 60 cm × 30 cm) was equipped with two computer fans (10 cm × 10 cm), thermocouple wire, and two small openings for air circulation, temperature, and gas exchange, respectively. The chamber was then fitted with portable infrared gas analyser (IRGA; EGM-4 PP systems USA) for CO_2 measurements (ppm). Prior to CO_2 measurements, the chamber was lifted, and headspace air was circulated by running fans with a 12 V battery to confirm ambient CO_2 concentration and temperature at the collar. Finally, the chamber was placed on a collar groove filled with water to ensure no gas leakage and CO_2 measurement was recorded at 15-second intervals from 0-2 minutes. Measurements were repeated if CO_2 concentrations were changing higher than 8-10 ppm after each 15-second interval. Together with CO_2 concentration, the air temperature inside the chamber was also recorded using digital temperature reader (Omega HH200). The linear change in CO_2 concentration was used to calculate SR and corrected for chamber temperature and volume. Non-linear fluxes were rejected at $R^2 < 0.80$. However, fluxes were retained if the overall change during measurement was less than 2 ppm. A minimum of five CO_2 measurements were required for analysis from each experimental unit. The conventional sign method was used where positive values indicated CO_2 emissions from the ecosystem to the atmosphere.

1.4.4 Environmental variables

During each SR measurement, water table (WT) level and soil temperature were also recorded close to the collars. For the WT, PVC wells, 2-inch diameter and > 1-meter length, were installed adjacent to the collars and monitored manually. Soil temperatures at -2 cm, -

5cm, -10 cm, -15 cm, and -20 cm peat depth were measured using thermocouple probes (Digi-Sense, Cole-Parmer) connected with a digital temperature reader (Omega HH200).

1.5 Chemical analyses

1.4.5.1 Peat samples

In the fall of 2022, a composite sample of peat (a mix of 6 individual collected samples separated by a 1 m distance in a transect line, marked at a distance of 15 m parallel to the ditch) was collected from the top 5-10 cm surface adjacent to the collars of each experimental unit. At the time of peat sampling, the soil temperature required for enzyme analysis was also recorded using thermocouple probes (Digi-Sense, Cole-Parmer) connected with a digital temperature reader (Omega HH200). During sample collection, vinyl gloves were used with 70% isopropyl alcohol to avoid contamination. Samples for phenolics and enzyme analyses were stored at 4 °C whereas, for other analyses samples were stored at -20 °C until further processing.

1.4.5.2 Enzyme, peat soluble phenolics, and elemental analyses

Peat extracellular hydrolase (β-D-glucosidase, arylsulphatase, N-acetyl-β-D-glucosaminidase, β-D-xylosidase, phosphatase) and phenol oxidase activities were measured according to Dunn et al. (2014) at Bangor University within two weeks of peat sampling. Peat soluble phenolics were measured using the water extraction method explained by Alshehri et al. (2020). Peat samples were oven dried at 105 °C for elemental analyses. Total carbon and nitrogen were analyzed in TruMAC CNS analyzer (LECO corporation, USA). Ammonium ion, nitrate ion, and sulphate ion analyses were done in QuikChem 8500 Series 2 FIA automated ion analyzer (Lachat Instruments, USA). Phosphate ion (P/PO_4^{3-}) was analyzed with 5110 ICP-OES analyzer (Agilent, USA). Peat slurries were made for pH and electrical conductivity analyses with accumet AB200 benchtop pH and EC meter (Fisherbrand, USA).

1.4.5.3 Water samples

Water samples from all experimental units were also collected in the fall of 2022 for dissolved organic carbon (DOC) analysis and stored at -20 °C till further processing. Before

DOC analysis, water samples were filtered using 0.45 µm syringe filter. Later, samples were analyzed in TOC-VCSN analyzer (Shimadzu, Japan).

1.6 Statistical analyses

The data, measured within the split-split plot design, were analyzed using linear mixed effects (LME) models from lme4 package (Bates et al., 2015) in R (R core team, 2023). To estimate differences in SR and enzyme activities, LME models were constructed with age, rewetting, phenolic treatments, and their interactions as fixed factors. Whereas random effects in LME models were based on repeated measurement presence or absence and error terms estimation for split-split plot design. For example, SR data from each experimental unit was comprised of several measurements over time (pseudo-replications). Therefore, random effects included were as "(1|age:rewetting:replicate) + (1|age:rewetting:replicate:phenolic treatments)". On the other hand, enzyme activities did not have pseudo-replications, so random effects included were as "(1|age:rewetting:replicate)". Normality and homogeneity of residuals were inspected visually for all models. For extracting model F-values and p-values, joint_tests function in emmeans package (Lenth, 2023) was used. With significant main effects, Tukey pairwise comparisons were completed using emmeans and compact letter display (cld) function in multcomp (Hothorn et al., 2008) packages. In case of the significant interaction term, a one-way Anova with Tukey pairwise comparisons were completed. Pearson correlation was used for estimating the correlations between response variables using the cor command in R. All figures were created with ggplot2 package (Wickham, 2016) and statistics were reported with a significant level of 0.05.

1.5 Results

1.5.1 Hydrological and physicochemical characteristics

1.5.1.1 Water table conditions

At UNR-1 yr sector rewetted plots showed significantly shallower mean WT (-48.0 ± 1.0 cm) than non-rewetted plots (-56.5 ± 1.2 cm; $p = 0.003$). While rewetted plots (-45.3 ± 1.1 cm) at the UNR-41 yr sector did not show a difference in mean WT than non-rewetted plots (Tables 1.1 & A1.1, -46.4 ± 2.5 cm, $p > 0.05$).

Table 1.1. Chemical properties of surface composite peat sample and water table conditions (measured from May to August) of two post-extracted unrestored (UNR) sectors (UNR-1 yr, and UNR-41 yr), that differed in age since the cessation of commercial peat extracting activities, among phenolic treatments. Control = no addition, Root = old roots from peat harrowing, and Wood = *Picea mariana* aboveground fresh wood chips. Observed variables represent average values ± standard errors of: the water table (WT; negative values indicate a WT below the peat surface, n = 3), potential of hydrogen (pH, n = 3), electrical conductivity (EC, n = 3), carbon and nitrogen ratio (C/N ratio, n = 3), ammonium ion (NH_4^+, n = 3), nitrate ion (NH_3^-, n = 3), sulphate ion (SO_4^{-2}, n = 3), and phosphate ion (PO_4^{3-}, n = 3). The presence of different lowercase letters indicates differences among phenolic treatments (One-way ANOVA, $p < 0.05$) based on the significant three-way interactive effect of age, rewetting, and phenolic treatments on observed variables. See Table A1.1 for detailed statistical analysis.

UNR sectors	Rewetting status	Phenolic treatments	WT (cm)	pH	EC (µS cm^{-1})	C/N ratio	NH_4^+ (ppm)	NH_3^- (ppm)	SO_4^{-2} (ppm)	PO_4^{3-} (ppm)
UNR-1 yr	Non-rewetted	Control	-56.1 ± 2.4	3.9 ± 0.1	74 ± 12	63 ± 1	98 ± 30	15 ± 1	885 ± 13	7 ± 1
		Root	-57.7 ± 0.3	3.9 ± 0.1	64 ± 13	59 ± 2	92 ± 33	11 ± 1	949 ± 51	8 ± 0.3
		Wood	-55.7 ± 3.4	4.1 ± 0.1	84 ± 16	61 ± 1	49 ± 15	11 ± 1	859 ± 51	9 ± 1
	Rewetted	Control	-46.4 ± 0.9	3.9 ± 0.1	94 ± 9	56 ± 1	107 ± 7	20 ± 3	1104 ± 93	7 ± 0.3
		Root	-48.3 ± 2.5	3.8 ± 0.02	84 ± 16	57 ± 1	100 ± 12	16 ± 1	1186 ± 47	7 ± 0.4
		Wood	-49.4 ± 1.3	4.0 ± 0.04	70 ± 4	56 ± 2	43 ± 13	13 ± 1	1159 ± 118	8 ± 0.1
		Control	-38.7 ± 2.5	3.8 ± 0.1	73 ± 5	51 ± 3	86 ± 19	12 ± 3	1238 ± 272	15 ± 2
		Root	-51.6 ± 1.2	3.8 ± 0.1	36 ± 8	57 ± 6	89 ± 12	11 ± 2	1033 ± 118	12 ± 2

UNR-41 yr	Non-rewetted	Wood	-48.9 ± 4.9	3.9 ± 0.1	54 ± 16	56 ± 7	62 ± 6	11 ± 0.3	904 ± 182	14 ± 1
	Rewetted	Control	-45.5 ± 3.2	4.0 ± 0.1	61 ± 14	57 ± 5	108 ± 14	**16 ± 4 b**	923 ± 159	**13 ± 1 ab**
		Root	-43.6 ± 1.0	3.9 ± 0.1	60 ± 7	56 ± 5	65 ± 14	**19 ± 3 b**	1010 ± 234	**16 ± 1 a**
		Wood	-46.7 ± 1.5	4.0 ± 0.1	61 ± 7	62 ± 3	94 ± 14	**36 ± 4 a**	1085 ± 93	**11 ± 1 b**

1.5.1.1 Physico-chemical conditions

Among phenolic treatments, the pH value was only 5.2% higher at wood addition subplots than the root subplots (Table A1.1). For EC, UNR-1 yr sector had a 36% higher value compared to UNR-41 yr sector (Table A1.1). A two-way interaction effect of age and phenolic treatment on NH_4^+ revealed that wood addition subplots had lower concentrations of NH_4^+ than control by 55% and root by 52% subplots at UNR-1 yr sector (Tables 1.1 & A1.1). In the case of NH_3^-, a three-way interactive effect among age, rewetting, and phenolic treatment was detected (Table A1.1). This interaction effect was explained by the wood addition at the rewetted plot of the UNR-41 yr sector where an increase in mean NH_3^- concentration from root (92%) and control (126%) subplots was observed (Table 1.1, $F = 21.5$, $p < 0.0001$). A least three-way interactive effect of age, rewetting, and phenolic treatment on PO_4^{3-} (Table A1.1) revealed that wood addition showed a reduction of 30.5% in mean PO_4^{3-} concentration from root addition at the rewetted plot of the UNR-41 yr sector (Table A1.1, $F = 6.8$, $p = 0.007$). At rewetted and non-rewetted plots of both UNR sectors, the mean values of pH, EC, C/N ratio, NH_4^+, and SO_4^{-2} were not different among the phenolic treatments (Tables 1.1 & A1.1, $p > 0.05$).

1.5.2 Soil respiration

At the UNR-41 yr sector, the mean value of soil respiration (SR) was 19% greater compared to the UNR-1 yr sector (Table 1.2, $F = 7.1$, $p = 0.03$). There was an interaction effect of age and rewetting on SR (Table 1.2, $F = 5.7$, $p = 0.04$). One-way ANOVA indicated that at the rewetted plots of UNR-1 yr sector, mean SR was 30% higher compared to non-rewetted plots (Fig. 1.2, $F = 6.8$, $p = 0.03$). Whereas, at the UNR-41 yr sector mean SR was not different between rewetted and non-rewetted plots. There was also an age-phenolic treatment interaction effect on SR (Table 1.2, $F = 4.4$, $p = 0.03$) with root (85%) and wood (76%) additions having increased mean value of SR relative to control at the UNR-41 yr sector (Fig. 1.2, $F = 22.5$, $p < 0.0001$), while wood addition at the UNR-1 yr sector showed a 46% increase in mean SR from the control (Fig. 1.2, $F = 5.8$, $p = 0.013$). No significant interaction between rewetting-phenolic treatments and nor any three-way interaction among age, rewetting, and phenolic treatments was detected on SR (Table 1.2).

Table 1.2. Linear mixed effects model to determine the main and interactive effects of age (UNR-1 yr and UNR-41 yr), rewetting (non-rewetted and rewetted plots), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) on soil respiration (SR, g CO_2 $m^{-2}d^{-1}$). In the source's column, A = age, R = rewetting, and P = phenolic treatments.

Sources	d.f	MS	*F*	*P*
Age[1]	1	60	7.1	**0.03**
Rewetting	1	15	1.7	0.23
A × R	1	48	5.7	**0.04**
Error a	8	8.5		
Phenolic treatments	2	203	23.9	**<.0001**
A × P	2	38	4.4	**0.03**
R × P	2	15	1.7	0.21
A × R × P	2	21	2.4	0.12
Error b	16	8.5		

[1]Age represents the number of years since the cessation of commercial peat extracting activities at post-extracted unrestored (UNR) sectors.

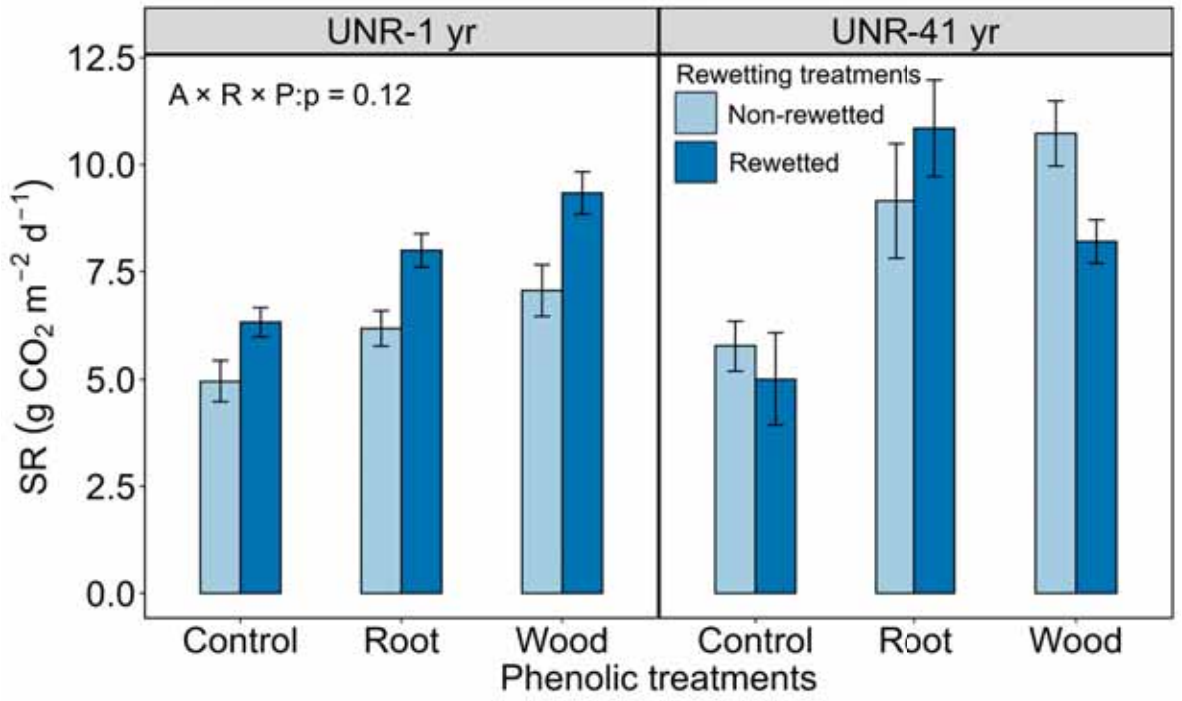

Fig. 1.2. Mean soil respiration (SR; g CO_2 $m^{-2}d^{-1}$, n = 3) measured during May to August of 2022 from phenolic treatments sub-plots (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) of non-rewetted and rewetted plots at two post-extracted unrestored (UNR) sectors (young and old) that differed in age (UNR-1 yr and UNR-41 yr) since the cessation of commercial peat extracting activities. Positive values represent a release of CO_2 from the ecosystem. Error bars represent the standard error of the mean. See Table 1.2 for detailed statistical analysis.

1.5.3 Peat soluble phenolics

The mean phenolic concentration was higher at the UNR-1 yr sector in relation to the UNR-41 yr sector by 33% UNR-41 yr (Table 1.3, $F = 72.7$, $p < 0.0001$). There was a significant main effect of rewetting, and phenolic treatments on peat phenolics (Table 1.3). The least interaction effect of age and phenolic treatments was detected on peat phenolics (Table 1.3, $F = 3.7$, $p = 0.046$), indicating that at the UNR-41 yr sector, root addition resulted in 25% more average peat phenolics compared to wood addition, but phenolic additions were similar to control in mean peat phenolics (Fig. 1.3, $F = 5.2$, $p = 0.018$). However, at the UNR-1 yr sector, wood addition showed lower mean peat phenolics by 23% from control and 17% from root subplots (Fig. 1.3, $F = 10.4$, $p = 0.0013$). There was also an interaction effect of rewetting and phenolic treatments on peat phenolics (Table 1.3). At non-rewetted plots, the mean peat phenolics were 20% more at root addition compared to control and wood subplots while the later two treatments were not different (Fig. 1.3, $F = 7.1$, $p = 0.006$). At rewetted plots, wood addition showed a decrease in mean peat phenolics by 18% from control and 10% from root subplots but later two treatments had no difference (Fig. 1.3, $F = 8.7$, $p = 0.003$).

Table 1.3. Linear mixed effects model to determine the main and interactive effects of age (UNR-1 yr and UNR-41 yr), rewetting (non-rewetted and rewetted plots), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) on peat soluble phenolics (mg g^{-1}). In the source's column, A = age, R = rewetting, and P = phenolic treatments.

Sources	**d.f**	**MS**	***F***	***P***
Age[1]	1	0.007	72.7	**<.0001**
Rewetting	1	0.0005	5.6	**0.045**
A × R	1	0.00009	1	0.36
Error a	8	0.00009		
Phenolic treatments	2	0.001	11.8	**0.001**
A × P	2	0.0003	3.7	**0.046**
R × P	2	0.0004	3.9	**0.04**
A × R × P	2	0.00003	0.3	0.73
Error b	16	0.00009		

[1]Age represents the number of years since the cessation of commercial peat extracting activities at post-extracted unrestored (UNR) sectors.

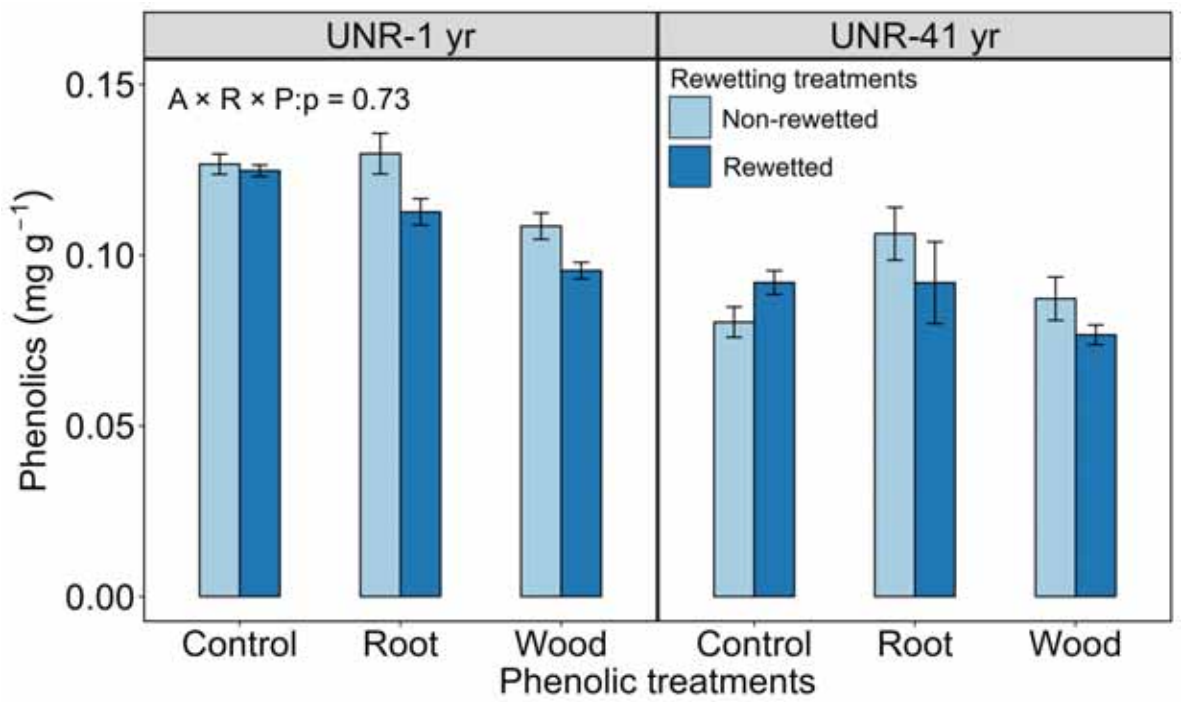

Fig. 1.3. Mean peat soluble phenolics (mg g^{-1}, n = 3) of surface composite peat sample, collected in the fall of 2022 from phenolic treatments sub-plots (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) of non-rewetted and rewetted plots at two post-extracted unrestored (UNR) sectors (young and old) that differed in age (UNR-1 yr and UNR-41 yr) since the cessation of commercial peat extracting activities. Error bars represent the standard error of the mean. See Table 1.3 for detailed statistical analysis.

1.5.4 Enzyme activities

1.5.4.1 Hydrolase enzyme activities

On average, hydrolase activities for all five enzymes were higher at the UNR-41 yr sector (6 ± 0.4 nmol g^{-1} min^{-1}) than UNR-1 yr sector (2 ± 0.1 nmol g^{-1} min^{-1}; Fig. 1.4, $p < 0.0001$). Specifically, mean β-D-glucosidase activities were higher at the UNR-41 yr sector compared to the UNR-1 yr sector by 197% (Table 1.4, Fig. 1.4A, $F = 78.8$, $p < 0.0001$). The mean β-D-glucosidase activities were least significantly higher at rewetted plots compared to non-rewetted plots by only 31% (Table 1.4). There was no interaction effect of age, rewetting, and phenolic treatments on β-D-glucosidase activities, indicating that β-D-glucosidase activities among phenolic treatments were non-significantly different at rewetted and non-rewetted plots of both sectors (Table 1.4). Like β-D-glucosidase activities, mean arylsulphatase activities were greater at the UNR-41 yr sector (300%) compared to the UNR-1 yr sector (Table 1.4, $F = 137.1$, $p < 0.0001$). A three-way interaction effect of age, rewetting,

and phenolic treatments was detected on arylsulphatase activities (Table 1.4, $F = 40.5$, $p < 0.0001$). At non-rewetted plots of the UNR-41 yr sector, wood addition showed greater mean arylsulphatase activities with an increase of 150% compared to control and root subplots, whereas later two treatments were similar in arylsulphatase activities (Fig. 1.4B, $F = 29.8$, $p = 0.001$). While at rewetted plots of the UNR-41 yr sector, mean arylsulphatase activities were lower at the root (83%) and wood (33%) subplots relative to control (Fig. 1.4B, $F = 57.7$, $p = 0.0001$). Phenolic treatments at rewetted and non-rewetted plots of the UNR-1 yr sector had similar arylsulphatase activities (Fig. 1.4B, $p > 0.05$). An increase in mean activities of β-D-xylosidase (126%), N-acetyl-β-D-glucosaminidase (276%), and phosphatase (179%) was observed at the UNR-41 yr sector compared to the UNR-1 yr sector (Fig. 1.4C-E, Table A1.2, for all, $p < 0.001$).

1.5.4.2 Phenol oxidase activities

In general, phenol oxidase (POX) activities were not different between UNR-1 yr (54 ± 6.6 nmol diqc g^{-1} min^{-1}) and UNR-41 yr (50 ± 6.3 nmol diqc g^{-1} min^{-1}) sectors (Table 1.4, Fig. 1.4F). Among phenolic additions, wood addition showed higher mean POX activities compared to the control with an increasing value of 120% (Table 1.4, $F = 6.9$, $p = 0.007$). The main effect of rewetting and interaction effects of age, rewetting, and phenolic treatments on POX activities were non-significant (Table 1.4).

1.5.5 Driving factors of the enzymic latch mechanism

A strong enzymic latch mechanism (ELM) was detected at rewetted plots of the UNR-1 yr sector indicated by the presence of negative correlation of peat phenolics with arylsulphatase ($r = -0.8$, $p = 0.02$) and SR ($r = -0.9$, $p = 0.003$, Fig. 1.5A and Fig. A1.2). SR exhibited positive correlation with arylsulphatase ($r = 0.7$, $p = 0.04$) and POX ($r = 0.8$, $p = 0.02$, Fig. 1.5A and Fig. A1.2) activities. A weak ELM, except for rewetting at the UNR-1 yr sector, existed that produced changes between the observed variables. For example, non-rewetted plots at the UNR-1 yr sector did not show any positive or negative correlations in favor of ELM (Fig. 1.5A, $p < 0.05$). Similarly, there was no clear pattern that SR was directly or indirectly affected through enzymes by peat phenolics (Fig. 1.5A). At rewetted plots of the UNR-41 yr sector, a negative correlation between pH and peat soluble phenolics was detected (Fig. 1.5B, $r = -0.7$, $p = 0.04$). Peat phenolics in turn were positively related with β-D-glucosidase

Table 1.4. Linear mixed effects model to determine the main and interactive effects of age (UNR-1 yr and UNR-41 yr), rewetting (non-rewetted and rewetted plots), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) on β-D-glucosidase (nmol g^{-1} min^{-1}), arylsulphatase (nmol g^{-1} min^{-1}), and phenol oxidase (nmol diqc g^{-1} min^{-1}) enzymes activities. In the source's column, A = age, R = rewetting, and P = phenolic treatments.

Sources	**d.f.**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
		β-D-glucosidase			***Arylsulphatase***			***Phenol oxidase***		
Age[1]	1	38	78.8	**<.0001**	0.01	137.1	**<.0001**	145	0.23	0.64
Rewetting	1	3	5.6	**0.045**	0.0003	2.9	0.13	120	0.2	0.67
A × R	1	1	1.6	0.246	0.000006	0.1	0.82	110	0.2	0.69
Error a	8	0.5			0.0001			629		
Phenolic treatments	2	0.02	0.04	0.96	0.005	51.6	**<.0001**	4332	6.9	**0.007**
A × P	2	0.2	0.5	0.65	0.005	50.3	**<.0001**	313.7	0.5	0.62
R × P	2	0.1	0.2	0.82	0.002	21.8	**<.0001**	192.7	0.3	0.74
A × R × P	2	0.03	0.1	0.94	0.004	40.5	**<.0001**	330.07	0.5	0.60
Error b	16	0.5			0.0001			628		

[1]Age represents the number of years since the cessation of commercial peat extracting activities at post-extracted unrestored (UNR) sectors

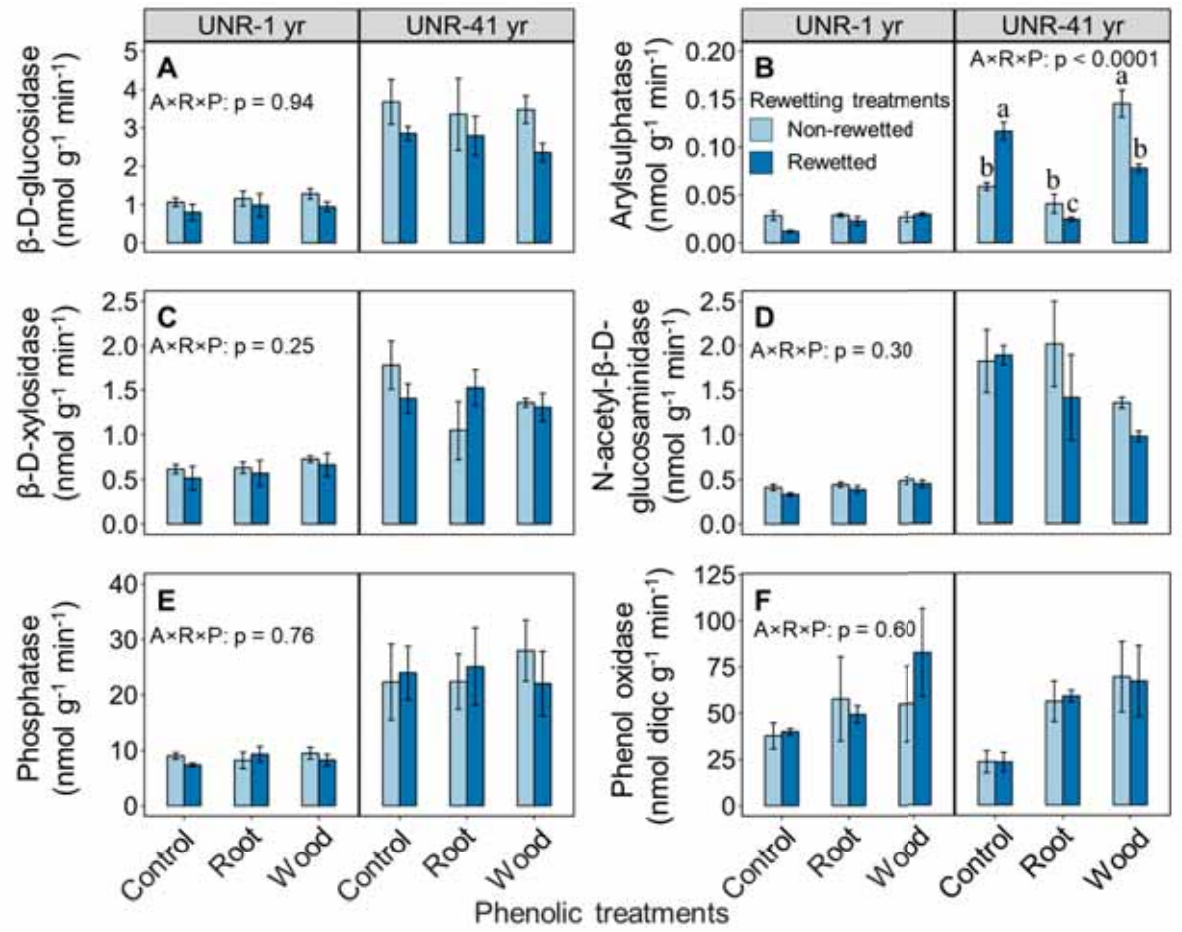

Fig. 1.4. Mean hydrolases (A to E, nmol g^{-1} min^{-1}, n = 3) and phenol oxidase (F, nmol diqc g^{-1} min^{-1}, n = 3) enzyme activities of surface composite peat sample, collected in the fall of 2022 from phenolic treatments sub-plots (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) of non-rewetted and rewetted plots at two post-extracted unrestored (UNR) sectors (young and old) that differed in age (UNR-1 yr and UNR-41 yr) since the cessation of commercial peat extracting activities. Error bars represent the standard error of the mean. The presence of different lowercase letters indicates differences among phenolic treatments (One-way ANOVA, $p < 0.05$) based on the significant three-way interactive effect of age, rewetting, and phenolic treatments on observed variables. See Tables 1.4 and A1.2 for detailed statistical analysis. Non-transformed data of N-acetyl-β-D-glucosaminidase is presented here, whereas statistical analysis was based on reciprocal transformed data.

(r = 0.8, p = 0.01) and phosphatase (r = 0.7, p = 0.044, Fig. 1.5B) activities. A clear significant negative relationship can be seen between SR and arylsulphatase activities (Fig. 1.5B, r = -0.8, p = 0.015), which is opposite to the concept of ELM. In contrast, non-rewetted plots of the UNR-41 yr sector resulted in a negative correlation between peat phenolics and β-D-xylosidase (Fig. 1.5B, r = -0.8, p = 0.008). Hydrolase enzymes, phenol oxidase enzymes, and

phenolics did not show any relationship with SR at the non-rewetted plots of the UNR-41 yr sector (Fig. 1.5B, $p > 0.05$). Overall, other observed variables, such as WT level, soil temperature, nutrients, soil organic matter, and dissolved organic carbon showed significant correlations but could not explain the ELM (Fig. A1.3 and A1.4).

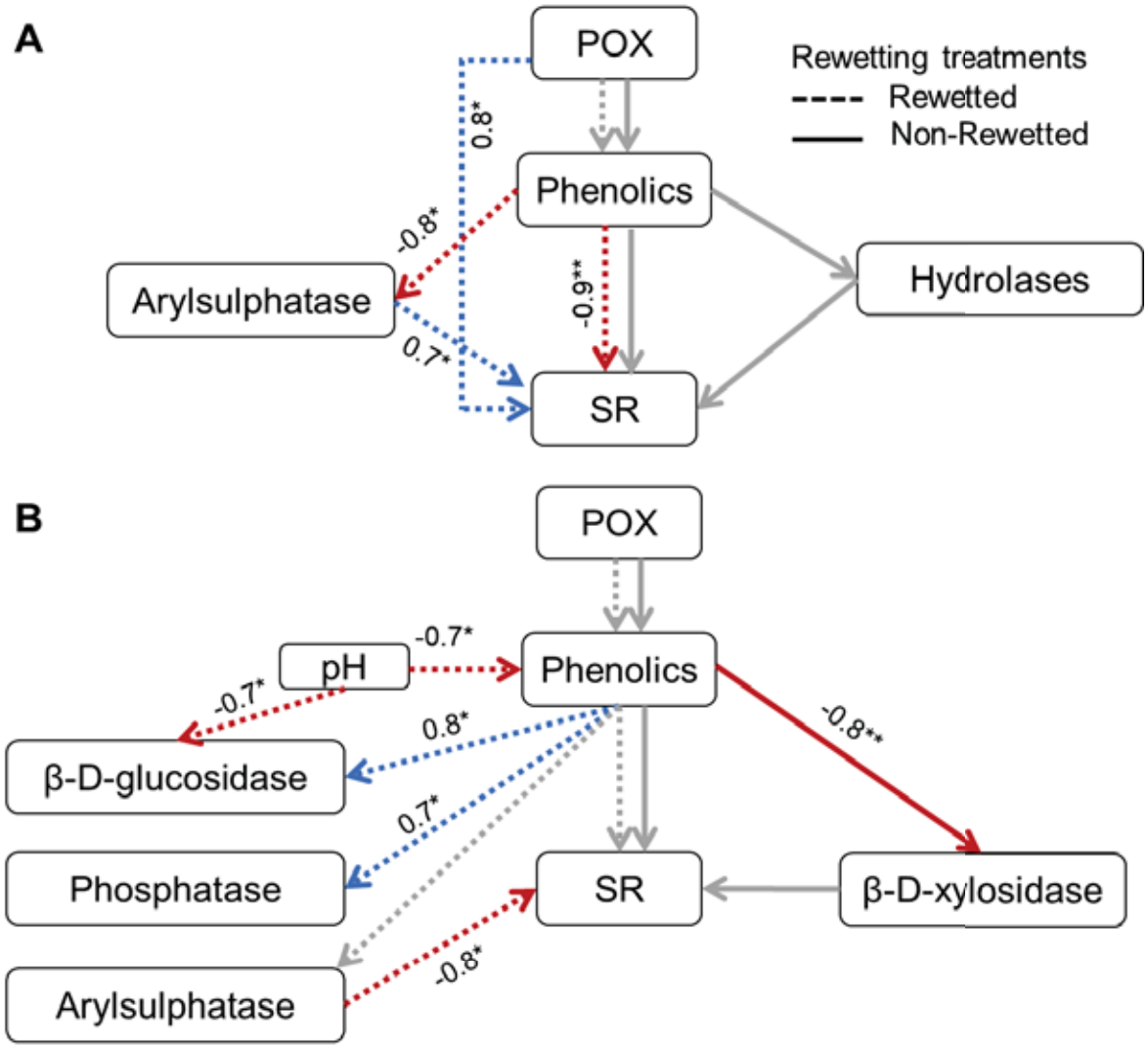

Fig. 1.5. Schematic diagram for a better understanding of the enzymic latch mechanism. Numbers indicate the Pearson correlation coefficient between observed variables from rewetted (dashed line) and non-rewetted (solid line) plots at two post-extracted unrestored (UNR) sectors, UNR-1 y (A) and UNR-41 yr (B), that differed in age since the cessation of commercial peat extracting activities. The arrow colour represents positive (blue), negative (red), and neutral (grey) correlation between the observed variables. Asterisks indicate significant correlations between observed variables: *$p < 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

Abbreviated variables are defined as phenol oxidase (POX), peat soluble phenolics (phenolics), potential of hydrogen (pH), and soil respiration (SR).

1.6 Discussion

Over the years, the enzymic latch mechanism (ELM) has been tested in different settings to understand its role in limiting enzyme activities specific to peat decomposition in oxic and anoxic environments. However, studies reported positive, negative, and neutral effects of phenolic concentration and water table (WT) level on enzyme activities (Fenner & Freeman, 2011; Wang et al., 2015; Fenner & Freeman, 2020; Alshehri et al., 2020; Urbanová and Bárta, 2016; Wang et al., 2017; Urbanová & Hájek, 2021). In this study, we investigated the response of rewetting and external phenolic additions on the ELM at a large scale. In general, we also found positive, negative, and neutral effects of rewetting and peat phenolics on enzyme activities. However, soil respiration was not suppressed by the combination of rewetting and phenolic addition, imposing a great concern in accepting such a concept for global peatlands. Such contrasting results create space for further research and identification of other factors that may be driving the ELM. For example, the 'iron gate' mechanism indicated the role of iron in manipulating enzyme activities against the ELM (Wang et al., 2017).

Drained peatlands are a major source of carbon dioxide (CO_2) emissions and are responsible for approximately 2 Gt CO_2-eq/year of global greenhouse gas emissions (Joosten et al., 2016; Günther et al., 2020). To reduce emissions from these degraded ecosystems, restoration involving rewetting and other nature-based solutions is required. However, it is important to understand key factors that can influence restoration such as peatland degradation age, remaining peat quality, peat depth, and time since rewetting. Considering the age factor, Waddington et al. (2002) found that seasonal CO_2 emission from the young degraded site (2-3 years old) was approximately 9% lower than the old degraded site (7-8 years old). Similarly, Rankin et al. (2018) in 2014 and 2015 estimated CO_2 emissions from a post-extracted unrestored site where peat extraction activities were halted in 1999. Rankin et al. (2018) found a rise of 50% in cumulative annual CO_2 emissions in the second year (2015) compared to the first year (2014), attributing the difference to variations in snow depth and snowmelt timing. In contrast, Renou-Wilson et al. (2019) estimated CO_2 emissions from drained peatland for consecutive four years and reported a decline of approximately 40% (in

the second year) and 30% (in the fourth year) in CO_2 emissions from the third year. In this current study, results are in line with former studies as CO_2 emissions from the UNR-41 yr sector were higher compared to the UNR-1 yr sector by 19%. We assumed that peat in the UNR-41 yr sector would be under a self-adaptive mechanism leading to lower CO_2 emissions than the UNR-1 yr sector where peat was recently stopped from harvesting. Contrary to our first hypothesis, underground root biomass from spontaneous plant regeneration at the UNR-41 yr sector could have potentially enhanced CO_2 emissions.

Rewetting is a well-known approach that can improve the hydrological, biogeochemical, and ecological functioning of the unrestored peatlands but its success depends on time and microclimatic conditions. We expected that rewetting would halt oxygen (O_2) penetration into the peat by increasing the WT level. As indicated in ELM, O_2 availability plays an important role in driving CO_2 emissions through peat soluble phenolics, phenol oxidase (POX), and hydrolase enzyme activities (Freeman et al., 2001). In our study, WT level was slightly higher at rewetted plots compared to non-rewetted plots at both UNR sectors, but still, more than one meter of oxic layer existed between the peat surface and WT level. Despite the higher WT level, rewetted plots at the UNR-1 yr sector emitted higher CO_2 emissions than their counterparts. Opposite to our expectations, peat soluble phenolics, POX, and hydrolase enzyme activities did not change between rewetted treatments at both UNR sectors. These results were inconsistent with our second hypothesis but somewhat consistent with the findings of Urbanová & Hájek (2021), which showed no difference between oxic and anoxic treatments regarding extracellular enzyme activities. However, Urbanová & Hájek (2021) did find a difference in phenolic concentration and CO_2 emissions between oxic and anoxic conditions. In the last decade, Waddington et al. (2010) compared pre-restoration (1999 yr) and post-restoration (2000, 2001, and 2002 yrs) data from Bois-des-Bel cutover peatland. Waddington et al. (2010) reported a higher WT level for the first three years of post-restoration (-31.5 cm, -30.4 cm, and -35.9 cm, respectively) compared to the reference UNR sector (-46.0 cm, -39.5cm, and -43.8 cm, respectively). Additionally, Waddington et al. (2010) reported lower bare peat CO_2 emissions from post-restoration plots (~ 4 g CO_2 $m^{-2}d^{-1}$) than pre-restoration plots (~ 6.9 g CO_2 $m^{-2}d^{-1}$). Another study by Wilson et al. (2013), showed lower and stable WT level after 9 years of rewetting compared to the 7 and 8 years of rewetting. Wilson et al. (2013) reported lower net ecosystem exchange (37.3 CO_2-C m^{-}

$^{2}yr^{-1}$) from the year with lower and stable WT level than the other two years (38.6 and 81.6 CO_2-C $m^{-2}yr^{-1}$, respectively). In literature, less data is available on large-scale rewetting post-extracted UNR peatlands that account for CO_2 emissions and enzyme activities from bare peat. Available studies that accounted for these factors presented lab-based results or small-scale experiments and hence ended up with contradictory results (Freeman et al., 2001a; Sun et al., 2010; Fenner & Freeman, 2011; Hall et al., 2014; Wang et al., 2015; Wang et al., 2017).

In this study, the role of peat soluble phenolics in response to external phenolic supplements and rewetting was tested to understand its inhibition role in the decomposition process that can limit enzyme activities leading to lower CO_2 emissions. A recent study by Urbanová & Hájek (2021) tested the key assumption of ELM that phenolics are potent inhibitors of extracellular enzyme activities and provided evidence under oxic and anoxic environments in a lab-based study. Urbanová & Hájek (2021) showed lower peat soluble phenolics in response to added phenolics in peat under oxic and anoxic conditions compared to the control. Added phenolics had no effect on POX and hydrolase enzyme activities compared to control under oxic and anoxic conditions. CO_2 emissions were similar in all added phenolic treatments but approximately four times lower under anoxic than oxic conditions. On the other hand, Fenner & Freeman (2020) found that different types of wood insertion into peat enhanced polyphenolic concentration and ultimately reduced global warming potential. They also revealed that wood insertion helped in exogenous carbon preservation compared to wood on the peat surface. Similarly, Alshehri et al. (2020) reported high peat phenolic concentration in response to added wood chips compared to the control. Furthermore, Alshehri et al. (2020) observed that spruce wood chips mixed with peat had higher peat phenolic concentration and 20 times lower β-glucosidase activity compared to the control treatment. Broadly, we were unable to see strong effects of external phenolic addition on peat soluble phenolics except for root addition at the non-rewetted plots where an increase of 20% was observed compared to control and wood addition subplots. Wood and root addition at the rewetted UNR-41 yr sector played an important role in limiting arylsulphatase activity but CO_2 emissions were not reduced due to external phenolic addition. POX is a crucial enzyme that is regulated by WT level and able to degrade peat phenolics (Freeman et al., 2004; Toberman et al., 2008). Overall, our results did not indicate any depletion of POX activity under rewetting and phenolic addition treatments at both UNR sectors, which is

opposite to the ELM concept. Dissimilar to our third hypothesis, rewetting and phenolic additions did not show marked changes in observed parameters in favour of ELM. Therefore, for such a complex mechanism, it is important to account for the direct and indirect relationships of all the conceptually linked variables within studied treatments.

1.6.1 Driving factors of enzymic latch mechanism

The relationship of phenolics with hydrolase enzyme activities (negative), soil respiration (negative), pH (negative), WT (positive) and links of hydrolase enzyme activities with soil respiration (positive), pH (positive), WT (negative), temperature (positive) and nutrients (positive) are essential driving relationships in ELM. In literature, ELM has been validated and criticized widely based on the presence or absence of driving factors. Freeman et al. (2004) validated ELM by showing a positive relationship between POX and oxygen availability and a negative relationship between hydrolase activities and phenolics. In contrast, Urbanová & Hájek (2021) found a difference in oxic and anoxic soil respiration but no relationship between hydrolase enzymes and phenolics. Urbanová & Hájek (2021) criticized ELM and linked slow decomposition rate with traditional factors such as anoxia, low pH, etc. Other studies partially confirmed ELM by indicating that hydrolase enzyme activities were linked with soil conditions, dissolved organic carbon (DOC), soil temperature, and peat depth (Sun et al., 2010; Romanowicz et al., 2015; Pinsonneault et al., 2016). Overall, in this study, only rewetting at UNR-1 yr sector showed strong ELM. Other factors, such as WT level, soil temperature, pH, DOC, and nutrients showed relationships with phenolics, enzyme activities, and soil respiration (SR) but these driving factors did not endorse ELM.

1.7 Conclusion

The goal of this study was to understand the effects of rewetting and phenolic additions at two post-extracted unrestored (UNR) sectors with different post-extraction ages, corresponding to the number of years since cessation of peat extracting activities, on enzyme activities, peat soluble phenolics, and soil respiration (SR) under enzymic latch mechanism (ELM). Water table (WT) level close to the surface is an indication of successful rewetting for effectively testing ELM. However, rewetting at both UNR sectors did not raise WT level close to the surface. No major difference was observed in WT level between rewetted (-45 cm) and non-rewetted (-46 cm) plots of UNR-41 yr sector, whereas at the UNR-1 yr sector,

WT was slightly higher at rewetted plots (-48 cm) compared to non-rewetted plots (-57 cm). In effective rewetting, due to lower water table level, at both UNR sectors showed no considerable changes in peat soluble phenolics, phenol oxidase, and average hydrolase enzyme activities, except for mean arylsulphatase activities. Rewetting along with phenolic addition showed limited arylsulphatase activities at the UNR-41 yr sector which is one of the key indications of the strengthening of ELM. However, rewetting along with phenolic additions were unable to produce any major effects that could lead to a reduction in SR, and we assumed that the phenolic product decomposition might have contributed to higher SR, which is opposite to ELM. In this current study, ELM was not validated. We think that it might be because the low WT level observed at both UNR sectors at more than one meter, did not create favorable anoxic conditions close to the peat surface, where the ELM is expected to function. Further research would be required to compare rewetting treatments based on time scale or different WT levels, along with sample analysis from different peat profile depths, to better understand the effects of rewetting and phenolic addition on peat decomposition under ELM.

1.8 Acknowledgements

We thank Canadian Sphagnum Peat Moss Association and its members for providing facility to conduct research work. We also appreciate Maria Strack for knowledge transfer related to carbon flux. We are also grateful for Peatland Ecology Research Group (PERG) colleagues and research assistants who helped during the field or laboratory work. In particular, special thanks to Andrew Albert Rutland, Meike Lemmer, Aamir Shehzad Khan, Laura Catalina Riano Pena, Charles Gignac, Kathy Pouliot, Brigitte Vimard Mathieu Ferland, Myriam Tougas-Dumesnil, Louis-Philippe Thériault, Siska Shooner, and Christina Tougas.

1.9 Appendices

Tables

Table A1.1. Linear mixed effects model to determine the main and interactive effects of age (UNR-1 yr and UNR-41 yr), rewetting (non-rewetted and rewetted plots), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = Picea mariana aboveground fresh wood chips) on water table (WT, cm), potential of hydrogen (pH), electrical conductivity (EC, µS cm^{-1}), carbon and nitrogen ratio (C/N ratio), ammonium ion (NH_4^+, ppm), nitrate ion (NH_3^-, ppm), sulphate ion (SO_4^{-2}, ppm), and phosphate ion (PO_4^{3-}, ppm). In the source's column, A = age, R = rewetting, and P = phenolic treatments.

Sources	**d.f.**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
			WT			***pH***			***EC***			***C/N ratio***	
Age[1]	1	917	20.8	0.002	0.001	0.1	0.79	3897	10.1	**0.013**	10	0.6	0.47
Rewetting	1	519	11.7	0.009	0.01	0.8	0.4	483	1.2	0.30	0.4	0.03	0.88
A × R	1	304	6.9	**0.03**	0.07	4.7	0.06	14	0.04	0.85	33	1.9	0.2
Error a	8	44	44.1		0.01			387			17		
Phen.[2]	2	126	2.9	0.09	0.08	5.4	**0.02**	626	1.6	0.23	12	0.7	0.5
A × P	2	43	1.0	0.4	0.01	0.7	0.49	62	0.2	0.85	27	1.6	0.24
R × P	2	100	2.3	0.1	0.01	0.7	0.53	527	1.4	0.28	2	0.1	0.88
A × R × P	2	121	2.7	0.09	0.002	0.1	0.90	523	1.4	0.29	28	1.6	0.23
Error b	16	44			0.01			387			17		
			NH_4^+			***NH_3^-***			***SO_4^{-2}***			***PO_4^{3-}***	
Age[1]	1	9	0.03	0.87	79	4.8	0.06	184	0.01	0.94	308	122.4	**<0.0001**
Rewetting	1	57	0.2	0.68	621	37.5	**0.0003**	22661	0.8	0.41	2	0.9	0.37
A × R	1	15	0.1	0.83	154	9.3	0.016	52676	1.7	0.22	0.3	0.1	0.74
Error a	8	291			17	0.0		18427			3		
Phen.[2]	2	4482	14.6	**0.0002**	36	2.2	0.15	6284	0.2	0.81	0.4	0.2	0.86
A × P	2	2087	6.8	**0.007**	181	10.9	**0.001**	14192	0.5	0.63	7	2.6	0.1
R × P	2	492	1.6	0.23	71	4.3	**0.03**	62434	2.1	0.16	9	3.5	0.06
A × R × P	2	969	3.2	0.07	123	7.4	**0.005**	33418	1.1	0.35	9	3.6	**0.046**
Error b	16	307			16.56			29924			3		

[1]Age represents the number of years since the cessation of commercial peat extracting activities at post-extracted unrestored (UNR) sectors (UNR-1 yr, and UNR-41 yr).

[2]Phenolic treatments

Table A1.2. Linear mixed effects model to determine the main and interactive effects of age (UNR-1 yr and UNR-41 yr), rewetting (non-rewetted and rewetted plots), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) on β-D-xylosidase (nmol g^{-1} min^{-1}), 1/N-acetyl-β-D-glucosaminidase (nmol g^{-1} min^{-1}), and phosphatase enzymes activities (nmol g^{-1} min^{-1}). In the source's column, A = age, R = rewetting, and P = phenolic treatments.

Sources	**d.f.**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
		β-D-xylosidase			***1/N-acetyl-β-D glucosaminidase***			***Phosphatase***		
Age[1]	1	5	64.7	**<.0001**	15	173.8	**<.0001**	1521	32	**<.001**
Rewetting	1	0.01	0.1	0.77	0.4	5.2	0.05	2	0.04	0.84
A × R	1	0.02	0.2	0.64	0.04	0.5	0.52	0.001	0.00002	0.99
Error a	8	0.1			0.1			47.5		
Phenolic treatments	2	0.1	0.6	0.54	0.04	0.4	0.66	5	0.1	0.9
A × P	2	0.1	1.6	0.23	0.6	6.6	**0.008**	1	0.03	0.97
R × P	2	0.2	1.8	0.20	0.01	0.2	0.84	23	0.5	0.62
A × R × P	2	0.1	1.5	0.25	0.1	1.3	0.30	13	0.3	0.76
Error b	16	0.2			0.1			50.0		

[1]Age represents the number of years since the cessation of commercial peat extracting activities at post-extracted unrestored (UNR) sectors UNR-1 yr, and UNR-41 yr).

Table A1.3. Linear mixed effects model to determine the main and interactive effects of age (UNR-1 yr and UNR-41 yr), rewetting (non-rewetted and rewetted plots), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) on peat methane flux (CH_4; mg CH_4 $m^{-2}d^{-1}$). In the source's column, A = age, R = rewetting, and P = phenolic treatments.

Sources	**d.f**	**MS**	***F***	***P***
Age[1]	1	96	8	0.02
Rewetting	1	0.1	0.01	1
A × R	1	76	6	**0.04**
Error a	8	12		
Phenolic treatments	2	3	0.2	1
A × P	2	13	1	0.4
R × P	2	0.3	0.02	1
A × R × P	2	4	0.3	1
Error b	16	15		

[1]Age represents the number of years since the cessation of commercial peat extracting activities at post-extracted unrestored (UNR) sectors.

Figures

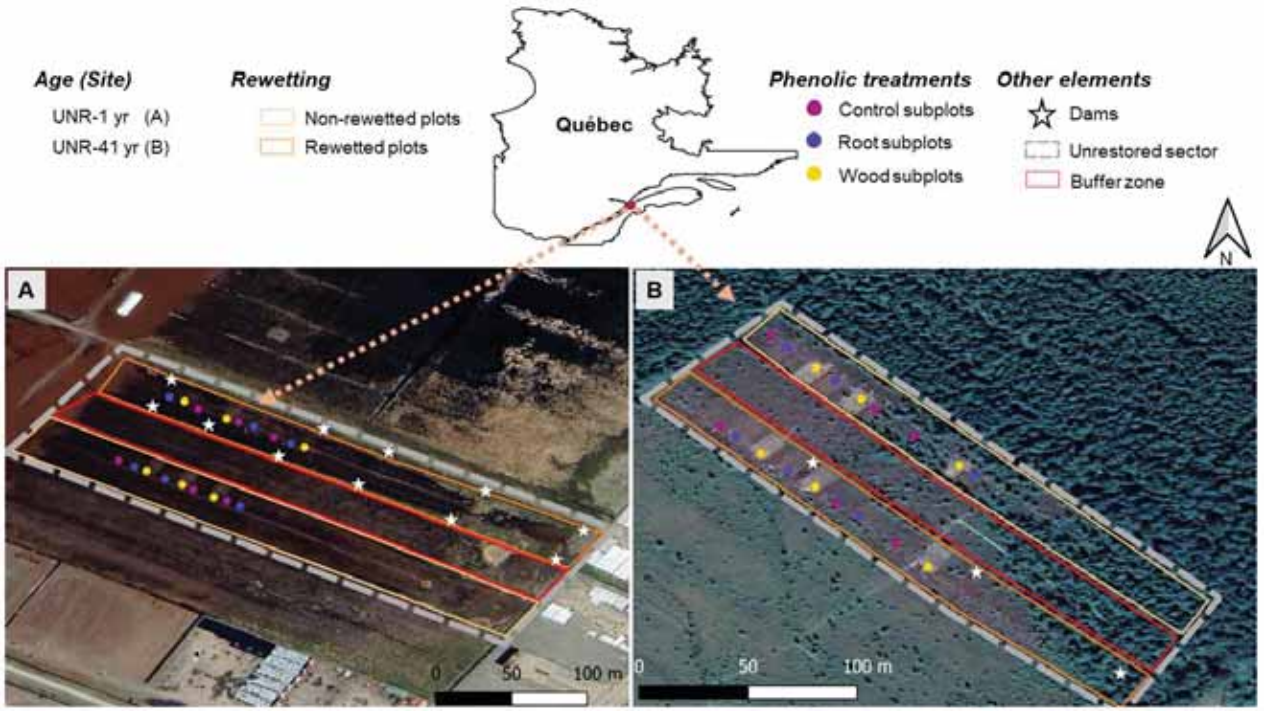

Fig. A1.1. Study area with experimental design in two post-extracted unrestored (UNR) sectors (gray dash line; A represents UNR-1 yr, and B represents UNR-41 yr), that differed in age since the cessation of commercial peat extracting activities. Rewetting treatments at both UNR sectors are represented by solid lines and separated by buffer zone (red line). Star shapes represent dams in rewetted plots (orange line). For phenolic treatments, purple dots represent control subplots, blue dots represent root subplots and yellow dots represent wood subplots.

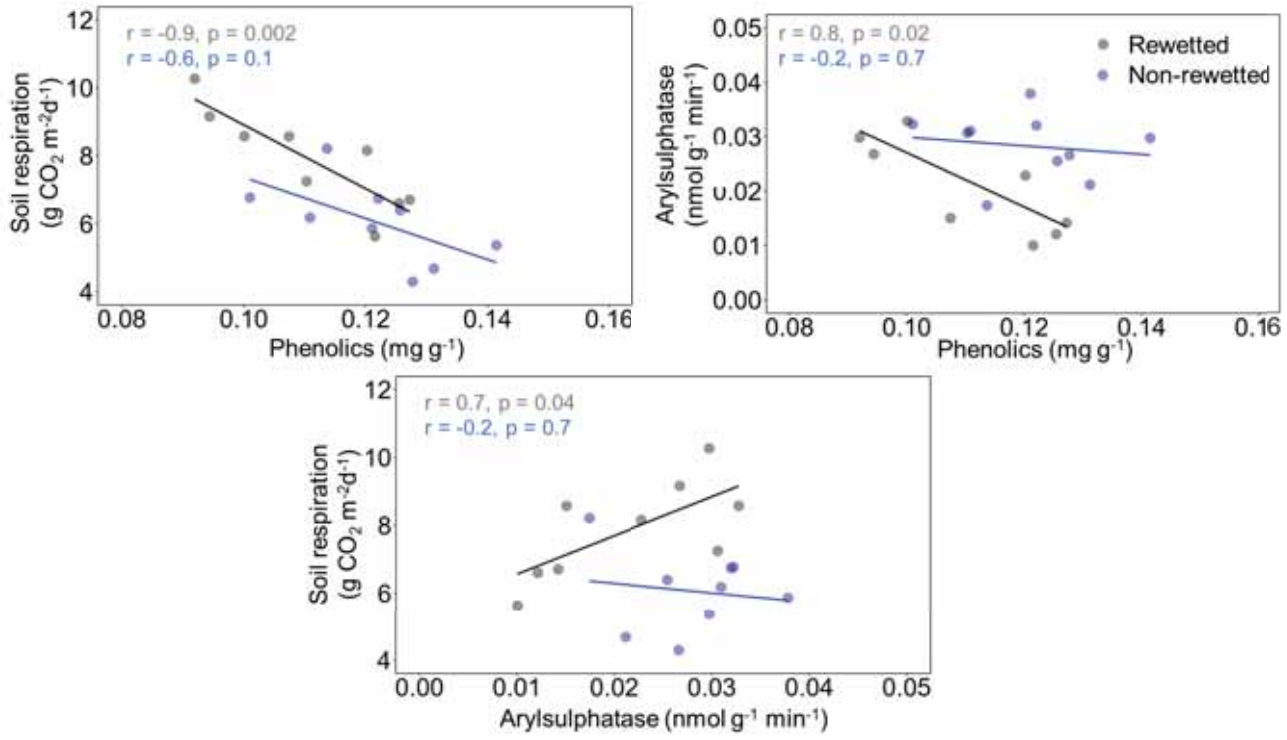

Fig. A1.2. Pearson correlation coefficient between observed variables at rewetted and non-rewetted plots of the UNR-1 yr sector.

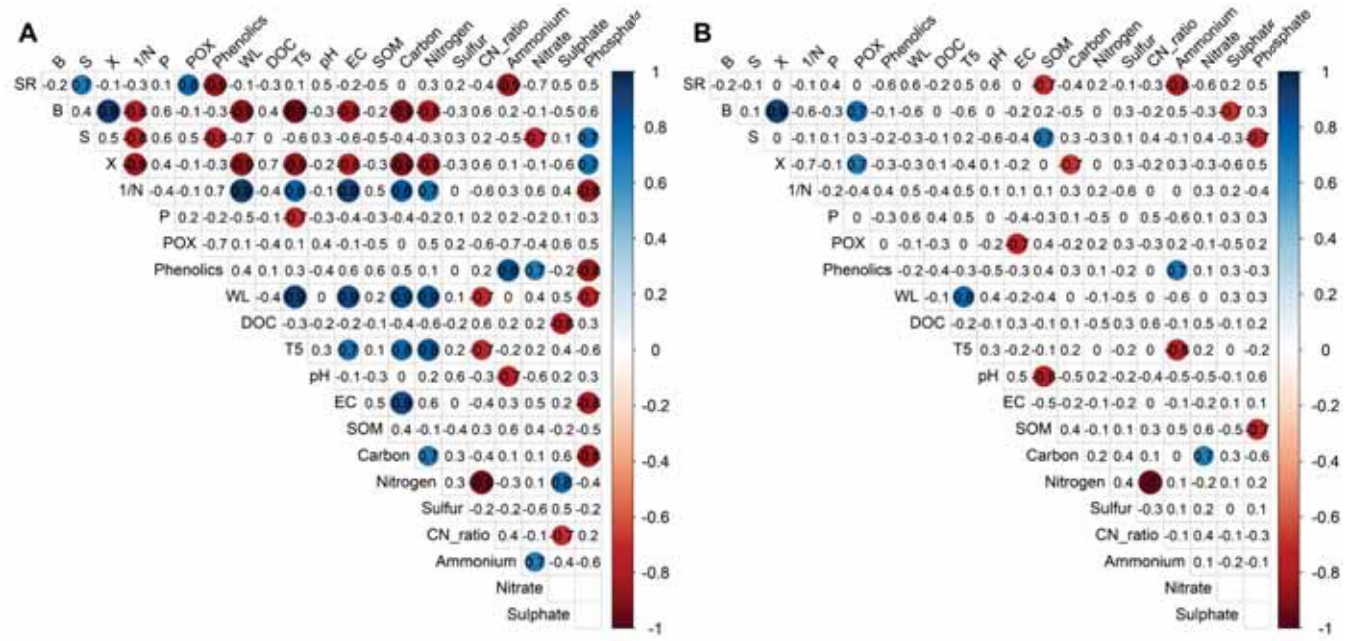

Fig. A1.3. Pearson correlation coefficient between observed variables at rewetted (A) and non-rewetted (B) plots of the UNR-1 yr sector. Highlighted coefficients indicate significant positive (blue) and negative (red) correlations between the observed variables ($p < 0.05$). Abbreviated variables are defined as soil respiration (SR), β-D-glucosidase (B), arylsuphatase (S), β-D-xylosidase (X), 1/N-acetyl-β-D-glucosaminidase (1/N), phosphatase (P), phenol oxidase (POX), peat soluble phenolics (phenolics), water table level (WL), dissolved organic carbon (DOC), peat temperature at -5 cm (T5), potential of hydrogen (pH), electrical conductivity (EC), and C/N ratio (CN_ratio).

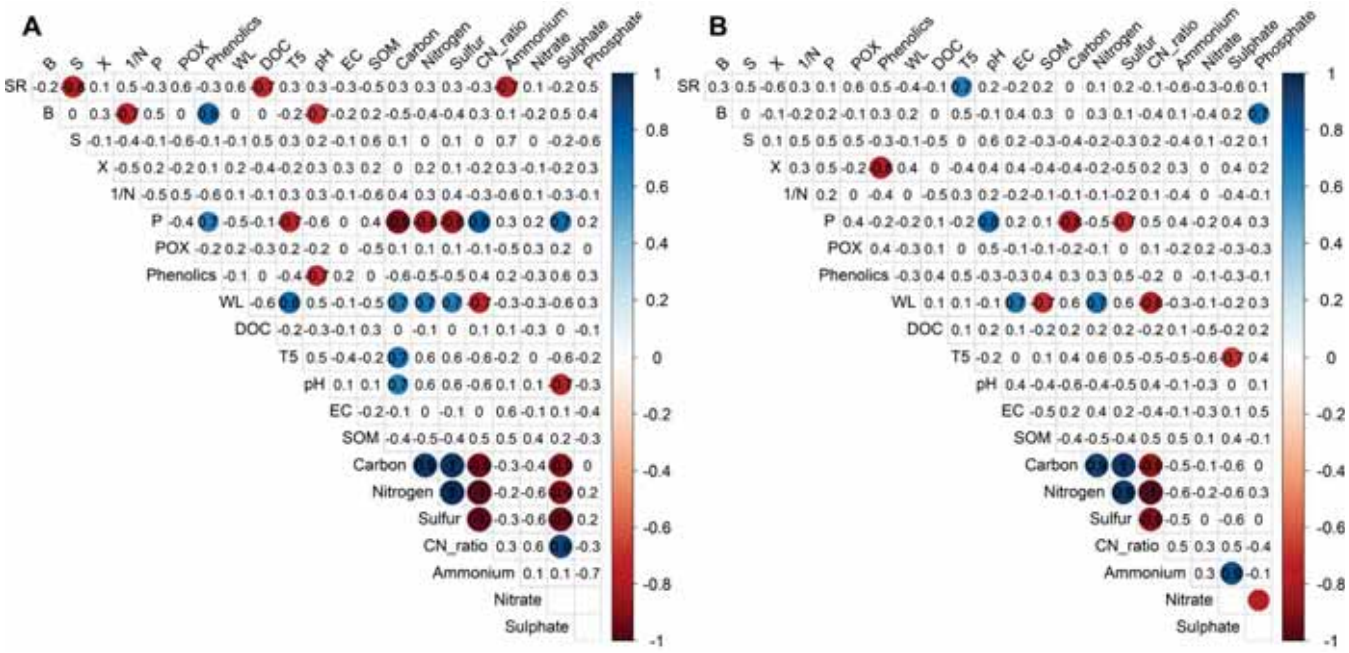

Fig. A1.4. Pearson correlation coefficient between observed variables at rewetted (A) and non-rewetted (B) plots of the UNR-41 yr sector. Highlighted coefficients indicate significant positive (blue) and negative (red) correlations between the observed variables ($p < 0.05$). Abbreviated variables are defined as soil respiration (SR), β-D-glucosidase (B), arylsuphatase (S), β-D-xylosidase (X), 1/N-acetyl-β-D-glucosaminidase (1/N), phosphatase (P), phenol oxidase (POX), peat soluble phenolics (phenolics), water table level (WL), dissolved organic carbon (DOC), peat temperature at -5 cm (T5), potential of hydrogen (pH), electrical conductivity (EC), and C/N ratio (CN_ratio).

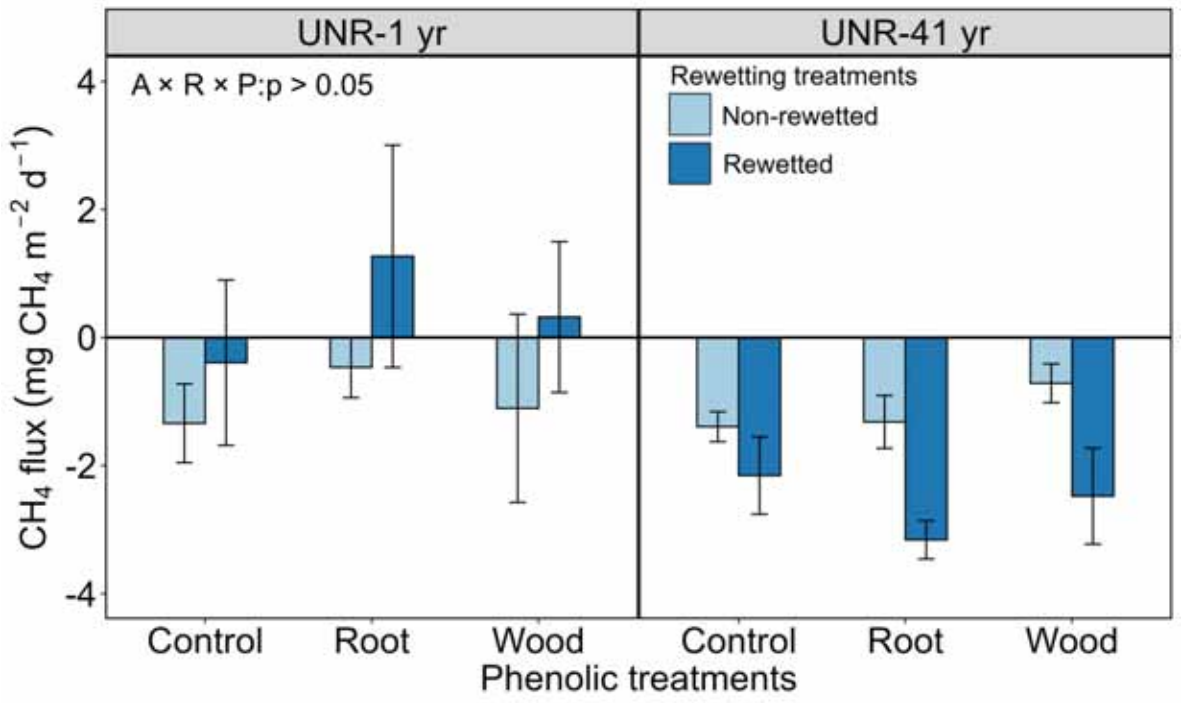

Fig. A1.5. Mean peat methane flux(CH_4; mg CH_4 $m^{-2}d^{-1}$, n = 3) measured during May to August of 2022 from phenolic treatments sub-plots (control = no addition, root = old roots from peat harrowing, and wood = *Picea mariana* aboveground fresh wood chips) of non-rewetted and rewetted plots at two post-extracted unrestored (UNR) sectors (young and old) that differed in age (UNR-1 yr and UNR-41 yr) since the cessation of commercial peat extracting activities. Positive values represent a release of CH_4 from the ecosystem and negative values indicate CH_4 sink. Error bars represent the standard error of the mean. See Table A1.3 for detailed statistical analysis.

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Chapter 2 Phenolic supplements: testing an approach to limit *Sphagnum* subgenus decomposition in a Sphagnum farming system

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2.1 Résumé

Dans un système de culture de sphaigne, nous avons examiné l'impact des traitements phénoliques sur le mécanisme de verrou enzymatique (MVE) pour limiter la décomposition de la sphaigne et améliorer sa productivité. Trois traitements ont été testés dans deux bassins de culture dominés par les sous-genres *Acutifolia* ou *Sphagnum*: granules de bois, vieilles racines et un témoin sans addition. Les deux sous-genres de sphaignes représentaient des puits de carbone faibles et similaires. Leurs émissions de dioxyde de carbone (CO_2) ont été plus élevées en réponse aux traitements phénoliques, probablement à cause de la décomposition des produits ajoutés. Les traitements n'ont pas modifié la concentration en composés phénoliques solubles, la productivité, la biomasse des sphaignes, ni l'activité enzymatique. L'inefficacité du MVE pourrait être due à l'absence d'effet inhibiteur des composés phénoliques sur les enzymes situées plus profondément. Des analyses supplémentaires à différentes profondeurs sont nécessaires pour mieux comprendre ces interactions.

2.2 Abstract

Phenolics limit decomposition by suppressing extracellular enzyme activities—a phenomenon explained in the enzymic latch mechanism (ELM). In this study, we tested the impact of phenolic treatments on ELM's effectiveness in limiting *Sphagnum* decomposition and thereby enhancing its productivity and biomass accumulation in Sphagnum farming system. A split-plot experiment with three phenolic treatments was implemented in two cultivation basins established with mosses dominated by the *Acutifolia* or *Sphagnum* subgenus. Phenolic treatments included wood pellets (wood), old roots from peat harrowing (root), and no addition (control). Our results indicate that both subgenera had approximately similar small sinks of carbon dioxide (CO_2) such as -2 ± 1 g CO_2 m^{-2} d^{-1} (*Acutifolia*) and -0.2 ± 0.8 g CO_2 m^{-2} d^{-1} (*Sphagnum*). Both subgenera under phenolic additions showed higher CO_2 values as net ecosystem exchange compared to control, which could be linked to emissions resulting from wood and root decomposition. On the other hand, phenolic additions had no impact on phenolics, productivity, and biomass for both subgenera. Similarly, for both subgenera, phenolic additions were unable to limit enzyme activities compared to control. The current study did not validate the strengthening of ELM and we assumed that it could be due to the absence of the inhibitory effect of phenolic products (applied at the surface) on enzyme activities (sampled ~10 cm below the surface). With surface phenolic application, various sample analyses from different depths will be required to better understand phenolics-enzyme interactions based on ELM.

2.3 Introduction

Sphagnum peat is important for growing substrates due to its low cost, easy availability, and unique characteristics for plant growth (Caron & Rochefort, 2013). However, the negative impact of peat extraction on peatland ecosystem services, such as carbon dioxide (CO_2) sequestration, water regulation, biodiversity, and more, has drawn significant focus toward more sustainable alternatives to minimize pressure on peatlands. A suitable after-use option for post-extracted peatlands is paludiculture, which involves the production of agricultural or forestry crops on rewetted peatlands (Wichtmann et al., 2016; Gaudig et al., 2017). Whereas cultivation of *Sphagnum* biomass on cyclical and renewable basis at rewetted peatlands is specifically termed Sphagnum farming, which is also a form of paludiculture (Pouliot et al., 2015; Gaudig et al., 2017). The production of undecomposed *Sphagnum* biomass has multiple end-uses, primarily as a donor material for restoration and as an alternative material for growing media (Emmel, 2008; Reinikainen et al., 2012; Jobin et al., 2014; Müller & Glatzel, 2021). Furthermore, Sphagnum farming can partially restore ecosystem services similar to pristine peatlands such as limited decomposition, enhanced CO_2 sequestration, regulation of water and nutrients, and provision of habitats for a wide variety of biodiversity (Joosten et al., 2012; Luthardt & Wichmann, 2016).

Experiments involving *Sphagnum* cultivation are currently underway in degraded and cutover peatlands in various countries, including Canada, Germany, Finland, Chile, Denmark, Lithuania, Latvia, Ireland, The Netherlands, South Korea, Japan, and New Zealand. Several factors to optimize *Sphagnum* yield have been tested, such as species selection based on decomposition and productivity comparison, regulation of external water supply, straw cover, and management of unwanted plant species (Guêné-Nanchen et al., 2017; Gaudig et al., 2017; Brown et al., 2017; Kim et al., 2020).

On the other hand, the enzymic latch mechanism (ELM) as a process in limiting decomposition; and phenolic compounds potential to inhibit the enzyme and microbial activities could be supplied to strengthen ELM. Nevertheless, the effectiveness of ELM is currently in a controversial stage, as certain studies have shown no effect of phenolics on limiting decomposition (Harris et al., 2020; Urbanová & Hájek, 2021). While others showed the role of phenolics in limiting enzyme activities leading to limited decomposition (Freeman

et al., 2001; Dunn et al., 2014; Alshehri et al., 2022). Also, ELM and its strengthening through ecological engineering tools in the form of phenolic additions have not been fully explored in the context of *Sphagnum* cultivation.

To improve *Sphagnum* yield in a farming system, productivity needs to be maximized, and the decomposition minimized. To achieve such objectives, ELM could play an important role which is based on two important factors, anoxia and enzyme activities, among the traditional abiotic and biotic factors of slow rate of decomposition in peatlands. According to ELM, anoxia is an initiation step in limiting phenol oxidase enzyme activity that is capable of degrading phenolic compounds in the presence of oxygen. Further, phenolic compounds abundance inhibits hydrolase enzymes which play an essential role in degrading organic matter. External phenolic supplementation could help in strengthening the process by further putting constraints on enzyme activities. All the above-mentioned constraints lead to restricted decomposition (Freeman et al., 2001; Freeman et al., 2012). However, ELM's role in enhancing *Sphagnum* yield is not well established.

Sphagnum species are rich in *Sphagnum* acids which are phenol derivative and act as a potent inhibition compound against enzymes (van Breeman, 1995; Wetzel, 1992; Freeman et al., 2001). Based on ELM, an external phenolic enrichment could be useful to further restrict the de novo synthesis of extracellular enzymes. Source of phenolic supplements can be natural products, such as root and wood chips, and commercial products, like phenols or by-products from paper and pulp industry (Dunn et al., 2014; Alshehri et al., 2020; Urbanová & Hájek, 2021). The ELM's potential to improve yield will be highly dependent on *Sphagnum* species at the subgenus level where they have different production and decomposition levels.

The three taxonomic distributions of *Sphagnum* (*Acutifolia*, *Sphagnum*, and *Cuspidata*) can have differences in stem densities, productivity, and decay patterns. Overall, *Acutifolia* subgenus has high stem densities, lower productivity, and decomposition. While *Sphagnum* subgenus has lower stem densities compared to *Acutifolia* subgenus but biomass per unit area is greater. *Sphagnum* subgenus is also associated with lower decomposition rates. Conversely, *Cuspidata* subgenus has lower stem densities but is characterized by high productivity and decomposition (Rochefort, 1990; Johnson & Damman, 1991). Species

selection in Sphagnum farming depends on the end use of the biomass. For example, moss species from *Acutifolia*, and *Sphagnum* subgenus could be selected if the farming goal is to produce raw material for horticultural growing substrates. Various plant cultivation experiments showed that several species of *Acutifolia*, and *Sphagnum* subgenus proved to be suitable components for growing substrates compared to species from *Cuspidata* subgenus (Gaudig et al., 2018). If, however, the goal is to harvest *Sphagnum* diaspores for peatland restoration, then species from *Acutifolia* and *Sphagnum* subgenus should be selected for Sphagnum farming, as they are more tolerant to desiccation and produce better quality products.

This study aims to evaluate the impact of surface phenolic additions on limiting *Sphagnum* decomposition, at the base of acrotelm, through strengthening of ELM and thereby increasing *Sphagnum* productivity and CO_2 uptake in the context of Sphagnum farming system. Based on our objective, we aim to answer the following statements: 1) Is there a difference in CO_2 exchange between cultivated mosses from *Acutifolia* and *Sphagnum* subgenus? 2) Do phenolic additions play any role in regulating CO_2 exchange, peat soluble phenolic, and enzyme activities? 3) Are phenolic additions an essential tool for optimizing productivity and biomass of mosses from *Acutifolia* and *Sphagnum* subgenus? 4) If ELM exists, what are the regulating factors, and do they align with the ELM's theoretical explanation for *Acutifolia* and *Sphagnum* subgenus?

2.4 Methods

2.4.1 Study site

This study was conducted in a Sphagnum farming site located approximately 13 km southeast of Rivière-du-Loup in Eastern Canada (47°49′ N and 69°27′ W). The *Sphagnum* farm covers an area of approximately 1 ha, established on a natural peatland area located beside a cutover bog. In 2013, six basins were built of 50 m × 10 m. Among the basins, three basins had a central irrigation canal, and others had a peripheral irrigation canal. Water in the irrigation canals was supplied from a nearby main drainage canal and maintained between -5 cm to 0 cm from the *Sphagnum* surface using automatic sensor systems. Mosses from *Acutifolia* and *Sphagnum* subgenus species were reintroduced to be cultivated using an adapted version of the Moss Layer Transfer Technique which is largely and specifically used

in the ecological restoration of post-extracted peatlands in North America (Rochefort et al., 2003). For a complete description of the study site area, refer to Pacheco et al. (2021) and for more details on general *Sphagnum* cultivation, *Sphagnum* farm design, and construction refer to Guêné-Nanchen & St-Hilaire (2022).

2.4.2 Experimental design

To investigate the effect of phenolic additions in a Sphagnum farming system, two basins having central irrigation canals and dominant moss carpets of *Acutifolia* (*S. rubellum*) and *Sphagnum* (*S. medium* and *S. papillosum*) subgenus were selected in June of 2021. A split block design was adopted with basins as the blocking factor, subgenus as the main plot factor (replicated four times across two basins), and phenolic supplements as the subplot factor. For phenolic treatments, two locally available products were chosen with different concentrations of soluble phenolics such as wood pellets (1.2 mg g^{-1} phenolics) and old roots from harrowing before peat extraction (0.2 mg g^{-1} phenolics). The wood pellets (later referred as wood) come from Granulco 100% natural softwood pellets made from species of the *Picea* and *Abies* genera. Approximately, each pellet had a length of 40 mm and a diameter of 6 mm. For root treatment, old roots (later referred as root) were chipped using a commercial chipping machine. The resulting root chips were fibrous, derived from *Picea mariana*, with sizes ranging from 0.3 mm × 0.1 mm ± 0.05. At both basins, phenolic treatments were randomly applied at 2 kg m^{-2} (fresh weight dosage) on top of the *Acutifolia* and *Sphagnum* subgenus plots of 4 m × 4 m sizes. The dosage of phenolic additions was based on previous greenhouse experiments and personal field experience. Besides phenolic additions, a control plot was also established for both subgenera for comparison within treatments. In the current study design, a total of 24 experimental units, two (subgenus) × four (subgenus replications) × three (phenolic treatments), were constructed (Fig. A2.1).

2.4.3 Samplings

2.4.3.1 Carbon dioxide exchange

In the middle of each experimental unit, 60 cm × 60 cm × 20 cm dimensions of stainless-steel collars having grooves on top were inserted into the *Sphagnum* carpet for carbon dioxide (CO_2) sampling. To avoid disturbance during CO_2 sampling, boardwalks, and platforms were installed at each experimental unit. After approximately one year of phenolic addition, CO_2

exchange (g CO_2 $m^{-2}d^{-1}$) was measured, from May to August/September 2022, using the closed chamber method (Alm et al., 1997). The net ecosystem exchange of CO_2 (NEE; g CO_2 $m^{-2}d^{-1}$) was determined with clear acrylic chamber (60 cm × 60 cm × 30 cm) connected with a portable infrared analyser (IRGA; EGM-4 PP systems USA). The clear acrylic chamber was equipped with two fans (10 cm × 10 cm) operated with batteries, holes for gas exchange, and thermocouple wire. Prior to each measurement, any vascular vegetation was clipped to meet the criteria of this study. Finally, the chamber was placed in the collar groove filled with water to avoid any gas exchange other than between the chamber and IRGA. The CO_2 exchange inside the chamber was measured for 0-2 minutes with data recording at every 15-second interval. At the same time, photosynthetically active radiation, temperature inside the chamber, and relative humidity inside the chamber were also recorded. Ecosystem respiration (ER; g CO_2 $m^{-2}d^{-1}$) was measured as a proxy for decomposition by covering the chamber with an opaque shroud. Gross ecosystem productivity (GEP) was calculated as the difference between NEE and ER. Before and during each measurement, the chamber was lifted from the collar to allow headspace air equal to ambient CO_2 concentration and temperature. The linear change in CO_2 concentration over time was used to calculate NEE and ER. Fluxes were rejected in case of a non-linear trend in CO_2 concentration ($R^2 < 0.80$). However, a flux with constant CO_2 concentration or change of less than 2 ppm over time was retained, an indication of NEE close to zero. The conventional sign method was used where negative values indicated CO_2 uptake by the ecosystem from the atmosphere and positive values indicated CO_2 emissions from the ecosystem to the atmosphere. To better understand NEE (NEE = GEP + ER), briefly negative NEE means that CO_2 captured (GEP, photosynthesis) by the *Sphagnum* species is higher than the CO_2 release (respiration) and opposite for the NEE positive values. At the end of the experiment, within each collar, several random height measurements were taken from the *Sphagnum* surface to the top of the collar for chamber volume correction. For statistical analysis, a minimum of five readings were required throughout the growing season from each experimental unit.

2.4.3.2 Sphagnum productivity and biomass

At both basins, *Sphagnum* productivity (P; g m^2 yr^{-1}) was determined by the following equation: P=AI ×D ×W ×C, where, AI = *Sphagnum* mean annual increment (cm), D = density of *Sphagnum* stem (stem m^{-2}), W = dry weight of one centimeter of *Sphagnum* shoot (g cm^{-}

^{1} stem^{-1}), and C = *Sphagnum* cover (%). *Sphagnum* mean annual increment (AI, cm) was measured with brush wires (Gunnarsson & Rydin, 2000) installed in each experimental unit in May of 2022. A total of 360 brush wires, five (brush wires) × three (cluster) × two (subgenus) × three (phenolic treatments) × four (phenolic treatment replications), were installed in 24 experimental units. The height of the brush wire was noted twice, one at the beginning (initial height, in May) and the other at the end of the season (final height, in October). *Sphagnum* height increment was calculated as the difference between the initial and final heights. At the end of the season, three *Sphagnum* biomass samples, each from an area of 35 cm^{2}. were collected close to the three brush wire clusters. Samples were used to count the number of *Sphagnum* capitulum for estimating the density of *Sphagnum* stem (D, stem m^{-2}). From the same sample, the top 3 cm of 40 *Sphagnum* stems with no capitulum were oven-dried and weighed. To estimate the dry weight of one centimeter of *Sphagnum* shoot (W, g cm^{-1} stem^{-1}), the above oven dry weight was divided by 3. *Sphagnum* cover (C, %) would be equal to 1 as our carpets had 100% *Sphagnum* cover.

At each experimental unit, three *Sphagnum* biomass (g m^{-2}) samples above the peat surface were collected using 25 cm × 25 cm quadrats in October of 2022. The samples were sorted to conserve only *Sphagnum* biomass, oven-dried at 70 °C for 72 hours and weighed for biomass dry weight.

2.4.3.3 Environmental conditions

After each CO_2 exchange measurement, the water table level was measured manually in a PVC well (2-inch diameter). Near the collar, temperature from the *Sphagnum* carpet surface to a depth of -2 cm, -5cm, -10 cm, -15 cm, and -20 cm was monitored using a thermocouple probe (Digi-Sense, Cole-Parmer) connected with a digital temperature reader (Omega HH200).

At the end of the growing season, five to six randomly spaced peat samples were collected from each experimental unit approximately 1-2 cm above the catotelm layer. Later, all peat samples were mixed to make a composite sample. During peat sampling, soil temperature for enzyme analysis was also recorded using thermocouple probes (Digi-Sense, Cole-Parmer) connected with a digital temperature reader (Omega HH200). During sampling, instruments

and hands were rinsed with 70% isopropyl alcohol to avoid contamination. For pH, and electrical conductivity (EC), samples were stored at -20 °C until further processing and later estimated by the methods explained in chapter 1. Samples for enzymes and soluble phenolics analysis were stored at 4 °C. Enzyme analysis such as hydrolase (β-D-glucosidase, Arylsulphatase, N-acetyl-β-D-glucosaminidase, β-D-xylosidase, Phosphatase) and phenol oxidase were measured at Bangor University by the methods explained by Dunn et al. (2014) within two weeks of sampling. Using the method illustrated by Alshehri et al. (2020), peat soluble phenolics were measured following the water extraction method.

2.5 Statistical analyses

All statistical analyses were completed in R software (R core team 2023) and reported with a significant level of 0.05. For our factorial experiment, the data was processed with a linear mixed effects (LME) model in the lme4 package (Bates et al., 2015). For all the response variables, LME models were formulated with subgenus, phenolic treatments, and their interactions as fixed factors. Random effects need special attention in the presence or absence of repeated measurements and to properly calculate error terms. For example, NEE data was collected several times from the same sampling unit, therefore, its LME model included random effects as (1|Basin) + (1|Basin: Subgenus) + (1|Replicate: Basin: Subgenus) (1|Phenolic: treatments: Replicate: Basin: Subgenus). On the other side, pH data did not have pseudo-replications, so random effects in the LME model were as: (1|Basin) + (1|Basin: Subgenus) + (1|Replicate: Basin: Subgenus). All the models were visually inspected for normality and homogeneity of residuals. For analyses of variances, joint_tests in emmeans package were used for all models (Lenth, 2023). Tukey multiple comparisons in emmeans and compact letter display (CLD) function in multcomp (Hothorn et al., 2008) packages were used to identify differences in significant main effects. For models with significant interaction effects, joint_tests with by function; later with Tukey multiple comparisons and CLD function were used to detect differences among different levels. To estimate the correlation between response variables, cor command was used. All the graphics were produced with ggplot2 package (Wickham, 2016).

2.6 Results

2.6.1 Carbon dioxide exchange

Broadly, *Acutifolia* and *Sphagnum* subgenus acted as carbon dioxide (CO_2) sink. However, mean values of net ecosystem exchange (NEE) between mosses of *Acutifolia* (-2 ± 1 g CO_2 m^{-2} d^{-1}) and *Sphagnum* subgenus (-0.2 ± 0.8 g CO_2 m^{-2} d^{-1}) were not different (Table 2.1). Among phenolic treatments, wood treatment (2 ± 0.6 g CO_2 m^{-2} d^{-1}) resulted in greater positive mean values of NEE compared to root (-1 ± 1 g CO_2 m^{-2} d^{-1}) and control (-4 ± 0.5 g CO_2 m^{-2} d^{-1}) treatments (Table 2.1, Fig. 2.1A). For mean gross ecosystem productivity (GEP), wood treatment augmented the mean GEP values for the mosses of the *Acutifolia* subgenus, while root and control treatments did not (significant interaction Table 2.1, Fig. 2.1B). For the mosses of the *Sphagnum* subgenus, mean GEP values among phenolic treatments were not different (Fig. 2.1B). The mean ecosystem respiration (ER) was similar between mosses of *Acutifolia* (10 ± 1 g CO_2 m^{-2} d^{-1}) and *Sphagnum* (9 ± 1 g CO_2 m^{-2} d^{-1}) and among phenolic treatments (Table 2.1, Fig.2.1C).

Table 2.1. Linear mixed effects model to determine the effects of subgenus (*Acutifolia & Sphagnum*), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on net ecosystem exchange (NEE; g CO_2 $m^{-2}d^{-1}$), gross ecosystem productivity (GEP; g CO_2 $m^{-2}d^{-1}$), and ecosystem respiration (ER; g CO_2 $m^{-2}d^{-1}$).

Flux component	Sources	df	MS	*F*	*P*
NEE	Subgenus	1	27	3.7	0.31
	Error a	6	7		
	Phenolic treatments	2	127	17	**< 0.001**
	S × P	2	6	0.8	0.47
	Error b	12	7		
GEP	Subgenus	1	21	2.8	0.34
	Error a	6	8		
	Phenolic treatments	2	76	10.2	0.003
	S × P	2	30	4.1	**0.045**
	Error b	12	7		
ER	Subgenus	1	4	0.5	0.62
	Error a	6	8		
	Phenolic treatments	2	26	3.2	0.08
	S × P	2	23	2.9	0.09
	Error b	12	8		

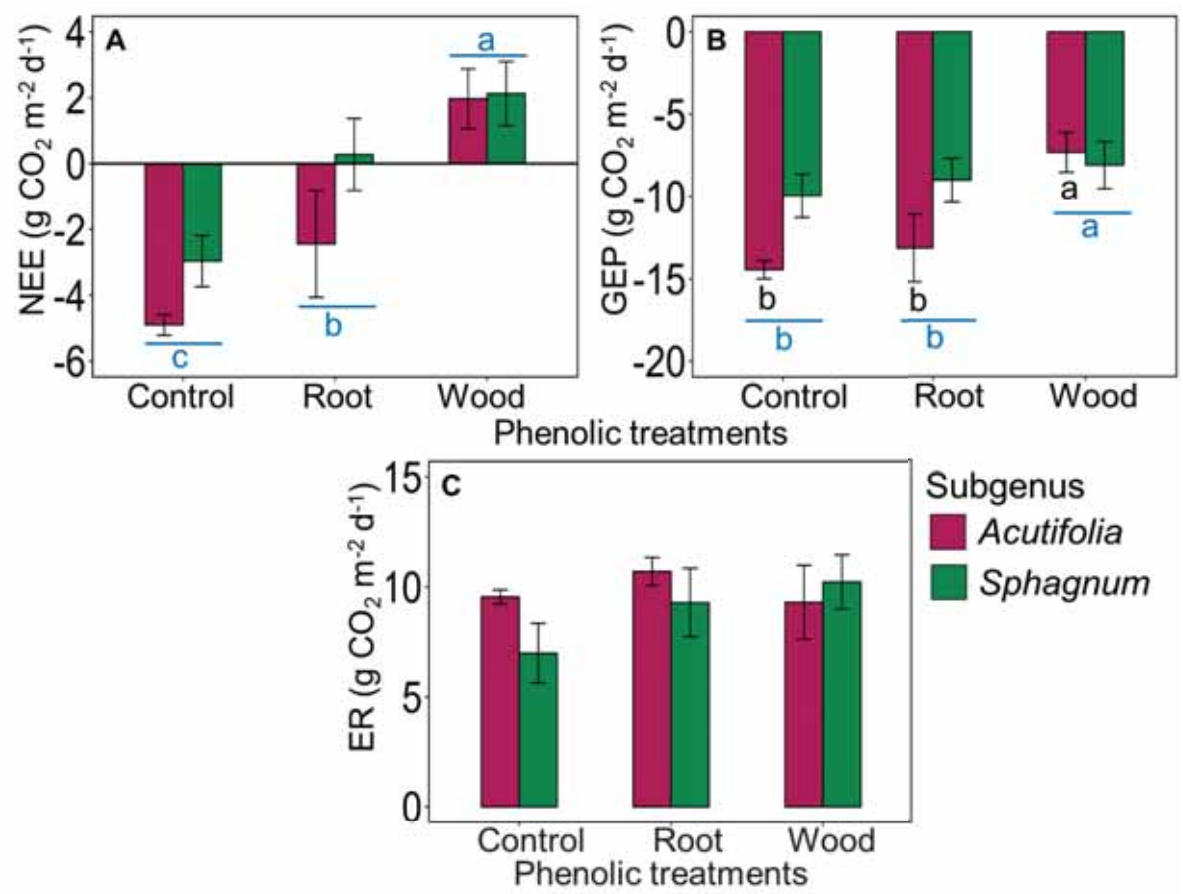


Fig. 2.1. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on mean net ecosystem exchange (A, NEE; g CO_2 $m^{-2}d^{-1}$, n = 4), mean gross ecosystem productivity (B, GEP; g CO_2 $m^{-2}d^{-1}$, n = 4), and mean ecosystem respiration (C, ER; g CO_2 $m^{-2}d^{-1}$, n = 4) at *Acutifolia* and *Sphagnum* subgenus. Negative values indicate CO_2 uptake by the ecosystem from the atmosphere and positive values indicate a release of CO_2 from the ecosystem to the atmosphere. Error bars represent the standard error of the mean. The presence of different lowercase letters indicates significant differences among phenolic treatments based on the significant two-way interactive effect of subgenus and phenolic treatments on observed variables ($p < 0.05$, Tukey's HSD). See Table 2.1 for detailed statistical analysis.

2.6.2 Peat soluble phenolics

The mean peat soluble phenolics content was not found different between both subgenera and independently of the phenolic addition treatments (Table 2.2, Fig. 2.2).

Table 2.2. Linear mixed effects model to determine the effects of subgenus (*Acutifolia* & *Sphagnum*), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on peat soluble phenolics (mg g^{-1}).

Sources	df	MS	*F*	*P*
Subgenus	1	1×10^{-3}	17	0.15
Error a	6	3×10^{-5}		
Phenolic treatments	2	3×10^{-5}	1	0.38
S × P	2	3×10^{-5}	1.1	0.37
Error b	12	3×10^{-5}		

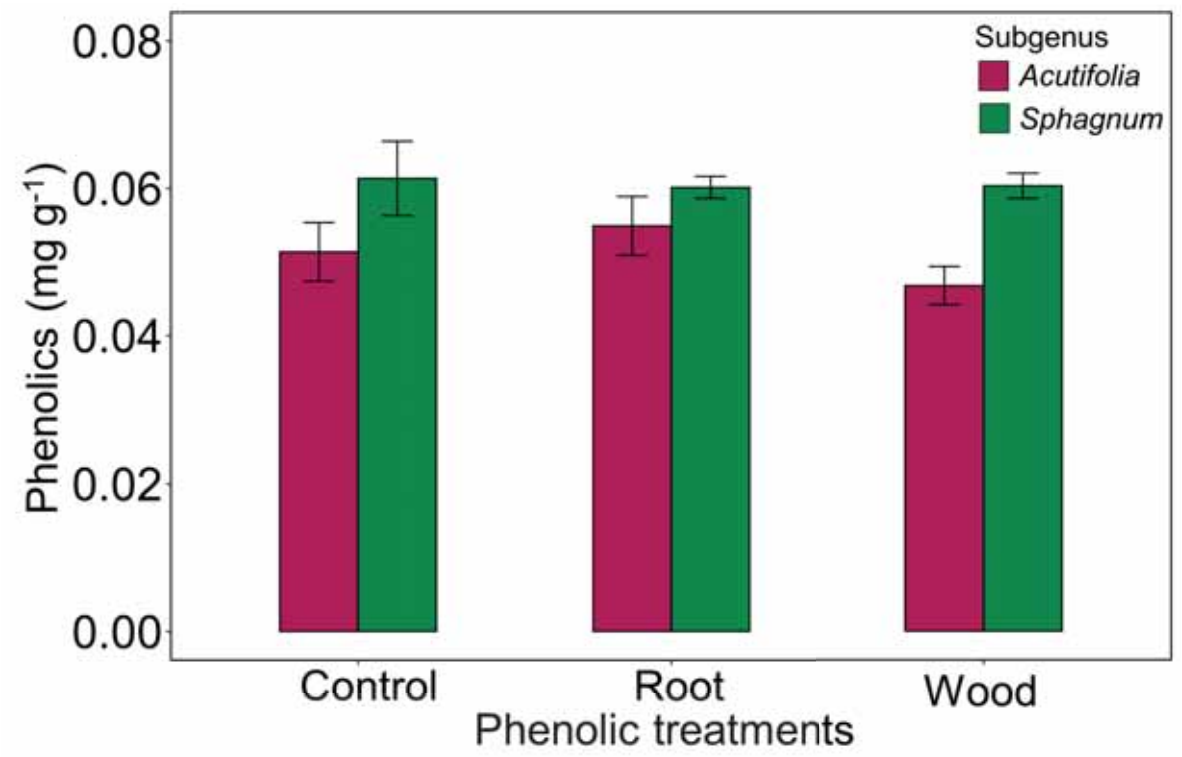


Fig. 2.2. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on mean peat soluble phenolics (mg g-1, n = 4) at *Acutifolia* and *Sphagnum* subgenus. Error bars represent the standard error of the mean. See Tables 2.2 for detailed statistical analysis.

2.6.3 Enzyme activities

2.6.3.1 Hydrolase activities

Among hydrolase enzymes, mean arylsulphatase activities were higher in wood (0.2 ± 0.08 nmol g^{-1} min^{-1}) treatment than control (0.08 ± 0.02 nmol g^{-1} min^{-1}) and root (0.08 ± 0.005 nmol g^{-1} min^{-1}) treatments for *Acutifolia* subgenus (Table 2.3, Fig. 2.3B). The opposite was observed at *Sphagnum* subgenus where root (0.3 ± 0.1 nmol g^{-1} min^{-1}) treatment showed

greater mean arylsulphatase activities than wood (0.2 ± 0.1 nmol g^{-1} min^{-1}) treatment, while control (0.2 ± 0.02 nmol g^{-1} min^{-1}) had similar mean arylsulphatase activities compared to root and wood treatments (Fig. 2.3B). Likewise, the interaction effect of subgenus and phenolic treatment on N-acetyl-β-D-glucosaminidase was significant (Table 2.3). This interaction effect was produced by the difference among phenolic treatments at *Acutifolia* subgenus, where wood (6 ± 1 nmol g^{-1} min^{-1}) treatment resulted in more mean N-acetyl-β-D-glucosaminidase activities compared to control (4 ± 0.3 nmol g^{-1} min^{-1}) and root (3 ± 0.01 nmol g^{-1} min-1, Fig. 2.3D). No difference in mean N-acetyl-β-D-glucosaminidase activities among phenolic treatments was observed for *Sphagnum* subgenus (Fig. 2.3D). In case of mean phosphatase activities, wood (56 ± 9 nmol g^{-1} min-1) treatment showed higher values compared to control (43 ± 2 nmol g^{-1} min^{-1}) and root (26 ± 3 nmol g^{-1} min^{-1}) treatments (Table 2.3, Fig. 2.3E). β-D-glucosidase and β-D-xylosidase activities were not different between mosses from the *Acutifolia* and *Sphagnum* subgenus and phenolic addition treatments (Table 2.3, Fig. 2.3).

2.6.3.2 Phenol oxidase activities

The mean phenol oxidase (POX) activities were similar between *Acutifolia* (64 ± 9 nmol diqc g^{-1} min^{-1}) and *Sphagnum* (110 ± 6 nmol diqc g^{-1} min^{-1}) subgenus (Table 2.3). Among phenolic treatments, wood (113 ± 9 nmol diqc g^{-1} min^{-1}) treatment resulted in greater mean POX activities compared to root (79 ± 12 nmol diqc g^{-1} min^{-1}) and control (69 ± 13 nmol diqc g^{-1} min^{-1}) treatments, and later two treatments had similar mean POX activities (Table 2.3, Fig. 2.3F).

Table 2.3. Linear mixed effects model to determine the effects of subgenus (*Acutifolia* & *Sphagnum*), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on average hydrolase (nmol g^{-1} $min^{-}1$, β-D-glucosidase, arylsulphatase, β-D-xylosidase, N-acetyl-β-D-glucosaminidase, and phosphatase) and phenol oxidase (nmol diqc g^{-1} min^{-1}) activities.

Sources	**d.f.**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
		β-D-glucosidase			***Arylsulphatase***			***β-D-xylosidase***		
Subgenus	1	1	0.4	0.65	0.08	12.8	0.17	8	20.7	0.14
Error a	6	1			0.01			0.4		
Phenolic treatments	2	0.5	0.2	0.85	0.01	0.9	0.4	1.5	3.6	0.06
S × P	2	3	1.1	0.34	0.05	8.2	**0.005**	0.3	0.7	0.50
Error b	12	1			0.01			0.4		
		N-acetyl-β-D-glucosaminidase			***Phosphatase***			***Phenol oxidase***		
Subgenus	1	0.3	0.3	0.68	329	1.2	0.47	13078	34.5	0.11
Error a	6	1			274			379		
Phenolic treatments	2	4	3.4	0.06	1825	6.63	**0.011**	4244	11.2	**0.002**
S × P	2	6	5.9	**0.016**	113	0.41	0.67	788	2.08	0.17
Error b	12	1			275			379		

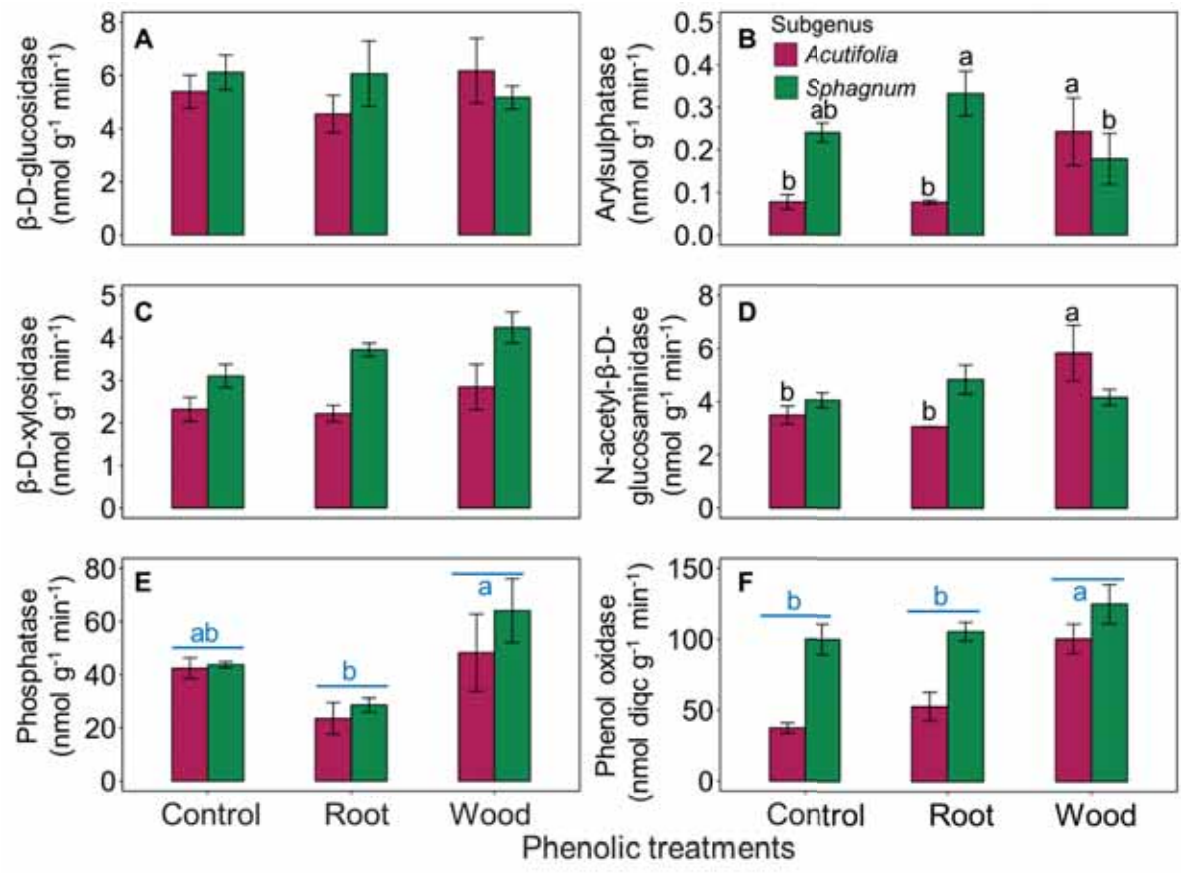


Fig. 2.3. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on mean hydrolase (A to E, nmol g^{-1} min^{-1}, n = 4) and phenol oxidase (F, nmol diqc g^{-1} min^{-1}, n = 4) enzyme activities at *Acutifolia* and *Sphagnum* subgenus. Error bars represent the standard error of the mean. The presence of different lowercase letters indicates significant differences among phenolic treatments based on the significant two-way interactive effect of subgenus and phenolic treatments on observed variables ($p < 0.05$, Tukey's HSD). See Table 2.3 for detailed statistical analysis.

2.6.4 *Sphagnum* productivity and biomass

The mean *Sphagnum* productivity between *Acutifolia* (378 ± 39 g m^{-2} yr^{-1}) and *Sphagnum* (363 ± 32 g m^{-2} yr^{-1}) subgenus was similar (Table 2.4). Alike, the mean values of *Sphagnum* biomass were not different between *Acutifolia* (998 ± 87 g m^{-2}) and *Sphagnum* (809 ± 83 g m^{-2}) subgenus (Table 2.4). Similarly, productivity and biomass were similar among phenolic treatments (Table 2.4, Fig.2.4).

Table 2.4. Linear mixed effects model to determine the effects of subgenus (*Acutifolia* & *Sphagnum*), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on *Sphagnum* productivity (g m^{-2} yr^{-1}) and biomass (g m^{-2}).

	Sources	**df**	**MS**	***F***	***P***
Productivity	Subgenus	1	530	0.05	0.86
	Error a	6	10600		
	Phenolic treatments	2	10168	0.9	0.43
	S × P	2	2346	0.2	0.82
	Error b	12	11298		
Biomass	Subgenus	1	54305	1.3	0.45
	Error a	6	41773		
	Phenolic treatments	2	157683	3.8	0.05
	S × P	2	35007	0.8	0.46
	Error b	12	41496		

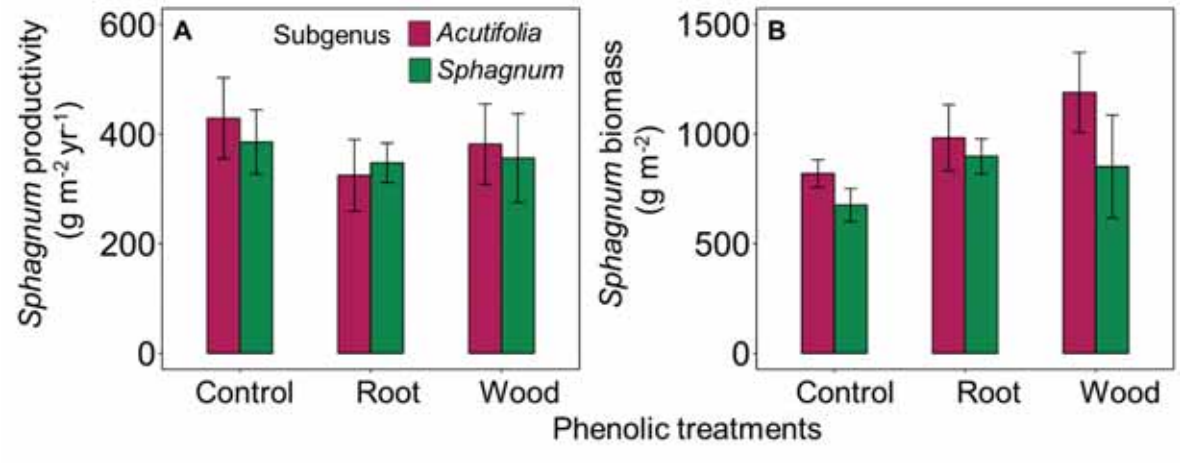


Fig. 2.4. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on mean *Sphagnum* productivity (A, g m^{-2} yr^{-1}, n = 4) and mean *Sphagnum* biomass (B, g m^{-2}, n = 4) at *Acutifolia* and *Sphagnum* subgenus. Error bars represent the standard error of the mean. See Tables 2.4 for detailed statistical analysis.

2.6.5 Regulating factors of the enzymic latch mechanism

Overall, *Acutifolia* and *Sphagnum* subgenus did not show a striking different pattern of enzymic latch mechanism (ELM) in response to phenolic additions. At *Acutifolia* subgenus, NEE was positively correlated with arylsulphatase (r = 0.7, p = 0.02), β-D-xylosidase (r = 0.6, p = 0.03), and N-acetyl-β-D-glucosaminidase (r = 0.6, p = 0.03) activities (Fig.2.5A & A2.2-A), which aligns with the ELM concept. Similarly, β-D-xylosidase activities at

Sphagnum subgenus also exhibited a positive correlation with NEE (Fig. 2.5A, $r = 0.9$, $p < 0.001$), supporting the concept of ELM. At *Acutifolia* subgenus, POX activities showed a positive correlation with NEE (Fig. 2.5B, $r = 0.9$, $p < 0.001$), supporting the concept of ELM. Whereas POX activities at *Sphagnum* subgenus were not correlated with NEE and ER (Fig. 2.5B & A2.2-B, $p > 0.05$). For both subgenera, pH was negatively correlated with peat soluble phenolics (Fig. A2.2). Despite all these, peat soluble phenolics were not correlated with NEE, ER, POX, and hydrolase enzyme activities ($p > 0.05$).

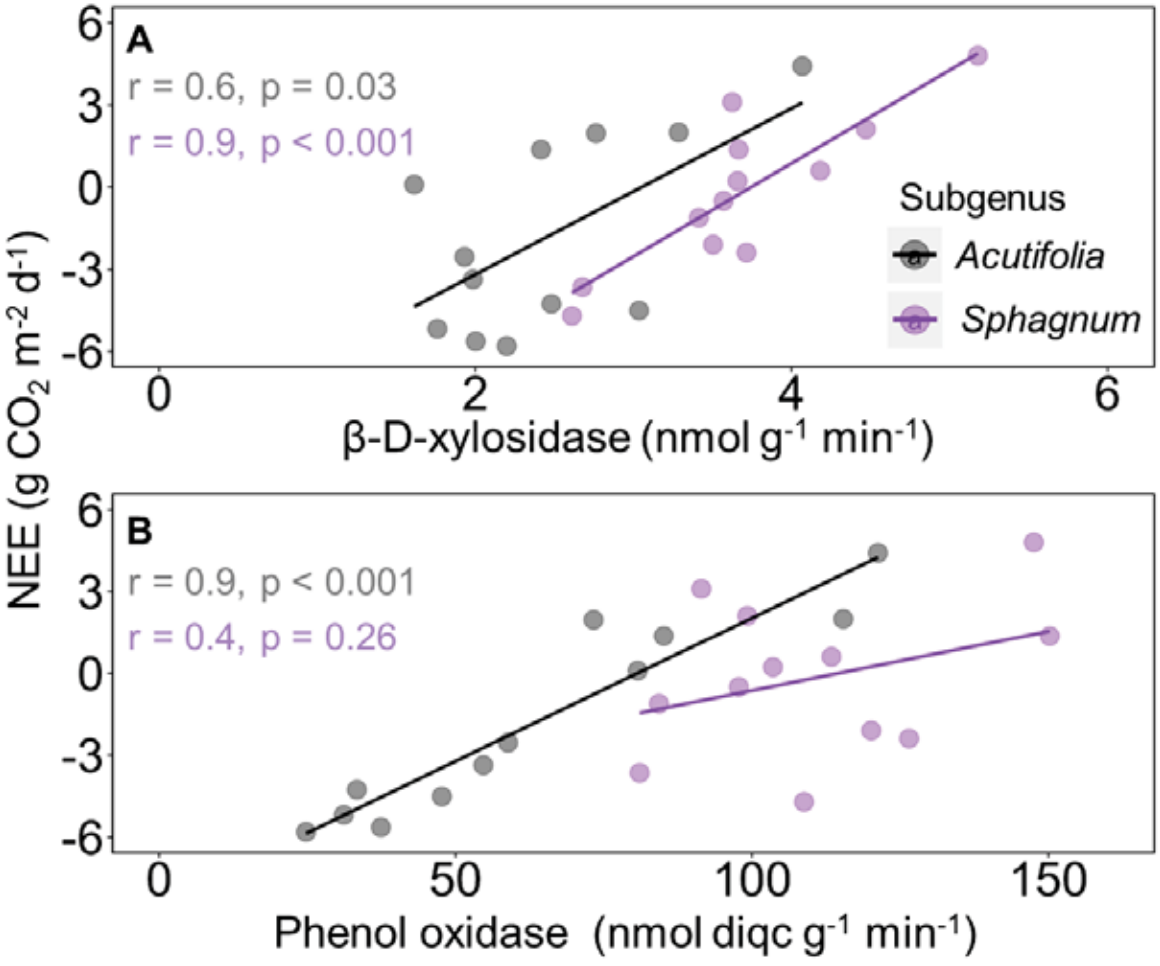


Fig. 2.5. Pearson correlation coefficient of β-D-xylosidase (A, nmol g^{-1} min^{-1}) and phenol oxidase (B, nmol diqc g^{-1} min^{-1}) activities with net ecosystem exchange (NEE, g CO_2 $m^{-2}d^{-1}$) at *Acutifolia* and *Sphagnum* subgenus level. For NEE, negative values indicate CO_2 uptake by the ecosystem from the atmosphere and positive values indicate a release of CO_2 from the ecosystem to the atmosphere.

2.7 Discussion

Phenolic (either natural or added externally) inhibition effect on extracellular enzyme activities emerged as a low-cost effective way to limit carbon dioxide (CO_2) emissions (used as a proxy for decomposition) in peatlands. However, its efficacy in the Sphagnum farming system among different *Sphagnum* species does not appear to support this process on a short-term evaluation in the field studied.

The first question of this paper investigated the difference in CO_2 exchange between cultivated mosses from *Acutifolia* and *Sphagnum* subgenus as biogeochemical differences between cultivated mosses in a Sphagnum farming system are not yet clear. Our results indicate that both subgenera, *Acutifolia* and *Sphagnum*, despite stable water table levels had identical results of ecosystem respiration — used as a proxy for decomposition. Likewise, net ecosystem exchange (NEE) values were also similar between *Acutifolia* (-2 ± 1 g CO_2 m^{-2} d^{-1}) and *Sphagnum* (-0.2 ± 0.8 g CO_2 m^{-2} d^{-1}) subgenus; though values representing a small sink of CO_2. In support to our results, Günther et al. (2017) estimated CO_2 exchange in a Sphagnum farming system in Germany for two growing seasons during the establishment phase and found no difference in the NEE values between *S. papillosum* (-15 ± 1 g CO_2 m^{-2} d^{-1}) and *S. palustre* (-15 ± 1 g CO_2 m^{-2} d^{-1}). The higher NEE values (more CO_2 release compared to CO_2 uptake) in current study compared to Günther et al. (2017) could be linked with the difference in the length of the growing season between Germany and Canada. Within the scope of the first question, it was clear that mosses from *Acutifolia* and *Sphagnum* subgenus did not show prominent differences in terms of CO_2 exchange. Therefore, it was important to account for the effect of externally added phenolics on decomposition of both subgenera measured through proxies such as CO_2 exchange and enzyme activities.

The second question in this study evaluated the role of externally added phenolics in understanding decomposition, based on the ELM, of mosses from *Acutifolia* and *Sphagnum* subgenus. With phenolic enrichment it was valuable to consider the change in peat phenolic content along with decomposition proxies. Unfortunately, the results indicated that phenolic treatments did not play a pivotal role in enhancing peat soluble phenolics, limiting extracellular enzyme activities, and reducing ecosystem respiration compared to the control for the tested *Sphagnum* species. The NEE outcome showed that, between the two phenolic

treatments, *Sphagnum* species under wood treatment subplots acted as a source of CO_2 whereas *Sphagnum* species under root treatment subplots were sink of CO_2. The wood chips decomposition might have contributed to higher NEE values (more CO_2 release compared to CO_2 uptake) compared to root additions. An indication that wood as an external phenolic supplement is not a suitable cost-effective method for reducing emissions in a short duration of experiment (one to two growing seasons) despite having higher soluble phenolic content (1.2 mg g^{-1}) compared to the root (0.2 mg g^{-1}) product. In a similar study conducted in a greenhouse mesocosms, Alshehri et al. (2020) did a phenolic supplement experiment where wood chips of *Larix laricina*, *Picea mariana*, and *Thuja occidentalis* as phenolic additions were added by two methods such as surface addition and phenolic products mixed within top 10 cm of peat surface (mixed treatment). Akin to our results, Alshehri et al. (2020) did not find differences in peat phenolics at surface addition treatments. However, differences in peat phenolics were found in mixed treatment, excluding *Larix laricina*, compared to the control. Surface addition also did not display any difference in enzyme activities but their mixed treatment, for *Picea mariana*, showed lower enzyme activity compared to control. For CO_2 exchange, between surface and mixed treatments, exceptionally *Larix laricina*, and *Picea mariana* under surface addition acted as sink of CO_2. Such results indicate the complex nature of the decomposition process and that could be different based on the method of phenolic addition applications and micro-site conditions. Therefore, it is important to conduct hit and trial lab-based or greenhouse experiments with phenolic additions ranging from surface application to the different levels of depth within *Sphagnum* carpet for better understanding decomposition process. Another experiment conducted in a controlled microcosm exhibited that wood insertion in peat (*Quercus robur* and *Liriodendron tulipifera* wood pieces of 2 cm^3 were inserted 5 cm below the peat surface under anerobic conditions) enhanced polyphenols that reduced extracellular enzyme activities and acted as a quadruple lock on decomposition (Fenner & Freeman, 2020). In the present study, lack of detection of the phenolic addition effect on peat soluble phenolics and extracellular enzyme activities could be attributed to the possibility that recalcitrant compounds from the phenolic products applied at the surface were not transferred to the base of acrotelm, from where peat sampling was conducted. The findings from the aforementioned two studies illustrated that ELM can be a determinative mechanism in controlling decomposition through phenolics-enzyme interaction but this

potential slowing down of decomposition processes was not supported in our short-term field study.

Comparable to short-term field experiments failing to support the ELM, Urbanová & Hájek (2021) conducted a lab-based study to understand the role of phenolics in the context of ELM, where they reported that added phenolics did not limit extracellular enzyme activities and indicated that phenolics could be a source of labile carbon. The researchers assessed that oxidative enzyme activities were similar between oxic and anoxic environments. Some other studies investigated the other aspects of biogeochemical process that could elaborate ELM, for instance Wang et al. (2017) examined the role of iron (Fe) for better understanding the ELM in a field based mesocosm and showed that a lower water table inhibited extracellular enzyme activities. The lower water table resulted in Fe oxidation that increased Fe-protected phenolics and acted as an iron gate against the opening of ELM, pointed out that iron gate mechanism is more important in mineral-rich or vascular plant dominated wetlands. Similarly, van Bodegom et al. (2005) conducted a lab-based study to understand the interaction of iron with phenol oxidase and CO_2 exchange in a water-logged soil where the results showed that added Fe^{2+} significantly increased phenol oxidase (POX) activities and CO_2 production, measured as a proxy for decomposition. The conflicting findings regarding ELM imply a need to test other sources of phenolic additions and to report parameters of vegetation like height increment, productivity, and biomass for better evaluation of treatment effects.

The third question in this experimental study administered the fate of phenolic addition in optimizing productivity and biomass of the *Sphagnum* species under investigation. The results of this study interpreted that phenolic additions did not enhance *Sphagnum* productivity and biomass. In the study of Alshehri et al. (2020), phenolic enrichment in the form of wood chips of *Larix laricina*, *Picea mariana*, and *Thuja occidentalis* did not enhance *Sphagnum* productivity and biomass. The role of phenolics has not been widely tested for *Sphagnum* biomass and productivity therefore current study results were compared with the studies that reported *Sphagnum* biomass and productivity in any manner. For example, Rochefort et al. (1990) compiled a comparison of different studies that reported productivity data. Briefly, different productivity values of *Sphagnum fuscum* were reported in Northern

Europe (70 to 290 g m^{-2} y^{-1}), Western Europe (424-80 g m^{-2} y^{-1}), England (270 g m^{-2} y^{-1}) and Canada (50-303 g m^{-2} y^{-1}). In the same way, different productivity values of *Sphagnum magellanicum* were reported in Northern Europe (70 g m^{-2} y^{-1}), England (50-230 g m^{-2} y^{-1}) and USA (540 g m^{-2} y^{-1}). Whereas, in the current study, productivity values between *Acutifolia* (378 ± 39 g m^{-2} yr^{-1}) and *Sphagnum* (363 ± 32 g m^{-2} yr^{-1}) subgenus were not different but aligns within the range of data reported by other studies. The biomass of *Acutifolia* (983 ± 201 g m^{-2}) and *Sphagnum* (819 ± 130 g m^{-2}) subgenus, estimated from the same experimental site in 2017 (PERG unpublished data), was similar to the biomass of both subgenera estimated in this study. Implying that *Sphagnum* biomass might have reached at a point where biomass production equals decomposition leading to constant biomass production. The results from this short duration study pointed out that surface phenolic additions were not an ideal tool for promoting *Sphagnum* species productivity and biomass.

Overall, phenolics additions did not help in limiting CO_2 emissions and enzyme activities that are essential in validating ELM. On the other hand, it is also essential to look for conceptual relationship between the observed variables that could lead to better interpretation. Therefore, the last question of this study investigated the existence of ELM through regulating factors and examined if they match with the ELM's theoretical explanation for mosses of both subgenera. In the present study, for both subgenera, extracellular enzyme activities showed a positive correlation with NEE (higher enzyme activities leading to higher CO_2 emissions) which is an essential relationship required to validate ELM. However, this pertinent result is of the least importance as enzyme activities were not inhibited by the phenolic additions or anoxic conditions. Contrary to our results, Freeman et al. (2004) showed that hydrolase and POX activities were limited in the presence of phenolic compounds. Overall, for both subgenera, our results clearly showed no evidence of strengthening of ELM in response to phenolic enrichments along with a stable water table close to the surface. In peatlands, a slow rate of decomposition is well established but how much it is regulated by ELM and impacted by phenolic additions remains uncertain.

2.8 Conclusion

This study examined the role of surface application of phenolics in strengthening enzymic latch mechanism (ELM) to limit decomposition at the base of acrotelm (~10 cm below the

Sphagnum surface), thereby boosting the productivity and biomass accumulation of *Acutifolia* and *Sphagnum* subgenus in Sphagnum farming system. Broadly, both subgenera showed indistinguishable responses to phenolic enrichment. Phenolic additions did not inhibit extracellular enzyme activities — an action required for ELM strengthening. Within this field study period (a season and half), phenolic additions did not emerge as a cost-effective method to limit *Acutifolia* and *Sphagnum* subgenus decomposition or optimizing their productivity and biomass accumulation. In this study, insignificant results were obtained to validate the strengthening of ELM through phenolic additions. Primarily we assumed that phenolic products, especially wood chip decomposition contributed to greater carbon dioxide emissions. Furthermore, we also assumed that the surface application of phenolic products for a short period (approximately one year) did not induce the inhibitory effects on extracellular enzyme activities at the *Sphagnum* carpet depth from where all sampling was taken. Consequently, further research is needed to explore other sources of phenolic products and their different methods of application. With surface application, long-term monitoring with sample analysis from various depths will be required to compare phenolic additions effect on the strengthening of ELM and its role in limiting decomposition and thereby optimizing productivity in Sphagnum farming system.

2.9 Acknowledgements

We thank Canadian Sphagnum Peat Moss Association and its members for providing facility to conduct research work. We also appreciate Maria Strack for knowledge transfer related to carbon flux. We are also grateful for Peatland Ecology Research Group (PERG) colleagues and research assistants who helped during the field or laboratory work. Special thanks to Andrew Albert Rutland, Meike Lemmer, Aamir Shehzad Khan, Laura Catalina Riano Pena, Charles Gignac, Kathy Pouliot, Brigitte Vimard Mathieu Ferland, Myriam Tougas-Dumesnil, Louis-Philippe Thériault, Siska Shooner, and Christina Tougas.

2.10 Appendices

Table

Table A2.1. Linear mixed effects model to determine the effects of subgenus (*Acutifolia* & *Sphagnum*), and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on peat methane flux (CH_4; mg CH_4 $m^{-2}d^{-1}$).

Sources	**df**	**MS**	***F***	***P***
Subgenus	1	138	5	0.3
Error a	6	28		
Phenolic treatments	2	47	2	0.2
S × P	2	9	0.3	1
Error b	12	24		

Table A2.2. Chemical properties of peat sample and water table conditions (measured from May to August) among phenolic treatments, control = no addition, root = old roots from peat harrowing, and wood = wood pellets, at *Acutifolia* and *Sphagnum* subgenus plots. Observed variables represent average values ± standard errors of: the water table (WT; negative values indicate a WT below the peat surface, n = 4), potential of hydrogen (pH, n = 4), electrical conductivity (EC, n = 4), carbon and nitrogen ratio (C/N ratio, n = 4), ammonium ion (NH_4^+, n = 4), nitrate ion (NH_3^-, n = 4), sulphate ion (SO_4^{-2}, n = 4), and phosphate ion (PO_4^{3-}, n = 4). The presence of different lowercase letters indicates differences among phenolic treatments (One-way ANOVA, $p < 0.05$) based on the significant two-way interactive effect of subgenus, and phenolic treatments on observed variables.

Sphagnum subgenus	Phenolic treatments	WT (cm)	pH	EC ($\mu S\ cm^{-1}$)	C/N ratio	NH_4^+ (ppm)	NH_3^- (ppm)	SO_4^{-2} (ppm)	PO_4^{3-} (ppm)
Acutifolia	Control	-13.2 ± 1.7	3.9 ± 0.1	79 ± 24	86 ± 10	**26 ± 5 ab**	12 ± 1	1520 ± 127	21 ± 2
	Root	-12.3 ± 1.7	3.8 ± 0.1	81 ± 11	88 ± 4	**21 ± 6 b**	10 ± 1	1537 ± 132	26 ± 3
	Wood	-11.4 ± 1.5	4 ± 0.04	69 ± 4	80 ± 12	**33 ± 2 a**	13 ± 1	1475 ± 260	19 ± 3
Sphagnum	Control	-9.4 ± 2.1	4.6 ± 0.2	61 ± 3	**89 ± 6 a**	34 ± 2	12 ± 0.4	1339 ± 76	36 ± 4
	Root	-8.8 ± 1.2	4.4 ± 0.2	77 ± 7	**76 ± 4 ab**	26 ± 1	9 ± 3	1368 ± 160	35 ± 1
	Wood	-11.1 ± 1.1	4.6 ± 0.2	58 ± 4	**67 ± 4 b**	32 ± 1	10 ± 1	1649 ± 218	32 ± 1

Figures

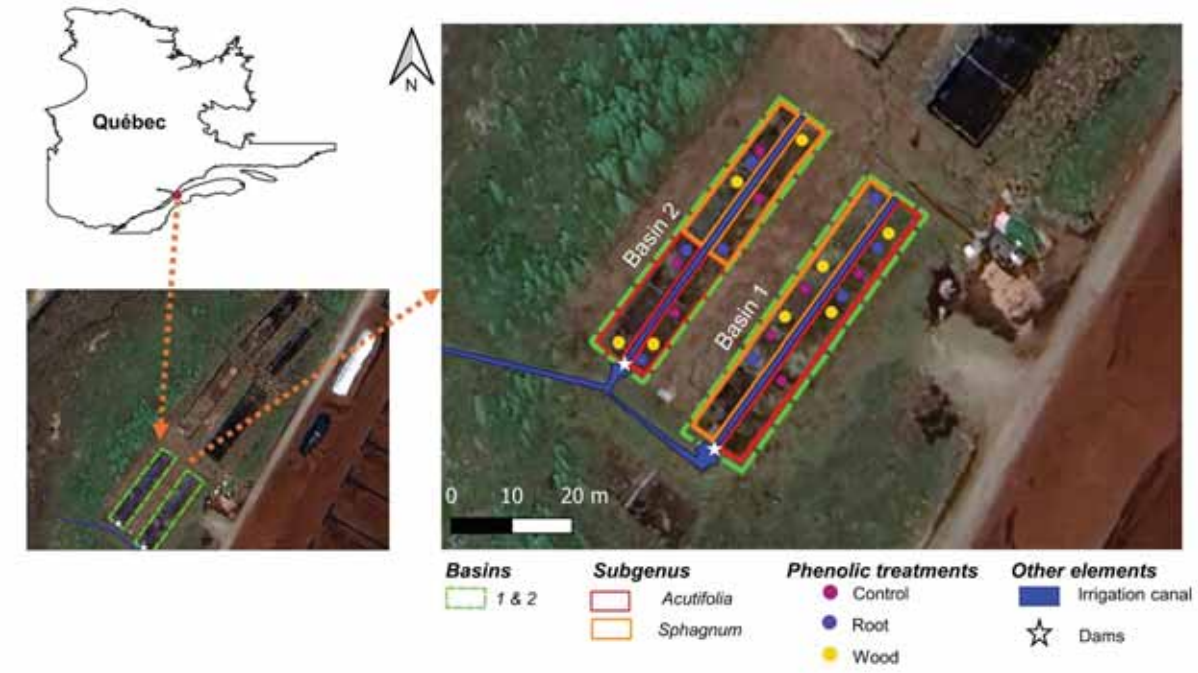
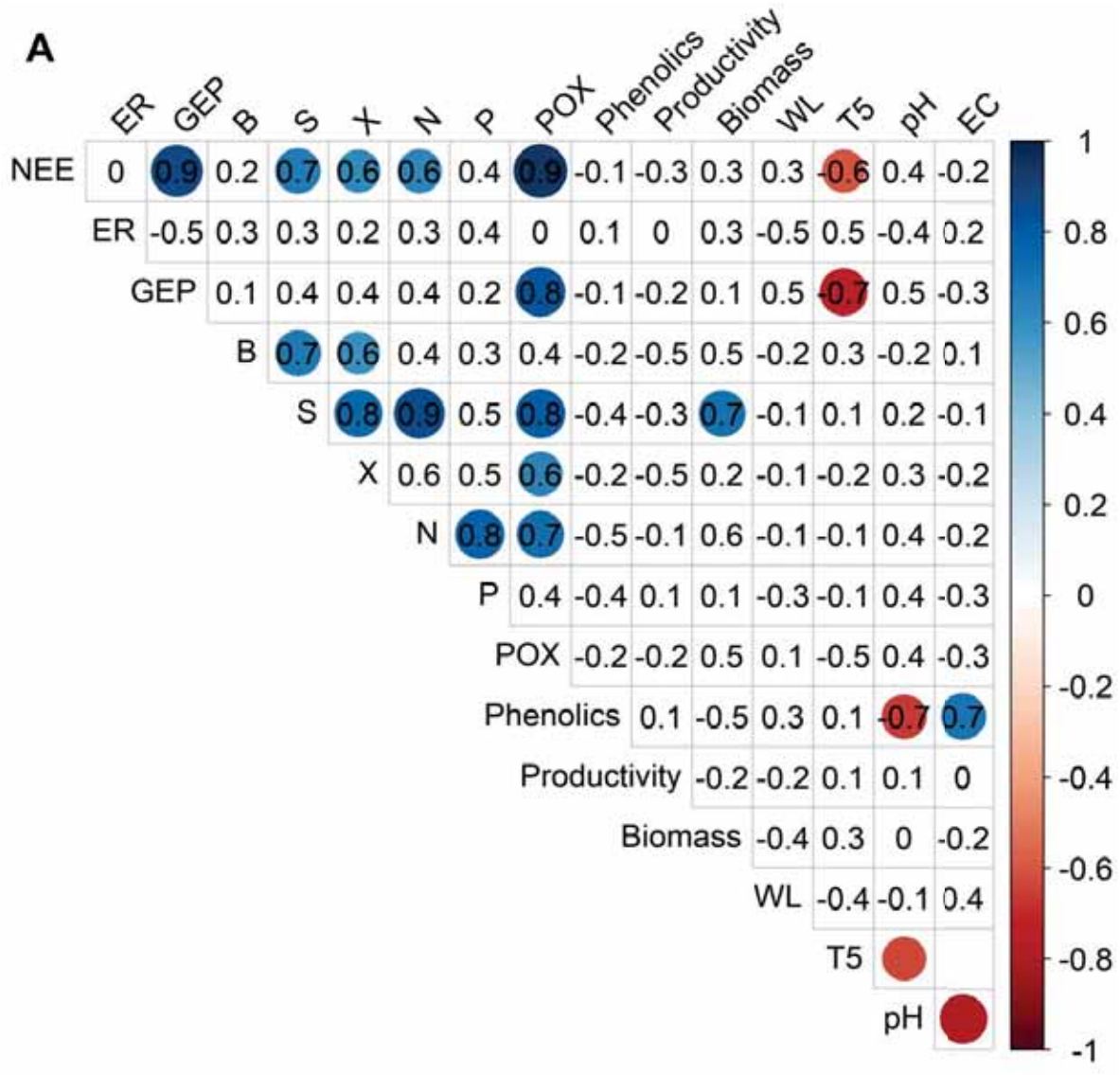


Fig. A2.1. Study area with experimental design in a Sphagnum farming system. Basins (green dash line,1-2) with *Acutifolia* (red lines) and *Sphagnum* (orange lines) subgenus were chosen to test phenolic treatments, where purple dots represent control, blue dots represent root, and yellow dots represent wood treatments. Blue lines represent irrigation canals and star shape represent dams.


A
ER
GEP
B
S
X
N
P
POX
Phenolics
Productivity
Biomass
WL
T5
pH
EC
NEE 0 0.9 0.2 0.7 0.6 0.6 0.4 0.9 -0.1 -0.3 0.3 0.3 -0.6 0.4 -0.2
ER -0.5 0.3 0.3 0.2 0.3 0.4 0 0.1 0 0.3 -0.5 0.5 -0.4 0.2
GEP 0.1 0.4 0.4 0.4 0.2 0.8 -0.1 -0.2 0.1 0.5 -0.7 0.5 -0.3
B 0.7 0.6 0.4 0.3 0.4 -0.2 -0.5 0.5 -0.2 0.3 -0.2 0.1
S 0.8 0.9 0.5 0.8 -0.4 -0.3 0.7 -0.1 0.1 0.2 -0.1
X 0.6 0.5 0.6 -0.2 -0.5 0.2 -0.1 -0.2 0.3 -0.2
N 0.8 0.7 -0.5 -0.1 0.6 -0.1 -0.1 0.4 -0.2
P 0.4 -0.4 0.1 0.1 -0.3 -0.1 0.4 -0.3
POX -0.2 -0.2 0.5 0.1 -0.5 0.4 -0.3
Phenolics 0.1 -0.5 0.3 0.1 -0.7 0.7
Productivity -0.2 -0.2 0.1 0.1 0
Biomass -0.4 0.3 0 -0.2
WL -0.4 -0.1 0.4
T5
pH
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

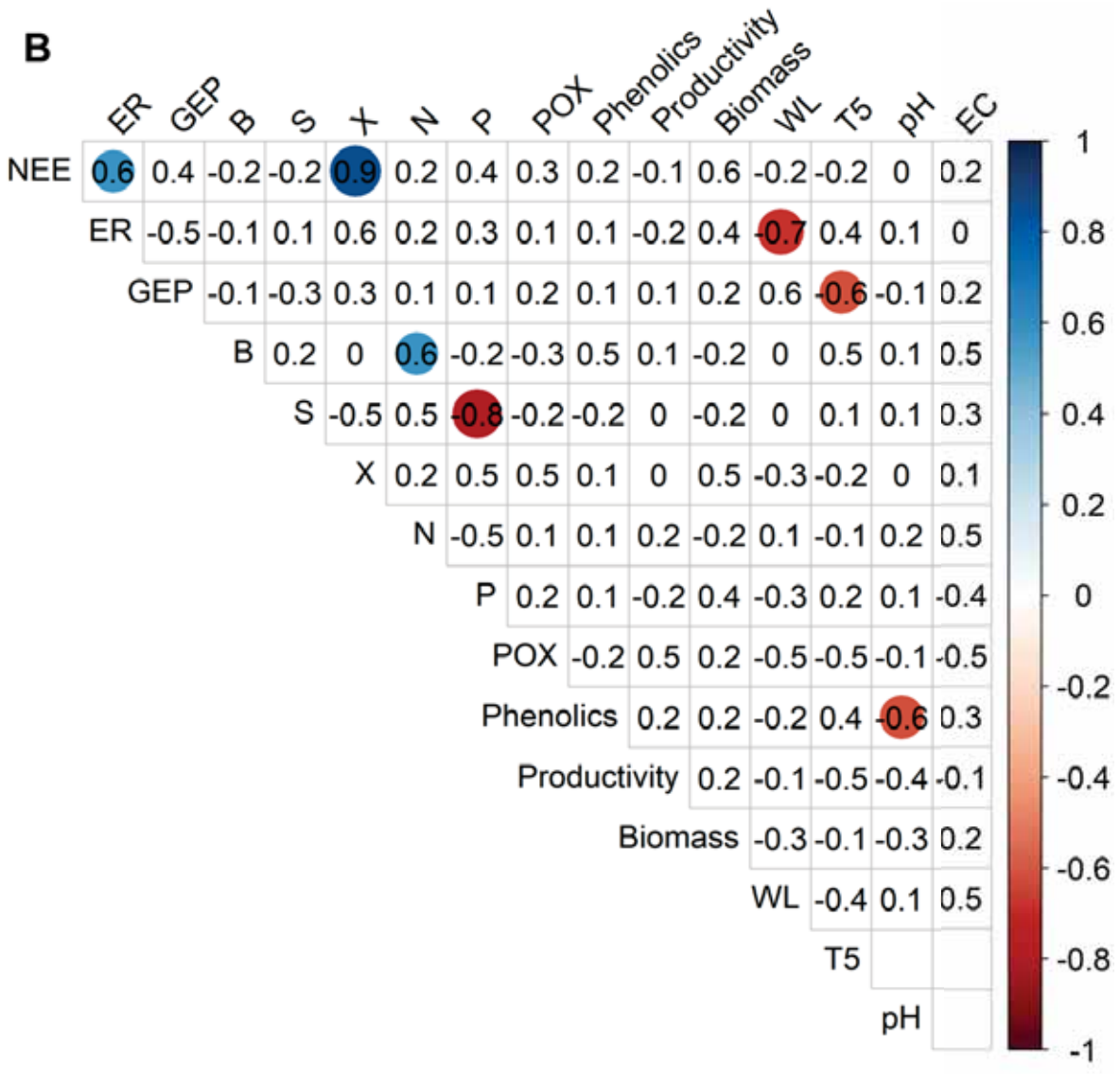


Fig. A2.2. Pearson correlation coefficient between observed variables at *Acutifolia* (A) and *Sphagnum* (B) subgenus level. Highlighted coefficients indicate significant positive (blue) and negative (red) correlations between the observed variables ($p < 0.05$). Abbreviated variables are defined as net ecosystem exchange (NEE), ecosystem respiration (ER), gross ecosystem productivity (GEP), *β-D-glucosidase* (B), arylsuphatase (S), β-D-xylosidase (X), N-acetyl-β-D-glucosaminidase (N), phosphatase (P), phenol oxidase (POX), peat soluble phenolics (phenolics), water table level (WL), peat temperature at -5 cm (T5), potential of hydrogen (pH), and electrical conductivity (EC).

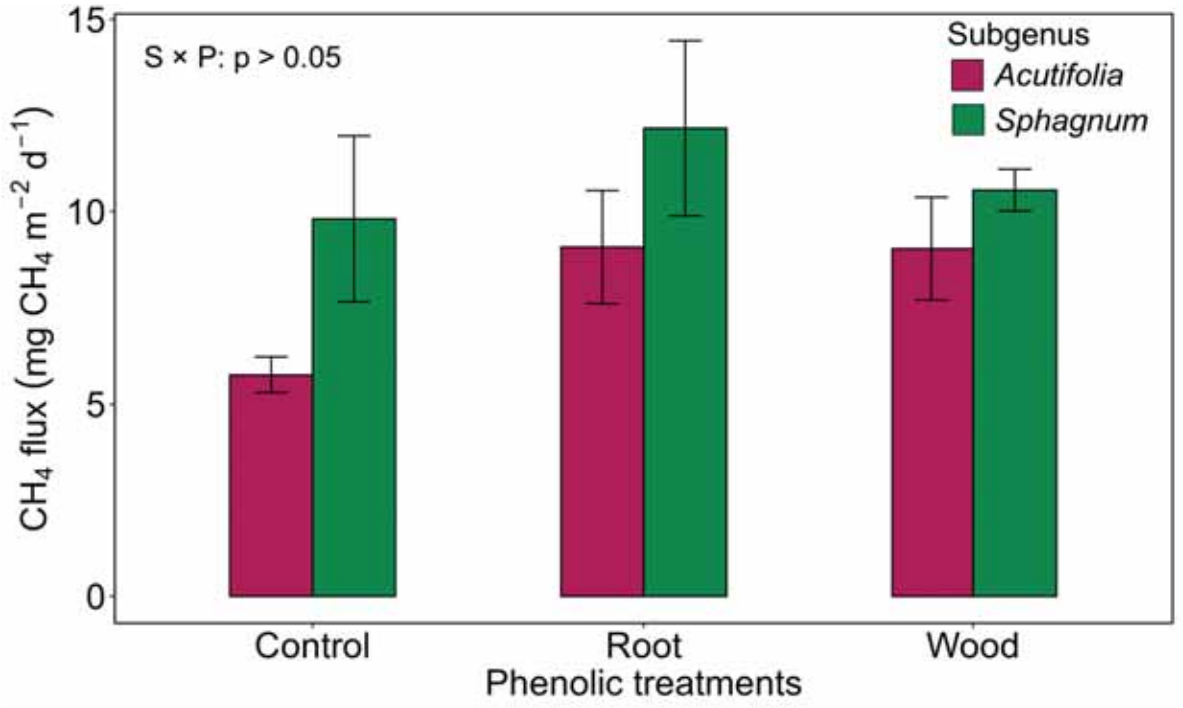


Fig. A2.3. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on peat methane flux (CH_4; mg CH_4 $m^{-2}d^{-1}$, n = 4) at *Acutifolia* and *Sphagnum* subgenus. Positive values represent a release of CH_4 from the ecosystem. Error bars represent the standard error of the mean. See Table A2.1 for detailed statistical analysis.

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Chapter 3 Testing phenolic enrichment effect on enzymic latch mechanism at two stages of *Acutifolia* establishment in a Sphagnum farming system

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3.1 Résumé

En culture de sphaignes, optimiser la biomasse nécessite de limiter la décomposition afin d'améliorer la productivité. Le mécanisme de verrou enzymatique (MVE) a été proposé pour inhiber la décomposition de la matière organique et réduire ainsi les émissions de dioxyde de carbone (CO_2). Dans ce contexte, des ajouts phénoliques ont été testés pour limiter la décomposition et augmenter la production de biomasse des espèces de sphaignes du sous-genre *Acutifolia* à deux stades de culture : un an et neuf ans de tapis établi. Trois traitements phénoliques ont été appliqués sur la surface supérieure des tapis : des granules de bois commerciaux, de vieilles racines et un témoin sans ajout. L'efficacité des traitements a été évaluée en estimant la biomasse des sphaignes, les composés phénoliques solubles dans la tourbe, les échanges de CO_2 et l'activité enzymatique. L'ajout de racines a augmenté les composés phénoliques solubles, mais les traitements phénoliques n'ont pas réduit significativement les émissions de CO_2 ni l'activité enzymatique. Le tapis de neuf ans avec granules de bois a montré une biomasse accrue par rapport au témoin, mais aucun changement significatif n'a été observé pour le tapis d'un an. La réponse de la biomasse résultant de l'ajout de composés phénoliques n'a pas contribué à la productivité brute de l'écosystème par rapport au témoin pour les deux stades des tapis de mousse. Dans cette étude, les enrichissements phénoliques n'ont pas renforcé le MVE, indiquant le besoin de recherches supplémentaires pour trouver les produits phénoliques ou les dosages qui pourraient limiter la décomposition et augmenter la production de biomasse.

3.2 Abstract

Optimization tools to limit decomposition in a Sphagnum farming system are welcome to improve economical productivity. The enzymic latch mechanism (ELM) has been proposed to inhibit organic matter decomposition leading to lower carbon dioxide (CO_2) emissions. In this context, phenolic additions were tested in limiting decomposition and thereby enhancing biomass production of *Acutifolia* subgenus species at two developmental stages — 1 yr-established carpet and 9 yr-established carpet — corresponding to the ages of cultivation of the *Sphagnum* moss in a Sphagnum farming system. Phenolic treatments, such as commercial wood pellets (wood), old roots (root), and control with no addition, were applied on top surface of *Acutifolia* subgenus carpets. The treatment effectiveness was assessed by estimating *Sphagnum* moss biomass, peat soluble phenolics, proxies for decomposition such as CO_2 exchange and enzyme activities. More soluble phenolics were measured with root addition, but overall phenolic additions did not reduce CO_2 emissions or the level of enzyme activities of the moss carpets. At 9 yr-old carpet, wood treatment stimulated the production of biomass compared to control while no difference was observed at 1 yr-young carpet among phenolic treatments. Biomass response from phenolic addition did not contribute to the gross ecosystem productivity compared to control for both ages of the moss carpets. In this study, phenolic enrichments were unable to strengthen ELM at the moss carpets. Further research is needed to identify phenolic products or dosage that could potentially limit decomposition and enhance biomass production through ELM strengthening and make a Sphagnum farming system more efficient.

3.3 Introduction

Peatlands cover approximately 500 million hectares around the globe, 12% of these being degraded and drained for purposes such as agriculture, mining, forestry, and peat extraction. Peatland degradation results in damaging biogeochemical and ecosystem functions. For instance, degraded peatlands contribute to approximately 2,000 megatons of carbon dioxide equivalent (Mt CO_2e) per year of greenhouse gas (GHG) emissions, circa 4% of global GHG emissions excluding those from fire (UNEP, 2022). Implementing peatland management strategies could mitigate the impact of peatland degradation and extraction.

Peatland restoration is recognized as a nature-based solution that re-establish carbon storage on long-term basis. For better understanding, a study by Nugent et al. (2019) compared net ecosystem exchange among unrestored (1 & 15 years), restored (1, 4, & 15 years), and reference (16 years) peatlands. They showed that unrestored and restored (1 & 4 years) peatlands were sources of carbon dioxide (CO_2), whereas, restored (15 years) peatlands had CO2 uptake higher than reference peatland. CO_2 emissions declined over time in restored peatlands due to reduced straw decomposition and increased biomass productivity. Similarly, a global comparison of CO_2 exchange from abandoned to restored peatlands revealed that abandoned and two-years old restored peatlands were sources of CO_2 (> 200 g C m^{-2} $year^{-1}$).Whereas, seven-years (-100 g C m^{-2} $year^{-1}$)) and older restored peatlands were on a trajectory towards net sink of CO_2. For detailed comparison refer to Nugent et al. (2018). For post-extracted and unrestored peatlands, paludiculture involving cultivation of Sphagnum on rewetted peatlands has been identified as an important land management strategy that could have positive effect on CO_2 capture and limit CO_2 emissions during the Sphagnum establishment (Mander et al., 2024).

Sphagnum farming is a type of paludiculture to produce non-decomposed *Sphagnum* fibers on a renewable and cyclical basis. In North America, Sphagnum farming is established for research and development purposes using an adapted version of the Moss Layer Transfer Technique (MLTT), a restoration method developed for cutover bogs (Rochefort et al., 2003). Briefly, it includes: 1) site preparation involving refreshing and reprofiling, 2) construction of basins and irrigation system, 3) donor material harvesting and spreading at a 1:10 ratio, 4) spreading of straw mulch, and 5) blocking of irrigation canals. In Sphagnum

farming, phosphorous fertilization is excluded as it could enhance undesired species of vascular plants, see Guêné-Nanchen & St-Hilaire, (2022) for an overview of Sphagnum farming in Canada.

Non-decomposed *Sphagnum* fibers cultivated in Sphagnum farming could provide donor material for restoration, replace perlite, and vermiculite in growing substrate mixes or be mixed with more decomposed peat to enhance the quality of a growing media (Emmel, 2008; Reinikainen et al., 2012; Jobin et al., 2014; Müller & Glatzel, 2021). As peat is not an infinite resource, sustainable alternatives in growing substrates are needed to reduce pressure on pristine peatlands. To ensure a responsible land management strategy and sustainable solution to peat extraction, optimizing tools will be required to enhance *Sphagnum* production and limit its decomposition.

Optimization experiments testing the impacts of several variables on *Sphagnum* production have been conducted, for example, testing water table level management, removal of unwanted plants, and straw cover (Guêné-Nanchen et al., 2017; Gaudig et al., 2017; Brown et al., 2017; Kim et al., 2020). Another important factor that must not be overlooked is time since establishment as the development of the *Sphagnum* carpet in terms of cover and thickness increases over time leading to enhanced gross ecosystem productivity. For example, Waddington et al. (2010) studied the effects of post-restoration of a peatland on CO_2 exchange from the current year of restoration, 1 and 2 years of post-restoration. The results indicated that vegetation cover increased as time progressed and reached 50% of the area by the end of third year, of which 90% was dominated by moss species. Over time, CO_2 sink function of restored peatland enhanced as measured net ecosystem exchange showed increased value: 1.67 ± 4.68 (the current year of restoration), 6.20 ± 4.80 (1 year of post-restoration), and 7.29 ± 7.75 (2 year of post-restoration) g CO_2 $m^{-2}d^{-1}$ — ecological sign convention was used where positive values indicate CO_2 sequestration from the ecosystem to the atmosphere. In another study, Nugent et al. (2019) compared CO_2 exchange from post-restoration sites that differ in restoration year. Results showed that sites after 15 years of restoration acted as sink of CO_2 compared to the sites that only had 1 and 4 years after restoration. In contrast to peatland restoration, CO_2 exchange measurements from different years of *Sphagnum* post-establishment in a Sphagnum farming system have not been studied.

However, Pouliot et al. (2015) estimated *Sphagnum* cover and *Sphagnum* biomass for seven years during the growing seasons in a Sphagnum farming system which was established in 2006. The results indicated that *Sphagnum* cover increased linearly over time from 13 ± 1% (one year of post-establishment) to 67 ± 5% (seven years of post-establishment). Similarly, a linear increase was observed in *Sphagnum* biomass from first year of post-establishment (42 ± 9 g m^{-2}) to the seven years of post-establishment (787± 86 g m^{-2}). It is important to understand that, similar to pristine peatlands, *Sphagnum* decomposition needs to be lower than productivity to optimize biomass production and yield in Sphagnum farming system.

In the last 20 years, a new biochemical process limiting decomposition in peatlands has been identified: the enzymic latch mechanism (ELM, Freeman et al., 2001; Dunn et al., 2014; Alshehri et al., 2020). In the ELM, decomposition is limited by the accumulation of phenolic compounds that limit microbial and extracellular enzyme activities, responsible for degrading organic matter. Briefly, Freeman et al. (2012) characterized the ELM as a decrease in phenol oxidase activity due to the absence of oxygen, resulting in the accumulation of phenolic compounds which in turn inhibits hydrolase enzyme activities. Such constraints on enzyme activities lead to the buildup of organic matter and reduced CO_2 emissions. Several studies published subsequently to the discovery of the ELM contradict this theory (Harris et al., 2020; Urbanová & Hájek, 2021; Hájek & Urbanová, 2024). However, all these studies, either supporting or refuting the ELM, were mostly relying on lab or greenhouse experiments, and do not fully represent outdoor cultivation of *Sphagnum*. Additionally, it has been documented that phenolics could have a crucial role in restricting de novo synthesis of extracellular enzymes and external supplements of phenolics could be beneficial in strengthening ELM, leading to lower decomposition (van Breeman, 1995; Wetzel, 1992; Freeman et al., 2001; Fenner & Freeman, 2020). As of now, the impact of phenolic addition to limit decomposition and optimize *Sphagnum* biomass production in the context of Sphagnum farming is not documented.

The current study aimed to assess the effect of external phenolics additions on limiting decomposition and enhancing *Sphagnum* biomass production by strengthening ELM at different times of post-establishment, 1 yr-established carpet vs 9 yr-established carpets (year correspond to the cultivation cycle of *Sphagnum*), in a Sphagnum farming system. Our goal

was to address the following questions: 1) Do CO_2 exchange, peat soluble phenolics, and enzyme activities vary depending on the time of post-establishment? 2) Is *Sphagnum* biomass production greater in response to phenolic additions? If yes, does it have a greater contribution to gross ecosystem productivity (CO_2 sequestration)? Irrespective of phenolic treatments, does ELM exist or differ between 1 yr and 9 yr-established carpets. We hypothesize that 1) phenolic additions would limit CO_2 emissions from 1 yr and 9 yr-established carpets, 2) enzyme activities in response to phenolic additions would be lower at 9 yr-established carpets compared to 1 yr-established carpets, 3) phenolic addition would increase biomass production leading to greater gross ecosystem productivity, and 4) a strong evidence of ELM would be observed at 9 yr-established carpets compared to 1 yr-established carpets irrespective of the phenolic additions.

3.4 Materials and methods

3.4.1 Study site

The Sphagnum farming site was established in 2013 on a natural peatland beside a cutover bog in the Rivière-du-Loup peatland complex (47°49′ N and 69°27′; Québec, Canada). Six basins were established: three with central irrigation canals (Basin 1, Basin 2, Basin 5) and three with peripheral irrigation canals (Basin 3, Basin 4, Basin 6), each measuring 50 m × 10 m. Water used for irrigating the *Sphagnum* cultivation basins was pumped from a sedimentation basin collecting the organic acidic water from peat field drainage ditches. The water table level in the cultivation basins was maintained between -5 cm to 0 cm from the *Sphagnum* surface by irrigation canals prompted by digital sensors. Different moss species of *Sphagnum* subgenus were cultivated on all basins according to the Moss Layer Transfer Technique with minor modifications. For further details on the site and Sphagnum farming please refer to Gutierrez Pacheco et al. (2021) and Guêné-Nanchen & St-Hilaire (2022).

3.4.2 Experimental setup

For this study, in June 2021, three 4 m × 4 m bare peat plots were replicated four times in different cultivation basins. Mosses from the *Acutifolia* subgenus were spread on these plots at a 1:10 ratio (1 m^2 donor area mosses spread over 10 m^2 area). To avoid measuring CO_2 release from straw decomposition, and thus isolate phenolic treatment effects, straw mulch was not spread on top of the *Acutifolia* mosses in these 12 plots (later referred to as ‘1 yr-

established carpet). Additionally, another twelve 4 m × 4 m plots within 9 years old established basins were selected, representing the 9th year of growth of the established carpet (later referred to as '9 yr-established carpet). The experimental setup was a split-plot with age as a main plot factor. In basins with 1 yr-established carpets, each basin represented a replication and had three phenolic treatments as a subplot factor. While, in basins with 9 yr-established carpets, four replications were established across two basins with three phenolic treatments as a subplot factor. Two locally available products with different soluble phenolic concentrations were chosen: commercial wood pellets (1.168 mg g^{-1} phenolics) and old roots extracted during peat harrowing (0.159 mg g^{-1} phenolics). The wood pellets (later referred to as 'wood') were 100% natural softwood pellets from Granulco, made from species of the *Picea* and *Abies* genera. Each pellet was approximately 40 mm in length and 6 mm in diameter. The old roots (later referred to as 'root') were chipped with the aid of a commercial wood chipping machine. The resulting root chips derived from *Picea mariana* were fibrous and ranged in size from 0.3 mm × 0.1 mm ± 0.05. At both carpets, the phenolic products were randomly applied at a rate of 2 kg m^{-2} (fresh weight dosage) on the top of the *Sphagnum* mosses, and a control plot with no phenolic addition was also established. Like previous chapters, dosage rate of phenolic products was chosen based on greenhouse experiments and personal experience. In total, 24 experimental units representing age (two levels) × phenolic treatments (three levels) and with the design replicated four times were constructed (Fig. A3.1).

3.4.3 Sampling and analyses

At both carpets, net ecosystem exchange (NEE) of carbon dioxide (CO_2; g CO_2 m^{-2}d^{-1}), *Acutifolia* biomass (g m^{-2}), water table level (cm), soil temperature (°C), and peat — for soluble phenolics (mg g^{-1}), hydrolase enzyme activities (nmol g^{-1} min^{-1}), phenol oxidase enzyme activities (nmol diqc g^{-1} min^{-1}), pH and electrical conductivity (EC, µS cm^{-1}) — were sampled and analyzed according to the methods outlined in chapters 1 and 2. Briefly, for *Acutifolia* biomass, three 25 cm × 25 cm quadrats were used to collect samples from each experimental unit in October 2022. Later, biomass samples were sorted in the laboratory to obtain oven-dried weight of *Acutifolia* fragments. In case of NEE measurement, stainless steel collars of 60 cm × 60 cm × 20 cm dimensions were inserted into *Acutifolia subgenus* carpets, and a clear acrylic chamber (60 cm × 60 cm × 30 cm) connected with a portable

infrared analyser (IRGA; EGM-4 PP systems USA) was used to measure NEE during the growing season of 2022. Ecosystem respiration (ER; g CO_2 $m^{-2}d^{-1}$) was measured by covering the clear acrylic chamber with an opaque shroud, serving as a proxy for decomposition measurement. Gross ecosystem productivity (GEP; g CO_2 $m^{-2}d^{-1}$) was calculated from the equation: GEP = NEE – ER. For CO_2 exchange, we used the conventional sign approach, where negative values indicate CO_2 sequestration and positive values indicate CO_2 emissions from the ecosystem to the atmosphere. Composite peat samples from each experimental unit were collected in October 2022 and analyzed for soluble phenolics through water extraction method explained by Alshehri et al. (2020). As a proxy for decomposition, peat samples were also used for the estimation of five key hydrolase enzymes (arylsulphatase, N-acetyl-β-D-glucosaminidase, β-D-glucosidase, β-D-xylosidase, and phosphatase) and phenol oxidases at Bangor University by the methods illustrated by Dunn et al. (2014).

3.4.4 Statistical analysis

For current study's factorial experiment, data was analyzed in R software (R core team, 2023) with a linear mixed effects (LME) model from the lme4 package (Bates et al., 2015). In the LME model, age, phenolic treatments, and their interaction were placed as fixed factors, Careful selection of random factors is crucial for addressing repeated measurements and appropriately calculating model error terms. For instance, NEE data consisted of pseudo-replication and its LME model included random effects as (1|Basin: Age) + (1|Replicate: Age: Basin) + (1|Phenolic treatments: Replicate: Age: Basin). In contrast, for biomass data without repeated measures, the random effects in the LME model were specified as (1|Basin: Age) + (1|Replicate: Age: Basin). The normality and homogeneity of residuals for all models were visually inspected. For all models, joint_tests in the emmeans package were used to extract ANOVA (Lenth, 2023). In case of significant factors, Tukey pairwise comparison in the emmeans package along with compact letter display function in multcomp package (Hothorn et al., 2008) were used. For significant interaction terms, one-way ANOVA was extracted using joint_tests with by function and later Tukey pairwise comparison was completed. To better understand linked factors in the enzymic latch mechanism, the Pearson correlation coefficient between the response variables for each level of age factor were estimated using correlation_matrix function in corrtable package (van der Laken, 2023) that provided Pearson correlation coefficient along with significance levels. All the figures were generated with the

ggplot2 package (Wickham, 2016) and statistical results were reported at a significant level of less than 0.05.

3.5 Results

3.5.1 Carbon dioxide exchange

The effect of phenolic treatments on the net ecosystem exchange (NEE) of carbon dioxide (CO_2) from wood (2 ± 0.5 g CO_2 $m^{-2}d^{-1}$) additions was higher compared to the root (-1 ± 1 g CO_2 $m^{-2}d^{-1}$) and control (-4 ± 0.4 g CO_2 $m^{-2}d^{-1}$) treatments (Table 3.1, Fig. 3.1A).

Table 3.1. Linear mixed effects model to determine the effects of *Acutifolia* carpet age (1 yr-established carpets & 9 yr-established carpets) and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on net ecosystem exchange (NEE; g CO_2 $m^{-2}d^{-1}$), gross ecosystem productivity (GEP; g CO_2 $m^{-2}d^{-1}$), and ecosystem respiration (ER; g CO_2 $m^{-2}d^{-1}$).

Flux component	Sources	df	MS	*F*	*P*
NEE	Age (A)	1	29	7.7	0.068
	Error a	6	4		
	Phenolic treatments (P)	2	99	26.2	**< 0.0001**
	A × P	2	7	1.8	0.210
	Error b	12	4		
GEP	Age (A)	1	171	48.3	0.006
	Error a	6	4		
	Phenolic treatments (P)	2	155	43.9	< 0.0001
	A × P	2	27	7.7	**0.007**
	Error b	12	4		
ER	Age (A)	1	202	60.6	**0.004**
	Error a	6	3		
	Phenolic treatments (P)	2	3	1	0.398
	A × P	2	3	0.8	0.486
	Error b	12	3		

In case of gross ecosystem productivity (GEP), an age-phenolic treatment interaction (Table 3.1) indicated that at 1 yr-established carpets, the mean flux of GEP from wood (-1 ± 0.2 g CO_2 $m^{-2}d^{-1}$) and root (-2 ± 0.2 g CO_2 $m^{-2}d^{-1}$) additions was higher compared to the control (-5 ± 0.2 g CO_2 $m^{-2}d^{-1}$, Table 3.1, Fig. 3.1B, $p < 0.001$). While, at 9 yr-established carpets, wood (-7± 1 g CO_2 $m^{-2}d^{-1}$) addition resulted in a greater flux of GEP than root (-13± 2 g CO_2 $m^{-2}d^{-1}$) and control (14 ± 0.5 g CO_2 $m^{-2}d^{-1}$) treatments (Fig. 3.1B, $p < 0.001$). The

mean CO_2 release value as ecosystem respiration (ER) was higher from 9 yr-established carpets (10 ± 1 g CO_2 $m^{-2}d^{-1}$) compared to the 1 yr-establishing carpets (3 ± 0.2 g CO_2 $m^{-2}d^{-1}$, Table 3.1, Fig. 3.1C).

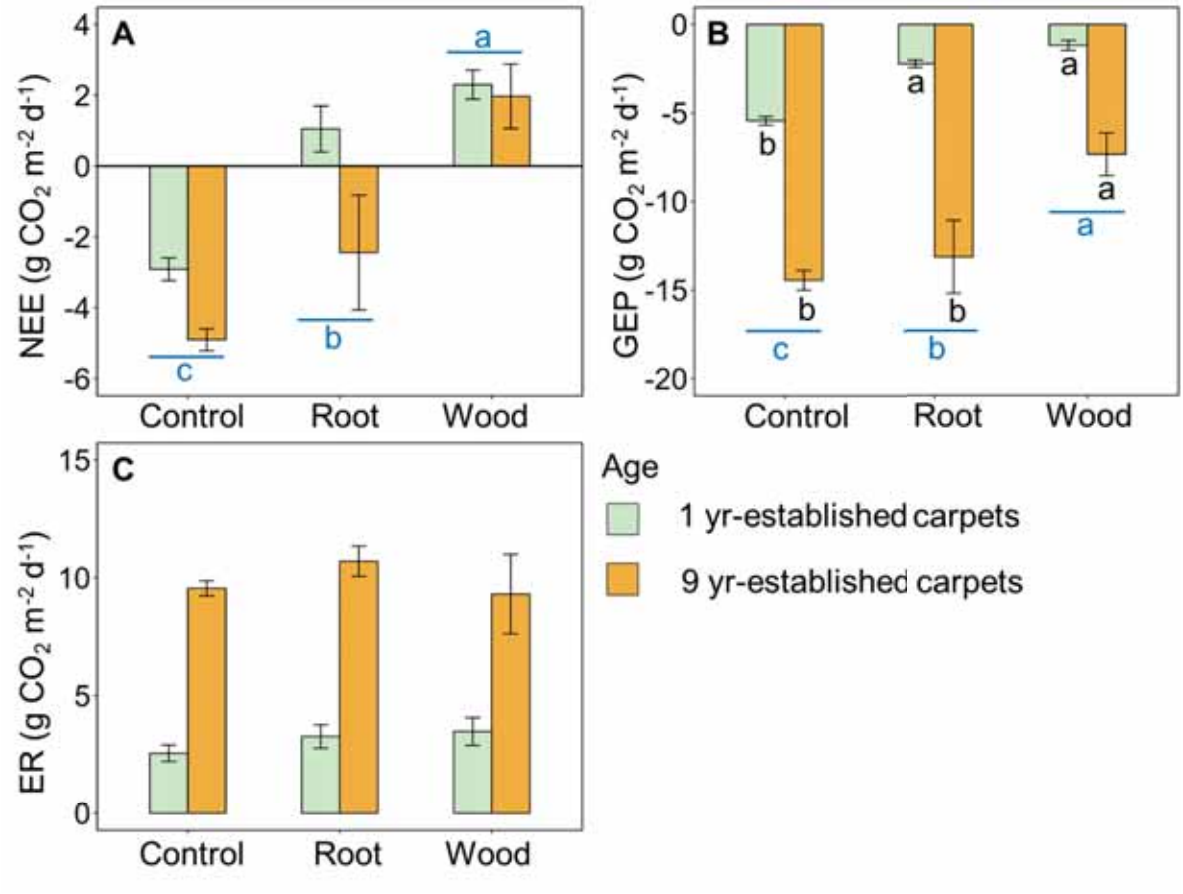

Fig. 3.1. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on mean net ecosystem exchange (A, NEE; g CO_2 $m^{-2}d^{-1}$, n = 4), mean gross ecosystem productivity (B, GEP; g CO_2 $m^{-2}d^{-1}$, n = 4), and mean ecosystem respiration (C, ER; g CO_2 $m^{-2}d^{-1}$, n = 4) at 1 yr-established carpets and 9 yr-established carpets. Negative values indicate CO_2 uptake by the ecosystem from the atmosphere and positive values indicate a release of CO_2 from the ecosystem to the atmosphere. Error bars represent the standard error of the mean. The presence of different blue colour lowercase letters indicates significant differences among phenolic treatments based on the significant main effect of phenolic treatments. Whereas the presence of different black colour lowercase letters indicates significant differences among phenolic treatments for each age level separately based on the interactive effect of age and phenolic treatments ($p < 0.05$, Tukey's HSD). See Table 3.1 for detailed statistical analysis.

3.5.2 Peat soluble phenolics

The mean content of peat soluble phenolics was greater in quantity at 1 yr-established carpets (0.2 ± 0.04 mg g^{-1}) compared to the 9 yr-established carpets (0.1 ± 0.002 mg g^{-1}, Fig. 3.2, $p < 0.001$). At 1 yr-established carpets, root (0.5 ± 0.01 mg g^{-1}) additions displayed higher amount of mean peat soluble phenolics compared to wood (0.1 ± 0.01 mg g^{-1}) and control (0.1 ± 0.01 mg g^{-1}) treatments, whereas, for the 9 yr-established carpets, mean peat soluble phenolics were identical among phenolic treatments (Table 3.2, Fig. 3.2).

Table 3.2. Linear mixed effects model to determine the effects of *Acutifolia* carpet age (1 yr-established carpets & 9 yr-established carpets) and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on peat soluble phenolics (mg g^{-1}).

Sources	**df**	**MS**	***F***	***P***
Age (A)	1	0.02	90	< 0.001
Error a	6	0.0002		
Phenolic treatments (P)	2	0.07	319	< 0.0001
A × P	2	0.06	296	**< 0.0001**
Error b	12	0.0002		

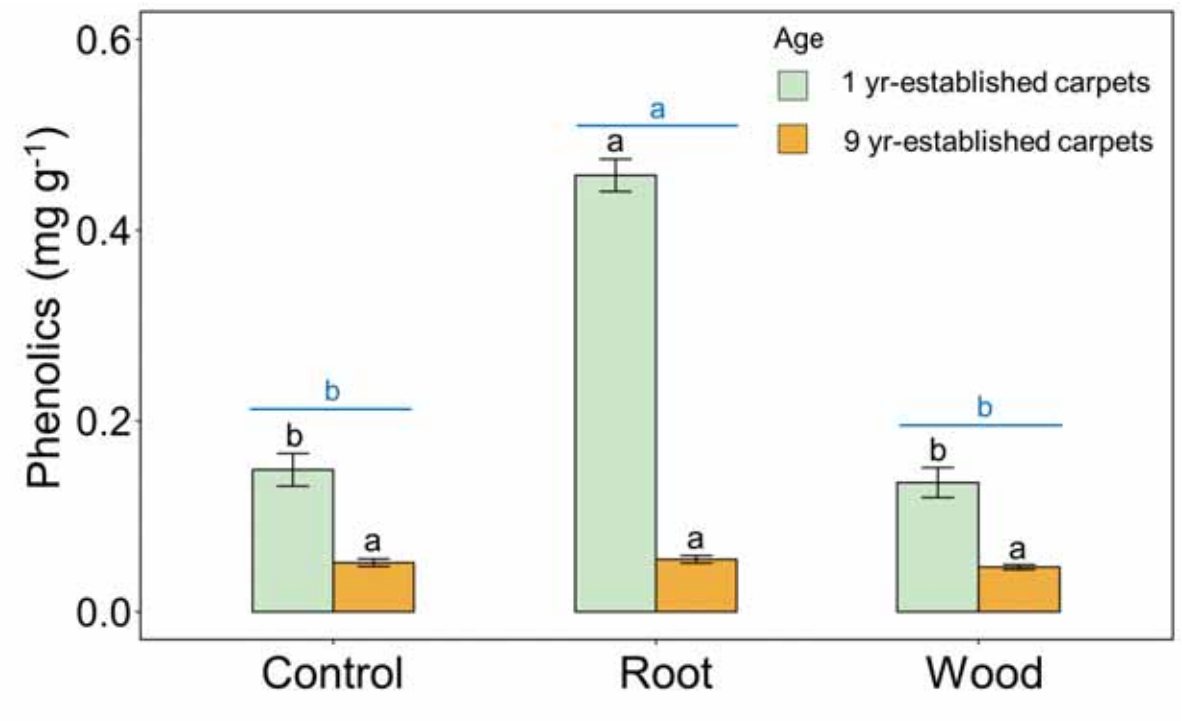

Fig. 3.2. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on mean peat soluble phenolics (mg g^{-1}, n = 4) at 1 yr-established carpets and 9 yr-established carpets. Error bars represent the standard error of the mean. The presence of different blue colour lowercase letters indicates significant differences among phenolic treatments based on the significant main effect of phenolic treatments. Whereas the presence of different black colour lowercase letters indicates significant differences among phenolic treatments for each age level separately based on the interactive effect of age and phenolic treatments ($p < 0.05$, Tukey's HSD). See Table 3.2 for detailed statistical analysis.

3.5.3 Enzyme activities

3.5.3.1 Hydrolases activities

All five hydrolase enzymes (arylsulphatase, N-acetyl-β-D-glucosaminidase, β-D-glucosidase, β-D-xylosidase, and phosphatase) activities were similar between 1 yr and 9 yr established carpets (Table 3.3, Fig. 3.3). Among hydrolase enzymes, mean arylsulphatase activities were higher at wood (0.3 ± 0.04 nmol g^{-1} min^{-1}) additions compared to control (0.1 ± 0.02 nmol g^{-1} min^{-1}) and root treatments (0.1 ± 0.02 nmol g^{-1} min^{-1}, Table 3.3, Fig. 3.3A). Likewise, greater mean N-acetyl-β-D-glucosaminidase activities were observed at wood (5 ± 0.7 nmol g^{-1} min^{-1}) additions compared to control (3.4± 0.2 nmol g^{-1} min^{-1}) and root treatments (3 ± 0.2 nmol g^{-1} min^{-1}, Table 3.3, Fig. 3.3B). Mean β-D-glucosidase, β-D-

xylosidase, and phosphatase enzyme activities were not different among phenolic treatments (Table 3.3, Fig. 3.3C-E).

3.5.3.2 Phenol oxidase activities

The mean phenol oxidase (POX) activities of 1 yr-established carpets (140 ± 16 nmol diqc g^{-1} min^{-1}) were found to be two-fold higher than the 9 yr-established carpets (64 ± 9 nmol diqc g^{-1} min^{-1}, Table 3.3). While no difference was seen in POX activities independently of phenolic treatments (Table 3.3, Fig. 3.3F).

3.5.4 *Sphagnum* biomass of *Acutifolia* species

The mean *Sphagnum-Acutifolia* biomass at 1 yr-established carpets (124 ± 9 g m^{-2}) was approximately 9 folds lower than the 9 yr-established carpets (998 ± 87 g m^{-2}, Fig. 3.4, p = 0.006). Among phenolic additions of 9 yr-established carpets, only wood addition resulted in higher *Sphagnum-Acutifolia* biomass compared to control (p = 0.0078, Fig. 3.4), while no difference among phenolic treatments was identified at 1 yr-established carpets (Table 3.4).

Table 3.3. Linear mixed effects model to determine the effects of *Acutifolia* carpet age (1 yr-established carpets & 9 yr-established carpets) and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on hydrolase (nmol g^{-1} $min^{-}1$; arylsulphatase, N-acetyl-β-D-glucosaminidase, β-D-glucosidase, β-D-xylosidase, and phosphatase) and phenol oxidase (POX; nmol diqc g^{-1} min^{-1}) enzyme activities.

Sources	**d.f.**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
		Arylsulphatase			***N-acetyl-β-D-glucosaminidase***			***β-D-glucosidase***		
Age (A)	1	0.03	3.3	0.168	9	7.8	0.066	10	5.1	0.108
Error a	6	0.01			1			2		
Phenolic treatments (P)	2	0.06	7.7	**0.007**	6	5.7	**0.018**	1	0.5	0.603
A × P	2	0.001	0.1	0.871	4	3.3	0.073	2	1.2	0.331
Error b	12	0.01			1			2		
		β-D-xylosidase			***Phosphatase***			***Phenol oxidase***		
Age (A)	1	2	6.7	0.08	1719	6	0.089	34955	23.5	**0.016**
Error a	6	0.3			287			1487		
Phenolic treatments (P)	2	0.8	3.3	0.074	654	2.3	0.144	4627	3.1	0.082
A × P	2	0.2	0.8	0.485	177	0.6	0.555	4986	3.4	0.07
Error b	12	0.2			295			1466		

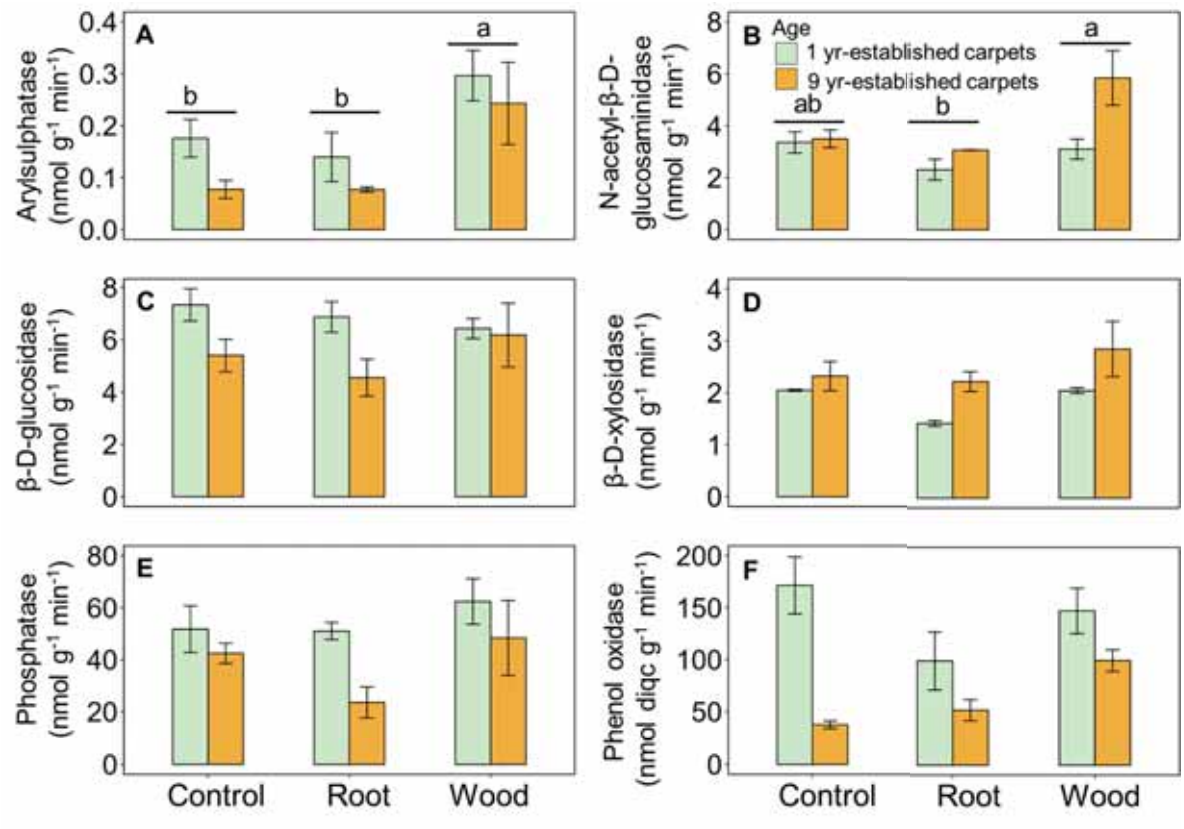

Fig. 3.3. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on mean hydrolase (A to E, nmol g^{-1} min^{-1}, n = 4) and phenol oxidase (F, nmol diqc g^{-1} min^{-1}, n = 4) enzyme activities at 1 yr-established carpets and 9 yr-established carpets. Error bars represent the standard error of the mean. The presence of different lowercase letters indicates significant differences among phenolic treatments for each age level separately based on the significant main effect of phenolic treatments ($p < 0.05$, Tukey's HSD). See Table 3.3 for detailed statistical analysis.

Table 3.4. Linear mixed effects model to determine the effects of *Acutifolia* carpet age (1 yr-established carpets & 9 yr-established carpets) and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on *Acutifolia* subgenus biomass (g m^{-2}).

Sources	**df**	**MS**	***F***	***P***
Age (A)	1	586928	42.6	0.007
Error a	6	13778		
Phenolic treatments (P)	2	66761	4.8	0.029
A × P	2	71057	5.2	**0.024**
Error b	12	13665		

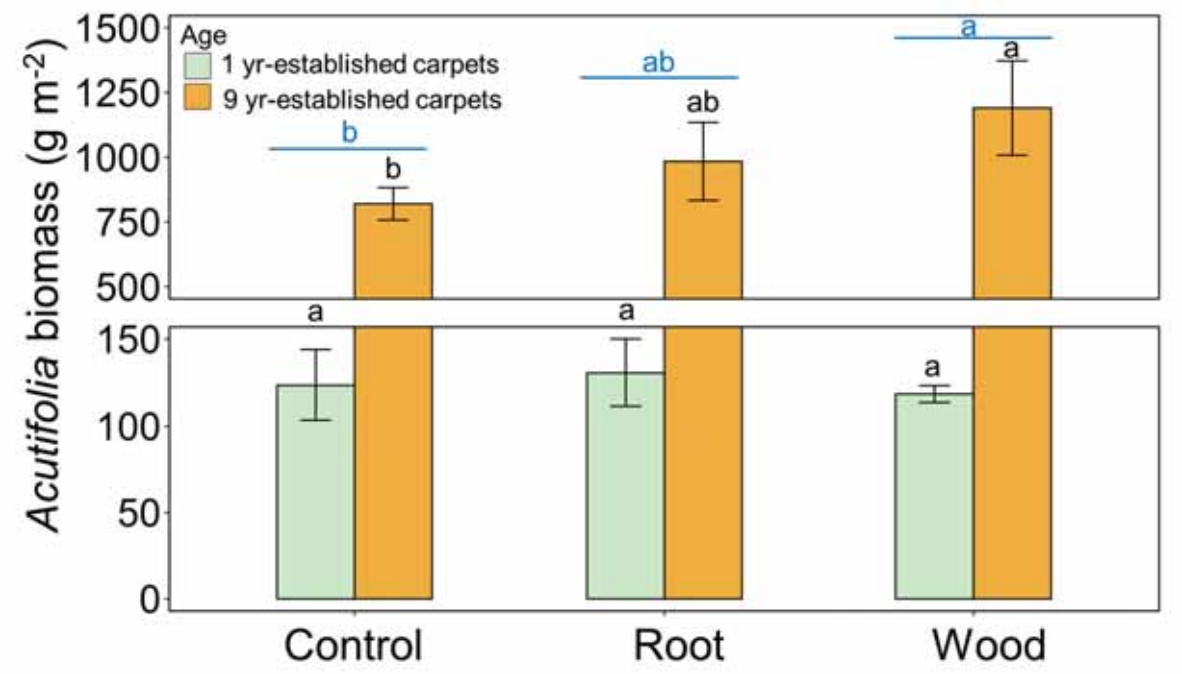

Fig. 3.4. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on mean *Acutifolia* biomass (g m^{-2}, n = 4) at 1 yr-established carpets and 9 yr-established carpets. Error bars represent the standard error of the mean. The presence of different blue colour lowercase letters indicates significant differences among phenolic treatments based on the significant main effect of phenolic treatments. Whereas the presence of different black colour lowercase letters indicates significant differences among phenolic treatments for each age level separately based on the interactive effect of age and phenolic treatments ($p < 0.05$, Tukey's HSD). See Table 3.4 for detailed statistical analysis.

3.5.5 Regulating factors of the enzymic latch mechanism

At large, Pearson correlation coefficients indicated no significant pattern of enzymic latch mechanism (ELM) that could lead to reduced ER at 1 yr and 9 yr-established carpets (Table A3.1 & A3.2). Precisely, no correlation was observed between POX and peat soluble phenolics at both carpets. Peat soluble phenolics showed a prominent negative correlation with pH ($r = -0.6$, $p = 0.03$) and β-D-xylosidase ($r = -1.0$, $p < 0.0001$, Fig. A3.3) enzyme activities at 1 yr-established carpets (Table A3.1). In contrast, at 9 yr-established carpets, peat soluble phenolics did not exhibit any correlation with hydrolase enzyme activities, whereas it did show a negative correlation with pH ($r = -0.7$, $p = 0.02$, Table A3.2). NEE was found positively correlated with arylsulphatase ($r = 0.7$, $p = 0.02$), β-D-xylosidase ($r = 0.6$, $p = 0.03$, Fig. A3.3), and N-acetyl-β-D-glucosaminidase ($r = 0.6$, $p = 0.03$) enzyme activities

at the 9 yr-established carpets (Table A3.2). Biomass and GEP were not correlated for both carpets.

3.6 Discussion

Sphagnum decomposition and growth are an ongoing process which could be influenced by ecological engineering techniques. In this study, external phenolics were tested to strengthen enzymic latch mechanism (ELM) in order to limit *Sphagnum* decomposition and enhance biomass production at different times of post-establishment in Sphagnum farming system. In this context, it was first hypothesized that phenolic additions would limit carbon dioxide (CO_2) emissions (used as a proxy for decomposition) for 1 yr and 9 yr established carpets (year correspond to the initiation of *Sphagnum* growth). Results of this study do not support this first hypothesis, as phenolic enrichments used as an ecological engineering tool did not play a significant role in limiting CO_2 emissions from both carpets. Largely, phenolic additions had higher mean CO_2 values (more CO_2 release compared to CO_2 uptake) as net ecosystem exchange (NEE) compared to control, which could be due to phenolic product decomposition. This result suggests that phenolic additions was not a suitable land management strategy to limit *Sphagnum* decomposition, at least with the products and means of application tested. A greenhouse study by Alshehri et al. (2020) examined the effect of wood chips of *Larix laricina*, *Picea mariana*, and *Thuja occidentalis* as phenolic additions, either applied to the surface or mixed with top 10 cm peat, on CO_2 emissions from *Sphagnum* mesocosm. Results indicated that only mesocosm with surface addition of *Larix laricina* and *Picea mariana* wood chips had lower values of CO_2 (CO_2 sequestration). In contrast to the phenolic addition there are studies that accounted CO_2 emissions of post-restoration peatlands as a result of straw decomposition. For example, Nugent et al. (2019) showed that one-year and four-years old restored sites were sources of CO_2 mainly due to straw decomposition. Later, CO_2 emissions from post-restoration sites lowered as straw decomposition diminished and biomass productivity increased over time, leading to sink of CO_2. With the scarcity of evidence testing phenolic enrichments in a Sphagnum farming system it is hard to develop solid conclusions and requires further investigation to test ELM effectiveness in limiting CO_2 emissions on a short- and long-term basis.

The key assumption in strengthening of ELM is the reduced activities of extracellular enzymes in response to phenolics. Likewise, the second hypothesis of this experiment was that phenolic additions would limit extracellular enzyme activities and in particular within the 9 yr-established carpets would have lower enzyme activities compared to 1 yr-established carpets. The results of this study indicated that root addition specifically at the 1 yr-established carpets showed higher soluble phenolics compared to the other treatments. Overall, root addition reduced mean arylsulphatase and N-acetyl-β-D-glucosaminidase activities compared to the wood addition, which is inconsistent with the second hypothesis as no difference was observed compared to control subplots. In contrast, phenol oxidase (POX) activities were not inhibited by the phenolic addition, but POX activities were two-fold lower at 9 yr-established carpets compared to the 1 yr-established carpets, lending partial support to the second hypothesis. Higher POX activities at 1 yr-established carpets could be linked with aerobic conditions as explained in ELM. Similarly, Freeman et al. (2004) found that increased oxygen levels resulted in 7-fold higher POX activities compared to lower oxygen levels. In literature, evidence related to the role of phenolics in limiting the decomposition process through enzyme activities is not consistent. For example, a study by Alshehri et al. (2020) showed that only *Picea mariana* in mixed treatment resulted in significantly lower β-glucosidase activities. Their results also showed that phenolic products mixed within top 10 cm peat (mixing treatment) might have the potential for sequestrating carbon in the long term. In another study, Dunn and Freeman, (2018) showed that enzyme activities decreased as the concentration (w/v %) of Ca lignosulphonate acid increased. They also showed that microbial respiration was lower for the phenolic products with higher molecular weight and vice versa. In contrast, few other studies were unable to find any significant role of phenolics in limiting enzyme activities and therefore did not corroborate the effectiveness of ELM in limiting decomposition (Sun et al., 2010; Urbanová & Hájek, 2021; Hájek & Urbanová, 2024).

Optimizing *Sphagnum* biomass on a renewable and cyclical basis in a Sphagnum farming system is challenging. Therefore, the third hypothesis in this study stated that phenolic additions would increase *Sphagnum* biomass leading to greater gross ecosystem productivity (GEP) for both carpets. The results from this study were partially consistent with the third hypothesis as wood addition resulted in higher *Sphagnum* biomass compared to other

treatments at 9 yr-established carpets, whereas phenolic additions were unable to induce positive effect on *Sphagnum* biomass at 1 yr-established carpets. To some extent, after a 10 month of greenhouse experiment Alshehri et al. (2020) also did not find difference in *Sphagnum* biomass in response to added phenolics (surface or mixed with top 10 cm peat) in the form of wood chips of *Larix laricina*, *Picea mariana*, and *Thuja occidentalis*. To date, literature is scarce with studies testing the effects of externally added phenolics on *Sphagnum* biomass in a Sphagnum farming system. However, certain studies are available that tested the shading effect on *Sphagnum* biomass and photosynthetic efficiency that could help in interpreting results (Laing et al., 2014; Ma et al., 2015). In this study, higher GEP values (less CO_2 sequestration) in response to phenolic additions did not comply with the third hypothesis. Based on the relationship between light availability and photosynthetic efficiency, it was assumed that phenolic products might have negatively impacted photosynthetic capacity of *Sphagnum* by blocking available light. Up to now, mixed evidence is available for a tradeoff — between light availability and photosynthetic efficiency — in bryophytes (Hájek et al., 2009; Waite and Sack, 2010; Hájek, 2014).

The last hypothesis of this study examined the fate of ELM at 1 yr and 9 yr-established carpets with the assumption of strong evidence to be observed at 9 yr-established carpet without considering the effects of phenolic additions. In contrast to the hypothesis, no prominent evidence was observed for both carpets that could validate ELM. However, there were certain cases that partially supported ELM. For example, NEE was positively correlated with arylsulphatase, β-D-xylosidase, and N-acetyl-β-D-glucosaminidase enzyme activities at the 9 yr-established carpets. These correlations are key in the ELM where positive relationship indicate that lower enzyme activities lead to lower values of NEE (more CO_2 uptake compared to CO_2 release). At 9 yr-established carpets no proof of a negative relationship between peat soluble phenolics and enzyme activities — an essential relationship as hypothesized in ELM — was found to support the last hypothesis of this study. Unexpectedly, another very important negative correlation between peat soluble phenolics and β-D-xylosidase activities was observed at 1 yr-established carpet. In contradiction to our results, Freeman et al. (2001) and Freeman et al. (2004) provided results that were in line with the ELM such as they showed that phenolics limit hydrolase enzyme activities of peat samples. Similar to our results, Sun et al. (2010), Urbanová & Hájek (2021), and Hájek &

Urbanová (2024) did not find significant relationship between phenolics and enzyme activities of peat samples that could validate ELM.

3.7 Conclusion

The aim of this study was "to understand the role of phenolic additions in limiting carbon dioxide (CO_2) emissions and enhancing biomass production through the strengthening of enzymic latch mechanism (ELM) at different stages of post-establishment of moss carpets in Sphagnum farming system". The results were inconsistent with the first two hypotheses of this study as phenolic additions were unable to limit CO_2 emissions and extracellular enzyme activities at 1 yr-established and 9 yr-established carpets. Such results were an indication of failure in strengthening and detection of ELM in response to phenolic additions at both ages since the start of the production cycle in a Sphagnum farming system. Though phenol oxidase (POX) activities were lower at 9 yr-established carpet compared to 1 yr-established carpet and we assumed that it could be due to the presence of higher oxygen levels at 1 yr-established carpet compared to 9 yr-established carpet, lending partial support to ELM. Partially consistent with our third hypothesis, wood and root additions (though only the former was significantly different than the control) enhanced *Sphagnum* biomass production at 9 yr-established carpet only. At both young and older carpets, *Sphagnum* biomass from phenolic additions did not contribute to greater gross ecosystem productivity as hypothesized. In contrast to the last hypothesis, no striking pattern was observed that could validate ELM at both carpets. Due to insufficient evidence, strengthening of ELM in response to phenolic additions was not validated at different stages of post-establishment of moss carpets in Sphagnum farming system. Literature is limited with field trials and require further investigation to better understand effects of phenolic addition in limiting decomposition under ELM.

3.8 Appendices

Tables

Table A3.1. Pearson correlation coefficient between observed variables at 1 yr-established carpets of *Acutifolia*. Variables included are; net ecosystem exchange (NEE; g CO_2 $m^{-2}d^{-1}$), ecosystem respiration (ER; g CO_2 $m^{-2}d^{-1}$), gross ecosystem productivity (GEP; g CO_2 $m^{-2}d^{-1}$), hydrolases enzymes activities (nmol g^{-1} min^{-1}; B = β-D-glucosidase, S = arylsulphatase, X = β-D-xylosidase, N = N-acetyl-β-D-glucosaminidase, P = phosphatase), phenol oxidase enzyme activities (POX; nmol diqc g^{-1} min^{-1}), peat soluble phenolics (Phen; mg g^{-1}), *Acutifolia* biomass (BM; g m^{-2}), water table level (WT; cm), carpet temperature at -5 cm depth (T5; °C), potential of hydrogen (pH) and electrical conductivity (EC; µS cm^{-1}). Highlighted coefficients indicate significant positive (blue) and negative (red) correlations between the observed variables ($p < 0.05$).

	NEE	ER	GEP	B	S	X	N	P	POX	Phen	BM	WT	T5	pH	EC
NEE	1														
ER	**0.7***	1													
GEP	**0.9*****	0.3	1												
B	-0.3	0.1	-0.5	1											
S	0.3	-0.03	0.4	-0.2	1										
X	-0.3	-0.2	-0.3	-0.03	0.5	1									
N	-0.3	-0.1	-0.3	0.2	0.2	0.5	1								
P	0.1	-0.2	0.2	-0.3	0.3	0.3	-0.2								
POX	-0.1	0.2	-0.3	0.3	0.2	0.4	**0.6***	-0.4	1						
Phen	0.3	0.2	0.2	0.03	-0.5	-1***	-0.5	-0.2	-0.5	1					
BM	-0.1	-0.3	-0.01	-0.1	-0.3	-0.1	0.04	0.3	-0.2	0.1	1				
WT	-0.3	**-0.7***	-0.1	0.1	-0.2	-0.1	-0.2	0.1	-0.5	0.1	0.04	1			
T5	0.3	0.01	0.4	0.3	-0.02	-0.4	-0.4	0.2	-0.4	0.5	-0.1	**0.6***	1		
pH	-0.2	-0.04	-0.2	-0.1	0.3	**0.6***	0.3	-0.02	0.4	-0.6*	-0.7*	0.02	-0.2	1	
EC	0.4	0.1	0.4	0.002	-0.1	-0.7**	-0.3	0.1	-0.5	**0.8****	0.04	0.2	**0.7***	-0.5	1

Table A3.2. Pearson correlation coefficient between observed variables at 9 yr-established carpets of *Acutifolia*. Variables included are; net ecosystem exchange (NEE; g CO_2 $m^{-2}d^{-1}$), ecosystem respiration (ER; g CO_2 $m^{-2}d^{-1}$), gross ecosystem productivity (GEP; g CO_2 $m^{-2}d^{-1}$), hydrolases enzymes activities (nmol g^{-1} min^{-1}; B = β-D-glucosidase, S = arylsulphatase, X = β-D-xylosidase, N = N-acetyl-β-D-glucosaminidase, P = phosphatase), phenol oxidase enzyme activities (POX; nmol diqc g^{-1} min^{-1}), peat soluble phenolics (Phen; mg g^{-1}), *Acutifolia* biomass (BM; g m^{-2}), water table level (WT; cm), carpet temperature at -5 cm depth (T5; °C), potential of hydrogen (pH) and electrical conductivity (EC; µS cm^{-1}). Highlighted coefficients indicate significant positive (blue) and negative (red) correlations between the observed variables ($p < 0.05$).

	NEE	ER	GEP	B	S	X	N	P	POX	Phen	BM	WT	T5	pH	EC
NEE	1														
ER	-0.02	1													
GEP	**0.9*** **	-0.5	1												
B	0.2	0.3	0.1	1											
S	**0.7***	0.3	0.4	**0.7***	1										
X	**0.6***	0.2	0.4	**0.6***	**0.8****	1									
N	**0.6***	0.3	0.4	0.4	**0.9*** **	0.6	1								
P	0.4	0.4	0.2	0.3	0.5	0.5	**0.8****	1							
POX	**0.9*** **	-0.01	**0.8****	0.4	**0.8****	**0.6***	**0.7****	0.4	1						
Phen	-0.1	0.1	-0.1	-0.2	-0.4	-0.2	-0.5	-0.4	-0.2	1					
BM	0.3	0.3	0.1	0.5	**0.7****	0.2	0.6	0.1	0.5	-0.5	1				
WT	0.3	-0.5	0.5	-0.2	-0.1	-0.1	-0.1	-0.3	0.1	0.3	-0.4	1			
T5	**-0.6***	0.5	**-0.7****	0.3	0.1	-0.2	-0.1	-0.1	-0.5	0.1	0.3	-0.4	1		
pH	0.4	-0.4	0.5	-0.2	0.2	0.3	0.4	0.4	0.4	**-0.7***	0.05	-0.1	**-0.6***	1	
EC	-0.2	0.2	-0.3	0.1	-0.1	-0.2	-0.2	-0.3	-0.3	**0.7***	-0.2	0.4	0.5	**-0.8****	1

Table A3.3. Linear mixed effects model to determine the effects of *Acutifolia* carpet age (1 yr-established carpets & 9 yr-established carpets) and phenolic treatments (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on peat methane flux (CH_4; mg CH_4 $m^{-2}d^{-1}$).

Sources	**df**	**MS**	***F***	***P***
Age (A)	1	365	8	0.1
Error a	6	46		
Phenolic treatments (P)	2	147	3	0.1
A × P	2	23	1	1
Error b	12	49		

Table A3.4. Chemical properties of peat sample and water table conditions (measured from May to August) among phenolic treatments, control = no addition, root = old roots from peat harrowing, and wood = wood pellets, at 1 yr and 9 yr-established carpets of *Acutifolia* plots. Observed variables represent average values ± standard errors of: the water table (WT; negative values indicate a WT below the peat surface, n = 4), potential of hydrogen (pH, n = 4), electrical conductivity (EC, n = 4), carbon and nitrogen ratio (C/N ratio, n = 4), ammonium ion (NH_4^+, n = 4), nitrate ion (NH_3^-, n = 4), sulphate ion (SO_4^{-2}, n = 4), and phosphate ion (PO_4^{3-}, n = 4). The presence of different lowercase letters indicates differences among phenolic treatments (One-way ANOVA, $p < 0.05$) based on the significant two-way interactive effect of age and phenolic treatments on observed variables.

Age	Phenolic treatments	WT (cm)	pH	EC ($\mu S\ cm^{-1}$)	C/N ratio	NH_4^+ (ppm)	NH_3^- (ppm)	SO_4^{-2} (ppm)	PO_4^{3-} (ppm)
1 yr-established carpets	Control	-10 ± 3	**4.4 ± 0.1 a**	**79 ± 24 b**	**47 ± 4 b**	**35 ± 2 a**	**12 ± 2 a**	**1263 ± 137 b**	**82 ± 10 a**
	Root	-9 ± 3	**4 ± 0.1 b**	**81 ± 11 a**	**73 ± 5 b**	**49 ± 8 a**	**9 ± 2 ab**	**3039 ± 575 a**	**26 ± 4 b**
	Wood	-13 ± 2	**4.4 ± 0.1 a**	**69 ± 4 b**	**160 ± 13 a**	**14 ± 2 b**	**6 ± 2 b**	**2429 ± 402 a**	**28 ± 10 b**
9 yr-established carpets	Control	-13 ± 2	3.9 ± 0.1	140 ± 7	86 ± 10	26 ± 5	12 ± 1	1520 ± 127	21 ± 2
	Root	-12 ± 2	3.8 ± 0.1	257 ± 23	88 ± 4	21 ± 6	10 ± 1	1537 ± 132	26 ± 3
	Wood	-11 ± 2	4 ± 0.04	175 ± 29	80 ± 12	33 ± 2	13 ± 1	1475 ± 260	19 ± 3

Figures

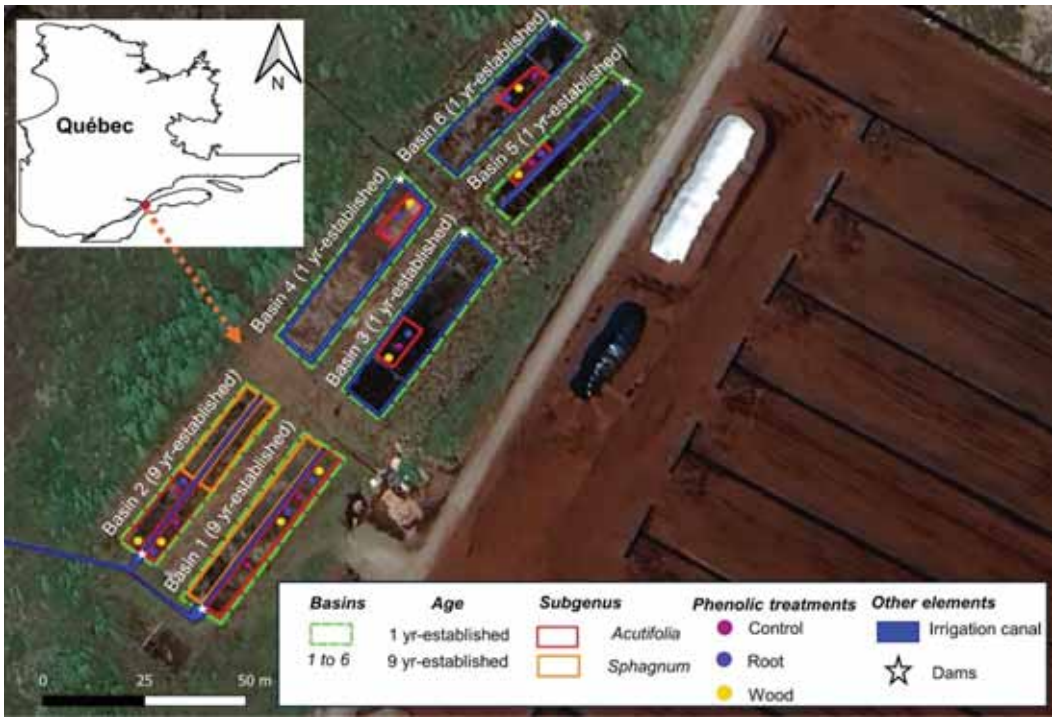

Fig. A3.1. Study area with experimental design in a Sphagnum farming system. Basins (green dash line,1-6) differ in age and represent growth of the established carpet (1 yr and 9 yr-established carpets). Only moss of *Acutifolia* subgenus (red solid line) was chosen to test phenolic treatments, where purple dots represent control, blue dots represent root and yellow dots represent wood treatments. Blue lines represent irrigation canals and star shape represent dams.

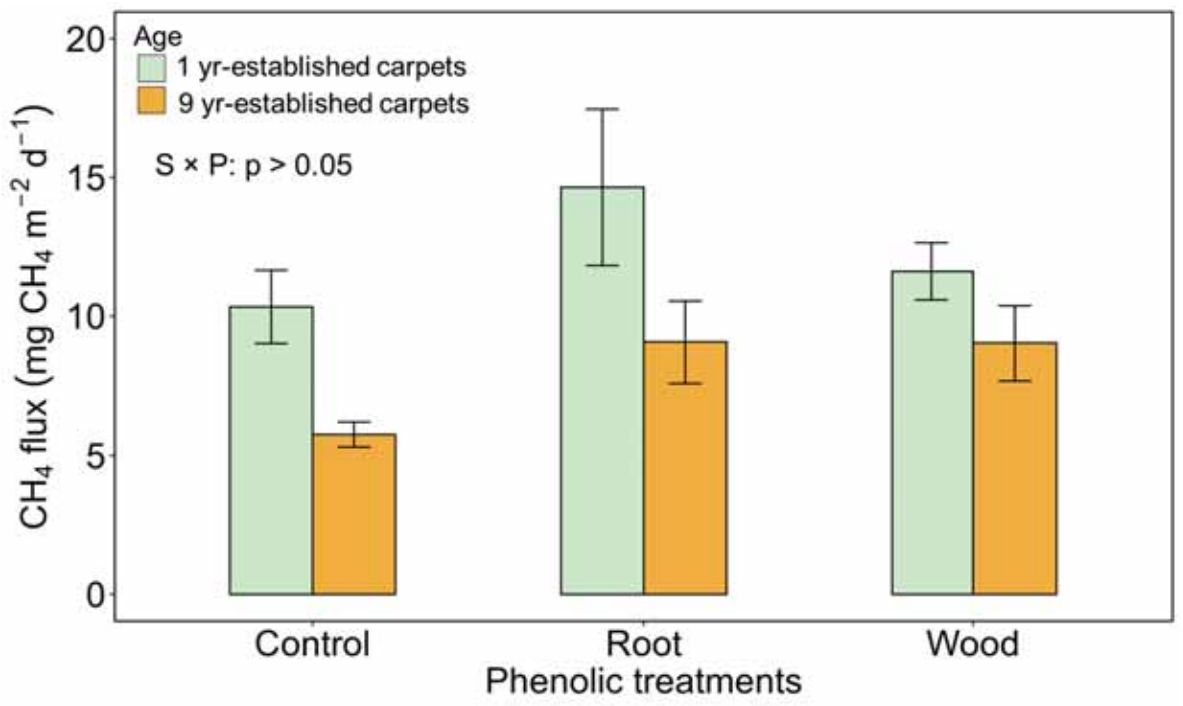

Fig. A3.2. Effects of external phenolic additions (control = no addition, root = old roots from peat harrowing, and wood = wood pellets) on peat methane flux (CH_4; mg CH_4 $m^{-2}d^{-1}$, n = 4) at 1 yr-established carpets and 9 yr-established carpets. Positive values represent a release of CH_4 from the ecosystem. Error bars represent the standard error of the mean. See Table A3.3 for detailed statistical analysis.

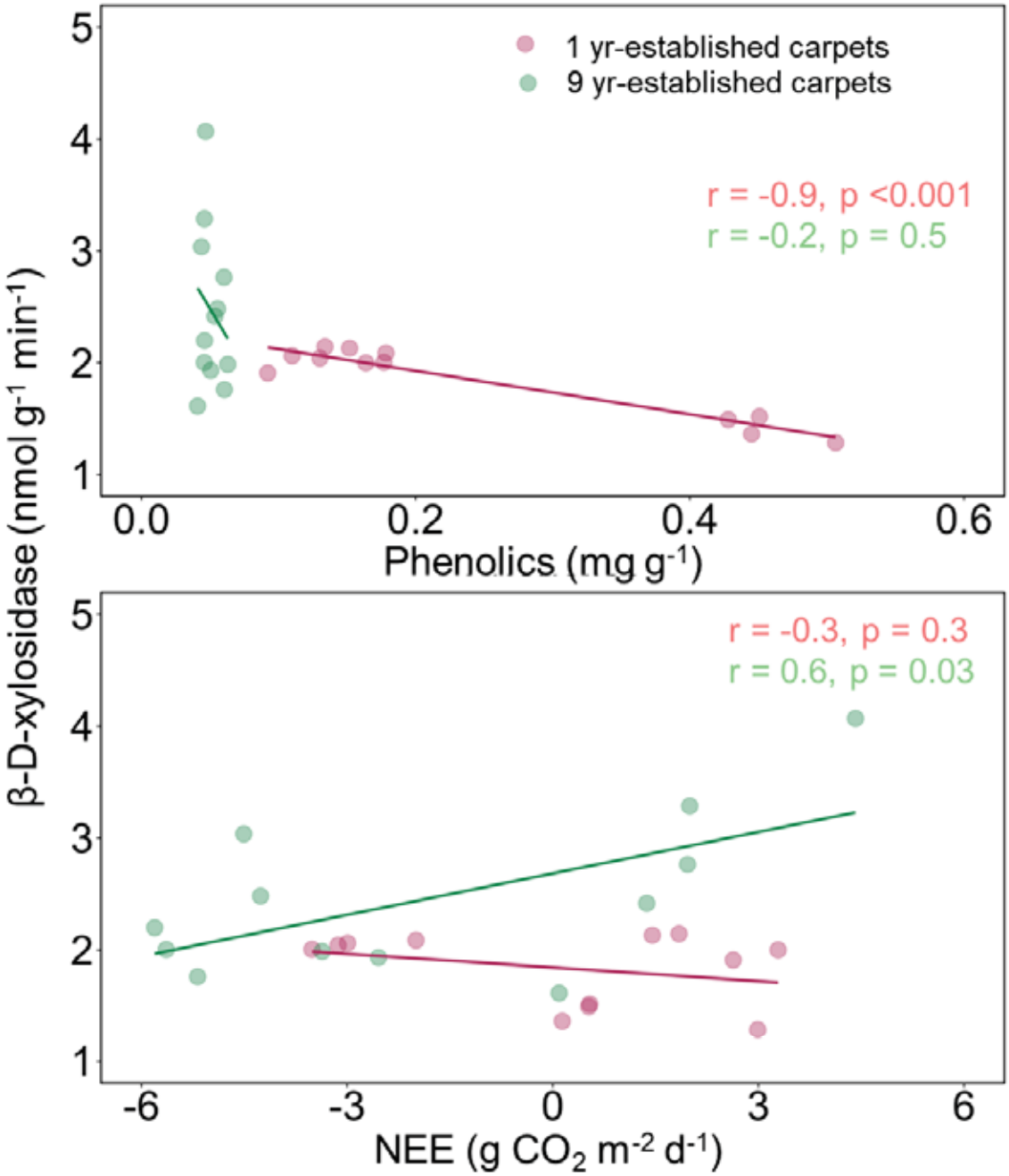

Fig. A3.3. Pearson correlation coefficient between observed variables at 1 yr and 9 yr-established carpets of *Acutifolia*.

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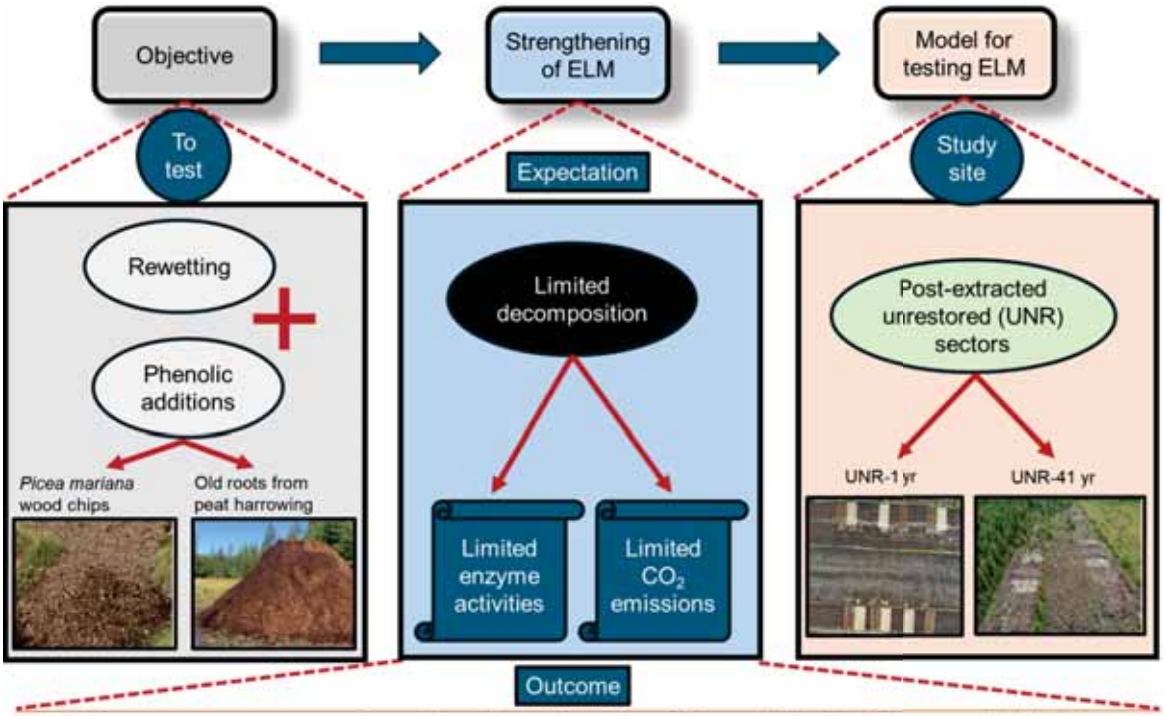
Conclusion

General summary

The main aim of this thesis was to test the effect of rewetting (blocking former ditches with dams) or enhanced rewetting (by irrigation system through channels) with phenolic addition on limiting peat decomposition through strengthening of enzymic latch mechanism (ELM) in two experimental models such as post-extracted unrestored peatlands and Sphagnum farming system in Québec, Canada. The overall theme of this thesis was subdivided into three chapters which also served as three sub-objectives. Main conclusions that could be extracted from this thesis based on each chapter are as follows:

In chapter 1, post-extracted unrestored sectors were the main experimental model for testing our first sub-objective aiming to evaluate the impact of large-scale rewetting associated with phenolic addition on the strengthening of the ELM. More specifically on the suppression of enzyme activities and decrease of CO_2 emissions at two post-extracted unrestored sectors (old and young), differing in age (UNR-1 yr and UNR-41 yr) since extraction activities ceased. We hypothesized that: "1) The young unrestored sector would have higher carbon dioxide (CO_2) emissions compared to the old unrestored sector. 2) Rewetting would limit CO_2 emissions and enzyme activities compared to non-rewetting. 3) Rewetting along with phenolic addition would strengthen the enzymic latch mechanism compared to non-rewetting." In contradiction to our first and second hypothesis, our field measurements show that UNR-41 yr sector (old unrestored sector) resulted in higher CO_2 emissions compared to the UNR-1 yr sector (young unrestored sector). Rewetting solely was unable to limit CO_2 emissions at both UNR sectors. Largely, mean enzyme activities were not limited in response to rewetting and phenolic additions except for mean arylsulphatase activities. At the rewetted plots of UNR-41 yr sector, mean arylsulphatase activities were reduced by 83% (for root treatment) and 33% (for wood treatment) compared to control treatment. Overall, rewetting along with phenolic addition was unsuccessful in strengthening of the ELM during the short-term (~ 1 year) field experiment. A conceptual framework with key expected and obtained outcome is illustrated in Fig. 0.14. In this experiment, two possible reasons could be linked for failure in detecting ELM and higher CO_2 emissions from peat surface; 1) absence of anoxic conditions at the rewetted plots which is required to put oxygen constraint on phenol

oxidase enzyme and 2) phenolic product decomposition together with oxygenated peat contributed to the higher CO_2 emissions.



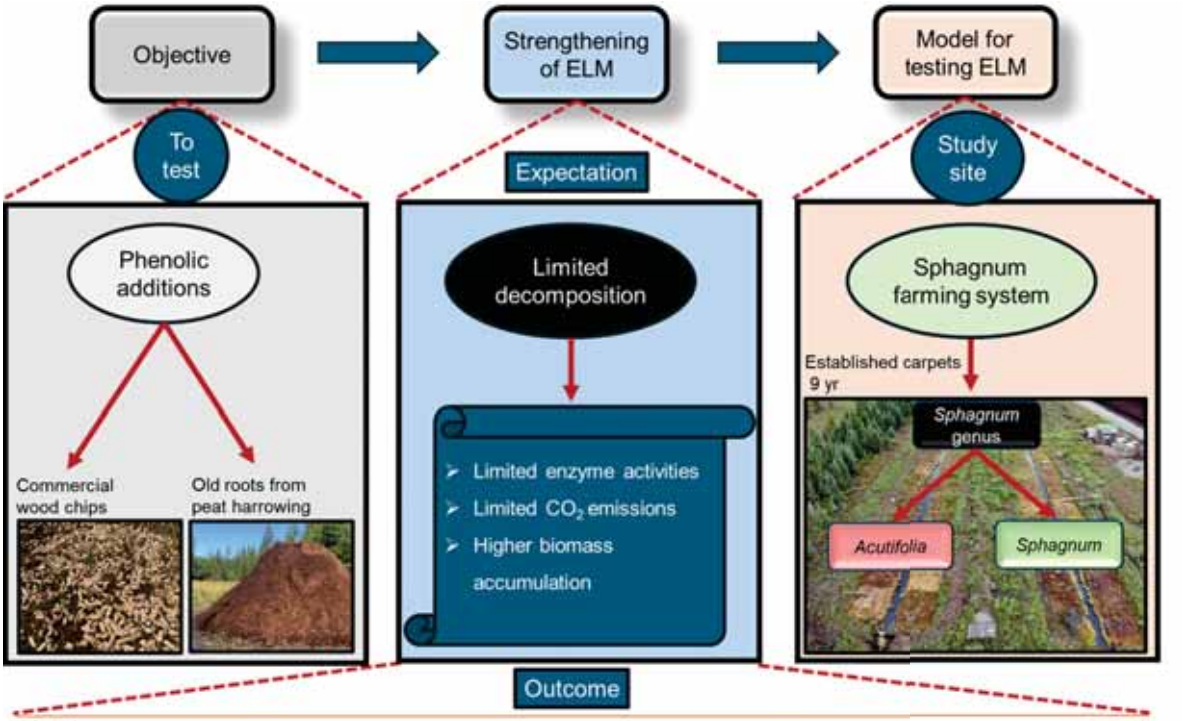
Variables	Rewetting (R)	Phenolic additions (PA)	R + PA	Study site
CO_2 emissions	×	×	×	UNR-1 yr & UNR-41 yr
Enzyme activities	×	×	×	UNR-1 yr & UNR-41 yr
Enzymic latch mechanism	×	×	×	UNR-1 yr & UNR-41 yr

× Indicates measured variable is not limited or strengthened (in case of enzymic latch mechanism) in response to applied treatments

Fig. 0.14. Conceptual framework of the first sub-objective focused on testing rewetting with phenolic addition (*Picea mariana* aboveground fresh wood chips and old roots from peat harrowing) on a large scale to strengthen enzymic latch mechanism (ELM) and test how ELM can suppress enzyme activities and limit CO_2 emissions at two post-extracted unrestored (UNR) sectors (old and young), differing in age (UNR-1 yr and UNR-41 yr) since extraction activities ceased. In the outcome section of the figure, cross mark indicates that variables were not limited or strengthened (in case of enzymic latch mechanism) in response to applied treatments in the mentioned study sites.

In chapter 2, Sphagnum farming system was the main experimental model for testing our second sub-objective that aimed to evaluate the impact of phenolic additions to strengthen ELM, decrease *Sphagnum* decomposition, and therefore increase *Sphagnum* productivity and CO_2 uptake in the context of the peat mosses from two *Sphagnum* subgenus differing in their

morphology (with the species tested). In this chapter, the goal was to seek answers for the following questions: "1) Is there a difference in CO_2 exchange between cultivated mosses from *Acutifolia* and *Sphagnum* subgenus? 2) Do phenolic additions play any role in regulating CO_2 exchange, peat soluble phenolic, and enzyme activities? 3) Are phenolic additions an useful tool for optimizing productivity and biomass of mosses from *Acutifolia* and *Sphagnum* subgenus? 4) If ELM exists, what are the regulating factors, and do they align with the ELM's theoretical



Variables	Phenolic additions	*Sphagnum* subgenus
CO_2 emissions	×	*Acutifolia* and *Sphagnum*
Enzyme activities	×	*Acutifolia* and *Sphagnum*
Biomass	×	*Acutifolia* and *Sphagnum*
Enzymic latch mechanism	×	*Acutifolia* and *Sphagnum*

× Indicates measured variable is not limited, enhanced (for biomass) or strengthened (in case of enzymic latch mechanism) in response to applied treatments

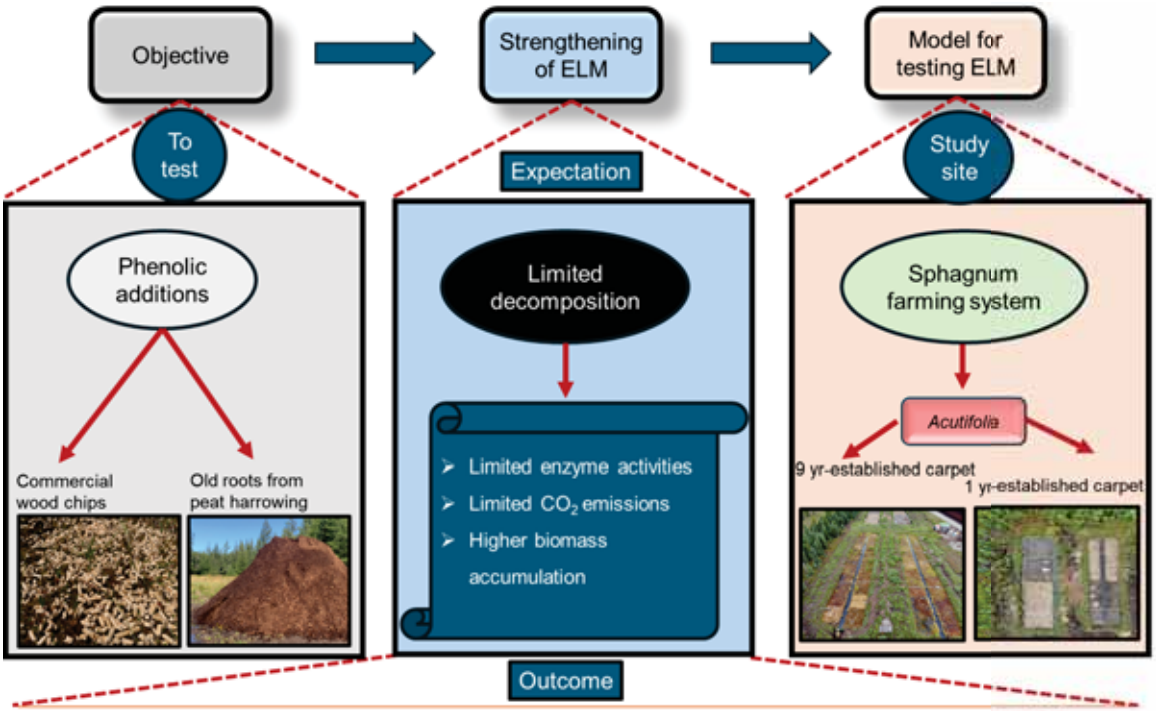
Fig. 0.15. Conceptual framework of the second sub-objective, which focused on testing phenolic addition (commercial wood pellets and old roots from peat harrowing) to strengthen enzymic latch mechanism (ELM) and test how ELM can reduce enzyme activities, limit CO_2 emissions, and promote *Sphagnum* biomass accumulation, thereby increasing *Sphagnum* productivity and CO_2 uptake in the context of the two widely structured *Sphagnum* subgenus (*Acutifolia* and *Sphagnum*) established in 2013 in a Sphagnum farming system (9 yr-established carpet). In the outcome section of the figure, cross mark indicates that variables

were not limited, enhanced (for biomass) or strengthened (in case of enzymic latch mechanism) in response to applied treatments for the mentioned *Sphagnum* subgenus.

explanation for *Acutifolia* and *Sphagnum* subgenus?" Our results indicated that peat mosses from *Acutifolia* and *Sphagnum* subgenus response to phenolic addition was statistically similar in terms of CO_2 exchange, soluble phenolics, enzyme activities, productivity and biomass accumulation. Phenolic additions were unable to limit CO_2 emissions as wood and root treatments compared to control treatment exhibited higher values of CO_2 (more CO_2 release compared to CO_2 uptake) measured as net ecosystem exchange. Furthermore, phenolic additions were unable to enhance peat soluble phenolics, limit enzyme activities, optimize productivity and biomass for both sub genera. In this experiment, phenolic supplementation was unable to strengthen ELM. A conceptual framework with key expected and obtained outcome is illustrated in Fig. 0.15. Mainly we assumed that two possible reasons could be accounted for such failure: 1) phenolic products might have created an inhibitory effect on enzyme activities at the *Sphagnum* surface (not accounted in this study) but failed to put constraints at the base of acrotelm (~10 cm below the *Sphagnum* surface) from where peat sampling was achieved and 2) phenolic product decomposition might have contributed to the higher CO_2 emissions.

In chapter 3, Sphagnum farming system was the main experimental model for testing our third sub-objective that aimed to evaluate the role of phenolic additions on ELM's effectiveness in limiting peat mosses from the *Acutifolia* subgenus decomposition and thereby enhancing biomass accumulation at two developmental stages — 1 yr-established carpet and 9 yr-established carpet — corresponding to the creation of the basins. In this chapter, we hypothesized that: "1) phenolic additions would limit CO_2 emissions from 1 yr and 9 yr-established carpets, 2) enzyme activities in response to phenolic additions would be lower at 9 yr-established carpets compared to 1 yr-established carpets, 3) phenolic addition would increase biomass production leading to greater gross ecosystem productivity, and 4) a strong evidence of ELM would be observed at 9 yr-established carpets compared to 1 yr-established carpets irrespective of the phenolic additions." Our measurements did not support the first hypothesis as phenolic additions compared to control treatment at 1 yr and 9 yr-established carpets resulted in higher values of CO_2 (more CO_2 release compared to CO_2 uptake) measured as net ecosystem exchange. Phenolic supplementation was unable to limit

enzyme activities at both young and old moss carpets. To our surprise, wood addition treatment at both moss carpets had higher mean arylsulphatase and N-acetyl-β-D-glucosaminidase activities. Wood treatment at 9 yr-established carpet resulted in greater moss biomass compared to control treatment but no link was found that gross ecosystem productivity was enhanced. Results from this experiment also did not support strengthening of ELM in response to phenolic additions at 1 yr and 9 yr-established carpets. A conceptual framework with key expected and obtained outcome is illustrated in Fig. 0.16. Broadly, it was assumed that chosen phenolic products were unable to induce inhibitory effects on enzyme activities.



Variables	Phenolic additions	Developmental stage
CO_2 emissions	✗	1 yr and 9 yr established carpets
Enzyme activities	✗	1 yr and 9 yr established carpets
Biomass	✓	9 yr-established carpets
Enzymic latch mechanism	✗	1 yr and 9 yr established carpets

✗ Indicates measured variable is not limitedor strengthened (in case of enzymic latch mechanism) in response to applied treatments

✓ Indicates measured variable is enhanced in response to applied treatments

Fig. 0.16. Conceptual framework of the third sub-objective addresses to test strengthening of enzymic latch mechanism (ELM) via phenolic additions (commercial wood pellets and old roots from peat harrowing) and to see how ELM can reduce enzyme activities, limit CO_2 emissions, and promote *Sphagnum* biomass accumulation at two developmental stages — 1

yr-established carpet and 9 yr-established carpet — corresponding to the number of years of *Sphagnum* growth in a Sphagnum farming system. In the outcome section of the figure, cross mark indicates that variables were not limited or strengthened (in case of enzymic latch mechanism), whereas tick mark indicates that variable is enhanced in response to applied treatments for the mentioned developmental stage of *Acutifolia* subgenus.

To sum up, literature is limited with field trials that tested rewetting and phenolic addition effect on ELM and the one's that exist present contradictory results. In this study, peat phenolics were not increased in response to rewetting and phenolic addition. Increasing the concentration of phenolics in peat is essential for effectively testing ELM. Though in this short-term (~ 1 year) field experiment ELM was not strengthened to such a degree that it is limiting CO_2 emissions, but it is believed that this research has added some new information to understand ELM in response to rewetting and phenolic additions.

Future recommendations

Peatland ecological engineering is crucial, particularly for strengthening the enzymic latch mechanism (ELM) through rewetting and phenolic additions to limit peat decomposition. More research in various peatland management settings is needed to better understand ELM and enhance its effectiveness in reducing organic matter decomposition and greenhouse gas emissions. The following recommendations are based on the gaps identified in this thesis.

Rewetting

To effectively test ELM, the ideal water table (WT) level should be close to the surface (0 to -5 cm), which is considered successful rewetting. As mentioned in chapter 1, the WT level in response to rewetting was not close to the surface. The lower WT level was likely due to drier conditions before rewetting, which requires more time and water inflow to raise the level close to the surface. In the context of rewetting, the following recommendations should be considered for future ELM research.

1. It is important to carefully assess the flow and direction of incoming water while ditch blocking. A cost-effective way to enhance rewetting after ditch blocking is by dumping vegetation or filling ditches with peat. This approach has the potential to stabilize WT level and promote water retention in the peatlands.

2. As discussed in Chapter 1, rewetting is influenced by the factors such as site conditions (remaining peat depth, oxic layer depth above water table level, and peat quality) and the time since rewetting. The current study was time-restricted; therefore, it is suggested to compare rewetting treatments over different time scales such as 5 years, 7 years, or 10 years of post-rewetting. In addition, future studies should test the effects of different WT levels, for example, 0 to -5 cm versus -5 to -10 cm versus -10 to -20 cm. This will provide valuable insights into how different rewetting strategies affect peat decomposition rates and carbon storage in peatlands.

Phenolic additions

Like rewetting, to effectively test the ELM, increasing the abundance of peat phenolics through phenolic additions is a prerequisite. In this study, phenolic additions did not increase the concentration of phenolics in peat, but no clear evidence was found for this issue. The following suggestions are made in the context of phenolic additions.

1. It is recommended to test phenolic products on a smaller scale in the laboratory and greenhouse before conducting large scale field experiments. This approach will help save time, labor, and resources.

2. The current study tested only wood chips, root chips and wood pellets with one dosage of 2 kg m^{-2}. Other phenolic products with different dosages and phenolic concentrations should be tested to determine their potential in effectively testing ELM. Some examples of phenolic products could be as below:

    a. Natural products like tree bark, sawdust, or other wood by products.

    b. Synthetic products including lignosulphonate, calcium lignosulphonate, primary sludge, secondary sludge, cinnamic acid, gallic acid or tannic acid.

    c. Biochar — produced from different wood types at different pyrolysis temperatures.

This approach will help identify phenolic products that might enhance the phenolic concentration in peat and reduce decomposition rates. It may also help explain why phenolic additions occasionally increase peat phenolic concentrations.

Other considerations

Given that ELM is a complex process, it is important to explore other biological mechanisms that may co-exist and control peat enzymes or phenolic concentration. One of the examples is 'iron gate mechanism' which controls enzymatic processes related to peat decomposition through ferrous iron. Studying these mechanisms could provide valuable insights into how biological processes interact with phenolic additions and affect peat decomposition. Another suggestion is to compare environmental variables between the top surface and vertical peat profile depth to better understand the effects of rewetting and phenolic additions on ELM.

The recommendations outlined above aim to address critical gaps in knowledge and might help to improve the effectiveness of peatland management strategies. By focusing on long-term studies with cost-effective approaches, future research can contribute to more sustainable and successful peatland management practices that could limit peat decomposition.

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