Effects of seismic lines on peatland carbon cycling in boreal Alberta, Canada

by

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Author's Declaration

This thesis consists of material authored by me: 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.

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Statement of Contributions

To reflect the results of this research, this thesis is written in manuscript format. Three independent manuscripts to be submitted for publication are presented as chapters 2, 3 and 4 of this thesis. As a result, repeated information may be included.

Tentative titles and authorship of future publications:

Manuscript 1: Effects of Seismic Lines on Microbial Community Function and Soil respiration in Boreal Peatlands. Authors: Korsah, P., Strack, M., Xu, B.

Manuscript 2: Seismic lines alter methane cycling in boreal peatlands. Authors: Korsah, P., Strack, M., Davidson, S.

Manuscript 3: Impact of seismic lines on net primary productivity and decomposition rates in boreal peatlands. Authors: Korsah, P., Strack, M., Xu, B.

Korsah P conducted the field design and setup, data collection and analysis and wrote the thesis chapters. Assistance with data collection was provided by O. Trudeau and S.J. Davidson in 2018, C. Etsey, J. Fanson and M. Lemmer in 2019, and M. Bird and B. Xu in 2020. M. V. Date, Trevor and Price labs provided support, reagents, and equipment for microbial analysis in 2018. Strack, S.J. Davidson and B. Xu assisted with study planning and design, data processing and manuscript review. Each manuscript, including figures and tables, was written in its entirety by P. Korsah and reviewed by M. Strack.

Abstract

Peatlands serve as long-term carbon (C) sinks as well as a significant source of methane (CH_4) to the atmosphere. Over 134,000 km^2 of peatlands are in northern Alberta, a part of the boreal region of Canada where extensive industrial exploration and extraction activities are ongoing to access vast oil sands deposits. These anthropogenic disturbances, including a vast network of linear disturbances, such as seismic lines and roads, could impact long-term peatland C storage by altering ecohydrological conditions. Prior studies reported changes to hydrology, microclimatic conditions, and vegetation communities. Yet, the cumulative impact of these changes on peatland functions, that is, microbial functional activity, peat accumulation rates and carbon dioxide (CO_2) and CH_4 exchange is not very well understood. Due to rising concerns related to climate change and the need to develop nature-based climate interventions, peatland management should be of utmost importance to Canada, which is home to the largest global peatland C stock.

We therefore measured in-situ and in-vitro soil respiration, net primary production (NPP) and litter decomposition, and CH_4 emissions on eight seismic lines across one fen and two bog peatland sites affected by seismic exploration in northern Alberta and compared the results to adjacent natural areas. Soil respiration was slightly lower on seismic lines than from natural peatlands, likely due to minimal contributions of tree root respiration on the lines. Ground layer NPP was higher on the lines, but this did not offset the loss of overstory NPP. The litter decomposition rate was similar on and off the seismic line, but a shift in plant community composition towards species with more easily decomposable litter, particularly at the fen site, resulted in greater loss of litter overall. The potential peat accumulation rate, calculated as the difference between NPP and litter loss to decomposition over two years, was therefore lower on the seismic lines. This implies that recovery of an overstory in these wooded peatlands is

necessary to achieve pre-disturbance C accumulation rates. Methane emissions were significantly higher on the seismic lines, increasing 176% (fens) and 261–308% (bogs) compared to the adjacent natural peatland. Higher CH_4 emissions on the seismic lines were associated with warmer, wetter conditions and, at the fen site, higher sedge cover.

Results from this study provide important baseline information about C cycling in peatlands affected by seismic line disturbance. Our findings contribute to accurate greenhouse gas (GHG) reporting for anthropogenic disturbances in boreal peatlands and can be used to assess the potential benefits, from a C storage perspective, of restoration efforts aimed at returning forest cover.

Acknowledgements

Dedication

This research is dedicated to my 3 awesome 'Ps'- Percy Jnr, Penielle and Preston. My efforts

to protect the environment is to guarantee you have a safe future.

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List of Abbreviations

C   Carbon

CC   Carmon Creek (Bog Site)

CH_4 Methane

CO_2 Carbon dioxide

GHG   Greenhouse Gas

GEP   Gross Ecosystem Productivity

HV   Harmon Valley (Bog Site)

NEE   Net Ecosystem Exchange

NPP   Net Primary Production

PAR   Photosynthetically Active Radiation

P   Precipitation

SIR   Substrate Induced Respiration

T   Temperature

WT   Water Table

Chapter 1: Introduction and Literature Review

1.1 INTRODUCTION

Peatlands are wetlands containing carbon (C)-rich soils formed from the accumulation of organic matter deposits over thousands of years (Blodau, 2002; Loisel *et al*., 2014). Peatlands cover 3–4% of the earth's land surface, approximately 500 million hectares, and contain up to 1/3 of the world's soil C, which is double the amount of C in all terrestrial forests (UNEP, 2022). Approximately 13% of Canada is covered by peatlands (Xu *et al*., 2018), mostly in the boreal ecozone (Tarnocai *et al*., 2011). Canadian peatlands form part of the northern peatlands, occurring in the high and mid latitudes of the northern hemisphere (Xu *et al*., 2018) and across the north, have a projected C storage of 300–550 Pg C (1 Pg C = 10^{15} g C) (Limpens *et al*., 2008; Yu, 2012; Hugelius *et al.,* 2020). Canada, therefore, possesses the world's largest peatland C stock (Harris *et al*., 2021) and has a very crucial role to play in global climate regulation. Peatlands are also responsible for significant methane (CH_4) source to the atmosphere estimated at 5–10% of global CH_4 emissions (Gorham 1991; Frolking *et al*., 2011).

Aside from the critical roles boreal peatlands play in C cycling, they also provide ecosystem services such as water storage, habitats for some endangered fauna and flora (Filicetti *et al*., 2019), and economic benefits including agriculture and forestry (e.g., horticultural peat and

timber production). However, the threats of a warming climate and increasing anthropogenic disturbances in peatlands, further amplifies the potential for peatlands to increase their greenhouse gas (GHG) emissions altering them from C sinks to C sources (Hugelius *et al.,* 2020; Harris *et al.*, 2021). Linear disturbances such as roads and seismic lines constitute some of the most predominant anthropogenic disturbances in Canada's western boreal forests (Pasher *et al.*, 2013) with estimates of 10 km of seismic lines per 1 km^2 of forest (Filicetti *et al.*, 2018). Boreal peatlands are increasingly impacted by anthropogenic disturbances because of natural resource exploration and extraction, but data on the effect of these disturbances on peatland C storage and exchange is scarce. Hence, this thesis investigated the impact of seismic lines on C cycling in boreal peatlands.

1.2 Relevant Literature

1.2.1 Peatlands

Wetland ecosystems with a minimum of 40 cm of organic soil (peat) accumulation are designated as peatlands as per the Canadian Wetland Classification System (NWWG, 1997). Canadian peatlands are characterized by prolonged dry cold winters, typically more than 5 months, and short cool to warm summers (1 to 4 months) (Larsen 1980). Peatlands connected to surface water and/or groundwater are known as fens (minerotrophic peatlands) and have

water table position (WT) at or above the surface with more humified peat and higher pH soils (Gorham 1991). The gradient of alkalinity can further segregate fens into rich fens and poor fens with the latter being less alkaline (Vitt *et al*., 2000). Fen vegetation is dominated by brown mosses, graminoids, trees (e.g., white cedar and tamarack) and non-ericaceous shrubs (Bridgham *et al*., 1998; Weltzin *et al*., 2003). When peatlands are disconnected from groundwater, and only receive water at the surface from precipitation, bogs (ombrotrophic peatlands) are formed that are generally low in nutrients, have lower pH, and WT often below the surface. Peat in bogs is less humified and vegetation in North America is dominated by *Sphagnum* mosses, black spruce trees and evergreen ericaceous shrubs (Vitt, 1994; Bridgham *et al*., 1998).

Changes in peatland hydrology and vegetation over time throughout peatland development lead to complex feedback mechanisms altering peatland types (Waddington *et al*., 2009) and modifying landscapes/microtopography (Clymo *et al*., 1998). Spatial variation in peat accumulation, arising from differences in plant productivity and litter decomposition (Pouliot *et al*., 2011) and water table fluctuations, result in disparities in peatland surface elevation leading to the formation of microforms/horizontal stratification (Foster & Fritz, 1987). Depressions in the peat surface are known as hollows, elevated microforms are described as hummocks (approximately 0.3 m or greater), while flat areas are designated as lawns (Nungesser, 2003). Hummocks have the deepest WT, whereas hollows and lawns occur at

elevations closer to the WT. The horizontal stratification in peatlands influences the vegetation community and microclimatic conditions hence varying gross primary productivity and decomposition rates (Belyea & Baird 2006; Bubier *et al.,* 2007).

Peatlands also exhibit vertical stratification into oxic and anoxic layers (Clymo *et al.* 1998). The depth of the vertical layers is primarily controlled by the WT, which is influenced by the amount of precipitation received and hydrology of the peatland affecting vertical and horizontal water movement. The depth of the oxic and anoxic layers are important controls on aerobic and anaerobic processes that exert their influence on C cycling.

1.2.2 Peatland Carbon Cycle

Carbon is cycled through the atmosphere, hydrosphere, lithosphere and terrestrially through soil and vegetation (Bhatti *et al*., 2012). Over the years, terrestrial and ocean C sinks have been indispensable in the regulation of atmospheric concentrations of greenhouse gases (GHGs; Ballantyne *et al*., 2012; Friedlingstein *et al*., 2020). Globally, peatlands contain very dense organic matter stocks and have accumulated up to 600 Pg C (Hugelius *et al*., 2020; UNEP, 2022), making them the largest terrestrial C sink. Compared to tropical forests, peatlands store twice as much C per unit area (Vitt *et al.,* 2009), making them an important component of the global C cycle; hence any disturbance risks destabilizing and potentially releasing large C stores and impacting the global climate (Ciais *et al*., 2013). Carbon accumulation in peatlands

occurs when net primary production (NPP) exceeds organic matter decomposition, which in northern peatlands is slow due to their natural characteristics of low pH, low temperature, anoxic waterlogged conditions, and vegetation with low nutrient content and recalcitrant litter (Johnson & Damman, 1993; Bridgham & Richardson, 2003). NPP rates are affected by the vegetation community structure and nutrient availability, especially nitrogen and phosphorus that are limiting nutrients in peatlands (Iversen *et al*., 2010). Boreal peatlands of North America are often treed (Turetsky *et al*., 2002), leading to greater living biomass in trees compared to open peatlands (Vitt *et al*., 2000). The presence of trees also contributes to higher accumulation rates resulting from higher NPP to decomposition ratios (Clymo *et al*. 1998). NPP rates respond to changes in peatland conditions; for example, shallow WT has been shown to decrease the productivity of trees due to high oxygen stress on roots (Dimitrov *et al*., 2014). Carbon availability from NPP has also been reported as a control on soil microbial growth which influences microbial mineralization of organic matter (Fisk *et al*., 2003).

The slow rate of decomposition in boreal peatlands has been outlined as the main driver of peat accumulation (Vitt, 1994; Bragazza *et al*., 2007). For example, the dominance of bryophytes in the understory vegetation of peatlands and their slow decomposition due to recalcitrant litter is essential for C sequestration. Factors that reportedly control decomposition rates include microbial community (Aerts, 1997; Andersen *et al.,* 2010), vegetation community and litter quality (Johnson & Damman, 1993; Strakova *et al.,* 2012), moisture content (Macrae *et al.,*

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2013), peat temperature, and WT (Wieder, 2006). Of the known factors, microbes exert a profound influence on the balance between storage and mineralization of organic matter (Fisk *et al*., 2003). Alteration to any of these conditions or disturbances which could lead to exposure of peat to air could potentially accelerate organic matter decomposition (Kemper & Macdonald, 2009; Caners & Lieffers, 2014).

Decomposition in waterlogged peatlands is mostly anaerobic and therefore supports methanogenesis (Bridgham *et al*., 2013) and the release of significant amounts of CH_4 (Gorham 1991; Frolking *et al*., 2011). The process involves the anaerobic degradation of labile C sources by methanogenic *Archaea* (Zinder, 1993; Lai, 2009; Bridgham *et al*., 2013). The amount of CH_4 released into the atmosphere is critical since CH_4 has a global warming potential (GWP) 27–30 times higher than CO_2 over a 100-year timescale (IPCC, 2021). Boreal and subarctic peatlands release an estimated 17–61 Tg of CH_4 annually (Bridgham *et al*., 2013). Abdalla et al. (2016) also reports an approximate emission of 36 Tg CH_4-C yr^{-1} from Northern peatlands. Daily CH_4 emission averages are estimated at 56.36 mg CH_4 m^{-2} d^{-1} (fens) compared to 25.98 CH_4 m^{-2} d^{-1} (bogs) (Turetsky *et al*., 2014). The production of CH_4 in peatlands is controlled by multiple factors including WT (Bubier *et al*., 1993; Pypker, 2013), peat temperature (Lai, 2009; Pypker, 2013), peatland vegetation community composition, and litter quality (Bridgham *et al*., 2013, Turetsky *et al*., 2014; Strack *et al*., 2017). The amount of CH_4 released into the atmosphere is, however, less than the amount of CH_4 produced due to

oxidation by methanotrophic bacteria, largely in the oxic zone (Valentine et al., 1994; Sundh et al., 1995). The depth of the WT is therefore critical in controlling the amount of CH_4 oxidized and eventually released to the atmosphere. Both methanogenesis and methanotrophy are temperature dependent, although methanogenesis has a steeper increase in response to temperature (Dunfield et al., 1993). CH_4 is released to the atmosphere via the following transport pathways: plant-mediated transport, ebullition, and diffusion (Limpens et al., 2008; Lai, 2009; Bridgham et al., 2013). Plant-mediated transport, which is influenced by the type of vegetation community, accounts for the bulk (30-100%) of CH_4 flux (Bridgham et al., 2013) and often bypasses oxic layers reducing the volume of CH_4 oxidized (King et al., 1998, Noyce et al., 2014). Vascular plants are therefore directly responsible for greater CH_4 fluxes in peatlands (Nugent, 2019) and indirectly produce root exudates (labile C) that can accelerate microbial processes such as methanogenesis (Bridgham et al., 2013). Nonetheless they also contribute to CH_4 consumption by enhancing oxidation in the rhizosphere through radial oxygen loss from roots facilitating the oxidation of CH_4 (Turetsky et al., 2014).

1.2.3 Microbial Activity in Peatlands

Peatland microbes drive soil C cycling. Aside the crucial role of organic matter decomposition and subsequent nutrient cycling (McGuire & Treseder, 2010), microbial activity has been identified as a direct control on CH_4 fluxes (Bubier et al. 1993). Peatland microbial

communities include a variety of fungal, bacterial, and archaeal organisms (Preston *et al*. 2012; Kitson & Bell, 2020), come with specialized functions, including CH_4 producers and oxidizers, sulfate reducers, nitrogen fixers and denitrifiers (Sundh *et al*., 1995; Peltoniemi *et al*., 2016). Microbial studies in wooded peatlands, especially low-latitude areas remain scarce (Wang *et al*., 2021a), with majority of boreal soil microbial studies focused on fungi (Allison & Treseder, 2011). Hence information available on the specific distribution and diverse functions of boreal bacteria and *Archaea* as well as the conditions that influence peatland microbial community decomposition function is limited (McGuire & Treseder, 2010; Allison & Treseder, 2011). There is a consensus though, on microbial associations with complementary enzymatic activities being responsible for nutrient cycling and decomposition under the influence of environmental variables (Anderson *et al*., 2013). The bulk of microbial activities are known to be influenced by C and nutrient availability, and environmental variables such as temperature, pH, and soil water availability, although the feedback mechanisms involved in the effects of the microbial controls, for example mosses acidifying their own environment, make it difficult to disentangle specific effects (Fisk *et al*., 2003). There is some agreement on the hypothesis of a positive correlation between microbial composition and plant species diversity (Robroek *et al*., 2021). Although complex, some authors attribute C substrates from root exudates and decomposing litter as the main drivers (Fisk *et al*., 2003; Strakova *et al.,* 2012). Recent studies under controlled conditions were indicative of shifting vegetation

communities and temperature increases driving microbial activity including methanogenesis (Wilson *et al*., 2021).

Despite the uncertainty about microbial community responses to peatland disturbances, microbial communities reportedly lagged in structural and functional recovery compared to other indicators (Anderson *et al.,* 2006, 2010). Since anthropogenic disturbances in peatlands affect above- and belowground communities and could alter CH_4 and CO_2 cycling (Strack *et al*., 2018), for example, warming temperatures could increase microbial decomposition rates (Wilson *et al*., 2021), updated techniques in microbiology can be leveraged to obtain more information on the diversity and composition of peatland microbes as well as how different environmental variables affect microbial function. Microbes are ideal candidates as environmental sensitivity indicators due to their large surface to volume ratio and physiological characteristics such as their permeable cell membranes (Chambers *et al*., 2016). These traits have been exploited over time to study microbial responses to local changes in conditions including substrate quality (Bossio & Scow 1998), nutrient availability (Troxler *et al*., 2012), community structure and function (Andersen *et al*., 2013) and these techniques including microbial profiling and sequencing are applicable to linear disturbances in peatlands.

1.2.4 Linear Disturbances

The Canadian province of Alberta has the world's third-largest proven oil reserves with many of these sites intersecting with boreal peatlands (Vitt *et al.,* 1996), since about 16% of Alberta is covered by peatlands (Tarnocai *et al*., 2011). Dominant anthropogenic disturbances created by the resource extraction industry in the region include access roads, seismic lines, pipelines and well pads (Vitt *et al.,* 1996). The most prevalent of these are seismic lines or cut lines (Schneider *et al*., 2010) constructed as long linear corridors of varying width (1.5 to 10 m) for geological surveys (Lee & Boutin 2006; Bayne *et al*., 2011). According to Strack *et al.,* (2019), over 345,000 km of seismic lines, covering an area of 1900 km^2, have been constructed in peatlands in Alberta alone. Seismic line creation involves the clearing of elongated narrow strips of forests, placing and detonating of explosives, and tracking the seismic wave frequency to detect and quantify hydrocarbon reservoirs (Dabros *et al*., 2018). The common types of seismic lines deployed are two-dimensional/legacy/conventional seismic lines and three dimensional seismic (3-D, also called low-impact) lines (Lee & Boutin, 2006). Legacy seismic lines are 5–10 m wide and cut with bulldozers and heavy machinery and are reported as some of the most extensive disturbance in Canadian peatlands (Kemper & Macdonald, 2009; Schneider *et al*., 2010; Pasher *et al*., 2013). These types of seismic lines were deployed across the boreal forest between 1950s and the early 2000s (Lee & Boutin 2006; Van Rensen *et al*., 2015). The impacts of legacy lines, linked to the heavy machinery use and clearing and

exploration methodology, on the boreal forest were documented over time including the failure

of felled trees to significantly regenerate, even on lines from the 1950s (Lee & Boutin, 2006),

followed by calls for reclamation (Komers & Stanojevic, 2013).

Modern, low-impact techniques, such as the introduction of 3-D seismic lines were therefore

introduced after 1995 as an intervention to mitigate some of the impacts (Lee & Boutin, 2006;

Dabros *et al*., 2018; Filicetti *et al*., 2019). However, although 3-D lines are narrower (1.5–5

m) and made with smaller equipment (e.g., chainsaws and mulchers), a greater density of lines

is required, with grid spacing between 50–300 m (Lee & Boutin, 2006; Dabros *et al*., 2018),

renewing questions about their low-impact status on the environment.

1.2.5 Research Gaps

Seismic lines crossing boreal peatlands are directly responsible for loss of biodiversity and

habitat destruction (Dyer *et al*., 2002; Venier *et al*., 2014) and the removal of tree canopy

biomass potentially impacting C uptake (Strack *et al*., 2018). Tree removal and opening of the

canopy has been linked to changes in the microclimatic conditions (Dabros *et al*., 2017;

Stevenson *et al*., 2019; Franklin *et al*., 2021) that likely affect understory vegetation and

microbial communities. Microforms have been eliminated and peat compressed (Caners &

Lieffers, 2014; Stevenson *et al*., 2019) by line creation with between 5–20 cm of organic peat

removed on legacy lines (Bliss and Wein, 1972). The effects and resulting changes from the

creation of lines in peatlands have reportedly increased peat temperature and moisture as well as CH_4 emissions (Strack *et al.,* 2018) and impacted vegetation communities (Davidson *et al.*, 2021). However, the impact of these changes in vegetation and microbial community function on C cycling or peat accumulation rates in boreal peatlands persist as significant research gaps. Very few direct measurements of C exchange from peatlands affected by seismic lines disturbance have been reported, making it difficult to assess whether the observed structural changes in the ecosystem are in fact altering C cycling. Yet, these changes could potentially convert peatlands from C sinks to sources (Limpens *et al.*, 2008; Strack *et al.,* 2018; Dabros *et al.*, 2018).

The need to improve caribou habitat and survival rates has triggered the implementation of ongoing restoration programs on seismic lines (Alberta Government, 2017; Dabros *et al.*, 2022). However, the lack of understanding on how seismic line disturbances have affected peatland functions, specifically C cycling, limits accurate assessment on the potential effects of these restoration activities further altering peatland C cycling or accelerating a return to pre-disturbance function. The effects of seismic lines on peatland C cycling and the biological and physical drivers of these effects are understudied and therefore require further investigation to enhance our comprehension of fundamental processes and relationships for accurate GHG emissions reporting, predicting ecosystem recovery, and designing restoration projects.

1.2.6 Objectives

Although only about 3–4 million hectares, or <2 % of total peatland area, of North America's peatlands are degraded, the biggest threats are currently posed by increasing oil and gas activity and warming temperatures that could lead to permafrost thaw and increased intensity and frequency of wildfires (Hugelius *et al.,* 2020; UNEP, 2022). Peatland management has therefore been highlighted as an important nature-based climate solution (Strack *et al*., 2022) and boreal peatlands are no exception considering they are more vulnerable to the additional impacts of global warming (Vitt *et al*., 2009). Earlier studies discussed above suggest that the controls on C sequestration and CH_4 production/emission in peatlands are affected by the construction of seismic lines in peatlands, but on the ground data is lacking. Hence this thesis addresses the knowledge gaps identified above and investigates the effect of seismic line disturbances on:

a) soil respiration and microbial aerobic organic matter turnover in a wooded fen and bogs (Chapter 2)

b) net primary productivity and decomposition rates of dominant species in vegetation communities in a wooded fen and bogs (Chapter 3)

c) peatland CH_4 exchange in a wooded fen and bogs (Chapter 4)

The experimental design and subsequent project execution are summarized in Figure 1-1.
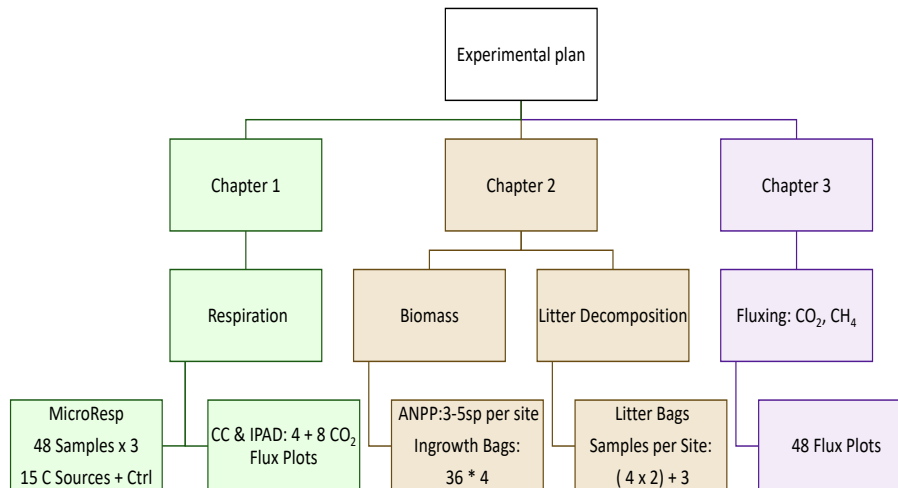
Figure 1-1 Experimental design showing workflow, processes, and related sample sizes. MicroResp (in-vitro microbial respiration measured via well plates). CC refers to Carmon Creek Bog site with 3-D seismic lines, whiles IPAD refers to fen site.

A general summary of the study sites and corresponding images are shown in Figure 1-2. Full details of the sites are provided in the methodology of subsequent chapters.
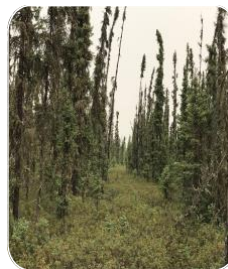
Figure 1-2 Description of study sites and plot summary.

Chapter 2: Effects of seismic lines on microbial aerobic organic matter turnover and soil respiration in boreal peatlands

2.1 ABSTRACT

Global focus on greenhouse gas emission reductions is currently at its peak due to overwhelming concerns over climate change. Peatlands, one of nature's most efficient and functional systems for carbon storage, are threatened by both natural and anthropogenic disturbances. The extensive network of linear disturbances in the Canadian boreal region, such as seismic lines and roads could alter carbon dioxide and methane exchange in peatlands, as well as long-term carbon storage. We investigated microbial community interactions driving carbon flow in peatlands by assessing changes in microbial aerobic organic matter turnover and measured in-situ soil respiration rates using automated chambers. Peat samples from seismic lines and surrounding natural areas were collected from different wooded peatlands near Peace River, Alberta, including bogs and a fen with wide and narrow seismic lines. The MicroResp technique was implemented using 15 carbon sources and Milli-Q Water as a control with samples run in triplicate. Soil respiration rates were reduced in disturbed areas accompanied by significant increases in peat temperature and moisture content. There were differences in CO_2 production rates from substrates between bogs and fens; however,

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significant shifts in substrate use on lines relative to natural areas were not detected. This could suggest that changes in plant community and microclimate are the main drivers of shifts in carbon exchange. Therefore, attributes related to the plant community, surface elevation and wetness should hold promise for mapping changes in peatland carbon cycling in response to seismic line disturbance.

2.2 INTRODUCTION

Northern peatlands serve as long-term carbon (C) sinks for an estimated 300–550 Pg C (Yu *et al.,* 2010; Hugelius *et al.,* 2020) while emitting an estimated 20–45 million metric tons of methane (CH_4) annually (Gorham, 1991; Frolking *et al*., 2011; Bridgham *et al*., 2013). Carbon sequestration in peatlands results from the difference between inputs via primary production and C losses including microbial decomposition (Bragazza *et al*. 2007). Peatlands are however, threatened by both natural and anthropogenic disturbances potentially resulting in the release of large amounts of C to the atmosphere. Linear disturbances such as seismic lines for oil and gas exploration and extraction constitute the bulk of these disturbances crossing large areas of boreal peatlands (Vitt *et al.,* 1996). The impact of seismic lines on peatland function, such as microbial community function and their overall impact on C cycling, is not very well understood although physical changes to topography and lack of tree re-establishment are well documented (Dabros *et al.,* 2017; Filicetti *et al.,* 2019). Previous studies have documented

vegetation community changes and increasing CH_4 emissions (Strack *et al.,* 2018; Davidson *et al*., 2021), but little on-the-ground data exists on microbial community responses to anthropogenic or more specifically, seismic line disturbances in peatlands. This study therefore assessed the effect of seismic line disturbances on boreal peatland microbial community functions.

Anthropogenic disturbances due to oil and gas development and mining activities dominate boreal Canada with an estimated 2875 km of disturbances added annually to the region of Alberta (Komers & Stanojevic, 2013), necessitating the need for better comprehension of their impact on the boreal zone. Seismic exploration needed for assessing oil and gas reservoirs, involves clearance of linear pathways (transmission and receiver lines for geological surveys) known as seismic lines/cutlines (Lee & Boutin, 2006; Bayne *et al*., 2011). The two main types are the two-dimensional/legacy lines which are 5–10 m wide lines cut with heavy machinery and the less intrusive 1.5–5 m wide narrow cut-lines/three dimensional seismic (3-D) lines made with smaller equipment and chainsaws (Lee & Boutin, 2006). Legacy seismic lines are the most extensive disturbance type related to the oil and gas industry in the Western Canadian Arctic (Kemper & Macdonald, 2009) and most likely, the entire boreal forest (Pasher *et al*., 2013). The density of seismic lines in northern Canadian forests range from 10 km/km^2 (Lee & Boutin) up to 40 km/km^2 (Schneider, 2002; Filicetti *et al.,* 2018) with Alberta alone accounting for over 1.7 million km of seismic lines (Brandt *et al*., 2013). The oldest lines are

from the early 1950s (Lee & Boutin, 2006) and felled trees are not as self-restorative as initially predicted, hence requiring the need for provincial restoration standards to be amended (Pigeon *et al*., 2016). Low-impact, 3-D lines were introduced as an intervention to mitigate some of the effects of legacy lines; however, there is lack of data to support the low impact claim, especially due to the higher density of lines required for seismic data (Lee & Boutin, 2006).

Linear disturbances such as seismic lines crossing boreal peatlands have been associated with loss of biodiversity directly through clearing/destruction of plant cover and indirectly through altered ecological interactions such as vegetation community successions (Dyer *et al*., 2002; Venier *et al*., 2014). Equipment used for cutting lines contributes to compression of peat, eliminated microforms, and cleared aboveground vegetation causing variation in microtopography and microhabitats (Caners & Lieffers, 2014). According to Venier *et al.* (2014) and Franklin *et al*. (2021) removal of the tree canopy in boreal forests drastically influences microclimatic factors such as light and soil temperature which affects the function of the understory vegetation and microbial community.

 Cutlines could also affect the organic matter layer, where the bulk of soil nutrients are located and potentially alter nutrient cycling contributing to changes in the vegetation and microbial community (Bliss & Wein, 1972). Resulting changes in peat temperature and moisture (Strack *et al.,* 2018) influence the release of soluble cations, e.g., sodium, calcium, etc. that could

trigger further changes in vegetation communities due to alteration in species-species interaction (Kemper & Macdonald 2009). Additionally, increasing temperature on lines and compressed peat encountering mineral soil or exposure of peat to air could potentially increase organic matter decomposition (Kemper & Macdonald, 2009; Caners & Lieffers, 2014; Wilson *et al*., 2021). This results in a feedback loop, where increased decomposition readily increases available nutrient supply resulting in vegetation succession as reported by (Davidson *et al*., 2021). However, an excessive increase in nitrogen may saturate the moss layer leading to greater microbial activity, and further decomposition driving vascular plant growth coupled with water table fluctuations (Li & Vitt 1997; Limpens *et al*., 2003). Changes in vegetation communities directly influence net primary productivity and hence the amount, type, and quality of litter inputs to the soil. These changes could further affect the production and emission of greenhouse gases (GHG), that is, CO_2, CH_4 and N_2O from microbial organic matter mineralization (Gorham, 1991; Bridgham *et al*., 1995).

Microbial processes greatly affect the balance between storage and mineralization of soil organic matter (Fisk *et al*., 2003). The level of microbial activity, oxygen availability due to water table position, peat temperature, chemical characteristics of the peat/litter, and the type of vegetation present are documented controls on the rate of C mineralization in peatlands (Yavitt *et al*., 1997; Blodau, 2002; Frolking *et al*., 2011; Robroek *et al*., 2021; Wilson *et al*., 2021). Microbes are drivers of the decomposition process, which is a product of physical,

chemical, and biological processes transforming plant residue into their elemental constituents (Bragazza *et al*., 2007). The vegetation community and thus litter availability has been reported to influence microbial community and functional activity (Fisk *et al*., 2003; Strakova *et al.,* 2012; Wilson *et al*., 2021). Litter availability determines substrate available for microbial use and this could be altered by seismic line disturbances. Apart from vegetation changes affecting productivity and litter decomposability, plant associations with microbes could also be affected by the environmental changes arising on seismic lines. This could cause a shift in microbial communities or functional group processes, for example, decomposition rates or abundance of methanogens and methanotrophs (Danilova *et al*., 2015), as well as substrate decomposition rates (Sundh *et al*., 1997). The large surface to volume ratio and physiological characteristics such as their permeable cell membranes and high sensitivity to environmental stressors make microbes ideal candidates as environmental sensitivity indicators (Chambers *et al*., 2016). These traits have been exploited over time to study microbial responses to environmental changes including substrate quality (Bossio & Scow 1998), nutrient availability (Troxler *et al*., 2012), community structure and function (Andersen *et al*., 2013) and these techniques are applicable to linear disturbances in peatlands.

Soil respiration releases C in the form of CO_2 to the atmosphere impacting the net exchange of C between the ecosystem and the atmosphere (Ryan & Law 2005). Soil respiration is made up of CO_2 emissions from belowground vegetation components (autotrophic respiration) and

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decomposition of soil organic matter/litter by microbes (heterotrophic respiration) (Lalonde & Prescott, 2007). Alterations in peat temperature in disturbed areas as reported by Van Rensen *et al.* (2015) and Strack *et al.*, (2018) could increase microbial decomposition rates. Lines have also been found to be wetter (Strack *et al.*, 2018), conditions which could lead to a reduction in autotrophic soil respiration but an increase in CH_4 production. It has been suggested that microbial activity directly controls CH_4 flux whiles vegetation type, soil temperature and water table position are the indirect controls (Bubier *et al.* 1993; Yavitt *et al.*, 1997).

Information is currently lacking on the impact of seismic lines on environmental controls and their cumulative role in altering soil respiration in disturbed peatlands. Changes to microbial communities could be investigated by assessing changes in aerobic processes that drive CO_2 flux and impact the very slow decomposition rates in natural peatlands, which is a major driver in preventing C losses (Limpens *et al.*, 2008). A viable technique that can readily provide information on mixed microbial community together with spatial and temporal microbial functional adaptation involves the analysis of microbial substrate utilization patterns through community level physiological profiling (CLPP). This relatively simple technique allows different communities to be classified and compared based solely on the utilization patterns of C sources (Weber & Legge, 2010). This technique when compared to classic cell culturing or molecular level RNA/DNA amplification techniques is less time consuming and does not

require specialized expertise. It could therefore be a relatively easy-to-deploy measure of ecosystem response to disturbance and recovery (Nwaishi *et al*., 2015).

The impact of seismic lines on boreal peatland functions is understudied, and there is much uncertainty about changes to microbial community in response to other peatland disturbances (Andersen *et al*., 2013). Saraswati *et al.* (2019), demonstrated that access roads crossing boreal peatlands alter enzymatic activity and could enhance organic matter decomposition rates, while Andersen *et al.,* (2006, 2010) confirmed that disturbance-impacted microbial communities lagged in structural and functional recovery and often required intervention in restoration projects. With concerns about linear disturbances transforming peatlands from C sinks to sources (Dabros *et al*., 2018) and increasing CH_4 emissions on boreal winter roads reported by Strack *et al*., (2018), additional research is essential to provide better understanding on implications of microbial community shifts, decomposition of substrates, and the gross effects of these changes on CO_2 and CH_4 fluxes in disturbed boreal peatlands. Therefore, the main objectives of our study were to i) investigate the impact of seismic lines on peatland microbial aerobic organic matter turnover and ii) assess the effect of seismic lines on soil respiration rates in boreal peatlands. We hypothesized that warmer conditions and more easily decomposable litter on seismic lines would increase soil respiration rates and overall substrate utilization, although this pattern would likely vary depending on the peatland type and line width.

2.3 MATERIALS AND METHODS

2.3.1 Study sites

The study was completed across three peatlands near the town of Peace River, Alberta, Canada. The first two study areas were approximately 40 km northeast of the town of Peace River, namely, Carmon Creek, a wooded bog (56°21′44″ N, 116°47′45″W) and Peace River complex (IPAD) a wooded poor fen (56°23′51.22″ N, 116°53'27.60″W). The last study site, Harmon Valley is a forested bog approximately 50 km southeast of Peace River, (56°12.10N, 116°56.04W). Site selection was primarily influenced by the age and type of seismic lines present, ease of access and availability of existing data on local vegetation and hydrological conditions (Dabros *et al.,* 2017; Strack *et al.,* 2018; Saraswati *et al.,* 2019). All study sites, shown in Figure 1-2, are actively connected to oil sands exploration/extraction and are therefore interspersed with additional disturbances such as seismic lines, well pads and roads.

Carmon Creek (CC) is characterized by low impact seismic lines (2–3 m width) that are 5–15 years old and sampling was subdivided into two sites based on age of lines: CC1 (<10 years) and CC2 (>10 years). Both IPAD and Harmon Valley (HV) have legacy seismic lines (6–8 m width) older than 20 years. At both CC and HV bogs, the natural areas were dominated by *Picea mariana* (black spruce) and a ground layer of *Pleurozium schreberi* (feathermoss), *Sphagnum fuscum* (rusty peat moss), *Vaccinium vitis-idaea* (lingon-berry), *Rhododendron*

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groenlandicum (Labrador tea), *Vaccinium oxycoccos* (bog cranberry) and lichens (e.g., *Cladina* spp*.*). The lines, although largely similar to the natural areas in composition, lacked both *P. mariana* and *P. schreberi* cover. At IPAD, vegetation at the natural fen was predominantly characterised by *P. mariana*, *Salix* spp. (willow), *P. schreberi* and lichens while lines were mostly covered by *Carex aquatilis* (water sedge), patches of *Sphagnum* spp*.,* brown mosses, and sparsely distributed *Salix* spp. (willow) saplings (Davidson *et al*., 2021). All seismic lines were devoid of trees taller than 1 m, irrespective of the age of the lines.

We collected data from two seismic lines per site with paired plots in the adjacent, natural peatland. At each site, the two study lines were at least 50 m and were instrumented with triplicate plots on the line and in the adjacent peatland (15 m from the seismic line). Therefore, this study consisted of 4 sites x 2 subsites x 6 plots (3 disturbed + 3 natural), totaling 48 plots. The aim of this study design was to capture impacts related to peatland and seismic line type across a range of ages of seismic lines. For analytical purposes, the different peatlands are denoted as sites (CC, HV and IPAD), while seismic lines are treated as a treatment factor called Position. Position, therefore, refers to a plot on the line or the surrounding peatland area (Line vs. Natural).

2.3.2 *In vitro* Microbial Respiration: MicroResp

We used the MicroResp method to determine substrate induced aerobic respiration potential of the microbial community as outlined in Campbell *et al.* (2003), with adaptations from Artz *et al.* (2006) and Daté *et al.* (2018). To ensure the integrity of the microbial samples, extraction and transportation of peat was restricted to a 24-hour window commencing on the 13th of July 2018. Peat sampling was conducted on plots from all eight seismic lines and their corresponding adjacent natural peatlands. The sampling location at each plot was chosen to represent the dominant plant communities at that location. Altogether a total of 148 peat samples (48 plots × 3 replicates) were collected by hand using the inverted plastic zipper bag technique. Peat samples were extracted from the oxic zone, up to a depth of 10 cm into sterile bags, sealed, labelled, and kept in coolers at ~ 4 °C (Anderson *et al.,* 2010). Samples were then flown from Peace River, AB to the Wetland Soils and Greenhouse Gas Exchange Lab in Waterloo, ON for processing and analysis.

We followed the MicroResp manufacturer instructions for sample and plate preparation. Approximately 0.3 g of homogenized peat was inoculated into pre-labelled deep-well microplates. Based on the 96 well configuration (2 mL volume per well), each plate had 16 wells exclusively filled with peat from the same plot. This was replicated three times for each location. The peat filled microplates were then incubated in the dark, at approximately 25 °C

(within sterile drawers in the laboratory) for 72 hours. We selected 15 C sources representing common root exudates and Milli-Q water (control) as per Daté *et al.* (2018). These substrates included amino acids: l-alanine, arginine, l-cysteine-HCl, N-acetylglucosamine, l-lysine; saccharides: l-arabinose, d-fructose, d-glucose, trehalose, galactose, and carboxylic acids: α-ketoglutaric acid, citric acid, γ-aminobutyric acid, l-malic acid, and oxalic acid. Carbon sources were dissolved into Milli-Q water at a concentration of 300 mg/mL, with the aim of delivering 30 mg substrate g^{-1} soil water when 25 µL of substrate solution is applied. The exceptions were N-acetylglucosamine and alanine which delivered 7.5 mg substrate g^{-1} soil water (Daté *et al.*, 2018). All substrate solutions were refrigerated at 4 °C prior to use.

Detection gel for the corresponding detection microplates were made from a solution of 150 mM potassium chloride and 2.5 nM sodium bicarbonate, with cresol red dye (12.5 ppm w/w) as the pH indicator. The solution was then mixed into 1% Noble agar in molten form, ensuring the temperature was maintained at 65 °C using a water bath. Each of the 96 wells in the detection plates was then filled with 150 µL of the resulting mixture and left to cool (30-60 minutes). The detection plates were then inserted into a microplate reader configured with a 570 nm filter (A570) to measure the absorbance of the gel across the entire plate. Detection plates with a coefficient of variance greater than 5% were discarded to ensure high spectrophotometric accuracy. All high accuracy detection plates were wrapped in aluminum foil and stored in a desiccator over a beaker full of sodium hydroxide pellets.

To initiate the reaction, 25 µL of substrate solution was added to each well of the deepwell plates. The detection microplates were then attached to the deepwell plates via a gasket and secured by clamps. The combined plates were initially read for absorbance at 570 nm and repeated after 6 hours of incubation in the dark. The chromatic variation in the gel color over the 6-hour incubation period triggered by CO_2 absorption was used to calculate the extent of substrate induced respiration, which directly correlates to microbial activity.

2.3.3 *In situ* Soil Respiration

In the field, soil respiration was measured using eosFDCO$_2$ forced diffusion chambers (EOSENSE, Dartmouth, NS, Canada) at IPAD (IP) and Carmon Creek (CC) during the peak of summer (July and August) of 2018. A total of four stand-alone automatic chambers were deployed to directly measure carbon dioxide (CO_2) flux from the peat surface over a circular, 10 cm diameter collar. Collars were pre-installed at an approximate peat depth of 1.5 cm, 24 hours preceding chamber deployment. Measured flux represents soil respiration as all surface vegetation was removed from each of the collars as per the instruction manual. During use, chambers were anchored in place by a mounting ring and steel line/pegs.

At both sites, two corresponding points on and off the seismic line were selected for continuous measurements (i.e., IPAD natural and IPAD line, CC natural and CC line). These chambers were programmed to automatically record hourly CO_2 flux for an average of 21 days. Each

unit was powered by a 35 W solar panel connected to a 12 V. 40-amp sealed lead acid battery housed in a marine battery box to withstand the outdoor conditions. To also capture spatial variation in soil respiration, at both the IP and CC sites, four additional points of reference were selected for short-term flux measurements with two additional chambers in each of the natural and two additional on each of the lines. The position of the flux transects were aimed at capturing low/high lying areas, as well as shaded and open areas (with no understory vegetation cover). Short-term chambers were programmed to record CO_2 fluxes at 5-minute intervals and operated for 30 minutes per day, between 8 am to 7 pm. These readings were taken daily for four different days before, during and after the continuous measurement phase resulting in 18 measurements for each plot during this period. Flux measurements stored by the CO_2 flux sensors were automatically processed using the eosLink-FD software (ver. 2.4.0) and downloaded for statistical analysis.

2.3.4 Environmental Conditions

Other field instruments were deployed on all sites to record data from May to August 2018. These included mounted HOBO U23 Pro v2 temperature/external temperature data loggers recording hourly 5 cm depth peat and air temperature at each subsite. A handheld probe from Delta-T devices (WET-2 Sensor attached to an HH2 readout meter) was used to record daily peat temperature and soil moisture at 5 cm depth alongside gas flux measurements at each plot.

Vegetation surveys were carried out by visually estimating percentage cover of plant species adjacent to soil sampling location in 60 cm × 60 cm quadrats in August 2018. Species of both vascular and non-vascular plants identified followed the USDA online plants database (http://plants.usda.gov) and their cover was estimated visually to the nearest 5% when greater than 5% cover and ranked as, 3 (occasional, more individuals), 1 (occasional, few individuals) or 0.1 (present) when cover was lower (Davidson *et al.*, 2021). Plant species were then compiled into growth forms, including Forbs, Graminoids, Dwarf shrubs, Trees, Bryophytes and Lichens.

2.3.5 Statistical Methods

Statistical analyses were undertaken in R Studio version 1.3.1056 (R Core Team 2020) and PC-ORD^{TM} version 6.0. The average well color development (AWCD) of MicroResp indicator plates provided a quantification for substrate induced aerobic respiration potential. Significant differences between respiration rates on/off lines and across study sites were determined using two-way analysis of variance (ANOVA), while the TukeyHSD function was used to test post-hoc differences (lsmeans packages) (Lenth, 2016). Multivariate Data Analysis was carried out using PC-ORD^{TM}. Non-metric multidimensional scaling (NMDS) was conducted for study plots ordinated by substrate use and environmental variables. The main matrix was the

substrate utilization rates. The second matrix (vegetation percent cover, peat temperature and moisture content) was square-root transformed and is presented as a vector fitted line ($r^2 > 0.2$).

Linear regression was used to assess the relationship between in-situ soil CO_2 flux rates from the peat surface and peat temperature on and off the lines, as well as between peatland types.

2.4 RESULTS

2.4.1 Environmental Conditions

At the time of peat sampling for the microbial respiration analysis, moisture content was higher on all lines (mean = 38.8%) compared to the natural peatlands (15.6%), with the highest and lowest moisture contents recorded at IPAD line and CC1 natural, respectively (Table 2-1). A similar trend was recorded for the average 5 cm peat temperature, as all lines were warmer than corresponding natural areas. The highest temperature was recorded at HV line (13.1 °C), while the lowest temperature was recorded at the natural site at IPAD (8.3 °C). A greater diurnal temperature variation was observed on the lines largely linked to the absence of shading. On average, all lines presented a vegetation shift to greater shrub cover, as well as greater graminoid cover for IPAD, and a reduction in bryophytes (except at HV) and lichen cover (except at CC2; Table 2-1).

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Table 2-1 Average (standard deviation) environmental conditions and vegetation cover across study plots at peat sampling time

Plot Location	Moisture (%vol)	Peat Temperature (°C)	Tree cover (%)	Lichen cover (%)	Bryophyte cover (%)	Dwarf shrub cover (%)	Graminoid cover (%)	Forb cover (%)
CC Line	37.25 (6.15)	11.6 (0.5)	0.1 (0)	18.6 (6)	65.9 (4)	18.9 (3)	0.8 (0.9)	2.1 (1)
CC Natural	15.8 (2.9)	10.2 (1.3)	0.1 (0)	25.95 (2)	76.75 (6)	14.35 (5)	0 (0)	2.1 (1)
HV Line	30.1 (6.8)	11.4 (1.8)	0.2 (0.2)	2.5 (3)	90.0 (2)	17.2 (2)	0 (0)	4.7 (0)
HV Natural	13.2 (0.5)	11.0 (1.6)	0 (0)	14.3 (1)	75.9(5)	14.7 (2)	0 (0)	8.7 (4)
IPAD Line	50.8 (14.9)	11.2 (1.5)	0 (0)	0 (0)	59.2 (8)	9.3 (8)	32.5 (20)	3.3(2)
IPAD Natural	17.6 (10.6)	10.6 (2.5)	0 (0)	1.7 (2)	81.8 (0)	8.8(5)	11.2 (10)	0.8 (1)

2.4.2 *In vitro* Microbial Respiration

Across all study sites, there was no clear pattern to shifts in substrate-induced respiration (SIR) on the seismic line compared to the natural plots (Figure 2-1). Therefore, the average rates of CO_2 production captured from substrate-induced respiration from all sites were not significantly different among sites or between line and natural plots (Appendix 1). Mean values of substrate induced respiration were most different between line and natural plots at HV, where the natural peatland recorded the highest potential microbial activity (Figure 2-1). Considering each study line (i.e., subsite) independently, substrate induced respiration was significantly different between the line and the natural peatland at only two subsites: HV1 ($F_{3,40} = 11.91$, $p < 0.0001$) and CC1 ($F_{3,40} = 9.69$, $p = 0.00128$).
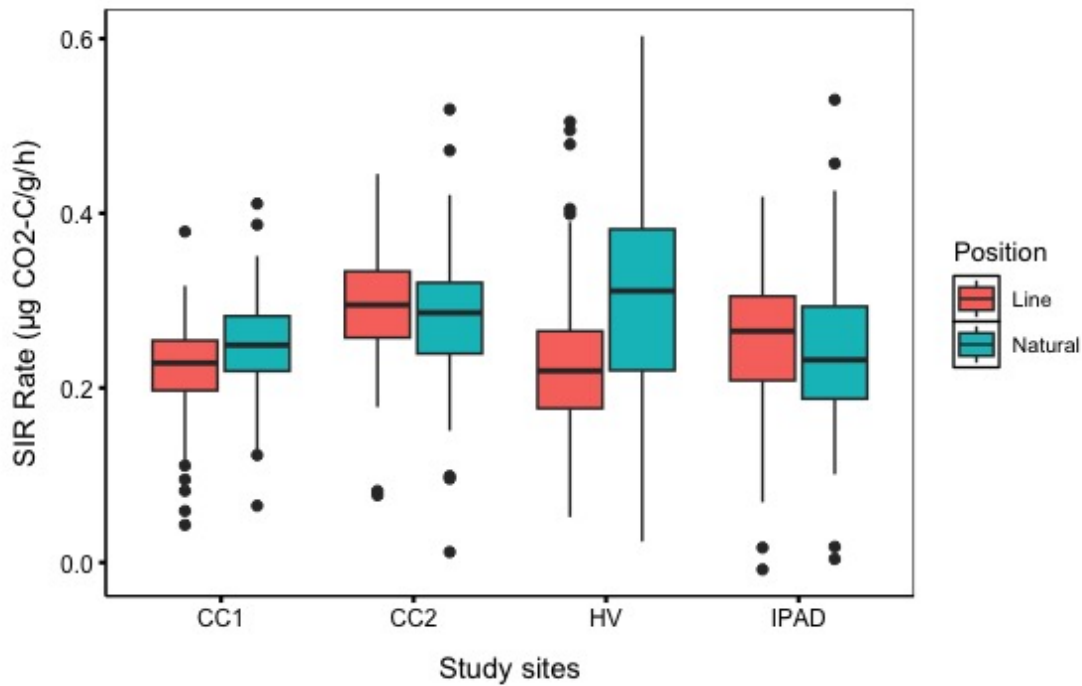
Figure 2-1 Differences in substrate-induced respiration (SIR) rate (μg CO_2-C/g/h) between study plots (Natural vs Lines), n= 48. The median is shown by the horizontal line within the boxplot; the upper and lower hinges represent the 75th and 25th percentiles, respectively. The smallest and largest values within the 1.5 times the interquartile range (IQR) are represented by whiskers, while dots outside the box and whiskers represent outliers (values > or < 1.5 x IQR).

Although the study design did not test for microbial preference for specific substrates, variations in utilization rates for the different C sources across the different peatland types and in response to seismic line disturbance were reviewed through ordination. Lysine utilization rates were highest at all sites, although not significantly different. As observed for mean substrate-induced respiration across all substrates, there were few significant differences in specific substrate utilization among peatlands or between seismic lines and natural sites. Most observed differences were among sites,

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with a general separation between the wooded poor fen (IPAD) and the wood bog sites. Site explained a significant amount of the variation in for substrate use for Arabinose ($F_{3,40}$ = 3.734, p = 0.01861), Alanine ($F_{3,40}$ = 5.458, p = 0.00306), Citric Acid ($F_{3,40}$ = 6.165, p = 0.00152), Fructose ($F_{3,40}$ = 2.956, p = 0.0438) and α-Ketoglutaric acid ($F_{3,40}$ = 2.927, p = 0.0453). The impact of the seismic line on peatland potential microbial functional activity was evidenced by the significant Position:Site interaction observed with the utilization rates for Glucose ($F_{3,40}$ = 4.209, p = 0.0112) and Arabinose ($F_{3,40}$ = 6.025, p = 0.00174); (Appendix 2, Figure 2-2A & B). Glucose induced respiration was higher in the natural areas compared to lines at HV and CC1, while CC2 and IPAD recorded the opposite trend with lines having higher rates than the natural peatland (Figure 2-2A). Arabinose utilization rates were also higher at natural areas of HV and CC1 compared to their corresponding lines, and higher at IPAD and CC2 lines compared to their corresponding natural areas (Figure 2-2B).

Substrate utilization rates for the control substance; Milli-Q water, as well as the following carbon sources: Arginine, Cysteine, Galactose, γ-Amino butyric acid, Lysine, Malic Acid, N-Acetyl Glucosamine, Oxalic Acid and Trehalose, showed no significant differences in utilization rates on/off the lines or between sites (Appendix 2).
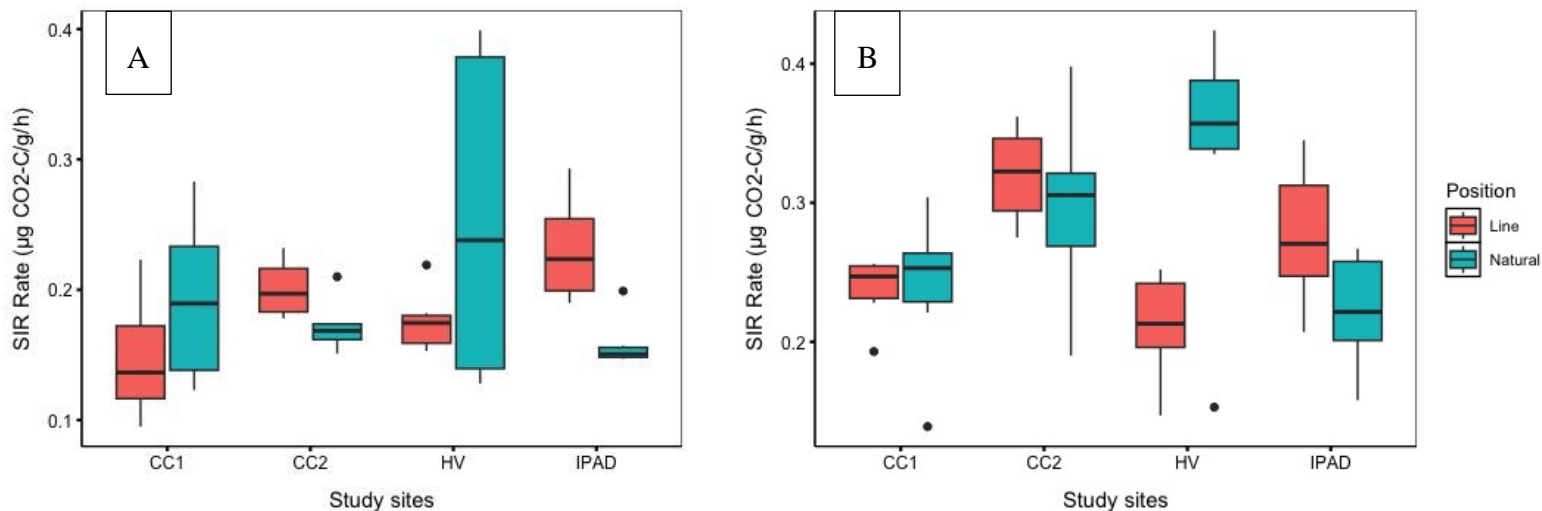
Figure 2-2 A) Differences in Glucose utilization rates (SIR) between sites; B) Site differences in Arabinose utilization rates (SIR). The median is shown by the horizontal line within the boxplot; the upper and lower hinges represent the 75th and 25th percentiles, respectively. The smallest and largest values within the 1.5 times the interquartile range (IQR) are represented by whiskers, whiles dots outside the box and whiskers represent outliers (values > or < 1.5 x IQR).

To further evaluate differences in substrate use across study plots and the potential environmental controls on this utilization, non-metric multidimensional scaling (NMDS) ordination was used (Figure 2-3 Non-metric multidimensional scaling (NMDS) biplot results (Euclidean distances, 500 iterations) for study plots' features (Line vs. Natural) ordinated by carbon utilization rates (dark blue lines with black dots) and environmental variables (vector fitted dark red line). The longer the substrate line, the stronger the association to microbial use. Results are presented in 2-D configurations (axis 1x2), axis 3 is not shown due to the variance explained being very low (less than 1%). Plots are clustered into on seismic lines and offline/natural and represented by red and green triangles, respectively.). A three-dimensional solution was reached with a final stress value of 0.088. Axis 1, 2 and 3 explained 63%, 15%, and less than 1% of the total variance, respectively. Except for lysine, all other carbon sources were clustered together, indicating similar aerobic microbial substrate utilization rates. Natural sites at CC are in closest proximity to the substrate cluster followed by HV and IPAD plots. However, there was no clear separation of plots on and off the seismic lines. Due to the controlled lab conditions, field environmental variables such as soil moisture, and pH had no direct effect on overall C substrate utilization rates by microbes in study samples. However, the recorded field variables were incorporated into the analysis to assess whether in-situ conditions contributed to variations in substrate utilization rates. The vector (dark red line in Figure 2-3) shows the environmental factors associated with the difference among plots. Peat temperature was therefore the most deterministic environmental variable aligning along axis 1 and accounted for most differences in the utilization rates of carbon substrates among different plots, with HV and IP having the most within-site variation.
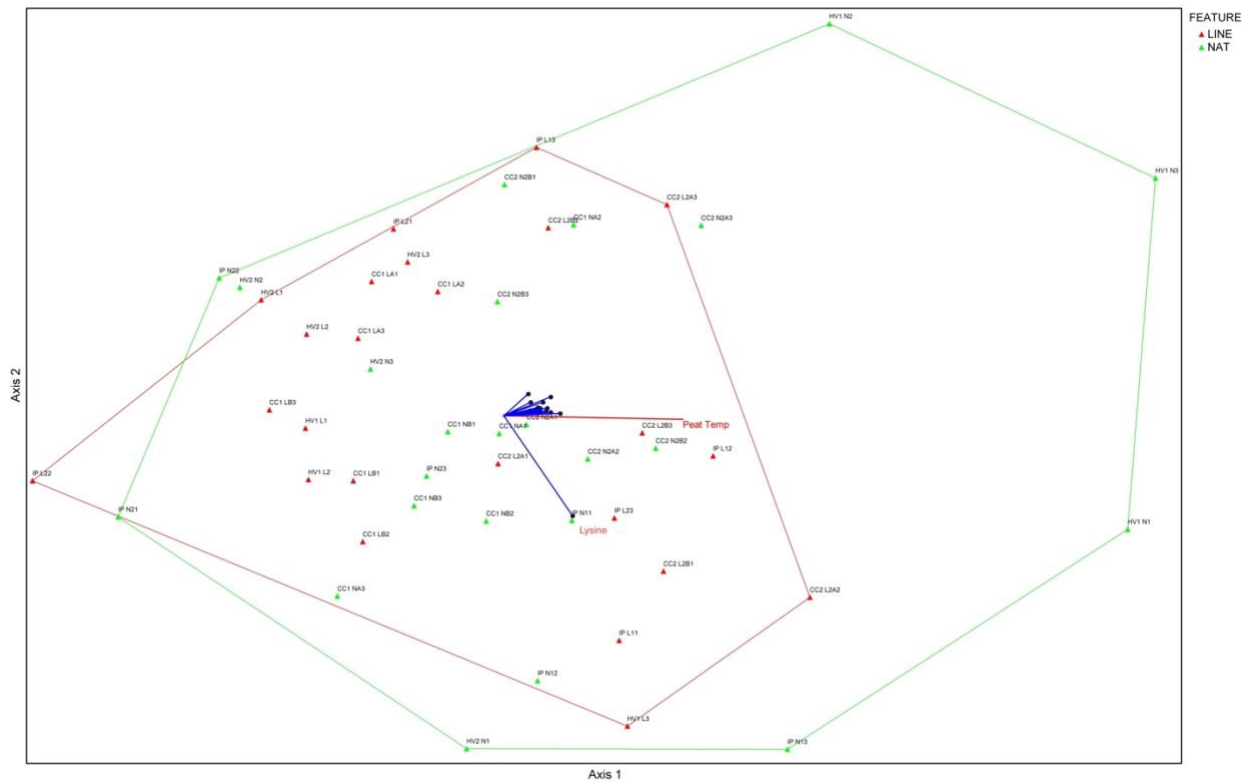
Figure 2-3 Non-metric multidimensional scaling (NMDS) biplot results (Euclidean distances, 500 iterations) for study plots' features (Line vs. Natural) ordinated by carbon utilization rates (dark blue lines with black dots) and environmental variables (vector fitted dark red line). The longer the substrate line, the stronger the association to microbial use. Results are presented in 2-D configurations (axis 1x2), axis 3 is not shown due to the variance explained being very low (less than 1%). Plots are clustered into on seismic lines and offline/natural and represented by red and green triangles, respectively.

2.4.3 Soil Respiration

The rates of CO_2 flux from soils at both IPAD and CC were slightly lower from plots on seismic lines compared to natural areas, but diurnal patterns of soil respiration also varied in response to seismic line disturbance (Figure 2-4A & B). Soil respiration at IPAD appears to be relatively consistent at both the natural area and line through the day, while CC presents a more inconsistent pattern especially on the line. Both sites showed the highest soil respiration rates between midday and midnight. Soil respiration was significantly impacted by the peatland type ($F_{1,1721} = 534.5$, p < 0.0001), the seismic line ($F_{1,1721} = 73.7$, p < 0.0001) as well as in the interaction of the peatland type and the seismic line disturbance ($F_{1,1721} = 39.4$, p < 0.0001). The relationship between soil CO_2 flux and peat temperature at 5 cm depth was also significant ($F_{1,723} = 19.9$, p < 0.0001). The natural area of IPAD had the least change in temperature through the day, and peat temperature was much higher on the line. At CC peat temperature on the line was much higher in the daytime, although diurnal range was much smaller. Peat temperature from the natural areas of CC exceeded line temperatures by midday and dropped off by midnight. The highest and lowest peat temperatures were recorded at the CC natural site (5 °C and 25 °C), while the line ranged between 10–20 °C. Soil fluxes at all sites increased/decreased with corresponding diurnal temperature variation (especially at CC) as illustrated in Figure 2-4 C & D. Therefore, there was a significant relationship between soil temperature and CO_2 flux for all site-position combinations, but the slopes varied with significantly higher slopes at IPAD.
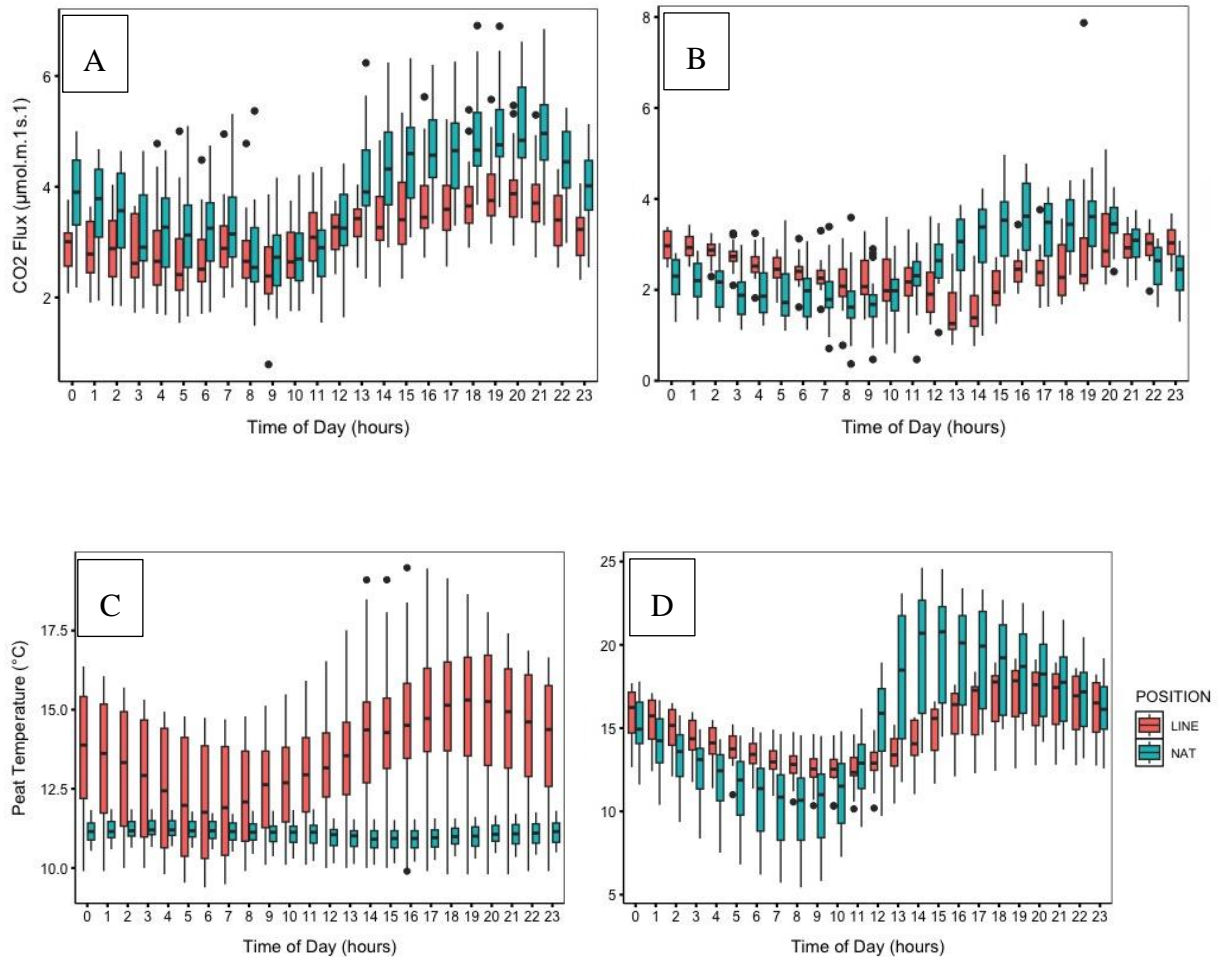
Figure 2-4 A/B/C/D Diurnal differences in Soil Respiration at sites: IPAD (A) and CC (B). Peat Temperature across sites: IPAD (C) and CC (D).

Short term readings (recorded over 30 minutes) from both CC and IPAD only captured brief fluxes but provided some additional spatial information. Mean (standard deviation) soil respiration (μmol m^{-1} s^{-1}) was higher on the lines at CC [line = 3.64 (2.8); natural = 2.97 (1.3)] and higher in the natural areas of IPAD [line = 3.64 (3.1); natural = 4.25(1.7)], although this was not statistically significant (Appendix 3; Figure 2-5). Variation in soil respiration was greater on seismic lines than in the natural areas. Since soil respiration rates were not significantly different between peatland

types or due to the existence of a seismic line, additional interactions with peat temperature and moisture content were explored for further process-based information. Neither peat temperature ($F_{1,136} = 0.0225$, p = 0.8811) nor moisture content ($F_{1,136} = 0.6151$, p = 0.4342) had a significant effect on soil flux. A model with the combined effect of peat temperature and moisture together also had no significant effects on soil fluxes for all sites. Additional results from statistical analyses are available in Appendix 3.
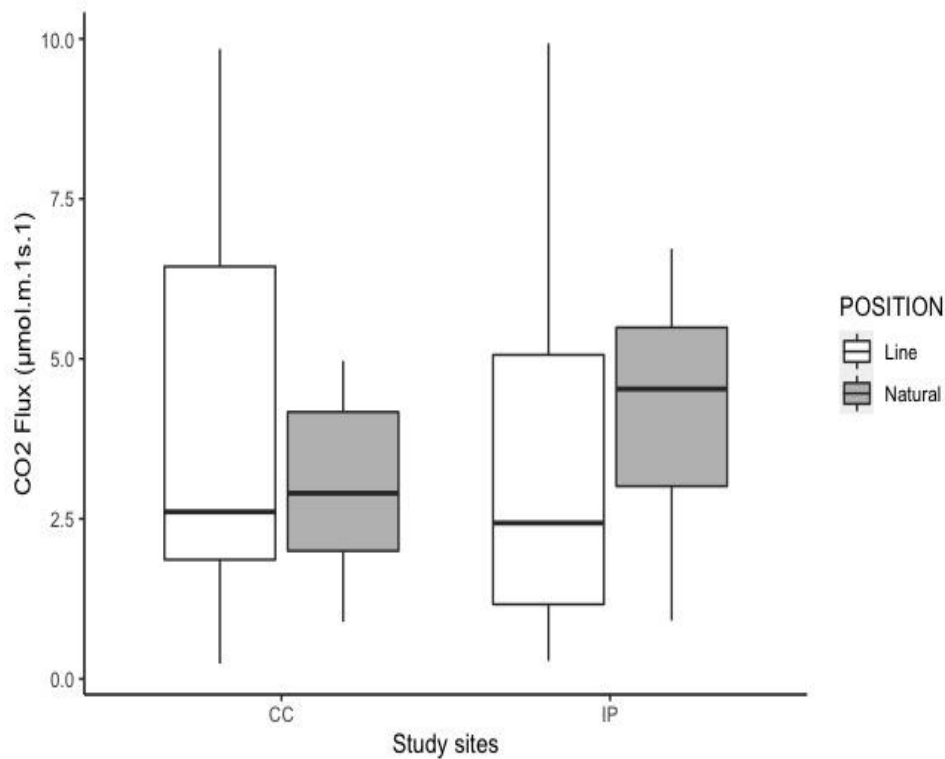
Figure 2-5 Soil respiration across multiple plots on IPAD and CC. The median is shown by the horizontal line within the boxplot; the upper and lower hinges represent the 75th and 25th percentiles, respectively. The smallest and largest values within the 1.5 times the interquartile

range (IQR) are represented by whiskers, whiles dots outside the box and whiskers represent outliers (values > or < 1.5 x IQR).

2.5 DISCUSSION

2.5.1 Microbial Soil Respiration

The release of CO_2 in peatlands is mainly controlled by plants and heterotrophic microorganisms, which are largely influenced by temperature, moisture, vegetation, and litter type (Artz *et al*. 2006; Peltoniemi *et al*., 2016). Our main aim was to investigate the effect of linear forest clearing on peatland microbial functional activity. This is imperative for a better understanding of the different roles microbial communities play in organic matter transformation contributing to the overall peatland C balance following disturbance. To the best of our knowledge this is the first study that has explored microbial community functional changes in boreal peatlands disturbed by seismic lines.

Disturbed peatlands in the study areas were characterized by increased temperature, higher soil moisture and changes in vegetation composition and CO_2 flux (Table 2.1, Figure 2-4). The disturbance significantly impacted soil respiration rates implying reduced microbial activity (Figure 2-4 A & B). However, the much higher respiration rates observed in the natural areas potentially could be also attributed to tree root respiration since lines were devoid of trees. Munir *et al*. (2017) showed a substantial contribution of autotrophic respiration to overall soil respiration in a forested boreal bog corroborating findings from our study. Yet, since our soil respiration measurements were carried out in relatively open sections of the natural peatland, this likely played

a minimal role in measured respiration. Apart from direct contribution from tree roots, root exudates provide labile carbon for microbial respiration (Glatzel *et al*., 2004) and this could also contribute to higher soil flux at natural plots. However, although trees were absent from the seismic lines, vegetation cover remained high, often with greater abundance of sedges that likely provide more labile root exudates than woody plants (Proctor & He, 2017), suggesting that differences in root exudation were unlikely the main driver for soil respiration on vs. off-line.

The process of line creation often removes the top layer of peat and any associated microbial and vegetation community in the upper horizon. Since microbial biomass and diversity decreases with depth (Jaatinen *et al*., 2008; Golovchenko *et al*., 2007), this surface disturbance may have impacted some community activity as demonstrated by the reduced microbial respiration rates on all lines. Soil disturbance can also reduce lability of near surface organic matter as indicated by greater decomposition status (Davidson *et al*., 2020), which could be leading to lower respiration rate on the lines (Glatzel *et al.,* 2003). It is, however, important to note that soil microbial communities typically adapt quickly to environmental changes (Schmidt *et al*., 2007) and hence could have undergone significant changes in the 10–50-year span following the creation of the seismic line.

 The significant differences in peat temperature recorded on and off the line could provide further explanation on the CO_2 respiration rates, although existing literature on the effect of temperature on microbial respiration is variable. For example, Kirschbaum (2013), Luo *et al*. (2001) and Giardina & Ryan (2000) report no impact, Bradford *et al*. (2008) report reduction and Wang *et al*. (2015), Kurbatova *et al*. (2013), Bonnett *et al*. (2006), and Davidson & Janssens (2006) report increasing respiration rates with higher temperature. Findings from this study also showed that

increasing temperature generally favors increased microbial respiration rates (Figure 2-4 C & D). Boreal peatlands are exposed to greater weather extremes compared to peatlands in other biomes, e.g., tropical peatlands and have been shown to respond differently to disturbances (UNEP, 2022). Lines were generally wetter than natural plots and this could have reduced soil respiration, despite the warmer temperatures. An increase of 0.5 °C in boreal soil temperature and a reduction of moisture content resulted in 50% reduction in fungal and bacterial populations and an active shift in microbial communities (Allison & Treseder, 2008). Our study encountered temperature increases coupled with an increase in moisture content, creating ideal conditions for higher methane emissions from lines (Strack *et al*., 2019), methane emissions were therefore measured in the final phase of this study.

2.5.2 Impact of Seismic Lines on Potential Microbial Activity

The recorded changes in potential microbial activity measured as C substrate utilization between study plots (natural vs. lines) due to the introduction of seismic lines in the peatlands, falsified our hypothesis of altered microbial functional activity. Although aerobic microbial functional activity was reduced on the seismic lines, the disturbance did not result in significant changes in contrast to our hypothesis. The MicroResp technique often elucidates potential respiration activity from primarily the r-strategists and more copiotrophic microorganisms present in the sample (Artz *et al.,* 2006). Peat by its nature has very high C content potentially encouraging higher numbers of copiotrophs at the detriment of oligotrophs and K-strategic microbes that may be more prevalent on the seismic lines, leading to similarities in measured community function. Even though samples were kept in the dark for three days at room temperature for microbes to presumably utilize any

labile C in the samples, a process more favorable to oligotrophs, a potential limitation in this study was the inability to quantify the carbon content of peat before the addition of substrates. However, all samples were organic soils and some previous studies have shown little change in organic matter content on peatland seismic lines (Davidson *et al*., 2020). Another constraint of the system is the potential anaerobic induction of individual wells during the incubation process (Artz *et al*., 2006). This is important since the concentration of carbon substrates added was based on the estimated available oxygen in each MicroResp well and as such, any oxygen limitation will affect overall microbial activity.

Only two subsites (HV1 and CC1) out of 24 paired plots exhibited significantly lower potential aerobic microbial functional activity on the lines compared to the surrounding peatlands. These plots were both located in wooded bogs with similar environmental characteristics and disturbance features. Higher microbial biomass has been recorded in upper layers of bogs compared to fens (Golovchenko *et al*., 2007) and could provide justification for the generally higher activity in the natural areas of HV and CC if some surface peat was removed during seismic line construction. An alternative explanation for the overall insignificant change in substrate utilization rates likely points to the availability of the C substrates not being a limiting factor in microbial community responses in peatlands or influenced by the ability of microbes to acclimatize to available substrate under conditions of stress (Bragazza *et al*., 2007). Since seismic line plots continued to be dominated by typical peatland plant species, albeit without tree cover, substrate availability was likely similar across disturbed and natural plots contributing to similar microbial community function.

Readily supplied C sources in this study eliminated potential influences such as selective utilization when nutrient stress is present under field conditions. Microbial preference for specific substrates were not tested but lysine and higher peat temperature were associated with the highest utilization rates on all sites (Figure 2-3 Non-metric multidimensional scaling (NMDS) biplot results (Euclidean distances, 500 iterations) for study plots' features (Line vs. Natural) ordinated by carbon utilization rates (dark blue lines with black dots) and environmental variables (vector fitted dark red line). The longer the substrate line, the stronger the association to microbial use. Results are presented in 2-D configurations (axis 1x2), axis 3 is not shown due to the variance explained being very low (less than 1%). Plots are clustered into on seismic lines and offline/natural and represented by red and green triangles, respectively.). The high utilization rates of lysine at all sites could indicate a microbial community preference for substrates. Typical microbial substrate utilization rates are highest for carbohydrates followed by amino acids and carboxylic acids then amides (Campbell *et al.*, 2003) which is in contradiction to the observed higher preference for lysine (amino acid) and in the present study. Even though oxalic acid, produced by living organisms and described by Palmieri *et al.* (2019) as the most common low molecular weight organic acid, could be associated with a high utilization rate due to availability, lysine has a much lower abundance in nature when compared to other free amino acids (Moe, 2013) and is more likely to be used based on microbial preference. Microbial communities and associated decomposition processes are vertically stratified in pristine peatlands (Sundh *et al.*, 1997; Morales *et al.*, 2006) hence, microbial samples from different sites at specific depths should have similar adaptation to substrate utilization rates. However, this trend in substrate use was observed on all sites despite changes to vegetation/litter quality, which impacts microbial

community stratification (Andersen *et al.,* 2013). If this observation was not due to lack of sensitivity to the substrates, then lysine could potentially be investigated as a cheap and reliable biomarker to assess disturbances to microbial communities in peatlands.

As expected, significant differences in the utilization rates of some substrates (arabinose, alanine, citric acid, fructose and α-Ketoglutaric acid) amongst sites were generally associated with peatland type. The controlled lab conditions eliminated the direct effects of environmental variables such as moisture, pH, and temperature, implying the observed differences could be related to differences imposed by vegetation type and nutrient content in the field (Fisk *et al.,* 2003). These findings correspond with Bragazza *et al*. (2007), Golovchenko *et al*., (2007) and Anderson *et al.* (2013) indicating that microorganisms present in bogs and fens have metabolic adaptations specific to litter chemistry of vegetation present in their specific habitat influencing decomposition rates of vegetation encountered by microbes. While vegetation shifts occurred in response to the seismic line disturbance (Davidson *et al*., 2021), all plots were dominated by peatland-specific species. Disturbed plots in the bog transitioned to a greater shrub cover, while at the fen there was more graminoid cover with a general loss of bryophyte and lichen cover. Such vegetation community shifts could generate more recalcitrant organic matter in the bogs or more labile substrates in the case of the fen (influencing litter substrate quality) and potentially impact microbial activity (Lafleur *et al*., 2011). This supports the generally higher potential utilization rates on the lines in the fen and the lower rates on lines in the bogs. Although, the only significant differences in substrate use observed in response to seismic line disturbances were for glucose and arabinose utilization rates in interactions with site where utilization increased on lines in some cases and

decreased in others, further investigation is warranted to understand the specific local controls and microbial community compositional shifts that alter the utilization of these substrates.

It is also important to be mindful of the 6 hr incubation window, which represents a short-term response to potential microbial substrate induced respiration as a measure of community activity (some studies including Artz *et al.* (2006) and Preston *et al*. (2012) had incubation periods up to 48 hours). Further, incorporation of 14C-labelled carbon sources (Artz *et al.,* 2006) or molecular techniques such as rRNA sequencing (Peltoniemi *et al*., 2016; Kitson & Bell, 2020) and terminal restriction fragment length polymorphisms (T-RFLP) profiles (Morales *et al*., 2006; Preston *et al*., 2012; Chambers *et al*., 2016) could provide a greater insight for comprehending impacts on labile C cycling in natural and disturbed areas of the peatland influenced by interactions between microbial diversity and function. Future applications of this study could also consider multiple sampling dates to capture seasonal variations as discussed by Anderson *et al*. (2013).

2.6 CONCLUSION

This study investigated changes to microbial community function driving carbon flow in peatlands disturbed by seismic lines. We assessed the effects of seismic lines on in-situ soil respiration rates and potential aerobic microbial activity in boreal wooded bogs and a wooded fen. Soil respiration rates were reduced in disturbed areas accompanied by significant increases in peat temperature and moisture content. Although significant changes to plant community structure were observed in disturbed areas, potential changes in microbial community function as measured by substrate utilization were not evident. Our findings therefore suggest that the impacts of seismic lines on

microbial functions impacting soil C cycling in peatlands is minimal, decades after disturbance. This indicates that the lack of trees on the line has minimal impact on the microbial community function suggesting that changes in plant community and microclimate are the main drivers of shifts in carbon exchange. Therefore, attributes related to the plant community, temperature variation and wetness should hold promise for mapping changes in peatland C cycling in response to seismic line disturbance. Measurements of ecosystem C flux in the field are therefore needed to understand the full impact of the environmental and structural changes to the vegetation community associated with seismic line disturbance on peatland C cycling.

Chapter 3: Impact of seismic lines on net primary productivity and decomposition rates in boreal peatlands

3.1 ABSTRACT

Peatland management has been highlighted as an important nature-based climate solution. Carbon sequestration in peatlands occurs when primary productivity exceeds decomposition. Earlier studies highlighted the impact of seismic line disturbances on vegetation and microbial community changes as well as microclimatic conditions. However, the cumulative impact of these changes on potential peat accumulation rates in boreal peatlands is yet to be investigated. This study assessed the impact of seismic line disturbances on net primary production (NPP) and litter decomposition rates in boreal peatlands and is the first study of its kind. This study was completed across three peatlands associated with oil sands exploration/extraction near the town of Peace River, Alberta. Four peatland subsites were selected based on the peatland type (bog, fen) and type of seismic line disturbance (legacy, low impact). Vegetation surveys, production (biomass) and decomposition (litter bags) data were collected between 2018 and 2021 from disturbed and adjacent natural areas. Measurements related to water table levels, physicochemical parameters and climatic conditions were recorded alongside. Total NPP remaining after two years was significantly reduced on all seismic lines, that is, from 165-239 to 69-108 ($g\,m^{-2}\,y^{-1}$). Although understory NPP rates increased on lines, it could not compensate for the overall NPP lost due to the absence of overstory woody vegetation, hence justifying the need for tree restoration in maintaining the C sink function of peatlands. Most of this NPP remained in the woody biomass and at some study sites, peat

accumulation potential considering only the soil layer was higher on seismic lines than the adjacent natural plots. Results from this research are novel and can serve as a foundation for new perspectives on designing and setting restoration targets in boreal peatlands.

3.2 INTRODUCTION

Peatlands sequester carbon (C) when primary productivity exceeds decomposition rendering these ecosystems as net C sinks (Clymo, 1998; Bragazza *et al*., 2007). Northern peatlands alone have been projected to store 300–550 Pg C (Yu, 2012; Hugelius *et al.,* 2020), accounting for a significant amount of the global terrestrial C pool (Gorham 1991) while providing unique habitats for some endangered fauna and flora (Filicetti *et al*., 2019). Natural conditions in peatlands, including low pH, low temperature, anoxic waterlogged environments, and vegetation with low nutrient quality and recalcitrant litter, favor low decomposition rates (Johnson & Damman, 1993; Bridgham & Richardson, 2003) supporting the imbalance between organic matter decomposition and net primary production (NPP). Since different vegetation types and plant parts have varying NPP and decomposition rates, any changes to the vegetation community could disrupt the peatland C balance. Natural and anthropogenic disturbances in peatlands can therefore impact their ability to remain effective C sinks (Turetsky *et al*., 2011; UNEP, 2022), justifying the need to investigate disturbances that impact NPP and/or decomposition rates.

Over 134,000 km^2 of peatlands can be found in northern Alberta (Strack *et al.,* 2019), a part of the boreal region of Canada with vast oil sand deposits. Resource exploration and extraction in this region has resulted in extensive anthropogenic disturbances such as access roads, well-pads,

seismic lines, and pipelines (Vitt *et al.,* 1996; Pasher *et al*., 2013). Predominant disturbance features in the region are seismic lines, which are long linear corridors of width between 1.5–10 m, constructed for petroleum exploration (Lee & Boutin 2006). Seismic lines are considered temporary access features and therefore were expected to naturally regenerate. However, studies have shown failed regeneration of woody species and slow return to forest cover on seismic lines decades after the creation (Lee & Boutin, 2006; Van Rensen *et al*. 2015). During the construction of seismic lines, trees and the top portion of understory vegetation are directly cleared contributing to loss of biomass and reduction in the C uptake potential of the ecosystem (Strack *et al*., 2018). Other physical changes, including compression of peat and microforms from construction and repeated use of lines (Strack *et al.,* 2019; Davidson *et al.,* 2020), further impact the local hydrology leading to sustained flooding during the growing season (Caners & Lieffers, 2014). More studies continue to highlight the direct and indirect impacts of seismic lines including habitat destruction (Dyer *et al*., 2002; Filicetti *et al.,* 2019), alteration of vegetation communities and microclimatic conditions (Venier *et al*., 2014; Van Rensen *et al.,* 2015; Franklin *et al.* 2021; Davidson *et al*., 2021) and impacts on C cycling (Strack *et al.,* 2018, Chapter 2).

There is a consensus linking peat accumulation to a low rate of decomposition rather than a high NPP in boreal peatlands (Vitt, 1990; Bragazza *et al*., 2007). It is important to note, however, that higher productivity rates are often positively correlated to higher decomposition rates, due to higher nutrient concentrations and better substrate quality in many highly productive species (Thormann, Szumigalski & Bayley, 1999). The quality of litter rather than the production rate, is a critical factor in the cycling and accumulation of C in peatlands, as litter inputs needed by microorganisms could either accelerate or slow down decomposition (Strakova *et al.,* 2012). The

vegetation community structure, therefore, plays an essential role in potential peat accumulation rates (Johnson & Damman, 1993). Nonvascular plants such as bryophytes, particularly *Sphagnum* mosses, produce recalcitrant biomass that are slow to decompose. In comparison vascular plant groups, such as herbaceous plants, are highly productive and decompose faster (Yavitt & Williams, 1997; Dieleman *et al.,* 2017). Although some studies suggest both bryophytes and vascular plants contribute equally to peat accumulation (Kubiw, Hickman & Vitt, 1998, Nicholson & Vitt, 1990), an assessment of about 341 peat cores in North America revealed bryophytes (*Sphagnum* in poor fens) as the major component of fen peat (Vitt *et al.*, 2000). In the same study, layers of the peat cores dominated by vascular plants decomposed faster and resulted in overall less peat accumulation. These findings contrast with other studies that highlighted vascular plants as the major component of fen peat (Hu & Davis, 1995). Belowground biomass can also play an important role in peat accumulation, particularly in fens (Chimner, Cooper & Patron, 2002; Hinzke *et al.* 2021). In peatlands, biomass distribution may be equal or in some cases greater belowground than aboveground (Murphy *et al.*, 2009). Estimation of belowground biomass, however, involves complex and time-consuming methodologies, hence limited data exists for boreal peatlands (Saarinen 1996; Finér & Laine, 1998; Bérubé & Rochefort, 2017). Studies suggest greater peat accumulation in bogs compared to fens, although very few studies compare potential peat accumulation rates directly between these peatland types (Thormann, Szumigalski & Bayley, 1999).

In addition to the effect of the plant community composition, differences in peat accumulation potential have been linked to the availability of essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K) (Reader, 1978; Blodau, 2002). As reported by Charman (2002), minor

changes in peatland nutrient availability can easily influence plant growth, with nutrient availability significantly impacting vegetation cover and composition in disturbed peatlands. N and P are limiting nutrients that influence photosynthetic carbon uptake, hence strongly impact NPP of plants (Iverson *et al*., 2010). Consequentially, N (Gorham, 1995) and P accumulation (*Loisel et al*., 2014) accompany the process of biomass accumulation exerting further influence on nutrient availability and subsequent processes linked to peat accumulation. The process of litter decomposition is predominantly controlled by biotic factors, that is, the activity of soil micro- and macro-organisms (Aerts, 1997; Andersen *et al.,* 2010) and litter chemistry (Thormann & Bayley, 1996). However, peatland nutrient levels influence the activity of microbial communities as well as the litter chemistry of the plant community. Therefore, the amount of C released or retained is correlated to the effect of physical, chemical, and biological processes converting plant residue into their elemental chemical constituents through decomposition (Bragazza *et al*., 2007).

Abiotic factors have also been highlighted as controls on NPP and decomposition rates in peatlands (Gorham, 1974). Specifically, NPP rates are impacted by the level of photosynthetic radiation received by plants and the water table depth of peatlands (Wieder, 2006), while decomposition rates are primarily controlled by water table depth, climate, and peat temperature (Moore *et al*., 2007). For example, increased tree productivity and root biomass were associated with lowering water table depths in a Canadian peatland (Lieffers & Rothwell, 1987) and a Finnish peatland (Heikurainen & Pakarinen, 1982). Additionally, deeper water tables have been linked to nutrient availability increases in a Quebec bog and fen (Macrae *et al*., 2013) as well as enhanced tree and shrub growth and overall plant productivity in peatlands (Moore *et al*., 2002; Weltzin *et al*., 2003; Munir *et al*., 2015). Decomposition rates are also increased due to reduced soil moisture

and greater aeration when the water table is lowered. There are suggestions of increasing carbon stores in surface peat after site drainage (Minkkinen & Laine, 1998) indicating the possibility of the inverse occurrence on flooded seismic lines, although this is yet to be investigated.

Although mature woody vegetation is conspicuously absent from the approximately 1900 km^2 seismic lines coverage of peatlands in Alberta (Strack *et al.,* 2019), the potential loss in biomass cannot accurately reflect changes in peat accumulation without assessing the thriving understory vegetation. Assessment of vegetation community changes in a disturbed peatland is a good way to predict potential changes to peat accumulation (Graf & Rochefort, 2009) due to the attributes of peatland flora in contributing to productivity and decomposition rates (Dieleman *et al.,* 2017). Further, changing temperature regimes and hydrologic conditions are also likely to affect peat accumulation on seismic lines through their impact on litter decomposition rates. For seismic lines, it is relatively easy to quantify the loss of woody biomass, but few studies have quantified the impact of changes in biophysical properties on vegetation community shift and the subsequent changes in NPP and decomposition. This will fill a knowledge gap of how seismic lines will affect overall peatland C balance. Therefore, the goal of this study was to assess the impact of seismic lines on NPP and decomposition rates of dominant plant species in a wooded fen and bogs. Specifically, we wanted to:

1) Quantify and evaluate changes in aboveground and belowground NPP rates between disturbed areas and the adjacent corresponding peatland. We hypothesized that the seismic lines would have reduced total ecosystem NPP (TNPP) due to the absence of trees but increased understory productivity from increased solar radiation and reduced competition.

We anticipate reduced belowground NPP (BNPP) on the lines resulting from the shallow WT limiting the depth of the rooting zone.

2) Estimate and compare rates of litter decomposition on the seismic lines to the surrounding peatland areas. We hypothesized that there would be an increase in litter decomposition rates on lines due to changes in vegetation community composition and warming temperatures.

3) Determine which site had a greater potential to accumulate organic matter in the ecosystem and soil. We hypothesized that loss of overstory NPP would reduce total organic matter accumulation on seismic lines, but that this would be partially offset by higher understory NPP.

3.3 MATERIALS AND METHODS

3.3.1 Study Sites

Sampling was conducted in a fen and two bogs located in the oil sands region near the town of Peace River, Alberta, Canada (Figure 1-2). The fen site is approximately 40 km northeast of the town of Peace River (56°23′51.22″N, 116°53'27.60″W), and can be classified as a wooded poor fen (NWWG, 1997) disturbed by a series of seismic lines and well pads, including a restored well pad site (IPAD) (Xu *et al*., 2022). It will be referred to as the "IPAD" fen site hereinafter. The dominant vegetation in the natural areas include *Picea mariana* (black spruce), *Salix* spp. (willow), *Pleurozium schreberi* (feather moss) and lichens (e.g., *Cladina* spp.) while seismic lines were dominated by *Carex aquatilis* (water sedge), patches of *Sphagnum spp.,* brown mosses, and

sparsely distributed willow saplings. The first bog site, referred to as Carmon Creek (CC) bog, is in the same vicinity (56°21′44″ N, 116°47′45″W) as IPAD fen site. The second bog, denoted as the Harmon Valley (HV) bog is located approximately 50 km southeast of Peace River (56°12.10N, 116°56.04W). Both bogs are classified as wooded bogs (NWWG, 1997) with a series of seismic lines running through both. Both bogs are dominated by *P. mariana*, *Vaccinium vitis-idaea* (lingon-berry), *Rhododendron groenlandicum* (Labrador tea)*, Vaccinium oxycoccos* (bog cranberry), lichens, and a ground layer of *Sphagnum fuscum* and *Pleurozium schreberi*, in the natural areas. The seismic lines had a similar vegetation profile except for the absence of both *P. mariana* and *P. schreberi* cover. Lines present at IPAD and HV are older, wider legacy lines 6–8 m in width whiles CC is characterized by "low impact" meandering lines 2–3 m in width. CC was further divided into CC1 and CC2 for data collection.

At each site (IPAD, CC1, CC2, HV), we picked two seismic lines that were at least 50 m apart. Along each line, we installed triplicate plots (60 x 60cm) on the line and in the adjacent peatland (15 m from the seismic line) for measuring carbon fluxes as reported in chapter four. Sample plots were selected from 4 sites × 2 subsites × 6 areas (3 disturbed + 3 natural) totaling 48 plots. For analytical purposes, seismic lines are treated as a treatment factor called Position. Position, therefore, refers to a plot on the line or the surrounding peatland area (Line vs. Natural). Data from CC1 and CC2 were combined as CC for analyses.

3.3.2 Vegetation surveys and species selection

Vegetation surveys were conducted in August 2018 at all 48 plots used for C flux measurements. Plots consisted of 60 cm × 60 cm stainless steel collars inserted 10–15 cm deep into the peat for greenhouse gas (GHG) measurements. Plots were installed in triplicates at each site on the line and in the adjacent peatland (15 m from the seismic line). Vegetation surveys were conducted by visually estimating percentage cover of all vascular and non-vascular plant species in each of the collars. Identification was carried out at the species level in line with the USDA online plants database (http://plants.usda.gov) and ranked based on abundance: 3 (occasional, more individuals), 1 (occasional, few individuals) or 0.1 (present) and then rounded to the nearest 5% for anything covering 5% or more of the plot (Davidson *et al*., 2021). Identified species were aggregated into growth forms (Forbs, Graminoids, Dwarf shrubs, Trees, Bryophytes and Lichens) for further analysis.

Based on the dominant species, five vascular plants and two bryophytes were assessed for decomposition and annual production rates. They are *Picea mariana* (black spruce), *Rhododendron groenlandicum* (Labrador tea), and *Sphagnum fuscum* for all sites, *Vaccinium vitis-idaea* (lingon-berry) and *Pleurozium schreberi* (feathermoss) for the bogs, while *Salix candida* (willow), and *Carex aquatilis* for the fen.

3.3.3 Production measurements

Annual primary production was measured to estimate peat accumulation potential of the species of interest highlighted above. Biomass samples were collected at the peak of plant productivity in

July 2021, as suggested by Davidson *et al.* (2021). Different methods were deployed to measure aboveground biomass of bryophytes and dominant vascular plants, and belowground biomass (root growth). More details are provided in the sections below.

3.3.3.1 Bryophytes

The wire brush technique, a modified version of the cranked wire according to Granath and Rydin (2013) was employed for measuring *Sphagnum* productivity at all sites. About 200 custom wire brushes were made in the laboratory from galvanized wire (150 guage) and bristles (from a bench brush) as per the Granath and Rydin (2013) protocol. Wire brushes were implanted at the beginning of the growing season in May 2018 alongside labelled flags for easy identification and recovery. Although other *Sphagnum* species such as *S. magellanicum* complex were present, selected patches where measurements were carried out were predominantly dominated by *S. fuscum*. Each wire brush was carefully inserted into the moss carpet through a truncated drinking straw at three random patches within a 1 m radius of the pre-installed C flux collars at each site. Nine wire brushes were installed per site yielding a total of 144 brushes across at all sites. They were anchored by their base bristles and the height of the wire above the moss carpet measured at the beginning (May) and end of the season (September). Wire brushes were checked and re-inserted at the beginning of the growing season in 2019, before measurements were taken. After final measurements were taken in September 2019, a *Sphagnum* core (5 cm x 5 cm) was extracted from each site for bulk density calculations. Extracted samples were stored in Ziploc bags, labelled and frozen before being shipped to the Wetland Soils and Greenhouse Gas Exchange Lab (Wetland GHG Lab) at the University of Waterloo, ON. The capitula was carefully clipped from all samples

as per Loisel *et al*. (2014), and the bulk density (dry mass divided by volume) was calculated from the stem section of the moss after oven drying at 60 ºC for 48–72 hrs (until constant weight). Recorded change in *Sphagnum* heights (decreasing distance from moss carpet to top of wire) were multiplied by their respective bulk density to generate estimates of *Sphagnum* production. Estimates for feather moss productivity were computed from cumulated averages of different peatland sites reported by Bona *et al.* (2013).

3.3.3.2 Understory productivity

Aboveground net primary production (ANPP) was measured by clipping understory vegetation (including small trees) at the base in triplicate 50×50 cm quadrats in July 2021 replicated on the lines and in the natural areas of the peatland. Samples were stored at 4 °C in Ziploc bags and shipped in coolers to the Wetland GHG Lab in Waterloo, ON for processing and analysis. Harvested vegetation was sorted into forbs, graminoids, shrubs and tree leaves and dried at 60 ºC for 48-72 hours until constant weight. Samples were then weighed, and the biomass calculated. Woody tissues were not included, and thus we assumed these annual leaf tissues represented NPP of the understory vegetation including small trees. Omission of annual growth of woody tissues in shrubs will slightly underestimate production while inclusion of all leaves will slightly overestimate production for evergreen shrubs and trees.

3.3.3.3 Trees

Multiple allometric equations based on regressions of dry biomass and diameter at breast height (DBH), or measured tree height were used to estimate aboveground biomass of trees (Munir *et al.*

2015). Biomass estimates were computed based on the tree species using the following equations (Grigal and Kernick 1984):

Biomass of *P. mariana* = 0.153(DBH)$^{2.248}$ (g) [1]

The following allometric equation from Carpenter (1983) was also used for:

Biomass of *Larix laricina* = 0.1359(DBH)$^{2.298}$ (g) [2]

The final equation used was derived from He *et al.* (2018) for:

Biomass of *Salix* spp = 55.85(BD)$^{2.325}$ (g) [3]

At each site, sampling was conducted in triplicate 5 × 5 m quadrats in the natural areas of the peatland. The total study area was relatively small, hence selected quadrats were within a 1 m radius to the C flux plots at each site. Lines were devoid of trees over 1 m hence tree biomass data was not calculated for lines, while small regenerating trees were captured in the understory productivity estimates. Biomass sampling involved measuring DBH and basal diameter (DB) of all trees in each quadrat, recording the height of the shortest and tallest trees, and cutting down three random trees in each plot and extracting 10 cm (width) tree cookies for analysis. In the lab, tree cookies were smoothed with coarse and fine grade sandpapers and scanned on a Canon pixma scanner. Images were processed using the java source code application Image J version 1.38e (https://imagej.nih.gov/ij/features.html). Measured tree rings were then incorporated into the species associated allometric equations to compute annual (incremental) biomass accumulation as

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per Szumigalski and Bayley (1996). To minimize disturbance to trees, incremental biomass of tree roots was estimated as per Li *et al*. (2003) using allometric equation (tree root biomass = 0.222* aboveground tree biomass). Tree litter fall for black spruce was also estimated based on derivations (17% of incremental biomass) from Szumigalski and Bayley (1996).

3.3.3.4 Roots

The root ingrowth bag method (Finér & Laine, 2000) was deployed to estimate belowground net primary production. Cylindrical mesh bags with a diameter of 10 cm and length of 50 cm were sewn from 1 mm gauge nylon mesh. The bags were filled with root-free commercial garden peat. In summer 2018, peat cores were extracted at each site within a 1 m radius from pre-installed C flux collars using PVC pipes measuring 50 cm long and 10 cm diameter. A total of 48 peat filled mesh bags were immediately installed into the holes after peat core extraction at all sites. Ingrowth bags were retrieved after two years in September 2020. One of the bags incubated at the IPAD line was disturbed by wildlife and therefore discarded. Excess roots and peat were carefully cleaned off the bags before processing the content. Each ingrowth bag was split into four depth increments (0-10, 10-20, 20-30, 30-50 cm). Newly formed roots in the peat were isolated by washing the bag contents through 1 mm and 0.5 mm mesh sieves. Separated roots were then dried at 60 ºC for 48–72 hours (until constant mass) and weighed.

3.3.4 Decomposition

In September 2018, feather moss and *Sphagnum* fragments were collected along with senesced aerial parts of target vascular plant specimen from all sites and processed as per Graf & Rochefort

(2009). Vascular plant materials were segregated into leaf litter and stems/twigs based on their potential to be broken down. Aboveground parts of sedges and bryophytes were, however, incubated as whole plants without segregation. *Sphagnum* samples were carefully de-capitulated and only the stems were kept. This resulted in a total of seven unique litter types for each site (*Sphagnum*, feather moss, sedges, willow, black spruce, Labrador tea, and lingon-berry). Samples were then oven dried at 40 °C until constant mass (minimum of 72 hours). Two grams of vascular plant material was loaded into pre-weighed individual nylon mesh bags (5 × 7.5 cm) with 1 mm mesh gauge, whiles 0.5 g of bryophyte samples were placed in pre-weighed nylon mesh bags with 0.25 mm mesh gauge (to prevent fragments from falling out). All sample-filled mesh bags were weighed to the closest 0.001 g. Sample-filled mesh bags were sorted into groups of 8 sets corresponding to target species identified above for fen and bog sites. Each group of 8 mesh bags were strung on a monofilament fishing line and attached to flags for easy recovery. Triplicate sets of mesh bags were inserted below the peat surface within a 1 m radius from pre-installed flux collars at each site. Bags containing bryophytes were vertically inserted between 5–10 cm depth, while bags with vascular plant materials were horizontally placed at about 5 cm below the peat surface. This was to mimic natural leaf fall and decomposition (Bérubé & Rochefort 2018). A total of 231 mesh bags were incubated at the three peatland sites corresponding to the dominant vegetation type at each site. All deployed mesh bags were recovered after two years of incubation in 2020. Retrieved bags were carefully rinsed in a water bath, and attached roots and vegetation removed with forceps. The cleaned mesh bags were frozen and shipped to the University of Waterloo, ON. Samples were later thawed, and oven dried at 40 °C until constant mass. Samples

were then weighed to the closest 0.001 g. The equation used for calculating the linear decay rate

(k) over the 2 growing seasons for each litter type was adapted from Reader and Stewart (1972),

$$k= [(X_0 – X)/X_0] * 100 \hspace{4cm} [4]$$

where X_0 is the mass (g) of the initial dry litter preceding decomposition and X represents the final

dry litter (g) after field incubation.

3.3.5 Environmental variables and vegetation survey

Peace River typically experiences warm summers and cold winters with a mean annual

precipitation of 287.3 mm and temperature of 13.3 °C as reported by Environment and Climate

Change Canada from 1981-2010 at the NAV Canada meteorological station (Peace River A)

(https://climate.weather.gc.ca/climate_data/daily_data_e.html?). Additional data was collected

from all sites including peat temperature, peat volumetric moisture content, and water table

position. A HOBO U23 Pro v2 temperature/external temperature data logger was mounted at each

site for recording hourly peat (5 cm depth) and air temperature at each subsite. In addition, daily

peat temperature and soil moisture at 5 cm depth was recorded alongside C flux measurements at

each plot, using a handheld probe from Delta-T devices (WET-2 Sensor attached to an HH2

readout meter). Wells were constructed of 1 m long PVC pipes with drilled holes and mesh

covering the base and installed adjacent to each plot for measuring water table position during

weekly C flux measurements.

3.3.6 Data Analysis

All statistical analysis was completed in R Studio version 1.3.1056 (R Core Team 2020). NPP and decomposition rates for the different species were computed based on the formulas above (Equation 1-4) and compared for differences between site and position (i.e., on the seismic line vs. the natural area) for the study period (2018 and 2019). The lm function and anova output were used to evaluate the effect of the peatland type and seismic line disturbance on decomposition rates and NPP. Where significant ($p < 0.05$) relationships existed, we conducted a Tukey pairwise comparison using the function 'lsmeans' (Lenth, 2016) to evaluate differences between groups.

3.4 RESULTS

3.4.1 Environmental conditions

Seismic lines were generally warmer and wetter than surrounding natural peatland areas (Table 3.1). The IPAD fen had the highest moisture levels with average volumetric moisture content (standard deviation) of 50.8 (14.9) % on the seismic line compared to 17.6 (10.6) % in the natural area, while the HV bog had the lowest moisture levels (Line: 30.1 (6.8); Natural: 13.2 (0.5) %). The highest average peat temperature was recorded on the seismic lines of CC at 11.6 (0.5) °C, and the natural peatland areas of CC presented the lowest average peat temperatures of 10.2 (1.3) °C.

Total vascular plant cover varied significantly ($F_{2,43} = 3.2880$, $p = 0.0469$) between the interaction of the seismic line disturbance and the peatland at all sites. Vegetation cover in disturbed areas generally presented a reduction in bryophytes and lichens with increasing shrub cover at the bogs and a greater graminoid cover at the fen (Table 3-1). These changes contributed to significant variation in vegetation cover due both to the position ($F_{1,43} = 5.6406$, $p = 0.0221$) and site ($F_{2,43} = 6.0116$, $p = 0.00496$)

Table 3-1 Average (standard deviation) environmental conditions and vegetation cover across study plots

Plot Location	Moisture (%vol)	Peat Temperature (°C)	Tree cover (%)	Lichen cover (%)	Bryophyte cover (%)	Dwarf shrub cover (%)	Graminoid cover (%)	Forb cover (%)
CC1 Line	38.1 (12.2)	10.6 (0.7)	0 (0)	25.1 (5)	63.4 (2)	10.5 (2)	0.8 (0.9)	2.3 (1)
CC1 Nat	10.2 (2.5)	9.3 (0.5)	0 (0)	46.0 (2)	68.0 (2)	9.2 (3)	0 (0)	1.3 (1)
CC2 Line	36.4 (0.1)	12.6 (0.2)	0.2 (0)	12.0 (6)	68.3 (6)	27.2 (3)	0 (0)	1.8 (1)
CC2 Nat	21.4 (3.2)	11.1 (0.8)	0.1 (0)	5.9 (1)	85.5 (9)	19.5 (7)	0 (0)	2.8 (1)
HV Line	30.1 (6.8)	11.4 (1.8)	0.2 (0.2)	2.5 (3)	90.0 (2)	17.2 (2)	0 (0)	4.7 (0)
HV Nat	13.2 (0.5)	11.0 (1.6)	0 (0)	14.3 (1)	75.9(5)	14.7 (2)	0 (0)	8.7 (4)
IPAD Line	50.8 (14.9)	11.2 (1.5)	0 (0)	0 (0)	59.2 (8)	9.3 (8)	32.5 (20)	3.3(2)
IPAD Nat	17.6 (10.6)	10.6 (2.5)	0 (0)	1.7 (2)	81.8 (0)	8.8(5)	11.2 (10)	0.8 (1)

3.4.2 Net primary production

Vegetation cover was multiplied by the NPP of each vegetation group to estimate the overall understory NPP of selected plant species per plot (Figure 3-1). Sphagnum mosses had the highest productivity at all sites followed by shrubs and trees (black spruce and willow). Productivity varied in relation to disturbance and site. For example, *Sphagnum* was more productive on lines at IPAD and HV, while the inverse was observed at CC. Shrubs and feather mosses were, however, more productive in natural areas of the peatland at all sites compared to the seismic lines. Forbs were, overall, the least productive plant group but did have higher productivity rates in natural areas at IPAD and CC, while in contrast at HV the lines were more productive. Sedges were mostly confined to the fen site and substantially increased in productivity on the seismic line.
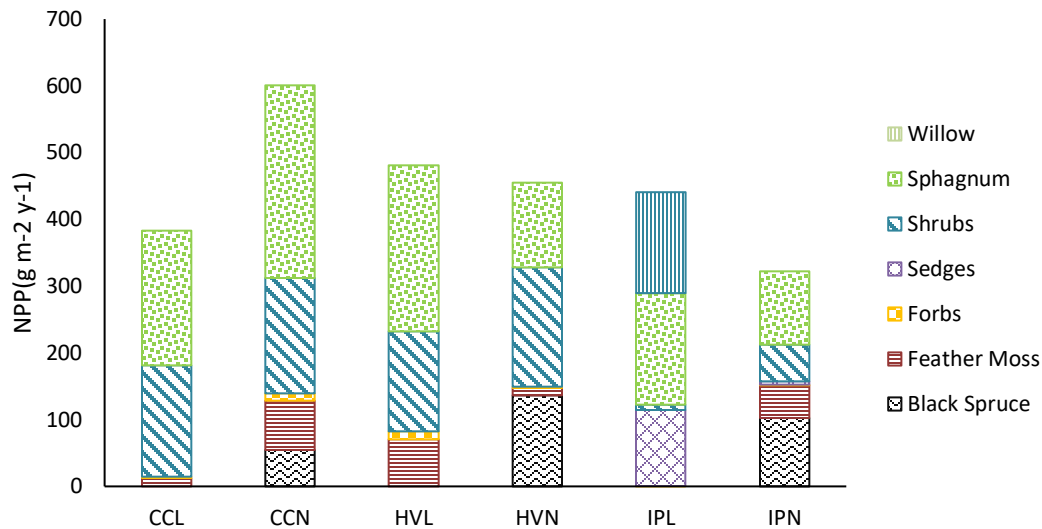
Figure 3-1 Distribution of average understory net primary production (NPP) of selected vegetation across study sites. Site nomenclature: Carmon creek (CC), Harmon Valley (HV) and IPAD (IP), Natural peatland area (N) and Seismic Line (L) respectively

At the IPAD fen site, NPP of the understory was remarkably higher on the lines compared to the natural areas (136.97 vs 30.05 g m^{-2} y^{-1}). Both bogs had higher ANPP in the natural areas compared to the seismic lines (Table 3-2). The lines at CC had the highest BNPP, followed by IPAD. BNPP was higher in the disturbed areas of both IPAD and CC by at least a factor of 1.5 times compared to the natural peatland areas, while at HV, BNPP rates were very similar across all sample areas although slightly greater in the natural areas (Table 3-2). Seismic lines at all sites are missing a substantial amount of overstory ANPP compared to the natural areas (IPAD-174.48; CC- 54.79 and HV-30.26 g m^{-2} y^{-1}) (Table 3-2). Total estimated tree NPP followed the same trend as the ecosystem NPP with the highest productivity recorded at the natural areas of IPAD followed by HV and then CC (Table 3-2).

Overall, the natural areas at the fen site had the highest total ecosystem NPP (299.3 g m^{-2} y^{-1}) closely followed by the HV bog (292.5 g m^{-2} y^{-1}) and then CC bog (194.7 g m^{-2} y^{-1}). Large differences between productivity rates were influenced by the NPP of the trees present in natural peatland areas. HV recorded the greatest variation in ecosystem NPP, with the natural areas being about three times more productive than the disturbed areas (292.5 vs. 97.6 g m^{-2} y^{-1}), whereas at the other study sites, ecosystem NPP in the natural peatland areas were closer to double the productivity rate on the seismic lines. Although the ecosystem NPP of disturbed sites varied between 97.6 to 162.9 g m^{-2} y^{-1} from HV to IPAD, the disturbed areas at IPAD, however, recorded much higher above and belowground NPP compared to the natural areas (excluding tree NPP), that is, 162.9 vs 44.8 g m^{-2} y^{-1}.

Table 3-2 The mean above- and belowground plant production, mass loss and total biomass remaining after 2 years

Site	ANPP[1]	BNPP[2]	Trees ANPP	Trees BNPP	Tree litter fall	Trees TNPP	Ecosystem NPP	Understory Mass loss[3]	Trees (Linear Decay)	Tree litter loss	Below ground Tree decomposition[3]	Below ground understory decomposition[3]	TNPP[3]	Soil Layer NPP[3]
							$(g\ m^{-2}\ y^{-1})$							
IPN[4]	30.05	14.57	174.48	38.74	41.50	254.72	299.3±21.5	21.34	17.13	7.11	29.63	6.42	234.85	60.37
IPL[5]	136.97	25.88	0.00	0.00	0.00	0.00	162.9±35.2	43.82	23.60	0.00	0.00	11.41	107.62	107.62
CCN[6]	99.55	18.63	54.79	12.16	9.53	76.49	194.7±5.01	13.03	18.95	1.81	6.81	8.22	164.81	110.02
CCL[7]	98.23	31.68	0.00	0.00	0.00	0.00	129.9±21.2	11.37	15.23	0.00	0.00	13.97	104.57	104.57
HVN[8]	91.81	10.37	136.3	30.26	23.72	190.28	292.5±39.6	27.69	19.22	4.56	16.93	4.57	238.71	102.41
HVL[9]	87.63	9.93	0.00	0.00	0.00	0.00	97.6±55.9	28.34	19.56	0.00	0.00	4.38	69.22	69.22

1-ANPP (understory)
2- BNPP (ingrowth bags)
3- Values are after 2y

4- IPAD Natural Peatland Area
5- IPAD Seismic Line
6- Carmon Creek Natural Peatland Area

7- Carmon Creek Seismic Line
8- Harmon Valley Natural Peatland Area
9- Harmon Valley Seismic Line

Overall, BNPP at CC presents a decrease with depth for both disturbed and natural areas with much higher productivity on the lines (Figure 3-2). The inverse is observed at IPAD, where productivity increases with depth and peaks at around 40 cm below the peat surface. The pattern of belowground productivity with depth between disturbed and natural peatland areas are similar at IPAD and HV in contrast to CC where the line had higher BNPP, particularly in the upper 20 cm (Figure 3-2).
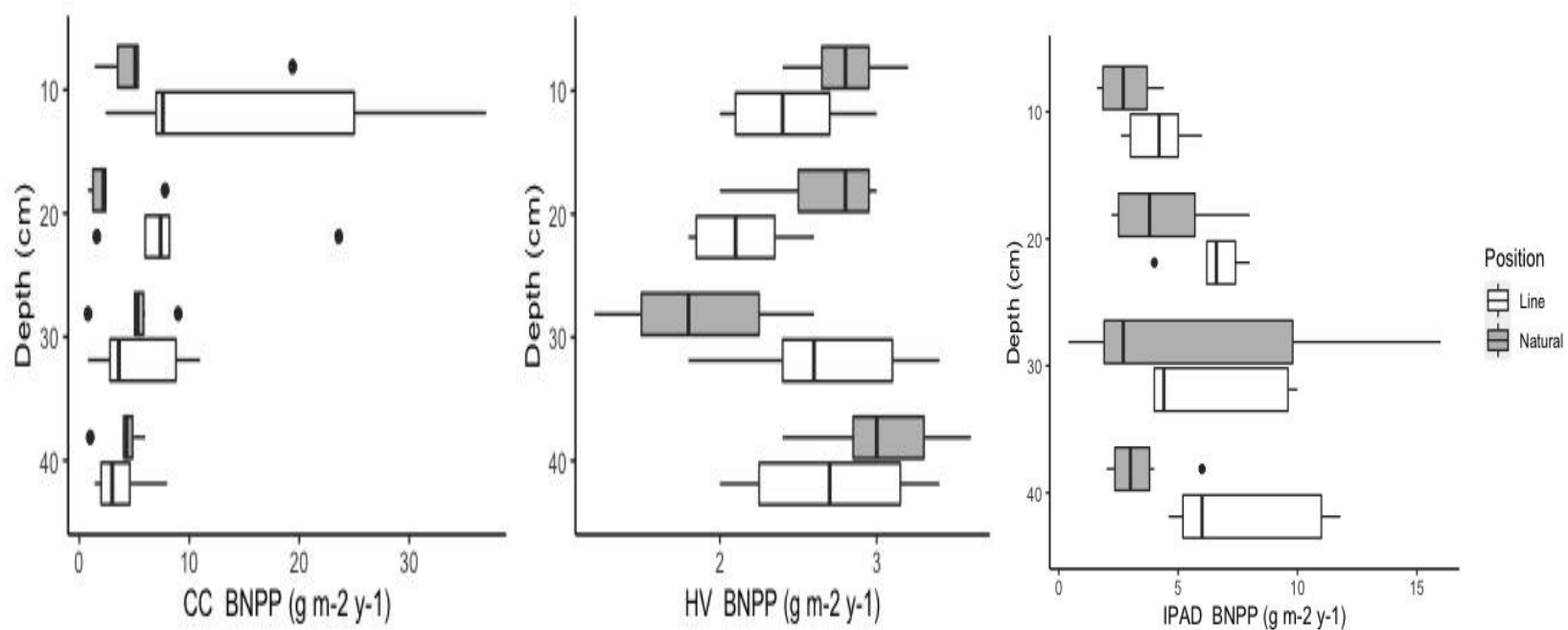
Figure 3-2 Average belowground net primary production (BNPP) per site over various depths. The median is shown by the horizontal line within the boxplot; the upper and lower hinges represent the 75th and 25th percentiles, respectively. The smallest and largest values within the 1.5 times the interquartile range (IQR) are represented by whiskers, whiles dots outside the box and whiskers represent outliers (values > or < 1.5 x IQR).

3.4.3 Decomposition

Sphagnum and Labrador tea were both incubated at all sites as common litter types to provide estimates for comparative decomposition rates across study sites. Overall linear decay coefficient (k) values for both Sphagnum and Labrador tea were higher in the natural peatland areas compared to the seismic lines at all sites (Figure 3-3 A & B). The highest rates for Sphagnum decay in both the disturbed and natural areas were recorded at HV. For Labrador tea, the highest k values were observed at CC followed by HV, then IPAD. Both the impact of the seismic line ($F_{1,24} = 8.27$, $p = 0.008$), and the peatland site ($F_{2,24} = 7.74$, $p = 0.003$) were significant on Sphagnum decomposition rates, while differences in Labrador tea decomposition rates were significantly impacted by the site ($F_{2,24} = 6.91$, $p = 0.004$). The interaction of position and site was not significant on the decomposition of either Sphagnum ($F_{2,24} = 0.634$, $p = 0.539$), or Labrador tea ($F_{2,24} = 0.235$, $p = 0.793$), as well as the impact of the disturbance on the decomposition of Labrador tea ($F_{1,24} = 0.209$, $p = 0.652$).
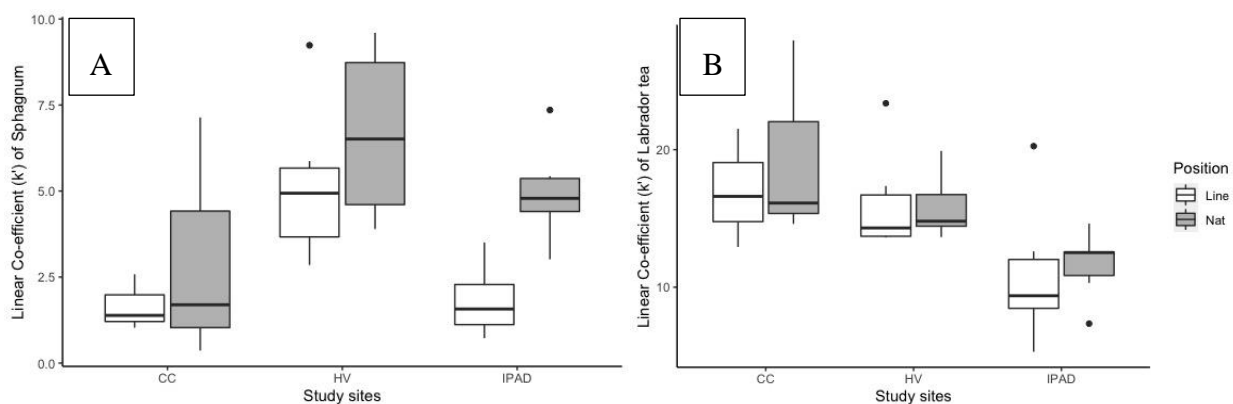
Figure 3-3 Linear decay coefficient (k) of Sphagnum (A) and Labrador tea (B) over 2 growing seasons. The median is shown by the horizontal line within the boxplot; the upper and lower hinges

represent the 75th and 25th percentiles, respectively. The smallest and largest values within the 1.5 times the interquartile range (IQR) are represented by whiskers, whiles dots outside the box and whiskers represent outliers (values > or < 1.5 x IQR).

Generally, the average k was dependent on site and position and varied between seismic lines and the adjacent forested peatland (Table 3-3). At CC, the natural peatland areas had higher k values for Sphagnum, black spruce, and shrubs than on the seismic lines while the natural and disturbed areas had with similar k values for feather mosses. At HV, higher k values were recorded at disturbed areas for Sphagnum and black spruce. Shrubs and feather mosses had approximately equal k values both on and off the seismic lines. At IPAD, sedges, black spruce, and shrubs had much higher k values on the seismic lines compared to the natural areas. In contrast, Sphagnum had higher k values at the natural areas than on the lines. At IPAD feather mosses grew only in the natural peatland areas while willows were only found on lines. Therefore, no comparison between position were made for these two litter types.

As plant species composition varied between natural areas and seismic lines, the variation in k for the different litter types could lead to differences in overall litter decay rates. For example, the highest k values were associated with sedges (at IPAD (0.23 (0.08) to 0.32 (0.04)), suggesting their increased abundance on the line would lead to an overall increase in litter decomposability (Table 3-3). Similarly, as Sphagnum had the lowest k values at all sites, ranging from 0.01 (0.02) to 0.05 (0.02), it can also be inferred that their presence would be associated with a decrease in litter decomposability.

Table 3-3 Linear decay co-efficient (k) (Standard deviation) of litter types over 2 growing seasons

Linear decay co-efficient (k)	Natural	SD	Line	SD
CC				
Sphagnum	0.03	0.03	0.02	0.01
Black Spruce	0.19	0.01	0.15	0.02
Shrubs	0.13	0.03	0.12	0.03
Feather Moss	0.06	0.01	0.06	0
HV				
Sphagnum	0.01	0.02	0.05	0.02
Black Spruce	0.19	0.02	0.20	0.02
Shrubs	0.12	0.02	0.12	0.01
Feather Moss	0.12	0.07	0.12	0.02
IPAD				
Sphagnum	0.05	0.01	0.02	0.01
Sedges	0.23	0.08	0.32	0.04
Willow	n/a	n/a	0.24	0.07
Black Spruce	0.17	0.02	0.20	0.03
Shrubs	0.09	0.02	0.20	0.06
Feather Moss	0.26	0.04	n/a	n/a

When litter type and k were combined at each location the total mass loss of litter after 2 years of incubation was generally higher in the natural peatland areas (Table 3-2). Deviations from this trend were associated with HV where mass loss was higher in the disturbed areas for Sphagnum (8.42 vs. 12.85 g $m^{-2}y^{-1}$ for natural and seismic line, respectively), feather mosses (1.30 vs. 8.59 g $m^{-2}y^{-1}$) and forbs (0.57 vs. 4.06 g $m^{-2}y^{-1}$). The litter with the greatest mass loss over the 2 growing seasons was sedges, followed by shrubs.

3.4.4 Potential accumulation of organic matter in the ecosystem and soil

After two years, the total NPP remaining in the ecosystem was consistently greater in natural sections of the forested peatlands than on the seismic lines, although the difference varied among the study sites. The total difference ranged from about 1.5 times greater in the natural area at CC compared to on the line to up to four times greater at HV, driven by differences in both tree and understory productivity. Although the tree productivity was highest at IPAD, a large increase in ground layer productivity on the line offset much of the loss of the overstory, resulting in only a moderate reduction in the total ecosystem NPP remaining on the line.

As most of the NPP remaining after two years in the natural areas was present as wood in the tree stand, we also computed the total NPP remaining after two years in the soil to get a better understanding of shifts in potential peat accumulation. After the two growing seasons, the total NPP remaining in the soil was higher at the disturbed areas for both IPAD (119.03 vs. 57.68 g $m^{-2}y^{-1}$ at seismic lines and natural, respectively) and CC (118.54 vs 112.88 g $m^{-2}y^{-1}$), while the inverse was recorded at HV, that is, 69.22 vs. 93.65 g $m^{-2}y^{-1}$ (Table 3-2)

3.5 DISCUSSION

To the best of our knowledge, this is the first study focused on assessing the impact of seismic line disturbances on net primary production and decomposition rates in boreal peatlands. This is essential in estimating potential peat accumulation, that is, the difference between annual litter production and decomposition and the overall impact on C storage. Our findings indicate a significant reduction in TNPP in disturbed areas accompanied by changes in litter decomposition rates. Although ground layer NPP was generally greater in the disturbed fen due to high aerial biomass production of graminoids, shrubs and tree saplings, this could not compensate for losses due to the absence of overstory vegetation.

Variation in productivity and decay was primarily driven by vegetation species and environmental characteristics as reported by Bridgham *et al*., (1996) and Szumigalski & Bayley, (1996). Species composition in our study area varied significantly between seismic lines and their adjacent reference peatland, potentially driven by significantly greater peat temperature and soil moisture content in the disturbed areas (Table 3-1). This is in line with our initial hypothesis on vegetation community changes and their contribution to changes in above (including all vascular plants and bryophytes) and belowground (roots) biomass and NPP (Table 3-2).

3.5.1 NPP

The higher TNPP rates associated with the natural areas (Table 3-2) was largely linked to the presence of overstory woody vegetation (Clymo *et al*., 1998; Bhatti *et al*., 2012; Berube & Rochefort, 2018). Natural sections of the peatland therefore sustained tree development leading to

increased tree productivity and development of root biomass in accord with previous findings (Lieffers & Rothwell, 1987; Hanson *et al*., 2000). Apart from the obvious absence of woody biomass of trees, seismic lines affect NPP in a complex way. For example, the higher WT on the lines contributed to a vegetation community shift to species preferring more waterlogged conditions (Caners & Lieffers, 2014; Lovitt *et al.,* 2018) with better substrate quality for decomposition (Thormann, Szumigalski & Bayley, 1999). The pattern of change in ground layer NPP in response to seismic line disturbance varied among study sites. In general, understory NPP remained higher in the natural areas when compared to the seismic lines in the bogs, while the lines in the fen were associated with significantly higher ANPP rates compared to their surrounding peatland areas (Table 3-2). The increased ANPP on the fen lines was associated with the elevated growth of highly productive graminoid species in the wetter and non-shaded areas with minimum competition from other vegetation (Caners & Lieffers, 2014; Strack *et al*., 2017). Increasing graminoid cover on wetter lines was also documented by Davidson *et al*. (2021) and Schmidt *et al*. (2022). Although lines were generally devoid of trees over 1 m, a few regenerating willow saplings present on lines at the fen (IPAD) were incorporated in the understory productivity estimates and contributed to the greater understory NPP rates on lines (Figure 3-1 and Table 3-2). The lines at IPAD were the warmest of the study areas and the change in temperature may have been a driving force in the high NPP rates observed. White *et al.* (2008) recorded significant increases in the NPP of a bog mesocosm study when the average peat temperature was raised by ~3 °C accompanied by lowering the WT by up to 32 cm, while Malhotra *et al*. (2020) found significant increases in fine root development in response to warming between +2 and 9 °C above ambient conditions.

Sphagnum moss had NPP rates in direct correlation with WT depths reported in chapter 4 (Table 4-2). Increasing light levels (Pouliot *et al*., 2011) and shallow WT depths (Granath *et al*., 2010; Strack *et al.,* 2018) negatively affect *Sphagnum* dominance and could justify the observed reduction in *Sphagnum* cover on all lines except at HV. The general increase in *Sphagnum* cover on the line at HV is in line with findings by Schmidt *et al.* (2022). The increase in *Sphagnum* cover at HV could account for the associated increase in NPP (Figure 3-1). The productivity of shrubs was consistently higher at natural peatland areas of all sites despite an increase in shrub cover on all lines. The inverse relationship between shrub NPP rates and vegetation cover could be explained by the omission of the annual growth of woody tissues in the estimation of shrub NPP leading to potential underestimation of NPP rates. However, it was more practical in our study to estimate shrub understory NPP based on only annual leaf tissues, albeit the potential risk of underestimating productivity rates. Again, because it was omitted at all study sites, it likely contributed little to the apparent differences observed on and off the lines.

Annual aboveground primary production of vascular plants was substantially higher than belowground production at all sites (Table 3-2). BNPP ranged between 10–48% of the associated ANPP, like values obtained in a poor fen by Graf & Rochefort (2009) and Berube & Rochefort (2018), and not too far from the reported 50% obtained in studies from both bogs and fens by Campbell *et al*. (2000) and Chimner *et al*. (2002). Although much higher values of 20–80% were obtained by Reader & Stewart (1972) from a bog, differences between studies are expected given that these ratios are influenced by factors such as vegetation community, hydrology, and peat chemistry. The root ingrowth bag method used in the present study is simpler and more cost effective (Graf & Rochefort, 2009; Malhotra *et al*., 2020) when compared to techniques involving

rhizosphere cameras. Nonetheless the importance of including root measurements in estimates for peat accumulation is validated as root production accounted for a substantial portion of TNPP (Chimner *et al*., 2002; Berube & Rochefort, 2018). Belowground productivity was correlated with vascular plant cover. Both CC and IPAD had greater BNPP on the lines compared to their surrounding peatlands (Table 3-2) while we measured slightly higher values in the natural peatland areas at HV. The greater root development at CC and IPAD on the lines were most likely because of the observed increase in shrub, forb and graminoid cover. Both sites were also associated with reduced Sphagnum cover on lines. In contrast, lines at HV were associated with greater Sphagnum cover, potentially outcompeting vascular plant root productivity, leading to the low BNPP recorded.

Although we expected that shallow WT could reduce BNPP by limiting the depth of the rooting zone, the effect of the WT depth on root productivity was not very consistent. CC had the driest conditions and had the highest mean BNPP for both disturbed and natural peatland areas (31.68 and 18.63 g m^{-2} y^{-1}, respectively (Table 3-2). Previous studies also report that root growth is generally greatest when the WT is deep (Lieffers & Rothwell, 1987; Campbell & Bergeron, 2012). Again, the root biomass distribution (Figure 3-2) in the depth profile at CC is in accord with findings from Lieffers & Rothwell (1987), where majority of root biomass in a drained bog occurred in the first 20 cm depth. In contrast, findings at HV deviated from this expectation, where conditions of higher WT position could account for the greater concentration of root biomass at greater depths (20–40 cm). The fen at IPAD had the shallowest recorded WT depths but the second highest BNPP rates for both lines (25.88 g m^{-2} y^{-1}) and natural peatland areas (14.57 g m^{-2} y^{-1}, Table 3-2). Root development here, that is, the high BNPP cannot be explained by the relationship

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to the WT, but rather vegetation cover type. The highly productive dominant graminoid cover on the lines are well adapted to wet conditions and can root in saturated soils due to aerenchyma. Data from more seismic lines are needed to better understand the variation in BNPP between bogs and fens and in response to disturbance because of hydrologic controls.

3.5.2 Decomposition

Litter decomposition rates at the different study sites would vary in response to differences in peat temperature, moisture content, WT position, and dominant vegetation cover (Bubier *et al.* 2007; Belyea & Baird 2006). Linear decay co-efficients for the sampled litter were generally lower at the natural areas compared to the seismic lines (Table 3-3). The conditions in the natural areas tended to favor plant communities that produce recalcitrant litter leading to lower decomposition rates over time (Waddington *et al.* 2001; Strack *et al.* 2006), providing a justification for the associated lower k values. However, changes to environmental conditions associated with seismic line disturbance also affected litter decay. Analysis of the standard litter (Figure 3-3) showed that Sphagnum incubated at all sites had significantly higher decomposition in the natural areas compared to the seismic lines. Differences in Labrador tea decomposition were less clear in response to disturbance although significant differences existed between the sites with higher decomposition in the bogs (CC followed by HV) compared to the fen (IPAD). Differences in the moisture content (Table 3-1) and WT, could account for the increased decomposition under oxic conditions observed at drier sites (natural vs. disturbed, and bog vs. fen), since aerobic decomposition is favored by a deeper WT due to increasing oxic layer thickness (Gazovic *et al.*, 2013).

Observed differences in k values could also be attributed to site level differences in climate, hydrology, and chemistry, and even mesh sizes of the litterbags. Smaller mesh sizes as used in this study, have been associated with slow litter decomposition (Johnson & Damman, 1993, Graf & Rochefort, 2009). IPAD had the highest k for vascular plant litter at both disturbed and natural peatland areas, in line with increasing understory vascular plant cover and warmer temperatures (Leroy *et al*., 2017). Graminoids on the lines had the highest k (mean (standard deviation) = 0.32 (0.04)) of all sampled vegetation, most likely because of the increased labile C. These values are slightly below measured k of the same genus from related studies in northern peatlands (0.37 – 0.70; (Szumigalski & Bayley, 1996; Graf & Rochefort, 2009). At HV, k values of litter followed a trend similar to IPAD with black spruce recording the highest k values on the lines (0.20 (0.02)). We measured the lowest k at CC with the natural peatland area slightly surpassing the seismic line for black spruce and shrub litter. Again, black spruce had the highest k values at CC. Sphagnum mosses recorded the lowest k at all sites in line with Strakova *et al*., (2012), although the other litter types were not necessarily in line with the decomposability reported by the same authors (herbs < graminoids < feathermoss < Sphagnum mosses). However, deviations in litter decomposability should be expected at different sites due to differences in litter chemical composition influenced by varying nutrient, cellulose, and lignin content (Strakova *et al*., 2012). The low k value of Sphagnum, that is, 0.01 (0.02) to 0.05 (0.02) and associated low variation within each site, points to the fact that Sphagnum decomposition is more controlled by intrinsic factors rather than habitat factors (Johnson & Damman 1991; Johnson, 1992; Graf & Rochefort 2009).

Although litter decay likely follows an exponential decay pattern, since we collected litter at only one time point the declining pattern of litter decay over time could not be calculated in the present study. Here we assumed linear decay over the two years incubation period and calculated the amount of litter remaining after two years. Total mass loss (Table 3-2) was estimated from the combination of the effects of litter decomposition rate, litter type and total plant production. Because litter production from understory vegetation was generally higher on the seismic lines and consistently of a higher proportion of more labile litter types, understory mass loss was similar to, or higher, on the seismic lines than in the adjacent peatland. Further, changes in nutrient availability or hydrologic conditions due to the seismic line disturbance could influence vegetation communities and impact decomposition rates, for example, by increasing N availability that favors tree and vascular plant growth while reducing Sphagnum cover (Limpens *et al.*, 2008). Weltzin *et al.* (2003) also reported on potential shifts to NPP and vegetation communities, highlighting nutrient levels as an important parameter that could further explain some of our observations. The impact of peatland nutrient levels on both NPP and decomposition were beyond the scope of the present study but should be investigated in future research.

3.5.3 NPP remaining after two years and potential peat accumulation.

The natural peatland areas at all sites generally had greater peat-accumulating potential compared to the seismic lines when considering the total NPP remaining after two years. Potential peat accumulation was influenced more by the associated slow decomposition rates rather than high rates of productivity in agreement with other findings (Vitt, 1990; Berube & Rochefort, 2018). The importance of Sphagnum in peatlands was emphasized here as it had the lowest decomposition

rate of all litter types at all sites, and further contributed the highest rate of productivity in the ground layer (Table 3-2, Figure 3-1), in line with other authors (Berube & Rochefort, 2018). Trees in the natural areas of the peatland contributed the greatest NPP with much of this remaining in standing biomass (Table 3-2), confirming initial speculations about the role of woody vegetation in long term ecosystem C storage.

Potential soil layer peat accumulation was observed to be higher on the lines compared to the natural areas at IPAD and CC, but lower on seismic lines at HV (Table 3-2). This was largely driven by altered understory vegetation cover on seismic lines and associated high ANPP and BNPP rates (Thormann, Szumigalski and Bayley, 1999). However, it is not very clear why these parameters were higher in the surrounding peatland areas of HV compared to the other sites, although as discussed earlier, HV showed the least variation in moisture and temperature differences between lines and the surrounding peatlands (Table 3-1). Given that much of the remaining biomass was in trees in the natural areas, the overall impact of seismic lines on peat accumulation rates in boreal peatlands presents additional questions, as not all the overstory biomass is destined to be accumulated into peat, for example, due to losses in wildfires. Therefore, for better comprehension on the impacts of seismic lines on ecosystem carbon storage, it is important for future studies to specifically investigate rates of peat accumulation using palaeoecological methods to provide better insight on the impacts of seismic lines on decadal rates of peat accumulation.

3.6 CONCLUSION

Seismic line disturbances in boreal peatlands remove trees, compact peat, and encourage sustained saturated soil conditions because of shallower water tables on lines. The open areas receive greater solar radiation leading to warmer temperatures on lines. These conditions shift vegetation communities on lines resulting in other complex interactions impacting peatland function.

Our study confirmed that seismic lines were associated with lower net primary production and variation in litter decomposition rates based on site conditions. The greatest changes to NPP rates were linked to the absence of overstory woody vegetation from all seismic lines at all sites. The loss of NPP from the overstory layer was not compensated for by the higher understory productivity observed on the seismic lines. The creation and persistence of seismic lines in boreal peatlands resulted in lower amounts of NPP remaining after two years, suggesting lower overall peat accumulation potential. This suggests that restoration of woody vegetation is important for maintaining the C storage function of boreal peatlands. It is however important to note that peat accumulation is a complex long-term process and longer-term studies may be required to for more accurate predictions to overall C budget in response to seismic line disturbance. This research has, however, provided the foundation for new perspectives on carbon cycling in disturbed peatlands via production and decomposition.

Chapter 4: Seismic lines alter methane cycling in boreal peatlands.

4.1 ABSTRACT

Boreal peatlands serve as long-term carbon (C) sinks as well as a significant source of methane (CH_4) to the atmosphere. However, peatlands are threatened by both natural and anthropogenic disturbances resulting in potential release of large amounts of C to the atmosphere. Linear disturbances such as seismic lines for oil and gas exploration and extraction constitute the bulk of these disturbances. The impact of seismic lines on peatland function, such as C cycling and hydrology, is not very well understood, although physical changes in topography and lack of tree re-establishment are well documented. Previous studies have suggested that these changes will increase CH_4 emission, but little on-the-ground data exists to test this hypothesis. This study measured growing season understory CH_4 fluxes from disturbed and surrounding peatlands and assessed environmental controls (e.g., vegetation cover and productivity, water table, peat temperature) on CH_4 dynamics. The closed chamber technique was deployed to measure fluxes of CH_4 and the net ecosystem exchange of CO_2 (NEE), from 48-paired plots distributed across a poor fen and two wooded bogs near Peace River, AB. Data was collected over two growing seasons (2018-2019) together with environmental variables such as water table, peat temperature and vegetation cover.

Sections of the peatland impacted by seismic lines were significantly warmer and wetter providing ideal conditions for increased CH_4 emissions at all sites. Methane emissions relative to natural plots were: 176% (fen, legacy), 261% (bog, low-impact) and 308% (bog, legacy) over the two

studied growing seasons. The persistence of the seismic lines and the elevated CH_4 emissions is a cause for concern due to CH_4 having a higher global warming potential compared to CO_2. Results from this study will contribute to accurate greenhouse gas (GHG) reporting for anthropogenic disturbance in boreal peatlands, which is lacking for many disturbance types, as well as provide a scientific foundation for integrated land management practices and policies related to boreal peatland restoration.

4.2 INTRODUCTION

Natural peatlands act as long-term carbon dioxide (CO_2) sinks by accumulating partially decomposed organic material, i.e., peat (Gorham 1991, Frolking & Roulet, 2007). Peatlands around the world store up to 600 Pg of carbon, with an estimated 300–550 Pg C held in northern peatlands (Hugelius *et al.,* 2020). Prevalent waterlogged, anoxic conditions in these environments lead to slow anaerobic decomposition releasing significant amounts of methane (Gorham 1991; Frolking *et al*., 2011). Peatlands account for 5–10% of global methane (CH_4) emissions (Blodau, 2002), making them a critical component of the global terrestrial carbon cycle.

Unfortunately, the occurrence of natural resources such as oil and gas deposits across peatland-rich regions have led to extensive anthropogenic disturbances, including mines, access roads, well-pads, pipelines, and seismic lines. Seismic lines, or cutlines, are long linear pathways constructed for petroleum exploration and extraction (Lee & Boutin 2006). There are multiple reports on the impacts of seismic lines on endangered fauna and habitat destruction (Dyer *et al*., 2002, Filicetti *et al.,* 2019), changes to vegetation and microclimatic conditions (Venier *et al*., 2014; Van Rensen

et al., 2015; Franklin *et al.,* 2021); however, not enough information is available on changes to carbon cycling in boreal peatlands (Strack *et al.,* 2019). Again, due to mounting concerns over climate change, and more importantly anticipated rapid changes in temperature and precipitation in northern climates (IPCC, 2021), investigating and quantifying potential increases in CH_4 emissions from disturbed peatlands (bogs and fens) is imperative for accurate reporting to better understand the overall impact of anthropogenic disturbance on atmospheric concentrations of CH_4. Hence, this study investigated the impacts of seismic lines on peatland CH_4 exchange.

Canada accounts for approximately 27% of global peatland area, located mostly in the boreal and sub-Arctic regions of the country (Xu *et al.*, 2018). About 16% of the province of Alberta is covered by peatlands (Tarnocai *et al.*, 2011), which intersect with some of the region's massive oil sands deposit. There are over 345,000 km of seismic lines reportedly crossing boreal peatlands in Alberta alone, with more in other provinces and territories (Strack *et al.*, 2019). These lines are required for geological surveys for the movement of exploratory equipment and often continue to serve as transportation routes once exploration is complete. There are two distinct types of cutlines used in the Alberta oil and gas industry, that is, conventional/legacy lines and three-dimensional (3-D) lines (Lee & Boutin 2006). Legacy lines are 5 –10 m wide corridors, constructed using heavy machinery including bulldozers (Bliss & Wein, 1972). Over time, legacy seismic lines have become the most prevalent disturbance type associated with the oil and gas industry in the Western Canadian Arctic (Kemper & Macdonald, 2009), and to a large extent, the entire boreal forest (Pasher *et al.*, 2013). The extensive footprint of legacy lines necessitated the need for "lower-impact" or 3-D seismic lines, that is, narrow 1.5 – 5 m wide lines cut with smaller equipment and chainsaws (Lee & Boutin, 2006). Evidence is lacking, however, in support of the low-impact

nature of 3-D seismic lines, especially due to the higher density of lines required for seismic data acquisition (Lee & Boutin, 2006).

The cutting of trees during the construction of lines leads to a reduction in tree net primary productivity and the removal of tree canopy biomass which may reduce the C uptake potential of the ecosystem (Strack *et al*., 2018, Chapter 3). Additional data has highlighted some impacts of cutlines on peatland soil and carbon dynamics such as increased CH_4 emissions linked to wetter lines and warmer temperatures (Strack *et al*., 2018); compression of peat and elimination/reduction of microtopography (Caners & Lieffers, 2014; Dabros *et al.,* 2018; Davidson *et al*., 2020), a potential cause for the sustained absence of trees on lines (Lee & Boutin, 2006; Filicetti *et al.,* 2019). These reported conditions on lines have culminated in vegetation community changes such as a reduction in bryophyte cover (Pouliot *et al*., 2011) or increasing sedge/reed populations (Lee & Boutin, 2006; Strack *et al*., 2018). The combined effects of these changes on C stocks and C cycling in peatlands is currently understudied and requires attention for accurate greenhouse gas (GHG) emissions reporting.

The estimated global warming potential (GWP) of methane is 27–30 times that of CO_2 over 100 years, highlighting its potential contribution to climate change (IPCC, 2021). CH_4 in peatlands is produced via methanogenesis, where the waterlogged conditions provide ideal conditions for anaerobic degradation of organic carbon by *Archaea* (Lai, 2009; Bridgham *et al*., 2013). Factors that influence the production of CH_4 include water table position (Bubier *et al*., 1993; Pypker, 2013), peat temperature (Lai, 2009; Pypker, 2013), peatland vegetation community composition and litter quality (Bridgham *et al*., 2013, Strack *et al*., 2017). The water table position acts as a

barrier for the diffusion of atmospheric oxygen, thereby controlling the "width" of the anoxic zone (Waddington *et al*., 1996), while peat temperature is essential in controlling decomposition rates, especially in northern peatlands (Valentine *et al.,* 1994; Wieder, 2006). CH_4 production rates increase with the availability of highly labile carbon, which is influenced by the vegetation community and litter type, as associated with fast growing graminoids in fens (Strack *et al*., 2017). The abiotic and biotic factors driving CH_4 production rates influence each other under variable conditions. For example, increasing temperature generally favors gross ecosystem productivity (GEP), leading to increased availability of labile carbon (root exudates) or additional litter inputs that can lead to greater CH_4 production (Lai, 2009; Waddington *et al*., 1996).

The amount of CH_4 produced that reaches the atmosphere is also dependent on the oxidation rate by methanotrophic bacteria and the transportation pathway. The WT position controls the amount of oxygen available for CH_4 breakdown (Couwenberg & Fritz, 2012; Andersen *et al.,* 2013) while the amount of CH_4 produced and physico-chemical parameters (pH, nutrient levels, temperature) drive the action of methanotrophs (Valentine *et al*., 1994; Sundh *et al*., 1995). The CH_4 produced is transported to the atmosphere through the following pathways: plant-mediated transport, ebullition, and diffusion through the soil (Lai, 2009). The bulk of CH_4 produced reaches the atmosphere through plant tissues and is favored by the presence of aerenchyma and thus vegetation type, and high transpiration rates (Couwenberg & Fritz, 2012). In other cases, CH_4 escapes in gas bubbles through the process of ebullition or slowly diffuses through the peat matrix to reach the atmosphere. When the water table is deep, much of the CH_4 diffusing through the peat is oxidized (Waddington *et al*., 1996).

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An estimated 17–61 Tg of CH_4 is annually released from boreal and subarctic peatlands (Bridgham *et al.*, 2013). Other reports indicate rates of 56.36 mg CH_4 m^{-2} d^{-1} for northern fens and 25.98 CH_4 m^{-2} d^{-1} for northern bogs (Turetsky *et al.*, 2014), while Strack *et al.* (2019) reported values of 1.4, 7.1, and 2.5 g CH_4 m^{-2} yr^{-1} for swamps, fens, and bogs, respectively, for western Canada. Wetter conditions resulting from seismic lines (Strack *et al.*, 2019; Davidson *et al.*, 2021; Chapter 2 & 3) could potentially increase CH_4 emissions by 4.4 to 5.1 thousand tons of CH_4 per year in Alberta (Strack *et al.*, 2019). However almost no studies have directly measured CH_4 emissions from peatlands disturbed by seismic lines, although similar studies from a winter road (Strack *et al.*, 2018) and measurements from lines in a fen during peak growing season conditions (Schmidt *et al.*, 2022) showed significant increases in CH_4 emissions. The actual effect of seismic lines on peatland CH_4 exchange remains largely unknown. Therefore, this study measured growing season understory CH_4 fluxes from disturbed and surrounding peatlands and assessed environmental controls (i.e., vegetation cover and productivity, water table, peat temperature) on CH_4 dynamics. We hypothesized that CH_4 emission would be higher on seismic lines compared to natural areas due to shallower water table, warmer soils, and more productive ground layer vegetation.

4.3 STUDY SITES AND METHODS

4.3.1 Site description

Multiple peatland sites disturbed by a series of seismic lines in the Peace River oil sands region of Alberta, Canada, were selected in May 2018 for this study (Figure 1-2), comprising a wooded poor fen and two wooded bogs (NWWG, 1997). The fen site (IPAD-56°23′51.22″N, 116°53’27.60″W)

and first bog site (Carmon Creek-56°21′44″ N, 116°47′45″W) are part of the Peace River Complex for oil exploration and production, located approximately 40 km northeast of the town of Peace River. The second bog site, (Harmon Valley-56°12.10N, 116°56.04W) is approximately 50 km southeast of the town of Peace River. Site selection was primarily influenced by the age and type of seismic lines present, ease of access and availability of existing data on local vegetation and hydrological conditions (Dabros *et al.,* 2017; Strack *et al.,* 2018; Saraswati *et al.,* 2019).

The predominant flora characteristic of both Harmon Valley (HV) and Carmon Creek (CC) included *Vaccinium vitis-idaea* (lingon-berry), *Rhododendron groenlandicum* (Labrador tea), *Vaccinium oxycoccos* (bog cranberry), lichens (e.g., *Cladina* spp.), and *Sphagnum fuscum* (rusty peat moss). In addition to the above vegetation, the natural areas included *Picea mariana* (black spruce), interspersed with patches of *Pleurozium schreberi* (feathermoss). At IPAD, *P. mariana*, *Salix* spp. (willow), *P. schreberi* and lichens dominated the natural areas of the fen, while lines were mainly dominated by *Carex aquatilis* (water sedge), patches of *Sphagnum* spp.*,* brown mosses, and sparse willow saplings (Davidson *et al.*, 2021). All lines were devoid of trees taller than 1 m, irrespective of the age of the lines. Lines present at IPAD and HV are older, wider legacy lines of width 6–8 m while CC is characterized by "low impact" lines of width 2–3 m. Two study areas were instrumented at CC to capture low-impact lines of varying ages (CC1 and CC2).

Data was collected from two seismic lines per site (CC1, CC2, IPAD and HV) with paired plots in the adjacent, natural peatland. At each site, two lines, at least 50 m apart, were instrumented with triplicate plots (60 × 60 cm) on the line and in the adjacent peatland (at least 15 m away), that is, 4 sites × 2 subsites × 6 plots (3 disturbed + 3 natural), totaling 48 plots. Plots consisted of

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permanent 60 cm × 60 cm stainless steel collars inserted 10–15 cm deep into the peat for greenhouse gas (GHG) measurements, alongside dip wells for water table measurements. The aim of this study design was to capture impacts related to peatland and seismic line type while capturing variation within each study site. Topography was incorporated by ensuring hummocks, hollows and lawns were captured at each site. For analytical purposes, the different peatlands are denoted as sites (CC, HV and IPAD), while seismic lines are treated as a treatment factor called Position. Position, therefore, refers to a plot on the line or the surrounding peatland area (Line vs. Natural). Data from CC1 and CC2 were combined as CC and represent low impact lines in a bog.

4.3.2 Methane flux

The closed chamber technique was used to determine CH_4 flux from plots over two growing seasons (May to September) in 2018 and 2019. CH_4 fluxes were measured weekly using opaque acrylic closed chambers (60 cm × 60 cm × 30 cm) placed over pre-installed stainless-steel collars. To minimize leaks during measurement, water was poured into the collar groove to provide an airtight seal, and a battery-operated fan in the chamber circulated air in the headspace. Tubing from the chamber was connected to an ultraportable GHG analyzer with precision 0.25 ppb (LGR model GGA-30p, from Los Gatos Research, California, USA) for simultaneous measurement of CH_4, CO_2 and water vapour concentrations in the headspace. The LGR analyzer was powered by a rechargeable 12 V, 40-amp lithium phosphate battery (Bioenno Power, California, USA) and recorded fluxes from each plot over a period of 15 minutes. On rainy days or when we encountered technical difficulties with power supply to the LGR, 20 ml of gas samples were manually collected into syringes from the chambers at intervals of 7-, 15-, 25- and 35-minutes post-closure, and stored

into 12 ml pre-evacuated glass vials (Exetainer, Labco Ltd, UK). To determine initial gas concentrations (time 0), control vials were filled with ambient air at least once per site measurement. All sample vials were then packaged and shipped to the Wetland Soils and Greenhouse Gas Exchange Lab in Waterloo, ON for analysis. A Shimadzu 2014 gas chromatograph (Mandel Scientific, Ontario, Canada) equipped with a flame ionization detector and an EST flex automatic sampler was used to determine the concentration of CH_4 in the vials. Manual collection of gas samples accounted for about 8% of samples in 2018 and 21% in 2019. Data integrity was not compromised as the same fluxing method was generally implemented across sites over the same week allowing for consistent comparison.

The linear change in CH_4 concentration over time was used to estimate the CH_4 flux, after corrections were made for actual chamber headspace volume and temperature. For samples collected manually, the flux was assumed to be below detection and set to 0, if the initial concentration of CH_4 was <5 ppm and the concentration over the closure time changed less than the precision of combined sampling, storage, and GC methods (+/-5%) (Strack *et al.*, 2018). Measurements were also discarded on the assumption of ebullition caused by a disturbance, if the initial CH_4 concentration was >5 ppm then followed by a decline in concentration over time. Fluxes were inspected for linearity and incorporated into the dataset when the R^2 of the decrease/increase in CH_4 concentration was > 0.75. This data cleaning procedure resulted in a data loss of 11% from 2018 and 13% from 2019.

4.3.3 Gross Ecosystem Productivity (GEP)

GEP of the understory vegetation was estimated for all sites by calculating the difference between the net ecosystem exchange of carbon dioxide (NEE) and ecosystem respiration (ER), as described in Munir *et al*. (2015). NEE was determined by placing a transparent acrylic closed chamber (60 cm $\times$ 60 cm $\times$ 30 cm) over pre-installed collars and measuring CO_2 concentrations every 15 seconds over a duration of 2 minutes using a portable infrared gas analyzer (EGM-4, PP Systems, Massachusetts, USA). NEE was calculated from the linear change of CO_2 over time. An opaque shroud was placed over the chamber and CO_2 concentrations measured over 2 minutes as for NEE to obtain ER values. As indicated above, chambers were equipped with battery-operated fans to circulate air in the head space while collar grooves were filled with water to provide a tight seal. In between each measurement, the chamber was lifted from the collar to permit equilibration to ambient temperature and CO_2 concentrations. Fluxes were inspected for linearity and accepted if $R^2 > 0.75$, the only exception being relatively unchanging fluxes and considered to represent a flux close to 0. 24% of fluxes from 2018 and 18% from 2019 were rejected after quality control.

4.3.4 Environmental variables and vegetation survey

Water wells made from 1-m long PVC pipes with drilled holes and mesh covering the base were installed at each plot for measuring water table position during C flux measurements. A handheld thermocouple probe (Omega HH200A, Spectris plc, Quebec, Canada) was also used to record peat temperature in increments of 5 cm to a depth of 30 cm. When ice was encountered in the top 30 cm of peat, the frost table was recorded. Soil moisture at 5 cm depth was also recorded during each

measurement using a capacitance meter (WET-2 Sensor attached to an HH2 readout meter). Climate data recorded by the NAV Canada meteorological station (Peace River A), was retrieved from Environment and climate change Canada (https://climate.weather.gc.ca/climate_data/daily_data_e.html?).

 In August 2018, a non-destructive vegetation survey was conducted in each of the 60 cm × 60 cm flux collars to obtain estimates of percentage cover for both vascular and non-vascular plants. Plant identification was conducted at the species level in accordance with the USDA online plants database (http://plants.usda.gov). Visual estimates of identified species were ranked by abundance, that is, 3 (occasional, more individuals), 1 (occasional, few individuals) or 0.1 (present) when cover was under 5% and then rounded to the nearest 5% for higher cover (Davidson *et al*. 2021). Identified species were further combined into one of the growth forms: Forbs, Graminoids, Dwarf shrubs, Trees, Bryophytes and Lichens.

4.3.5 Data Analysis

All statistical analyses and figures were completed in R Studio version 1.3.1056 (R Core Team 2020). Linear mixed effects models (lme) built with the "nlme" package (Pinheiro *et al*. 2017) were used to analyze variability between C fluxes and environmental conditions on and off seismic lines. To evaluate whether fluxes varied between study sites and position (i.e, on the seismic line versus in the adjacent peatland) models incorporated average daily fluxes as the response, location, position and their interaction as fixed effects. To evaluate potential environmental controls on CH_4 flux, fixed effects of WT, soil temperature (T5-T30) and Vegetation cover were investigated. To

account for repeated measurements, all models included plot as a random factor. To improve the normality of residuals, CH_4 flux data was log transformed 314159 and used as the dependent variable in conjunction with environmental data (independent variables) for analysis. Additional lme functions were used to evaluate the effect of the peatland type and position on controls such as peat temperature, WT position and vegetation cover. Where significant ($p < 0.05$) relationships existed, we conducted a Tukey pairwise comparison using the function 'lsmeans' (Lenth 2016) to evaluate differences between groups.

4.4 RESULTS

4.4.1 Environmental conditions

During the growing seasons of the study period (2018-2019), overall conditions were relatively drier and colder compared to the 30-year (1981-2010) average for the same May to August season (Table 4-1). The average temperature was very similar for 2018 and 2019, at 12.8 °C and 12.7 °C, respectively in comparison to 13.3 °C as the mean from 1981-2010. Although both seasons commenced with much higher temperatures than average, a peak was reached in July and much colder temperatures ended both seasons.

Total precipitation from 2019 (255.2 mm) was much closer to the long-term seasonal average of 287.3 mm, while 2018 (208.4 mm) was comparatively drier.

Table 4-1 Mean monthly temperature (T) and total precipitation (P)

Month	2018		2019		1981-2010	
	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)
May	13.8	6	10.6	5.2	9.9	36.5
June	14.7	75.6	14.5	57.4	14.6	73.3
July	16.1	81.4	15.4	90.5	17.1	80.7
August	15.1	21.6	13.1	60	15.4	57.1
September	4.2	23.8	9.9	42.1	9.5	39.7
Growing Season	12.78	208.4	12.7	255.2	13.3	287.3

In general lines were warmer and wetter than their surrounding peatland areas (Table 4-2). Recorded line temperatures at the peat surface and predetermined depths were on average, a minimum of 1 °C greater than that of the surrounding natural peatland. Disturbed peatland areas at IPAD and CC maintained higher WT positions (minimum 3 cm closer to the surface) compared to the natural areas. However, the average WT position for the natural plots at HV was slightly lower than the seismic line in both 2018 and 2019. WT position was more variable in the 2019 growing season and statistically significantly shallower on the seismic lines compared to the surrounding peatland ($F_{1,42} = 5.82$, $p = 0.02$), and varied among the study sites ($F_{2,42} = 5.61$, $p = 0.007$); however, for the 2018 growing season only differences among peatland sites were significant ($F_{2,372} = 8.86$, $p = 0.0002$). Statistical output for all models is shown in Appendix 4.

Table 4-2 Descriptive statistics for measured variables over the study period.

Sites	WT (cm)			T2 (°C)[a]			T15 (°C)[a]			CH_4 flux (mg CH_4 m^{-2} day^{-1})			GEP (g CO_2 m^{-2} day^{-1})		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
2018															
CC Line	92	-9.2	7.7	91	16.7	4.3	91	10.4	2.9	69	22.8	65.1	52	-9.4	5.8
CC Nat	90	-12.5	12.7	91	15.6	4.2	91	9.4	3.5	75	12.3	49.5	48	-7.1	5.6
HV Line	72	-12.3	10.1	71	15.3	5.2	72	10.2	4.1	54	28.2	138.9	39	-18.3	7.6
HV Nat	68	-10.6	11.4	69	12.7	5.6	69	8.2	4.5	57	5.1	19.7	41	-9.3	5.8
IPAD Line	60	-1.7	4.9	62	13.7	3.6	61	10.4	3.2	46	69.3	94.6	42	-14.5	7.6
IPAD Nat	61	-4.3	7.0	61	13.1	4.3	61	8.7	4.4	49	71.7	169.6	38	-5.3	5.2
2019															
CC Line	117	-15.7	7.5	120	16.0	4.7	120	9.5	3.7	104	13.8	35.0	93	-16.6	8.2
CC Nat	125	-24.3	9.7	125	14.7	5.2	123	8.8	3.5	100	1.8	4.0	95	-9.4	5.9
HV Line	54	-17.7	7.4	54	15.7	4.5	54	9.9	3.6	38	4.5	4.8	42	-12.1	7.2
HV Nat	54	-17.6	12.6	54	14.3	5.7	54	8.1	3.5	36	5.5	18.5	41	-6.0	5.8
IPAD Line	77	-8.6	7.3	78	13.7	4.4	78	8.9	3.4	50	116.7	183.0	60	-19.6	13.1
IPAD Nat	78	-13.3	9.0	78	11.8	4.7	74	6.1	3.4	44	33.6	161.4	60	-6.8	6.8

a. T2 & T15 represents peat temperature at 2 cm and 15 cm depth respectively, GEP gross ecosystem photosynthesis.

Differences in total vascular plant cover were significant between all sites ($F_{2,43} = 6.02$, $p = 0.005$), as well as between the disturbed area and surrounding peatland ($F_{1,43} = 5.64$, $p = 0.02$) and finally there was a significant interaction of site and the disturbance ($F_{2,43} = 3.29$, $p = 0.046$). Hence, both the position and study site accounted for significant variation in vegetation cover, but the changes were site specific. Other important relationships identified were the significant ($F_{2,43} = 6.4372$, $p = 0.003585$) differences in forb cover among sites as well as graminoid cover having a significant interaction with all sites ($F_{2,43} = 20.5433$, $p < 0.0001$), the seismic line ($F_{1,43} = 4.25$, $p < 0.045$), and the interaction of both the line and site ($F_{2,43} = 20.5433$, $p < 0.00296$). Hence graminoid cover was most dominant on lines at IPAD. Overall differences in vegetation cover were marked by a general reduction in bryophytes and lichen cover on all seismic lines coupled with a transition to greater shrub cover at the bogs and a shift to greater graminoid cover at the fen site (Table 3-1).

4.4.2 GEP

During the study period, understory productivity was generally higher on the seismic lines at all sites compared to the surrounding peatland areas (Figure 4-1). The significant variation in GEP ($F_{1,491} = 17.163$, $p < 0.0001$) at the disturbed areas of the peatland was strongly linked to total vascular plant cover ($F_{1,47} = 6.6644$, $p = 0.013$) and significantly related to WT ($F_{1,586} = 17.843$, $p < 0.0001$) and peat temperature ($p < 0.0001$) at all measured depths from T2-T30 (Appendix 4). Hence, higher vascular plant cover was associated with greater GEP and warmer and wetter conditions. The IPAD fen site therefore had the most productive lines (Line 17.05 g CO_2 m^{-2} day^{-1}, Natural 6.05 g CO_2 m^{-2} day^{-1}) followed by HV (Line 15.2 g CO_2 m^{-2} day^{-1}, Natural 7.65 g CO_2

m^{-2} day^{-1}), while CC had the most productive natural areas (Line 13 g CO_2 m^{-2} day^{-1}, Natural 8.25 g CO_2 m^{-2} day^{-1}).
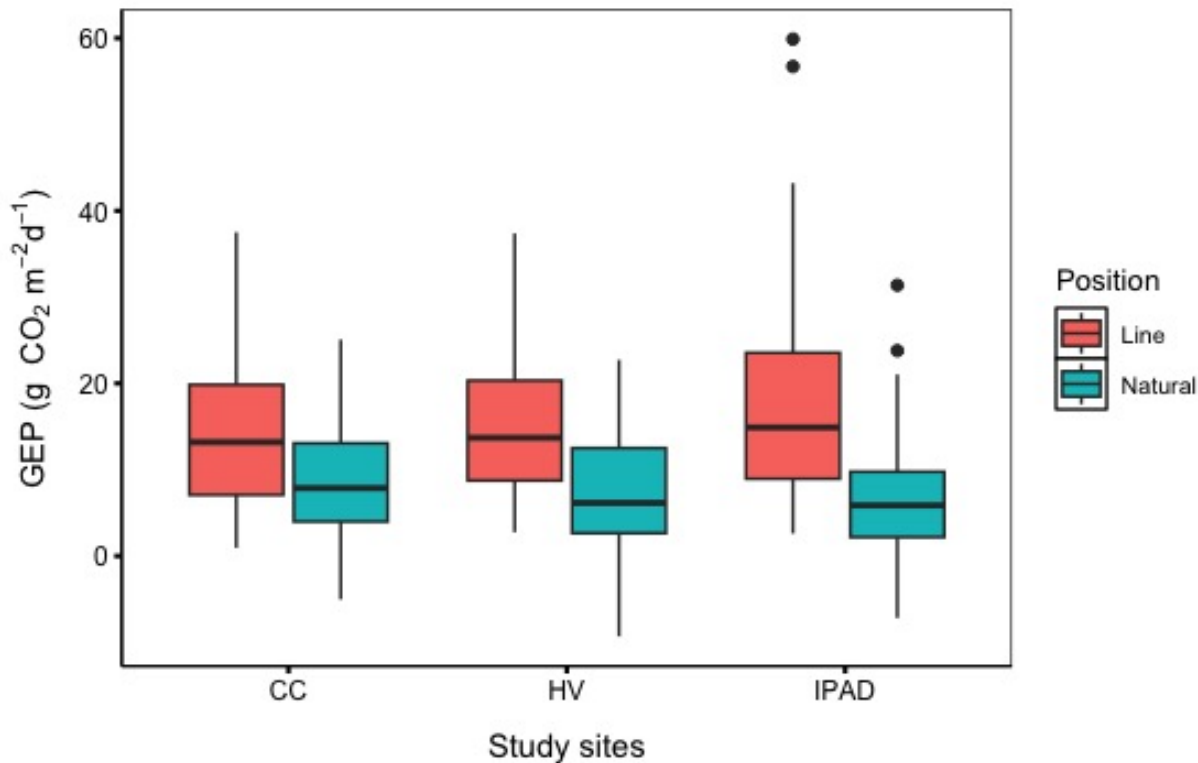
Figure 4-1 Understory gross ecosystem photosynthesis (GEP) from all sites over 2 growing seasons. The sign convention for GEP is reversed so positive values indicate carbon sinks. GEP values are the mean of all measured fluxes in full light conditions. Positive values indicate release of CO_2 to the atmosphere. The median is shown by the horizontal line within the boxplot; the upper and lower hinges represent the 75th and 25th percentiles, respectively. The smallest and largest values within the 1.5 times the interquartile range (IQR) are represented by whiskers, whiles dots outside the box and whiskers represent outliers (values > or < 1.5 x IQR).

4.4.3 CH$_4$

In both years, we recorded a general increase in CH$_4$ fluxes from May until August across all sites (Figure 4-2), following the seasonal pattern of warming temperatures and increasing precipitation (Table 4-1). During the 2019 growing season, CH$_4$ fluxes varied significantly between peatland types ($F_{2,42} = 11.679$, $p < 0.0001$) and were significantly impacted by the existence of the seismic lines ($F_{1,42} = 11.679$, $p < 0.0001$). However only variation in fluxes between different peatland types ($F_{2,286} = 14.353$, $p < 0.0001$) was significant for the 2018 growing season. Notably, much higher fluxes were recorded in May 2018 in comparison to May 2019, and this trend overlaps with the higher than seasonal temperatures (13.8 °C vs. 9.9 °C) recorded for the same period. Although average CH$_4$ fluxes exceeded 100 mg CH$_4$ m^{-2} day^{-1} at IPAD (116.7 mg CH$_4$ m^{-2} day^{-1}) in 2019 and reached 69.3 mg CH$_4$ m^{-2} day^{-1} in 2018, all other sites recorded fluxes lower than 30 mg CH$_4$ m^{-2} day^{-1} during the study period (Table 4-2). HV showed the least variation in CH$_4$ fluxes in 2019, with greater emissions in the natural peatland areas compared to the lines (5.5 vs. 4.5 mg CH$_4$ m^{-2} day^{-1}).
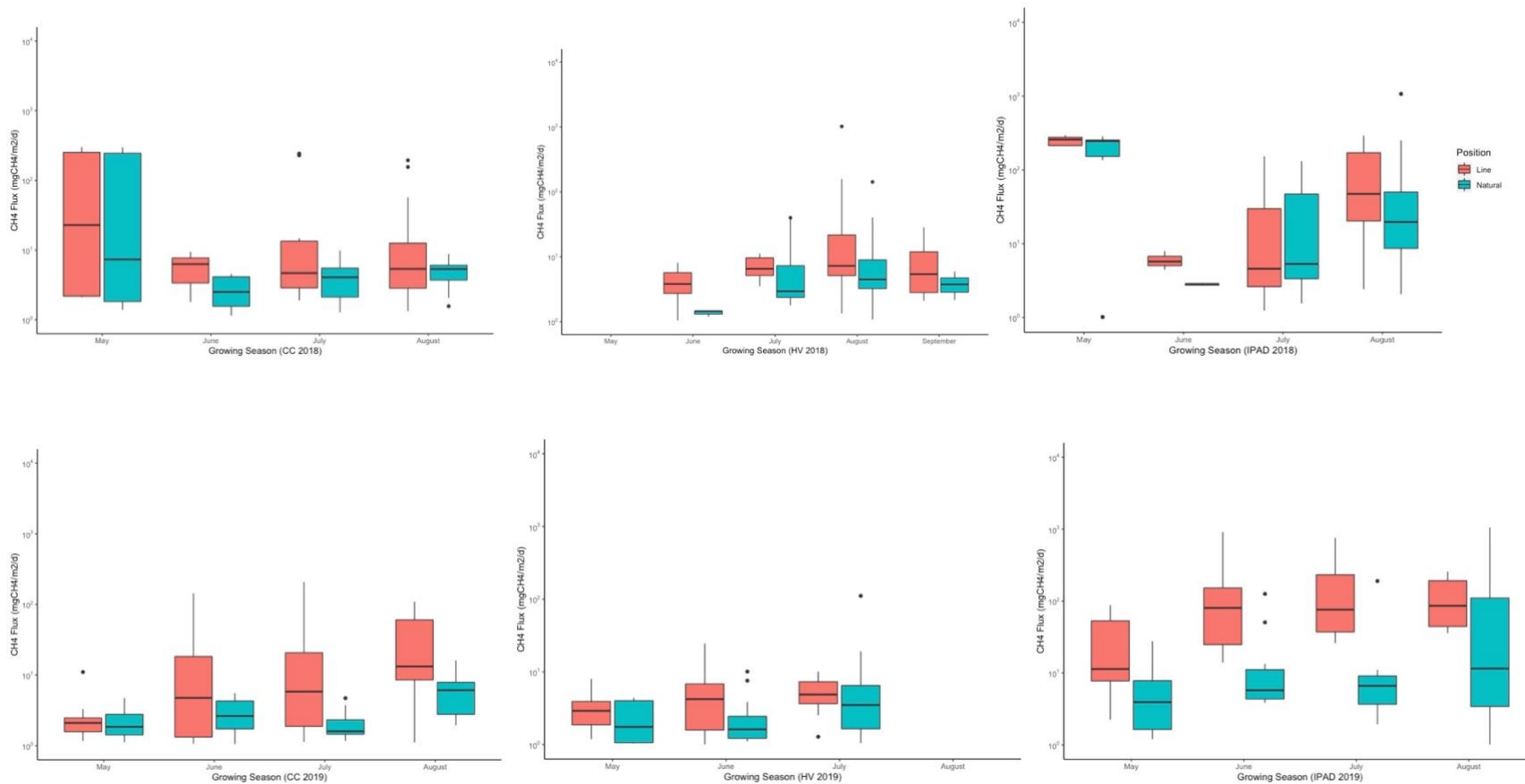
Figure 4-2 Average monthly CH$_4$ fluxes from all sites over 2018 and 2019 growing seasons. The median is shown by the horizontal line within the boxplot; the upper and lower hinges represent the 75th and 25th percentiles, respectively. The smallest and largest values within the 1.5 times the interquartile range (IQR) are represented by whiskers, whiles dots outside the box and whiskers represent outliers (values > or < 1.5 x IQR).

Over the entire 2-year study period, CH4 emissions were significant between study sites ($F_{2,660} = 15.010$, $p < 0.0001$), were impacted by the seismic line disturbance ($F_{1,59} = 9.219$, $p = 0.0036$), and the interaction of the peatland type and disturbance ($F_{2,660} = 3.121$, $p = 0.0448$). CH4 fluxes were generally higher on all lines compared to the surrounding peatland during the study period (Figure 4-3). The average CH4 flux rates were similar at the bog sites, although there were still overall differences among sites. The fen recorded the highest fluxes, followed by CC and HV in correlation to temperature and moisture levels. A shallower WT resulted in a higher log (CH4 flux) ($F_{1,656} = 7.584$, $p = 0.0061$), while warmer peat temperatures significantly increased log (CH4 flux) at the following depths T2 ($F_{1,663} = 4.56$, $p = 0.0331$), T15 ($F_{1,659} = 5.426$, $p = 0.0201$), T25 ($F_{1,588} = 7.130$, $p = 0.0078$), T30 ($F_{1,558} = 17.163$, $p < 0.0001$). Graminoid cover was the only significant ($F_{1,47} = 6.4437$, $p = 0.0145$) vegetation control related to variation in log (CH_4 flux).
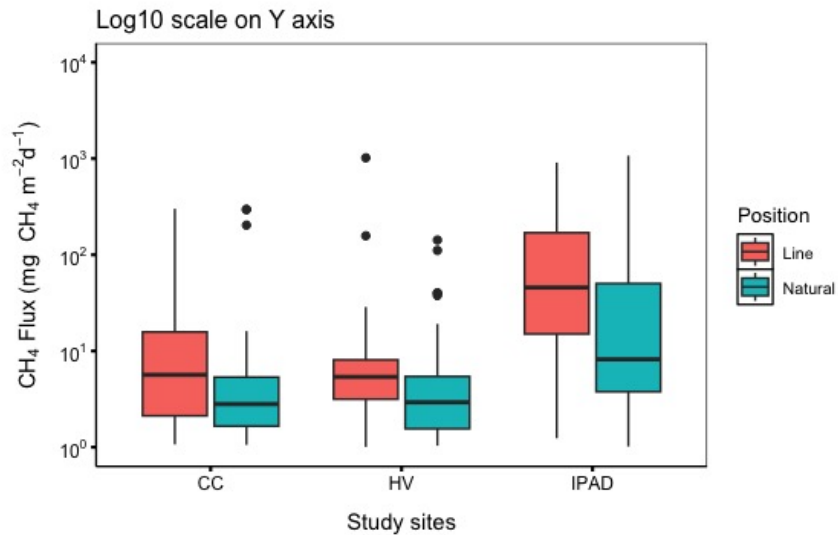
Figure 4-4 CH$_4$ fluxes at each study site over 2 growing seasons. The median is shown by the horizontal line within the boxplot; the upper and lower hinges represent the 75th and 25th percentiles, respectively. The smallest and largest values within the 1.5 times the interquartile range (IQR) are represented by whiskers, whiles dots outside the box and whiskers represent outliers (values > or < 1.5 x IQR).

Overall CH$_4$ fluxes and understory GEP at all the sites over the study period show that highly productive sites are associated with higher CH$_4$ fluxes (Figure 4-4A). The significant (F$_{1,491}$ = 6.320, p = 0.0123) relationship between variation in log CH$_4$ flux and GEP highlight the changes to carbon cycling due to the persistence of the seismic line disturbance. Both CH$_4$ emissions and CO$_2$ uptake are greater on the lines than their surrounding peatlands, with IPAD showing the highest fluxes. This is correlated with the change in vegetation cover and general increase in moisture and temperature on the lines (Figure 3-1/Table 2-1).

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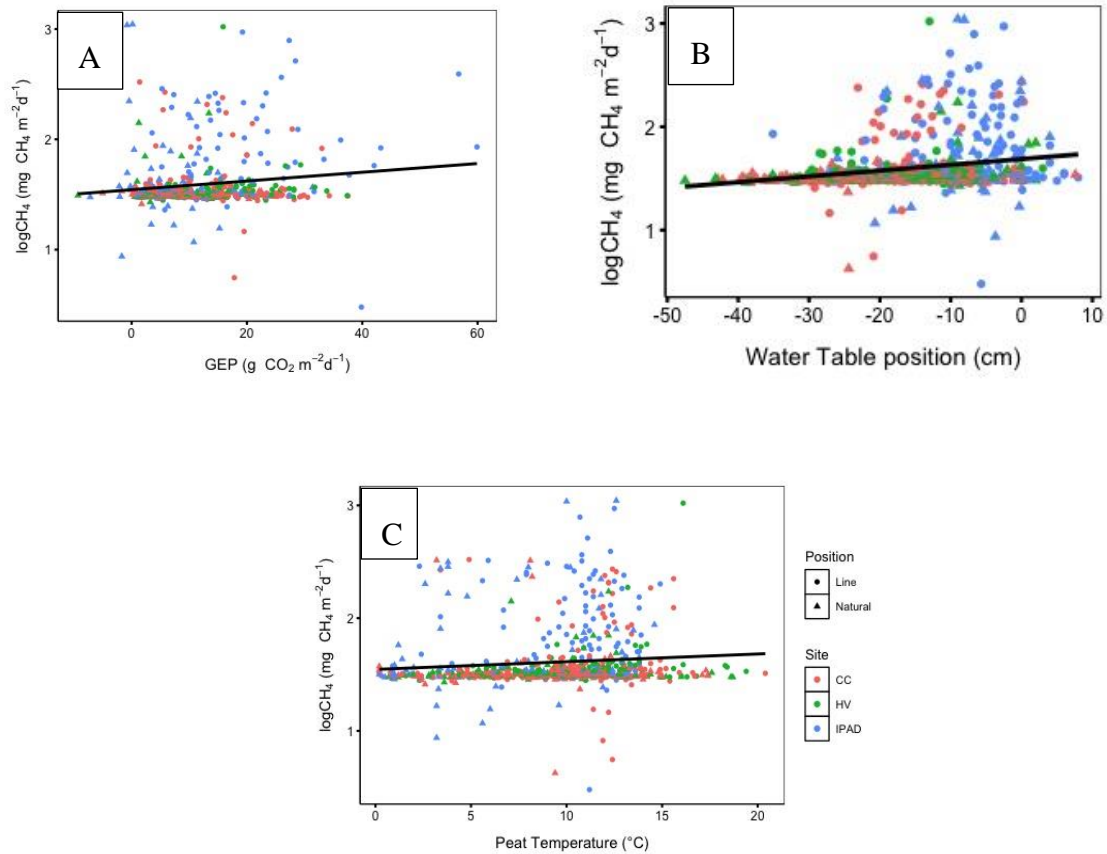
Figure 4-5 Log CH4 flux Vs GEP (A); Log CH$_4$ flux vs Water Table position (B) and Log CH$_4$ flux vs Temperature at 15 cm depth (C). The log scale was used on the y-axis (log$_{10}$(CH$_4$ flux+30)), where 30 was added to accommodate negative values. The sign convention for GEP is reversed so positive values indicate carbon sinks.

4.5 DISCUSSION

The persistence of seismic lines across our study areas resulted in a significant increase in CH_4 emissions at both bog and fen sites, as per our hypothesis. The higher CH_4 fluxes in the disturbed areas were associated with wetter, warmer conditions, vegetation community changes and higher productivity. These findings justify concerns raised by Strack *et al.* (2019) with regards to under reporting of CH_4 emissions due to the unavailability of data on changes to carbon dynamics in disturbed peatlands, especially in Alberta.

4.5.1 Changes to physical conditions and plant communities on seismic lines

The absence of shading on the lines resulted in increased solar radiation potentially accounting for the observed significant temperature differences where the average temperature on all lines at measured depths were at least 1 °C warmer than the surrounding peatland (Table 4-1). The changing thermal conditions at disturbed areas are in accord with other studies (Van Rensen *et al*. 2015; Strack *et al*. 2018) and potentially influenced organic matter breakdown and/or the rates of CH_4 production/oxidation/transportation (Valentine *et al.* 1994, Lai 2009). Although additional lines in this study would be useful in capturing the effect of the line width and orientation on soil warming, findings from this study (Table 4-2, Figure 4-2) corroborate Franklin *et al*. (2021). CH_4 fluxes from the natural areas of both HV and CC were very similar although fluxes from the 3D lines at CC were generally higher than the fluxes from the 2D at

HV (Figure 4-6). This trend was driven by their corresponding line temperatures which may have been influenced by the orientation of the lines as well.

The construction of seismic lines and the repeated use of the lines have been linked to compression of peat leading to higher bulk density (Strack *et al*. 2018). Although not measured in this study, an increase in bulk density would restrict water movement and lower the peat surface level, hence, the most probable explanation for the shallow WT position observed on lines at IPAD and CC. Seismic lines compared to surrounding peatland areas were generally wetter (Table 4-2) in line with predictions by Strack *et al.* (2019) and reports by Lovitt *et al.* (2018). The absence of tree cover on the lines and the associated reduction in tree transpiration could further support the wetter conditions as proposed by Vitt *et al*. (1999). In contrast, we observed slightly deeper WT positions on the line at HV with the least variability between the seismic line and the surrounding peatland (Table 4-2). Differences in human use and re-use of seismic lines may limit soil compaction (Kleinke *et al.,* 2022) and this may play a role in the conditions observed at HV. Further, the longtime duration since the introduction of the disturbance (Dabros *et al.,* 2022) may have allowed recovery of WT position in response to new moss growth. The effect of increased evapotranspiration driven by warming-induced vapour pressure deficit shown to exceed evapotranspiration rates in forests by up to 30%, could also account for the drier wider lines at HV (Helbig *et al*., 2020). Since the study involved only one bog site with legacy seismic lines, further hydrological/ecological studies may be needed

to further explain the observations at HV. Despite a different pattern for WT position at HV, surprisingly, moisture levels and peat temperature recorded at that site followed the same trend observed on and off the lines at IPAD and CC (Table 3-1, Table 4-2).

Plants have preferences for optimal growing conditions coupled with tolerance limits and physiological adaptations for survival in peatlands (Dabros *et al*., 2017) and therefore responded to changes imposed by the seismic line (Table 3-1). Lines at the IPAD fen increased in shrub, forb and graminoid coverage likely due to more abundant sunlight, and shallow WT (Caners & Lieffers 2014; Strack *et al*., 2017). The increase in graminoid cover likely led to them outcompeting *Sphagnum* and lichens on lines, which are negatively impacted by shallow WT or flooding (Granath *et al*., 2010) and high light levels (Pouliot *et al*., 2011). The bog at CC followed a similar vegetation community shift although change in graminoid cover was limited, that is, from 0 to 0.8%, which was expected, considering the limited abundance of graminoids in bogs. The vegetation community changes observed at IPAD, and CC were consistent with findings by Strack *et al*. (2018) and Davidson *et al*. (2021). However, HV followed a different pattern with a general increase in *Sphagnum* cover, similar to findings by Schmidt *et al.* (2022), and a reduction in forb cover on the lines. The relatively deeper WT (Table 4-2) compared to other sites, may have been more favorable to Sphagnum moss (Dieleman *et al.,* 2017). Also, seismic lines at the HV bog are legacy lines, constructed about

35–50 years ago (Dabros *et al*. 2022). The site is currently devoid of any oil and gas exploration activity and that could account for some level of natural recovery.

The understory of all lines were significantly more productive compared to understory of the surrounding peatland areas (Figure 4-1), with plant cover, peat temperature and WT position as the strongest controls (Table 4-2). The transition to greater vascular plant cover and the warmer conditions on the lines with limited shading increased photosynthetic activity hence greater net CO_2 uptake. Again, the persistence of the shallow WT on lines provided a readily available water supply for plant uptake. Findings by Davidson *et al*. (2021) confirmed higher GEP on seismic lines in boreal Alberta. As these productivity estimates do not include carbon uptake by the overstory trees, whole ecosystem estimates are still needed to assess how the loss of tree cover affects overall CO_2 uptake, although net primary productivity measurement indicate that the overstory makes a significant contribution to ecosystem productivity (Chapter 3). The focus on the understory vegetation in this study stems from the understanding that the understory is likely the essential source of substrate supply for methanogenesis given the recalcitrant nature of woody litter produced by trees (Thormann *et al.*, 1999; Waddington and Day, 2007). Again, the importance of the understory vegetation, especially vascular plants, in plant-mediated transport pathways for CH_4 emissions, which can range from 30 to 100% of total CH_4 flux, is of great significance in peatlands (Couwenberg & Fritz, 2012; Bridgham *et*

al., 2013). This was supported by our results that indicated significantly higher CH$_4$ emissions with greater graminoid cover and understory GEP (Figure 4.4) and described further below.

4.5.2 Response of peatland CH$_4$ emissions to seismic line disturbance

CH$_4$ fluxes measured in this study are similar to findings from other Canadian peatlands. The seismic line and surrounding peatland average fluxes (standard deviation) in mg CH$_4$ m^{-2} day^{-1} ranged from HV [16.3 (71.8) vs 5.3 (19.1)], CC [18.3 (50.1) vs 7.0 (26.8)] and IPAD [93.0 (138.8) vs 52.7 (165.5) respectively, compared to -1.3 to 23 mg CH$_4$ m^{-2} day^{-1} for bogs (Bubier *et al.,* 1993; Pelletier *et al*., 2007) and 99 mg CH$_4$ m^{-2} day^{-1} for a poor fen (Liblik *et al*., 1997). Our findings, however, varied slightly from reports from Turesky *et al.* (2014), that reported lower average emissions from fens (56.36 mg CH$_4$ m^{-2} d^{-1}) but comparable emissions from bogs (25.98 mg CH$_4$ m^{-2} d^{-1}). Although conditions present at our study fen favored higher CH$_4$ emissions, the bogs experienced the greatest proportional change from their natural state, that is, close to triple the rate of CH$_4$ emissions in bogs versus not quite a doubling of emissions in fens.

Peat temperature and WT were strong predictors of CH$_4$ flux (Table 4-2) at our study sites in agreement with multiple reports (Pelletier *et al*., 2007; Strack *et al*., 2018) and meta-analysis (Turetsky *et al*., 2014). A deeper WT position results in increasing thickness of the oxic zone limiting CH$_4$ production and simultaneously increasing the potential oxidation rate for CH$_4$

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produced. The IPAD fen was therefore associated with the highest fluxes followed by CC (Figure 4-2) in correlation with shallower WT positions and warmer conditions at these study sites. The lines at HV released the least amount of CH_4 corresponding to the relatively drier conditions on the lines. The higher CH_4 rates emitted from lines at HV despite drier conditions compared to the natural areas, were more likely driven by the higher peat temperatures as proposed by Pypker (2013). Similarly, when investigating an increase in CH_4 emissions on a winter road near the IPAD site, Strack *et al*. (2018) reported that soil temperature was the best predictor of the observed CH_4 flux. Understory CH_4 fluxes were significantly influenced by peat temperature at all sites (Table 4-2).

Other factors explaining the higher fluxes on lines were the presence of large amounts of fresh substrate through root exudates and litter accumulation provided by graminoids and shrubs (Strack *et al*., 2017). As vegetation in disturbed areas transitioned to increased graminoids/shrubs which are highly productive, CH_4 production was increased, and emission was further facilitated by the aerenchymatous conduits of the graminoids. The availability of more labile organic matter and changes in substrate quality due to changes in plant community (shrubs and/or graminoids) on all lines supports the higher CH_4 fluxes at all sites as reported in other studies (e.g., Tuittila *et al*., 2000; Bridgham *et al*., 2013). IPAD and CC presented a greater increase in vascular plant cover compared to HV. With warming temperatures through the growing season (Table 4-1), transpiration rates potentially increased at these sites.

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Increased transpiration rates have been shown to favor plant-mediated CH_4 transport (Couwenberg & Fritz, 2012). The relatively lower rates of CH_4 fluxes recorded at HV, may be attributed to the reduced shrub and greater Sphagnum cover (Strack *et al.*, 2017; Dieleman *et al.,* 2017). The observed relationship between vegetation and CH_4 is further corroborated by the measured understory productivity as all sites exhibited strong positive correlation between GEP and CH_4 (Figure 4-4A) as also reported by Lai (2009).

Due to logistical and time constrains, this study excluded non-growing season measurements of CH_4 fluxes. Non continuous plot scale measurements of understory C fluxes were restricted to only the growing season potentially underestimating the C fluxes at the study sites. Pelletier *et al.* (2007) report CH_4 emissions outside the growing season at 13% from a Canadian peatland, Saarnio *et al.* (2007) indicated 15%, and Alm *et al.* (1999) reports a range of 8 to 17%. However, these numbers are conservative and exclude the impact of disturbances. For example, Treat *et al.* (2019) report non-growing season CH_4 emissions of up to 47% of overall annual CH_4 fluxes. Working with any of these assumptions, the impact of seismic lines will only exacerbate the rates of CH_4 emissions from boreal peatlands if wintertime emissions were also included emphasizing the need to have assessments and models to accurately predict CH_4 dynamics in these sensitive environments. Further studies involving non growing season measurements will be important for accurate GHG estimation in boreal peatlands since

different controls may be relevant in the cold season and under freeze-thaw cycles (Chen *et al.*, 2021).

The use of a portable GHG analyzer increased the accuracy and reliability of the data set and is highly recommended for future studies. Measured understory fluxes were generally steady, with little evidence of ebullition, likely due to the relatively low CH_4 fluxes measured at our bog study sites. We recommend follow-up studies to incorporate ebullition measurements especially on lines, as studies have reported rates from 7% (Bieniada & Strack, 2021) to 20% (Strack & Waddington, 2008) in peatlands.

4.6 CONCLUSION

This study measured growing season understory CH_4 fluxes from two wooded bogs and a fen disturbed by seismic lines in the vicinity of Peace River, Alberta. CH_4 flux measurements were correlated with environmental controls to interpret findings and provide baseline data for assessing and predicting the impact of seismic lines on boreal peatlands. The creation of the seismic lines resulted in increased average CH_4 emissions by 176% (IPAD), 261% (CC) and 308% (HV) over the two studied growing seasons. The changes in CH_4 emission rates in the disturbed areas are consistent with previous studies and rates were largely driven by shallower WT positions, warmer peat temperatures and shifts in vegetation community to more

graminoid/shrub cover. The understory plant communities on the seismic lines had greater net CO_2 uptake than the understory of the adjacent forested peatlands, potentially partially compensating for the higher CH_4 emissions. However, the absence of trees on the lines and the associated loss in potential C storage should not be discounted (Chapter 3) as the shallow WT position (Caners & Lieffers, 2014; Van Rensen *et al.*, 2015) and the absence of microforms for anchorage of tree seedlings (Lieffers *et al.*, 2017; Filicetti *et al.*, 2019) presents a low likelihood of trees returning to the seismic lines in the near future. The persistence of the seismic lines and altered conditions over long periods of time (up to 50 years in the case of HV) should warrant concerns over the elevated CH_4 emissions likely persisting for prolonged periods. Results from this study will contribute to updated accurate C reporting for anthropogenic disturbance in boreal peatlands, which as lacking for many disturbance types (Harris *et al.*, 2021), as well as provide a scientific foundation for integrated land management practices and policies related boreal peatland disturbance and restoration.

Chapter 5: Conclusions

5.1 Summary of main findings

Seismic line disturbances in boreal peatlands encourage saturated near-surface soil conditions impacting tree recovery and altering vegetation communities and microclimatic conditions. Overall, there was a general increase in temperature and soil moisture content in disturbed areas compared to the surrounding peatland. This was accompanied by a reduction in bryophyte and lichen cover on all seismic lines, while bog sites had increased shrub cover and fen sites had increased graminoid cover. These changes we linked to altered rates of carbon cycling, summarized below as aligned with the three objectives of this thesis.

5.1.1 Microbial functional responses to seismic line disturbances

Soil respiration was slightly reduced in the disturbed areas of the peatland, potentially linked to lower contributions from tree roots on the lines associated with the low tree cover rather than a reduction in microbial community activity. This observation was consistent at both bog and fen sites, where the measured CO_2 fluxes were positively correlated with the peat temperature. Aside from the consistent changes in respiration rates, there were no conclusive findings on shifts in microbial community responses to aerobic utilization of substrates.

Potential microbial activity was seemingly unaffected by the presence of seismic lines since no significant differences were detected between soils collected on and off seismic lines for the utilization of supplied substrates. However, the possibility of the microbial community adapting to the disturbance conditions, as well as, the possibility of the supplied C sources not being limiting factors in microbial community responses, could have contributed to these findings. In the present study, we were more concerned with whether the function of the microbial community related to organic substrate use was affected by seismic lines and did not investigate shifts in microbial community composition or nutrient cycling. Overall, the insignificant relationship between the seismic line disturbance and potential microbial functional activity provides more credence to the suggestion that the main drivers of shifts in carbon cycling are due to changes in the plant community and microclimatic conditions.

5.1.2 Impact of seismic lines on net primary productivity and decomposition rates

The potential peat accumulation rates, represented by the difference between NPP and litter loss to decomposition over two years, was lower in the disturbed peatland areas for both fen and bogs. Although the vegetation community changes led to higher ground layer NPP on the lines, this could not compensate for the loss of tree NPP. Hence, the absence of matured trees on the lines resulted in a significant loss of overstory, and total ecosystem NPP. Litter

decomposition rates in disturbed and surrounding peatland areas were similar and were influenced by changes in the vegetation community structure. For example, the fen site experienced greater overall litter loss due to increasing graminoid cover (i.e., less recalcitrant litter with more labile exudates). These findings can provide information needed for assessing and modeling conditions in disturbed peatlands and for planning and optimizing peatland restoration projects.

5.1.3 Alteration to Methane Cycling

Significantly higher CH_4 emissions were associated with all lines compared to their surrounding peatland areas. Increased CH_4 fluxes were driven by warmer, wetter conditions and increasing vascular plant cover on lines. Greater increases were recorded on seismic lines in the bogs (261–308%) compared to the fen (176%), although average CH_4 emissions were lower overall at the bog sites. Legacy lines had the highest emission increases followed by 3D lines (308 vs. 261%). The large increases associated with lines in the bogs may also be linked to a significant reduction in the depth of the oxic zone and the subsequent loss of CH_4 oxidation by methanotrophs. Majority of the recorded CH_4 fluxes were steady fluxes, hence the obtained values could change if ebullition is fully accounted for in the estimates, particularly at very wet sites (e.g., Schmidt *et al*., 2022). Previous estimates of the effect of seismic lines on peatland CH_4 emissions based on estimated changes in water table position and literature-

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derived water table – CH_4 flux empirical relationships predicted (100 to 150%) increase in CH_4 fluxes on seismic lines (Strack *et al*. 2019). Comparing this to field measured changes suggests measurements from the fen were similar to projected values, while emissions from the bogs are much higher. These findings support claims of under-reporting of actual CH_4 emissions induced by disturbance in peatlands (Strack *et al.,* 2019; UNEP, 2022).

5.1.4 Future Research & Recommendations

Since peat chemistry has an impact on microbial abundance, diversity and function, future microbiological studies should incorporate peat chemical analyses for a greater understanding of in-situ conditions and potential limiting factors to microbial activity. Although techniques in microbiology have improved, they are still relatively expensive and are complicated to implement in peatland studies; however, further studies incorporating more advanced molecular techniques identified earlier, such as rRNA sequencing (Peltoniemi *et al*., 2016; Kitson & Bell, 2020) and terminal restriction fragment length polymorphisms (T-RFLP) profiles (Morales et al., 2006; Chambers *et al*., 2016) could be useful in proper identification of microbial community shifts. The transition on the lines to largely anoxic conditions due to WT variations could impact fungal communities. Since fungi have consistently accounted for predominant decomposition of peat in the upper unsaturated layers (Thormann, 2006; Wang

et al., 2021a), further studies on seismic lines will provide much useful information about potential shifts in fungal communities.

We acknowledge that peat accumulation is a complex long-term process and future studies should consider extending the time scale of data collection or measuring recent rates of peat accumulation (e.g., over 50–150-year time scales; Primeau & Garneau 2021). In this research, linear decay of litter was assumed over the two growing seasons and the mass of litter remaining after two years calculated. Values calculated for the exponential decay rates over the 2-year growing season, were not incorporated due to lack of time points to accurately fit a decay curve. Future studies should consider additional sampling points over longer periods to capture exponential litter decay.

To improve the reliability of CH_4 emissions data, diurnal and wintertime flux measurements are recommended for accurate compilation of annual emissions from peatland sites. Although measured understory fluxes were generally steady, with little evidence of ebullition, potentially linked to the relatively low CH_4 fluxes measured at our bog study sites, incorporating ebullition measurements into follow-up studies will enhance accuracy and reliability of the dataset, especially on seismic lines, as studies have reported ebullition rates from 7–20% in peatlands (Bieniada & Strack 2021; Strack & Waddington 2008).

5.2 Significance of the Research

Our research is the first to show how peatland microbial communities respond to seismic line disturbances, as well as how potential peat accumulation rates are impacted by lines. These changes alter C turnover and affect peat accumulation rates and long-term C storage in boreal peatlands. Our findings therefore provide important baseline information about C cycling in peatlands disturbed by seismic lines, applicable to integrated land management practices and policies related to boreal peatland restoration. From a C storage perspective, our results also suggests that restoration efforts must include recovery of an overstory in these wooded peatlands to return pre-disturbance C accumulation rates.

The pervasiveness of seismic lines in boreal peatlands and the elevated CH_4 emissions is a cause for concern due to CH_4 having a higher global warming potential compared to CO_2. Accurate GHG reporting is lacking for many disturbance types in the boreal region and results from this study can contribute useful data on GHG cycling in boreal peatlands impacted by anthropogenic disturbance. This can inform the development of emission factors that can be used for national GHG reporting and development of GHG offset protocols.

Although no major changes in peatland microbial community substrate utilization were identified because of the seismic line disturbance, lines were associated with lower soil respiration, shifts in moisture and temperature, as well as vegetation community changes.

Collectively, this suggests that the impacts of seismic lines on boreal peatland soil C cycling, decades after disturbance are minimal despite the continuous structural changes to the vegetation community. Measurements of ecosystem-scale C flux in the field are however needed to better understand the full impact on C cycling.

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Appendices

Appendix 1: ANOVA results for substrate induced respiration (SIR) for all sites.

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: Carbon source	15	0.051	0.00343	0.675	0.810
Site: Carbon source	45	0.210	0.00467	0.920	0.623
Position: Site: Carbon source	45	0.228	0.00507	0.999	0.476

Residuals   639 3.244 0.00508

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

Appendix 2: ANOVA results for SIR of individual carbon sources.

> l-Alanine

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: l-Alanine	1	0.001	0.00001	0.003	0.961
Site: l-Alanine	3	0.055	0.01819	5.458	0.003
Position: Site: l-Alanine	3	0.021	0.00701	2.103	0.115

Residuals   40 0.13330 0.003333

> l-Arabinose

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: l-Arabinose	1	0.002	0.00188	0.603	0.442
Site: l-Arabinose	3	0.035	0.01160	3.734	0.019
Position: Site: l-Arabinose	3	0.056	0.01872	6.025	0.002

Residuals   40 0.12429 0.003107

> Arginine

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: Arginine	1	0.003	0.00276	0.748	0.392
Site: Arginine	3	0.019	0.00619	1.678	0.187
Position: Site: Arginine	3	0.017	0.00569	1.542	0.219

Residuals   40 0.14755 0.003689

> Citric Acid

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: Citric Acid	1	0.000	0.00018	0.033	0.856
Site: Citric Acid	3	0.098	0.03270	6.165	0.002
Position: Site: Citric Acid	3	0.029	0.00960	1.809	0.161

Residuals   40 0.21217 0.00530

> l-Cysteine-HCl

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: l-Cysteine-HCl	1	0.002	0.00183	0.548	0.463
Site: l-Cysteine-HCl	3	0.023	0.00773	2.323	0.089
Position: Site: l-Cysteine-HCl	3	0.013	0.00440	1.322	0.281

Residuals   40 0.13317 0.003329

> d-Fructose

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: d-Fructose	1	0.005	0.00508	1.364	0.250
Site: d-Fructose	3	0.033	0.01102	2.956	0.044
Position: Site: d-Fructose	3	0.009	0.00297	1.798	0.503

Residuals   40 0.14914 0.003728

> Galactose

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: Galactose	1	0.009	0.00878	2.359	0.132
Site: Galactose	3	0.011	0.00373	1.002	0.402
Position: Site: Galactose	3	0.027	0.00896	2.407	0.081

Residuals   40 0.14880 0.003720

> d-Glucose

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: d-Glucose	1	0.000	0.00041	0.122	0.729
Site: d-Glucose	3	0.013	0.00441	1.300	0.288
Position: Site: d-Glucose	3	0.043	0.01427	4.209	0.011

Residuals   40 0.13565 0.003391

> γ-Aminobutyric acid

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: γ-Aminobutyric acid	1	0.014	0.01418	3.760	0.059
Site: γ-Aminobutyric acid	3	0.026	0.00881	2.336	0.088
Position: Site: γ-Aminobutyric acid	3	0.015	0.00498	1.321	0.281

Residuals   40 0.15084 0.003771

> l-Malic Acid

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: l-Malic Acid	1	0.006	0.00546	0.249	0.620
Site: l-Malic Acid	3	0.011	0.00349	0.160	0.923
Position: Site: l-Malic Acid	3	0.055	0.01847	0.844	0.478

Residuals   40 0.8759 0.021898

> l-Lysine

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: l-Lysine	1	0.019	0.01888	3.658	0.063
Site: l-Lysine	3	0.029	0.00956	1.851	0.153
Position: Site: l-Lysine	3	0.015	0.00510	0.988	0.408

Residuals   40 0.20648 0.005162

> α-Ketoglutaric acid

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position α-Ketoglutaric acid	1	0.008	0.00811	2.601	0.115
Site: α-Ketoglutaric acid	3	0.027	0.00913	2.927	0.045
Position: Site: α-Ketoglutaric acid	3	0.023	0.00774	2.483	0.075

Residuals   40 0.12474 0.003118

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> N-acetylglucosamine

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: N-acetylglucosamine	1	0.008	0.00832	2.168	0.149
Site N-acetylglucosamine	3	0.024	0.00788	2.054	0.122
Position: Site: N-acetylglucosamine	3	0.031	0.01021	2.662	0.061

Residuals   40 0.15353 0.003838

> Oxalic Acid

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: Oxalic Acid	1	0.006	0.00646	1.698	0.200
Site: Oxalic Acid	3	0.025	0.00838	2.202	0.103
Position: Site: Oxalic Acid	3	0.012	0.00395	1.038	0.386

Residuals   40 0.15223 0.003806

> Trehalose

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: Trehalose	1	0.004	0.00364	0.926	0.342
Site: Trehalose	3	0.010	0.00330	0.849	0.480
Position: Site: Trehalose	3	0.028	0.00923	2.349	0.087

Residuals   40 0.15718 0.003929

> Milli-Q water (Control)

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position: Milli-Q water	1	0.025	0.02502	3.732	0.061
Site: Milli-Q water	3	0.034	0.01126	1.679	0.187
Position: Site: Milli-Q water	3	0.013	0.00417	0.621	0.605

Residuals   40 0.26819 0.006705

Appendix 3: ANOVA results for soil respiration.

Results for continuous soil respiration measurements:

Soil Respiration at CC and IPAD (CO_2 flux)

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position:	1	454.17	454.17	534.510	0.001
Site:	1	62.69	62.69	73.776	0.001
Position: Site:	1	33.46	33.46	39.383	0.001

Residuals   1721   1462.34   0.85

Soil Respiration against Peat Temperature

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Peat Temperature	1	22.99	22.9866	19.906	0.001

Residuals   1723 1989.67 1.1548

Short term readings:

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Soil Respiration at all sites (CO$_2$ flux)

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Position:	1	14.46	14.4601	2.599	0.109
Site:	1	0.02	0.0212	0.0038	0.951
Position: Site:	1	14.23	14.2314	2.5574	0.112

Residuals   134 745.68 5.5647

Soil Respiration Against Peat Temperature

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Peat Temperature	1	0.13	0.1279	0.023	0.881

Residuals   136 774.26 5.6931

Soil Respiration Against Peat Temperature and Moisture

	Degrees of freedom (df)	Sum of squares	Mean square	F	p
Peat Temperature	1	0.13	0.1279	0.023	0.881
Moisture	1	3.39	3.3941	0.600	0.434
Peat Temperature: Moisture:	1	12.49	12.4942	2.208	0.112

Residuals   134 758.37 5.6595

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Appendix 4: ANOVA results for Chapter 4

ANOVA for all Sites (2018-2019)

CH$_4$ Fluxes (LogCH4~Site*Position) with Collar as random intercept in lme model

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	660	15.010	0.001
Position:	1	59	9.219	0.004
Site: Position:	2	660	3.121	0.045

CH$_4$ vs WT (LogCH4~WL) with Collar as random intercept in lme model

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
WL	1	656	7.584	0.006

CH4 vs Peat temperature (LogCH4~T depth) with Collar as random intercept in lme model

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
T2	1	663	4.56	0.001
T15	1	659	5.426	0.004
T25	1	588	7.130	0.045
T30	1	588	17.163	0.001

CH4 vs GEP (LogCH4~GEP) with Collar as random intercept in lme model

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
GEP	1	491	6.320	0.012

GEP vs WT (GEP~WL) with Collar as random intercept in lme model

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
WL	1	586	17.843	0.001

GEP from all sites (GEP~Site*Position) <u>with Collar as random intercept in lme model</u>

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	589	0.146	0.865
Position:	1	58	56.247	0.001
Site: Position:	2	589	1.984	0.014

GEP vs Peat temperature (GEP ~T depth) <u>with Collar as random intercept in lme model</u>

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
T2	1	663	20.428	0.0001
T5	1	659	47.180	0.0001
T10	1	588	77.114	0.0001
T15	1	588	94.976	0.0001
T20	1	588	80.236	0.0001

ANOVA for all Sites 2018 CH$_4$ Fluxes (LogCH4~Site*Position)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	286	14.353	0.0001
Position:	1	58	2.810	0.0990
Site: Position:	2	286	0.083	0.9205

2018 CH$_4$ vs WT (LogCH4~WL)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
WL	1	285	3.723	0.055

2018 CH$_4$ vs Peat temperature (LogCH4~T depth)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
T20	1	283	6.198	0.013
T30	1	264	7.887	0.005

2018 GEP from all sites (GEP~Site*Position)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	197	9.233	0.0001
Position:	1	57	34.376	0.0001
Site: Position:	2	197	4.536	0.012

2018 GEP vs WT (GEP~WL)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
WL	1	197	48.447	0.0001

2018 GEP vs Peat temperature (GEP ~T depth)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
T2	1	663	7.863	0.0055
T5	1	659	25.034	0.0001
T10	1	588	38.915	0.0001
T15	1	588	48.514	0.0001
T20	1	588	60.230	0.0001

ANOVA for all Sites 2019 CH$_4$ Fluxes (LogCH4~Site*Position)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	42	11.679	0.0001
Position:	1	42	7.901	0.008
Site: Position:	2	42	4.568	0.016

2019 CH$_4$ vs WT (LogCH4~WL)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
WL	1	320	3.688	0.056

2019 CH$_4$ vs Peat temperature (LogCH4~T depth)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
T2	1	323	4.095	0.0441
T5	1	322	8.722	0.0027
T10	1	320	14.966	0.0001
T15	1	297	12.066	0.0001
T20	1	264	13.393	0.0001
T30	1	243	13.235	0.0001

2019 GEP from all sites (GEP~Site*Position)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	42	3.532	0.0382
Position:	1	42	42.613	0.0001
Site: Position:	2	42	2.069	0.1390

2019 GEP vs WT (GEP~WL)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
WL	1	339	0.188	0.665

2019 GEP vs Peat temperature (GEP ~T depth)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
T2	1	342	20.004	0.0001
T5	1	342	37.872	0.0001
T10	1	340	71.044	0.0001
T20	1	307	86.339	0.0001

WT from all seasons: WL~Site*Position with Collar as random intercept in lme model

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	812	4.623	0.010
Position:	1	61	4.387	0.040
Site: Position:	2	812	1.573	0.208

#2018 WL

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	372	8.865	0.0002
Position:	1	65	0.723	0.3982
Site: Position:	2	372	1.694	0.1852

#2019 WL

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Site:	2	42	5.609	0.007
Position:	1	42	5.821	0.020
Site: Position:	2	42	1.209	0.309

Vegetation Analyses

CH$_4$ vs Veg (LogCH4~ plant group)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Graminoid	1	47	6.444	0.0145
Dwarf Shrub	1	47	3.559	0.0654
Bryophyte	1	47	0.006	0.9364
Forb	1	47	0.212	0.8836
Lichen	1	46	0.004	0.9479
Total Vascular	1	47	1.826	0.1831

GEP vs Veg (GEP~ plant group)

	Numerator Degrees of freedom (numDF)	Denominator Degrees of freedom (denDF)	F	p
Graminoid	1	47	3.273	0.0768
Dwarf Shrub	1	47	0.661	0.4202
Bryophyte	1	47	1.688	0.2002
Forb	1	47	1.478	0.230
Lichen	1	46	3.506	0.0675
Total Vascular	1	47	6.664	0.0130