Organic matter decomposition at a constructed fen in the Athabasca Oil Sands Region of Alberta, Canada

By

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A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Geography

Waterloo, Ontario, Canada, 2019

Author's declaration

This thesis consists of material all of which I authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of contribution

To reflect the results of this research, this thesis is written in manuscript format. Two independent manuscripts to be submitted for publication are presented as chapter 2 and 3 of this thesis. As a result, repeated information may be included.

This manuscript reports on data collected at the constructed fen and reference sites near Fort McMurray, AB over the 2017 growing season. It also contains data collected at the Ecohydrology Lab and Wetland Soils and Greenhouse Gas Exchange Lab at the University of Waterloo in Waterloo, Ontario. Chapter 2 and 3 of this manuscript will be submitted for publication as such:

Chapter 2: Coulas, M.; Strack, M.; Saraswati, S.; Parsons, C. 2018. Effect of substrate type and environmental constraints on organic matter decomposition at a constructed fen.

Chapter 3: Coulas, M.; Strack, M.; Saraswati, S.; Parsons, C. 2018. Microbial decomposition and the 'priming effect': carbon dioxide production from decomposition of substrates relevant to fen reconstruction in the Athabasca oil sands.

M. Strack assisted with the design of the study sites and laboratory incubations, as well as manuscript revisions and funding. C. Parson provided access to lab resources and S. Sarswati helped with lab procedures. M. Smith assisted with the soil and water sampling, and D. Miller assisted with incubation sampling. The manuscript, including tables and figures, was written in its entirety by M. Coulas and reviewed by the co-authors prior to submission.

Abstract

Resource mining and extraction in northern Alberta has resulted in large disturbances across a variety of ecosystems, including fen peatlands. Provincial regulations require companies to reclaim disturbed areas similar to their pre-existing function, with fen reclamation only being attempted in recent years. Fen peatlands store tremendous amounts of carbon (C) due to organic matter accumulation exceeding decomposition. Due to the length of time required for the development of these landscapes it is imperative to identify potential opportunities to minimize decomposition, thereby maximizing peat accumulation. To meet this objective, sufficient understanding of the biogeochemical and environmental controls of organic matter (OM) degradation is a priority.

This research estimates decomposition rate using the litter bag method and tea bag index at a constructed fen (Nikanotee Fen) in the Athabasca Oil Sands Region (AOSR) near Fort McMurray, Alberta. Throughout the growing season in 2017, environmental conditions including volumetric water content (VWC), electrical conductivity (EC), pH, and soil temperature were measured to determine controls on decomposition. Additionally, soil and water samples were collected to determine biogeochemical controls on decomposition, namely phenolic compound concentration and extracellular enzyme activities. Laboratory incubations under oxic and anoxic conditions were also used to determine microbial respiration rates under varying treatments of peat, *Carex aquatilis*, *Juncus balticus*, straw, and wood-strand mulch, which (with the exception of straw) were all utilized in the construction of the Nikanotee Fen. Mixed results were obtained from these two studies. Our field study suggests that *Carex aquatilis* biomass decomposes faster than *Juncus balticus*, and that decomposition is higher under plots planted with *Carex aquatilis* as opposed to *Juncus balticus* or left bare. Furthermore, we did not observe increased concentration of phenolics as a result of the wood-strand mulch, nor did we observe any significant evidence to support the enzymatic latch hypothesis at the constructed fen. Although we observed an inhibitory effect of phenolics on OM-degrading hydrolase enzymes at the reference sites, it

was not observed at the constructed fen, nor was there a significant correlation between phenol oxidase (PO) activity and decomposition rate. Lastly, we found increased decomposition under higher pH, higher soil temperature, lower VWC and lower EC. Contrary to our field study, our laboratory findings suggest *Juncus balticus* may be of higher lability relative to the other treatments including *Carex aquatilis*. We also observed negative priming rates under oxic conditions from treatments containing *Juncus balticus*, while positive priming effects under anoxic conditions were observed from the *Carex aquatilis* treatments, which could significantly impact long-term C sequestration. Similar to the findings from our field study, our results from our laboratory incubation do not support the enzymatic latch theory. Phenolics were not readily leached from the wood-strand mulch, and we observed a negative interaction between PO activity and microbial respiration. Despite this, wood-strand mulch remains preferable over straw during fen reclamation due to its reduced lability and potential negative priming effect under anoxic conditions; however, if not required for successful vegetation establishment, wood-strand mulch is not a recommend amendment as it has little effect on decomposition rates.

Acknowledgments

First and foremost, I would like to thank Dr. Maria Strack for her tremendous guidance and mentorship. Her patience and willingness to help me succeed has been paramount. All those who've worked with her know how truly exceptional she is and I'm forever grateful for the opportunity I had to be a part of her research group. Thanks to Dr. Chris Parsons and the rest of the Ecohydrology group, including Amanda and Marianne, who were instrumental in a major portion of my lab work. Also, special thanks to Saru for showing me the do's and don'ts of phenolics and enzyme research, and to Dr. Scott Davidson for holding my hand through the impromptu meetings on stats and figure creations.

A big thanks goes to all those that were involved in the Fort McMurray project, including Dr. Jonathon Price, Dr. Rich Petrone, and Dr. David Cooper. Thank you to all the other grad students, lab techs, and especially research assistants who helped with data collection and mitigation of countless field problems. Also, thank you to my colleagues in the Wetland Soils and GHG Exchange lab for the feedback and assistance throughout the past two years.

Lastly, I would like to thank all of my friends and family who have supported me. Thank you to my roommates Peter, Amy, Alex, and Lindsay for the comradery and fun times at Winchester. Thank you to all the other friends I met in the Faculty of Environment as well as in the Kitchener/Waterloo community – lots of great times were had that made all the difficult points worth it. Thanks to mom, dad, my brother Patty, and the rest of my family for believing in me and supporting me. Also, thanks to all my friends back home for the chirps and laughs that made me feel like I never left home. Last but not least, thanks to Hill for always being by my side and being the light at the end of all the long, dark days I faced.

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Chapter 1: Introduction and context

The Athabasca Oil Sands Region (AOSR) in northern Alberta contains the third largest reserve of crude oil in the world, with an estimated initial reserve of 15 billion barrels (Alberta Energy Regulator, 2019). Extraction of oil occurs through surface mining or in-situ recovery processes. Surface mining involves the complete removal of the overlying soil and vegetation, and is only viable to a depth of approximately 75 m. Both methods of extraction require subsequent reclamation activities to equivalent land capabilities at closure. Equivalent land capability is defined as "the ability of the land to support various land uses after conservation and reclamation is similar to the ability that existed prior to an activity being conducted on the land, but that the individual land uses will not necessarily be identical" (Alberta Environment & Parks, 2015). Furthermore, reclamation differs from restoration, which is defined as "the process of restoring site conditions as they were before the land disturbance" (Powter, 2002).

Approximately 142 000 km^2 of the AOSR overlies the Western Boreal Plain (WBP), and about 62% of this landscape is occupied by peatlands (Vitt et al. 2016). Historically, open-pit mining has resulted in the replacement of peatlands to tailings storage lakes and upland forests, representing a loss of >29 000 ha of peatlands (Rooney et al. 2012). However, as of 2015, companies are now responsible to meet peatland-specific reclamation criteria (Environment & Parks, 2015), and oil sands companies are conducting research into the feasibility of peatland reclamation (Ketcheson et al. 2016). The lack of restored fen peatlands within the AOSR is largely due to the difficulties associated with successful techniques (Audet et al. 2015). Peatlands have also been presumed to require thousands of years to develop (Price et al. 2010). As a result, most wetland reclamation efforts pertain to the establishment of marshes and open water wetlands (Ketcheson et al. 2016; Scarlett et al. 2017); however, a significant proportion of the surface mineable landscape is underlain by fen peatland systems (Price et al. 2010; Borkenhagen & Cooper, 2016; Vitt et al. 2016). To date, peatland reclamation following AOSR

development has not been fully achieved, and only 104 ha of the >900 km^2 disturbed area has been fully reclaimed (i.e., reclamation certified; Dietrich et al. 2017). However, two ongoing pilot projects located at Syncrude's Sandhill Fen and Suncor's Nikanotee Fen have shown signs of success with fen re-establishment. Both sites were designed to optimize hydrological requirements and utilized various re-vegetation and surficial treatments to establish fen properties within a post-mining landscape (Ketcheson et al. 2016; Vitt et al. 2016). Ongoing evaluation of peatland functions (e.g., carbon sink, water storage, etc.; Vitt et al. 2000; Nwaishi et al. 2015) is necessary to determine the level of success of these projects, as well as the potential to identify improvements that will enhance peat formation, and thus more timely reclamation (Ketcheson et al. 2016). The areas of evaluation must include hydrologic properties, vegetation assessments, biogeochemical and microbial processes, and greenhouse gas fluxes (Nwaishi et al. 2015). The focus of the present study is to improve understanding of organic matter decomposition processes within a constructed fen.

1.1 Fen Reclamation

Peatlands, in Canada, are defined as wetlands containing >40 cm of organic material (i.e., peat; Daigle & Gautreau-Daigle, 2001). Peatlands are further classified as bogs and fens, with the former obtaining hydrological inputs strictly from precipitation (Halsey et al. 1998). Fens are considered minerotrophic due to multiple hydrological inputs (i.e., surface and subsurface flows), and are further classified as either rich or poor fens (Vitt & Chee, 1990; Bedford & Godwin, 2003). Both bogs and fens are important ecosystems for water storage and carbon sequestration, as well as habitat for various plants and animals. In terms of carbon sequestration, northern peatlands contain 450 – 550 Gt of carbon, or 1/3 of the world's total soil carbon (Clymo et al. 1998; Belyea & Clymo, 2001; Turetsky, 2002), with fens potentially containing twice as much carbon as bogs (Vitt et al. 2000).

Restoration of fen peatlands is not a new practice (e.g. Cobbaert et al. 2004); however, procedures leading to a fully reclaimed peatland within a post-mined landscape are still being developed

(Borkenhagen & Cooper, 2016). These landscapes require extensive engineering and design to allow for the necessary hydrological regime to support the reestablishment of peat-accumulating species (Vitt et al. 2000; Ketcheson et al. 2016). Historically, reclamation projects within the AOSR have focused on small marshes and open water wetlands (Scarlett et al. 2017), and only recently have efforts been made to investigate viable techniques for peatland construction (Ketcheson et al. 2016). The ultimate goal of fen reclamation is to restore a functioning ecosystem including a net carbon sink component. Previous studies from eastern Canadian peatlands following peat harvesting suggest a possible return to natural functions within two decades (Lucchese et al. 2010; Nugent et al. 2018). Strategies that include the introduction of peat-forming moss and vascular plant species, as well as mulching and herbaceous plant cover, could lead to more timely return to peat accumulation function (Borkenhagen & Cooper, 2016). In addition, strategies that reduce the decomposition of peatland biomass will inherently promote more timely reclamation through greater peat accumulation rates (Graf & Rochefort, 2009).

1.2 SOM decomposition

Peat accumulation, and thus carbon sequestration, is a result of biomass accumulation rates exceeding peat decomposition rates. This occurs predominantly due to cool conditions and a shallow water table, leading to suppressed decomposition rates (Turetsky, 2002; Basiliko et al. 2012). Acidic conditions found in bogs and some fens also aid in suppressed decomposition rates (Pind et al. 1994), and promote the development of peat forming species like *Sphagnum* and brown mosses (Turetsky et al. 2008; Graf & Rochefort, 2009).

As mentioned above, decomposition in peatlands is most inhibited by anaerobic conditions and cooler temperatures (Belyea & Clymo, 2001; Basiliko et al. 2012), and has also been shown to be impacted by pH, soil moisture, nutrient availability, and species composition (Turetsky et al. 2008; Linkosalmi et al. 2015; Walker et al. 2016). However, organic matter decomposition is ultimately controlled by microbial activity (Preston et al. 2012). Microorganisms break down organic matter to

access energy sources in order to maintain cell function and for reproduction. In addition, microorganisms are also responsible for the release of nutrients locked inside decaying soil organic matter (SOM) into the surrounding environment (i.e., mineralization; Freeman et al. 2012), which can then be taken up by other microorganisms and plants (Turcotte, 2009). As SOM is decomposed primarily by fungi and bacteria, CO_2 is released through respiration, with higher rates of CO_2 respiration indicating more labile material (Belyea, 1996).

Peat is initially formed through the burial of partially decomposed plant matter that builds up over time, with substrate composition and microbial activity varying based on overlying vegetation composition (Walker et al. 2016). Labile substrates (i.e., more easily decomposable material) provide easily accessible energy sources required for microbial growth and reproduction (Nwaishi et al. 2016). Therefore, labile substrates are preferentially used, and may enhance peat decomposition through additional processes such as 'priming' (Kuzyakov et al. 2000). Substrates containing more recalcitrant materials, such as woody biomass containing more complex compounds, like lignin or other phenolic-containing compounds, are much more difficult to breakdown and require additional microbial processes (i.e., extracellular enzymes) to degrade structures and provide energy (Romanowicz et al. 2015). When conditions change, such as water table drawdown leading to an expanded oxic zone, a rapid increase in extracellular enzyme activity could be induced, leading to an unlocking of peat-degrading hydrolase enzymes (Preston et al. 2012), resulting in a positive feedback on peat decomposition and subsequent carbon loss.

1.3 Priming and the 'Enzymatic Latch'

When labile substrate is added to the soil, microbial respiration rates increase, and this increase may exceed the mass of the material added. This increased rate of respiration from the surrounding microbial community by the addition of a substrate is often referred to as priming. The 'priming effect' was first recognized in the 1920's and has since been the subject of numerous studies showing increased

rates of carbon (C) and nitrogen (N) mineralization following various substrate additions (Kuzyakov et al. 2000). The microorganisms responsible for the induced mineralization are still debated (Kuzyakov, 2010), but in general the activity from both bacteria and fungi have shown two distinct types of priming: positive/negative priming, and real/apparent priming (Kuzyakov, 2010).

Not all additions of organic substrates lead to induced C and N mineralization, and in fact substrate additions have also shown to impede mineralization rates or even result in net immobilization (Hamer & Marschner, 2002). This has been referred to as negative priming (Kuzyakov et al. 2000). Additions of substrates like glucose, wheat straw, cellulose, and sewage have shown negative priming effects (Kuzyakov et al. 2000). It has been suggested that negative priming could be a result of a shift towards a more efficient microbial community, leading to reduced nutrient losses (Kuzyakov et al. 2000). Additionally, negative priming could be caused by the replacement of C or N as opposed to being released into the surrounding environment, again leading to reduced nutrient availability (Kuzyakov et al. 2000).

Of greater importance in terms of preventing access to the massive C storage in the WBP is positive priming. Positive priming is a result of increased rates of mineralization due to a stimulation or change in the surrounding microbial community (Hamer & Marschner, 2002). Various studies have shown that adding substrates like glucose, aspartate, amino acids, glutamate and even compost (Hamer & Marschner, 2002) can alter or stimulate the microbial community, inducing higher rates of mineralization and nutrient release. In fact, CO_2 release has been found to increase as much as 11 times that of untreated substrates (Kuzyakov et al. 2000). The additional respiration is generally associated with a greater energy input from the more labile energy source, which is often a limiting factor amongst some substrates (Kuzyakov et al. 2000). Other reasons for induced mineralization of C include increased microbial activity via co-metabolism, biomass turnover, or a direct shift in the microbial community itself including activation of a previously dormant microbial community (Kuzyakov et al. 2000; Hamer &

Marschner, 2002; Blagodatskaya & Kuzyakov, 2008). The overall strength, direction and duration of priming ultimately depends on substrate composition and the lability of the organic material (Kuzyakov et al. 2000; Hamer & Marschner, 2002; Blagodatskaya & Kuzyakov, 2008), often reflected by lower C/N ratios (Richert et al. 2000; Windham, 2001).

Positive and negative priming can be further broken down to either 'real' or 'apparent' priming. Real priming refers to the direct stimulation of the microbial community leading to an observed impact on SOM decomposition (Blagodatskaya & Kuzyakov, 2008). This differs from apparent priming, where the stimulated microbial activity (i.e., observed increase in microbial respiration) is due to changes in biomass turnover or from the added substrate itself (i.e., no change in SOM decomposition (Blagodatskaya & Kuzyakov, 2008). In these instances, there is no change in the nutrient status of the soil pool – rather, there is an exchange of nutrients between the microbial community and the added substrate (Kuzyakov et al. 2000). This can be a result of either short-term microbial metabolic increase from trace amounts of the substrate, or long-term pool substitution accompanied by microbial turnover (Blagodatskaya & Kuzyakov, 2008). Since real priming can occur simultaneously as apparent priming, they can be difficult to distinguish, especially for real priming (Blagodatskaya & Kuzyakov, 2008). Apparent priming will never result in release of C or N exceeding the mineralized amount that was added by the substrate, contrary to real priming where mineralization can easily exceed the amount added, depending on SOM composition (Kuzyakov et al. 2000; Blagodatskaya & Kuzyakov, 2008). Furthermore, apparent priming tends to occur immediately upon substrate addition, while real priming can occur within days to even weeks later (Blagodatskaya & Kuzyakov, 2008; Kuzyakov, 2010).

One of the major controlling mechanisms for the preservation of the organic C pool in peatlands has been attributed to the 'enzymic latch' theory (Freeman et al. 2001). This theory suggests that an accumulation of phenolic compounds (i.e., aromatic structures bounded by at least one hydroxyl group; Appel, 1993) under anoxic conditions inhibit SOM-degrading enzymes (Dunn & Freeman, 2018).

However, a stimulation of phenol oxidase (PO) enzymes results in the removal of phenolics, releasing the 'latch' mechanism and thus inducing enhanced SOM turnover with subsequent carbon loss, and further enzyme production (Freeman et al. 2004). While several studies have shown evidence of the enzymatic latch in a variety of peats (Sun et al. 2010; Fenner & Freeman, 2011; Brouns et al. 2014; Saraswati et al. 2016), few studies exist on WBP fen peatlands as it relates to the theory. For this reason, it is imperative to assess the presence of phenolic compounds, as well as the potential enzyme activities in order to identify opportunities to reduce rates of decomposition, and therefore enhance peat formation.

During fen construction, additions of material during the reclamation activities (e.g., mulches, planted species) and subsequent organic matter additions from the establishing plant communities are expected to influence decomposition processes. Improved understanding of decomposition rates and associated processes related to priming and extracellular enzyme activities under various reclamation treatments will be useful in potentially influencing peat accumulation in peatland construction projects in the AOSR.

1.4 Research Objectives

Peatland reclamation in the AOSR requires a complete understanding of peat formation, which includes SOM decomposition processes. Thus far there has been a limited understanding of decomposition dynamics, particularly from constructed fens, and therefore further investigation into the biogeochemical and microbial processes is required as they play a significant role in peatland reclamation. The re-establishment of vegetation and hydrological conditions, specifically at the constructed fen, impact these processes, leading to a void in a complete understanding of peat accumulating properties related to the overall success of the reclamation project. The goal of this paper is to address the gaps in understanding decomposition rates across the fen, and to look for potential

opportunities to minimize SOM decomposition, leading to faster rates of peat accumulation. The main objectives of this thesis are to:

1. Evaluate environmental and biogeochemical functions at the constructed fen and reference sites, and compare to estimated decomposition rates at each to determine controls on decomposition and assess progress (Chapter 2)
2. Assess microbial respiration rate from treatments of peat containing *Carex aquatilis*, *Juncus balticus*, straw, and wood-strand mulch to determine patterns in SOM degradation, potential priming, and carbon sources (Chapter 3)
3. Form inferences on decomposition dynamics related to fen reclamation within a post-mined landscape, and make recommendations that present opportunities to enhance peat accumulation (Chapter 4)

1.5 Format and Project Role

The format of this thesis includes four chapters that address the decomposition characteristics of peat and related materials used in the construction of the Nikanotee Fen at the Suncor Energy Inc. Oil Sands Base . Following this first chapter, which provides a literature review on organic matter decomposition in peatlands, the second chapter reports direct estimation of decomposition rates using the tea bag index (TBI) and litter bags across the constructed fen. In addition, environmental and biogeochemical controls were analyzed including potential extracellular enzyme activity and phenolic compound concentration. The third chapter contains a laboratory assessment of microbial respiration rates using substrates related to the Nikanotee Fen. Isotopic signatures, phenol oxidase activity and phenolic compound concentrations were also assessed to determine potential controls on SOM degradation. The final chapter includes key findings and provides concluding recommendations to consider during fen reclamation as it pertains to SOM degradation and potential for peat accumulation enhancement.

My role within the project was to assess decomposition rate among varying vegetation and mulch treatment plots across the constructed fen through peat and water sampling, as well as measuring environmental conditions such as soil temperature, pH, electrical conductivity (EC), and volumetric water content (VWC). I was responsible for the design and implementation of the sampling regime, as well as ensuring timely collection of data throughout the summer of 2017. Litter bags were constructed and buried at the constructed fen in 2016 prior to my involvement in the project, including biomass collection, but I completed retrieval and final processing. All other laboratory work and data processing was completed by me with the help of field assistants and colleagues in the research group.

Chapter 2: Effect of Substrate Type and Environmental Conditions on Organic Matter Decomposition at a Constructed Fen in the Athabasca Oil Sands Region

2.1 Introduction:

Approximately 62% of the Oil Sands Region in northern Alberta is overlain by peatlands providing critical carbon and nutrient storage as well as habitat for various animals and plants (Vitt et al. 2016). To date, disturbed peatlands have posed a significant reclamation challenge, despite a legal obligation to return land to "equivalent land capability" (Environment & Parks, 2015). Although peatlands require thousands of years to develop, evidence suggests some functions can be returned to damaged peatlands within decades (Lucchese et al. 2010; González & Rochefort, 2014; Nugent et al. 2018). However, current methods to re-establish a fully functioning peatland are still being reviewed (Borkenhagen & Cooper, 2016). Oil sands companies are conducting research into the feasibility of peatland reclamation (Ketcheson et al. 2016). The Nikanotee Fen is one of only two experimental fens on a post-mining landscape, and is designed to support vegetation and hydrological processes that maintain peat accumulating functions (Ketcheson et al. 2016; Scarlett et al. 2017).

Peatlands, and more specifically, fen peatlands, consist of organic matter (peat) that accumulates due to net primary productivity (NPP) exceeding organic matter (OM) decomposition (Vitt et al. 2000), largely driven by slow decomposition rates (Bartsch & Moore, 1985). The supressed decomposition rate is primarily the result of wet, anoxic and cool conditions (Basiliko et al. 2012; Bonnett et al. 2017) typically found in peatlands of the Western Boreal Plain (WBP). Other local factors that slow decomposition, such as acidity and nutrient availability, depend on the soil organic matter (SOM) pool present (Carrasco et al. 2006). The degree of lability/recalcitrance of SOM dictates microbial community composition and enzymatic activities, thus impacting the rate of peat formation and the bioavailability of nutrients for the overlying vegetation (Turcotte, 2009). More labile material will degrade much faster (Bradford et al. 2017) by providing microorganisms with an easily accessible energy

source (Turcotte, 2009) and expediting carbon mineralization (Fontaine et al. 2003; Rooney et al. 2012). Additionally, a process known as 'priming' may further enhance carbon mineralization rates. Priming refers to the addition of an organic substrate that can lead to enhanced microbial activity, resulting in increased carbon mineralization (Kuzyakov et al. 2000).

In contrast, recalcitrant material composed of highly complex organic structures (e.g., phenolic compounds) will require specific extracellular enzymes (i.e., phenol oxidase (PO)) to break down (Bonnett et al. 2017). Failure to break down these complex structures leads to the accumulation of phenolic compounds that inhibits peat degradation by hydrolase enzymes under anoxic conditions (Min et al. 2015); a process often referred to as the "Enzymic Latch" (Freeman et al. 2001). However, if the oxic zone is expanded, for example through water table draw down, PO activity is stimulated and phenolic compounds are removed (Bonnett et al. 2017). This induces carbon mineralization and subsequent nutrient release, providing a positive feedback on microorganism production and furthering the release of stored carbon (Fenner & Freeman, 2011).

Considering this, the addition of phenolic compounds to the soil via soil amendments or overlying vegetation could foreseeably lead to reduced hydrolase enzyme activity, reduced OM decomposition rates, and hence increased peat accumulation. Additionally, the overlying plant community will dictate the lability of the material available for decomposition, and may pose a risk to OM storage through the 'priming' effects of the remaining material. This study aims to investigate various reclamation practices related to fen construction and their impact on enzymatic activity and OM decomposition. Findings from this study could be used to generate recommendations for preferred overlying vegetation and mulch treatments during oil sands reclamation to peatland that will reduce decomposition and therefore optimize peat accumulation rates.

The specific objectives of this study were to:

1. Assess current decomposition rates and environmental conditions under different plant cover types (*Juncus balticus, Carex aquatilis*, bare peat) and mulching treatments at the constructed fen and compare them to nearby undisturbed (reference) fens;
2. Determine relative concentrations of phenolic compounds and associated enzyme activities under each of the treatment plots and compare them to nearby reference fens;
3. Determine which combination of factors (i.e., plant type, mulch, soil temperature, soil water content, pH, phenolic concentration, enzymatic activity) significantly impact decomposition and develop recommendations for best practices to minimize organic matter decomposition rate.

2.2 Methods

2.2.1 Study Site

The Nikanotee Fen (56.932° N, 111.417° W) is a 2.9 ha constructed fen located within a 32 ha constructed watershed on the Suncor Energy Inc. Oil Sands Base, approximately 20 km north of Fort McMurray, Alberta, Canada (Figure 2.1). Construction of the fen and watershed was completed in January 2013, with planting occurring in June 2013 (see Ketcheson et al. (2016) for details regarding the constructed fen design). Peat harvested from a dewatered fen on lease was placed to an approximate depth of 2 m and was vegetated with a factorial design to assess the success of various vegetation types and treatment methods (Figure 2.2). These treatment methods included mulching and weeding

Figure 2.1 View of the Nikanotee Fen from the southwest looking northwest

treatments over plots of bare peat (i.e., control), moss, seedlings, seedlings and moss, and seeds. Each plot was divided into 9 x 9 m sub-plots, half of which was covered by a wood-strand mulch. Wood-strand mulch (WoodStraw® ECM 2012) is made from a low grade-veneer, soaked and cut to pieces ~ 10 cm long, 0.5 cm wide, and 0.1 cm thick (Borkenhagen, Personal Communication, 2015). For the moss treatment, the top 10 cm of a nearby rich treed fen was harvested mechanically and applied to the site following the moss-layer transfer technique (Quinty & Rochefort, 2003). The moss was spread by hand to approximately 1 cm in thickness. Seedlings were germinated in a greenhouse and hand-planted on site. Seedlings included freshwater (*Carex aquatilis, Betula pumila*) and salt-tolerant (*Juncus balticus, Triglochin maritima, Calamagrostis inexpansa*) species.

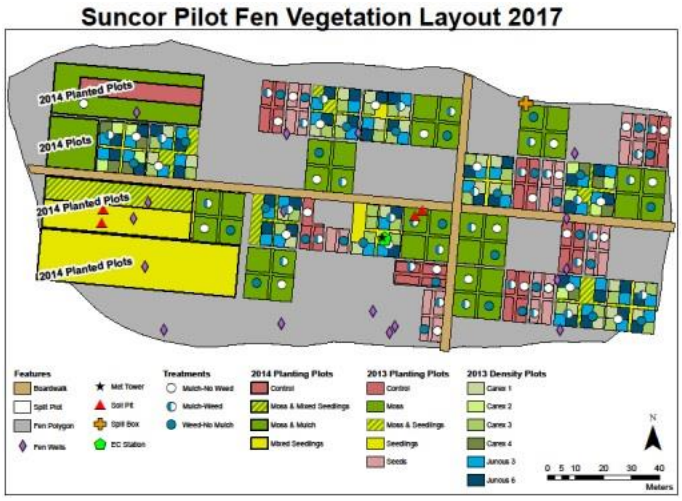


Figure 2.2 Vegetation and mulch treatment plots at the Niaknotee fen. Note: Ponding isn't shown on diagram.

By 2016, the site was largely covered by *Carex aquatilis* with some large *Juncus balticus* patches and *Typha latifolia* in wet areas. To investigate the effects of plant type and mulch, we chose sampling locations dominated by either *C. aquatilis* or *J. balticus* and areas with minimal vegetation cover (bare) in both mulched and non-mulched plots. Six locations were selected under each vegetation type and mulch treatment combination for a total of 36 sampling locations. Results from the constructed fen

were compared to three nearby reference fens (Figure 2.3): poor fen (i.e., Pauciflora; Wells et al. 2017), rich fen (i.e., Poplar; Elmes et al. 2018), and saline fen (i.e., Saline; Wells & Price, 2015). The poor fen (56° 22.610 N, 111° 14.164 W) is situated within a forested upland consisting of plant species that included *Sphagnum* spp., *Chamaedaphne calyculata, Carex* spp., *Picea mariana* and *Betula pumila*. The rich fen (56° 56.330 N, 111° 32.934 W) is a treed moderately-rich fen dominated by *Larix laricina, B. pumila, Equisetum fluviatile, Smilcina trifolia, Carex* spp. and*Tomenthypnum nitens.* The saline fen (56° 34.398 N, 111° 16.518 W) receives abnormally high salinity groundwater due to its geological setting, and contains *Juncus balticus, Calamagrostis stricta* and *Triglochin maritima* (Khadka et al. 2015). In addition to the different fen types, differences between microforms (i.e., hummocks/hollows) within each site were also assessed. Six hummock/hollow pairs at each of the three sites were selected for a total of 36 sampling locations across the three reference fens.

Figure 2.3: poor fen (a), rich fen (b), and saline fen (c) used as reference sites.

2.2.2 Environmental conditions

Data from the constructed fen and reference sites was collected from May – August 2017. Soil temperature at depths of 5 and 10 cm, electrical conductivity (EC), pH, and volumetric water content (VWC) measurements were obtained every 2-3 weeks at both the constructed fen and reference sites. Soil temperature was obtained using an Omega copper-constantan thermocouple. EC and pH were measured in water samples collected at 15 cm and 30 cm depths using a portable porewater sampler made from a slotted polyvinyl chloride (PVC) pipe, with separate tubing positioned at each depth inside

the pipe and wrapped in filter sock to prevent peat build up, and measured using a Thermo Scientific Orion Economy series pH Combination Electrode that was calibrated monthly. VWC was measured directly using a WET-Sensor (Delta-T Devices) and corrected using a gravimetrically-determined calibration curve specific to soil obtained from each study site.

2.2.3 Decomposition rates: Litter Bags and Tea Bag Index

We used litter bags (Moore, 1984) and the tea bag index (Keuskamp et al. 2013) to estimate decomposition rate at all sites. Four litter bags, each containing one type of either above- and below-ground biomass of *Carex aquatilis* (*Carex*) or *Juncus balticus* (*Juncus*), were buried in August 2016 following Moore (1984). Each litter bag consisted of 10 cm x 10 cm 1-mm mesh screen sewn together with homogenized plant material inside the bag and was dried in an oven for 24 hours at 60°C and weighed prior to burial. At the three reference sites, only above-ground biomass of *Juncus* and *Carex* was buried due to limited availability of belowground material. All litter bags were retrieved approximately 1 year later, placed in a drying oven for 48 hours at 60°C, roots and debris removed from the surface and inside of the mesh bag, and weighed for mass-loss. Mass-loss from the litter bags was used to estimate a decomposition rate (*k'*) using equation 1:

$$\boldsymbol{k'} = \mathbf{ln}\frac{X_0/X_t}{t} \quad [1]$$

where, X_0 is the initial mass (g), X_t is the final mass (g), t is the time buried (days), and k' is the estimated decomposition rate (day^{-1}).

Additionally, four tea bags (two green tea and two rooibos tea bags), as per the Tea Bag Index (TBI; Keuskamp et al. 2013), were buried at depths of 1 cm and 8 cm at the same litter bag locations in May 2017. At each of the three reference sites only two sets of each were buried at depths of 1 cm and 8 cm in May 2017. At the constructed fen, four tea bags (i.e., one green and one red tea bag at both 1 cm and 8 cm depths) were retrieved after ~90 days. Tea bags were placed in a drying oven for 48 hours at 60°C,

roots and debris removed, and bags weighed for mass-loss. Since exponential decay rate is not consistent over time (i.e., rate changes as the lability of the material changes; Keuskamp et al. 2013), the TBI method includes a stabilization factor to account for the chemical change in material. The mass-loss from the green tea is used to determine a stabilization factor, *S* (Equation 2):

$$\boldsymbol{S = 1 - \frac{ag}{Hg}} \quad [2]$$

where, *ag* is the fraction of green tea that was decomposed, and *Hg* is the hydrolysable fraction of green tea (Keuskamp et al. 2013). Once *S* is determined, Equation 3 can be used to determine an estimated decomposition rate, k_{TBI}:

$$\boldsymbol{k_{TBI} = ln \frac{\frac{ar}{WR-(1-ar)}}{t}} \quad [3]$$

where, *WR* is the fraction of rooibos tea remaining, *t* is time spent buried (days), and *ar* is the predicted labile fraction of rooibos tea, calculated using Equation 4:

$$\boldsymbol{ar = Hr \times (1 - S)} \quad [4]$$

where, *Hr* is the hydrolysable fraction of rooibos tea and *S* is the stability factor. The final k_{TBI} value is an estimate of the approximate decomposition rate (day^{-1}) over the course of the burial period.

2.2.4 Peat and water sample collection:

Water sampling was completed once a month, while peat was collected in mid-July, at the constructed fen and reference sites. Peat samples were obtained at 18 locations (three replicates of six treatments) at 15 cm and 30 cm depths using a hand auger. Peat samples were frozen and sent to the lab for phenolics and enzyme analysis at a later date. Pore water samplers were installed at the same 18 locations and were used to collect water samples at 15 cm and 30 cm depths. Pore water samplers were constructed of PVC pipe containing two separate tygon tubing segments positioned at the appropriate depths (i.e., 15 cm and 30 cm). Water was collected using a 60 ml syringe and placed in 60 ml containers

and stored in a cooler. Water samples were then filtered with Flipmate 50 ml filtration cups made of PES/PTFE with 0.45 μm filter (Environmental Express, RK-35202-33) and stored at 4 °C until analysis.

2.2.5 Phenolic compounds and potential enzyme activities

Phenolic compound concentration as well as hydrolase (β-glucosidase, Arylsulphatase, β-D-xylosidase, N-acetyl-β-D-glucosaminidase, and Phosphatase) and phenol oxidase enzyme activities were assayed because of their role in organic matter decomposition in peatlands (Freeman et al. 2001). Phenolic compound concentrations were assessed following a modified version of the method of Box (1983). Hydrolase enzymes and phenol oxidase were assessed following Dunn et al. (2014). The details of phenolic compound concentration and enzyme assessments are provided in the Supplemental information section in Appendix A.

2.2.6 Statistical analysis

In order to compare environmental conditions, *k*, and biogeochemical factors (i.e., phenolics and extracellular enzyme activities) between the constructed fen and its associated vegetation and mulch treatment plots with the reference sites and their associated microforms, a linear regression model was used. Within the constructed fen, a linear regression model was used to compare the seasonal means of environmental conditions to the biogeochemical factors obtained in July and *k* based on the vegetation and mulch treatments along with their interactions with each. Within the reference sites, a linear regression model was also used to compare the seasonal means of environmental conditions to the biogeochemical factors obtained in July and *k* based on site types and microforms along with their interactions with each. For all significant results a Tukey HSD pairwise comparison was performed to determine significant differences between treatment plots or sites. Additionally, to determine environmental controls on *k* and biogeochemical factors, a multiple linear regression was utilized using seasonal means of environmental conditions and biogeochemical data obtained in July. Lastly, multiple linear regressions were also used to determine biogeochemical controls on *k* using

biogeochemical data obtained in July as well as interactions with environmental conditions using seasonal means. All statistical analysis was conducted using the statistical program R (R Core Team, 2017), and a significance of $\alpha = 0.05$ was applied.

2.3 Results:

2.3.1 Environmental conditions

Environmental conditions (Table A.1) and statistical results (Table A.2) at both the constructed fen and reference sites are provided in Appendix A. Volumetric water content (VWC) varied significantly across the constructed fen depending on mulch treatment ($F_{1,30} = 12.98$, $p < 0.01$) but not the vegetation type ($F_{2,30} = 0.054$, $p = 0.95$). Mulch plots had a lower VWC (77.7%) as compared to no-mulch plots (84.6%). Additionally, VWC varied significantly across the reference sites, differing among the study fens ($F_{2,30} = 20.32$, $p < 0.001$) and microforms ($F_{1,30} = 86.60$, $p < 0.001$). From the reference sites, the saline fen had the highest VWC (73.7%), while the rich fen had the lowest (43.4%), and hollows showed a consistently higher VWC (75.1%) as compared to hummocks (38.1%). Across all plots, the constructed fen had a significantly higher VWC than the reference sites (81.2% vs. 56.6%; $F_{1,70} = 25.35$, $p < 0.001$).

Electrical conductivity (EC) at 15 cm and 30 cm depths is provided in Appendix A. At the constructed fen, average EC did not vary significantly across either the vegetation ($F_{2,30} = 1.83$, $p = 0.18$) or mulch ($F_{1,30} = 2.92$, $p = 0.098$) treatment plots. The highest average EC at the constructed fen was at the *Juncus* plots (2970 µS/cm) and the lowest was at the bare plots (2153 µS/cm), with mulch plots having a lower EC (2262 µS/cm) as compared to no-mulch plots (2732 µS/cm). At the reference sites, the average EC varied significantly across both site type ($F_{2,30} = 3203.99$, $p < 0.001$) and microforms ($F_{1,30} = 9.03$, $p < 0.01$). The highest EC was at the saline fen (12720 µS/cm) and the lowest was at the poor fen (86 µS/cm), with hollows having a higher EC as compared to hummocks. In general, it was found that the average EC was higher at the constructed fen (2497 µS/cm) as compared to the reference sites ($F_{1,70} = 3.39$, $p = 0.07$).

The pH at 15 cm and 30 cm depths are provided in Appendix A. At the constructed site, the average pH did not vary significantly across the vegetation ($F_{2,29} = 0.029$, $p = 0.97$) or mulch treatment plots ($F_{1,29} = 0.045$, $p = 0.83$). At the reference sites, pH varied significantly across the site types ($F_{2,30} = 182.57$, $p < 0.001$), but not across the microforms ($F_{1,30} = 1.26$, $p = 0.27$). The highest average pH was at the rich fen (6.98) and the lowest was at the poor fen (4.44). In general, the constructed fen showed a higher average pH (7.13) as compared to the reference sites ($F_{1,69} = 37.10$, $p < 0.001$).

Soil temperature at the 5 and 10 cm depths are provided in Appendix A. The average soil temperature at the constructed fen varied significantly only across the vegetation ($F_{2,30} = 49.059$, $p < 0.001$) and not the mulch treatment plots ($F_{1,30} = 0.17$, $p = 0.68$). The highest average soil temperature was at the bare plots (14.7 °C) and the lowest was at the *Carex* plots (11.5 °C). The reference sites also varied significantly in average soil temperature across both site types ($F_{2,30} = 33.014$, $p < 0.001$) and microforms ($F_{1,30} = 5.68$, $p = 0.024$). The highest average soil temperature was at the saline fen (13.8 °C) and the lowest was at the rich fen (9.7 °C), with hollows showing a lower temperature (11.5 °C) compared to hummocks (12.5 °C). In general, the constructed fen had a higher average soil temperature (13.2°C) than the reference sites (12.0 °C; $F_{1,70} = 6.92$, $p < 0.05$).

In summary, the constructed fen had on average warmer soil with higher VWC than reference fens with a pH closer to neutral. The EC measured in soil water was higher than the poor fen and rich fen sites, but lower than the saline fen.

2.3.2 Decomposition rate – litter bags

Figure 2.1 shows the average *k'* of measured litter types across the constructed fen and reference sites ($F_{3,182} = 26.2$, $p < 0.001$). Figure 2.2 shows *k'* from each litter type at the constructed fen. Only the above-ground biomass of *Carex* and *Juncus* varied significantly across the vegetation treatment plots ($F_{2,26} = 5.01$, $p < 0.05$; $F_{2,26} = 4.09$, $p < 0.05$), but not across the mulch treatment plots ($F_{1,26} = 0.0002$,

p = 0.99; $F_{1,26}$ = 0.085, p = 0.77). Average above-ground biomass decomposition rate (*k'*) is provided in Figure 2.3. The highest *k'* from the above-ground biomass was at the *Carex* plots (0.00145 day^{-1}) and the lowest was at the bare plots (0.000933 day^{-1}). The decomposition of below-ground biomass of *Carex* and *Juncus* (Appendix A, Figure 2.17) did not vary significantly across vegetation treatment plots ($F_{2,28}$ = 1.22, p = 0.31) or mulch treatment plots ($F_{1,25}$ = 0.95, p = 0.34). However, there was a significant difference between the above-ground and below-ground biomass decomposition rates ($F_{1,22}$ = 10.34, p < 0.01) across the vegetation plots ($F_{2,22}$ = 4.16, p < 0.05), but not across the mulch plots ($F_{1,22}$ = 0.97, p = 0.3364).

At the reference sites, *k'* from the above-ground biomass of *Carex* and *Juncus* varied significantly across both fen types ($F_{2,25}$ = 8.44, p < 0.01) and microforms ($F_{1,25}$ = 12.34, p < 0.01). The highest decomposition rate was at the rich fen (0.00232 day^{-1}) and the lowest was at the poor fen (0.00162 day^{-1}), and hollows had a lower decomposition rate (0.00168 day^{-1}) compared to hummocks (0.00209 day^{-1}). Below-ground biomass of *Carex* and *Juncus* was not included in the study at the reference sites and therefore is not considered here. Based only on decomposition of above-ground litter, the constructed fen had a significantly lower average decomposition rate (0.00127 day^{-1}) than the reference sites (0.00189 day^{-1}; $F_{1,63}$ = 23.98, p < 0.001).

To evaluate environmental controls on decomposition rate, the average estimated decomposition rate from the above-ground biomass was tested with VWC, average EC, average pH and average soil temperature, and the only significant relationships were with pH across the reference sites (Figure 2.7a; R^2 = 0.16, $F_{1,29}$ = 6.76, p < 0.05), and with the average temperature at the constructed fen (Figure 2.7b; R^2 = 0.24, $F_{1,32}$ = 11.51, p < 0.01).

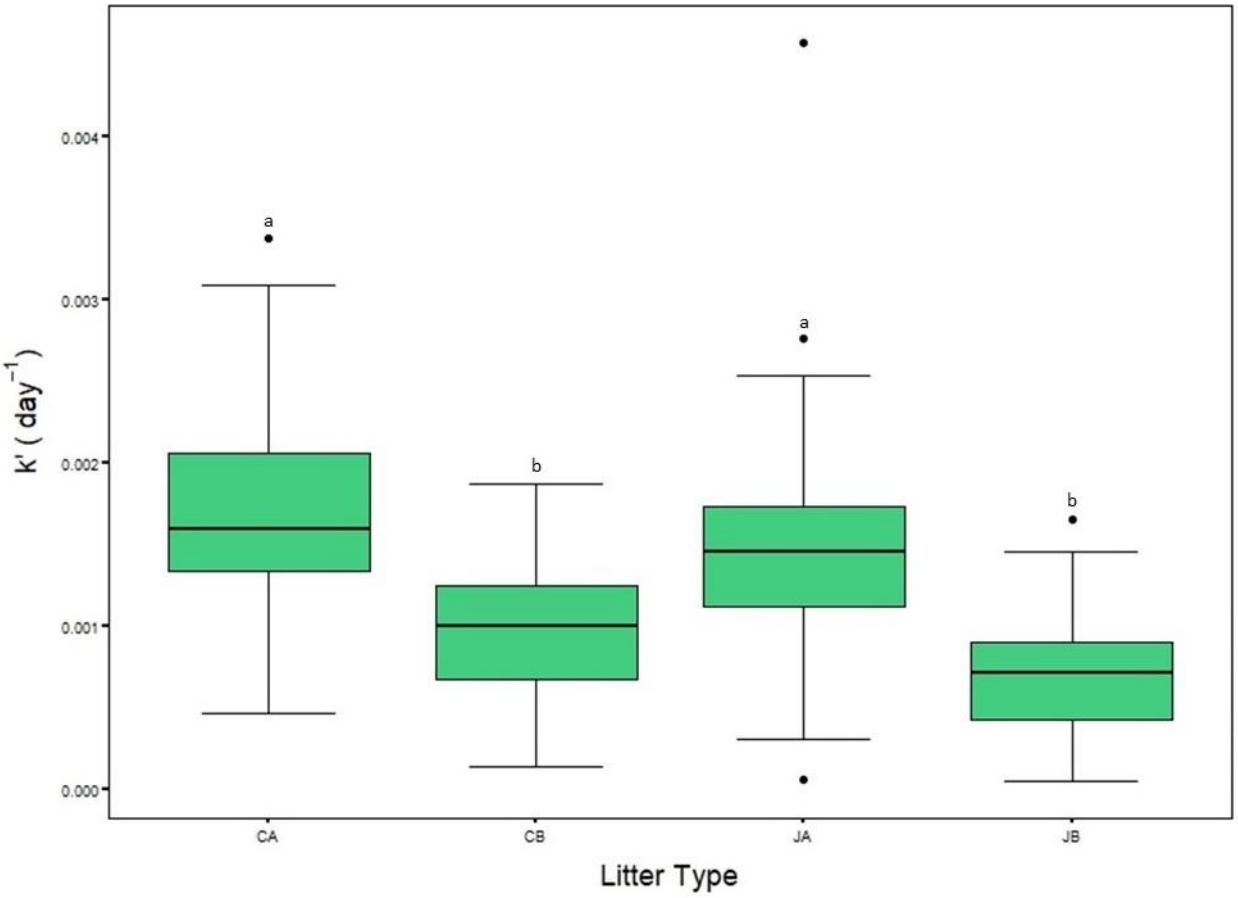


***Figure 2.3**: Estimated decomposition rate from the litter bags using above-ground* Carex aquatilis *(CA), below-ground* Carex aquatilis *(CB), above-ground* Juncus balticus *(JA), and below-ground* Juncus balticus *(JB) across the constructed fen and reference sites. Litter types with the same letter(s) indicates a lack of significant difference.*

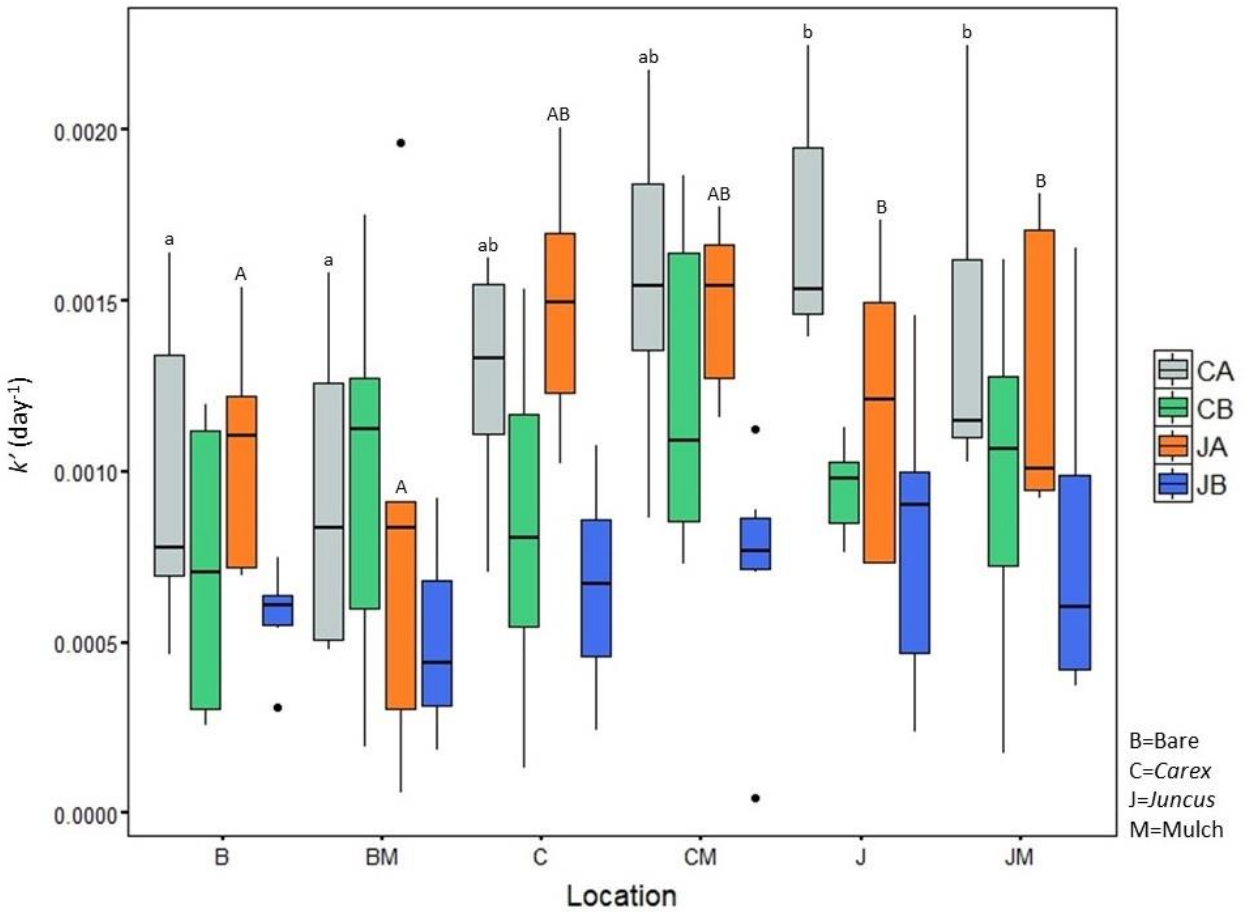


Figure 4.2: *Estimated decomposition rate from the litter bags using above-ground* Carex aquatilis *(CA), below-ground* Carex aquatilis *(CB), above-ground* Juncus balticus *(JA), and below-ground* Juncus balticus *(JB) across the constructed fen. Differences were only statistically significant for the above-ground biomass, and are represented by the lower-case letters (Carex) and upper-case letters (Juncus). Locations with the same letter(s) indicates a lack of significant difference.*

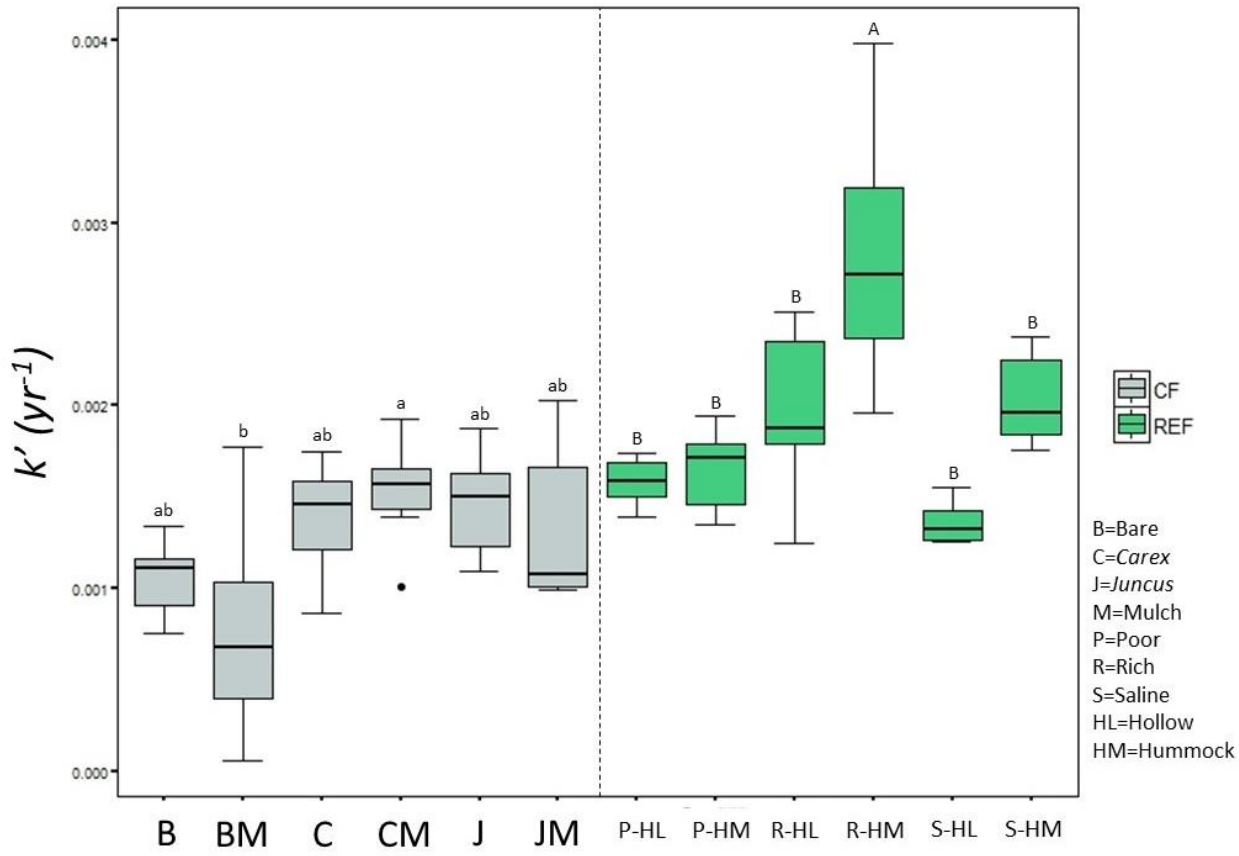


Figure 2.5: *Estimated decomposition rate using the average above-ground biomass of* Carex aquatilis *(Carex) and* Juncus balticus *(Juncus) from the litter bags at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Locations with the same letter(s) indicates no significant difference.*

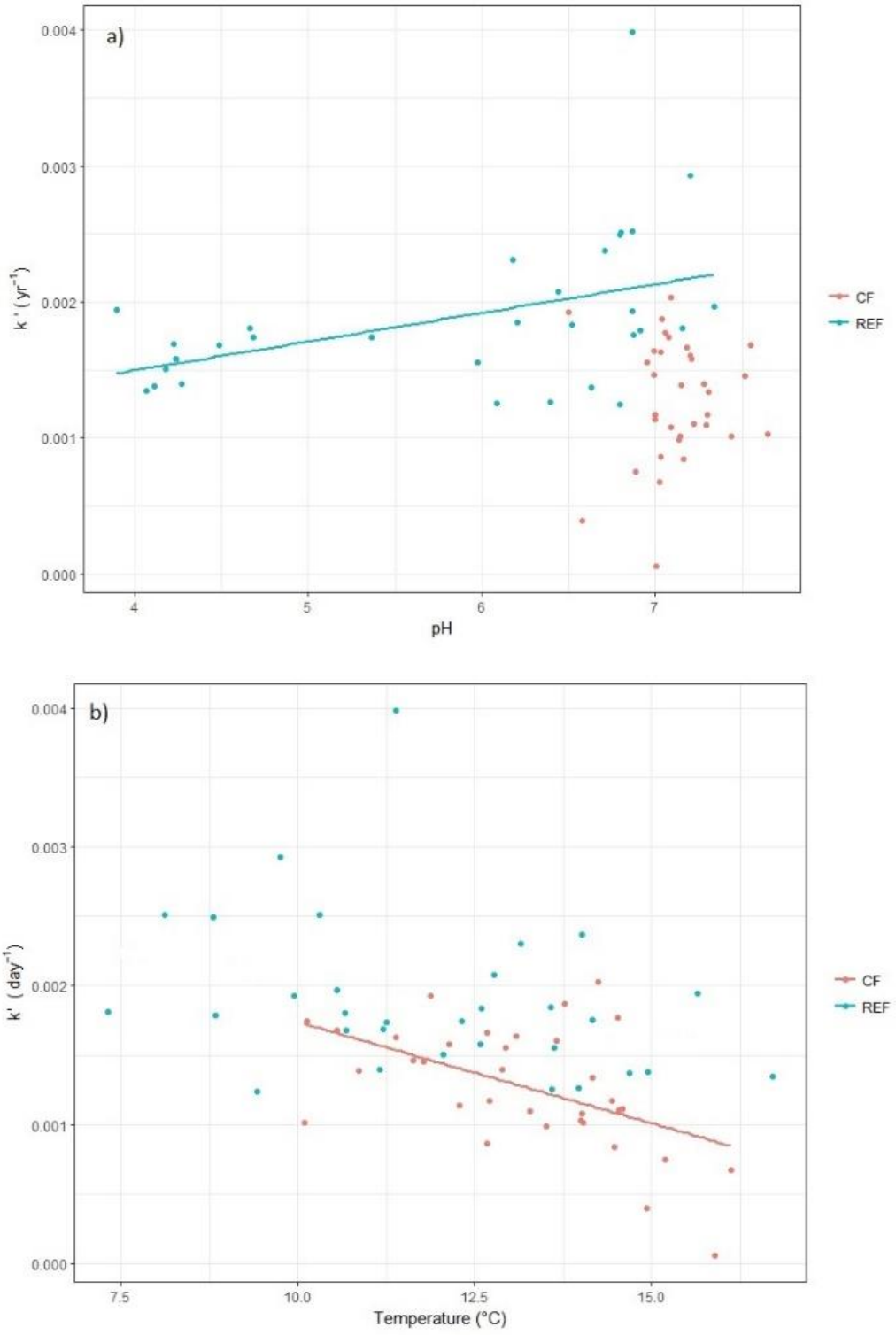


Figure 2.6: *Average estimated decomposition rate from above-ground biomass with average pH (a; $R^2 = 0.16$, $F_{1,29} = 6.76$, $p < 0.05$) and average soil temperature (b; $R^2 = 0.24$, $F_{1,32} = 11.51$, $p < 0.01$). Average pH was obtained at 15 cm and 30 cm depths and soil temperature was obtained from the 5 cm and 10 cm depths. Measurements were taken every 2-3 weeks during the growing season (May-September) at the constructed fen and reference sites Trendlines are shown only for significant regressions.*

2.3.3 Decomposition rate – tea bags

The estimated decomposition rates from tea bags buried at 1 cm and 8 cm depths from the constructed fen and reference sites are provided in Appendix A. Since values from both burial depths were similar, the average was used in further analysis (Figure 2.5). It was found that k_{TBI} did not vary significantly across either the vegetation treatment plots ($F_{2,27} = 0.77$, $p = 0.47$) or the mulch treatment plots ($F_{1,27} = 0.23$, $p = 0.64$). Unlike the findings from the litter bags, the highest k_{TBI} was at the bare plots (0.0187 day^{-1}) and the lowest was at the *Carex* plots (0.0126 day^{-1}). At the reference sites, k_{TBI} did not vary significantly across the fen types ($F_{2,12} = 2.52$, $p = 0.12$) and microforms ($F_{1,12} = 0.45$, $p = 0.30$). Similar to the results from litter bags, the highest k_{TBI} was at the rich fen (0.0133 day^{-1}) and the lowest was at the poor fen (0.0105 day^{-1}), with hollows (0.0116 day^{-1}) and hummocks (0.0123 day^{-1}) showing a similar trend to the litter bags. In general, the constructed fen had a higher average estimated decomposition rate (0.0153 day^{-1}) as compared to the reference sites (0.0119 day^{-1}), but the difference was not statistically significant ($F_{1,50} = 1.50$, $p = 0.2889$).

All environmental variables were tested in regressions with k_{TBI} and significant relationships were found with VWC at both the constructed fen (Figure 2.9a; $R^2 = 0.15$, $F_{1,31} = 6.58$, $p < 0.05$) and reference sites ($R^2 = 0.17$, $F_{1,16} = 4.53$, $p = 0.049$), as well as with the average EC at the constructed fen (Figure 2.9b; $R^2 = 0.41$, $F_{1,31} = 23.32$, $p < 0.001$), and with the average pH at the reference sites (Figure 2.9c; $R^2 = 0.21$, $F_{1,16} = 5.47$, $p < 0.05$). When we compared the average k_{TBI} to the average k' from the above-ground biomass in litter bags, there was only a significant relationship found at the reference sites (Figure 2.10; $R^2 = 0.38$, $F_{1,13} = 9.5$, $p < 0.01$).

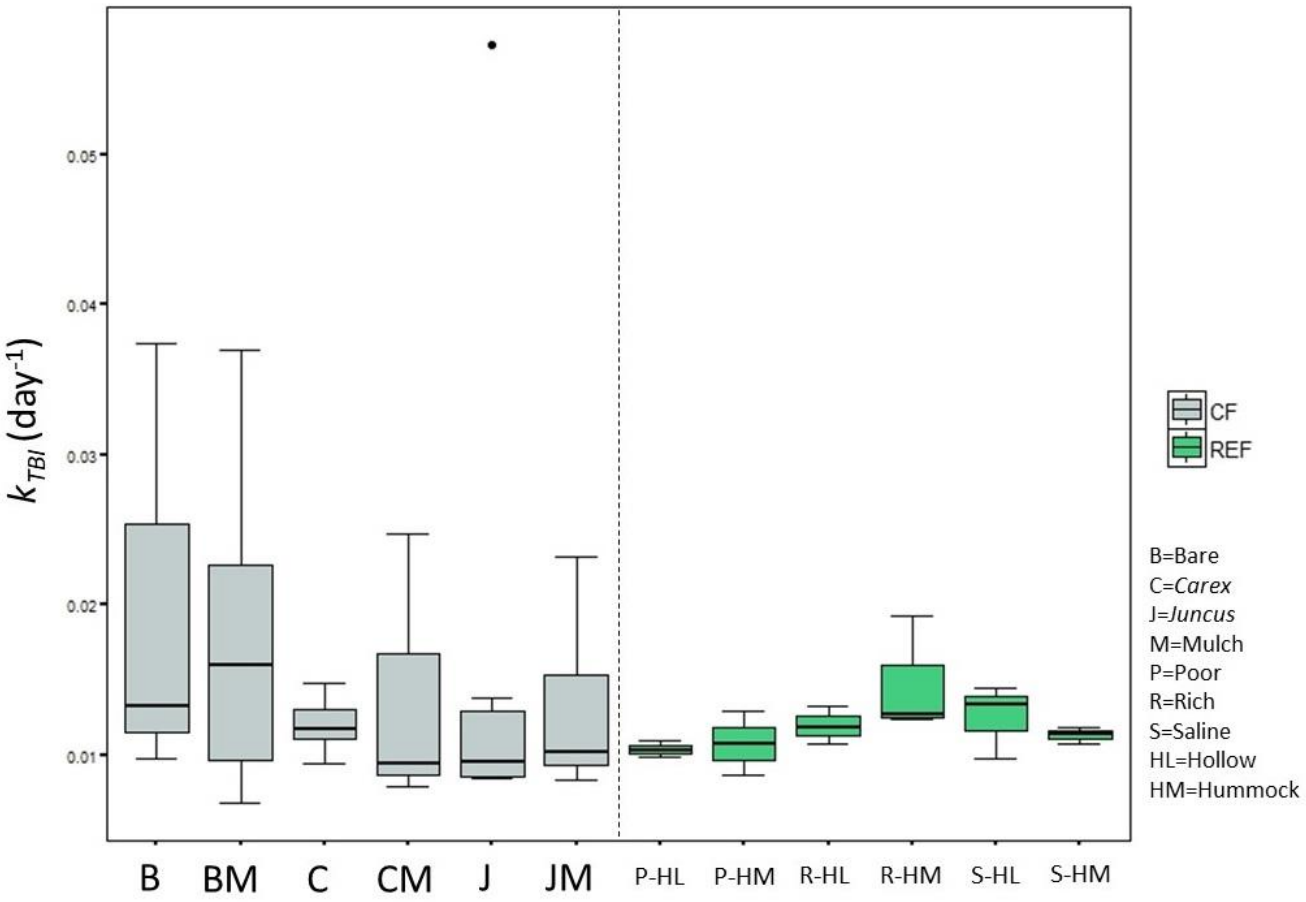
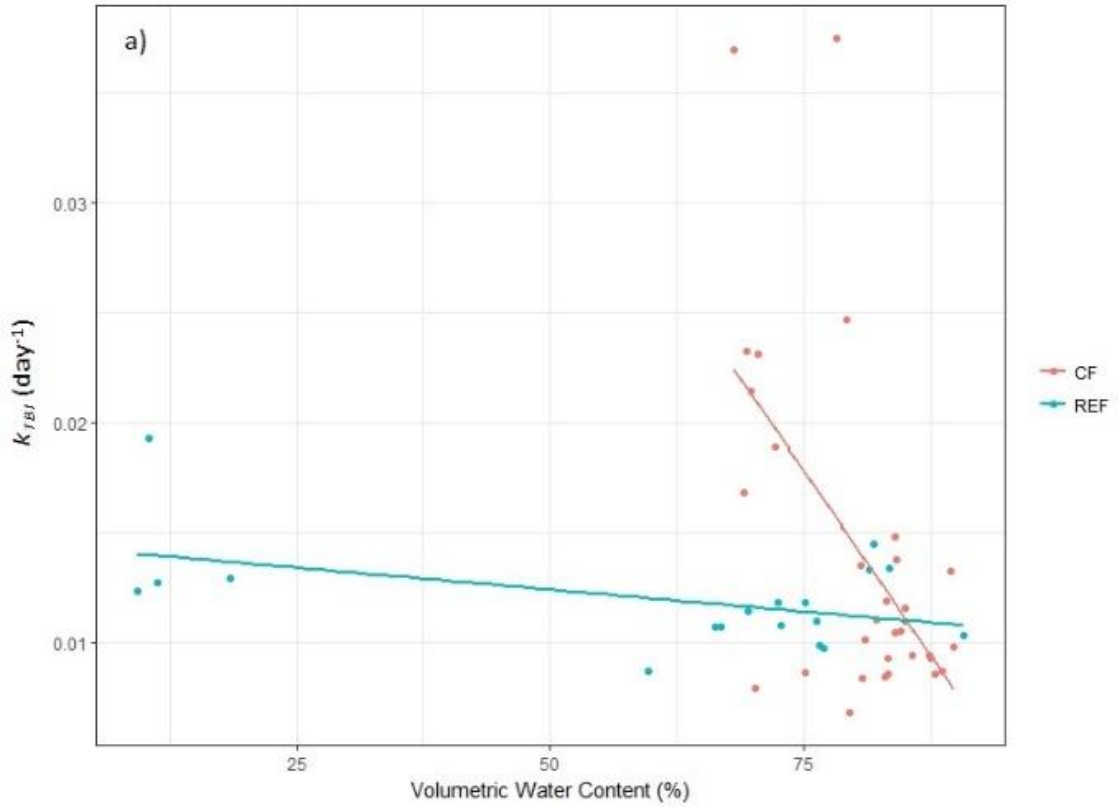
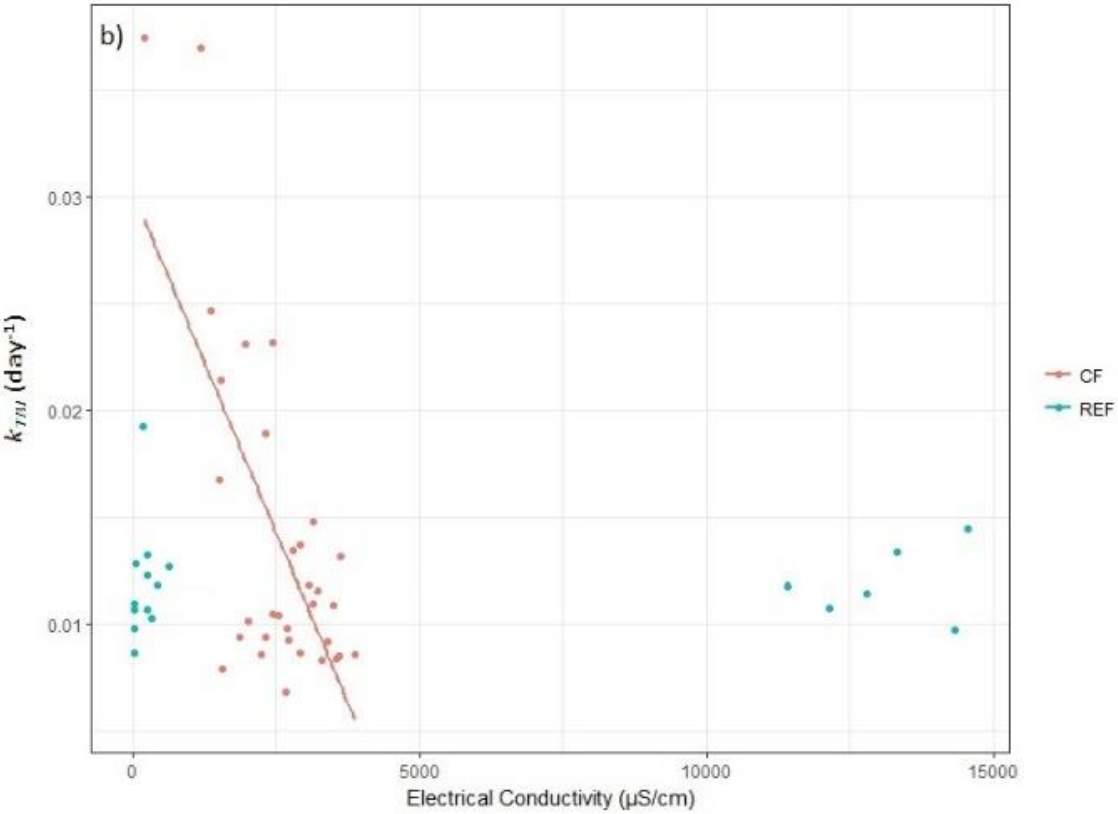


Figure 2.7: *Average estimated decomposition rate using the tea bags (TBI) at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Averages from the TBI included 1 cm and 8 cm depths. TBI includes decomposition only during the growing season (May – September).*


a)
0.03
0.02
0.01
k_{TBI} (day^{-1})
25
50
75
Volumetric Water Content (%)
CF
REF


b)
0.03
0.02
0.01
k_{TBI} (day^{-1})
0
5000
10000
15000
Electrical Conductivity (µS/cm)
CF
REF

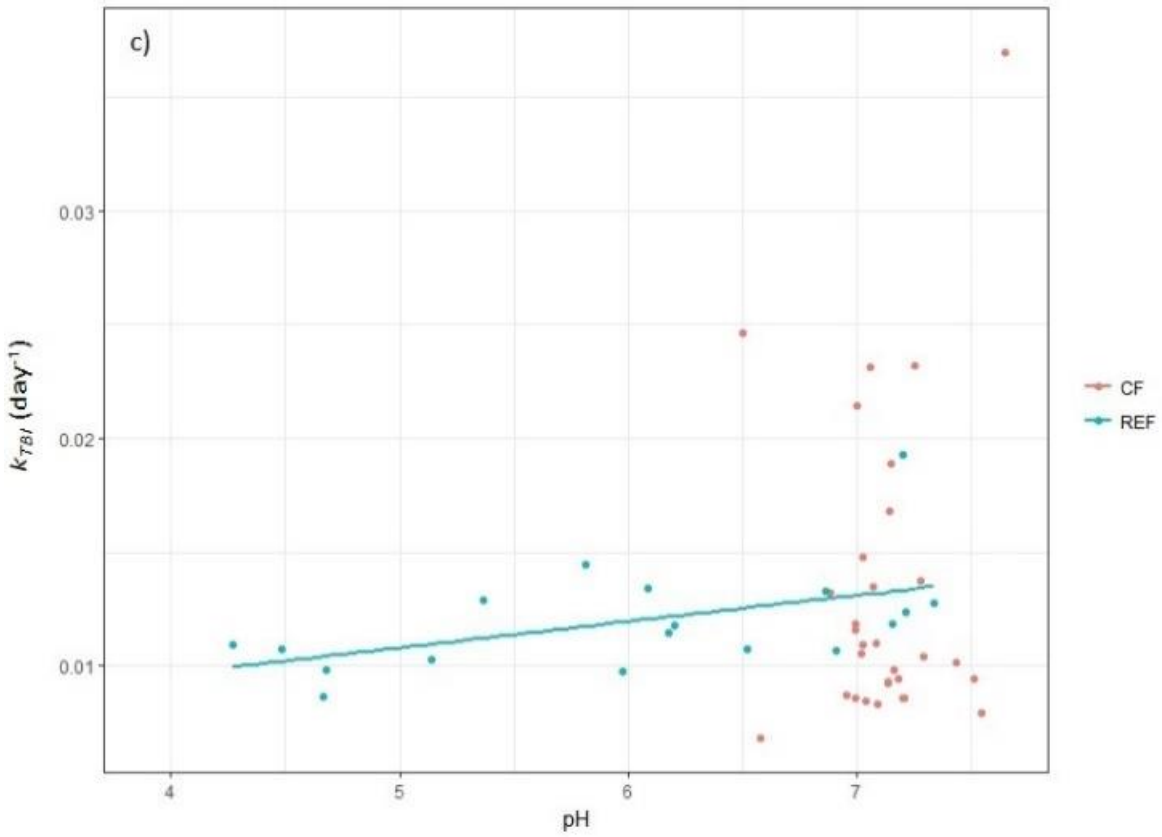


Figure 2.8: *Average estimated decomposition rate from the tea bags buried at 1 cm and 8 cm depths vs VWC (a; $R^2 = 0.15$, $F_{1,31} = 6.58$, $p < 0.05$; $R^2 = 0.17$, $F_{1,16} = 4.53$, $p = 0.049$), average EC (b; $R^2 = 0.41$, $F_{1,31} = 23.32$, $p < 0.001$) and average pH (c; $R^2 = 0.21$, $F_{1,16} = 5.47$, $p < 0.05$). Average EC and pH were obtained at 15 cm and 30 cm depths, and VWC was obtained just below the surface. Measurements were collected very 2-3 weeks during the growing season (May – September).*

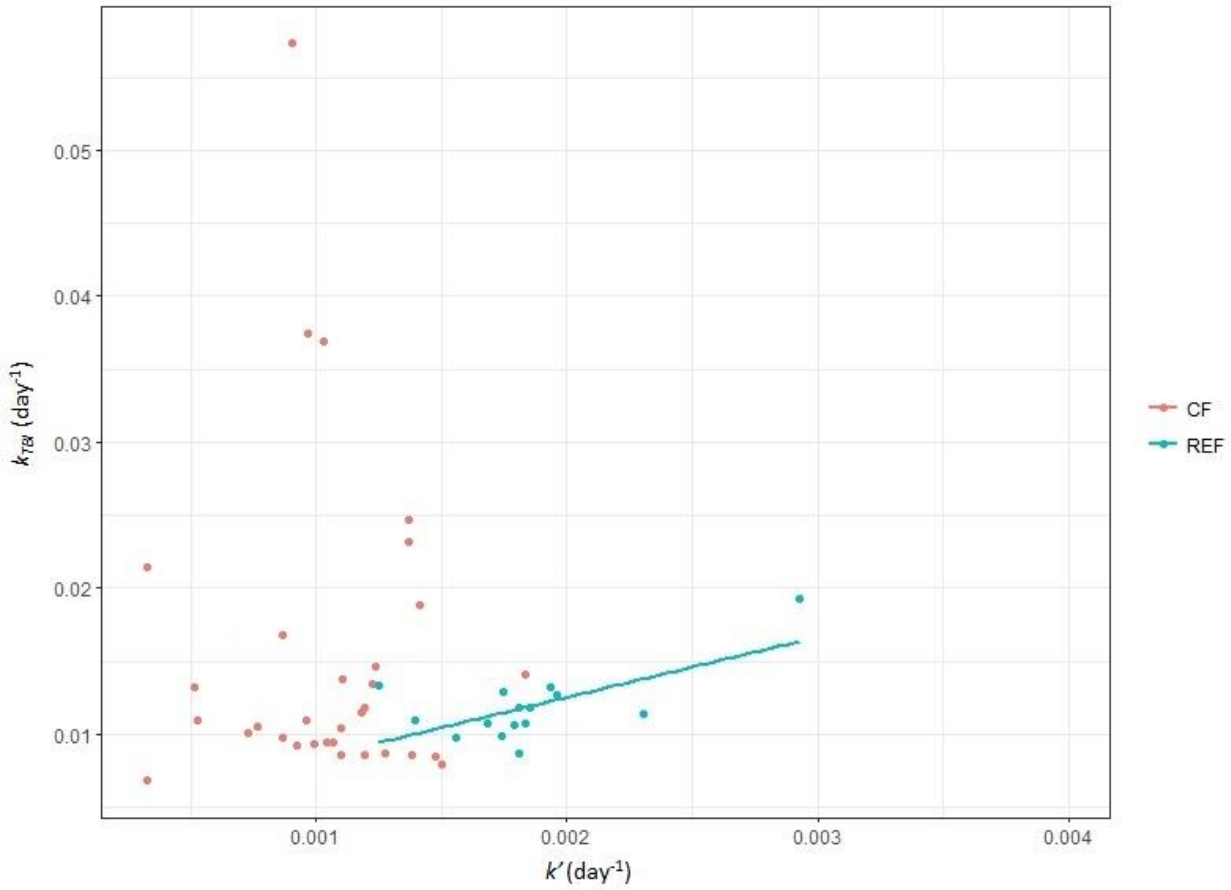


Figure 2.9: *Average estimated decomposition rate (k') from the above-ground biomass vs average estimated decomposition rate from the tea bags (k_{TBI}) at the 1 cm and 8 cm depths (R^2 = 0.38, $F_{1,13}$ = 9.5, p < 0.01).*

2.3.4 Phenolic compounds

Phenolic compound concentrations did not vary significantly at the constructed fen at 15 cm (Figure 2.8a) or 30 cm (Figure 2.8b) depths across the vegetation (15 cm: $F_{2,11}$ = 1.01, p = 0.3957; 30 cm: $F_{2,12}$ = 0.14, p = 0.87) or mulch treatment plots (15 cm: $F_{1,11}$ = 0.16, p = 0.69; 30 cm: $F_{1,12}$ = 0.0042, p = 0.95). At 15 cm depth, the highest concentration was at the *Juncus* plots (0.190 mg/ml) and the lowest was at the *Carex* plots (0.176 mg/ml), with mulch treatment plots showing a slightly higher concentration (0.187 mg/ml) compared to no-mulch plots (0.183 mg/ml). At the reference sites, phenolic compound concentration at the 15 cm depth did vary significantly across both fen types ($F_{2,12}$ = 18.71, p < 0.001) and microforms ($F_{1,12}$ = 11.57, p < 0.01). The highest concentration was at the saline fen (0.187 mg/ml) and the lowest was at the poor fen (0.128 mg/ml), with the hollows showing a lower

concentration (0.152 mg/ml) than the hummocks (0.183 mg/ml). Overall, phenolic concentrations were higher at 30 cm and varied significantly across the fen types ($F_{2,10} = 9.21$, $p < 0.01$) but not across the microforms ($F_{1,12} = 11.57$, $p = 0.1743$) with similar patterns to 15 cm depth. Compared to the constructed fen, the reference sites had a lower concentration of phenolics at both the 15 cm (0.167 mg/ml vs. 0.185 mg/ml) and 30 cm (0.418 mg/ml vs 0.543 mg/ml) depths, being significantly different at the latter (15 cm: $F_{1,33} = 3.12$, $p = 0.086$; 30 cm: $F_{1,32} = 14.76$, $p < 0.001$).

Only phenolic compound concentrations at the 15 cm depth were used in comparison to other variables as this was closer to the depth of litter decomposition measured at the surface. Phenolic compound concentration showed a positive relationship with increasing pH, but this was only significant at the reference sites (Figure 2.12a; $R^2 = 0.53$, $F_{1,16} = 20.47$, $p < 0.001$) and not at the constructed fen ($R^2 = -0.071$, $F_{1,14} = 0.0074$, $p = 0.93$). In general, higher concentrations of phenolic compounds were associated with higher *k'* (Figure 2.12b), although the regression was only significant for reference sites ($R^2 = 0.28$, $F_{1,13} = 6.51$, $p < 0.05$) and not across the constructed fen ($R^2 = 0.13$, $F_{1,15} = 3.45$, $p = 0.083$).

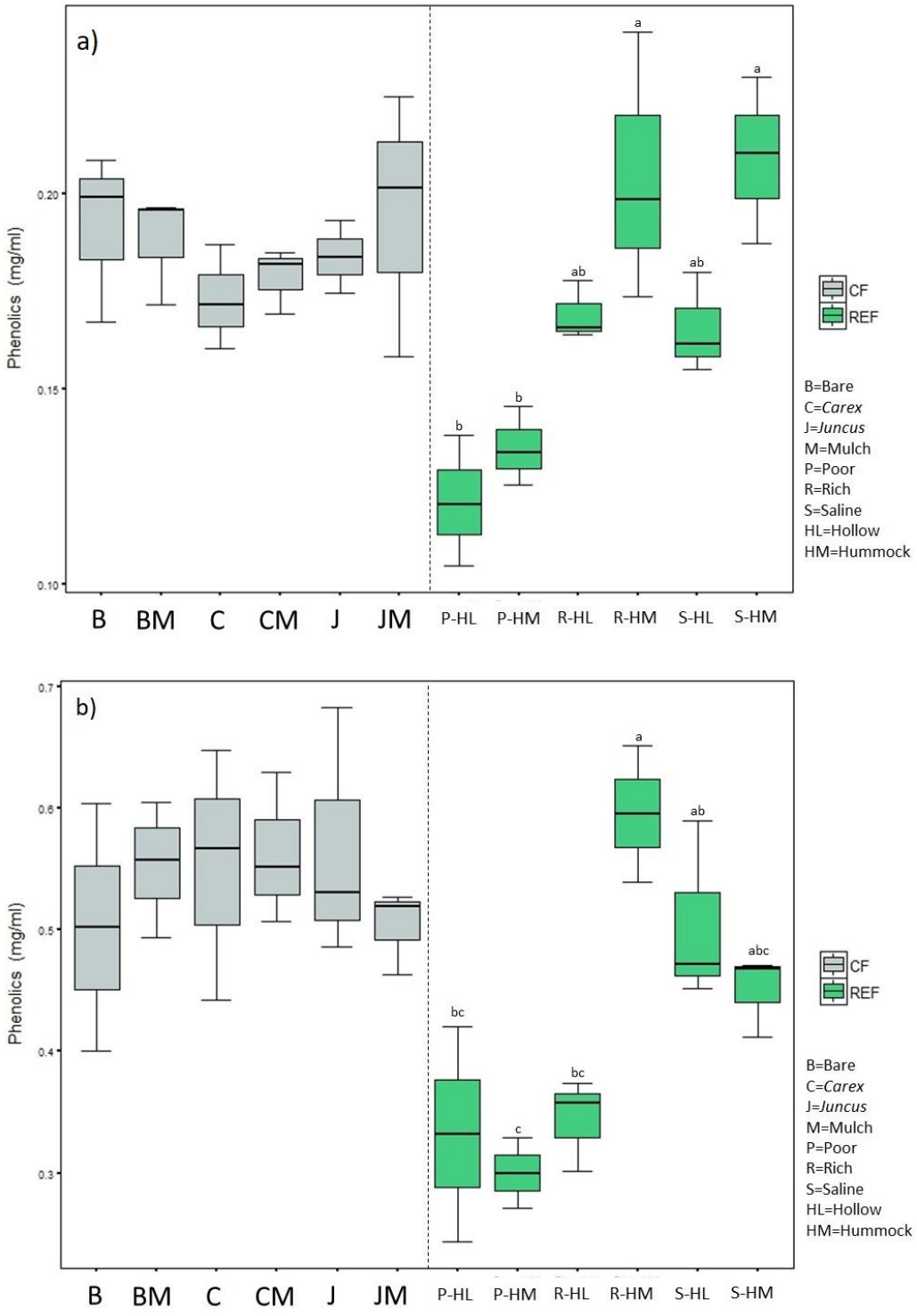


Figure 2.10: *Phenolic compound concentration collected at 15 cm (a) and 30 cm (b) at the Constructed Fen (CF) and Reference Sites (REF). CF locations include plots of* Carex *(C),* Juncus *(J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Concentrations from the 15 cm depth were obtained from water extractions performed on the organic material while 30 cm depth concentrations were obtained from water samples collected directly from the site. Letters indicate significant differences between each location. Locations with the same letter(s) indicates no significant difference.*

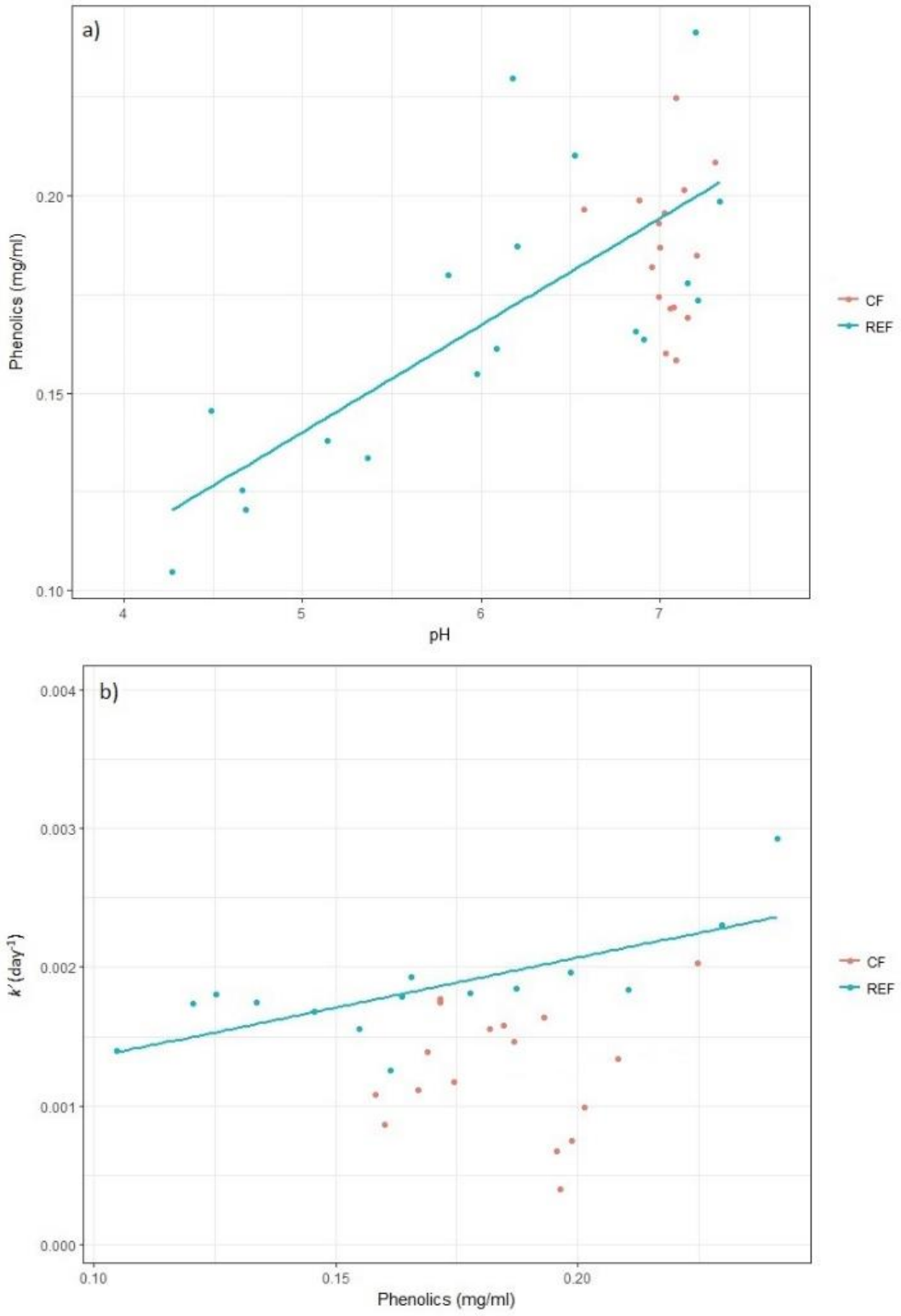


Figure 2.11: *Phenolic compound concentration (15 cm depth) vs average pH (a; $R^2 = 0.53$, $F_{1,16} = 20.47$, $p < 0.001$) and average estimated decomposition rate (k'; b; $R^2 = 0.28$, $F_{1,13} = 6.51$, $p < 0.05$) at the constructed fen and reference sites. Average pH was obtained from the 15cm and 30 cm depths every 2-3 weeks during the growing season (May – September). Estimated decomposition rate from the litter bags included above-ground biomass of* Carex aquatilis *and* Juncus balticus*. Phenolic compound concentration was retrieved through water extractions from organic matter collected at site.*

2.3.5 Enzyme activities – phenol oxidase

Potential phenol oxidase (PO) activity at the constructed fen and reference sites is provided in Figure 2.10. At the constructed fen, there was a significant interaction between the vegetation and mulch treatment plots ($F_{2,9} = 4.80$, $p = 0.038$) where mulched plots had lower PO activity for the bare treatment, but higher PO activity under *Juncus* and *Carex*. Therefore, there was no significant difference across the vegetation ($F_{2,9} = 1.19$, $p = 0.35$) or mulch treatment plots ($F_{1,9} = 0.17$, $p = 0.69$). The highest PO activity was at the *Carex* plots (0.0937 µmol dicq/min) and the lowest was at the bare plots (0.0768 µmol dicq/min), with mulch treatment plots showing higher activity (0.0851 µmol dicq/min) as compared to no-mulch treatment plots (0.0794 µmol dicq/min). At the reference sites, PO activity did vary significantly across the fen types ($F_{2,12} = 9.74$, $p < 0.01$) but not across the microforms ($F_{1,12} = 0.013$, $p = 0.91$), and was highest at the rich fen (0.124 µmol dicq/min) and lowest at the poor fen (0.0285 µmol dicq/min). In general, the constructed fen had higher, but not significantly different, PO activity (0.0825 µmol dicq/min) as compared to the reference sites (0.0632 µmol dicq/min; $F_{1,31} = 1.43$, $p = 0.24$).

When tested against environmental variables, PO activity had a positive relationship with pH (Figure 2.14a) and a negative relationship with temperature (Figure 2.14b), both of which were only significant across the reference sites ($R^2 = 0.31$, $F_{1,16} = 8.54$, $p < 0.01$; $R^2 = 0.19$, $F_{1,16} = 4.97$, $p = 0.04$). There was no significant relationship between PO activity and phenolic compound concentration (Figure 2.15a), nor was there a significant relationship with *k'* (Figure 2.15b).

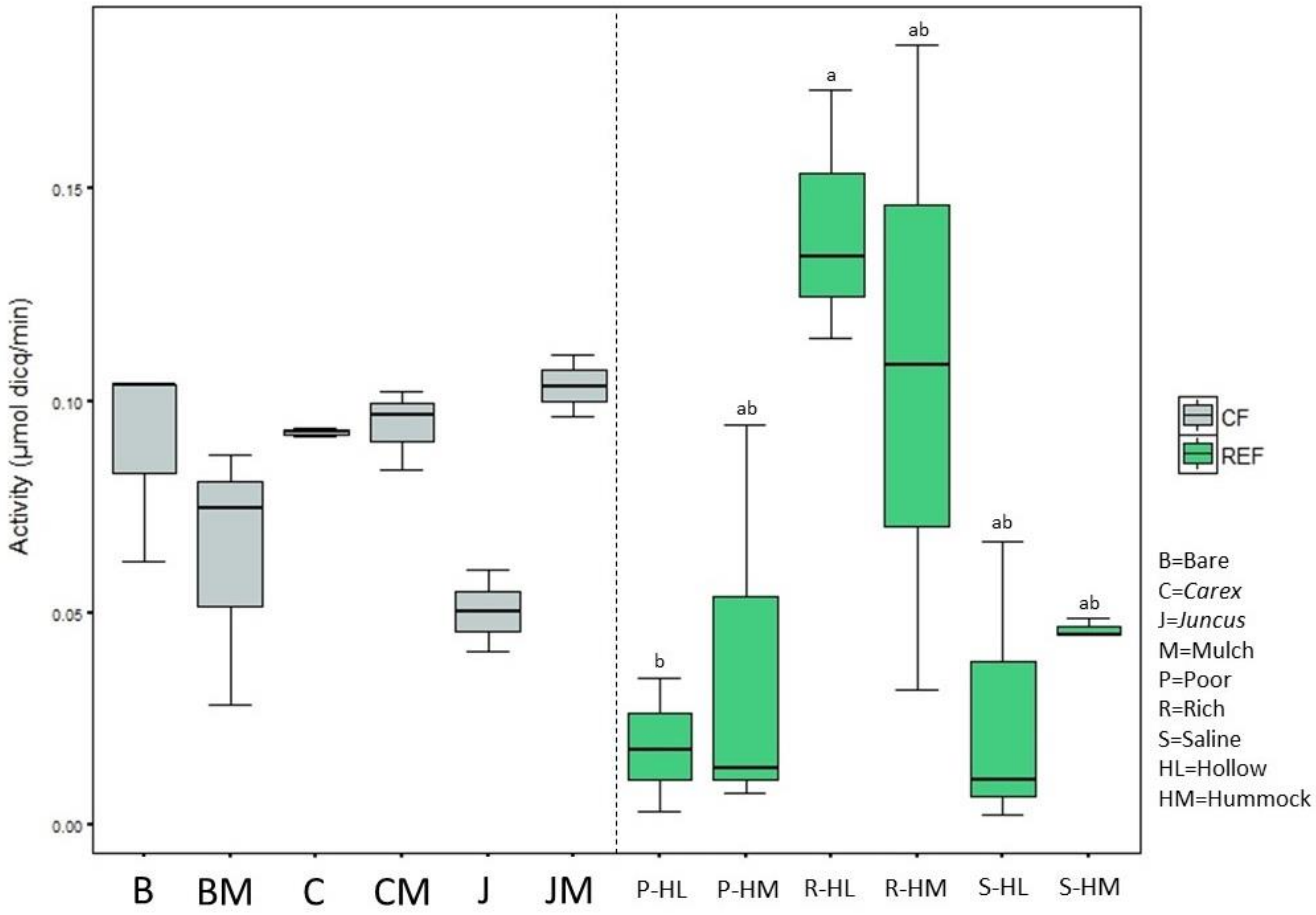


***Figure 2.12**: Phenol oxidase enzyme activity at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Activities were obtained from organic matter collected at a 15 cm depth from each location. Letters indicate significant differences between each location. Locations with the same letter(s) indicates no significant difference.*

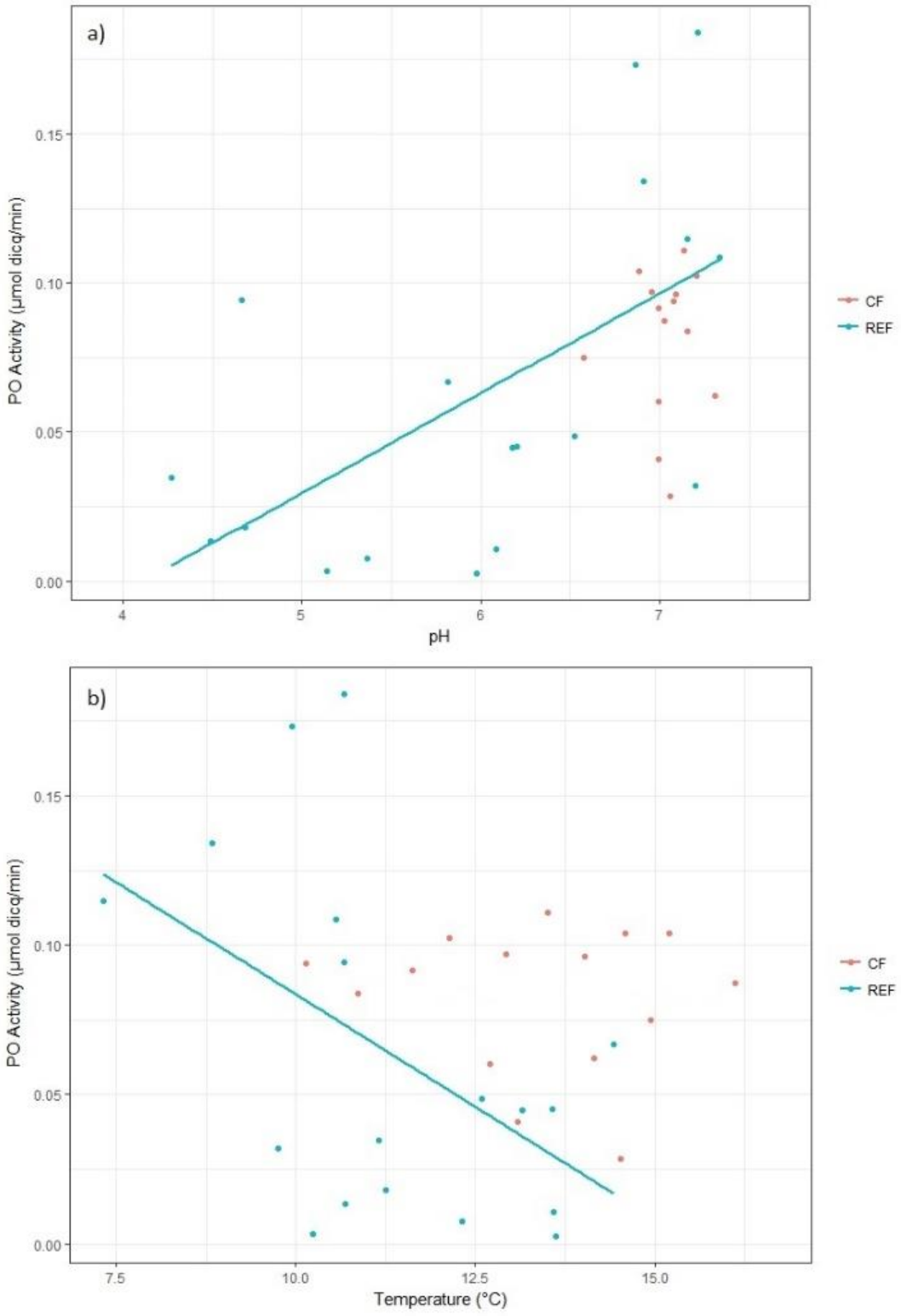


Figure 2.13: *Phenol oxidase activity vs average pH (a; R^2 = 0.31, $F_{1,16}$ = 8.54, p < 0.01) and average soil temperature (b; R^2 = 0.19, $F_{1,16}$ = 4.97, p = 0.04) at the constructed fen (CF) and reference sites (REF). Average pH was obtained from the 15 cm and 30 cm depths, while average soil temperature was obtained at 5 cm and 10 cm depths. Measurements were taken every 2-3 weeks during the growing season (May – September). Activities were analysed from organic matter obtained at 15 cm depth from each site.*

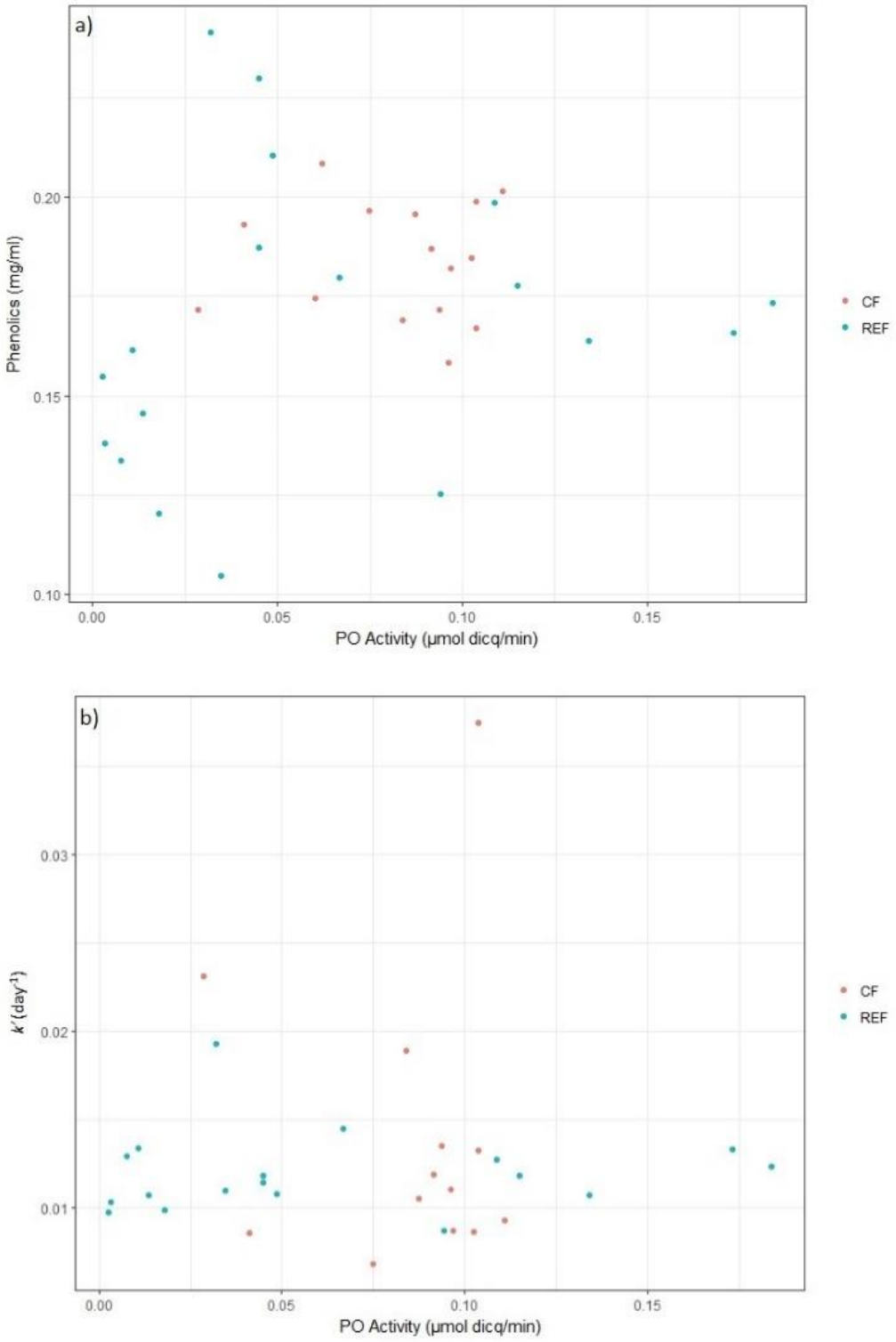


Figure 2.14: *Phenol oxidase activity vs phenolic compound concentration (a) and the estimated decomposition rate from the above-ground litter bags (k'; b) at the constructed fen (CF) and reference sites (REF). Phenolic compound concentration was obtained through water extractions of organic matter collected from the 15 cm depth at each location. Estimated decomposition rate from the litter bags included above-ground biomass of* Carex aquatilis *and* Juncus balticus. *Activities were assessed from the same organic matter collected at the same depth.*

2.3.6 Enzyme activity – hydrolase enzymes

Figure 2.13 shows the averages of each potential hydrolase enzyme activity at the constructed fen and reference sites. Figures of individual enzyme activities at both the constructed fen and reference sites along with relationships to environmental variables are provided in Appendix A. At the constructed fen, the sum of hydrolase enzyme activities did not vary significantly across the vegetation ($F_{2,9} = 0.18$, $p = 0.83$) or mulch treatments ($F_{1,9} = 1.18$, $p = 0.31$). The highest hydrolase enzyme activity at the constructed fen was phosphatase and the lowest was arylsulphatase. At the reference sites, hydrolase enzyme activity varied significantly across the fen types ($F_{2,12} = 11.54$, $p = 0.0016$) but not across microforms ($F_{1,12} = 2.26$, $p = 0.16$). Hydrolase enzyme activity was the highest at the poor fen (81500 µmol/g/min) and the lowest was at the saline fen (48300 µmol/g/min). As at the constructed fen, at the reference sites, the highest hydrolase enzyme activity was phosphatase and the lowest was arylsulphatase. On average, the reference sites showed significantly higher activity (304418 µmol/g/min) as compared to the constructed fen (63803 µmol/g/min; $F_{1,31} = 87.65$, $p < 0.001$).

All environmental variables were tested against hydrolase enzyme activity, with negative relationships being observed with pH (Figure 2.17a) at both the constructed fen ($R^2 = 0.38$, $F_{1,12} = 8.91$, $p < 0.05$) and reference sites ($R^2 = 0.39$, $F_{1,16} = 11.90$, $p < 0.01$), as well as a negative relationship with EC (Figure 2.17b) that was significant at the reference sites only ($R^2 = 0.041$, $F_{1,16} = 4.93$, $p = 0.041$). There was also a significant negative relationship between hydrolase enzyme activity and phenolic compound concentrations at the reference sites (Figure 2.18; $R^2 = 0.43$, $F_{1,16} = 13.98$, $p < 0.01$). There was no significant relationship between hydrolase enzyme activity and k_{TBI}, however there was a negative relationship with *k'* (Figure 2.18b) that was significant at the constructed fen ($R^2 = 0.39$, $F_{1,13} = 9.81$, $p < 0.01$), but not at the reference sites.

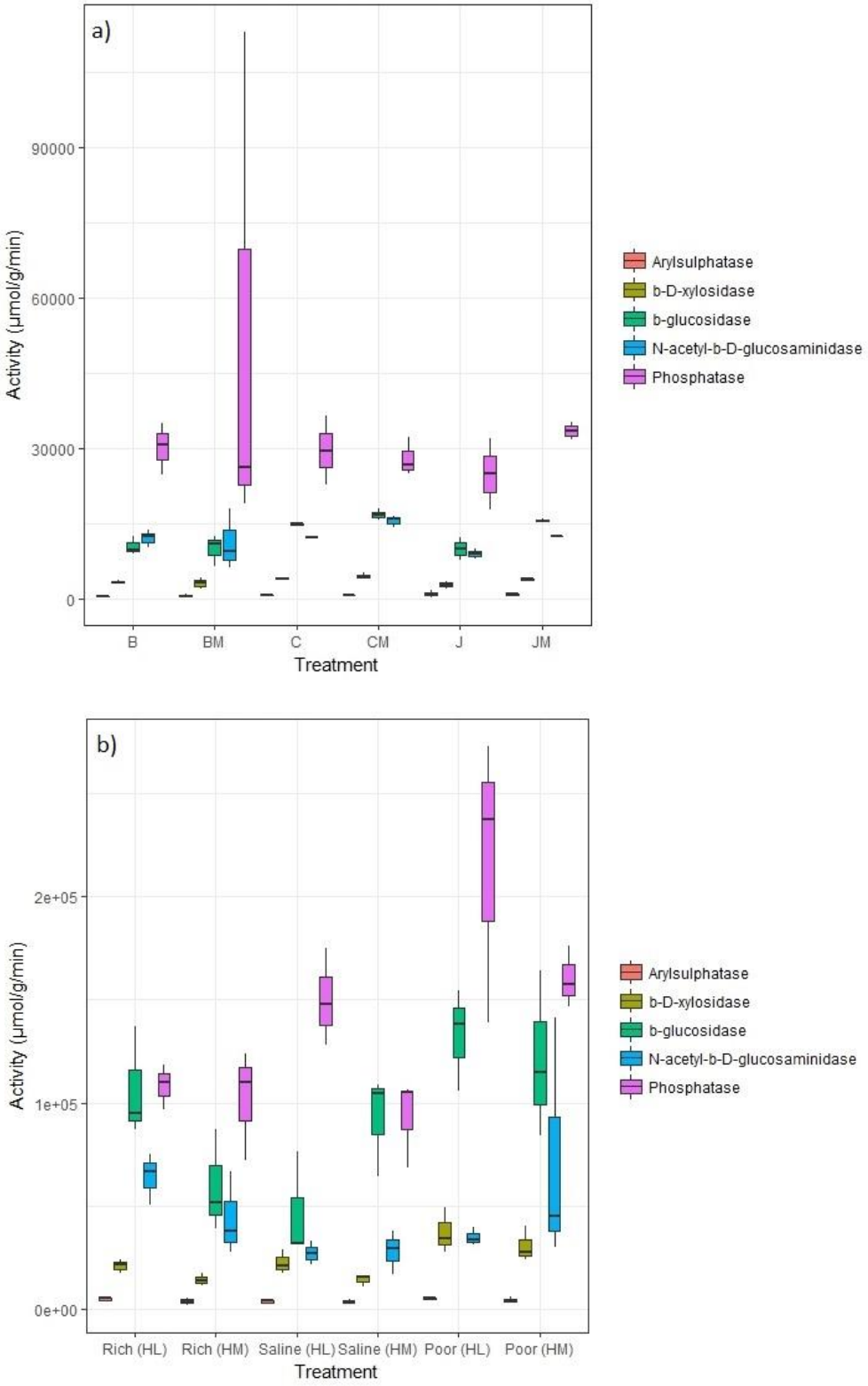


Figure 2.15: *Hydrolase enzyme activities at the constructed fen (a) and reference sites (b). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Activities were obtained from organic matter collected at a 15 cm depth from each location.*

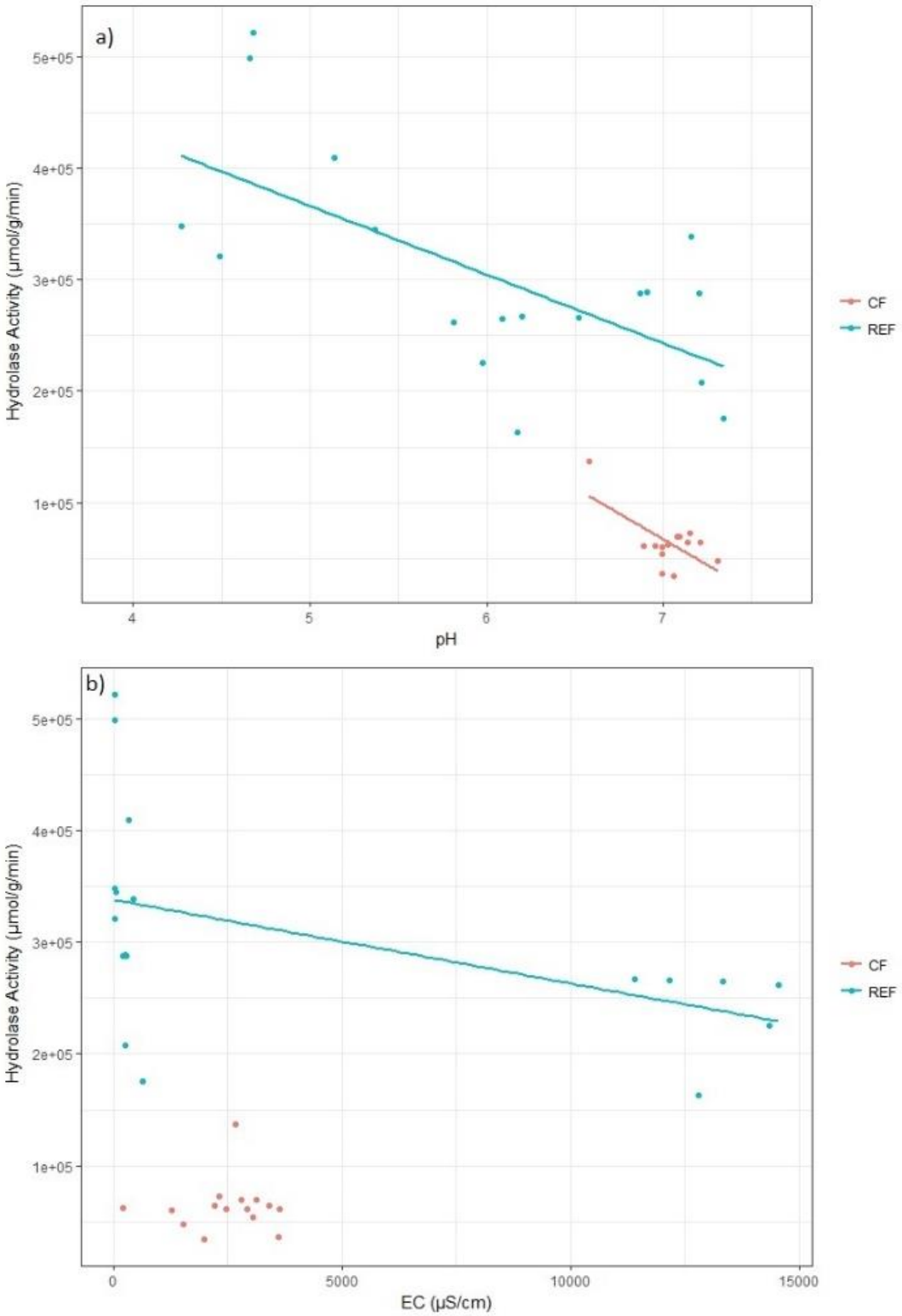


Figure 2.16: *Hydrolase enzyme activity (sum) vs average pH (a; R_2 = 0.38, $F_{1,12}$ = 8.91, $p < 0.05$; R_2 = 0.39, $F_{1,16}$ = 11.90, $p < 0.01$) and average EC (b; R_2 = 0.041, $F_{1,16}$ = 4.93, $p = 0.041$) at the constructed fen (CF) and reference sites (REF). Average pH and EC were obtained at 15 cm and 30 cm depths, with measurements being collected every 2-3 weeks during the growing season (May – September). Activities were obtained from organic matter collected from the 15 cm depth at each of the locations.*

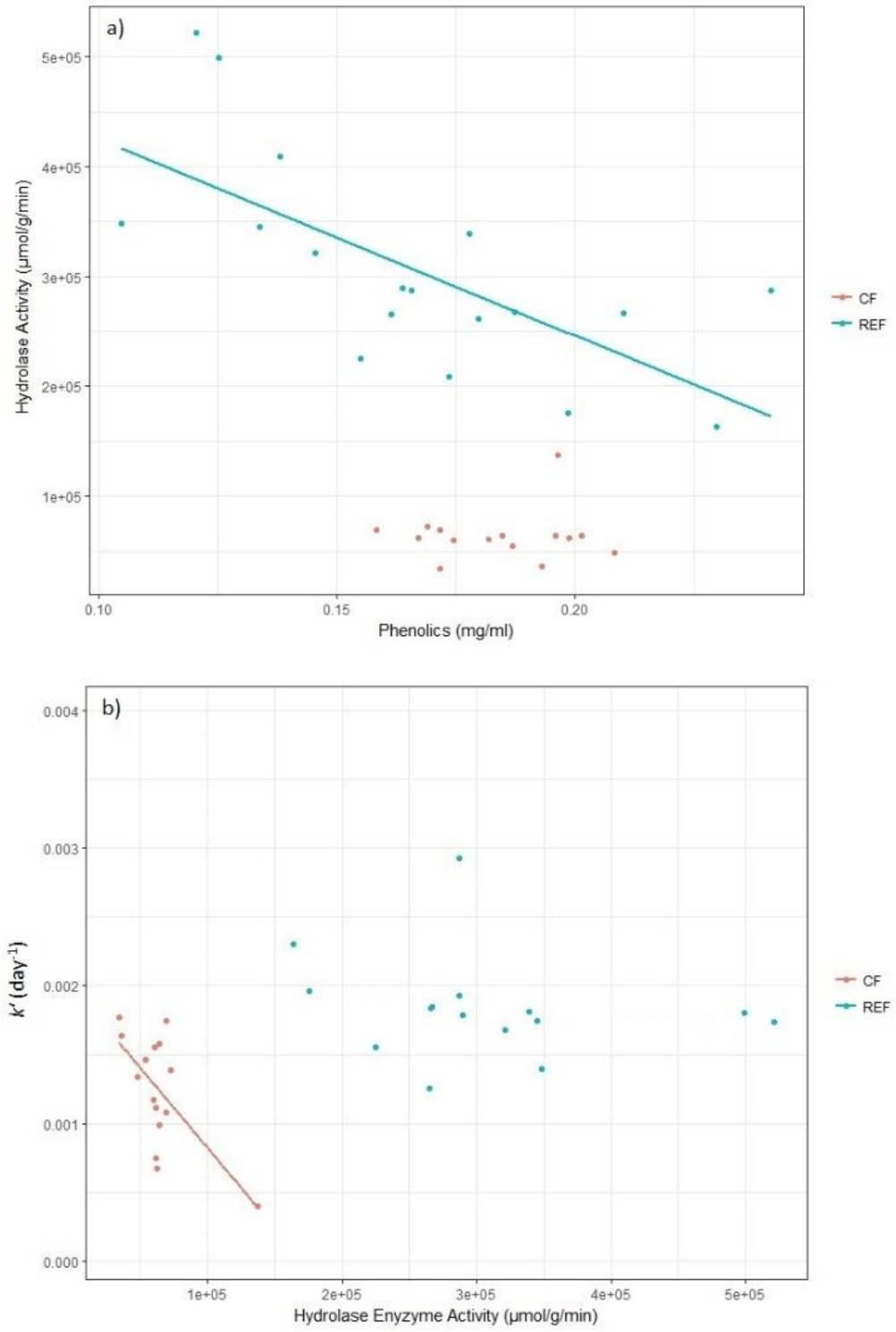


Figure 2.17: *Hydrolase enzyme activity (sum) vs phenolic compound concentration (a; $R^2 = 0.43$, $F_{1,16} = 13.98$, $p < 0.01$) and average estimated decomposition rate from the above-ground litter bags (k'; b; $R^2 = 0.39$, $F_{1,13} = 9.81$, $p < 0.01$) at the constructed fen (CF) and reference sites (REF). Phenolic compound concentration was obtained through water extractions of organic matter collected from the 15 cm depth at each location. Estimated decomposition rate from the litter bags included above-ground biomass of* Carex aquatilis *and* Juncus balticus. *Activities were assessed from the same organic matter collected at the same depth.*

2.4 Discussion

Critical to the preservation of the peatland carbon pool is the suppressed decomposition rate as a result of the wet, cool, and acidic conditions combined with recalcitrant substrate (Turcotte 2009; Carrasco et al. 2006; Basiliko et al. 2007). Re-establishing these conditions on a post-mined landscape has only recently been attempted (Ketcheson et al. 2016). In order to achieve more timely reclamation objectives, a better understanding of the dynamics of soil organic matter (SOM) decomposition under conditions present within constructed peatlands is required. In this study, decomposition rate was directly estimated at a recently constructed fen (< 5 years) and compared with estimates from several different undisturbed fen types to evaluate progress. Furthermore, various biogeochemical properties known to have key roles in SOM decomposition were assessed to form inferences on these processes and provide recommendations leading to enhanced peat accumulation and more timely reclamation.

Although our k_{TBI} produced similar rates to previous TBI studies in boreal peatlands (e.g., Touchette, 2017; MacDonald et al. 2017), as a result of rapid growth of roots at the constructed fen and frequent penetration into the tea bags that likely contributed to mass loss due to tearing, we did not use these decomposition rates in comparison to other site factors. This was further supported by the fact that k' and k_{TBI} were not well-correlated (Figure 2.7). Instead, our litter bag experiment maintained and produced the most reliable and representative yearly decomposition rates. Estimated decomposition rate using the above-ground biomass litter bags (k') at the constructed fen (0.0013 day^{-1}) was lower than the nearby rich fen (0.0023 day^{-1}), poor fen (0.0016 day^{-1}) and saline fen (0.0018 day^{-1}). These results are slightly higher than most studies of *Carex* spp. across a variety of peatlands (0.00047 – 0.002 day^{-1}; Bartsch & Moore, 1985; Verhoeven & Arts, 1992; Aerts & Caluwe, 1997; Graf & Rochefort, 2009); however, some previous studies do report higher rates (0.002 – 0.0061 day^{-1}; Danell & Sjöberg, 1975; Brinson et al. 1985). Our results from the reference fens were also in partial agreement with previous studies. Verhoeven and Arts (1992) also found decomposition rate highest in rich fens as compared to

poor fens. On the contrary, Szumigalski & Bayley (1996) found poor fens to have a higher decomposition rate as compared to wooded-rich fens, while Bartsch & Moore (1985) found no significant difference. *Sphagnum*-dominated peatlands can show even lower *k* values (0.00022 day^{-1}; Bragazza et al. 2009), with *Sphagnum* litter types showing the lowest *k* values ranging from 0.00 – 0.000027 day^{-1} (Filippova & Glagolev, 2018). The large variance in the rates obtained and literature values could be the result of (1) depth of burial or spatial differences within sites (Barreto et al. 2018), (2) differences in mesh size leading to exclusion of certain faunal groups (Brinson et al. 1981; Bradford et al. 2002), (3) temporal differences in incubation time (Filippova & Glagolev, 2018), (4) drying temperature of the plant litter (Clymo, 1965), (5) the degree of plant litter senescence prior to burial (Ohlson, 1987), and/or (6) regional differences in climate and hydrology (Clymo, 1965).

2.4.1 Environmental controls on decomposition

At the constructed fen, VWC was higher under plots without the mulch treatment (85%) as compared to plots with mulch (78%; Table 2.1). This finding contradicts the intent behind mulch treatment during reclamation as it is intended to prevent water loss via evaporation (Price et al. 1998; Cobbaert et al. 2004). Nevertheless, wetter sites have been associated with lower decomposition rates (Szumigalski & Bayley, 1996), and water-saturated conditions have been proposed as one of the main drivers of suppressed decomposition in northern boreal peatlands (Moore & Dalva, 1993; Laiho, 2006; Bonnett et al. 2017). A lower water table expands the oxic zone, raising decomposition rate. However, it has also been found that moisture deficiency within the surface layer of a peatland may impede decomposition (Laiho, 2004). Furthermore, Berg et al. (1975) found that decomposition of cellulose strips was higher when the pieces were wet. From the above-ground litter bags, higher moisture content was associated with lower decomposition at all the reference sites, but only at the *Carex* plots at the constructed fen. These findings, as well as similar rates between treatment plots or microforms despite the difference in VWC, particularly at the reference sites, suggest that moisture content was not

the main driver of decomposition in our study. Although similar patterns exist between decomposition rates and VWC across the hummock/hollow pairs at the reference sites, Barreto & Lindo (2018) showed no significant differences in decomposition rate due to moisture differences across these microforms.

Another component of slow decomposition in northern peatlands is the cool temperatures found in the northern boreal climate (Carrasco et al., 2006). At the reference sites, with the exception of the saline fen, hummocks had higher soil temperature as compared to hollows (+1.0°C; Table 2.1) and also higher *k*'. These results are in agreement with previous studies that show increased rates of vascular plant decomposition with higher soil temperature (Bragazza et al. 2009; Barreto & Lindo, 2018). However, low soil temperature did not correspond to low decomposition, as the rich fen was coldest, yet had the highest decomposition rate (Table 2.1; Figure 2.3). Additionally, despite showing the highest average soil temperature, bare plots had the lowest estimated decomposition rate from the litter bags at the constructed fen (Appendix A, Figure 2.16). This would support the findings of Laiho et al. (2004) that higher temperatures could also enhance moisture stress, resulting in lower decomposition rate. However, we assumed bare plots did not experience moisture stress as they occupied some of the highest VWC plots (81%) at the constructed site, and supported similar decomposition rates as the other treatment plots. Therefore, we conclude that, although deemed important (Aerts & Caluwe, 1997; Basiliko et al. 2007), climatic responses (i.e., moisture and temperature) are not driving variation in decomposition rates in our study.

Chemical conditions, such as salinity and pH, have been shown to have a profound effect on the microbial communities responsible for the decomposition of organic matter (Brouns et al. 2014a; Bonnett et al. 2017). At the constructed fen, EC values (2497 µS/cm) were higher than the rich fen (283 µS/cm) and poor fen (86 µS/cm), but lower than at the saline fen (12720 µS/cm; Table 2.1); yet, decomposition rate observed was relatively similar to the reference sites (Figure 2.3). Although limited research exists on the effect of salinity on decomposition rates in boreal peatlands, several other studies

on wetlands and coastal peatlands have shown varying effects of salinity under oxic and anoxic conditions (Mendelssohn et al. 1999; Rejmánková, 2007; Chambers et al. 2011; Brouns et al. 2014a;). Under oxic conditions, Brouns et al. (2014a) observed a reduction in decomposition of about 50% following salinization during an incubation experiment. Others have also reported a negative correlation between salinity and the activities of SOM-degrading enzymes (Rejmankova, 2007), potentially due to osmotic stress (Brouns et al. 2014a). However, under anoxic conditions, sulfate salts could potentially stimulate decomposition (Brouns et al. 2014a), acting as an alternative electron acceptor. Therefore, evidence suggests that decomposition could be higher or lower under saline conditions (Chambers et al. 2011), or exhibit no direct relationship at all (Mendelssohn et al. 1999). On the contrary, there is a general consensus that increasing pH results in an increase in fungal and bacterial activity (Ivarson, 1977; Bonnet et al. 2006), and is accompanied by an increase in decomposition rate (Gorham et al., 1987). This is supported by the significant relationship between *k'* and pH at the reference sites (Figure 2.4a). At the constructed fen, however, no significant relationship between pH and *k'* among vegetation and mulch treatment plots existed. Furthermore, trends in *k'* (Figure 2.3) were not reflected by the trends in pH (Table 2.1) at the reference sites, therefore we conclude pH is not the main driver of between site differences in this study.

2.4.2 Vegetation controls on decomposition

Despite the importance of favourable environmental controls on decomposition there is an overwhelming agreement that vegetation input and substrate composition may ultimately dictate this process (Bartsch & Moore, 1985; Szumigalski & Bayley, 1996; Thormann et al., 2003; Laiho, 2007). In general, *Carex* biomass decomposed faster than *Juncus*, and above-ground biomass decomposed faster than below-ground biomass (Figure 2.1). Both of these findings are in line with previous research (Rosswall et al. 1975; Hartmann, 1999; Scheffer & Aerts, 2000; Graf & Rochefort, 2009). Thormann et al. (2004) measured total carbon (TC) and total nitrogen (TN) concentrations in leaves and rhizomes of *C.*

aquatilis and found that the leaves had a lower TC:TN quotient, potentially resulting in a favourable supply of nitrogen (N), which is often a limiting nutrient in peatlands (Thormann & Bayley, 1997). Furthermore, *C. aquatilis* leaves have a high concentration of cellulose (180-240 mg/g; Thormann et al. 2004), which comprises about 70% of plant tissue and is readily degraded, potentially even under anoxic conditions (Mendelssohn et al. 1999; Agethen and Knorr, 2018).

Our results also support previous findings that *Carex* material decompose relatively fast in relation to other species, particularly mosses (Laiho, 2007; Hall & Hopkins, 2015). Although we did not investigate decomposition rates under plots with moss alone (this did not exist at the constructed site), our results are in line with other studies of sedge species (e.g., Bartsch & Moore, 1985; Aerts and de Caluwe, 1997; Keuhn et al. 2000; Kuehn & Suberkropp, 1998). Our *k'* from *Carex* biomass (0.0012 day^{-1}) across the constructed fen was similar to several previous studies, which have shown the relative lability of *C. aquatilis* decomposition in peatlands (e.g., Aerts and de Caluwe, 1997; Thormann and Bayley, 1997; Thormann et al. 2001, Thormann et al. 2004). Limited research has been conducted on *J. balticus* and few studies exist on decomposition dynamics of *Juncus* spp. Kuehn et al. (2000) found the decomposition rate of *J. effusus* leaves at a wetland in Alabama to be approximated 0.00099 day^{-1}. Similarly, Kuehn & Suberkropp (1998) found a slightly faster rate of the same species at 0.0011 day^{-1}. These rates are slightly higher than our *k'* from *Juncus* biomass across the constructed fen (0.00092 day^{-1}), which could be attributed to the climatic differences. Nevertheless, our results suggest slower decomposition of *Juncus* as compared to *Carex*.

The plant community growing at the site may also affect litter decomposition by altering below-ground processes (Basiliko et al. 2012; Wang et al. 2014; Keiluweit et al. 2015). At the constructed fen, *Carex* plots showed a higher above-ground *k'* (0.0015 day^{-1}) as compared to the *Juncus* (0.0014 day^{-1}) and bare plots (0.000933 day^{-1}). This suggests potential inputs from the overlying plant community leading to varying substrate and nutrient availability and subsequent microbial activity. One of the initial

stages of decomposition is plant leachate of water-soluble compounds that can that can provide microorganisms with an easily accessible energy source (Wang et al. 2014). Additionally, active root exudation of organic compounds have been found to strongly stimulate microbial activity (Bradford et al. 2017; Brummell et al. 2017) and could potentially lead to enhanced decomposition (i.e., priming; Basiliko et al. 2012). Furthermore, the microbial community composition is preferentially established based on overlying inputs and substrate composition (Berg et al. 1975). Our results partially support this as we found the below-ground biomass of *Juncus* decomposed faster under *Juncus* plots (0.00081 day^{-1}) as compared to *Carex* (0.00069 day^{-1}) or bare plots (0.00056 day^{-1}). However, this finding was restricted to below-ground *Juncus* biomass and was not observed for any of the above-ground biomass.

At the reference sites, decomposition rate was slightly higher at hummocks (0.0061 day^{-1}) as compared to hollows (0.0058 day^{-1}; Figure 2.3). This is in partial agreement with previous studies (Farrish & Grigal, 1985; Johnson & Damman, 1991; Belyea, 1996). Hummocks consist of built up peat with less decomposable species that establish in drier areas (Belyea, 1996). As a result, hummocks persist well above the water table therefore having an expanded oxic zone and greater temperature variation (Barreto & Lindo, 2018), potentially leading to the higher decomposition rate observed.

2.4.3 Biogeochemical controls on decomposition

In addition to the vegetation treatments at the constructed fen, we also looked at the effect of mulching treatments using a wood-strand mulch. The purpose of this material was to aid in moss re-establishment and prevent water loss (Price et al. 1998). We hypothesized that water-soluble phenolics (e.g., lignin) could be leached from the material and inhibit SOM-degrading enzymes (i.e., hydrolase enzymes), resulting in a reduced decomposition rate in the underlying peat. Phenolic compound concentrations were similar at mulch treatment plots (0.187 mg/ml) and no-mulch plots (0.183 mg/ml), and both were lower than concentrations observed under *Juncus* plots (0.190 mg/ml), suggesting the mulch treatment did not lead to higher phenolic compound concentrations in the underlying substrate.

More importantly, however, our results show that higher phenolics did not lead to reduced decomposition rate at the constructed fen, nor the reference sites (Figure 2.9b). In fact, at the reference sites, there was a slight positive correlation between decomposition rate and phenolic compound concentrations.

Several studies have shown that phenolic compounds, and in particular, low molecular weight (LMW) phenolics, can readily be degraded and assimilated as a C source (Müller et al. 1988; Bernhard-Reversat, 1999; Fierer et al. 2001; Thormann et al. 2003). It is surprising this trend was absent from the constructed fen, considering phenolics concentrations were similar to if not higher than concentrations observed at the reference sites (Figure 2.8a). Given the infancy of the constructed fen and the lability of the vegetation inputs (Thormann & Bayley, 1997), we hypothesize that a greater portion of the concentration of phenolics could consist of easily degradable, LMW phenolics (Bernhard-Reversat, 1999; Bonnett et al. 2017; Meier & Bowman, 2008). However, we only tested for total phenolics and cannot distinguish between high and low molecular weight compounds, therefore cannot confirm this hypothesis.

At the reference sites, it is possible that over time the microbial community has been able to adapt to the utilization of phenolics under low nutrient availability. Use of LMW phenolics as substrate by microbes could enhance microbial biomass, resulting in the observed positive relationship between phenolics and decomposition rate (Figure 2.9b). A potential reason for no significant correlation of phenolics and *k'* at the constructed fen could be due to the lack of an established microbial community. Fungi and bacteria account for approximately 95% of the decomposer biomass (Chapin et al. 2002) and are instrumental in the breakdown of recalcitrant material (Belyea, 1996; Thormann et al. 2004; Brant et al. 2006; Carney et al. 2007). Fungi play a more prominent role than bacterial in SOM decay, especially pertaining to the breakdown of phenolics (Brant et al. 2006). It has also been suggested that there exists a successional pattern, particularly of fungal species, that requires time to establish before the most

decay-resistant polymers can be broken down (Knöbel-Knubner, 2002; Thormann et al. 2003; Thormann et al. 2004). If this was the case, we could expect to see rates of decomposition of *Carex* under *Carex* plots, or *Juncus* under *Juncus* plots, be substantially higher than the other species due to an already established microbial community best suited for the breakdown of the overlying material (Berg et al. 1975). However, we observed similar trends in decomposition of *Carex* and *Juncus* material between the *Carex* and *Juncus* plots (Figure 2.2), which would support our hypothesis that more time is needed for the successional development of fungal assemblages across the constructed fen. The low rates of hydrolase enzyme activities observed at the constructed fen compared to the reference fens also supports the hypothesis that the microbial community development is limiting decomposition rates.

Fungi are integral to organic matter decomposition because they are part of a small group of microorganisms that can secrete oxidative enzymes responsible for the majority of the breakdown of more complex phenols in peatlands (Brant et al. 2006; Sun et al. 2010; Romanowicz et al. 2015). The two classes of these enzymes include peroxidases and phenol oxidases (PO), with the latter being of particular importance in peatlands due to the oxygen limitation and high phenolic content (Criquet et al. 2000; Sinsabaugh, 2010). Phenol oxidases use oxygen as an electron acceptor to catalyze the oxidation of phenolic compounds, reducing them to simple polymers and making them available for microbial uptake (Sinsabaugh et al. 2008; Sinsabaugh, 2010). In our study, phenol oxidase was not significantly related to phenolic compound concentration (Figure 2.12a), nor we observe a relationship with decomposition rate (Figure 2.12b). We did, however, see a positive correlation between pH and phenol oxidase activity at the reference sites (Figure 2.11a), which has been frequently reported (Williams et al. 2000; Sinsabaugh et al. 2008; Sun et al. 2010; Sinsabaugh, 2010). Phenol oxidase has an optimal pH of 8 – 10 (Pind et al. 1994), which is higher than the constructed fen and much higher than the reference sites (Figure 18), suggesting pH could be limiting PO in our study, but is not a determining factor particularly at the constructed fen. Additionally, a negative correlation between phenol oxidase activity

and temperature was also observed at the reference sites (Figure 2.11b). Some studies have shown extracellular enzyme activity being positively correlated to temperature (Laiho, 2007; Romanowicz, 2015), while others have shown oxidases unresponsive to warming treatments (Henry, 2012). Higher temperatures result in greater enzyme efficiency (Bell et al. 2010) as well as lower microbial efficiency (Frey et al. 2013) that lead to reduced production of extracellular enzymes due to metabolic costs (Allison, 2005). Although soil temperature on average was higher at the constructed fen, it was far below the temperature optimum for enzyme synthesis (20°C – 35°C; Thormann et al. 2004), and therefore the lack of significant relationship could be a result of differences in microbial community composition or temporal variations in PO activity (Henry, 2012). Nevertheless, our findings do not support the enzymic latch theory (Freeman et al. 2001) and suggests a minimal role of PO on decomposition rate at these sites.

Hydrolase enzymes are also important for organic matter decomposition. This suite of extracellular enzymes plays a critical role in nutrient supply, leading to the degradation of SOM by the surrounding microbial community (Allison, 2005). In our study there was a negative correlation between hydrolase enzyme activity and the concentration of phenolics at the reference sites (Figure 2.15a). This is in support of previous studies showing the inhibitory effect of phenolics on hydrolase enzyme activity (Freeman et al. 2001; Limpens et al. 2008; Fenner & Freeman, 2011). Interestingly, there was no significant relationship between decomposition rate and hydrolase enzyme activity at the reference sites; however, there was a negative relationship between hydrolase enzyme activity and decomposition rate at the constructed fen, despite there being no significant relationship between phenolics and hydrolase enzyme activity at that site (Figure 2.15a). This suggests that there is an interaction between hydrolase enzyme activity and the decomposition rate, but it is unlikely related to the accumulation of phenolics as per the enzymatic latch theory.

Enzyme production itself requires nutrient investment, particularly of C and N (Schimel & Weintraub, 2003), and under conditions of severe nutrient limitation, microbes may not utilize extracellular enzymes as a strategy if production exceeds retention (Mooshammer et al. 2014). Thus, increased hydrolase enzyme activity results in increased nutrient supply for SOM degradation, leading to additional enzyme synthesis (Freeman et al. 2012). However, if sufficient nutrient supply and accessibility provided by the surrounding substrate already exists, potentially as a result of belowground inputs or leaching from the overlying material (Brinson et al. 1981; Basiliko et al. 2012), then utilization of these extracellular enzymes may not be required. Therefore, a shift to the production and utilization of hydrolase enzymes indicates a lack of nutrient availability (Sinsabaugh et al. 2008), which could explain the inverse relationship between hydrolase enzyme activities and decomposition rate observed in our study at the constructed fen.

Looking at the individual hydrolase enzyme activities (Figure 2.13), phosphatase activity is substantially higher than the other hydrolase enzymes, particularly at the constructed fen. Phosphatase is responsible for releasing phosphate ions through the hydrolyzation of phosphoric acid monoesters (Dunn et al. 2016), which suggests phosphorus (P) may be limiting at this site. Several studies have shown peatlands to be P-limited (e.g. Sinsabaugh et al. 1993; Aerts et al. 2001; Güsewell & Gessner, 2009). Although we did not analyze nutrient concentrations in our study, we can assume sufficient C and N availability since relative phosphatase activity is high (P is not required for enzyme synthesis; Mooshammer et al. 2014) and C and N acquiring enzyme activities (i.e., β-glucosidase and N-acetyl-β-D-glucosaminidase, respectively) are relatively low. This may be due to the input of labile organic material (i.e., *Carex* and *Juncus* plots), which has been found to harness a fast-growing microbial community with substantial P demand (Güsewell & Gessner, 2009). With the input of labile substrates, N becomes more readily available which occasionally leads to priming effects and enhanced SOM decomposition (Blagodatskaya & Kuzyakov, 2008). At the constructed fen, however, lower rates of decomposition were

observed which fails to support any priming effects. Mooshammer et al. (2014) suggests that at P-limited sites, labile material may be utilized for the mobilization of P rather than for SOM decomposition, possibly explaining the slightly lower rates of decomposition at the constructed fen compared to the reference sites.

At the reference sites, hydrolase enzyme activity was substantially higher than at the constructed fen (Figure 2.13). This is to be expected given the age and recalcitrant nature of the surrounding substrate. Additionally, extracellular enzymes have also been known to be adsorbed or immobilized by clay or particles of organic matter (Mooshammer et al. 2014; Bonnett et al. 2017), which could potentially be more prominent at the constructed fen. When compared to *k'*, we found no relationship to hydrolase enzyme activity. This is likely due to vegetation differences used in our litter bag experiment and the surrounding plant community, leading to an overestimation of *k'*. *Carex* and *Juncus* are highly labile substrates that were buried amongst relatively recalcitrant material, which likely induced priming effects (Mooshammer et al. 2014) and supplied the microbial community with an easily accessible energy source. Since the established microbial community has adapted to low nutrient availability (specifically, N) commonly associated with *Sphagnum* species (Aerts et al. 2001), this labile material would be preferentially decomposed, leading to this overestimation. The rates observed at these sites would therefore be subject primarily to environmental conditions, which is apparent as the majority of these relationships observed have only been witnessed at the reference sites.

2.5 Conclusion

In this study, estimated decomposition rate, phenolic compound concentrations, potential extracellular enzyme activities, and environmental conditions were measured at a constructed fen and compared to rich fen, poor fen, and saline fen reference sites following fen construction in the AOSR. The findings of this study suggest that varying vegetation and mulching treatments at the constructed fen have not led to appreciable differences in decomposition, and overall the site does not reflect the

biogeochemical conditions observed at the undisturbed natural fens. Additionally, the enzymatic latch theory was not supported by our findings as potential extracellular enzyme activities and their interactions with phenolic compounds did not lead to suppressed decomposition as the theory states. The controls on decomposition dynamics are not limited to a select few variables; rather, decomposition is site-specific and requires an extensive understanding of the complex interactions between the environmental conditions and biogeochemical processes. Furthermore, appropriate field methods and a wider range of parameters (e.g., nutrient status, phenolic compound chemistry and representative litter) are needed to effectively assess decomposition and possible limitations of enhanced peat accumulation and thus more timely reclamation. Ultimately more time is required for ecosystem functions to establish at the constructed fen, and ongoing assessment of the aforementioned variables is needed to identify and recommend industry practices that will lead to the slowest decomposition, and greatest peat accumulation rates.

Chapter 3: Microbial decomposition and the 'Priming Effect': Carbon dioxide production from decomposition of substrates relevant to fen construction in the Athabasca oil sands

3.1 Introduction:

Peatlands account for an estimated 1/3 of global terrestrial soil carbon due to their peat accumulation and slow decomposition rate (Fenner et al. 2005). However, concerns regarding future carbon storage under a changing climate have risen due to an incomplete understanding of peat accumulation/decomposition dynamics (Orwin et al. 2006; Bonnett et al. 2017). Shifts in temperature and moisture regimes could alter species composition (Dieleman et al. 2015) and nutrient availability leading to abrupt changes in decomposition rates (Bonnett et al. 2017). Microorganisms are responsible for soil organic matter (SOM) decomposition resulting in nutrient release for continued plant and microbial uptake (Turcotte, 2009). The degree of microbial activity is subject to environmental conditions, but is ultimately determined by substrate composition. Contrasting results from previous SOM decomposition studies (Orwin et al. 2006) indicate a need to further investigate how changes in plant species composition may alter the carbon accumulating function so critical to peatlands.

Soil organic matter decomposition processes are highly complex and remain poorly understood. Microorganisms in oxic and anoxic environments preferentially secrete extracellular enzymes to breakdown substrates and release nutrients for growth and reproduction. Highly labile material provides an easily accessible energy source (Turcotte, 2009) that may also aid in the breakdown of much older, recalcitrant material (i.e., 'priming'; Hamer & Marschner, 2002). However, an accumulation of enzyme-inhibiting material (e.g., phenolic compounds) may prevent microbes from accessing nutrients (Meier & Bowman, 2008), resulting in the long-term protection of the carbon pool.

Short- and long-term changes, such as land-use change or a changing climate, can induce large shifts in environmental conditions and plant communities (Weltzin et al. 2000), leading to changes in

peat accumulation rate. One such land-use change is resource extraction, including oil sands mining, where whole landscapes are disturbed. In order to return the post-mining landscape to equivalent land capability, oil sands companies have recently attempted fen reconstruction. During fen reclamation within a post-mined landscape, a variety of vegetation and surficial techniques are used to recreate conditions representative of regional peatland ecosystems. A common reclamation technique is to apply straw or wood-strand mulch to the site to reduce evapotranspiration losses and leave the site wetter (Scarlett et al. 2017). Planting or seeding the site is used to initiate native sedge species, while the moss-layer transfer technique is used to reintroduce moss species (Graf & Rochefort, 2009). These organic additions could potentially "prime" the protected organic pool (i.e., peat used in the fen construction process), and/or release additional enzyme-inhibiting phenolic compounds.

While many studies have been conducted on the decomposition of various organic substrates under a variety of conditions, contrasting results show the need to independently investigate processes specific to peatland reclamation. In this study, the objective was to investigate decomposition dynamics of peat in combination with substrates representative of the various mulching and vegetation treatments used in fen construction through a two-week laboratory incubation period. The hypothesis was that treatments containing more labile organic substrates with lower amounts of phenolic compounds (i.e., lignin) would prime the microbial community leading to higher microbial respiration rates and thus induced decomposition.

3.2 Methods:

3.2.1 Experimental Design

To assess aerobic and anaerobic decomposition of various fen reclamation treatments, combinations of peat, straw, wood-strand mulch, *Carex aquatilis* (*Carex*), and *Juncus balticus* (*Juncus*) were incubated for 14 days. *Carex* and *Juncus* were selected due to their utilization at the reconstructed Nikanotee Fen located on Suncor Energy Inc.'s Oil Sands Base approximately 40 km north of Fort

McMurray, Alberta. The incubation was split into single, double and triple treatments of each substrate, with double and triple treatments containing peat with straw or wood-strand mulch and/or *Carex* or *Juncus. Carex, Juncus*, and wood-strand mulch was collected from the Nikanotee Fen, while the wheat straw was harvested near Peace River, Alberta in 2011. As fens are common throughout the boreal landscape, the peat was collected from an undisturbed rich-treed fen (Elmes et al. 2018) and is considered the type of peat that would be used during fen reconstruction (Nwaishi et al. 2015). Each substrate, excluding the peat, was dried in an oven for 60°C for 48 hours to prevent premature decomposition and cut into pieces < 1 cm in length. Peat was frozen and homogenized by hand prior to the incubation. Triplicates of each treatment combination were used for a total of 36 replicates per incubation (aerobic and anaerobic incubations were run separately). Jars (60 ml) with a silicon-seal lid were flushed with ambient air (aerobic) or nitrogen (anaerobic) prior to the experiment. Approximately 2.5 g of total carbon (dry weight biomass) was used in each jar. Gravimetric water content of each amendment was calculated by dividing the mass of the wet weight by the mass of the dry weight, then multiplied by 5 g to determine the amount of amendment in each single treatment jar (assumed 50% of the dry weight as carbon). For double and triple treatments, the 2.5 g of carbon was divided evenly amongst the number of amendments and again added based on gravimetric water content. Distilled water was added to achieve equivalent moisture content in each jar (87.3%) and jars were stored in the dark at approximately 23°C.

During the aerobic incubation period jars were left open and sampling occurred on day 1, day 3, day 6, day 9, day 12, and day 14. Jars were sealed for one hour prior to sampling, followed by sampling 20 ml from the headspace four times, every 5 minutes using a needle and syringe. Immediately upon sampling, 20 ml of nitrogen was backfilled in each jar. During the anaerobic incubation, jars remained sealed and only one sample was obtained on each sampling date, occurring on day 0, day 1, day 3, day 6, day 7, day 8, and day 13. After seven days of the incubation jars were opened to flush the system with

nitrogen. Samples were run on a gas chromatograph (GC; Shimadzu GC2014, Mandel Scientific, Canada) with a thermal conductivity detector to determine carbon dioxide (CO_2) concentration. For both aerobic and anaerobic incubations, the rate of respiration was determined by the linear change in concentration during the time the jars were sealed, and concentrations were corrected for volume of headspace, temperature and nitrogen dilution.

Rates of priming were obtained by comparing observed rates of respiration to expected rates calculated by the means of respiration rates for individual substrates (Equation 5). Rates resulting in a positive value indicate positive priming, while negative values reflect negative priming.

$$\boldsymbol{Priming} = \bar{\boldsymbol{y}} - \left(\frac{\boldsymbol{\Sigma x}}{\boldsymbol{nx}}\right) \qquad [5]$$

where, $\bar{y}$ is the observed respiration rate from the double and triple treatments and the expected rate of respiration is the sum of the respiration rates from the single substrates (x) divided by the number of substrates (nx).

3.2.2 $\delta^{13}C$ Signatures

To identify the source of respiration, samples of the treatment substrates were submitted to the Environmental Isotope Lab at the University of Waterloo for $\delta^{13}C$ analysis following the principles outlined in Fry at al. (1992). Each substrate was ground to a fine powder using a ball and mill grinder, and approximately 0.7 mg of each were submitted for analysis. Isotopic ratio measurements were determined through the combustion conversion of sample material to gas through a 4010 Elemental Analyzer (Costech Instruments) coupled to a Delta Plus XL (Thermo) continuous flow isotope ratio mass spectrometer (CFIRMS). Results are reported in per mil (‰) units, against the primary reference scale of Vienna Pee Dee Belemnite (VPDB). To obtain the $\delta^{13}C$ of the headspace from each jar, the last sampling event from both incubations were submitted for isotopic analysis. Air samples were analyzed using

Gilson 222XL microgas auto-sampler coupled with Isoprime mass spectrometer (Isoprime LTD, UK). Means of substrate $\delta^{13}C$ values of substrates were used for double and triple treatments to compare to respired $\delta^{13}CO_2$. Isotopic discrimination (ϵ) was calculated using Equation 6:

$$\boldsymbol{\epsilon = 1000 \times \left(1 - \frac{1}{\alpha}\right)} \quad [6]$$

where, α is the fractionation factor, calculated as:

Equation 7: $$\boldsymbol{\alpha = {Rsom}/{RCO2}} \quad [7]$$

where, *Rsom* is the $\delta^{13}C$ of the organic matter and *RCO2* is the $\delta^{13}C$ of the respired CO_2.

3.2.3 Phenolic Compounds

Prior to analyzing for water soluble phenolic compound concentrations, water extraction from each treatment was performed by adding ultra pure water to 1 g of material at a 5:1 ratio. For double and triple treatments, 0.5 g of each substrate type was collected from the double treatments, and 0.33 g of each substrate was collected from the triple treatments. The mixtures of the substrate and ultra pure water were placed into 60 ml centrifuge tubes. The tubes were then placed on a rotator for 48 hours and centrifuged at 5000 rpm for one hour and the supernatant was used as the water sample to be analyzed. Phenolic compound concentration was determined following a modified version of the Box (1983) method. Three separate phenol concentration standards were created using 1000 ppm phenol stock solution, ranging from 0.25 ppm to 30 ppm, and were selected based on water sample color (i.e., clear, light brown, or dark brown). Once selected, 1 ml of phenol standard was put into a 3 ml centrifuge vial using a pipette. Approximately 1 ml of the supernatant was filtered using a 0.45 µm syringe filter, then filtered again using a 0.2 µm syringe filter, and placed into a separate 3 ml centrifuge vial. Next, 50 µl of Folin & Ciocalteu's phenol reagent was added to each standard and sample vial, followed by 0.15 ml of 200 g/L of Na_2CO_3. The vials were mixed for approximately two minutes then incubated at room

temperature for 75 minutes. Three replicates of each standard and water sample, as well as three ultra pure water blanks were added to a microplate and were run for absorbance at 750 nm using a Flexstation Multimode Microplate Reader (Flexstation). Results were obtained as a concentration in mg/ml (±0.003 OD ±1.0%, 0-2 OD).

3.2.4 Enzyme Activity – Phenol Oxidase

Potential phenol oxidase (PO) activity was assessed following Dunn et al. (2014) under oxic and anoxic conditions. Prior to the analysis, ultra pure water and soil samples were stored at field temperature (~15°C) for at least 24 hours before beginning the analysis. All anaerobic samples were prepared immediately at room temperature (~23°C) using an anoxic chamber with oxygen levels less than 5%. To perform the analysis, approximately 1 g of material was placed into separate Stomacher bags. For double and triple treatments, 0.5 g and 0.33 g of each substrate was used, respectively. Using a pipette, 9 ml of ultra pure water was put in each bag and homogenized by hand for 30 seconds. An additional 10 ml of ultra pure water was added to one of the bags labelled as a blank, and 10 ml of 10 mM of phenolic amino acid L-3,4-dihydroxy phenylalamine (L-DOPA) solution was added to the other Stomacher bag. Both bags were homogenized again by hand for 30 seconds and incubated at field temperature (~15°C) for 10 minutes. Following incubation, approximately 1.5 ml of each solution were transferred to separate centrifuge tubes, and were centrifuged for 5 minutes at 14 000 rpm. Three replicates of both the blank material and L-DOPA material was analyzed using the Flexstation. Finally, 300 µl of supernatant from each tube was pipetted into a clear-bottom 96 well microplate and was run for absorbance at 475 nm (±0.003 OD ±1.0%, 0-2 OD). Equation 9 was used to convert the absorbance value to rates of activity (µmol dicq/g/min)

$$\boldsymbol{Enzyme\ Activity} = \frac{\left(\frac{\boldsymbol{std-L}}{\boldsymbol{37000}*\left(\frac{\boldsymbol{1500}}{\boldsymbol{750}}\right)*\boldsymbol{10000}}\right)}{\boldsymbol{w}*\boldsymbol{9}} \qquad [9]$$

where, *std* is the absorbance of the blank sample, *L* is the absorbance of the L-DOPA treated sample, and *w* is the dry weight of the sample (g)

3.2.5 Statistical Analysis

In order to compare average aerobic and anaerobic respiration rates between single, double and triple treatments (treatments) of peat, *Carex*, *Juncus*, straw and wood (substrates), a linear regression model was used. Under both oxic and anoxic conditions, average microbial respiration rate, average rates of priming, isotopic signatures determined from bulk material, isotopic discrimination of CO_2 collected on the final day of incubation, and phenolic compound concentrations and potential PO enzyme activities collected from the remaining material post-incubation, were compared with treatments and substrates using a linear regression model. For all significant interactions a Tukey HSD pairwise comparison was performed to determine significant differences between treatments. To determine controls on average aerobic and anaerobic respiration rates, as well as average rates of priming and isotopic discrimination of CO_2 collected on the final day of incubation, a multiple linear regression model was used to compare treatments and substrates with phenolic compound concentration and PO enzyme activity collected from the material post-incubation. Finally, to determine the effect of treatments and substrates on average microbial respiration rates and average rate of priming, as well as isotopic discrimination of CO_2 collected on the final day of incubation, and phenolic compound concentrations and PO enzyme activities collected from the remaining material post-incubation, a linear regression model as used for both oxic and anoxic incubations. All statistical analysis was conducted using the statistical program R (R Core Team, 2017), and a significance of $\alpha = 0.05$ was applied.

3.3 Results

3.3.1 Microbial respiration

Microbial respiration rates were approximately twice as high under oxic conditions as compared to anoxic conditions (Figure 3.1). Under oxic conditions, the highest rates of respiration were from the *Juncus* treatments ($F_{1,37} = 4.53$, $p = 0.04$), which were twice the rate of the wood treatments ($F_{1,37} = 4.75$, $p = 0.036$). Single treatments produced substantially higher rates (3.1 $\mu gCO_2\ gC^{-1}\ min^{-1}$) as compared to double (1.22 $\mu gCO_2\ gC^{-1}\ min^{-1}$) and triple (1.61 $\mu gCO_2\ gC^{-1}\ min^{-1}$) treatments ($F_{2,36} = 6.928$, $p = 0.003$). Average respiration steadily decreased under oxic conditions for the first half of the incubation before stabilizing for several days, then continuing to drop by the latter stages of the incubation (Appendix B, Figure B.1). Under anoxic conditions, the highest and lowest respiration rates were also produced by treatments containing *Juncus* and wood. Triple treatments produced much higher rates (1.44 $\mu gCO_2\ gC^{-1}\ min^{-1}$) as compared to single (0.795 $\mu gCO_2\ gC^{-1}\ min^{-1}$) and double (0.863 $\mu gCO_2\ gC^{-1}\ min^{-1}$) treatments ($F_{2,36} = 7.677$, $p = 0.002$). Respiration rates remained consistent under anoxic conditions until the latter half of the incubation where there was a slight decrease in CO_2 production (Appendix B, Figure B.2).

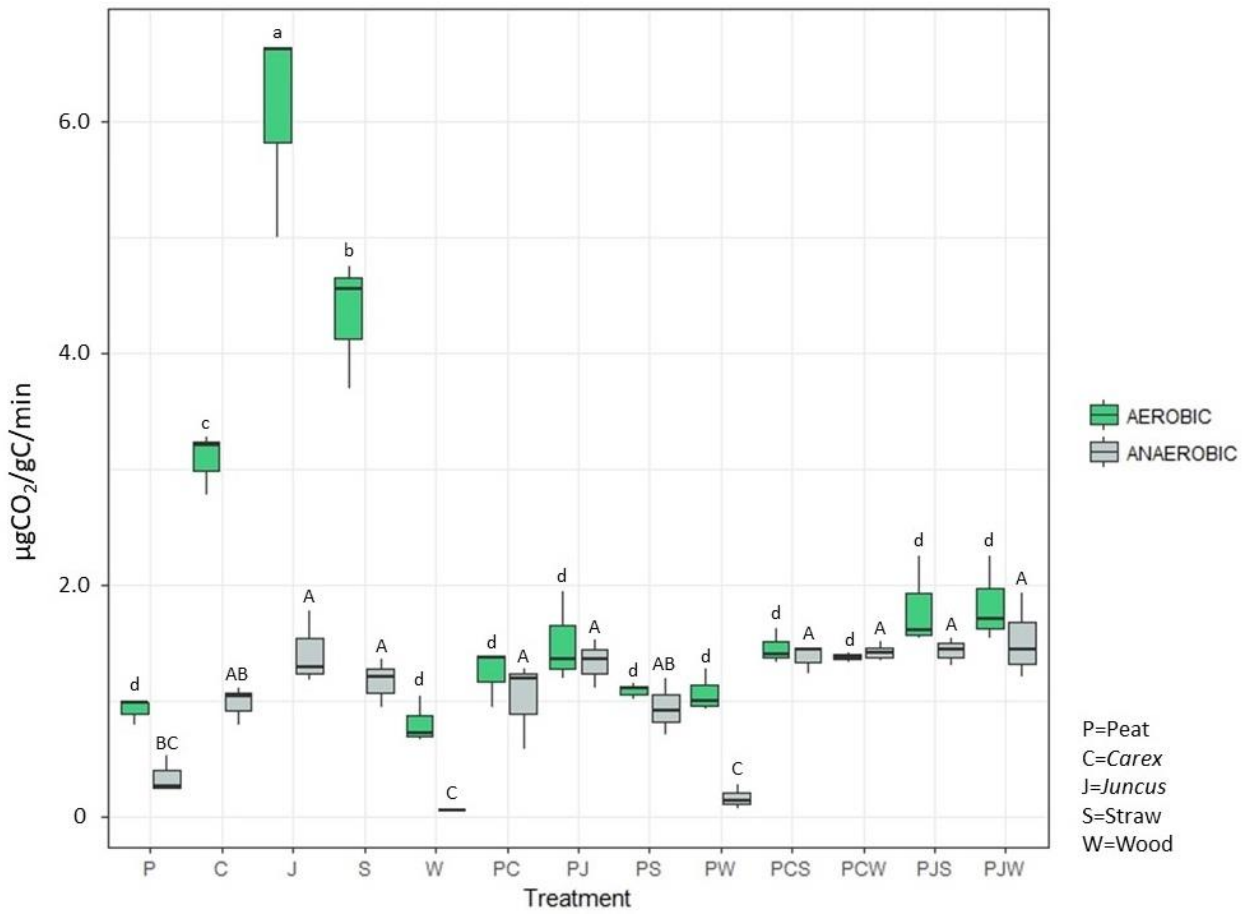


Figure 3.1: *Microbial aerobic and anaerobic respiration rates. Single treatments included one of each substrate (i.e., peat), sedge species (i.e.,* Carex aquatilis *or* Juncus balticus*), and amendment (straw or wood). Double treatments contained peat and one plant species or amendment. Triple treatments contained peat, one plant species, and one amendment. Letters indicate significant differences between each treatment under aerobic (lower case) and anaerobic (upper case) conditions. Treatments with the same letter(s) are not significantly different from each other.*

3.3.2 Priming

All treatments under oxic conditions, with the exception of the double treatment of peat-wood, experienced negative priming, but rates varied between treatments (Figure 3.2; $F_{7,16} = 24.67$, $p < 0.001$). The highest rate of negative priming under oxic conditions came from the double treatment of peat-*Juncus*, while the lowest rate of negative priming came from the triple treatment of peat-*Carex*-wood. Triple treatments showed a slightly more negative rate of priming (-1.08 $\mu gCO_2\ gC^{-1}\ min^{-1}$) as compared to double treatments (-1.02 $\mu gCO_2\ gC^{-1}\ min^{-1}$), but the difference was not significant ($F_{1,22} = 0.035$, $p = 0.85$). Treatments containing straw showed approximately 10 times the negative priming that was

observed from treatments containing wood. Trends in priming rate over the course of the oxic incubation are provided in Appendix B.

Under anoxic conditions, all treatments, with the exception of the double treatment of peat-wood, experienced positive rates of priming (Figure 3.2). Rates of priming under anoxic conditions varied between treatments ($F_{7,16} = 6.41$, $p < 0.001$) with the highest rate of positive priming from the triple treatment of peat-*Carex*-wood while the lowest came from the double treatment of peat-straw. Triple treatments showed three times the average rate of priming as compared to double treatments ($F_{1,22} = 16.47$, $p < 0.001$). Treatments containing *Carex* had the highest rates of priming (0.62 $\mu gCO_2\ gC^{-1}\ min^{-1}$), while treatments with straw showed the lowest (0.40 $\mu gCO_2\ gC^{-1}\ min^{-1}$).

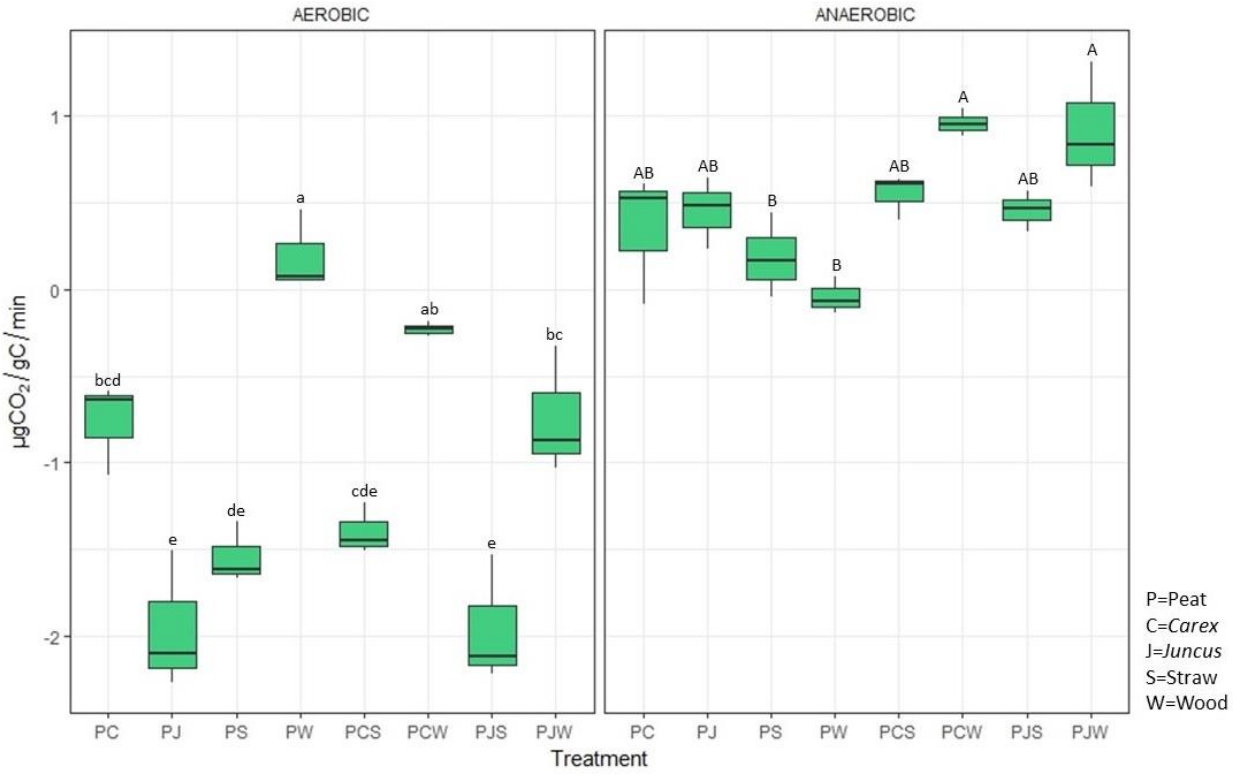


Figure 3.2: *Rates of priming from a 14-day incubation under aerobic and anaerobic conditions. Positive values indicate positive priming while negative values indicate negative rates of priming. Priming was calculated according to equation 5. Letters indicate significant differences between each treatment under aerobic (lower case) and anaerobic (upper case) conditions. Treatments with the same letter(s) were not significantly different from each other.*

3.3.3 $\delta^{13}C$ Signatures

Results from the $\delta^{13}C$ analysis during the aerobic and anaerobic incubations are provided in Table 3.1. Of the substrates, peat was the most ^{13}C-depleted, followed by *Juncus*, straw, *Carex*, and wood. On average, $\delta^{13}C$ of respired CO_2 was slightly less ^{13}C-depleted under oxic conditions (-21.8 ‰) as compared to anoxic conditions (-22.3 ‰), although the difference was not statistically significant ($F_{1,76}$ = 1.06, p = 0.31). Under oxic conditions, treatments containing *Juncus* led to respired CO_2 being the most ^{13}C-depleted ($F_{1,37}$ = 6.7, p = 0.014), while treatments with wood resulted in respired CO_2 that was the least ^{13}C-depleted ($F_{1,37}$ = 6.93, p = 0.012). Single treatments produced CO_2 that was highly depleted of ^{13}C while double treatments produced CO_2 that was the least ^{13}C-depleted ($F_{2,36}$ = 3.19, p = 0.053). Under anoxic conditions, treatments containing straw produced the most ^{13}C-depleted CO_2 ($F_{1,37}$ = 0.34, p = 0.56) while treatments with wood, similar to oxic conditions, produced CO_2 that was the least ^{13}C-depleted ($F_{1,37}$ = 4.72, p = 0.036). Likewise to oxic conditions, there was no significant difference in ^{13}C depletion between the highest (single treatments) and lowest (triple treatments) observations under anoxic conditions ($F_{2,36}$ = 0.35, p = 0.71).

***Table 3.1**: Isotopic signatures of organic matter ($\delta^{13}C$), respired CO_2 ($\delta^{13}CO_2$), and isotopic discrimination (ϵ) under aerobic and anaerobic conditions. $\delta^{13}C$ values are reported as per mil (‰). $\delta^{13}C$ of organic matter is the average from three replicates from each substrate, and double and triple treatments are averages from those substrates used based on dry weight mass. Letters indicate significant differences between each treatment under aerobic (lower case) and anaerobic (upper case) conditions. Treatments with the same letter(s) indicates treatments with no significant difference.*

Aerobic				**Anaerobic**			
Treat	**$\delta^{13}C$**	**$\delta^{13}C$ (CO_2)**	**ϵ**	**Treat**	**$\delta^{13}C$**	**$\delta^{13}C$ (CO_2)**	**ϵ**
P	-29.8 ± 0.15	-23.0 ± 2.77^{cde}	228 ± 93^{abcde}	**P**	-29.8 ± 0.15	-25.4 ± 1.38^{D}	146 ± 46^{AB}
S	-28.0± 0.16	-22.0 ± 0.072^{cd}	213 ± 3^{bcde}	**S**	-28.0 ± 0.16	-24.5 ± 0.13^{CD}	125 ± 5^{B}
W	-25.2 ± 0.36	-17.9 ± 2.21^{ab}	289 ± 88^{abc}	**W**	-25.2 ± 0.36	-19.1 ± 2.29^{A}	241 ± 91^{A}
C	-26.9± 0.13	-24.8 ± 0.65^{de}	78 ± 24^{de}	**C**	-26.9 ± 0.13	-21.7 ± 0.27^{ABC}	194 ± 10^{AB}
J	-29.7 ± 0.12	-26.8 ± 0.074^{e}	99 ± 2^{e}	**J**	-29.7 ± 0.12	-22.3 ± 0.49^{BC}	250 ± 16^{A}
PC	-28.3 ± 0.0029	-21.7 ± 0.99^{bcd}	234 ± 35^{abcde}	**PC**	-28.3 ± 0.0014	-21.3 ± 0.81^{AB}	249 ± 29^{A}
PJ	-29.8 ± 7.8E-05	-22.5 ± 1.28^{cd}	246 ± 43^{abcd}	**PJ**	-29.8 ± 5.14E-05	-23.1 ± 0.56^{BCD}	226 ± 19^{AB}
PS	-28.9 ± 0.0011	-19.8 ± 0.22^{abc}	314 ± 8^{ab}	**PS**	-28.9 ± 0.0022	-21.8 ± 0.74^{ABS}	246 ± 26^{A}
PW	-27.5 ± 0.0031	-17.7 ± 1.18^{a}	358 ± 43^{a}	**PW**	-27.5 ± 0.0037	-22.9 ± 1.48^{BCD}	166 ± 54^{AB}
PCS	-28.2 ± .00095	-21.3 ± 0.64^{abcd}	246 ± 23^{abcd}	**PCS**	-28.2 ± 0.0066	-21.9 ± 1.42^{ABC}	223 ± 50^{AB}
PCW	-27.3 ± 0.0045	-21.7 ± 0.90^{bcd}	204 ± 33^{bcde}	**PCW**	-27.3 ± 0.0068	-21.3 ± 0.39^{AB}	218 ± 14^{AB}
PJS	-29.2 ± 0.0014	-20.6 ± 1.07^{abc}	292 ± 37^{abc}	**PJS**	-29.2 ± 0.0035	-22.5 ± 0.36^{BCD}	230 ± 12^{AB}
PJW	-28.2 ± 0.0026	-23.6 ± 1.87^{cde}	166 ± 66^{cde}	**PJW**	-28.2 ± 0.011	-22.5 ± 0.36^{BCD}	204 ± 13^{AB}

3.3.4 Isotopic discrimination

Isotopic discrimination (ϵ) was higher, but not significantly different, under oxic conditions as compared to anoxic conditions (Table 3.1; $F_{1,76} = 1.39$, $p = 0.24$). The aerobic decomposition of peat-wood treatment showed the highest discrimination while aerobic decomposition of *Juncus* showed the lowest ($F_{12,26} = 8.54$, $p < 0.0001$). Although not statistically significant, treatments containing straw led to the highest ϵ ($F_{1,37} = 3.57$, $p = 0.067$) while treatments with *Carex* led to the lowest ($F_{1,37} = 3.47$, $p = 0.070$). Isotopic discrimination was the highest from double treatments and lowest from the single treatments ($F_{2,36} = 6.39$, $p = 0.004$). Treatments also varied for isotopic discrimination under anoxic

conditions ($F_{12,26} = 3.47$, $p = 0.0038$), but the pattern between substrates varied. Contrary to the aerobic incubation, ϵ from the single treatment of *Juncus* was twice that of the single treatment of straw under anoxic conditions. Treatments containing *Juncus* showed the highest ϵ ($F_{1,37} = 2.37$, $p = 0.13$) while treatments with straw showed the lowest ($F_{1,37} = 0.062$, $p = 0.80$), with double treatments having the highest and single treatments showing the lowest ϵ ($F_{2,36} = 1.59$, $p = 0.22$), but none of these differences were significant. We observed a negative relationship between ϵ and CO_2 production rate (Figure 3.3) that was only significant under aerobic conditions ($R^2 = 0.36$, $F_{1,37} = 22.66$, $p < 0.001$).

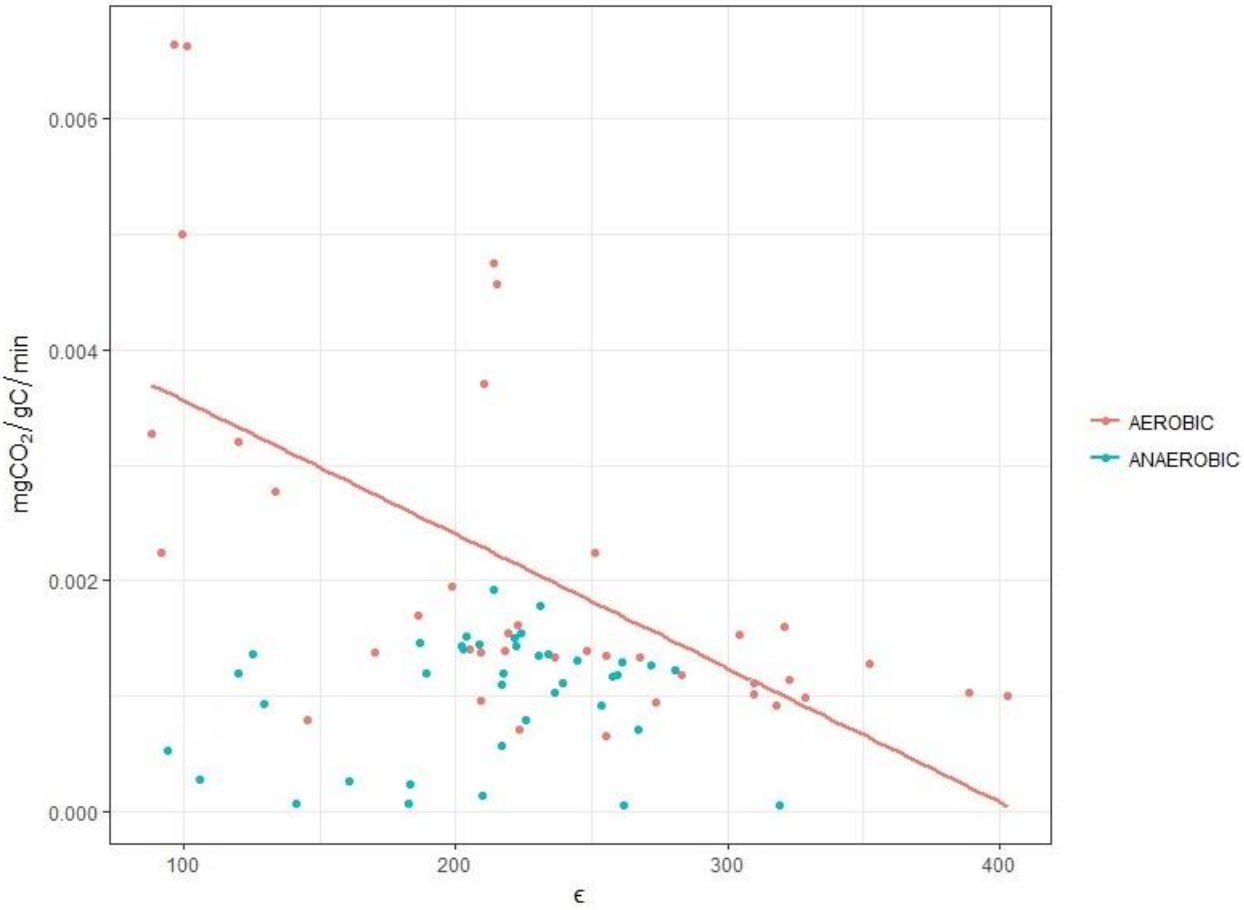


Figure 3.3: *Isotopic Discrimination (ϵ) vs microbial CO_2 respiration rate from a 14-day incubation under aerobic ($R^2 = 0.36$, $F_{1,37} = 22.66$, $p < 0.001$) and anaerobic conditions. Respiration rates are averages from each of the 13 treatments consisting of single, double and triple combinations of peat,* Carex aquatilis *or Juncus* balticus*, and wood or straw. ϵ values were obtained from samples collected on the final sampling date during each of the incubations.*

3.3.5 Phenolic Compounds

Average water soluble phenolic compound concentrations (Figure 3.4) were higher under oxic conditions (1.93 mg/ml) as compared to anoxic conditions (1.70 mg/ml; $F_{1,67} = 1.27$, $p = 0.26$). In the aerobic incubation, the highest concentration of phenolics came from the single treatment of straw and the lowest came from the single treatment of wood ($F_{12,23} = 9.75$, $p < 0.001$). Across all treatments, those containing straw had the highest concentration of phenolics ($F_{1,34} = 24.18$, $p < 0.001$) while treatments containing wood had the lowest ($F_{1,34} = 23.3$, $p < 0.001$). Under oxic conditions, triple treatments led to the largest accumulation of phenolics while double treatments produced the lowest, although the differences were not significant ($F_{2,33} = 1.02$, $p = 0.37$). Similar to oxic conditions, the single treatment of straw showed the highest concentration of phenolics under anoxic conditions, while the double treatment of peat-wood produced the lowest ($F_{12,20} = 63.08$, $p < 0.001$). Treatments containing straw also had the highest concentration of phenolics ($F_{1,31} = 18.61$, $p < 0.001$) and treatments containing wood had the lowest ($F_{1,31} = 33.12$, $p < 0.001$), with single treatments producing the highest concentration of phenolics while double treatments produced the lowest ($F_{2,30} = 2.59$, $p = 0.092$).

Comparing phenolic compound concentrations to microbial respiration rates of CO_2 revealed a positive relationship (Figure 3.5); however, the relationship was only significant under oxic conditions ($R^2 = 0.13$, $F_{1,34} = 6.32$, $p < 0.05$). Similarly, phenolic compound concentration showed a negative relationship with rates of priming (Figure 3.6) that was only significant under oxic conditions ($R^2 = 0.29$, $F_{1,21} = 9.96$, $p < 0.01$). No significant relationship was observed between phenolic compound concentration and isotopic discrimination (Appendix B, Figure B.18).

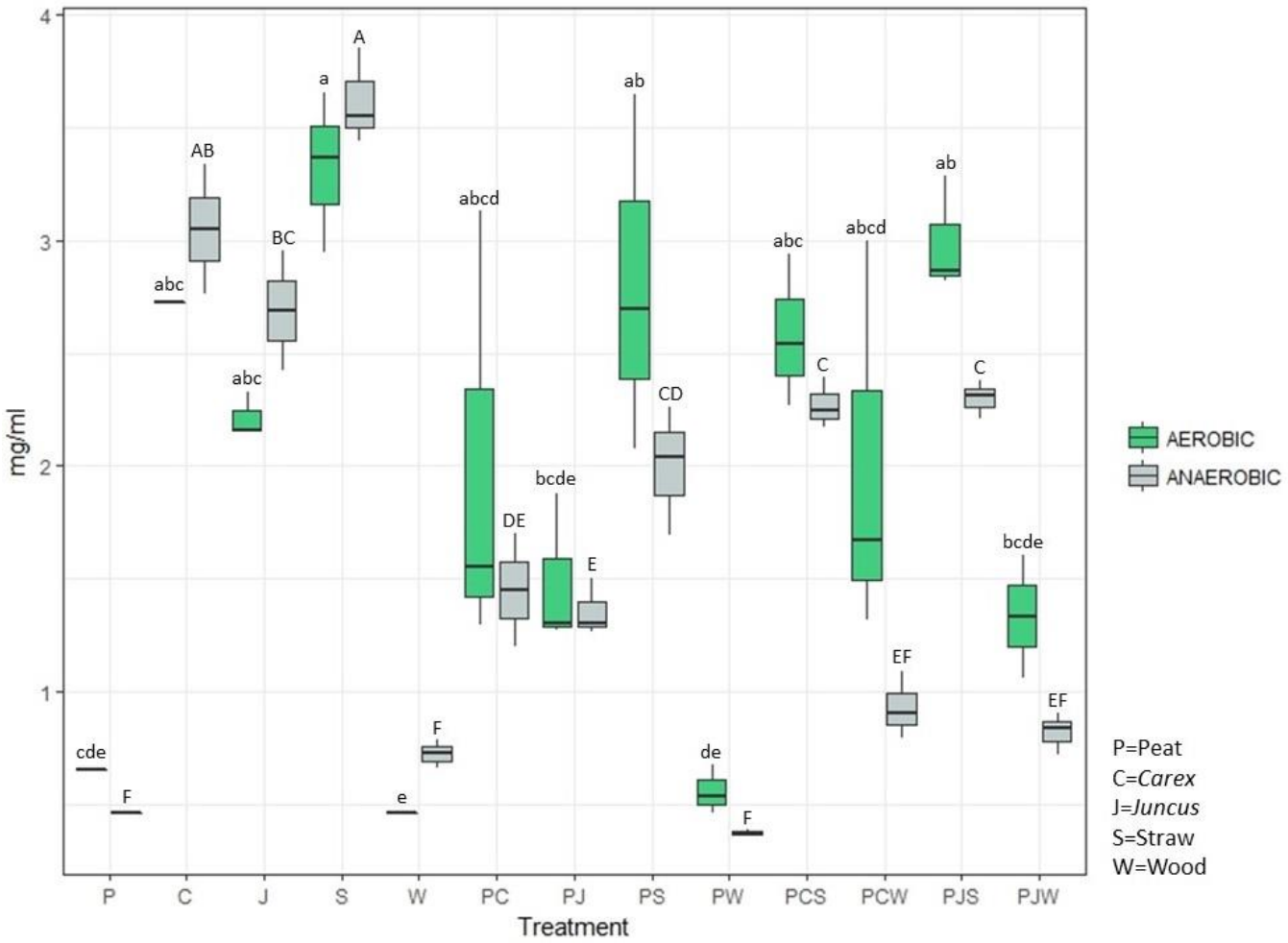


Figure 3.4: *Water soluble phenolic compound concentrations (mg/ml) from a 14-day incubation under aerobic and anaerobic conditions. Concentrations were obtained from water extractions performed on the material following the incubations. Treatments consisted of single, double and triple combinations of peat,* Carex aquatilis *or* Juncus balticus*, and wood or straw. Letters indicate significant differences between each treatment under aerobic (lower case) and anaerobic (upper case) conditions. Treatments with the same letter(s) indicates treatments with no significant difference.*

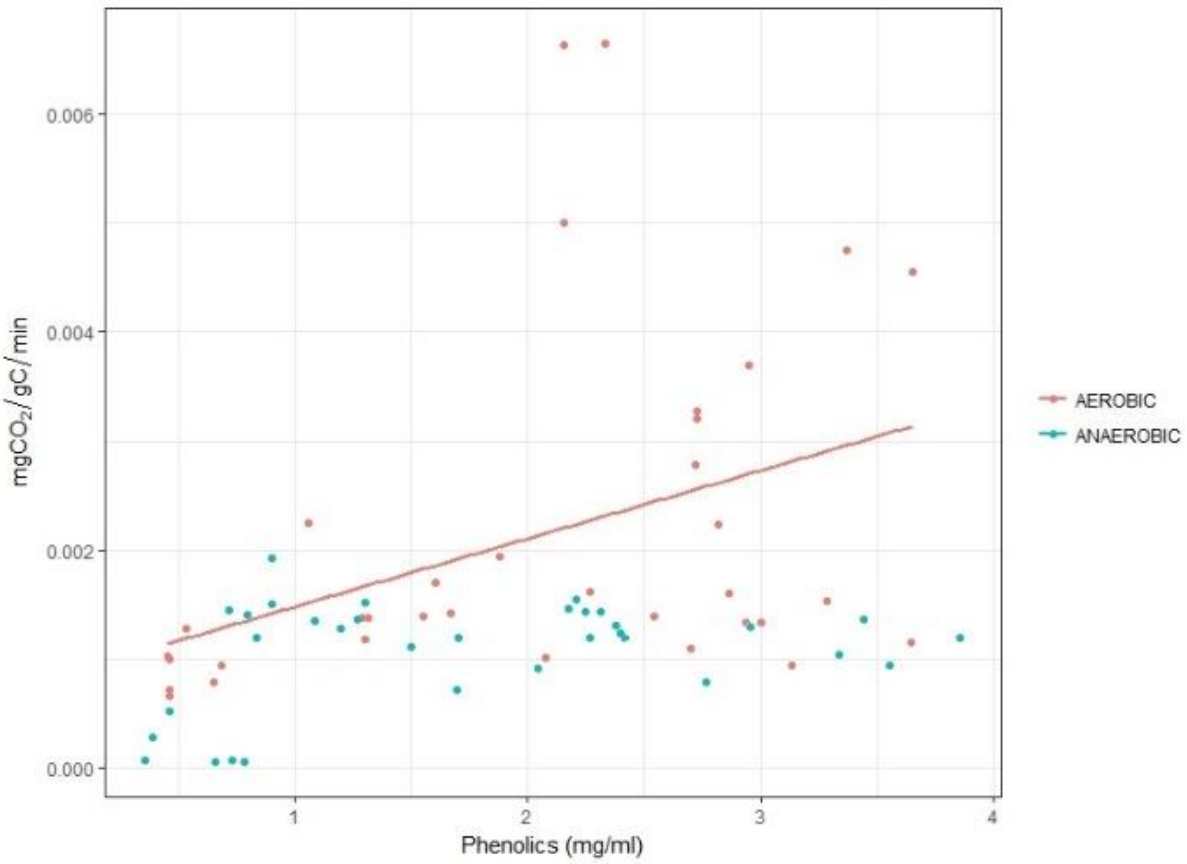


Figure 3.5: *Phenolic compound concentrations vs microbial CO_2 respiration rate from a 14-day incubation under aerobic (R^2 = 0.13, $F_{1,34}$ = 6.32, $p < 0.05$) and anaerobic conditions. Respiration rates are averages from from each of the 13 treatments consisting of single, double and triple combinations of peat*, Carex aquatilis *or* Juncus balticus*, and wood or straw. Phenolic compound concentrations were obtained from water extractions performed on the remaining material post-incubation.*

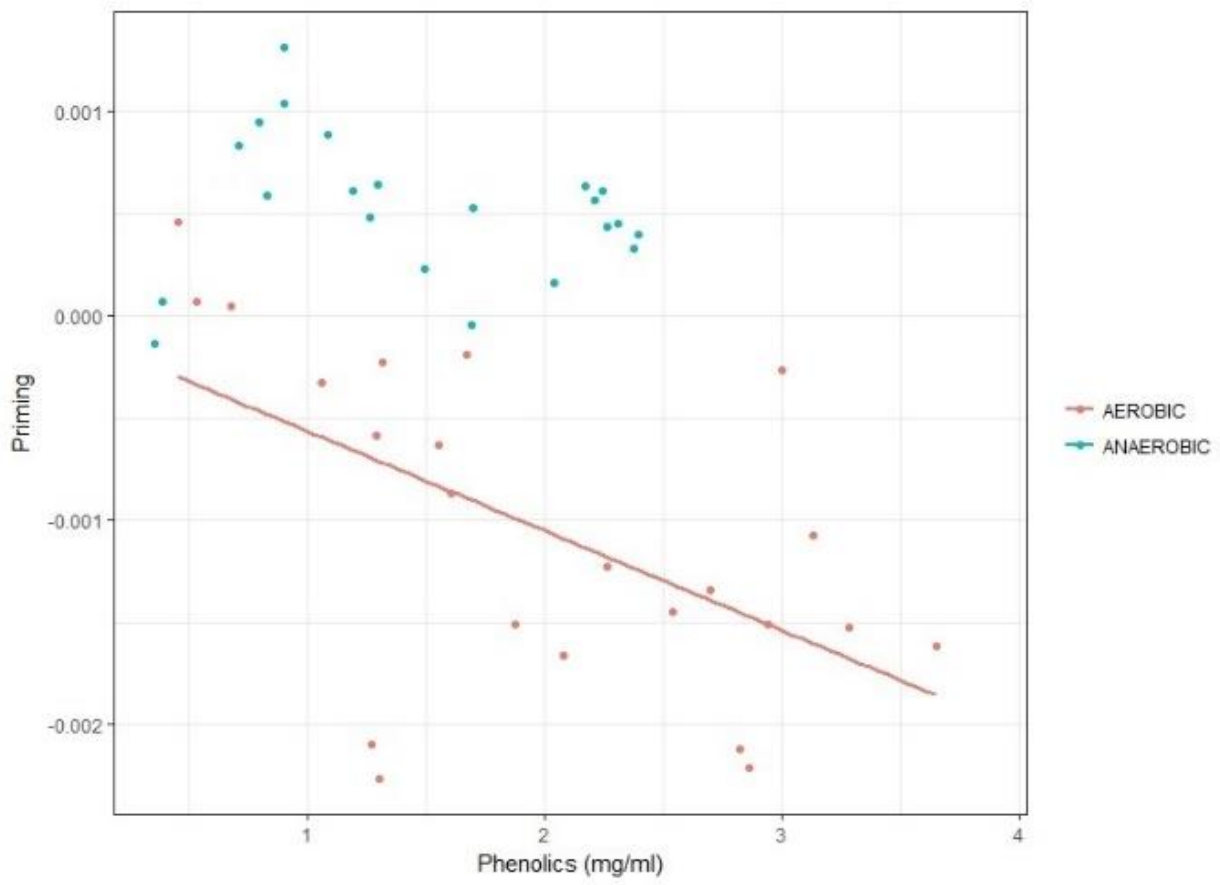


Figure 3.6: *Phenolic compound concentration vs rates of priming ($\mu gCO_2\, gC^{-1}\, min^{-1}$) from a 14-day incubation under aerobic (R^2 = 0.29, $F_{1,21}$ = 9.96, p < 0.01) and anaerobic conditions. Priming rates are averages from each of the 13 treatments consisting of single, double and triple combinations of peat,* Carex aquatilis *or* Juncus balticus*, and wood or straw. Positive values indicate positive priming while negative values indicate negative rates of priming. Phenolic compound concentrations were obtained from water extractions performed on the remaining material post-incubation.*

3.3.6 Phenol oxidase activity

Phenol oxidase activity (PO; Figure 3.7) was twice as high under oxic conditions as compared to anoxic conditions ($F_{1,72}$ = 7.80, p < 0.01). Under oxic conditions, PO activity varied between treatments ($F_{12,24}$ = 45.46, p < 0.001) being highest from the single treatment of peat and lowest from the single treatment of wood. In general, treatments with peat had the highest PO activity ($F_{1,34}$ = 8.47, p < 0.01), while treatments with *Carex* had approximately half the activity. Under anoxic conditions, the single treatment of peat had the highest PO activity and the single treatment of wood had the lowest. Similar to oxic conditions, under anoxic conditions, treatments with peat showed the highest PO activity ($F_{1,34}$ = 9.78,

$p < 0.01$), but treatments with wood showed the lowest, although the presence of wood did not lead to significantly lower PO activity ($F_{1,34}$ = 1.37, p = 0.25). When compared to phenolic compound concentrations and rates of priming, PO activity did not have a significant relationship with either (Appendix B, Figure B.19; Figure B.20). However, there was a significant, but weak negative relationship between PO activity and CO_2 respiration rates (Figure 3.8) under oxic conditions only (R^2 = 0.083, $F_{1,34}$ = 4.15, p = 0.05).

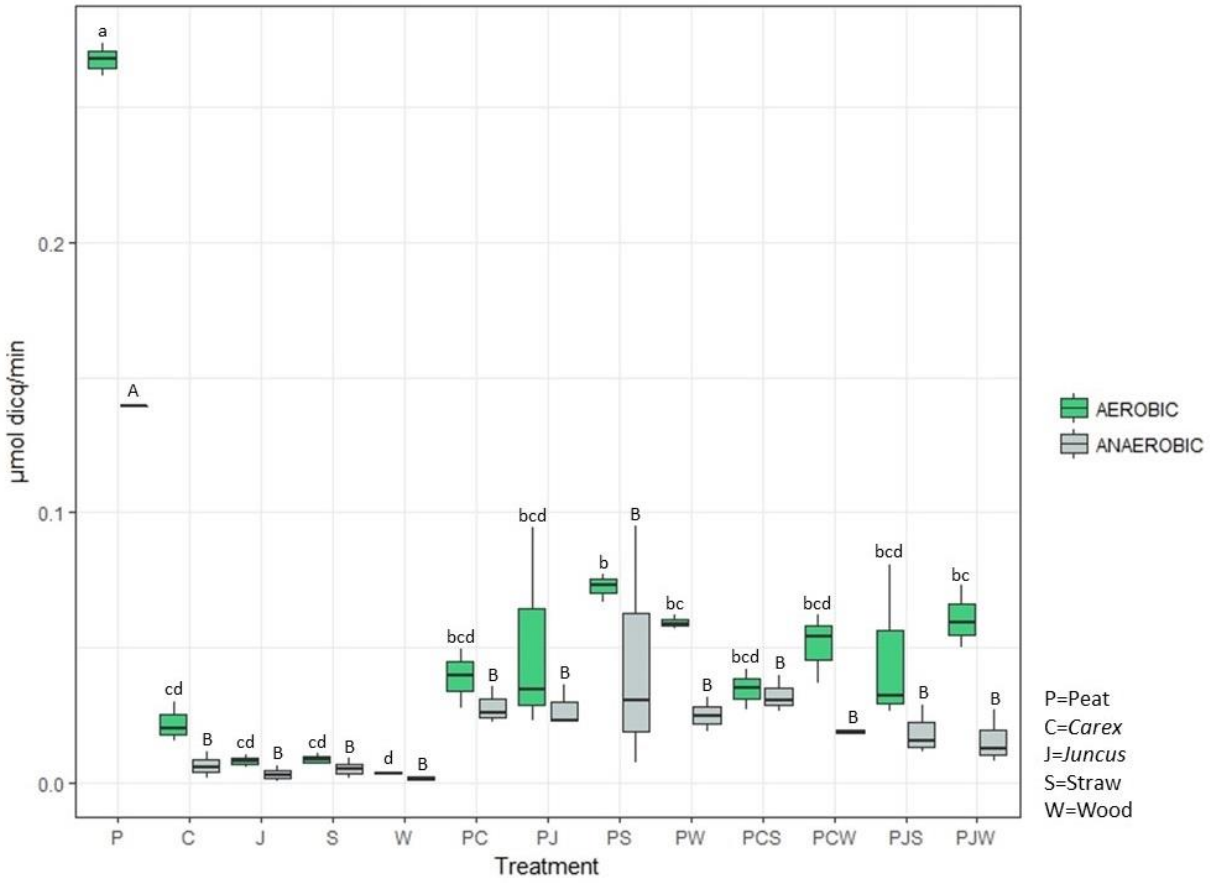


Figure 3.7: *Phenol oxidase activity following a 14-day incubation under aerobic and anaerobic conditions. Activity was obtained from the material following the incubations. Treatments consisted of single, double and triple combinations of peat,* Carex aquatilis *or* Juncus balticus*, and wood or straw. Letters indicate significant differences between each treatment under aerobic (lower case) and anaerobic (upper case) conditions. Treatments with the same letter(s) indicates treatments with no significant difference.*

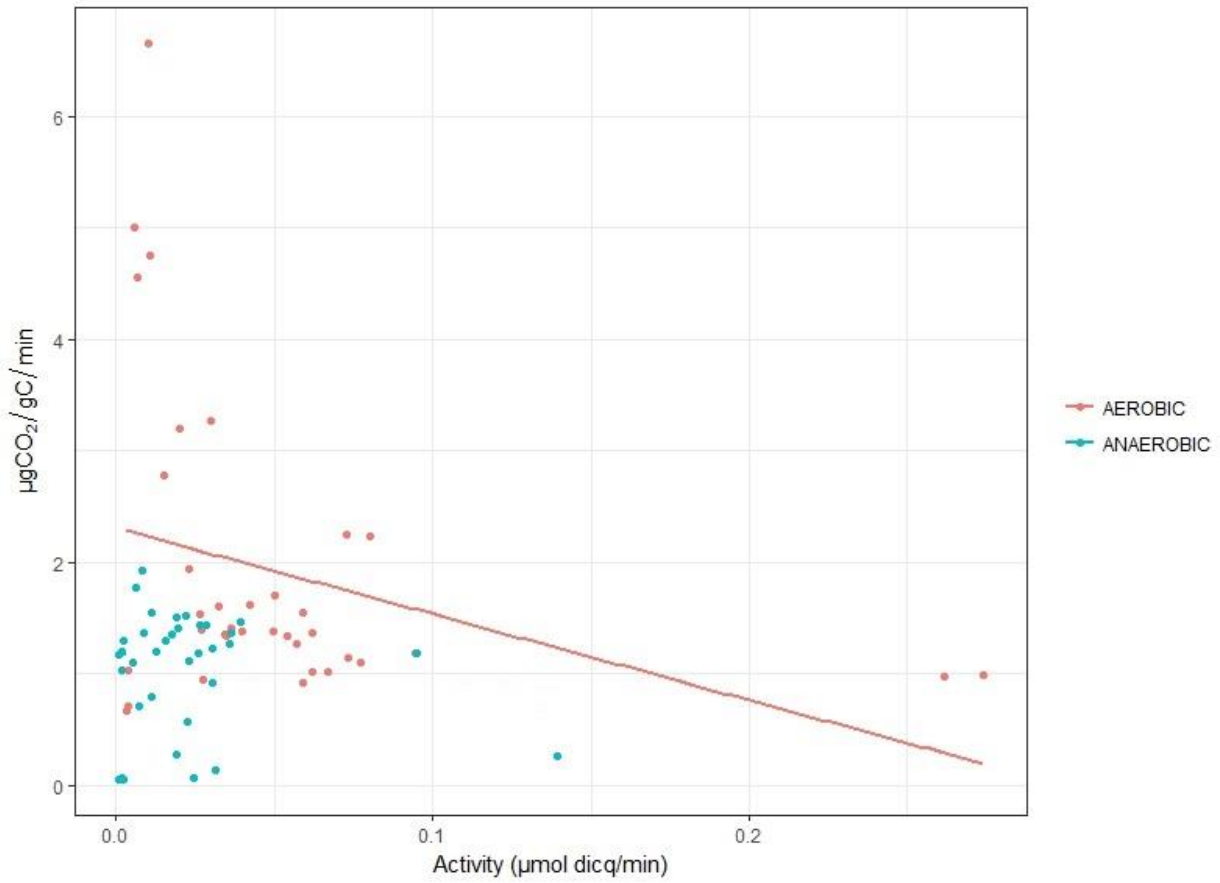


Figure 3.8: *Phenol oxidase activity vs microbial CO_2 respiration rate from a 14-day incubation under aerobic (R^2 = 0.083, $F_{1,34}$ = 4.15, p = 0.05) and anaerobic conditions. Respiration rates are averages from each of the 13 treatments consisting of single, double and triple combinations of peat,* Carex aquatilis *or* Juncus balticus*, and wood or straw. Activity was obtained from each of the treatments following the incubations.*

3.4 Discussion

Preservation of the critical carbon pool in peatlands can be partly attributed to organic matter (OM) composition and the relative lability of vegetation input. Microorganisms are responsible for OM decomposition and are subject to a variety of controls including temperature, moisture, pH, oxygen availability and substrate composition. Since fen reclamation within a post-mined landscape has only been recently attempted (Ketcheson et al. 2016), it is important to investigate current techniques in order to evaluate potential improvements or limitations to peat accumulation and thus reclamation timeline objectives. In this study we performed two laboratory incubations under oxic and anoxic

conditions to assess the decomposition of two common sedge species, as well as mulch treatments often utilized during reclamation. Results of this study will be used as an assessment of the decomposition of materials used in reclamation in order to postulate improvements to limit decomposition and enhance peat accumulation during fen construction.

While direct measurements of OM decomposition in the field are predominantly performed by weight loss (Bernhard-Reversat, 1999), carbon dioxide (CO_2) production has also been frequently used as an indicator of OM degradation (Bridgham & Richardson, 1992; Laiho, 2007; Brouns et al. 2014; Dunn et al. 2016). The latter allows for the observation of shorter time lags and leads to the interpretation of varying stages of decomposition (Bernhard-Reversat, 1999), while excluding additional forms of respiration (i.e., autotrophic and root respiration) that come with in-situ measurements (Basiliko et al. 2012; Dunn et al. 2016). The degradation of OM occurs as microbes break down organic compounds and release products including CO_2, methane (CH_4), and dissolved organic carbon (DOC; Moore et al. 2018). We only assessed respired CO_2 due to a lack of substrate required for all other analyses (including DOC), and because CH_4 can be several orders of magnitude less than CO_2 production, particularly under oxic conditions (Reddy & Patrick, 1975; Bridgham & Richardson, 1992; Kayranli et al. 2010). This degradation of OM is subject to temperature and moisture constraints, which we were able to control under laboratory conditions, thus isolating the impact of substrate composition (Moore & Dalva, 1993; Carrasco et al. 2006; Bonnett et al. 2017).

3.4.1 Controls on OM breakdown

Following the 14-day incubations, respiration rates were twice as high under oxic conditions as compared to anoxic conditions (Figure 3.1). This is in agreement with the vast majority of incubation studies on decomposition dynamics in peatlands (Reddy & Patrick, 1975; Brinson et al. 1981; Bridgham & Richardson, 1992; Brouns et al. 2014a). Under oxic conditions, oxygen is the terminal electron acceptor (TEA) and provides a thermodynamically favourable pathway to OM degradation as compared

to other TEAs available under anoxic conditions (Brouns et al. 2014a). These alternative TEAs include nitrate (NO_3^-), manganese (Mn^{4+}), ferric iron (Fe^{3+}), humic substances, sulfate (SO_4^{2-}), and finally CO_2 leading to CH_4 production (Brouns et al. 2014a; Agethen & Knorr, 2018). Under anoxic conditions, organic C is mineralized through fermentation into short chain fatty acids or hydrogen, which are oxidized to CO_2 and H_2O, or utilized as substrates for methanogenesis (Agethen & Knorr, 2018). Under oxic conditions, carbohydrates, amino acids and proteins are reduced for biomass maintenance and reproduction, resulting in CO_2 respiration (Kayranli et al. 2010; Agethen & Knorr, 2018).

Although peat contains approximately 95% OM (Bridgham & Richardson, 1992), and roughly 45% - 50% of organic C (Moore et al. 2018), microorganisms are limited by labile C sources (Bridgham & Richardson, 1992). The lability of a substrate is often characterized by C:N ratios (Windham 2001), with ratios below 25-30:1 indicating more labile material (Richert et al. 2000; Bonanomi et al. 2015). Although we did not run a detailed N analysis, test samples showed *Juncus* (32:1) having a higher ratio than *Carex* (30:1), which were both higher than straw (23:1). Previous literature has also shown the relative lability of *Carex* and *Juncus* species compared to other litter types in peatlands (e.g., Szumigalski & Bayley, 1996; Kuehn et al. 2000; Thormann et al. 2004; Graf & Rochefort, 2009; Hall & Hopkins, 2015). This is supported by the higher aerobic respiration rates of treatments containing *Carex*, *Juncus*, or straw as compared to peat or wood, which we assume was less labile (Figure 3.1). Furthermore, double and triple treatments containing *Carex*, *Juncus*, or straw were observed to have higher rates of respiration than from peat or wood alone. The higher respiration rates observed for *Carex*, *Juncus*, and straw is likely due to the labile nature of the material, and differences among these could be due to leaf thickness, as has been found in a previous study showing a negative correlation between respiration rates and leaf thickness (Bernhard-Reversat, 1999). Significantly lower respiration rates for the peat and wood treatments confirms a lack of lability, potentially as a result of lignin content.

Lignin is a primary component of woody species that consists of a complex network of polyphenol units that are difficult to break down (Turcotte, 2009). Lignin has also been shown to prevent microbial access to more labile structures like cellulose (Bernhard-Reversat, 1999). Although *Carex* and *Juncus* do contain some lignin (Thormann et al. 2004), we assumed the wood-strand mulch contains a much higher proportion of lignin, thus resulting in the lower respiration rates observed (Figure 3.1). Lignin, as well as and other phenolic compounds, have been extensively researched and their role in inhibiting OM degradation is widely recognized (Kleber, 2010; Bonnett et al. 2017; Dunn & Freeman, 2018). Phenolics have been found to immobilize essential nutrients and extracellular enzymes, in addition to their recalcitrant properties, thereby reducing decomposition rate (Meier & Bowman, 2008; Min et al. 2015; Dieleman et al. 2016).

In our study, treatments with straw contained the highest concentration of soluble phenolics under both oxic and anoxic conditions (Figure 3.4), yet considerable respiration rates were observed (Figure 3.1). Contrary to previous studies, the concentration of phenolics was positively correlated to respiration rate under oxic conditions (Figure 3.5). In addition to inhibitory effects, phenolic compounds, and more specifically, low molecular weight (LMW) phenolics, have been found to be a source of labile C for microbial activities (Thormann et al. 2003; Bonnett et al. 2006; Dieleman et al. 2016; Bonnett et al. 2017). Crucial to their role in decomposition dynamics is the form, rather than the total concentration, of phenolics (Min et al. 2015), and attempts to distinguish between simple and complex phenolics have been made (Kuiters, 1990). Although only total concentration was analyzed in this study, we can assume a proportion of these water soluble phenolics are labile leading to stimulated microbial activity. Out of the substrates analyzed, peat contained the lowest concentration of water soluble phenolics in both aerobic and anaerobic incubations (Figure 3.4). Peat has undergone considerable decomposition with highly recalcitrant compounds remaining, including complex phenolics (Brant et al. 2006; Kleber, 2010), which is reflected by the accompanying lower rates of respiration observed. Interestingly, with the

exception of peat, all single treatments showed higher concentrations of phenolics under anoxic conditions. Since we analyzed for phenolics at the end of the incubation, the lower concentrations under oxic conditions could be the result of water soluble and LMW phenolics being utilized as an energy source throughout the duration, thus resulting in less accumulation.

The breakdown of phenolics requires a suite of oxidative extracellular enzymes (i.e., phenol oxidases (PO)) that are only produced by a small group of decomposers (Thormann et al. 2003; Brant et al. 2006). These PO enzymes promote the oxidation of phenolics to simple polymers that can be taken up by the surrounding microbial community (Criquet et al. 2000). Our results show that PO activity was higher under oxic conditions as compared to anoxic conditions for all treatments (Figure 3.7). This is in agreement with previous studies demonstrating the oxygen limitation of PO enzymes (Freeman et al. 2001; Dunn et al. 2016). Microorganisms preferentially secrete PO enzymes when nutrient availability is low, potentially due to direct or indirect effects of phenolics (Meier & Bowman, 2008). Our results support this, as PO activity was substantially higher from the single treatment of peat as compared to the other single treatments. Due to the lack of nutrient availability and higher proportion of complex phenolic compounds, microbes secrete PO enzymes in attempt to satisfy nutrient demand. This can also be observed in the present study, as all treatments that contain peat (i.e., double and triple treatments) showed higher PO activity than the single treatments of the more labile substrates on their own (*Carex*, *Juncus*, or straw). This secretion of PO, however, only occurs when the benefit of enzyme production outweighs the associated metabolic costs (Mooshammer et al. 2014), and could explain the lack of production and activity occurring from the single treatment of wood. Although we observed a positive correlation between phenolics and microbial respiration under oxic conditions, surprisingly we also observed a negative correlation between PO activity and microbial respiration under oxic conditions (Figure 3.8). In the presence of oxygen, PO could be cleaving off side-chains of aldehyde monomers, which lowers pH (Turcotte, 2009) and other complex phenols that are then immobilizing nutrients or

other extracellular enzymes (Kuiters, 1990; Kuehn & Suberkropp, 2002; Fenner et al. 2005) resulting in lower respiration. A drop in heterotrophic respiration despite an increase in other extracellular enzymes has been reported before (Bonnett et al. 2017). When peat is present, PO activity is stimulated so that decomposition is increased, however this trend is masked by the availability of more labile substrates from the double and triple treatments.

3.4.2 Microbial adaption to OM degradation

Microbial communities responsible for the breakdown of OM are subject to environmental conditions and substrate availability. A wide-range of studies have looked at the microbial response to variables such as oxygen availability, pH, temperature and moisture, and to a lesser extent, substrate adaptation and utilization (Orwin et al. 2006). One of the methods to address how a microbial community responds to a given substrate is through isotopic discrimination. Isotopic discrimination (ϵ) refers to the preferential uptake or release of a particular elemental isotope through, in this case, C mineralization (Bostrom et al. 2007). Substrates will vary in ^{13}C, and microbes that breakdown OM typically respire CO_2 that is ^{13}C-depleted relative to the OM, resulting in a slight enrichment of ^{13}C in the remaining biomass (Boström et al. 2007). Our results show the opposite, however, as the respired CO_2 was less depleted as compared to the substrate used in all treatments under both oxic and anoxic conditions (Table 3.1). This could be due to microbial biomass turnover, as we analyzed the CO_2 at the end of the incubation. Although OM can vary greatly in ^{13}C signatures (Schweizer et al. 1999), lignin-C tends to be more ^{13}C-depleted, while cellulose and other labile compounds are ^{13}C-enriched, leading to an enriched biomass of ^{13}C as microbes preferentially use these labile compounds (Boström et al. 2007). When this biomass dies it becomes a source of C, particularly towards the end of the incubation when resources are limited, leading to respired CO_2 that is less ^{13}C-depleted than the original substrate. Unexpectedly our isotopic analysis shows wood being less ^{13}C-depleted as compared to *Carex*, *Juncus*,

and straw, which could be due to the varying isotopic composition not completely reflective of the bulk material (Schweizer et al. 1999).

Of considerable importance, particularly regarding the stable C pool in peatlands, is the potential 'priming effect' on microbial degradation of OM (Kuzyakov et al. 2000). The addition of labile material has the potential of inducing mineralization rates of recalcitrant material through the added nutrient availability and/or C source (Hamer & Marschner, 2002). This could promote OM degradation, particularly in nutrient-poor environments (Brant et al. 2006) and the increased loss of C through respiration. Interestingly, we predominately observed negative priming under oxic conditions and positive priming under anoxic conditions (Figure 3.2). Positive and negative rates of priming have both been previously reported (Kuzyakov, 2010). Under oxic conditions, with the exception of the peat-wood treatment, we observed a reduction in respired CO_2 that could be due to the microbial community response to the change in substrate. Our incubation duration (i.e., 14 days) was relatively short compared to other incubations, particularly for degradation of materials such as lignin (Bernhard-Reversat, 1999). Although the effects of priming can occur within just a few hours of adding the substrate (Hammer & Marschner, 2005), more time may be needed for microbes to adapt to the additional substrate. It has been suggested that microbial assemblages are established over time (Thormann et al. 2004) that are best suited for the substrate available. A change in composition induces a change in microbial community, thus resulting in a 'lag' response. Furthermore, we observed a negative correlation between rates of priming and phenolic compound concentration under oxic conditions (Figure 3.6). It is likely that increased phenolics is resulting in increased competition or immobilization of C or N when trying to utilize the more labile substrate under oxic conditions (Kuehn & Suberkropp, 2002; Meier & Bowman, 2008). It has also been suggested that in C-limited soils, glucose can be stored in microbial cells and utilized at a later time (Brant et al. 2006) thus supporting the respiration peaks towards the end of the incubations (Appendix B, Figure B.9; Figure B.17). Over the

course of the incubation, with the exception of the peat-wood treatment, absolute values of rates of priming generally decreased in the aerobic incubation (Appendix B; Figure B.17), which supports our theory of microbial adaptation and nutrient competition.

Similarly, under anoxic conditions with the exception of the peat-wood treatment, we observed an increase in respiration that is an indication of microbial adaption to the added substrate. Under anoxia, resource utilization is limited, and this is reflected by the substantially lower rates of respiration (Figure 3.1). Previous studies have suggested that positive priming is induced by microbial biomass turnover with the addition of C sources, as opposed to the decomposition of the recalcitrant material (Brant et al. 2006). Since phenolics can be toxic to some microorganisms (Brant et al. 2006), and a lack of oxygen results in a lack of oxidative extracellular enzymes removing these phenolics (Freeman et al. 2004), then our results would support this hypothesis of increased biomass turnover potentially due to phenol toxicity. Furthermore, higher rates of priming have been observed following the addition of more complex C substrates as compared to simple polymers (Orwin et al. 2006). Rates of priming under anoxic conditions were higher under triple treatments containing wood as compared to the more simple, double treatments of *Carex*, *Juncus*, and straw. This could be the result of an increase in demand of N-acquiring enzymes as a result of the complex C substrates made available to the microbial community present (Orwin et al. 2006). Additionally, mixing of C substrates have also been found to produce a greater variety of enzymes, leading to higher rates of mineralization (Orwin et al. 2006). Since OM degradation in peatlands persists predominantly under anoxic conditions (Bonnett et al. 2017), these potential effects of priming could lead to mineralization of the stable C pool.

3.5 Conclusion

In this study we evaluated microbial CO_2 respiration, phenolic compound concentration, potential phenol oxidase enzyme activity, and $\delta^{13}C$ signatures from laboratory incubations under oxic and anoxic conditions to determine the potential impact on OM degradation during fen reclamation. We hypothesized that the wood-strand mulch would leach more phenolics thus resulting in supressed decomposition. However, higher concentrations of phenolics were observed with more labile substrates. This suggests that the concentration of phenolics alone does not reduce decomposition, and in fact we observed a positive correlation between phenolics and respiration under oxic conditions. In the presence of labile substrates, phenolics are unlikely to hinder decomposition as per the enzymatic latch theory, and the form of phenolics is critical to determine its role in decomposition. Furthermore, although the presence of wood with peat alone had the lowest rate of priming under anoxic conditions, when combined with labile substrates, there is no evidence that the wood-strand mulch reduces decomposition. Additionally, previous research has shown that the wood-strand mulch deterred species establishment in areas not planted with moss (Price et al. 2017), and therefore its use in reclamation does not promote peat accumulation. The findings from this study also suggest that *Juncus* species may decompose more readily as compared to *Carex* species based on respiration rates and isotopic discrimination observed. The controls of this decomposition extend beyond the environmental constraints, as demonstrated by the results of the incubation, and complex biogeochemical interactions dictate these processes. Our results also suggest that under anoxic conditions, combinations of wood-mulch and *Juncus* treatments could stimulate C mineralization through the priming effect, potentially leading to enhanced C losses and reduced peat accumulation.

Chapter 4: Implications on fen reclamation and final recommendations

The critical C-sequestering properties known of boreal fen peatlands exist due to biomass accumulation exceeding SOM decomposition largely in part of the climatic and anoxic conditions. Re-establishing these conditions within a post-mined landscape remains a significant challenge and has only recently been attempted despite legal obligations to return disturbed areas to "equivalent land capability" (Government of Alberta, 1993). Due to the substantial time required for these ecosystems to develop naturally, preferential techniques utilized during fen reclamation may enhance peat accumulating functions that lead to increased C storage and/or more timely return of C sink potential. One method of enhancing peat accumulation is through the suppression of SOM decomposition. The results from our field and laboratory studies show how decisions made during fen reclamation impact decomposition rates, and therefore further investigation into these processes is required.

Species composition is of considerable importance to the decomposability of SOM. In our study we selected *Carex aquatilis* and *Juncus balticus* due to their presence at the constructed fen and their lability relative to mosses (Graf & Rochefort, 2009). We obtained mixed results and marginal differences when comparing *Carex* and *Juncus* species. The results from our field study suggest *Carex* biomass decomposes faster than *Juncus* biomass, and that the decomposition rate is on average higher under plots planted with *Carex* compared *Juncus* or bare plots. In addition to species composition, environmental conditions such as higher pH, higher soil temperature, lower VWC, and lower EC could also lead to a higher decomposition rate. Contrary to our field study, results from our aerobic and anaerobic laboratory incubations suggest *Juncus* is of higher lability as compared to *Carex*. Microbial respiration rate was twice as high for *Juncus,* suggesting *Carex* may be the favourable species to utilize during reclamation. However, higher rates of negative priming were observed with *Juncus* treatments under oxic conditions, which could improve C retention during periods of water table drawdown and

subsequent rewetting (Fenner & Freeman, 2011). Under the more predominate anoxic conditions, treatments with *Carex* induced higher rates of positive priming, which could potentially promote C loss. Additionally, straw treatments had higher respiration rates, even higher than *Carex*, but induced slightly less positive priming as compared to the wood treatments.

In addition to estimating decomposition rate, we assessed key functions involved in SOM turnover, including potential extracellular enzyme activities and concentrations of recalcitrant phenolic compounds. At the constructed fen, hydrolase and phenol oxidase enzyme activities did not vary significantly between *Carex* and *Juncus* plots, and phenolic compound concentrations were only slightly lower under *Carex* plots. From our lab incubations, slightly higher concentrations of phenolics and phenol oxidase activity were observed from the *Carex* treatments. These findings suggest that *Carex* may possess the more favourable biogeochemical conditions to restrict decomposition; however, more research is required to support these findings. In contrast, our isotopic analysis suggests *Juncus* may be less labile and the better species to prevent decomposition. These contradicting findings show the need for ongoing research to determine the long-term trends of decomposition from these two species.

We hypothesized that applying wood-strand mulch would result in lower decomposition rate due to phenolic inhibition and reduced lability. At the constructed fen, no appreciable differences were observed in the estimate decomposition rate across mulch or no-mulch treatments. We expected phenolic compound concentrations would be higher under the mulch treatments due to leaching of water soluble phenolics, however no significant differences were observed, nor were there appreciable differences in extracellular enzyme activities. Therefore, we conclude that the enzymatic latch theory is not supported by our study and could be due to the abundance of labile substrate that is driving decomposition. However, over time the enzymatic latch theory could become relevant as the fen continues to develop and ecosystem function is restored. From our lab incubations, we observed the lowest concentration of phenolics from the wood treatments, suggesting phenolics are not readily

leached from this substrate in the short term. Likewise, wood treatments sustained lower PO activities under anoxic conditions as compared to straw, and we found a negative correlation between PO activity and microbial respiration. Therefore, the wood treatment has not contributed higher phenolic concentration or lower decomposition rates. Treatments with wood had relatively lower microbial respiration rates, and the peat-wood treatment was the only one to experience negative priming under anoxic conditions. Thus, the wood-mulch provides the added advantage of reduced lability and, when mulch is required, its use is recommended over straw as it pertains to decomposition dynamics.

The constructed fen did not fully reflect the biogeochemical conditions present in reference fens. This was reflected in varying decomposition rates. Although we measured a lower decomposition rate at the constructed fen for our tested litter types, we may have overestimated the actual litter decomposition rate at the reference fens by decomposing species not native to these areas (Johnson & Damman, 1991; Barreto & Lindo, 2018). Nevertheless, we showed substantially lower rates of hydrolase enzyme activities at the constructed fen, potentially indicating higher nutrient availability. We also observed similar phenolic compound concentrations at the constructed fen to the rich and saline fen, as well as similar PO enzyme activity as the rich fen. The constructed fen also showed the highest activity from phosphatase, however relative rates of β-glucosidase and N-acetyl-β-D-glucosaminidase were much lower at the constructed fen, also supporting sufficient nutrient availability. More time is required for phenolics to leach and the microbial assemblages to establish as the lability of SOM deteriorates. Ongoing research is recommended to continue to investigate decomposition dynamics at a constructed fen as newly deposited litter leads to new peat accumulation and vegetation succession occurs.

In addition to continued research of the aforementioned constituents, research into other parameters would help to gain knowledge on SOM decomposition dynamics. An in-depth look into the type of phenolic compounds, rather than total concentration, would work towards distinguishing inhibitory effects versus labile sources of C. Furthermore, analysis of the type and count of bacteria and

fungi present at the constructed fen and reference sites could help identify progress in establishing natural fen conditions. Investigating additional forms of organic and inorganic nutrients, such as dissolved organic carbon/matter (DOC/DOM), C/N ratios, and P availability, as well as redox potentials and the impact of clay particles, could prove beneficial as they all relate to SOM decomposition. Lastly, of particular importance is the peat accumulating potential under the vegetation and mulch treatment plots. We showed that bare plots had the lowest decomposition rate; however, this is only favourable if it is accompanied by a high peat accumulating rate, which has yet to be determined but would require net primary production for organic matter inputs.

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APPENDIX A:

Supplemental information:

Phenolic compounds

Three separate phenol concentration standards were created using 1000 ppm phenol stock solution, ranging from 0.25 ppm to 30 ppm, and were selected based on water sample color (i.e., clear, light brown, or dark brown). Once selected, 1 ml of phenol standard was put into a 3 ml centrifuge vial using a pipette. Approximately 1 ml of the water sample was filtered using a 0.45 µm syringe filter, then filtered again using a 0.2 µm syringe filter, and placed into a separate 3 ml centrifuge vial. 50 µl of Folin & Ciocalteu's phenol reagent was added to each standard and sample vials, followed by 0.15 ml of 200 g/L of Na2CO3. The vials were mixed for approximately two minutes then incubated at room temperature for 75 minutes. Three replicates of each standard and water sample, as well as three ultra pure water blanks were added to a microplate and ran for absorbance at 750 nm using a Flexstation Multimode Microplate Reader. Results were obtained as a concentration in mg/ml (±0.003 OD ±1.0%, 0-2 OD).

Hydrolase enzymes

Prior to the analysis for the five hydrolase enzymes, a 400 uM of each enzyme substrate and 200 uM of phosphtase was prepared using the following substrates: 4-Methylumbelliferyl β-D-glucopyranoside, 4-Methylumbellifery sulfate potassium salt, 4-methylumbelliferyl β-D-xylopyranoside, 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide, 4-Methylumbelliferyl phosphate (free acid). 20 ml of Cellosolve (2-ethoxyethanol) and 1 L of ultra pure water was mixed with each substrate and left in the dark for two hours. Hydrolase substrate solutions were stored at field temperature (14°C) and replaced monthly. A stock solution of 1000 µM MUF solution was also prepared in order to generate a calibration curve. The solution was prepared daily by dissolving 0.0881 g of 4-methylumbelliferone sodium salt (98%, M1508; Sigma Aldrich Ltd, Dorset, UK) in 20 ml of Cellosolve solvent and placed in the dark for 10

minutes. The solution was than diluted with 500 ml of ultra pure water to a final concentration of 1000 µM and stored at field temperature at least 12 hours prior to analysis. Approximately 1 g of peat soil collected from the 18 fen locations and subsequent reference sites was placed into separate Stomacher bags, as well as an additional sample used to generate a calibration curve. 7 ml of the corresponding enzyme substrate solution was pipetted into each Stomacher bag and homogenized by hand for 30 seconds. All enzyme reactions were then incubated at field temperature for one hour, with the exception of phosphatase and the stock solution (phosphatase was incubated for 45 minutes and the stock solution did not require incubation). During the incubation period, standard solutions of the MUF-free acid were prepared using ultra pure water to generate a calibration curve at concentrations of 0,5, 10, 20, 40, 60 and 80 µM. These solution standards were combined with 20 µL of ultra pure water and transferred to a 96 well opaque microplate. Upon completion of the incubation period, approximately 1.5 mL of enzyme substrate solution was centrifuged for five minutes at 14,000 rpm, and three replicates of each solution was transferred to the microplate wells along with 50 µL of ultra pure water. Using the Flexstation Multimode Microplate Reader, fluorescence was run under 330 nm (excitation) and 450 nm (emission) and used in conjunction with Equation 5 to generate rates of activity in nmoles/g/min (< 5 mP standard deviation @ 1 nM fluorescein).

Equation 5: $$Enzyme\ Activity = \frac{F}{w*t} \times 8$$

Where, *F* is the fluorescence, *w* is the weight of peat (dry), and *t* is the incubation period (minutes).

Phenol oxidase:

To analyze phenol oxidase activity, two 1-g samples of peat stored at field temperature >12 hours prior was placed into separate Stomacher bags. 9 ml of ultra pure water maintained at field temperature was put in each bag using a pipette and homogenized by hand for 30 seconds. An additional 10 ml of ultra pure water was added to one of the bags labelled as a blank, and 10 ml of 10 mM of phenolic amino acid

L-3,4-dihydroxy phenylalamine (L-DOPA) solution was added to the other Stomacher bag. Both bags were homogenized again by hand for 30 seconds and incubated at field temperature for 10 minutes. Following incubation, approximately 1.5 ml of each solution were transferred to separate centrifuge tubes, and were centrifuged for 5 minutes at 14 000 rpm. Three replicates of both the blank material and L-DOPA material was analyzed using the flexmachine. 300 µl of supernatant from each tube was pipetted into a clear-bottom 96 well microplate and ran for absorbance at 475 nm (±0.003 OD ±1.0%, 0-2 OD). Equation 6 was used to convert the absorbance value to rates of activity (µmol dicq/g/min):

Equation 6: $$Enzyme\ Activity = \frac{\left(\frac{std-L}{37000*\left(\frac{1500}{750}\right)*10000}\right)}{w*9}$$

Where, *std* is the absorbance of the blank sample, *L* is the absorbance of the L-DOPA treated sample, and *w* is the dry weight of the sample (g).

Environmental Conditions:

Table A.1*: Environmental conditions at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Parameters include volumetric water content (VWC); electrical conductivity (EC) @ 15cm, 30cm and the average; pH @ 15cm, 30cm and the average; soil temperature (Soil Temp) @ 5cm, 10cm and the average. Measurements were obtained every 2-3 weeks during the growing season (May – September). Letters indicate significant differences between each site at the CF (lower case) and REF (upper case) sites. Sites with the same letter(s) indicates treatments with no significant difference.*

	B	BM	C	CM	J	JM	Rich (hl)	Rich (hm)	Poor (hl)	Poor (hm)	Saline (hl)	Saline (hm)
VWC (%)	86 ± 5	76 ± 7	84 ± 2	78 ± 7	84 ± 3	79 ± 8	76 ± 7	11 ± 2	74 ± 9	31 ± 26	75 ± 7	73 ± 2
EC 15cm (µS/cm)	2014 ± 1734	1519 ± 1450	3102 ± 718	1487 ± 1141	2943 ± 1212	2289 ± 1279	366 ± 103^{C}	398 ± 347^{C}	147 ± 231^{C}	42 ± 40^{C}	16065 ± 746^{A}	14192 ± 904^{B}
EC 30cm (µS/cm)	2848 ± 583	2671 ± 622	2928 ± 663	2668 ± 250	2996 ± 863	2939 ± 531	225 ± 28^{B}	184 ± 38^{B}	30 ± 9^{B}	81 ± 115^{B}	11753 ± 1612^{A}	10954 ± 1796^{A}
EC avg (µS/cm)	2211 ± 1287	2095 ± 630	3015 ± 406	2077 ± 561	2970 ± 956	2614 ± 800	295 ± 65^{C}	271 ± 182^{C}	88 ± 128^{C}	83 ± 115^{C}	13370 ± 898^{A}	12069 ± 551^{B}
pH (15cm)	7.1 ± 0.2	6.9 ± 0.2	7.1 ± 0.2	7.2 ± 0.2	7.1 ± 0.1	7.1 ± 0.1	6.9 ± 0.2^{AB}	7.3 ± 0.2^{A}	4.5 ± 0.4^{D}	4.6 ± 0.7^{D}	5.9 ± 0.3^{C}	6.3 ± 0.3^{BC}
pH (30cm)	7.2 ± 0.2	7.1 ± 0.4	7.1 ± 0.2	7.1 ± 0.4	7.2 ± 0.2	7.2 ± 0.2	6.9 ± 0.1^{AB}	6.9 ± 0.1^{A}	4.4 ± 0.4^{C}	4.5 ± 0.5^{C}	6.4 ± 0.2^{B}	6.5 ± 0.2^{AB}
pH (avg)	7.2 ± 0.2	7.1 ± 0.3	7.1 ± 0.2	7.1 ± 0.4	7.1 ± 0.1	7.1 ± 0.1	6.9 ± 0.1^{A}	7.1 ± 0.2^{A}	4.5 ± 0.4^{C}	4.4 ± 0.5^{C}	6.2 ± 0.3^{B}	6.5 ± 0.3^{AB}

Soil Temp 5cm (°C)	14.9 ± 0.4^{ab}	15.4 ± 1.2^{b}	12.2 ± 0.9^{cd}	11.9 ± 1.0^{d}	13.7 ± 0.5^{bc}	13.9 ± 0.9^{b}	8.9 ± 0.9^{D}	11.3 ± 0.6^{CD}	11.9 ± 1.0^{BC}	14.2 ± 2.9^{AB}	14.8 ± 0.8^{A}	13.9 ± 0.6^{AB}
Soil Temp 10cm (°C)	14.2 ± 0.3^{a}	14.3 ± 0.8^{a}	11.1 ± 0.9^{c}	10.9 ± 1.1^{c}	12.8 ± 0.4^{b}	13.1 ± 0.6^{ab}	8.6 ± 1.0^{D}	10.0 ± 0.6^{CD}	10.9 ± 0.7^{BC}	12.8 ± 2.3^{AB}	13.8 ± 0.9^{A}	12.9 ± 0.7^{AB}
Soil Temp avg (°C)	14.6 ± 0.3^{ab}	14.9 ± 1.0^{a}	11.6 ± 0.9^{c}	11.4 ± 1.1^{c}	13.2 ± 0.4^{b}	13.5 ± 0.8^{ab}	8.7 ± 0.9^{D}	10.6 ± 0.6^{CD}	11.4 ± 0.8^{BC}	13.5 ± 2.6^{AB}	14.3 ± 0.8^{A}	13.4 ± 0.6^{AB}

Table A.2*: Statistical summary of environmental variables at the constructed fen and reference sites. Measurements were obtained every 2-3 weeks over the course of the growing season (May – September). Parameters at the constructed fen were tested against vegetation (veg) treatment plots (*Carex aquatilis, Juncus balticus*, and bare), as well mulch treatment plots (mulch, no-mulch). At the reference sites, parameters were tested against site types (saline, rich and poor), as well as microtopography (hummocks/hollows). (p < 0.05)* (p < 0.01)** (p < 0.001)****

Constructed Fen			**Reference Sites**		
VWC	**F**	**P**	**VWC**	**F**	**P**
Veg	$F_{2,30}$ = 0.0541	0.947412	Site	$F_{2,30}$ = 20.322	2.635e-06 ***
Mulch	$F_{1,30}$ = 12.9773	0.001124**	Micro	$F_{1,30}$ = 86.597	2.378e-10 ***
Veg*Mulch	$F_{2,30}$ = 0.7737	0.470282	Site*Micro	$F_{2,30}$ = 21.846	1.398e-06 ***
EC (15 cm)	**F**	**P**	**EC (15 cm)**	**F**	**P**
Veg	$F_{2,30}$ = 1.3211	0.28192	Site	$F_{2,22}$ = 3134.5596	< 2.2e-16 ***
Mulch	$F_{1,30}$ = 4.5707	0.04079*	Micro	$F_{1,22}$ = 8.8109	0.0070963 **
Veg*Mulch	$F_{2,30}$ = 0.6606	0.52388	Site*Micro	$F_{2,22}$ = 11.6099	0.0003614 ***
EC (30 cm)	**F**	**P**	**EC (30 cm)**	**F**	**P**
Veg	$F_{2,29}$ = 0.4006	0.6736	Site	$F_{2,30}$ = 517.7454	<2e-16 ***
Mulch	$F_{1,29}$ = 0.6251	0.4345	Micro	$F_{1,30}$ = 0.6395	0.4302
Veg*Mulch	$F_{2,29}$ = 0.0822	0.9213	Site*Micro	$F_{2,30}$ = 0.6702	0.5191
EC (avg)	**F**	**P**	**EC (avg)**	**F**	**P**
Veg	$F_{2,30}$ = 1.8266	0.17842	Site	$F_{2,30}$ = 3203.9923	< 2.2e-16 ***
Mulch	$F_{1,30}$ = 2.9153	0.09808	Micro	$F_{1,30}$ = 9.0280	0.005327 **
Veg*Mulch	$F_{2,30}$ = 0.7864	0.46463	Site*Micro	$F_{2,30}$ = 8.4302	0.001244 **
pH (15)	**F**	**P**	**pH (15 cm)**	**F**	**P**
Veg	$F_{2,22}$ = 0.6474	0.5331	Site	$F_{2,21}$ = 118.6934	3.585e-12 ***
Mulch	$F_{1,22}$ = 0.1195	0.7329	Micro	$F_{1,21}$ = 3.9018	0.06153
Veg*Mulch	$F_{2,22}$ = 2.0672	0.1504	Site*Micro	$F_{2,21}$ = 0.4531	0.64172
pH (30 cm)	**F**	**P**	**pH (30 cm)**	**F**	**P**
Veg	$F_{2,29}$ = 0.2219	0.8023	Site	$F_{2,30}$ = 232.2331	<2e-16 ***
Mulch	$F_{1,29}$ = 0.0634	0.8029	Micro	$F_{1,30}$ = 1.0209	0.3204
Veg*Mulch	$F_{2,29}$ = 0.2949	0.7468	Site*Micro	$F_{2,30}$ = 0.1994	0.8203
pH (avg)	**F**	**P**	**pH (avg)**	**F**	**P**
Veg	$F_{2,29}$ = 0.0288	0.9716	Site	$F_{2,30}$ = 182.5737	<2e-16 ***
Mulch	$F_{1,29}$ = 0.0454	0.8327	Micro	$F_{1,30}$ = 1.2550	0.2715
Veg*Mulch	$F_{2,29}$ = 0.2279	0.7976	Site*Micro	$F_{2,30}$ = 0.4882	0.6185
Temp (5 cm)	**F**	**P**	**Temp (5 cm)**	**F**	**P**
Veg	$F_{2,30}$ = 38.2796	5.533e-09***	Site	$F_{2,30}$ = 29.1227	9.364e-08 ***
Mulch	$F_{1,30}$ = 0.1777	0.6764	Micro	$F_{1,30}$ = 7.0043	0.012826 *
Veg*Mulch	$F_{2,30}$ = 0.5368	0.5901	Site*Micro	$F_{2,30}$ = 5.4274	0.009732 **
Temp (10 cm)	**F**	**P**	**Temp (10 cm)**	**F**	**P**
Veg	$F_{2,30}$ = 58.1928	4.724e-11***	Site	$F_{2,30}$ = 35.0622	1.408e-08 ***
Mulch	$F_{1,30}$ = 0.1465	0.7046	Micro	$F_{1,30}$ = 3.9305	0.05664
Veg*Mulch	$F_{2,30}$ = 0.3956	0.6767	Site*Micro	$F_{2,30}$ = 4.7728	0.01586 *
Temp (avg)	**F**	**P**	**Temp (avg)**	**F**	**P**
Veg	$F_{2,30}$ = 49.0589	3.489e-10 ***	Site	$F_{2,30}$ = 33.0139	2.635e-08 ***
Mulch	$F_{1,30}$ = 0.1702	0.6829	Micro	$F_{1,30}$ = 5.6802	0.02369 *
Veg*Mulch	$F_{2,30}$ = 0.4328	0.6527	Site*Micro	$F_{2,30}$ = 5.2861	0.01080 *

Decomposition:

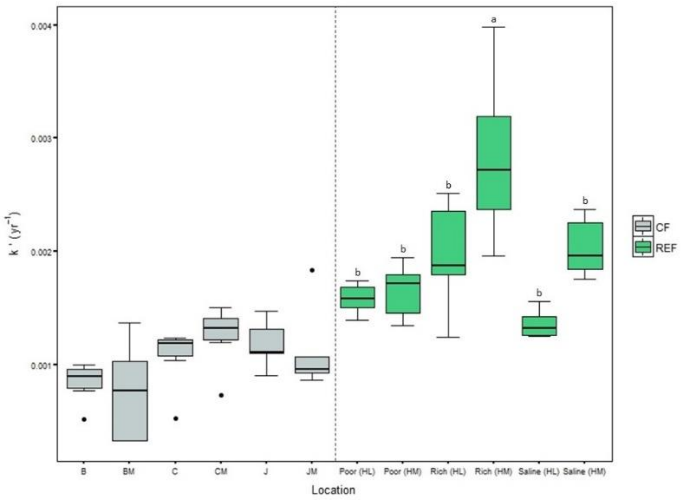

Figure A.18*: Average estimated decomposition rate from the above- and below-ground biomass litter bags (k') at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL).*

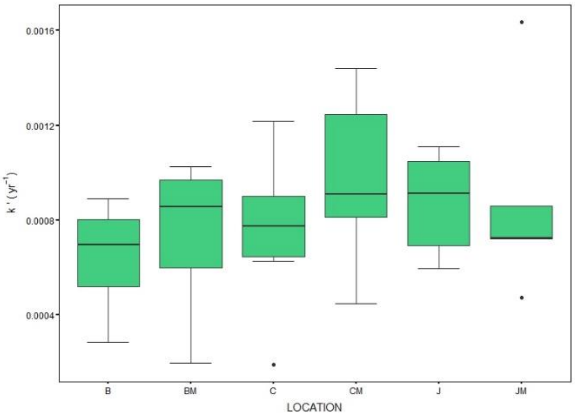

Figure A.19: *Average estimated decomposition rate from the below-ground biomass (k') at the constructed fen (CF). locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments.*

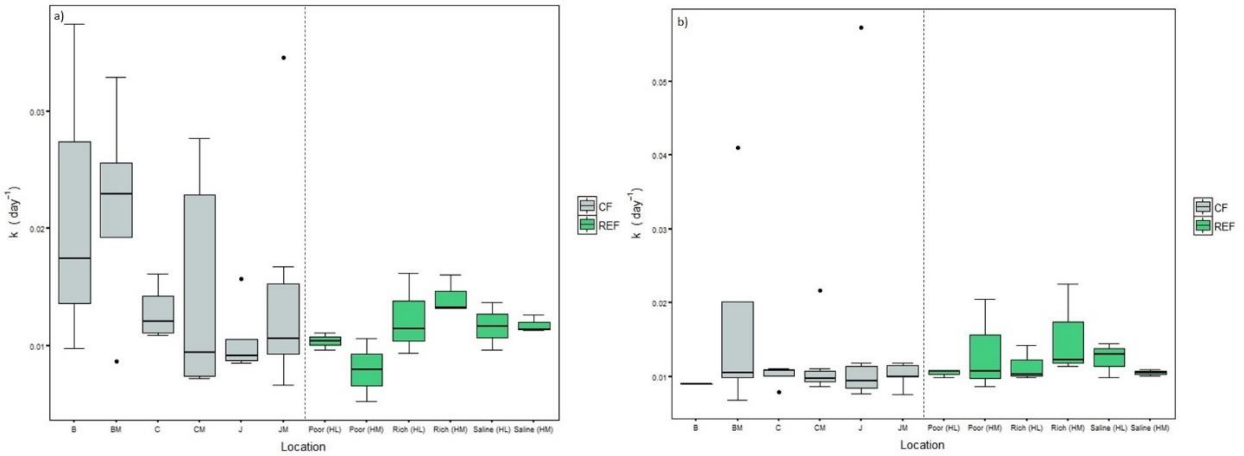

Figure A.20: *Estimated decomposition rate @ 1 cm (left) and 8 cm (right) depths using tea bags at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL).*

β-glucosidase

β-glucosidase activity varied significantly across the vegetation treatment plots ($F_{2,9}$ = 10.49, p = 0.00445) but not across the mulch treatment plots. β-glucosidase activity was highest at the *Carex* plots (16116 µmol/g/min) and the lowest was at the bare plots (10279 µmol/g/min). At the reference sites, there was a significant interaction between microforms and site types ($F_{2,12}$ = 4.09, p = 0.04424) and only a significant difference in β-glucosidase activity across site types ($F_{2,12}$ = 11.54, p = 0.0016). At the reference sites, β-glucosidase activity was the highest at the poor fen (126778 µmol/g/min) and lowest at the saline fen (69675 µmol/g/min). In general, β-glucosidase activity was lower at the constructed fen (12916 µmol/g/min) as compared to the reference sites (93135 µmol/g/min; $F_{1,31}$ = 59.9, p < 0.001). β-glucosidase activity showed a negative relationship with EC and pH that was significant at the reference sites (R^2 = 0.18, $F_{1,16}$ = 4.81, p = 0.04338; R^2 = 0.17, $F_{1,16}$ = 4.51, respectively). β-glucosidase also showed a negative relationship with temperature that was only significant at the constructed fen (R^2 = 0.22, $F_{1,13}$ = 4.96, p = 0.04424).

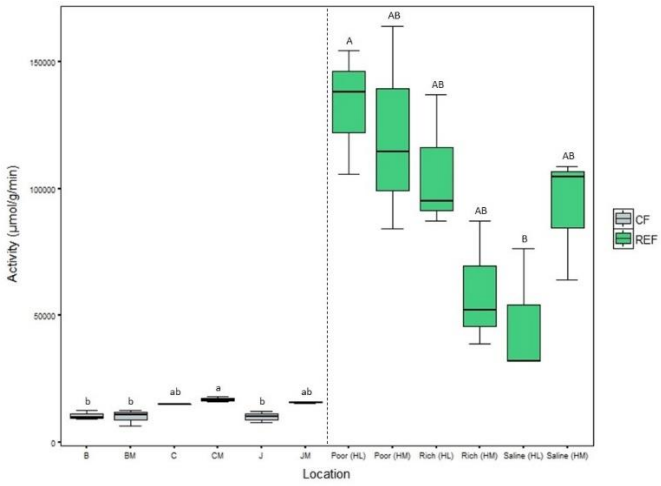

Figure A.21: *β-glucosidase activity at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Activity was determined from organic matter collected at 15 cm from each location. Letters indicate significant differences between each treatment plot at the CF (lower case) and REF (upper case). Locations with the same letter(s) indicates no significant difference.*

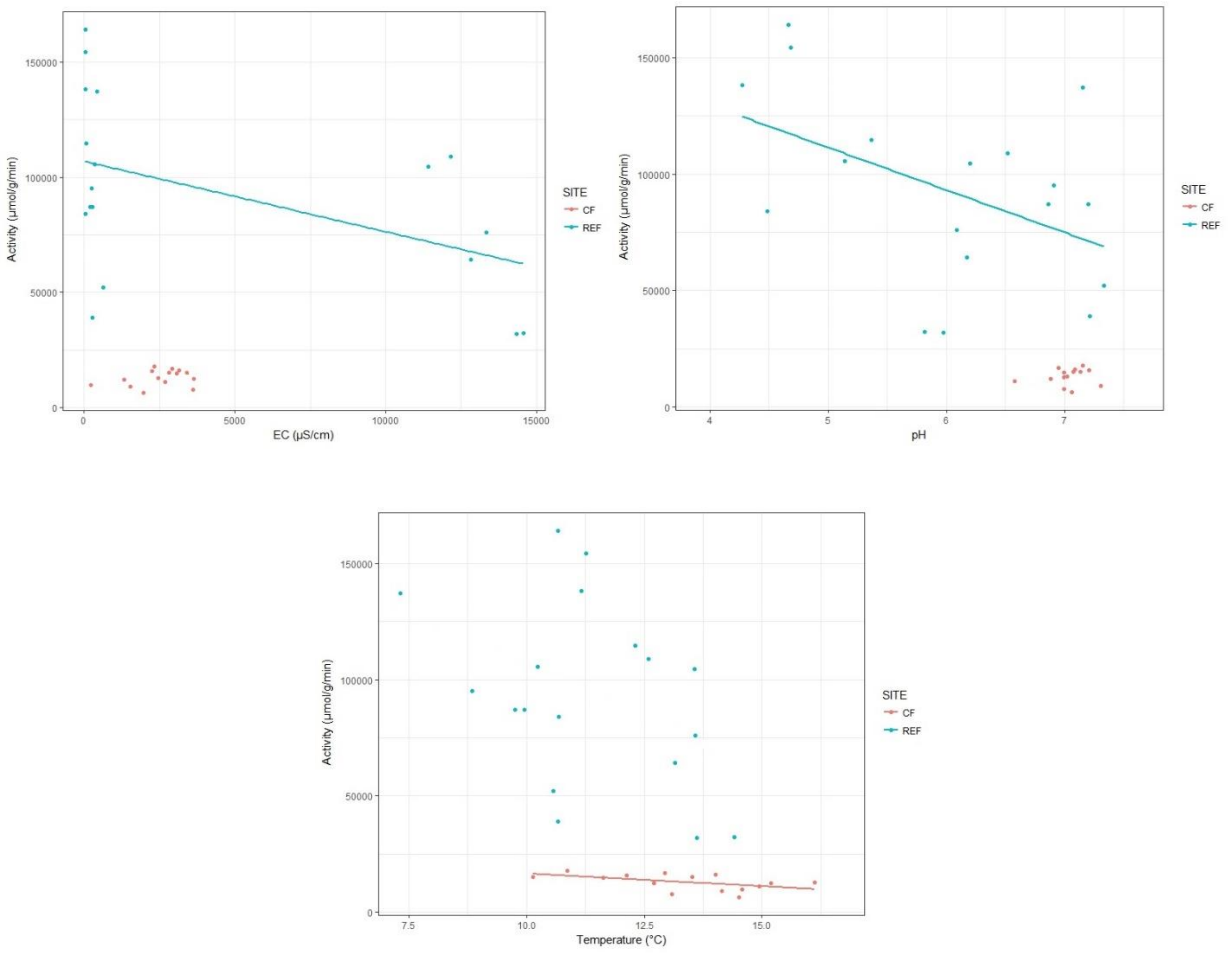

Figure A.22: *β-glucosidase activity vs average electrical conductivity (EC; top left), average pH (top right), and average soil temperature (℃; bottom centre) at the constructed fen (CF) and reference sites (REF). Activity was determined from organic matter collected at 15 cm depth at each site. The EC and pH was obtained at 15 cm and 30 cm depths, and soil temperature was obtained at 5 cm and 10 cm depths. Measurements were collected every 2 – 3 weeks during the growing season (May – September).*

Arylsulphatase

Arylsulphatase activity did not vary significantly across the vegetation ($F_{2,9} = 0.99$, $p = 0.4090$) or mulch treatment plots ($F_{1,9} = 0.0074$, $p = 0.9332$) at the constructed fen. Arylsulphatase activity also did not vary significantly across either site types ($F_{2,12} = 1.0$, $p = 0.3981$) or microforms ($F_{1,12} = 0.11$, $p = 0.2663$) at the reference sites. In general, Arylsulphatase activity was lower at the constructed fen (834 µmol/g/min) as compared to the reference sites (4335 µmol/g/min; $F_{1,31} = 114.4$, $p < 0.001$). Arylsulphatase activity did not show a significant relationship with any of the environmental variables.

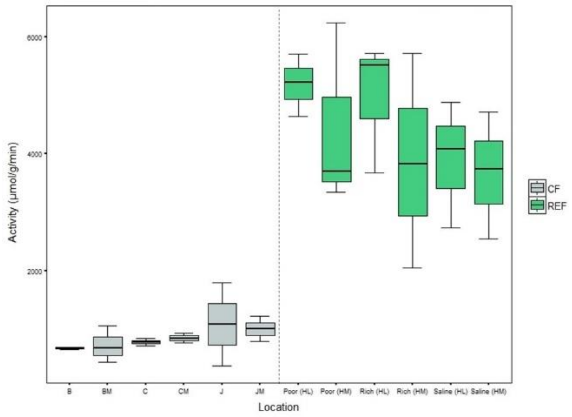

Figure A.23: *Arylsulphatase activity at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Activity was determined from organic matter collected at 15 cm from each location.*

β-D-xylosidase

β-D-xylosidase did not vary significantly across the vegetation ($F_{2,9} = 2.96$, $p = 0.1031$) and mulch treatment plots ($F_{1,9} = 0.76$, $p = 0.4067$) at the constructed fen. β-D-xylosidase activity did vary significantly across the site types ($F_{2,12} = 11.85$, $p = 0.001445$) and microforms ($F_{2,12} = 5.39$, $p = 0.03860$). β-D-xylosidase activity was the highest at the poor fen (33903 µmol/g/min) and lowest at the rich fen (17651 µmol/g/min), with hollows showing higher activity (26928 µmol/g/min) as compared to hummocks (18930 µmol/g/min). In general, β-D-xylosidase activity was lower at the constructed fen (3701 µmol/g/min) as compared to the reference sites (23370 µmol/g/min; $F_{1,31} = 56.09$, $p < 0.001$). β-D-xylosidase activity only showed a negative relationship with pH that was significant at the reference sites ($R^2 = 0.54$, $F_{1,16} = 20.82$, $p < 0.001$) and not at the constructed fen.

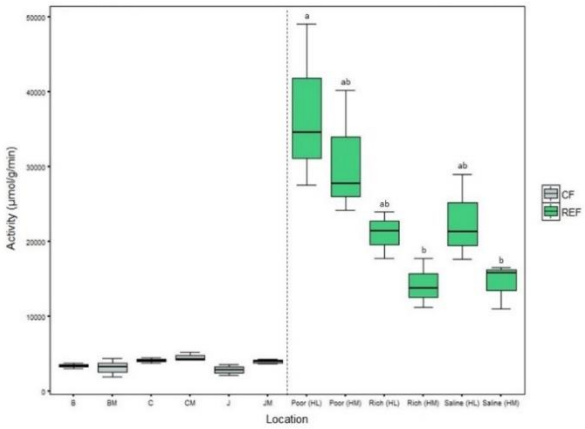

Figure A.24: *β-D-xylosidase activity at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Activity was determined from organic matter collected at 15 cm from each location. Letters indicate significant differences between each treatment plot at the reference sites. Locations with the same letter(s) indicates no significant difference.*

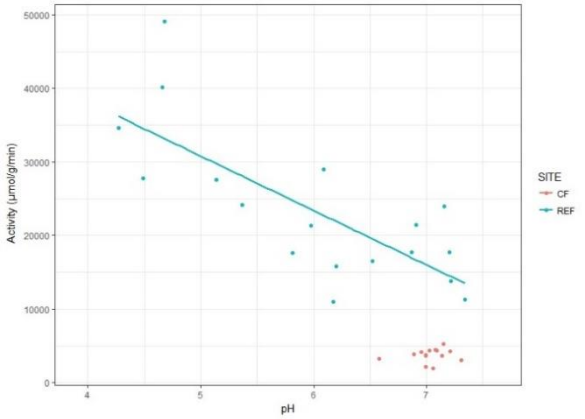

Figure A.25: *β-D-xylosidase activity vs average pH at the constructed fen (CF) and reference sites (REF). Average pH was determined from 15 cm and 30 cm depths measured every 2 – 3 weeks during the growing season (May – September). Activity was obtained from organic matter collected from 15 cm depth at each location.*

N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase activity did not vary significantly across the vegetation ($F_{2,9} = 1.61$, $p = 0.2527$) or mulch treatment plots. ($F_{1,9} = 1.02$, $p = 0.3389$) at the constructed site. N-acetyl-β-D-glucosaminidase activity also did not vary significantly across the site typess ($F_{2,12} = 1.89$, $p = 0.1941$) and microforms ($F_{1,12} = 0.23$, $p = 0.6431$) at the reference sites. In general, N-acetyl-β-D-glucosaminidase activity was lower at the constructed fen (12367 µmol/g/min) as compared to the reference sites (45188

µmol/g/min; $F_{1,31} = 19.07$, $p < 0.001$). N-acetyl-β-D-glucosaminidase activity only showed a negative relationship with temperature that was only significant at the reference sites ($R^2 = 0.18$, $F_{1,16} = 4.62$, $p = 0.04726$) but not at the constructed fen.

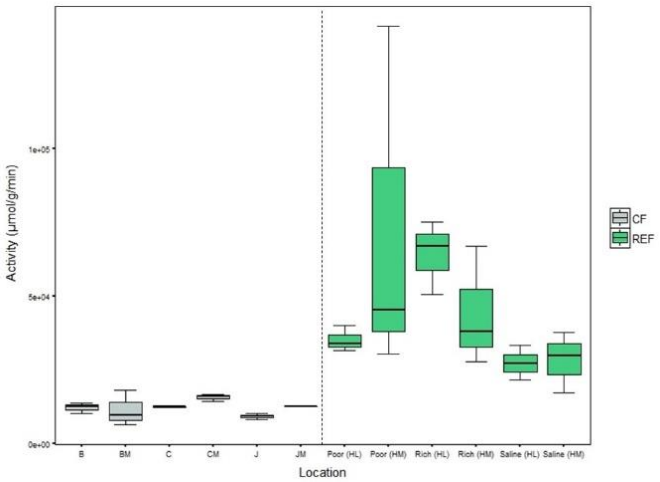

Figure A.26: *N-acetyl-β-D-glucosaminidase activity at the constructed fen (CF) and reference sites (REF). CF locations include plots of* Carex *(C),* Juncus *(J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Activity was determined from organic matter collected at 15 cm from each location.*

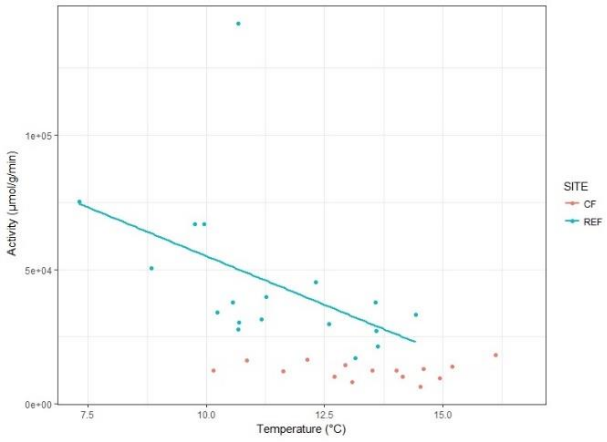

Figure A.27: *N-acetyl-β-D-glucosaminidase activity vs average soil temperature (℃) at the constructed fen (CF) and reference sites (REF). Soil temperature was collected at 5 cm and 10 cm depths during the growing season (May – September). Activity was obtained from organic matter collected at a 15 cm depth.*

Phosphatase

Phosphatase activity did not vary significantly across the vegetation ($F_{2,9} = 0.45$, $p = 0.6525$) and mulch treatment plots ($F_{1,9} = 0.70$, $p = 0.7412$). Phosphatase activity did vary significantly across both the site

types ($F_{2,12}$ = 10.10, p = 0.00268) and microforms ($F_{1,12}$ = 6.22, p = 0.02824). Phosphatase activity was the highest at the poor fen (188153 µmol/g/min) and lowest at the rich fen (105333 µmol/g/min), with hollows showing higher activity (158264 µmol/g/min) as compared to hummocks (118518 µmol/g/min). In general, phosphatase activity was lower at the constructed fen (33985 µmol/g/min) as compared to the reference sites (13891 µmol/g/min; $F_{1,31}$ = 51.61, p < 0.001). Phosphatase activity showed a negative relationship with pH that was significant at both the constructed fen (R^2 = 0.50, $F_{1,12}$ = 13.86, p < 0.01) and reference sites (R^2 = 0.42, $F_{1,16}$ = 13.43, p < 0.01).

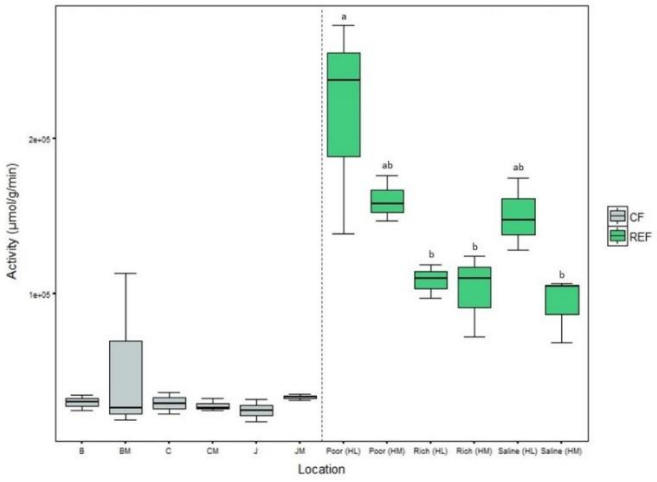

Figure A.28: *Phosphatase activity at the constructed fen (CF) and reference sites (REF). CF locations include plots of Carex (C), Juncus (J) and bare (B), as well as mulch (M) treatments. REF locations include saline fen, rich fen, and poor fen, as well as hummocks (HM) and hollows (HL). Activity was determined from organic matter collected at 15 cm from each location. Letters indicate significant differences between each treatment plot at the reference sites. Locations with the same letter(s) indicates no significant difference.*

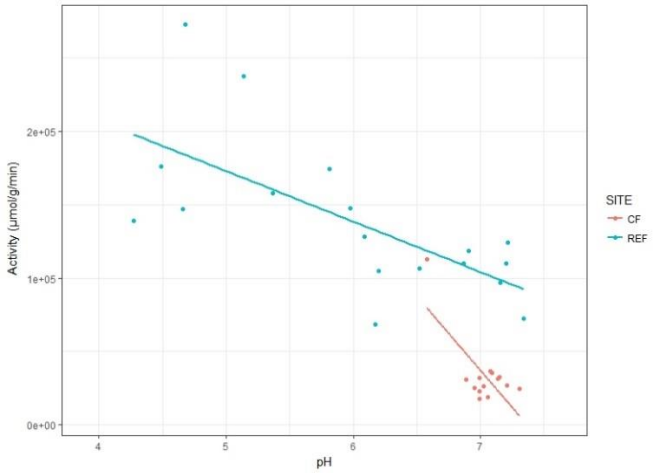

Figure A.29: *Phosphatase activity vs average pH at the constructed fen (CF) and reference sites (REF). Average pH was measured at 15 cm and 30 cm depths every 2 – 3 weeks during the growing season (May – September). Activity was obtained from organic matter collected at a 15 cm depth from each location.*

APPENDIX B:

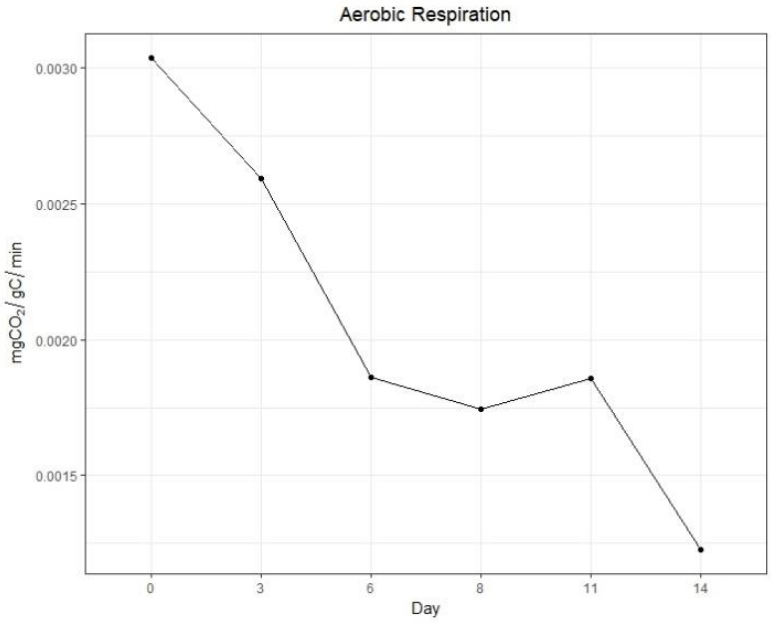

Figure B.9: *Microbial respiration rates under aerobic conditions through the course of a 14-day incubation. Respiration rates were obtained through the linear change in concentration from sampling occurring every three days. Averages from three replicates of each of the 13 treatments consisting of single, double and triple combinations of peat, Carex aquatilis or Juncus balticus, and wood or straw are represented.*

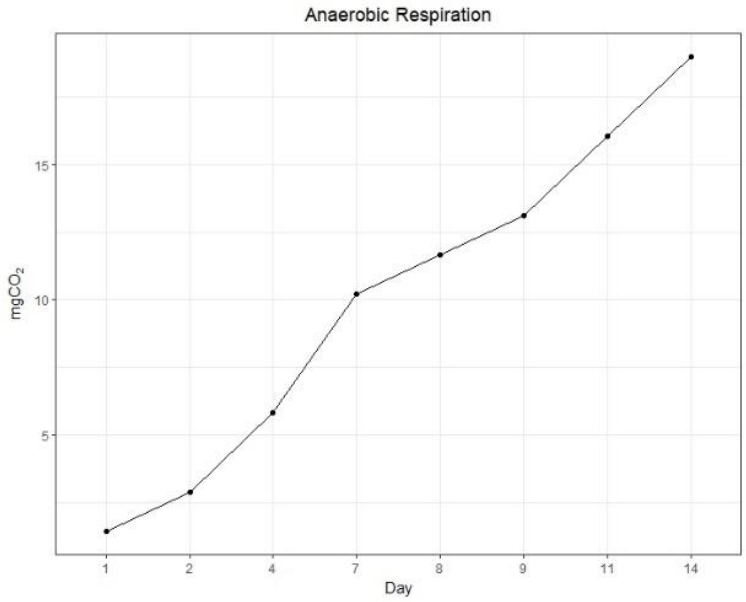

Figure B.10: *Microbial respiration rates under anaerobic conditions through the course of a 14-day incubation. Respiration rates were obtained through the linear change in concentration from eight sampling events. Averages from three replicates of each of the 13 treatments consisting of single, double and triple combinations of peat, Carex aquatilis or Juncus balticus, and wood or straw are represented.*

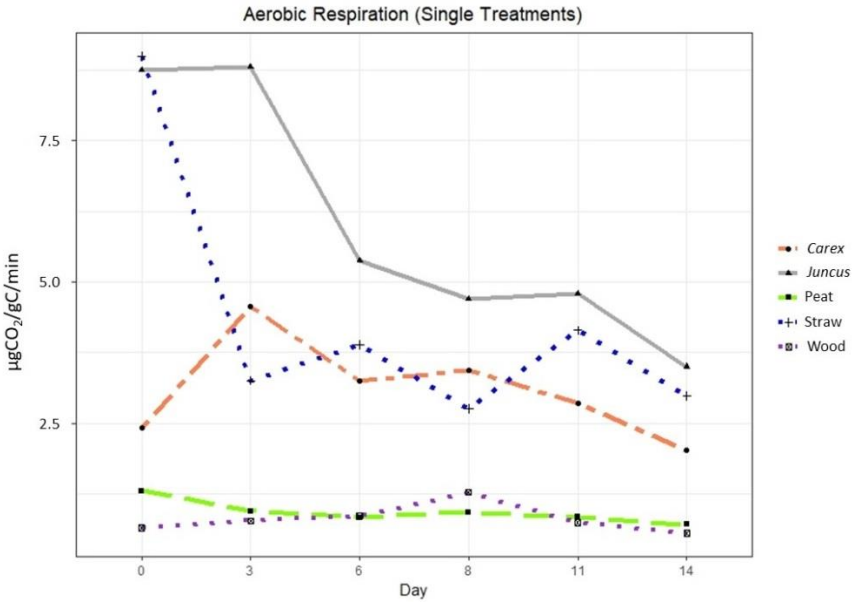

Figure B.11 *Microbial respiration rates under aerobic conditions through the course of a 14-day incubation. Respiration rates were obtained through the linear change in concentration from four sampling events occurring every three days. Averages were obtained from the single treatments of the substrate (peat), sedges (Carex aquatilis and Juncus balticus), and amendments (straw and wood).*

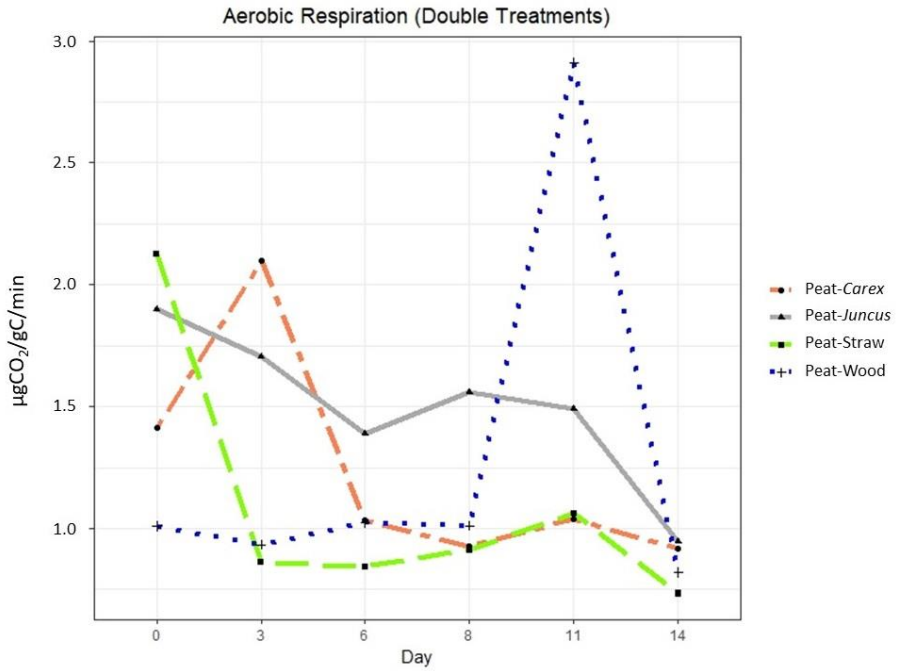

Figure B.12*: Microbial respiration rates under aerobic conditions through the course of a 14-day incubation. Respiration rates were obtained through the linear change in concentration from four sampling events occurring every three days. Averages were obtained from the double treatments of the substrate (peat) and one of each of the sedges (Carex aquatilis or Juncus balticus) or amendments (straw or wood).*

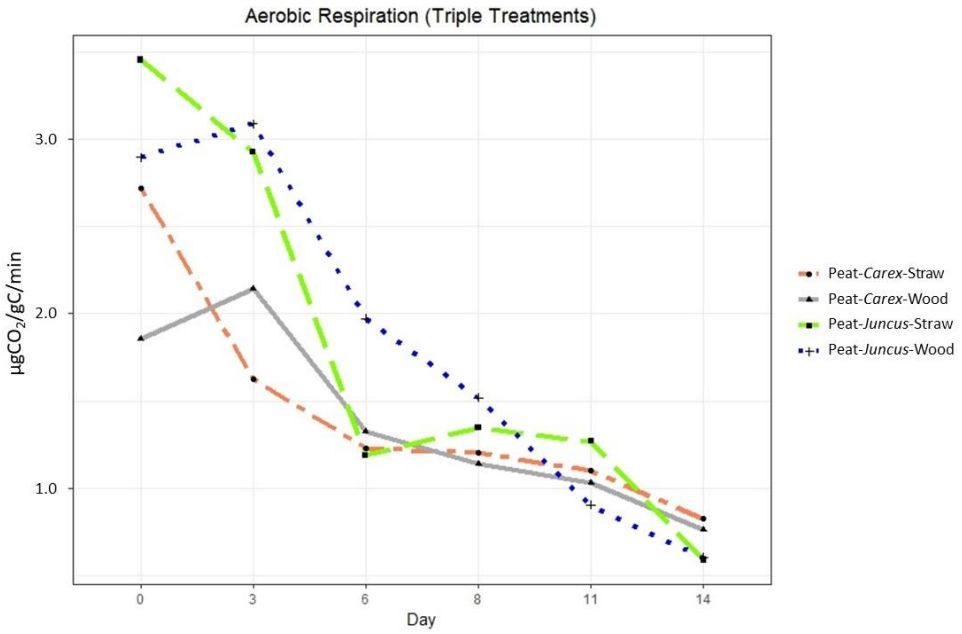

Figure B.13*: Microbial respiration rates under aerobic conditions through the course of a 14-day incubation. Respiration rates were obtained through the linear change in concentration from four sampling events occurring every three days. Averages were obtained from the triple treatments of the substrate (peat) and one of each of the sedges (Carex aquatilis or Juncus balticus) and amendments (straw or wood).*

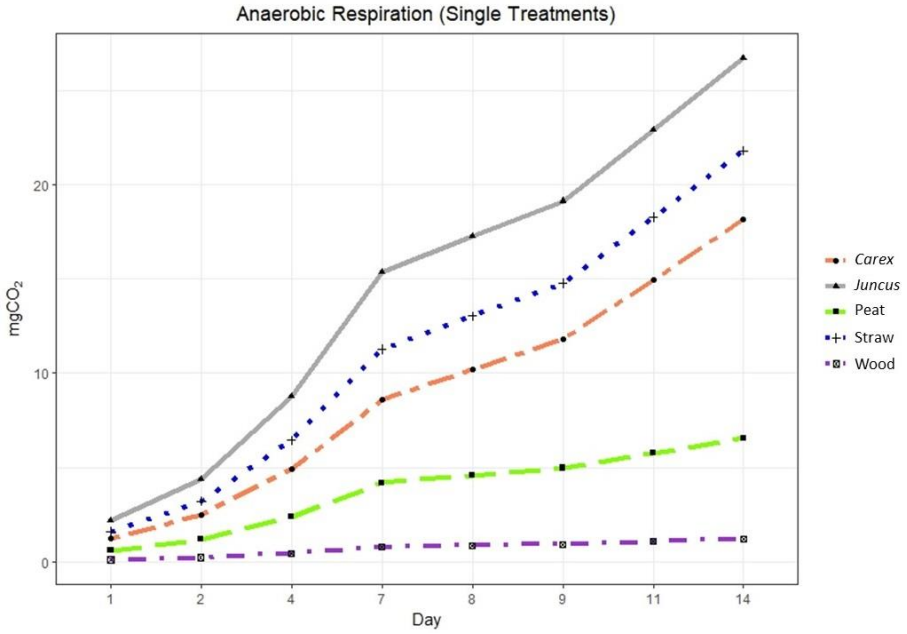

Figure B.14: Microbial respiration rates under anaerobic conditions through the course of a 14-day incubation. Respiration rates were obtained through the linear change in concentration from eight sampling events. Averages were obtained from the single treatments of the substrate (peat), sedges (Carex aquatilis and Juncus balticus), and amendments (straw and wood).

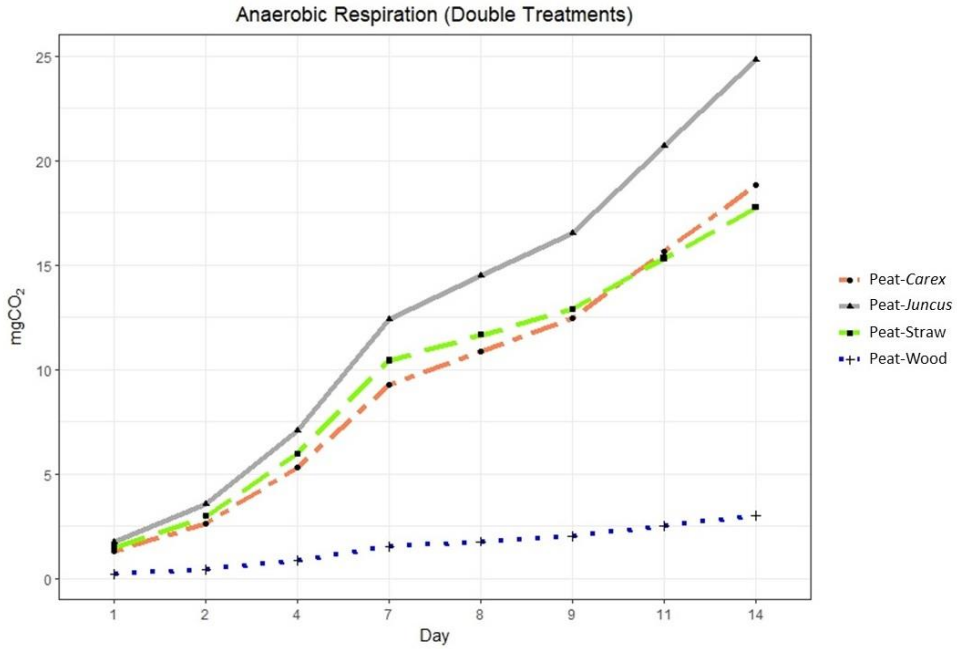

Figure B.15: Microbial respiration rates under anaerobic conditions through the course of a 14-day incubation. Respiration rates were obtained through the linear change in concentration from eight sampling events. Averages were obtained from the double treatments of the substrate (peat) and one of each of the sedges (Carex aquatilis or Juncus balticus) or amendments (straw or wood).

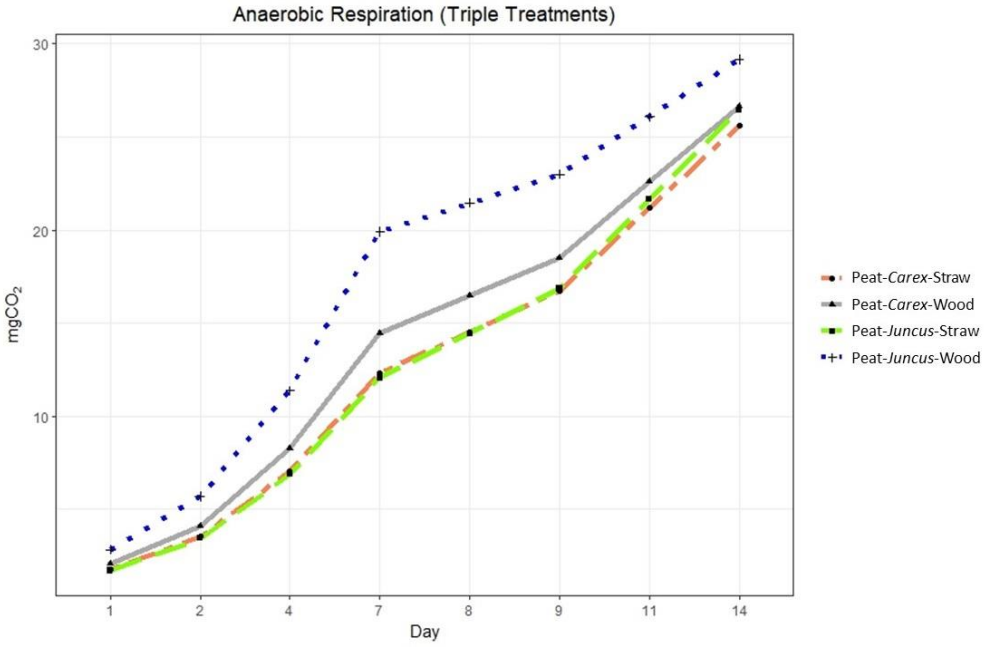

Figure B.16: *Microbial respiration rates under anaerobic conditions through the course of a 14-day incubation. Respiration rates were obtained through the linear change in concentration from eight sampling events. Averages were obtained from the triple treatments of the substrate (peat) and one of each of the sedges (Carex aquatilis or Juncus balticus) and amendments (straw or wood).*

Table B.1 *Anaerobic respiration rates during 14-day incubation ($mgCO_2/gC/min$)*

Day	P	C	J	S	W	PC	PJ	PS	PW	PCS	PJS	PCW	PJW
0-6	0.000414	0.000852	0.001522	0.001117	7.687E-05	0.000922	0.001231	0.001035	0.000152	0.001224	0.001196	0.001434	0.001976
7-14	0.000277	0.001105	0.001314	0.001218	5.201E-05	0.001105	0.001437	0.000848	0.000170	0.001534	0.001666	0.001410	0.001070

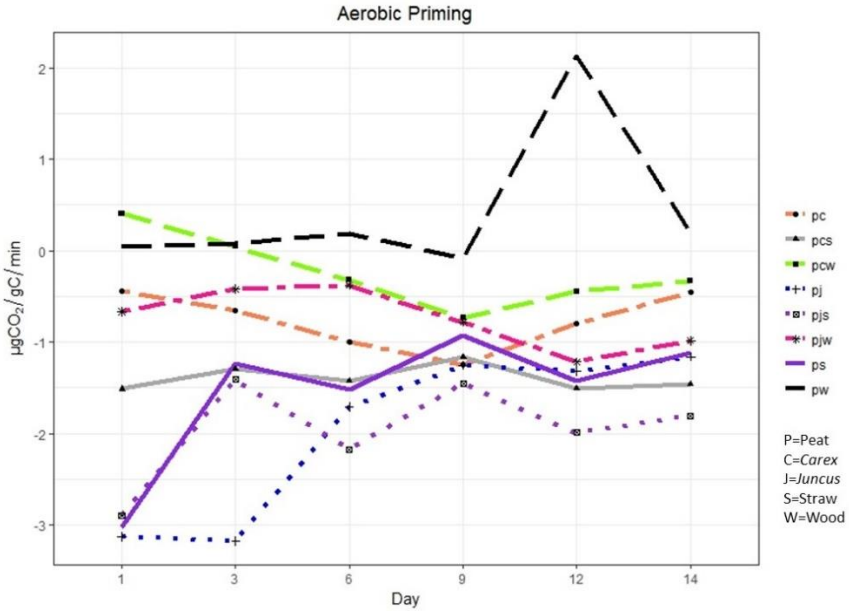

Figure B.17: *Priming rates under oxic conditions through the course of a 14-day incubation. Positive values indicate positive priming while negative values indicate negative rates of priming. Priming was calculated according to equation 5.*

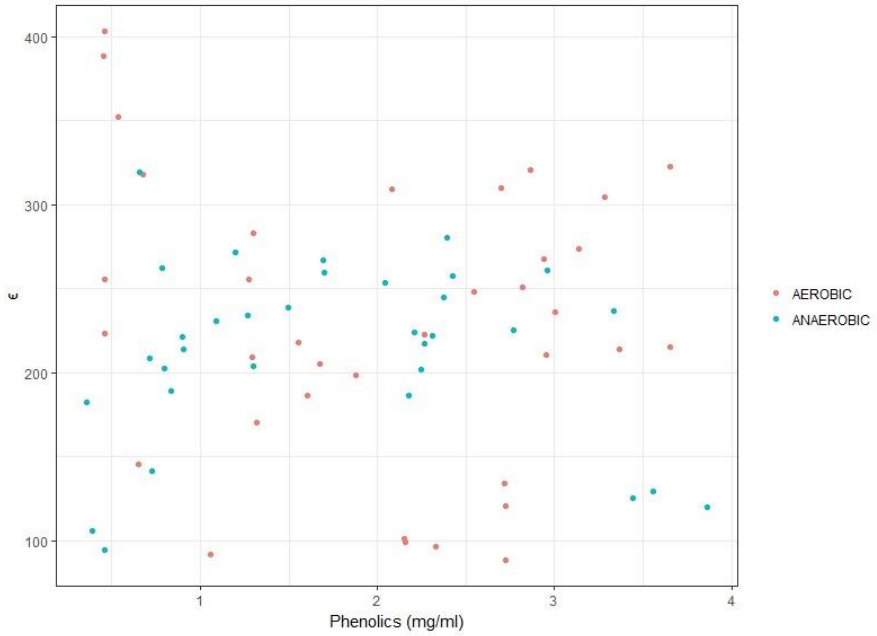

Figure B.18: *Phenolic compound concentration vs isotopic discrimination (ϵ) from a 14-day incubation under aerobic and anaerobic conditions. Treatments consisted of three replicates of 13 treatments consisting of consisting of single, double and triple combinations of peat,* Carex aquatilis *or* Juncus balticus*, and wood or straw. Phenolic compound concentrations were obtained from water extractions performed on the remaining material post-incubation. ϵ values were obtained from samples collected on the final sampling date during each of the incubations.*

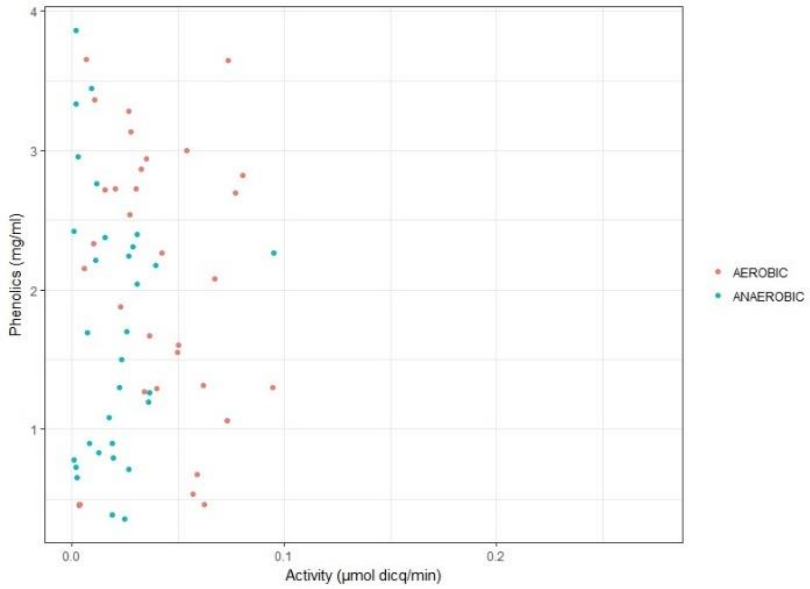

Figure B.19: *Phenolic compound concentration vs phenol oxidase activity from a 14-day incubation under aerobic and anaerobic conditions. Treatments consisted of three replicates of 13 treatments consisting of consisting of single, double and triple combinations of peat,* Carex aquatilis *or* Juncus balticus*, and wood or straw. Phenolic compound concentrations were obtained from water extractions performed on the remaining material post-incubation, as was phenol oxidase activity.*

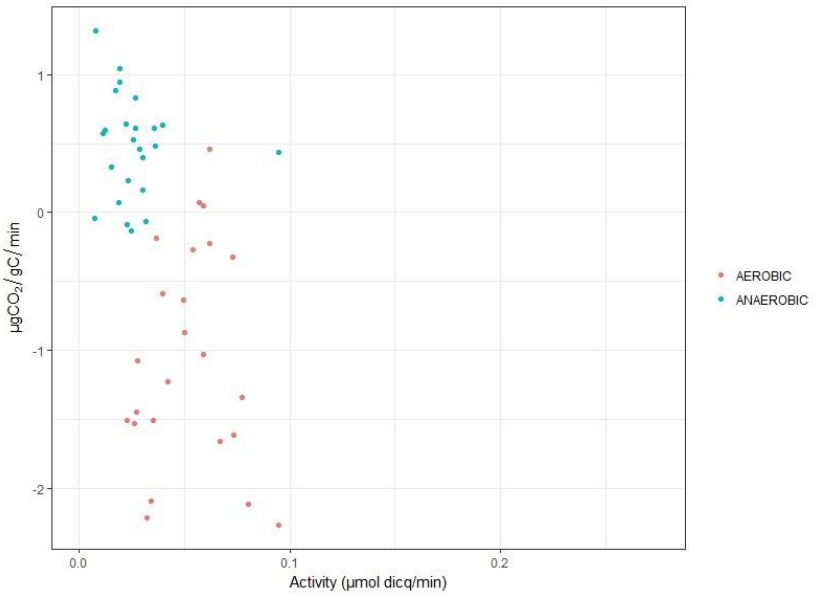

Figure B.20: *Phenol oxidase activity vs rates of priming from a 14-day incubation under aerobic and anaerobic conditions. Priming rates are averages from from each of the 13 treatments consisting of single, double and triple combinations of peat,* Carex aquatilis *or* Juncus balticus*, and wood or straw. Activity was obtained from each of the treatments following the incubations. Rates were determined by dividing the observed rate by the expected rate of respiration, which was calculated from each of the single treatments. Phenol oxidase activity was obtained from the remaining material post-incubation.*