Impacts of resource access road crossings on ecohydrology and carbon dynamics of boreal peatlands

by
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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis as accepted by my examiners.

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

Statement of Contributions

This thesis has been prepared as a manuscript-based thesis. Chapter two and four have been submitted to peer-review Journals. Chapter three has been published in the journal Science of the Total Environment. Chapter five has been prepared for the submission but has not yet been submitted. The published paper may differ from the chapter presented here based on the comments from the peer review process. Repetition between chapters in background and methodology may occur to some extent. Also, figure formatting elements may differ, and abbreviations may be repeated between chapters as per the requirements of individual journals.

Dr. Maria Strack was the advisor for this thesis, and helped with the research design, data analyses and provided feedback on the analysis. Saraswati was the first author of the all chapters within this thesis and wrote the initial draft of each chapter. As a thesis advisor, Dr. Maria Strack reviewed all chapters and provided comments and suggestions where needed.

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Abstract

Resource access road crossings are expected to alter peatland ecohydrological properties by obstructing surface and sub-surface water flows, providing favorable conditions to stimulate microbial activity, and ultimately impacting greenhouse gases (GHGs) emissions. A multi-year study at two boreal peatlands (a forested bog and a shrubby rich fen) was conducted near Peace River, Alberta to study the impacts of all-season resource access roads on the local ecohydrology, soil enzyme activities, methane (CH_4) emission and carbon (C) dynamics of adjacent peatland. Field measurements (bi-weekly depth to water table (WT), hydraulic head, peat temperature, CH_4 flux and CO_2 flux, and one-time hydraulic conductivity and peat sampling for enzyme assays, understory vegetation and tree survey, and tree disk/core collection) during the snow free period of 2016 and 2017 were conducted from sampling plots located at road impacted (RI) areas representing: 1) side of the road (upstream and downstream); 2) distance from the road (lateral; 2, 6, and 20 m); and 3) distance from culverts (longitudinal; < 2 and > 20 m).

Results showed that the resource access roads disturbed the surface and sub-surface water flow at the bog, but the effect was minimal at the fen as the road orientation was nearly parallel to the flow direction at the fen. At the bog, the shallowest depth to WT position was observed at upstream areas close to the road, when culverts were located > 20 m distance from transects. In contrast, when culverts were present < 2 m from the transects, variation in hydrological conditions between upstream and downstream areas were greatly reduced. The highest enzyme activities in the bog occurred on the downstream side of the road at plots located far from the culvert (> 20 m distance from transects). Two of six investigated enzymes had significantly higher activities in the road impacted areas compared to undisturbed areas, suggesting that road construction may alter organic matter degradation pathways and potentially enhance organic matter decomposition rates. Bog

plots located upstream of the road on transects located at > 20 m from culverts and closer to the road emitted significantly more CH_4 (124.6 mg CH_4 m^{-2} d^{-1}) than other areas due to shallower water table position and warmer peat temperature. Estimated CH_4 emissions from road-disturbed peatlands were 90.8 and 212.2 kg CH_4 y^{-1} for each km of road, in 2016 and 2017, respectively.

At the bog, the road impacted (RI) areas were sources of CO_2 and understory flux was significantly different than the undisturbed areas in full light conditions. The average tree above ground net primary productivity (NPP_{ag}) at the reference areas of both study sites were significantly higher in both years compared to RI areas, respectively. In both study sites, the plots located at vegetation clearance areas were devoid of tree NPP_{ag}. Overall, at the bog, the RI areas were sources, but reference areas were sinks of C in both years. In contrast, at the fen, both RI and reference areas were sinks of C in both years. The estimated road induced C losses in 2016 and 2017 were ~ 8.0 and 7.4 Mg C y^{-1} for each km of the road, respectively.

In conclusion, the construction of all-season access roads has the potential to severely impact the local ecohydrology and enzyme activities in peatlands. The road induced CH_4 emissions and C losses from adjacent peatlands indicate that road construction across peatlands creates additional sources of anthropogenic GHGs. However, adequate hydrologic connections through culverts and road construction parallel to the water flow when and where possible can minimize the road induced impacts.

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Dedication

I dedicate this thesis to my parents and my loving husband for their immense support, love and care.

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List of abbreviations

C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
CO_2/H_2	Acetate
DTW	Depth to water table
DOC	Dissolved organic carbon
ER	Ecosystem respiration
ET	Evapotranspiration
GHG	Greenhouse gases
GEP	Gross ecosystem photosynthesis
GWL	Ground water level
h	hydraulic head
HI	High impacted areas
K_{sat}	Saturated hydraulic conductivity
LAI	Leaf area index
LI	Low impacted areas
LMEM	Linear mixed effects model
NPP	Net primary productivity
NEE	Net ecosystem exchange
NECB	Net ecosystem carbon balance
PAR	Photosynthetically active radiation
PMC	Peat moisture content
P	Precipitation
POC	Particulate organic carbon
q_{hor}	Specific groundwater discharge
SOM	Soil organic matter
RI	Road impacted areas
TEAs	Terminal electron acceptors
WBP	Western Boreal Plains
WBR	Western boreal region
WT	Water table position

Chapter 1: Introduction

Peatlands are wetlands accumulating a deep layer of partially decomposed organic materials (i.e. peat; depth > 40 cm by definition in Canada; National Wetlands Working Group, 1997) over a course of centuries (Clymo, Turunen, & Tolonen, 1998; Mitsch & Gosselink, 2015; Vitt et al., 2000). They have the potential to be one of the key drivers of global climate change as they are long-term sinks of carbon (C) in the form of peat - making them a reservoir of one-third of global soil C, or twice the amount of C stored in global forest biomass (Kaat & Joosten, 2009; Parish et al., 2008). Despite covering only 3% of the land surface (Freeman, Fenner, & Shirsath, 2012; Limpens et al., 2008), peatlands store over 600 Pg of organic C (Page, Reiley, & Banks, 2011; Yu, Loisel, Brosseau, Beilman, & Hunt, 2010). Peatland ecosystems also provide various ecological services, which contribute to the conservation of biodiversity, human welfare, climate regulation, water purification, educational opportunities, and recreational options (Kimmel & Mander, 2010; Millennium Ecosystem Assessment, 2005; Parish et al., 2008).

Canada has the second largest (i.e., 30% of global total) peatland coverage in the world (Tarnocai, 2006; Tarnocai, 2009). Of the estimated 1.136 million km^2 area of peatland coverage in Canada (i.e., 12% of the total land area), the boreal region has approximately 64% of the total Canadian peatlands (Tarnocai, Kettles, & Lacelle, 2011), making the region an important storage of organic C in its peat (Vitt et al., 2000). The stored peat in these peatlands forms a vertical profile with upper (acrotelm) and lower (catotelm) layers (Ingram 1978). The acrotelm is usually < 50 cm thick, with fluctuating WT, variably oxic conditions, high hydraulic conductivity due to a large pore size (Boelter, 1965; Hoag & Price, 1995), and has higher availability of fresh litter to aerobic microbes (Boelter, 1965; Ingram, 1978). The catotelm, the peat layer below the acrotelm and underlain by mineral soils, is water-saturated with anoxic conditions, restricts aerobic metabolism, and has

lower hydraulic conductivity due to highly decomposed organic matter resulting in small pore size (Ingram, 1978; Price, 2003; Price & Schlotzhauer, 1999; Whittington, 2005).

However, peatland properties, including soil biogeochemistry, ecohydrology and vegetation have been changed or altered by various disturbances, causing them to become potential contributors to global warming because of increased GHG emissions. The disturbances can be grossly categorized into linear and non-linear disturbances. While non-linear disturbances include land use changes, drainage and peat extraction (Cleary, Roulet, & Moore, 2005), linear disturbances include winter road construction, all-season resource road construction, and the use of seismic lines linked to resource extraction including the oil and gas industry and forestry (Williams, Quinton, & Baltzer, 2013). These disturbances influence both temporal and spatial patterns of GHG emissions from Canadian peatlands.

While, it is clear that road crossings are likely to alter peatland function, a comprehensive study investigating the impact of all-season resource access roads on hydrology, enzyme activity, and C dynamics of adjacent peatlands has not been conducted yet. Therefore, to fill the gap, I conducted a multi-year study in a boreal forested bog and a shrubby rich fen located at Carmon Creek, near Peace River, Alberta, Canada. Before presenting findings of the research, the background on peatland types, C cycling in peatlands, and anticipated road impacts on adjacent peatlands have been summarized below.

1.1 Peatland types

Main peatland types in the boreal region are bogs and fens (National Wetlands Working Group, 1997). Bogs are nutrient poor ombrotrophic peatlands located at local topographically high areas that are mostly disconnected from the ground water, and they are characterized by acidic peat

(mostly pH < 4; Hemond, 1980; Mitsch & Gosselink, 2015). The bog ecosystems are mainly shaped by *Sphagnum* spp. though their floral diversity includes bryophytes combined with a variable proportion of dwarf shrubs and trees (Clymo & Hayward, 1982; Fenner & Freeman, 2011; Oechel & Van Cleve, 1986; Rydin & Jeglum, 2013; Zoltai & Vitt, 1995). In comparison, fens are minerotrophic as they receive nutrients from precipitation, surface flow and groundwater flow from surroundings (Zoltai & Vitt, 1995). In fens, pH is higher compared to bogs and vegetation is dominated by sedges, grasses, or reeds (Conway, 1937; Marinier, Glatzel, & Moore, 2004; Mitsch & Gosselink, 2015; Wheeler & Proctor, 2000). While vegetation diversity in North American ombrotrophic bogs and poor fens includes *Picea mariana* (black spruce), *Sphagnum* spp., *Rhododendron groenlandicum* (labrador tea), *Vaccinium vitis-idaea* (large bog cranberry), *Eriophorum vaginatum* (cotton grass) and *Rubus chamaemorus* (cloudberry), the main vegetation cover in moderate to rich fens are *Betula glandulosa* (dwarf birch), *Larix laricina* (larch), *Carex* spp. (sedges), *Polytrichum* spp., *Sphagnum* spp., *Equisetum fluviatile* (swamp horsetail) and brown mosses (Ben Bond-Lamberty, Wang, & Gower, 2004; Moore & Basiliko, 2006; Wood et al., 2016; Zoltai & Vitt, 1995).

Owing to differences in terms of biogeochemical properties between bog and fen, the rate and drivers of peat accumulation in both bog and fen vary as described in the following sub-sections.

1.2 Peat accumulation and C cycling

Peat accumulation in peatlands is the balance between carbon dioxide (CO_2) taken up by plants during photosynthesis (gross ecosystem photosynthesis; GEP) and release of C during autotrophic and heterotrophic respiration (ecosystem respiration; ER), CH_4 emission, and leaching in dissolved and particulate forms (Clymo et al., 1998; Frolking et al., 2010; Lafleur, Roulet, Bubier, Frolking, & Moore, 2003; Potter, Bubier, Crill, & Lafleur, 2001; Turunen, Tomppo, Tolonen, & Reinikainen, 2002). Though peatlands have lower annual primary productivity compared to forests and grasslands (Bubier, Frolking, Crill, & Linder, 1999; Frolking et al., 1998; Ruimy, Jarvis, Baldocchi, & Saugier, 1995; Vasander & Kettunen, 2006), peat accumulation in peatlands is favored by the slowly decomposing litter and saturated soils impedes decomposition. Peatlands take hundreds to thousands of years to accumulate thick layers of peat because of the small annual difference between C gain and C loss in peatland ecosystems (Clymo, Pearce, & Conrad, 1995; Clymo et al., 1998; Gorham, 1991; Turunen et al., 2002; Vitt et al., 2000). However, peatlands may not accumulate peat every year because of variability observed in the annual growing season conditions (e.g., temperature and precipitation; Waddington & Roulet, 2000). Estimated C storage rates in peatlands range from 15 to 380 g m^{-2} y^{-1}, and the large variation is attributed to methodological differences in the estimation, peatland types and peatland age, interannual environmental variabilities, and disturbances (Ali, Ghaleb, Garneau, Asnong, & Loisel, 2008; Clymo et al., 1998; Roulet et al., 2007).

In boreal peatlands, organic matter decomposition is constrained by a typically deep anoxic soil layer (Freeman, Ostle, Fenner, & Kang, 2004; Freeman, Ostle, & Kang, 2001), low nutrient availability, low pH (Williams, Shingara, & Yavitt, 2000), distinctive vegetation composition, low enzyme activities (phenol oxidase and hydrolases; Freeman, Ostle, & Kang, 2001), and low

temperature. However, heterogeneity, both in peat accumulation rate and peatland properties, occurs between peatland types (e.g. bogs and fens) and within the peat profile.

1.2.1 GEP and ER in peatlands

Photosynthesis is the initial step in primary productivity by which a plant or other primary producer converts inorganic C from the atmosphere (gaseous CO_2) to organic C that then accumulates in the form of biomass in the presence of photosynthetically active radiation (PAR; Chivers, Turetsky, Waddington, Harden, & McGuire, 2009; Gerdol, Bonora, Gualandri, & Pancaldi, 1996). The total photosynthesis (GEP) performed by primary producers, mainly bryophytes and vascular plants, determine the overall peatland ecosystem productivity. However, there is a huge variation among peatland types in terms of GEP, and the variation can be attributed to vegetation diversity and functional group type as influenced by peat moisture content (PMC) and WT position, and nutrient availability in the peat (Alm, et al., 1999; Gorham, 1991; Griffis, Rouse, & Waddington, 2000; Petrone, Solondz, Macrae, Gignac, & Devito, 2011; Weltzin et al., 2000).

The GEP is positively correlated with leaf cover or leaf area index (LAI), but increased canopy cover of trees can reduce the photosynthesis of ground vegetation (shrubs, bryophytes and graminoids) by reducing PAR exposure (Bergamini, Pauli, Peintinger, & Schmid, 2001; Chivers et al., 2009; Harley, Tenhunen, Murray, & Beyers, 1989; Zhao, Peichl, Öquist, & Nilsson, 2016). Therefore, in some cases, vascular plants can contribute more to GEP of a peatland compared to bryophytes due to higher growth forms dominating the canopy and having higher LAI (Munir, Perkins, Kaing, & Strack, 2015). In general, GEP is lower in ombrotrophic bogs and poor fens than rich because of low nutrient availability and higher WT that favor mainly *Sphagnum* spp. (Vitt, 2006), which get first access to available nutrients (Malmer, Albinsson, Svensson, & Wallen, 2003).

Studies in boreal peatlands have shown that a deeper WT (e.g., drought conditions or experimentally lowered WT) can lead to lower GEP due to water stress on the productivity of primary producers (Griffis et al., 2000; Rinne et al., 2007; Sonnentag, Kamp, Barr, & Chen, 2009; Weltzin et al., 2000). Similarly, the prolonged drought or deeper WT position is harmful to the peatland plant growth as that makes nutrients less plant available through immobilization (Chivers et al., 2009; Gorham, 1991). However, the PMC can be modified by the vegetation types, though PMC is positively correlated with WT position. For example, PMC can be higher at or near to the surface of peat in places where *Sphagnum* spp. are present compared to the presence of other bryophytes (Limpens, Heijmans, & Berendse, 2006).

An estimated > 85% of gross primary productivity (GPP) is lost through ER, leaching and methanogenesis, and the remaining 2-16% gets deposited as peat (Laiho, 2006; Reader & Stewart, 1972). In boreal peatlands, much of the C efflux as ER occurs in the growing season, but the winter season also contributes to ER through oxygen availability to microbes and release of CO_2 stored below the frozen surface layer (Alm, Saarnio, Nykanen, Silvola, & Martikainen, 1999). ER involves CO_2 efflux from peatlands through living plant-mediated C loss, including plant respiration, microbial respiration in the rhizosphere, and root exudate mineralization by microbes, as well as microbial decomposition of litterfall and organic matter in peat (Beverly & Franklin, 2015; Crow & Wieder, 2005; Hanson, Edwards, Garten, & Andrews, 2000; Matteucci, Gruening, Ballarin, Seufert, & Cescatti, 2015; Turetsky et al., 2014).

Plant-mediated respiration can contribute up to 90% of the total ER during a growing season depending on vegetation type and ecosystem properties (Crow & Wieder, 2005; Hanson et al., 2000), and < 25% during the winter season (Aurela, Laurila, & Tuovinen, 2001; Beverly & Franklin, 2015; Lafleur, Moore, Roulet, & Frolking, 2005). Plant-mediated soil respiration can be

observed in the top 30 cm of peat, where the greatest root biomass of shrubs and sedges are found (Crow & Wieder, 2005; Saarinen, 1996). Since vascular plants have extensive growth forms (root, shoot and leaves), their share in ER can be higher compared to mosses, which do not have advanced growth forms (Dorrepaal, Cornelissen, Aerts, Wallen, & Logtestijn, 2005; Walker, Ward, Ostle, & Bardgett, 2015). In open peatlands, vascular plants can contribute 35 to 57% of ER (Crow & Wieder, 2005). During plant growth and development, the photosynthetically produced carbohydrates exuded from plant roots may provide a source of labile carbon to microbial communities and increase associated ER (Högberg et al., 2001; Johsen et al., 2007). Higher *Sphagnum* spp. cover can reduce decomposition due to their decay-resistant litter and low nutrient content (Limpens & Berendse, 2003; Thormann, Bayley, & Currah, 2001). *Sphagnum* can also reduce peat temperature by drawing water up to the surface (Dorrepaal et al., 2005) and hence, higher peat accumulation can be observed in areas with *Sphagnum – Picea mariana* combination than feather moss (e.g. *Pleurozium schreberi* and *Hylocomium splendens*) – *Picea mariana* dominant sites (Bisbee, Gower, Norman, & Nordheim, 2001; Harden, O'Neill, Trumbore, Veldhuis, & Stocks, 1997; Trumbore & Harden, 1997). On the other hand, graminoids (e.g., *Eriophorum vaginatum*) grow faster and their litter decomposes easily (Moore & Basiliko, 2006) and that helps to cause plant-induced peat respiration (Trinder, Artz, & Johnson, 2008). This suggests that vascular plants can contribute to the priming effect in peatlands i.e., increase microbial activity and ER (Kuzyakov, Friedel, & Stahr, 2000; Kuzyakov, 2010; Lindén et al., 2014).

Microbial decomposition of litterfall and soil organic matter is a very slow process in peatlands and depends on soil microbial diversity, biomass, electron acceptor availability and enzyme activities (Ballantyne, Hribljan, & Pypker, 2014; Gorham, 1991), which in turn are impacted by

environmental conditions such as biogeochemical properties including peat temperature, anoxic conditions, peat pH, redox potential, nutrient availability, vegetation structure, and organic matter properties (Dalva & Moore, 1993; Freeman et al., 2001; Laiho, 2006; Mäkiranta et al., 2009; Munir et al., 2015; Silvola, Alm, Ahlholm, Nykanen, & Martikainen, 1996; Wheeler & Proctor, 2000). Temperature is another important factor determining the microbial decomposition in peatlands as increased soil temperature, e.g., due to global warming, influences the thermodynamic favorability of metabolic reactions and provides a suitable condition for the microbial communities to flourish and help to enhance ER (Chivers et al., 2009; Christensen, Jonasson, Callaghan, & Havström, 1999; Dalva & Moore, 1993; Pinsonneault, Moore, & Roulet, 2016; Silvola, Alm, Ahlholm, Nykanen, & Martikainen, 1996). Also, the oxidation of some organic substrates coupled to nitrate reduction is not thermodynamically favorable at low temperature, but becomes thermodynamically favorable above a critical temperature so that microbial communities can derive energy from the metabolism (Reddy, & Delaune, 2008).

Peatlands provide habitat for microbial communities, which produce various enzymes (e.g. phenol oxidase and hydrolases) under favorable conditions to liberate nutrients from organic matter (Freeman, Nevison, Hughes, Reynolds, & Hudson, 1998; Kang, Kim, Fenner, & Freeman, 2005; Nathalie, Freeman, & Reynolds, 2005; Pind, Freeman, & Lock, 1994; Sinsabaugh, 2010). Microbial communities produce extracellular enzymes to break large organic molecules into small molecules before cellular uptake (Luo, Meng, & Gu, 2017). Microbes release extracellular enzymes in response to nutrient limitation, but it also requires investment from the microbial communities. For example, in the presence of oxygen, phenol oxidase can degrade phenolics (McLatchy & Reddy, 1998). Nevertheless, oxygen limitation due to water-saturated conditions and low pH decreases phenol oxidase enzyme activity in peatlands and increases phenolic material

accumulation, which restricts the microbial activity and ultimately slows down the decomposition (Fenner & Freeman, 2011; Freeman et al., 2004; Sinsabaugh, 2010).

1.2.2 CH_4 emission from peatlands

In addition to ER, peatlands release C in the form of CH_4, which is produced by methanogenesis through anaerobic decomposition of organic matter in the catotelm and anoxic zone in acrotelm of peatlands (Cao, Marshall, & Gregson, 1996; Galand, Fritze, Conrad, & Yrjälä, 2005; Garcia, Patel, & Ollivier, 2000; Matthews & Fung, 1987). Methanogenesis occurs under highly reduced conditions (i.e., anoxic conditions; redox potential below -200 mv) when methanogens use CO_2 as an electron acceptor to produce CH_4 after fermentation of organic matter in the absence of other available terminal electron acceptors (TEAs; Bellisario, Bubier, Moore, & Chanton, 1999; Mitsch & Gosselink, 2015). The methanogens utilize limited substrate types such as root exudates (e.g., acetate) or H_2/CO_2 in order to produce CH_4, and the type of substrate available defines the pathways of methanogenesis (Zinder, 2001). When methanogens utilize H_2/CO_2 as a substrate, the pathway is known as hydrogenotrophic methanogenesis, which occurs predominantly in ombrotrophic bogs (Galand et al., 2005; Horn, Matthies, Küsel, Schramm, & Drake, 2003; Hornibrook, Longstaffe, & Fyfe, 1997; Lansdown, Quay, & King, 1992), whereas, when methanogens use acetate as a substrate the pathway is known as acetoclastic methanogenesis, which is dominant in nutrient rich, minerotrophic fens (Bridgham, Cadillo-Quiroz, & Keller, 2013; Duddleston, Kinney, Kiene, & Hines, 2002; Galand et al., 2005). The presence of more labile C in moderate to rich fens, compared to less labile forms of organic matter from *Sphagnum* spp. and woody plant dominated bogs, causes higher CH_4 emissions in fens (Chanton et al., 2008). Once produced, CH_4 either remains into peat pores or passes to the atmosphere. However, in this process, the produced CH_4 may oxidize in the unsaturated acrotelm layer or in the deeper roots of plants

due to transportation of oxygen to the plants roots (Laiho, 2006) or by the anerobic oxidation of CH_4 by methanotrophic organisms (Bridgham et al., 2013; Reddy & DeLaune, 2008).

WT position is a dominant control on CH_4 emission from peatlands as it determines the size of the acrotelm and catotelm (Bubier, 1995; Dalva & Moore, 1993). In general, as WT falls, the thickness of the acrotelm increases, which contributes to lower CH_4 production (Christensen et al., 2003; Turetsky, Treat, & Waldrop, 2008), but higher CH_4 oxidation. However, the control of WT position on CH_4 emission can be influenced by the vegetation types of peatlands. For example, the deeper WT position can increase the belowground productivity of emergent plants (e.g. *Carex rostrata*) providing more labile C (from root exudates) to the catotelm (Weltzin et al 2000; Chanton et al 2008), and in turn, may increase CH_4 production (Crow & Wieder, 2005; Saarinen, 1996; Strack, Waller, & Waddington, 2006). In contrast, there would be less CH_4 release in tree and shrub-dominated sites as they root at shallow depth to avoid saturated soil (Mitsch & Gosselink, 2015). Plants with roots reaching to saturated soil transport oxygen to the rhizosphere and that can inhibit methanogenesis and reduce CH_4 release (Watson, Stephen, Nedwell, & Arah, 1997).

In addition the impact of shallow WT position on CH_4 production can be accelerated by 80-300% when both temperature and WT are increased together (Turetsky et al., 2008). Therefore, CH_4 production can be higher during wet summers (Saarnio et al., 1997). However, as peat temperature increases, the solubility of CH_4 in pore water decreases (Yamamoto, Alcauskas, & Crozier, 1976), and hence, some part of the produced CH_4 diffuses into peat pores and the rest releases into atmosphere in the gaseous form by following three pathways: diffusion through the peat, ebullition or bubbles release and plant-mediated transport through the aerenchymatous tissues of emergent vascular plants (Bridgham, Cadillo-Quiroz, Keller, & Zhuang, 2013; Chanton, Martens, Kelley, Crill, & Showers, 1992; Conrad, 1989; Joabsson, Christensen, & Wallén, 1999; Mitsch &

Gosselink, 2015). The ebullition pathway, which accounts for 18-64% of total CH_4 emissions (Christensen et al., 2003; Tokida et al., 2007) from peatlands, is dominant at some vegetated areas (van der Nat et al 1998) where WT is above the peat surface (Beckwith & Baird, 2001; Reeve, Evensen, Glaser, Siegel, & Rosenberry, 2006). During the winter season, CH_4 production can still occur in the water saturated catotelm and can contribute 10 to 30% of annual gas emission (Alm, et al., 1999). Since the release of produced CO_2 and CH_4 can be constrained by an upper frozen layer, the spring season can see a surge of gas emission at thaw.

pH is another important factor that can influence CH_4 production. The study conducted by Ye et al., (2012) showed that low pH inhibits CH_4 production by directly impacting methanogenesis and indirectly impacting fermentation. Soil pH manipulation studies of peatlands showed that acidic conditions in peatlands contribute to less CH_4 production as substrates (acetate, CO_2/H_2) become limited with the increased acidity (Dunfield, Knowles, Dumont, & Moore, 1993; Kotsyurbenko et al., 2004; Valentine, Holland, & Schimel, 1994; Ye et al., 2012). Also, trace metals are important controls on CH_4 production. For example, a study conducted in North American *Sphagnum* dominated peatlands showed enhanced CH_4 production due to addition of trace metals (Fe, Ni, Co) and sodium by enhancing the metabolism and growth of methanogens (Basiliko & Yavitt, 2001).

1.2.3 Waterborne C losses from peatlands

In addition to the loss of C in gaseous forms (CO_2 and CH_4), studies have shown that waterborne C loss, particularly dissolved organic carbon (DOC), contributes ~10 % of C losses at a rate of 5 to 50 g m^{-2} y^{-1} from peatlands (Billett et al., 2004; Roulet et al., 2007). While DOC production can be enhanced by lowered WT, vascular plant growth and nutrient availability (Freeman et al 2004a; Clark et al 2005; Strack et al 2008), export of DOC only occurs when mixed with ground water, or when streams drain through or originate from peatlands, unless drained by humans.

1.3 Anthropogenic disturbances in peatlands

Boreal peatlands are facing challenges due to ongoing anthropogenic climate change coupled with various direct anthropogenic disturbances that include conversion to agricultural land, peat extraction, drainage, winter road construction, resource access road construction, resource extraction (e.g. oil, gas), pipeline installations, seismic lines, forest clearance, and dust/nutrient deposition – all impacting peatland properties and C cycling (AECOM, 2009; Campbell & Bergeron, 2012; Cleary et al., 2005; Glatzel, Basiliko, & Moore, 2004; Strack, Keith, & Xu, 2014; Smith et al., 2008; Turetsky & Louis, 2006; Williams et al., 2013; Willier, 2017). For example, open pit mining (e.g., for oil sands extraction), which involves complete removal of peat and surficial geology, has resulted into the complete removal of 600 km^2 area of peatland in Alberta alone (Government of Alberta, 2011).

An important anthropogenic activity that results in peatland disturbance in the boreal region is the construction of resource access road networks (e.g., winter roads, all season roads) for natural resource exploration, extraction, and transportation (Partington & Clayton, 2012). The boreal region of Canada has over 217,000 km of roads with > 50% passing through peatlands (Pasher, Seed, & Duffe, 2013). Usually, the construction of access roads involves the placement of external material with a geosynthetic material on the surface of peat to improve the bearing capacity of peatland. The external materials may include glacial till, corduroy, lightweight fill, wood fiber, or temporary access mats made from soft or hardwood. The geosynthetics may include geotextiles, geogrids, and geocells to avoid mixing of external material with peat, providing reinforcement, drainage, filtration, and confinement (Partington, Gillies, Gingras, Smith, & Morissette, 2016). The constructed road modifies the hydrological properties in the upstream and downstream (Nielsen, Noble, & Hill, 2012) part of the fragmented peatlands and that can directly and indirectly

influence the physical, biological, and chemical properties that are responsible for the peat accumulation (Miller, Benscoter, & Turetsky, 2015; Partington, Gillies, Gingras, Smith, & Morissette, 2016; Strack, Softa, Bird, & Xu, 2018; Willier, 2017).

Road construction across peatlands may impact ecohydrology (e.g., WT and temperature) and biogeochemistry of adjacent peatlands. Although the specific impact of roads on peatland carbon cycling has been quantified by Plach, Wood, Macrae, Osko, & Petrone, 2017; Strack et al., (2017), the resulting road construction associated changes in WT position, peat temperature, substrate availability, and vegetation types may ultimately impact CO_2 and CH_4 production and emission pathways in peatlands (Bergman, 2000; Chanton et al., 1995; Huttunen, Nykänen, Turunen, & Martikainen, 2003; Moore et al., 2011; Treat, Bubier, Varner, & Crill, 2007) – that is yet to be quantified. Though the road may not directly lead to differences in the peat temperature in a peatland, it is possible that the deforested and degraded portion of the fragmented peatlands would allow more radiation to reach the peat surface. Therefore, adjacent areas along the road might have higher ER and CO_2 emissions, particularly if the WT is also deeper. However, the level of local topography/slope of peatlands, underlying substrate, culvert effectiveness in connecting water flow, and orientation of the road may bring variations in the intensity of road induced impacts on peatlands (Gillies, 2011; Partington, Gillies, Gingras, Smith, & Morissette, 2016; Willier, 2017).

The anticipated impacts of resource access road crossings on adjacent peatland hydrology, enzymatic activities, and C cycling are summarized below.

1.3.1 Potential road impacts on hydrology

The construction of resource access road crossings involves the laydown of external materials (mentioned above) over the peat layer to enhance the bearing capacity of peat. The placed external material compresses the peat beneath the road. As a result, the resource access road itself

fragments the hydrology of peatlands by obstructing surface and sub-surface water flows (Bocking, Cooper, & Price, 2017; Gillies, 2011; Partington et al., 2016; Patterson & Cooper, 2007; Plach, et al., 2018). This normally creates flooded conditions in the upstream areas and dry conditions on the downstream side (Willier, 2017). The subsidence of the road edges is also possible (Gillies, 2011).

1.3.2 Potential road impacts on enzymes

As mentioned above, the construction of resource access roads fragments peatlands into two sections, i.e., flooded upstream and dry downstream areas. The deeper WT on the downstream side of the road could enhance the diffusion of oxygen into deeper layers of peat, and that results in more energetically favorable heterotrophic metabolism (Reddy & DeLaune, 2008) and therefore the microbial community would be more able to devote energy to produce enzymes such as phenol oxidase and hydrolases (Reddy & DeLaune, 2008), consequently, enhancing microbial decomposition of the peat. Simultaneously, a quorum sensing response can also stimulate enzyme production (Cezairliyan & Ausubel, 2017). For example, when oxygen is permanently or periodically present the investment in phenol oxidase production results in better conditions for the microbial community to flourish so they continue producing more phenol oxidase (Burns & Dick, 2002). Conversely, at the more saturated, anoxic sites of the upstream side of the road, the microbial communities would get no benefit from producing phenol oxidase as there is no oxygen available for it to function, so they would downregulate phenol oxidase production (Burns & Dick, 2002; Olander & Vitousek, 2000). Overall, within disturbed areas, the enzyme activities will likely be higher on the downstream plots than upstream plots (Peacock et al., 2015; Pinsonneault et al., 2016).

1.3.3 Potential road impacts on CH_4 emissions

The hydrological perturbation in the peatland surrounding the access road crossing may alter plant community, peat temperature, substrate availability, microbial activity, and soil biogeochemistry (Bocking et., 2017; Campbell & Bergeron, 2012; Plach et al., 2017; Saraswati et al., 2019; Strack et al., 2018), all factors known to impact CH_4 emissions from peatlands. Under flooded anoxic conditions in the upstream areas, CH_4 emission may be increased as a result of increased anaerobic decomposition and rapid release of CH_4 (Asada, Warner, & Schiff, 2005; Turetsky et al., 2008). CH_4 emission will likely be reduced in the downstream areas due to a smaller anoxic methanogenic zone with less labile C and increased depth of oxic peat, where CH_4 oxidation can happen under lowered WT conditions (Strack et al., 2014; Watson et al., 1997; Weiss et al., 2006; White, Shannon, Weltzin, Pastor, & Bridgham, 2008).

1.3.4 Potential road impacts on CO_2 cycling

Road crossings can substantially impact the CO_2 cycling in the adjacent peatland both directly (as a result of vegetation clearance) or indirectly (as a result of changes to hydrological and thermal regime, vegetation and microbial community, and biogeochemical function including enzymatic activities). Immediately after road construction through a bog or a poor fen, the GEP may decrease as lowered WT in the downstream area could start inhibiting the growth of keystone *Sphagnum* spp. (Miller et al., 2015; Price & Ketcheson, 2009; Taylor & Price, 2015). In the longer term, the GEP may increase in treed bog and fen because deeper WT could favor the growth of woody plants (e.g., *Picea mariana*, *Larix laricina*, *Betula* spp.) by enhancing aeration in the root zone and nutrient availability (Chivers et al., 2009; Gorham, 1991; Weltzin et al., 2000). Peatlands with a low PMC and WT position > 20 cm below the surface (Devito & Hill, 1997) tend to have high nutrient mineralization (Holden, Chapman, & Labadz, 2004; Plach et al., 2017; Thormann &

Bayley, 1997; Wood et al., 2016). However, the upstream flooded areas may experience a substantial reduction in the GEP as a result of reduced vegetation cover and dying of vascular plants, particularly trees, in response to inundation.

The road can also alter rates of ER when spatial and temporal WT level variation and differences in the depth of aerobic layers are generated in the fragmented peatlands (Plach et al., 2017; Straková et al., 2011). The flooded upstream areas may have lower ER due to anoxic conditions, which reduces aerobic decomposition (Pinsonneault et al., 2016). In contrast, the downstream area can have increased ER due to lower WT and increased oxygen diffusion into deeper layers of peat (Alm, et al., 1999; Weiss et al., 2006). This will also make substrates and nutrients available for microbial activity, which will accelerate the decomposition of organic matter (Ballantyne et al., 2014; Dunfield et al., 1993; Silvola et al., 1996; Strack et al., 2008) and exhibit more enzyme activities (Freeman et al., 2001; Kang et al., 2005; Reddy, 1998). This further provides favorable conditions to the microbial communities to grow and further break down organic matter, and consequently produces a biochemical cascade of CO_2 production and emissions (Freeman et al., 2001; Freeman et al., 2012).

1.4 Objectives

The goal of this study was to quantify the magnitude and direction of impacts of all-season resource access road crossings on ecohydrological properties, enzymatic activity, CH_4 emissions and C dynamics of boreal forested peatlands by capturing the specific effect of the road in a forested bog and a shrubby rich fen. At these two study sites, the specific objectives were:

1) To determine the effect of resource access roads on the WT position and sub-surface water flow in the study sites.

2) To investigate the extent, magnitude, and direction of access road impacts on enzymatic activities in the study sites.

3) To determine the extent, magnitude, and direction of the impact of the resource access road crossings for the study sites' CH_4 emissions.

4) To investigate the impacts of access road crossings on the study sites' carbon dynamics.

5) To investigate the role of culverts in mitigating road induced impacts on the adjacent peatland's hydrology, enzymatic activities, CH_4 emissions and C dynamics.

1.5 Organization of the thesis

This thesis is organized into six chapters. The first chapter introduces peatlands, various factors impacting peatlands, research gaps and objectives of the research related to the impact of road crossings on peatland function. Chapter two addresses the primary objective of our research, by investigating the impact of resource access roads on hydrological properties at the selected study sites. Chapter three addresses the impact of resource access road crossing on enzymatic activities of two boreal forested peatlands. Chapter four addresses the impacts of resource access roads on CH_4 emissions from adjacent peatlands, which is followed by chapter five that focuses on the impacts of access road crossings on C dynamics of adjacent peatlands. The respective mitigation potential of culverts (objective five) is described in all chapters (two to five). The last chapter summarizes the study and suggest future research directions. Appendixes A1 to A3 provide supporting information and site photographs.

Chapter 2: Hydrological effects of resource access road crossings on boreal forested peatlands

2.1 Introduction

Despite relatively low annual precipitation levels (Marshall et al 1999), the Western Boreal Plains (WBP) of Canada are comprised of ~21% (365 000 km^2) peatland cover. Types include poor to extremely rich fens, saline fens, ombrotrophic bogs, and minerotrophic swamps (Chee and Vitt, 1989; Vitt et al., 2000; Wells et al., 2017). Peatlands receive water by either precipitation (P; snowfall and rainfall) or by runoff from surrounding areas; a portion of which is lost by evapotranspiration (ET) and runoff. The remaining stays either in living organisms or in soil-water storage/groundwater (Waddington et al., 2015). Water sources and hydrological conditions (e.g., water-table position and soil moisture) affect a variety of peatland ecosystem functions, including photosynthesis, microbial or plant respiration, organic matter decomposition, thermal gradient (Gong et al., 2012), nutrient dynamics (Waddington and Roulet,1997), and ultimately the rates of peat accumulation and greenhouse gas emission (Mitsch and Gosselink, 2015; Price and Waddington, 2000; Ulanowski and Branfireun, 2013; Waddington et al., 2015).

Horizontal groundwater movement in peatlands is controlled by the depth to water table (DTW), hydraulic-head variation, hydraulic conductivity, and terrain slope (Ivanov, 1981). The groundwater flow patterns (hydraulic gradient) could either be from the surface of the peat towards deep layers of the peat (recharge) or the reverse (discharge; Devito and Hill, 1997). However, drought conditions associated with water-level drawdown can reverse the flow patterns of peatlands from recharge to discharge areas (Devito & Hill, 1997).

In general, fens are more resistant to drought because the groundwater discharge from adjacent areas can partially offset the water losses (Siegel & Glaser, 1987). However, this depends on hydrogeologic setting (Elmes & Price, 2019). Also, the spatial connection and strength of groundwater connectivity impacts hydraulic head distribution, which in turn influences flow direction and discharge rate (Tóth, 1999; Winter, Rosenberry, Buso, & Merk, 2001; Winter, Rosenberry, & LaBaugh, 2003). In the absence of precipitation (P), peatlands that are mainly influenced by local flow systems (as opposed regional groundwater flow) are more susceptible to vertical flow reversals (Devito, Waddington, & Branfireun, 1997; Fraser, Roulet, & Lafleur, 2001), and become recharge zones during periods of water deficiency or water stress. Alternatively, peatlands receiving discharge from the regional flow systems are less influenced by seasonal and annual hydrometeorological variations (Smerdon, Devito, & Mendoza, 2005).

When peat formation occurs above low-permeability mineral soil, the vertical flow of groundwater becomes almost negligible. Under these conditions, horizontal flow becomes dominant in the upper layers of the peatland (Reeve et al., 2000). On the other hand, moderate-rich fens usually have coarse-grained substrates that help improve groundwater connectivity and sub-surface water table maintenance (Reeve, Siegel, & Glaser, 2000). These systems form on groundwater-discharge areas (Winter et al., 2003) and receive solutes and nutrients from surrounding areas (Reeve et al., 2000).

Across the boreal region – and particularly in the WBP – peatlands are facing disturbance by the construction of various anthropogenic linear disturbances, including seismic lines, pipelines, winter roads, power lines, and resource access roads (Lee & Boutin, 2005; Lovitt et al., 2018; Plach et al., 2017; Saraswati et al., 2019; Strack et al., 2018). The present study focuses in particular on resource access roads, which are constructed to explore and extract natural resources

(e.g., oil-gas and forestry; Lee and Boutin, 2005). In addition to removing vegetation and the laydown of mineral soil on top of a geotextile layer over peat, the compressing of the peat beneath the road itself leads to resource access roads fragmenting the hydrology of peatlands by obstructing surface and sub-surface water flows (Gillies, 2011; Partington et al., 2016; Plach et al., 2017; Paterson and cooper 2007; Strack et al., 2017). This normally creates flooded conditions in the upstream areas and dry conditions on the downstream side (Willier, 2017). Subsidence of the road edges is also possible (Gillies, 2011). A few studies have documented potential hydrological impacts of road crossings in peatlands (Partington et al., 2016). However, to our knowledge, no comprehensive multi-year study is available that has quantified these hydrological changes in different peatland types linked to (or caused by) all season resource access roads. Furthermore, it remains unclear whether culverts, structures made to connect water flow, are mitigating the hydrological impacts of the road in adjacent peatland. Therefore, the objective of this research was to determine the effect of resource access roads on the DTW position and sub-surface water flow in two boreal forested peatlands by instrumenting transects perpendicular to access road crossings in both a forested bog and shrubby fen. We hypothesized that:

1) In general, there would be higher DTW position in upstream areas, and lower DTW in downstream areas. These DTW-position differences would be greatest closest to the road.
2) Hydraulic gradients would follow local topography in the peatland, but when roads are in place, the blockage of water will alter these gradients, potentially resulting in flow reversal.
3) Finally, when culverts are in place, some connection will be re-established, and the predicted hydrologic alterations will be reduced.

2.2 Methods

2.2.1 Study sites

This research was conducted in two study sites – a shrubby rich fen (56°22'09" N and 116°46'12" W) and a forested bog (56°21'44" N and 116°47'45" W) – located in the Carmon Creek watershed, 40 km northeast of the town of Peace River, Alberta, Canada (Fig 2.1). The dominant vegetation in the bog included *Picea mariana* (black spruce), *Rhododendron groenlandicum* (Labrador tea), *Vaccinium vitis-idaea* (small craneberry), *Vaccinium oxycoccos* (bog craneberry), *Rubus chamaemorus* (cloudberry), *Sphagnum* spp. and lichens (e.g., *Cladina stellaris*, *Cladina rangiferina,* and *Cladina mitis*). The dominant vegetation in the fen included tall shrubs (e.g., *Salix* spp. (willow), *Alnus incana* (grey alder), and *Betula papyrifera* (paper birch), sedges (e.g., *Carex utriculata* (beaked sedge), *Carex aquatilis* (water sedge), *Carex canescens* (silvery sedge)), and grasses (e.g., *Calamagrostis canadensis*). The average peat depth recorded in the bog and fen was 130 and 110 cm, respectively.

The 30-year (1986-2015) normal for daily average air temperature and total rainfall during the growing season (May to August) was 14.1 ^{o}C and 213.5 mm, respectively, at the Peace River Airport Station located at ~40 km from study sites (Environment and Climate Change Canada, 2018). Compared to 30-year normal climate data, recorded growing season rainfall in 2016 growing season (444.2 mm) was substantially wetter than average. The 2017 growing season (137.0 mm) was drier than average.

Although, the watershed was bisected by a pipeline, and contained a network of seismic lines and inversion pads, our study sites were only bisected by resource access roads that were constructed in 2013 and 2014 at the fen and bog, respectively. For the construction of roads, glacial till material

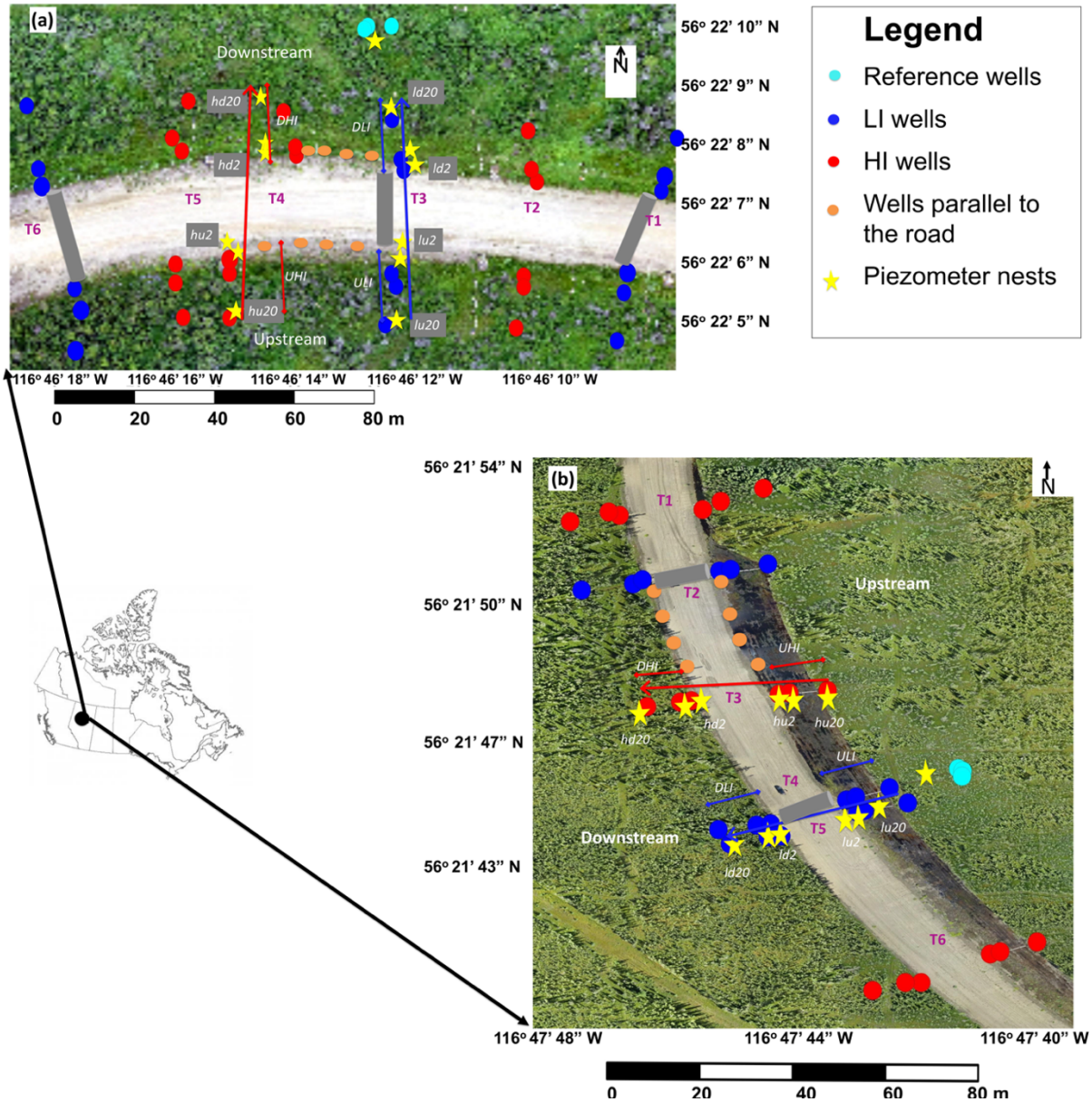

Figure 2.1 Maps showing study areas: (a) fen, and (b) bog. Grey pipes represent culverts, and numbers (T1 to T6) represent transects. lu2, lu20, ld2, and ld20 represent well/piezometer located on the low impact (LI; < 2 m away from a culvert) transect at upstream 2 m, upstream 20 m, downstream 2 m, downstream 20 m away from the road, respectively. hu2, hu20, hd2, and hd20 represent well/piezometer located on the high impact (HI; > 20 m away from a culvert) transect at upstream 2 m, upstream 20 m, downstream 2 m, downstream 20 m away from the road, respectively.

was placed on a semi-permeable geotextile material over compressed peat. Culverts were installed at an interval of ~140 and ~50 m in bog and fen, respectively. The upstream and downstream sides

of the road were determined based on the average elevation differences across the sites, as explained in Saraswati et al. (2019).

2.2.2 Field layout and setup

To collect data required to examine the first hypothesis (i.e., DTW variation across the road), six transects perpendicular to the road (20 m long on each side of the road) were established at both study sites in 2015 (Figure 2.1). At each transect, six water wells constructed of polyvinyl chloride tubing with holes throughout and mesh screen (3 cm internal diameter and a minimum of 1 m long) were installed at 2, 6, and 20 m from the edge of the road on both sides (Figure 2.1). In addition, we installed triplicate wells at 50 m from the road on both sides, and six additional wells between 150 and 300 m away from the road on both sides. In both study sites, transects located close to culverts (<2 m away) were considered *low impact* (LI), while transects located far from culverts (>20 m away) were considered *high impact* (HI). We compared measurements between these transects to test the third hypothesis (i.e., effectiveness of culverts). In May/June 2016, pressure transducers (Levelogger, Solinst, Canada) were installed in additional wells located at 1, 15, and 25 m on both sides of the road representing both HI and LI transects, and one each at 50 m on both study sites for recording DTW position every 30 minutes.

We used nests of piezometers to measure hydraulic heads and saturated hydraulic conductivity (K_{sat}) at depths of 50, 100 and 125 cm in the bog, and 50, 75, and 100 cm in the fen. These instruments were installed along a central HI transect (transect 3 in both bog and fen) and LI transect (transect 5 in bog and transect 4 in the fen) at 2, 6 and 20 m from the road on both sides (Figure 2.1). Similar piezometer nests were installed at 50 m on both sides of the road in the bog, and on one side of the road in the fen. The water intake length of each piezometer was 20 cm (i.e.,

the length of perforated areas of the pipe), centred at the depth of interest. Data collected from those instruments were used to test the second hypothesis.

In 2017, additional wells (1 m long and 3 cm diameter) were installed on parallel transects on both sides of the road to evaluate the effect of culverts on DTW positions along the road, further testing the third hypothesis. At both sites, the parallel transects were laid at 1 m perpendicular distance from the edge of the road on both sides. In the bog, wells were spaced at 10 m intervals extending 70 m from a culvert along the road. In the fen, wells were spaced at 5 m intervals, extending 25 m from a culvert. The spacing of the wells was based on the existing gap between adjacent culverts at each site (i.e., 140 m in bog and 50 m in fen).

2.2.3 Field measurements

The groundwater table position in peatlands is represented either as the DTW position, when the groundwater position is measured with reference to the surface, or ground water level (GWL), when the height of the groundwater surface is measured with reference to sea level (Harris and Bryant 2009). We measured DTW bi-weekly from May to August/September in 2016 and 2017 for all wells except the ones installed in 2017 (wells parallel to the road, where bi-weekly measurements were taken in 2017 only) using a hand-held blow-pipe.

Field-controlled aquifer tests (slug test; Surridge et al., 2005) were conducted to determine the K_{sat} of each piezometer in July 2017, with K_{sat} calculated following the method of Hvorslev (1951).

Ground elevation for the piezometer nests and wells was surveyed in 2016 with a Trimble R8s real-time kinematic global navigation satellite system (RTK GNSS) GPS. A base station at a nearby known location (X, Y, and Z) was also installed to increase the accuracy of the GPS. The

average horizontal (x, y) and vertical (z) errors recorded during surveys were 0.87 cm and 1.47 cm, respectively.

A meteorological (MET) station was setup at ~ 4 km away from the study sites. The MET station measured precipitation (ECRN-100, METER, USA), temperature (VP-3, METER, USA), solar radiation (PYR, METER, USA), and soil temperature 5 cm below the surface (GS3, METER, USA). All variables were recorded by a datalogger (EM50, METER, USA) hourly. For this study, daily rainfall data (summed to 24 hours each day) from May to August of both study years was used.

2.2.4 Topographical variations

To prepare topographic maps of the study sites (Figure 2.2), we classified (ground and non-ground) airborne LiDAR data with a point density of ~4 pts m^{-2}. The LiDAR (provided by Shell Canada) was collected and pre-processed by Airborne Imaging Inc. in May 2013. The ground points were converted to a Digital Terrain Model (DTM) in ArcMap (Version 103.1) using a 'triangulation with linear interpolation' technique (Akima, 1975). Finally, contour lines with 0.5 m elevation/contour interval were generated from the terrain model by using Contour tool of ArcMap (10.3.1) that converts raster (elevation) into contour lines at a specified interval.

2.2.5 Impact of roads on shallow groundwater flow

The sub-surface water flow analysis, which was performed to test the second hypothesis, included calculation of vertical and horizontal hydraulic gradients, and horizontal hydraulic fluxes (specific groundwater discharge, q_{hor}, mm d^{-1}) calculated using Darcy's Law (Hendriks, 2010). For each hydraulic gradient and q_{hor} analysis, the bi-weekly hydraulic head measurements (h, May-August)

were used. Vertical hydraulic gradient of each nest was calculated by taking h differences between two piezometers representing peat layers (50 and 100 cm) in both study sites.

Calculations of q_{hor} included the h differences between nests located along the same transect and side of the road (e.g. h difference between nests at 2 m and 20 m on the upstream side of the HI transect). For each nest, h_{avg} was calculated as the arithmetic means of h measured from all piezometers of that nest. Due to differences in DTW, culvert distance, and average surface elevation, I calculated weighted harmonic mean K_{sat} separately (Freeze & Cherry, 1979) for both upstream HI and LI areas and downstream HI and LI areas in both study areas. The calculated K_{sat} was then used to calculate q_{hor} for that segment of the transect by multiplying it by the horizontal hydraulic gradient (Freeze & Cherry, 1979).

To estimate the impact of the road on subsurface water flow, the q_{hor} of the entire transect was first calculated (by determining the hydraulic gradient between upstream 20 m to downstream 20 m) separately in both HI (q_{HI}) and LI (q_{LI}) areas (Figure 2.1) by using the respective K_{sat} and hydraulic gradients. We assume that this represents subsurface discharge through this section of peatland if no road was present, assuming the h at 20 m is relatively unaffected by the road. Secondly, the q_{hor} of the upstream area (between 20 and 2 m upstream of the road; q_{UHI} or q_{ULI}) of the respective transects was calculated and subtracted from the q_{HI} or q_{LI} to determine the impact of the road (IR_{HI}, IR_{LI}) and effectiveness of the culvert (IR_{HI} - IR_{LI}) as follow:

$$Road\ impact\ (IR) = \left(-K_{sat} * \frac{(hd20\ - hu20)}{50}\right) - \left(-K_{sat} * \frac{(hu2\ - hu20)}{18}\right) \ldots\ldots\ldots\ldots\ldots Eq.1$$

Where,

K_{sat} = Average (upstream and downstream) hydraulic conductivity

*hd*20 = Hydraulic head at 20 m downstream of the road

*hu*20 = Hydraulic head at 20 m upstream of the road

*hu*2 = Hydraulic head at 2 m upstream of the road

2.2.6 Statistical analyses

Statistical analyses were performed with R (R Core Team, 2017) and Microsoft Excel 2016. Differences between group means were tested either by t-tests for comparing two groups (e.g., reference vs. disturbed areas, 2016 vs. 2017 etc.) or one-way analysis of variance (ANOVA) for more than two groups (e.g., 2, 6 and 20 m from the road). For testing the first and third hypotheses, linear mixed effect models were used to investigate the contribution of road associated factors (i.e., sides of the road, culvert distance, and distance from the road) to variation in hydrological conditions (e.g. DTW, K_{sat}) using a significance level of $\alpha = 0.05$. In each model, the plots or wells were taken as a random factor to account for repeated measurements. Each model was followed by graphical testing of the homogeneity and normality of residuals (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Post hoc analyses of the significant main and interaction effects were performed using Tukey pairwise comparisons with either the Tukey's HSD test (ANOVA) or *emmeans* function (linear mixed effects model) of *emmeans* package (Lenth, 2018).

2.3 Results

2.3.1 Site topography

Both study sites differed in terms of the topography surrounding the roads. The bog site showed a gradual decrease in surface elevation from the upstream (east) to downstream (west) areas, with the slope (~0.5%) more clearly perpendicular to the road on the northern end of the study site

(Figure 2.2a). In contrast, the elevation difference between upstream (south) and downstream (north) areas in the fen was minimal (Figure 2.2b), and slope in the upland on the east side of the fen was nearly parallel to the road.

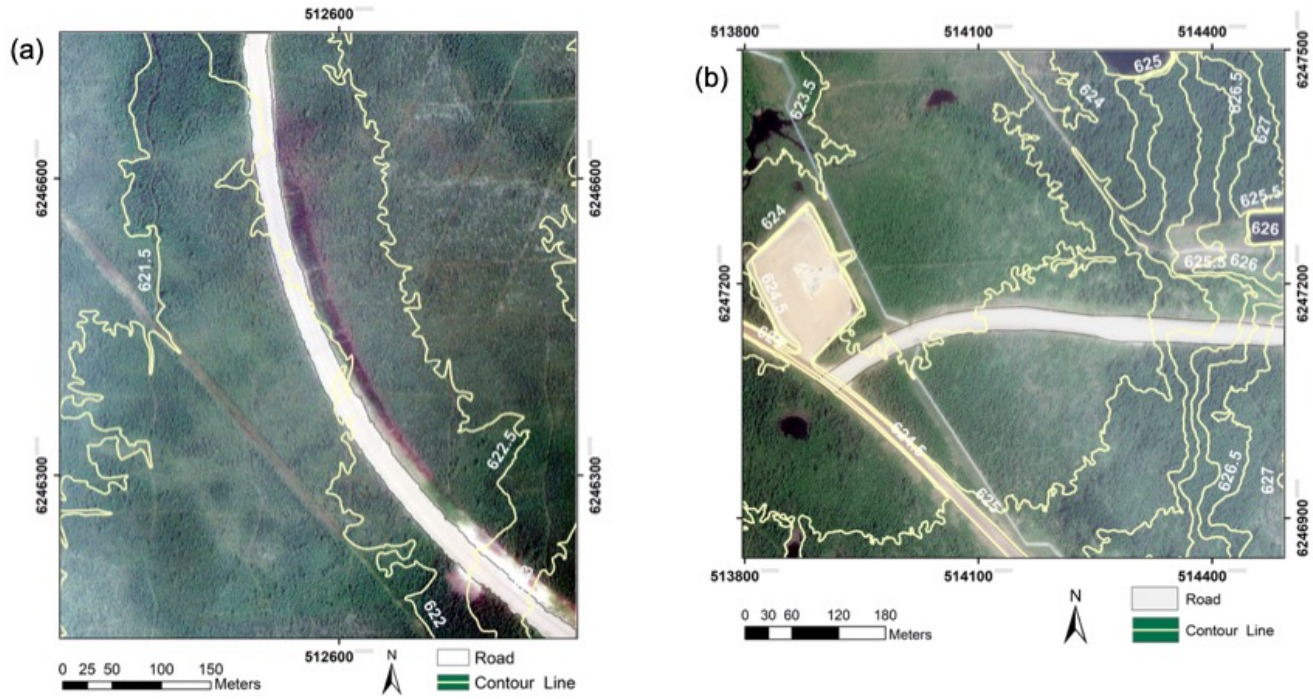

Figure 2.2 Map of topographical slope gradient at study sites: (a) bog, (b) fen, in Carmon Creek watershed, Peace River, Alberta, Canada.

2.3.2 DTW variations

Mean DTW of bog (-11.3 cm) and fen (-2.5 cm) in 2016 were significantly shallower compared to DTW of bog (-19.2 cm, $z = 7.58$, $p < 0.001$) and fen (-17.9 cm, $z = 13.14$, $p < 0.001$) in 2017. The comparison between study sites showed that DTW in the fen was significantly shallower compared to bog in 2016 ($z = 12.04$, $p < 0.001$), but not in 2017 ($z = 0.91$, $p = 0.364$).

Using bi-weekly DTW observations in the bog, conditions within the detailed study area ≤ 20 m (road disturbed areas) from the road were compared to areas 50 or > 150 m away (reference areas) from the road. Average DTW at ≤ 20 m (-11.0 ± 1.6 cm) and 50 m (-16.4 ± 1.7 cm) away from the

road were significantly shallower than areas > 150 m from the road in 2016 ($z = 2.86$, $p < 0.01$; $z = 3.27$, $p < 0.001$, respectively). In contrast, average DTW at ≤ 20 (-2.6 ± 1.5 cm) and 50 m (-2.6 ± 0.7 cm) from the road in 2016 were not significantly different compared to areas located at > 150 m from the road ($z = 0.09$, $p = 0.925$) in the fen. Also, in 2017, average DTW at ≤ 20 m (-18.5 ± 0.9 cm) from the road was significantly shallower than areas at 50 m away from the road (-24.3 ± 1.6 cm; $z = 3.27$, $p < 0.001$) in the bog. In the fen, in 2017, no significant differences ($z = 1.15$, $p = 0.26$) were observed between average DTW at ≤ 20 m (-16.4 ± 1.2 cm) and 50 m (-22.7 ± 4.4 cm) from the road. However, we did not measure DTW data of wells located at > 150 m from the road in 2017.

At sites within 20 m of the road (disturbed areas) in the bog, the interaction of side of the road, culvert position, and distance from the road was significant in 2016, with interactions of side of road and culvert position also significant in both 2016 and 2017 for describing variation in DTW position (Table 2.1, Figure 2.3a). In 2017, bog DTW was also significantly related to the interactions of culvert position and distance from the road ($F_{2,23} = 10.47$, $p = 0.02$). In general, post-hoc comparisons showed that, at the bog, the average DTW on the upstream areas, independent of distance from the culvert, were significantly shallower than the downstream areas in both 2016 and 2017 ($p < 0.001$, Table 2.2). Within upstream areas, the 2 and 6 m plots were wetter than the 20 m plots in both HI and LI transects (Figure 2.3a). This supported the first hypothesis that DTW would vary across the road, with greater differences close to the road. The shallowest and deepest DTW were observed at the HI upstream 2 m and HI downstream 2 m plots, respectively (Figure 2.3a). Post-hoc comparisons also revealed that DTW at LI downstream 2 m plots was significantly shallower compared to 6 and 20 m plots at LI downstream areas and all

plots of HI downstream areas. Consistent with our third hypothesis, this suggested that culverts improved hydrologic connection across the road.

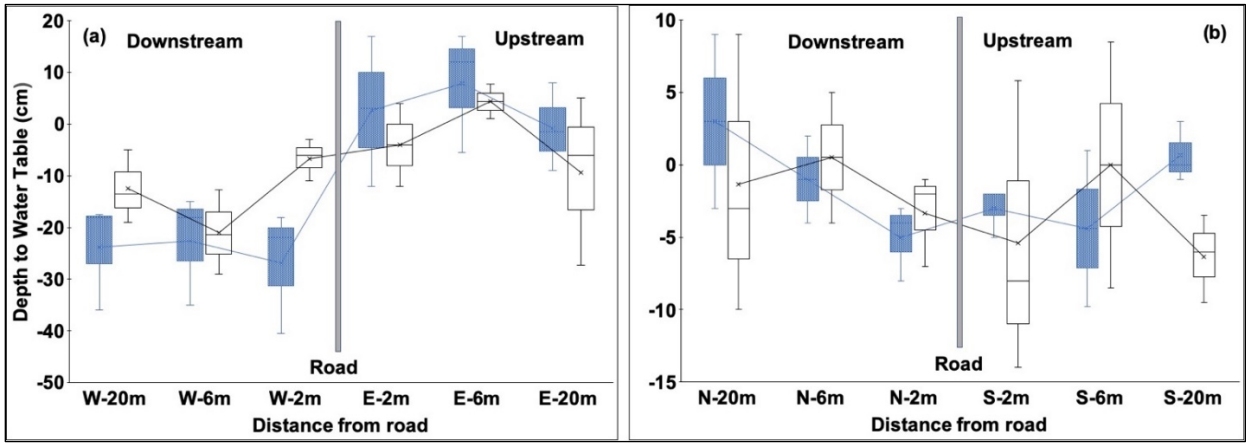

Figure 2.3 Depth to water table (DTW) variation created by road construction in the (a) bog and (b) fen, in Carmon Creek Alberta, Canada. Box plots (pattern filled) represent HI transects (> 20 m away from the culverts) and box plots (no fill) represent LI transects (< 2 m away from the culverts) in 2016.

In contrast, at the fen little impact of the road on DTW was observed in both years, except that DTW was significantly shallower on the downstream side in 2017 ($F_{1,24} = 5.96$, $p = 0.02$; Table 2.1, Figure 2.3b).

The growing season rainfall pattern had a direct link with DTW, as DTW was shallower in 2016 than 2017, corresponding with greater rainfall in 2016 (Figure 2.4). Similar to the results obtained from the bi-weekly DTW measurements, the continuous levelogger data recorded along the select HI and LI transects also indicated that DTW on the HI upstream areas were shallower close to the road (DTW at 1 >15 > 25 m from the road) with the opposite trend observed on the downstream side (DTW at 1 < 15 < 25 m from the road; Figure 2.4a to 2.4h) in the bog. Along the LI transects in the bog, irrespective of side of the road, the DTW position close to the road varied less than that measured at wells further from the road. However, DTW variations in the fen did not follow a distinct trend related to side of the road or distance from the road and fluctuated in parallel across measured wells (Figure 2.5).

Table 2.1 Linear mixed effects model results showing the impacts of side of the road, culvert distance, and distance from the road on depth to water table (DTW) in 2016 and 2017 at bog and fen, Carmon Creek, Peace River, Alberta.

Treatments	**Bog**		**Fen**	
	F values	**P-values**	**F values**	**P- values**
Depth to Water Table (2016)				
Intercept	**43.81** $_{(1, 411)}$	**<0.001**	**6.40** $_{(1, 441)}$	**0.01**
Side[1]	**36.88** $_{(1, 32)}$	**<0.001**	0.86 $_{(1, 24)}$	0.36
Culvert[2]	**7.24** $_{(1, 32)}$	**0.01**	0.001 $_{(1, 24)}$	0.97
Dist[3]	2.23 $_{(2, 411)}$	0.11	0.88 $_{(2, 24)}$	0.42
Side ×Culvert	**5.66** $_{(1, 32)}$	**0.03**	2.3 $_{(1, 24)}$	0.14
Side ×Dist	0.56 $_{(2, 411)}$	0.57	0.71 $_{(2, 24)}$	0.50
Culvert ×Dist	0.49 $_{(2, 411)}$	0.61	1.60 $_{(2, 24)}$	0.22
Side*Culvert ×Dist	**0.84** $_{(2, 411)}$	**<0.001**	0.07 $_{(2, 24)}$	0.93
Depth to Water Table (2017)				
Intercept	**154.21** $_{(1,323)}$	**< 0.001**	**181.30** $_{(1,417)}$	**<0.001**
Side[1]	**51.74** $_{(1, 23)}$	**<0.001**	**5.96** $_{(1, 24)}$	**0.02**
Culvert[2]	**7.39** $_{(1, 23)}$	**0.04**	0.02 $_{(1, 24)}$	0.89
Dist[3]	2.14 $_{(2, 23)}$	0.28	0.14 $_{(2, 24)}$	0.87
Side ×Culvert	**9.43** $_{(1, 23)}$	**0.03**	3.30 $_{(1, 24)}$	0.08
Side ×Dist	1.74 $_{(2, 23)}$	0.38	0.04 $_{(2, 24)}$	0.95
Culvert ×Dist	**10.47** $_{(2, 23)}$	**0.02**	0.62 $_{(2, 24)}$	0.54
Side ×Culvert ×Dist	7.43 $_{(2, 23)}$	0.09	0.21 $_{(1, 24)}$	0.81

[1] Side of the road; [2] Distance from a culvert; [3] Distance from the road; bold numbers represent statistically significant results

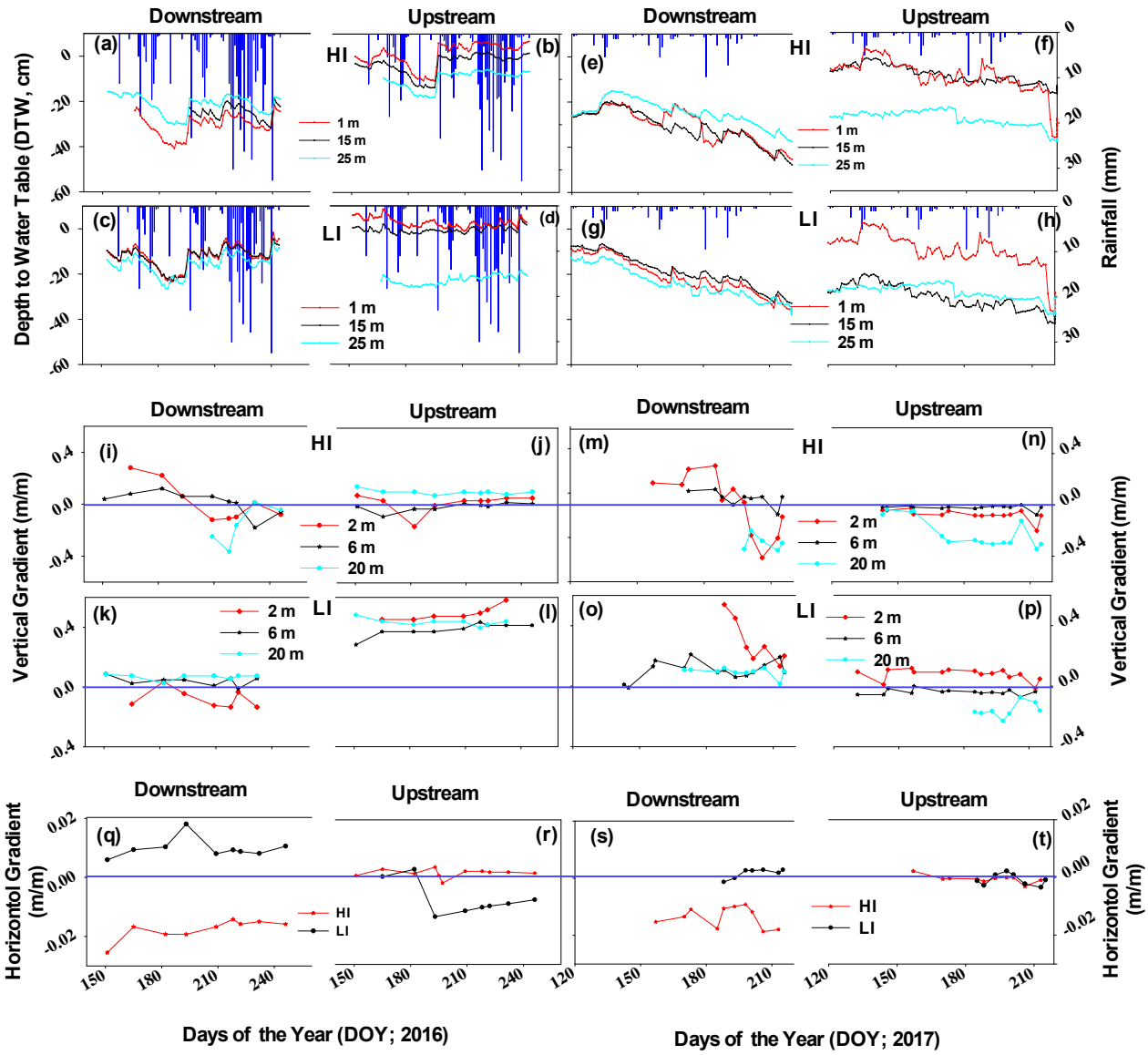

Figure 2.4 Average sub-surface hydrological variations observed during the growing seasons (May-August) of 2016 and 2017 in the bog site: (a) to (h) seasonal DTW changes (line) and rainfall (bar); (i) to (p) average vertical hydraulic gradients; (q) to (t) average horizontal hydraulic gradient on transects located at ≥ 20 m from culverts (T3) and at ≤ 2 m from culverts (T5). Negative vertical gradient denotes recharge to the ground water (downward movement) and +ve denotes discharge from the groundwater (upward movement). Negative horizontal gradient indicates flux towards the downstream and the +ve denotes the reverse flow (flow towards the upstream).

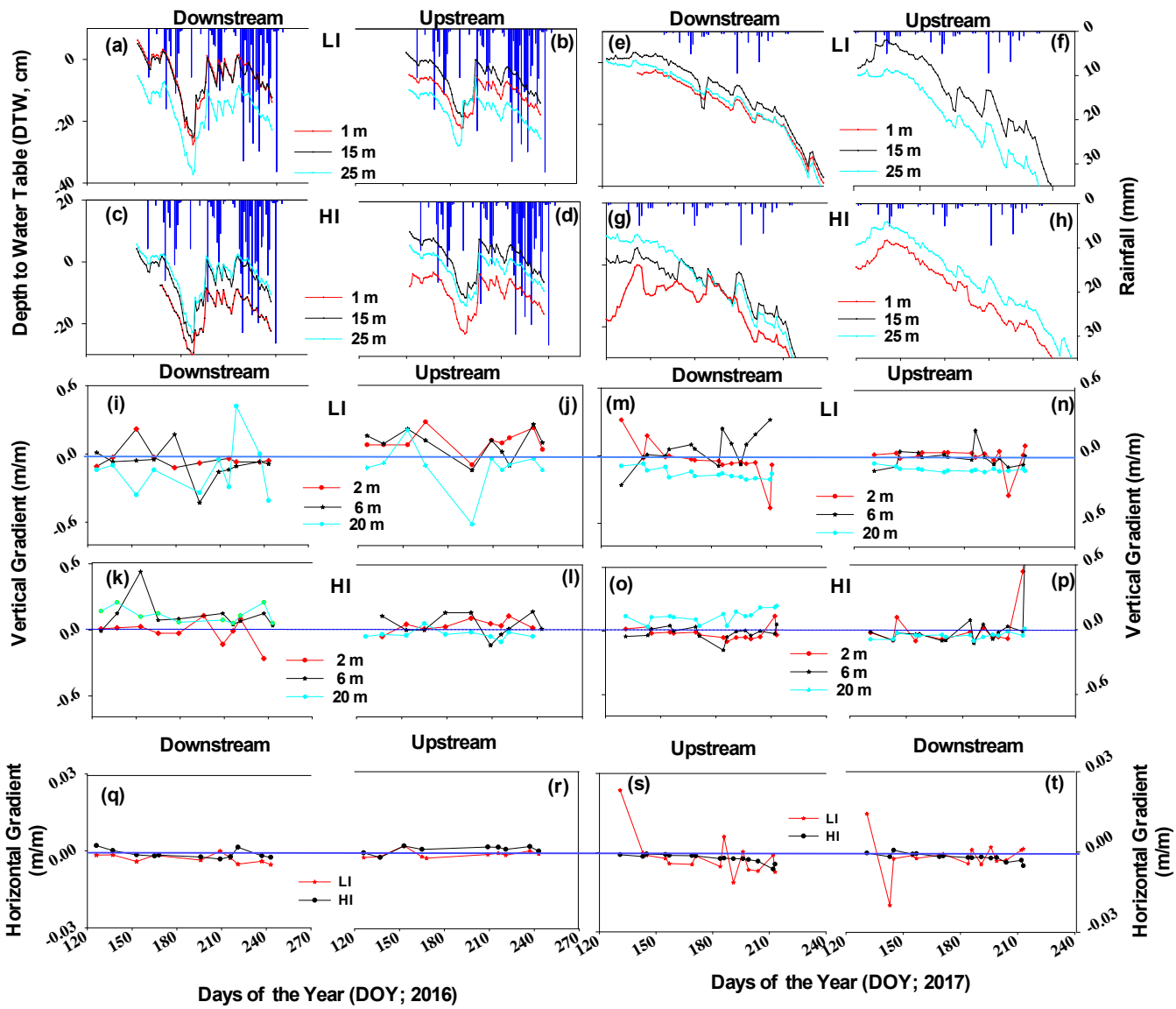

Figure 2.5 Average sub-surface hydrological variations observed during the growing season (May-August) of 2016 and 2017 in the fen site: (a) to (h) seasonal DTW changes (line) and rainfall (bar); (i) and (p) average vertical hydraulic gradient; (q) and (t) average horizontal hydraulic gradient on transects located at ≥ 20 m from culverts (T4) and at ≤ 2 m from culverts (T3). Negative vertical gradient denotes recharge to the ground water (downward movement) and +ve denotes discharge from the groundwater (upward movement). Negative horizontal gradient indicates flux towards the downstream and the +ve denotes the reverse flow (flow towards the upstream).

In order to explore the effect of culverts (third hypothesis) on water table position adjacent to the road, DTW was measured in a road-parallel transect laid at 1 m perpendicular distance from the edge of the road on both sides. Since small differences in surface topography made interpreting DTW difficult, the absolute elevation of the water table (ground water level: GWL) was

considered. There was a significant interaction between side of the road and distance from the culvert on GWL position ($F_{6,110} = 23.42$, $p < 0.001$) in the bog. Post-hoc analysis showed a significantly shallower GWL (-7.5 cm; $p < 0.001$) on the upstream areas of the road compared to the downstream areas (GWL = -27.1 cm), with the difference declining closer to the culvert. The effectiveness of the culvert for reducing GWL difference between upstream and downstream areas of the bog was greatly reduced ~ 15 m from a culvert (Figure 2.6). In contrast, a significant pattern of the GWL variation away from the culvert in the fen site was not clear (data not shown).

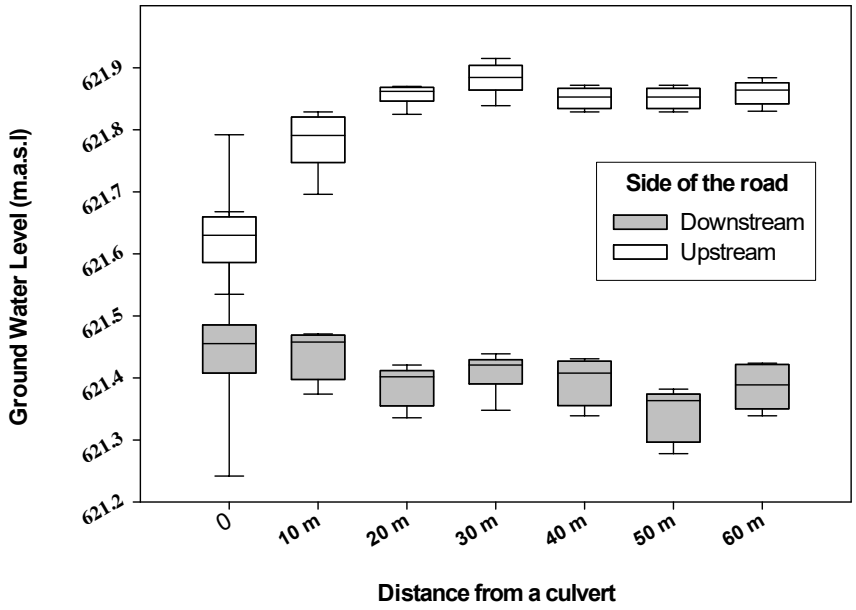

Figure 2.6 Ground water level (GWL) variation among water wells located parallel to the road in the bog in 2017, Carmon Creek, Alberta.

2.3.3 Sub-surface water flow

The average K_{sat} values across all depths and nests of the bog and fen were 2.18 ± 1.24 and 1.85 ± 0.47 cm h^{-1}, respectively. At the bog, high K_{sat} was measured at some 50 cm piezometers, but variation at this depth was high (Table 2.2). Therefore, although there was a general pattern of decline of K_{sat} with depth at the bog, there was no significant effect of depth of piezometer ($F_{2,29}$

= 1.82, p = 0.19). There was also no significant impact of side of the road ($F_{1, 29} = 2.23$, $p = 0.15$), distance from culvert ($F_{1,29} = 0.50$, $p = 0.61$), or distance from road ($F_{2,29} = 0.63$, $p = 0.35$) in the bog. However, in the fen, the K_{sat} values varied with depth ($p < 0.01$, $F_{2,24} = 19.7$), but did not vary by side of the road, culvert distance and distance from the road ($p > 0.05$). Fen K_{sat} values were significantly higher in the 50 cm piezometer and gradually decreased with depth (Table 2.2).

Table 2.2 The saturated hydraulic conductivities (K_{sat}) of piezometer nests in the bog and fen, Carmon Creek, Peace River, Alberta in 2017.

Bog piezometer depth (cm)	Hydraulic conductivity (range) (K_{sat}, cm h^{-1}) x 10^3	Fen piezometer depth (cm)	Hydraulic conductivity (range) (K_{sat}, cm h^{-1}) x 10^3
50	5.37 (0.001, 33.04)	50	4.97 (0.002, 11.31)
100	0.22 (< 0.001, 2.40)	75	0.44 (0.001, 2.66)
125	0.03 (< 0.001, 0.13)	125	0.004 (< 0.001,0.008)

Throughout the study seasons of both 2016 and 2017, *h* corresponded with rainfall, increasing following rainfall events (observed in 2016) and gradually declining when rainfall was not frequent, as observed in 2017. At the bog, *h* followed a linear trend with a gradual decrease in magnitude from upstream areas towards the downstream areas far from the road in the bog. Similar, trends were observed in the fen in 2016 and 2017.

In general, prominent vertical discharge (groundwater moving to the surface) was evident until the end of June in both study sites. At the bog, there was a greater upward flux on the upstream side and close to the road areas, and even greater at the LI transects. Whereas, after mid-summer, the vertical gradient was downward (groundwater recharge) from 50 cm to 100 cm depth with changes corresponding to rainfall events both in bog (Figure 2.4i to 2.4p) and fen (Figure 2.5i to 2.5p), with the exception of the bog HI transect (T3) in 2016, which remained mostly unchanged throughout the season. Also, in the fen in 2017, vertical discharge corresponded to little or no rainfall in the late season (Figure 2.5p).

Differences in vertical hydraulic gradients relative to the road were further supported by evidence that horizontal flow was disturbed by the road with instances of flow reversals observed on both upstream and downstream areas of both bog and fen, particularly during the dry period (Figure 2.5a and 2.5b). This was consistent with our second hypothesis that the road would alter local hydraulic gradients. The general slope of the water table across the sites, particularly at the bog, indicated that flow should occur from upstream to downstream areas. However, the q_{hor} analyses showed that water was reversing back from the edges of the road on the upstream areas, while on the downstream areas, the water was reversing towards the edge of the road when culverts were not connecting upstream and downstream areas. In the bog, the reverse flow was also observed on the downstream side of the road at the LI transect in both years. Shifts in hydraulic gradient greatly reduced subsurface discharge through this section of the bog. Impacts of the road on the subsurface water flow were observed on both sides of the bog in 2017, but the effects varied between HI and LI transects. The impact on the HI transect (IR_{HI}, 5.53 mm d^{-1}) was nine times higher ($t = 8.09$, $p < 0.001$) than the impact on the LI transects (IR_{LI}, 0.63 mm d^{-1}). In contrast, the impact of the road on the subsurface water flow was minimal in the fen in 2017 ($IR_{HI} = 1.03$ mm d^{-1}, $IR_{LI} = 0.31$ mm day^{-1}, $t = 0.96$, $p = 0.17$).

2.4 Discussion

2.4.1 The effect of access-road crossings on peatland DTW

The construction of roads across peatlands results in disturbed hydrological properties including altered surface and sub-surface water flow between the peatland on either side of the road (Gillies, 2011; Partington et al., 2016; Partington & Clayton, 2012; Plach et al., 2017; Strack et al., 2018). In our study sites, the impacts of the road on the DTW and sub-surface water flow were detected

up to 20 m from the road in the bog in both study years, but not in the fen mainly due to the flow direction in the fen being nearly parallel to the road. However, the lateral extent of this impact varies within and between study sites due to local conditions including culvert position, and road alignment with respect to the flow direction.

Interactive effects of side of the road and culvert position were observed in both 2016 and 2017, and culvert position and distance from the road in 2017, on DTW in the bog. The observed average DTW positions on the upstream areas (plots at 2 and 6 m from the road) were significantly shallower than the 20 m plots in both HI and LI downstream. This implies that the presence of a road impeded surface and sub-surface water flow and disconnected the upstream and downstream areas. Consequently, flooded conditions on the upstream areas closer to the road and the drying of the downstream areas were evident in HI transects. However, in LI transects of the bog, the observed shallower DTW on the downstream areas closer to the road indicate the culverts helped in connecting surface and sub-surface water flow. However, the water was not effectively distributed further from the culvert openings. The elevation was lower surrounding the culvert openings due to peat removal while installing culverts and this made the channelled water moving through the culvert pool near the culvert opening. This suggests that while culverts are partially effective in reducing hydrologic impacts, they may be too far spaced and/or installed at improper elevation to maintain hydrologic connectivity at the bog studied here.

Results at the bog were in line with Plach et al. (2017), who also observed impeded water flows between road fragmented parts of a peatland, with the significantly shallower DTW in the upstream areas compared to DTW in the downstream areas. In contrast to the bog, the impact of the road on the surface and sub-surface water flow was not significant at the fen in both years and that could be because the road in the fen is aligned nearly parallel to the water flow direction (Figure 2.2b).

Therefore, orientation of the road relative to local topography is more important than peatland type for determining the hydrological impact.

2.4.2 The effect of access-road crossings on hydraulic gradients in adjacent peatland

In undisturbed peatlands, hydraulic gradients generally follow local topography (Siegel & Glaser, 1987). In contrast to that, we observed frequent flow reversals in both upstream and downstream areas of the bog. Under normal conditions, the water should always flow towards the road edges in the upstream areas as the road edges are at lower elevation than areas upstream of them (by natural orientation and also as a result of compaction). However, during high rainfall periods, the damming effect of the road resulted in elevated *h* close to the road, particularly at HI upstream areas because of the absence of culverts. This damming effect also resulted in much drier conditions on the downstream areas nearest to the road causing low *h.* Thus, those areas received water from further downstream areas in the absence of rainfall. Bog LI downstream areas also experienced a flow reversal that was likely caused primarily by peat removal surrounding the culvert opening compared to the downstream areas away from the road. This resulted in lower ground elevation on the road edges around the culverts (up to 1 m).

The observed variation in the vertical recharge/discharge among transects in both study sites could be linked with rainfall, culvert position, and peat-thawing patterns throughout the growing seasons, except in road-adjacent (2 m) areas. In road-adjacent areas, the compression and subsidence of the peat beneath the road may block the surface and sub-surface water flow leading to shallow conditions (Gillies, 2011; Patterson & Cooper, 2007). Mainly, the discharge observed until mid-growing season may be associated with the frozen peat layers below 50 cm of the peat, which obstruct downward water movement. Also, enhanced radiative fluxes because of the vegetation clearance during road construction on the upstream areas could resulted in early thawing of the

upper peat layers, which could contribute more to surface than vertical flow (Quinton, Hayashi, & Chasmer, 2009). As deeper peat layers start thawing, the water might be moving towards surface peat layers – contributing to more discharge (Connon, Quinton, Craig, & Hayashi, 2014). We saw prominent vertical discharge (groundwater moving to the surface) in the fen in 2017, which could instead be due to no rainfall in the late growing season of 2017 (Figure 2.5p), and water deficiency may lead to groundwater discharge, as fens get water from both P and groundwater flow.

Vertical reversals due to water deficiency, or DTW drawdown have also been found in peatlands isolated from regional groundwater flow systems (Devito et al.,1997). However, the groundwater recharge observed later in the growing season could correspond mainly to rainfall events and high hydraulic gradients both in bog and fen (i.e. rainfall events resulted in groundwater recharge). Fraser et al. (2001) also found a shift in groundwater flow patterns at Mer Bleue bog mainly due to low P, high ET, and differential peat depth *h* response. We found that the upstream areas of the bog in 2016 had DTW close to the surface and were mostly vertical discharge zones irrespective of the rainfall amounts. This is because peat layers in those areas were mostly water-saturated, leading to surface flow and no further recharge (Holden & Burt, 2003).

2.4.3 Effectiveness of culverts for mitigating road-associated hydrologic changes

Although likely too widely spaced – particularly in the bog – our study showed that culverts help connect the surface and sub-surface water flow between fragmented peatland areas. The road was calculated to have greatly reduced daily flow (5 mm d^{-1}) in the bog when culverts were not present. However, the water storage difference on the upstream areas was only 2.56 mm d^{-1}, indicating approximately half of the blocked water was lost from the upstream areas. Greater ET and flow reversals as observed in our study could have contributed to the enhanced loss of the water from this zone. Studies have shown that the ET, which may range from 4.8 mm d^{-1} to 14.4 mm d^{-1} in

WBP peatlands (Brown et al., 2010), significantly lowers the DTW in WBP peatlands in the late growing season (Price, 2003;Reeve et al., 2006). Increased ET in the bog HI upstream areas can be associated with the greater solar radiation, warmer conditions, and windier conditions on the open, flooded areas (Petrone et al., 2007; Waddington et al., 2009), and colonisation by sedges resulting in higher transpiration losses (Waddington et al., 2009).

The effectiveness of the culverts depends on micro-topographical variations, proper installation and adequate numbers of culverts, orientation of the road, water flow direction, and wetland type (e.g., bog, fen, swamps etc.; Willier, 2017). In general, culverts in peatlands are installed at an interval of 100 to 250 m if ponding conditions are expected (Partington et al., 2016). However, this interval distance cannot provide effective hydrological connection across the road as results here showed that culverts may connect water movements up to ~ 15 m distance provided that the water flow direction is perpendicular to the access road. The specific spacing and size of culverts will ultimately depend on expected water flow perpendicular to the road, which will be driven by local slope and hydraulic conductivity. Therefore, in the fen, culverts had little effect on water flow as the slope of the road was mostly parallel to the flow direction. Also, though not observed in our study sites, the effectiveness of culverts can be significantly reduced when the culverts are blocked by factors such as beavers (Bocking et al., 2017), mineral soil deposition, or the subsidence of the road edges (Gillies, 2011; Partington & Clayton, 2012) or uneven compression of the culverts (Partington et al., 2016). Further, the compression of the peat and/or additions of dust and fine particles due to the road can lower K_{sat} on the surface of the peat resulting in further reductions in surface and sub-surface flow.

2.5 Chapter conclusions

In this study, we hypothesized that resource access road crossings would lead to DTW variation between fragmented parts of the adjacent peatland with the possibility of flow reversals, but that the culverts would reduce the hydrological impacts. We observed that the construction of resource access roads disturbed surface and sub-surface water flow at the bog, but the effect was minimal at the fen. The observed hydrological variation in bog supported all the hypotheses examined in this study and the variations were primarily linked to the road alignment perpendicular to the water flow direction, and the culvert position. Overall at the bog, the shallowest and deepest DTW positions were measured in transects far from culverts, at the HI upstream 2 m and HI downstream 2 m plots, respectively. In the bog, the road greatly reduced daily flow (5 mm d^{-1}) in HI areas. Although culverts were shown to improve hydrologic connection across the road, we also observed that culverts provide a point source of water to downstream areas in peatlands, with chances of water not redistributing well. Therefore, the first option is to avoid the construction of access roads across peatlands; however, if this is not possible, using more permeable road designs (permeable fills), building on a corduroy base, or brush mats, and rock fills (Gillies, 2011) would be beneficial. Such options will improve hydrologic connections as well as enhance the bearing capacity of roads. Therefore, there is a need for further hydrological studies on more permeable road designs across peatlands.

Chapter 3: Access roads impact enzyme activities in boreal forested peatlands

3.1 Introduction

Peatlands have the potential to be one of the key drivers of global climate change because they are long-term sinks of carbon in the form of peat. Peatlands account for one-third of global soil carbon which is equivalent to twice the amount of carbon stored in forest biomass (Kaat & Joosten, 2009; Parish et al., 2008). Despite covering only 3% of the land surface (Freeman et al., 2012; Limpens et al., 2008), peatlands store ~550 Gt of organic carbon (Yu et al., 2010). Globally, the majority of peatlands (~89% of the total or 3.6 million km^2 area) are distributed in boreal and temperate regions (Page et al., 2011). Of the estimated 1.14 million km^2 of peatland in Canada, 64% occurs in the boreal region (Tarnocai, 2006; Tarnocai et al., 2011) and a significant fraction (up to 50%) is distributed in the northwest boreal region (Zoltai & Vitt, 1995).

Boreal peatlands have been accumulating a deep layer of peat (depth > 40 cm by definition in Canada; National Wetlands Working Group, 1997) over millennia (Belyea & Clymo, 2001; Bhatti, Apps, & Tarnocai, 2002; Clymo et al., 1998; Gajewski et al., 2000; Turunen et al., 2002). This accumulation is due to sustained organic matter production rates that exceed rates of decomposition. A combination of various environmental conditions in peatlands are responsible for the restricted decomposition rate of organic matter (Fenner & Freeman, 2011; Freeman et al., 2012, 2004). These typically include water saturation, resulting in deep layers of anoxic soil (Freeman et al., 2004, 2001), low nutrient availability, low pH (Williams et al., 2000), distinctive vegetative composition, elevated concentrations of phenolic compounds (phenolics) and low extracellular enzyme activities (phenol oxidase and hydrolase; Freeman et al., 2001).

Extracellular enzymes are produced and released by fungi, bacteria (Fenner, Freeman, & Reynolds, 2005) and archaea (Bell et al., 2013). These extracellular enzymes play a significant role in nutrient cycling (e.g. nitrogen, sulfur, phosphorus and carbon; Luo et al., 2017). Microorganisms release extracellular hydrolase enzymes to cleave the polymers that make up particulate organic matter into dissolved organic matter monomers, which serve as substrates for metabolism (Bell et al., 2013; Luo et al., 2017; Sinsabaugh & Moorhead, 1994; Sinsabaugh, 2010). In peatlands, mainly five hydrolase enzymes, i.e. β-D-glucosidase (glucosidase), arylsulfatase (sulfatase), β-D-xylosidase (xylosidase), N-acetyl β-D-glucosaminidase (glucosaminidase) and phosphatase, play a crucial role in carbon and nutrient cycling (Dunn, Jones, Girard, & Freeman, 2014; Freeman et al., 2001; Pinsonneault et al., 2016). Glucosidase is responsible for the breakdown of complex carbohydrates into simpler glucose fragments. Sulfatase hydrolyses sulfate ester bonds, which are present in a large number of biomolecules including proteins and carbohydrates. Sulfatases, therefore, are an essential component of the biogeochemical sulfur cycle in soils (Press, Henderson, & Lee, 1985). Glucosaminidase breaks down chitin (a derivative of glucose) into amino sugars and plays a significant role in nitrogen and carbon cycling (Kang, Kim, Fenner, & Freeman, 2005). Phosphatase helps to liberate phosphate from organic matter by hydrolyzing phosphomonoester bonds present in phosphosugars, mononucleotides and phospholipids (Nannnipieri, Pedrazzini, Arcara, & PiovanelliI, 1979; Turner, Frossard, & Baldwin, 2005). This process is ecologically critical as the availability of phosphate often limits primary production in freshwater environments. Phenol oxidase has the capacity to fully degrade phenolics (e.g., lignin) in the presence of oxygen (McLatchey & Reddy, 1998). Further, under higher temperatures (> 30 ºC), which may be experienced in tropical regions, phenolics can also be degraded in the absence of oxygen using alternative electron acceptors at a much slower rate

(Bakker, 1977; Elder & Kelly, 1994; Levén, Nyberg, & Schnürer, 2012). Therefore, phenol oxidase plays an important role in the decomposition of organic matter when peat is aerated. In undisturbed boreal peatlands, phenol oxidase activity is mainly restricted to the surface, aerated layer (Fenner & Freeman, 2011; Freeman et al., 2004, 2001) as deeper layers remain anoxic. In undisturbed peatlands, oxygen availability limits the degradation of phenolics by phenol oxidase below the surface. The accumulated phenolics can then inhibit hydrolase activities in a process known as the 'enzymatic latch' mechanism (Freeman et al., 2001). However, various anthropogenic disturbances can open this enzymatic latch (Fenner & Freeman, 2011).

Many studies have investigated various anthropogenic disturbances to peatlands and their impact on carbon cycling including land conversion, drainage, peat extraction, wildfires, and permafrost thaw (Cleary et al., 2005; Glatzel et al., 2004; Haapalehto et al., 2014; Holden et al., 2004; Laiho, 2006; Lee and Boutin, 2005; Pasher et al., 2013; Price et al., 2002; Saarnio et al., 1997; Turetsky and St. Louis, 2006; Williams et al., 2013;). However, few studies have investigated the impacts of linear disturbances, such as construction of access roads, on peatland ecosystems (Campbell and Bergeron, 2012; Miller et al., 2015; Plach et al., 2017; Strack et al., 2017; Willier, 2017). Furthermore, to the best of our knowledge no studies exist on the impact of access roads on the dynamics of enzymatic activities.

The construction of access roads for the exploration and extraction of natural resources (e.g., oil and gas, forest harvesting), pipeline installation, and geologic exploration is an important anthropogenic disturbance in the boreal region; the Canadian boreal region alone has a network of over 217,000 km of roads with >50% passing through peatlands (Pasher et al., 2013). Road construction across peatlands involves vegetation clearing, laying of logs and/or geotextile, and mineral soil deposition (Graf, 2009; Partington & Clayton, 2012). Under the footprint of the roads

themselves, and within the road modified upstream and downstream areas, the physical, biological, and chemical properties that are responsible for peat accumulation are directly and indirectly altered (Kowalski and Wilcox, 2003; Miller et al., 2015; Pasher et al., 2013; Williams et al., 2013; Willers 2017). Access roads can act as dams, preventing the flow of water from one side of the road to the other, often resulting in flooding to upstream, and drying to downstream areas (Bocking et al., 2017; Willier, 2017).

This study was designed to investigate the extent, magnitude, and direction of access road impacts on enzymatic activities in two boreal forested peatlands. It was anticipated that:

1) The construction of access roads would lower the water table (WT) on the downstream side of the road. 2) That the lowered WT would enhance the diffusion of oxygen into deeper layers of peat, 3) that this would stimulate the production and activity of phenol oxidase and hydrolase enzymes.

In contrast, we postulated that on the upstream side of the road, the raised WT position would suppress enzyme activities. We hypothesized that factors representative of road impacts (distance to a culvert, side of the road, distance from the road) would interactively effect enzyme activities due to disruption of the water table and consequent changes to chemical parameters e.g. redox potential, pH, electrical conductivity (EC) and phenolic concentration (Figure 3.1). We tested if enzyme activities were higher in areas disturbed by road construction (e.g., < 20m from the road) compared to undisturbed areas (reference sites). Within disturbed areas, we tested if the activities were higher in downstream areas compared to upstream areas and whether enzyme activities varied with distance from the road. We also examined whether culverts minimized the enzyme activity variation in nearby areas by connecting upstream and downstream areas, allowing for water flow (Figure 3.1).

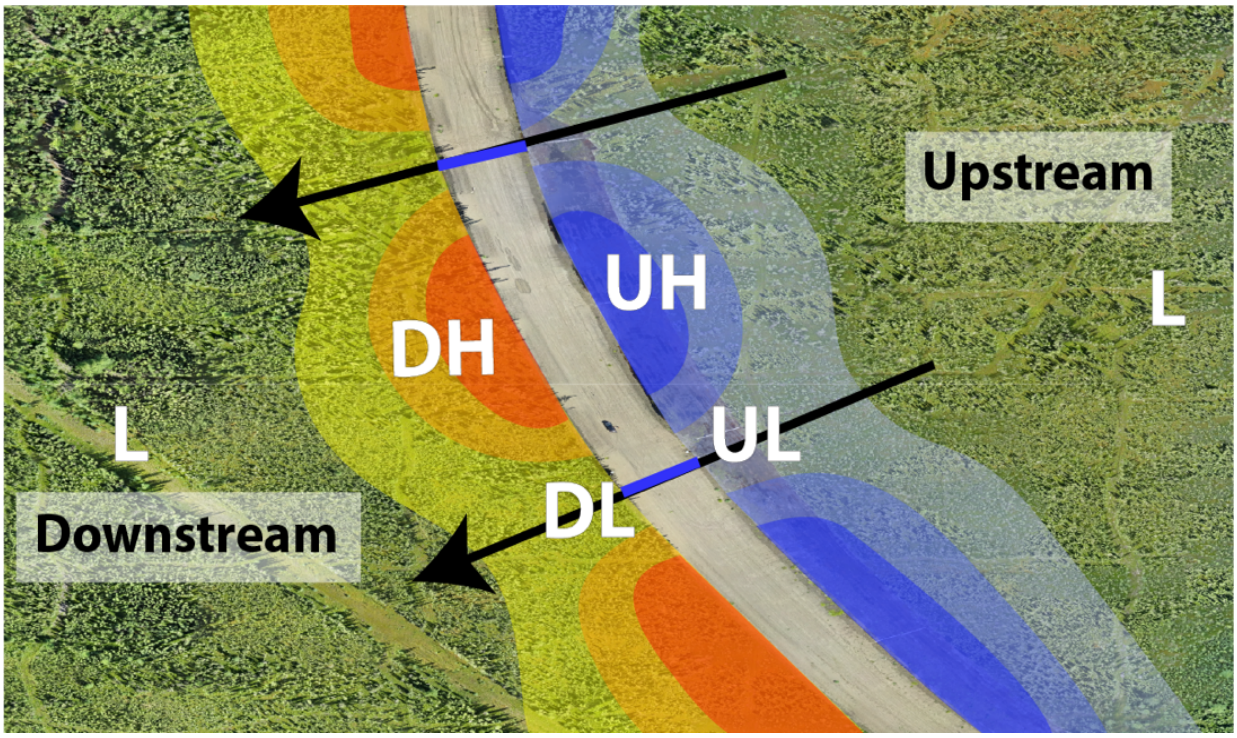

Figure 3.1 A conceptual diagram showing hypothesized impact zones in the study site. Black arrow lines represent culverts and the arrow heads show the water flow direction. We hypothesize that the impact of the road will be higher closer to the road (dark blue shaded; upstream high impact (UH; flooding) and dark orange shaded; downstream high impact (DH; drying) areas) and the impact decreases further away from the road. However, I also hypothesized that the impact will be minimal in areas nearby culverts (light shaded; upstream (UL) and downstream low impact (DL) areas). Reference (undisturbed by road) areas represented by L.

3.2 Methods

3.2.1 Study sites

The study sites (Figure 3.2), a forested bog (56°21’44” N and 116°47’45” W) and a shrubby rich fen (56°22’09” N and 116°46’12” W) are located within the Carmon Creek watershed, Peace River, Alberta. The vegetation in the bog is dominated by *Picea mariana*, *Rhododendron groenlandicum*, *Vaccinium oxycoccos*, *Vaccinium vitis-idaea*, *Sphagnum* mosses and lichens (e.g., *Cladina stellaris*, *Cladina rangiferina,* and *Cladina mitis*). The dominant plants in the fen are tall shrubs (e.g., *Salix* spp., *Alnus incana*, and *Betula papyrifera*), sedges (e.g., *Carex utriculata*, *Carex*

aquatilis, *Carex canescens*), and grasses (e.g., *Calamagrostis canadensis*). The average pH and EC in the bog and fen were 5.4 and 7.5, and 102.1 µS cm^{-1} and 285.2 µS cm^{-1}, respectively.

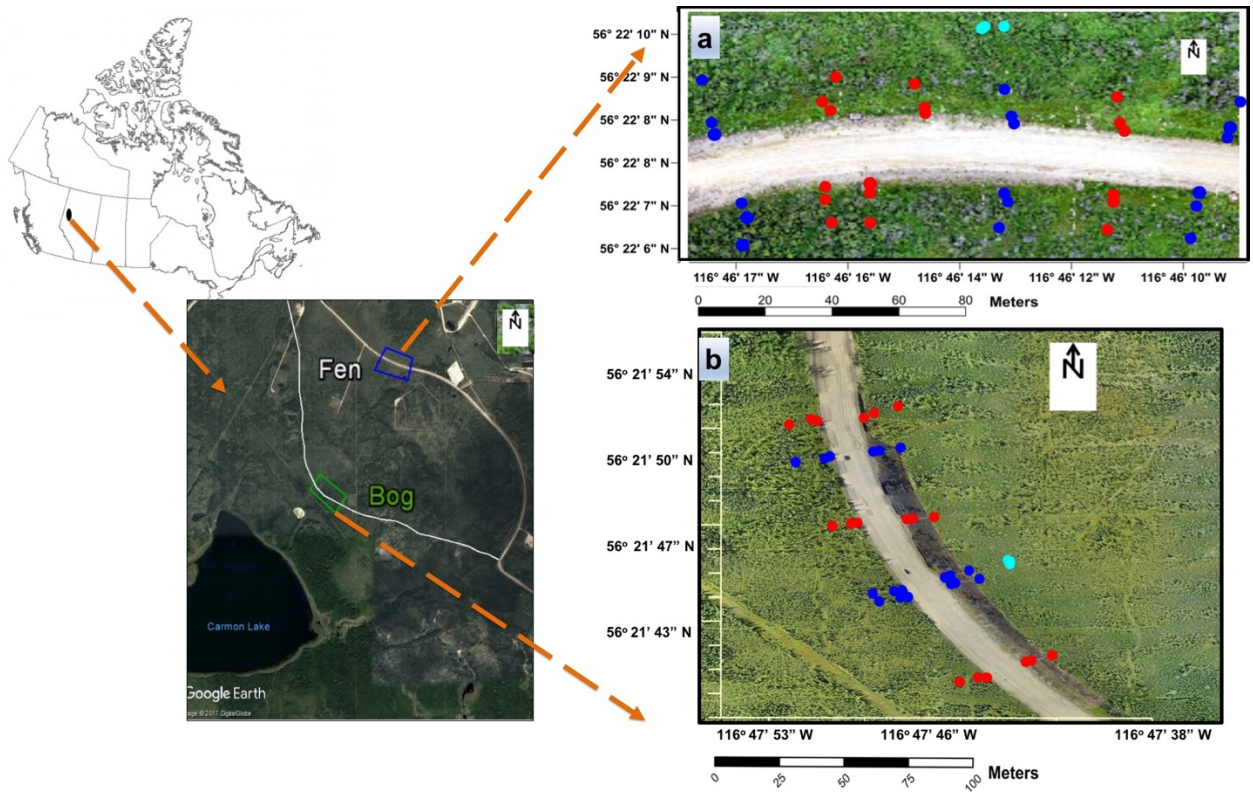

Figure 3.2 Study sites, a) a fen and b) a bog, in Carmon Creek watershed, Peace River, Alberta, Canada. Where red dots represent transects > 20 m from culverts position, blue dots represent transects <2 m away from culverts position and teal dots represent undisturbed reference areas.

Fen and bog sites were bisected by resource access roads in 2013 and 2014, respectively. Both roads are elevated above the peat surface by glacial till material which was deposited over semi-permeable geotextile layers (Gillies, 2011). Culverts, at irregular intervals (> 20 m), were installed beneath the roads. I did not find any documented reason for the irregular distance between culverts. To improve visibility, all vegetation was completely cleared on the east side (upstream) of the road in the bog, and on the north side (downstream) of the road in the fen. As the road in the bog was constructed more recently (2014), the clearing, extending up to 18 m from the road, was largely devoid of vegetation during the study. However, some herbaceous plants were beginning to re-

establish themselves in the cleared area. At the fen, the cleared area was devoid of tall shrubs; however, compared to the bog, the growth of sedges provided almost complete surface cover. The 'upstream' and 'downstream' sides of the road at each site were determined based on an elevation survey and topographic maps (see Rahman et al., 2017). In the bog, there is a gradual decrease in slope from the upstream areas (average elevation 622.5 m.a.s.l.) to the downstream areas (621.5 m.a.s.l.). In the fen, the elevation difference between the upstream (625.4 m.a.s.l.) and downstream (625 m.a.s.l.) areas was much less pronounced than in the bog.

3.2.2 Field layout and Sampling

A total of twelve sampling transects perpendicular to roads were laid out, six at the bog site and six at the fen site. Three of the transects at each site were located < 2 m from a culvert, the remaining three transects at each site were located > 20 m away from the nearest culvert (Figure 3.2). All transects extended to 20 m from the edge of the road on both sides of the road. In August 2016, peat samples were collected at six locations on each transect (at 2 m, 6 m and 20 m from both sides of the road) and at three undisturbed locations >50 m from the road at each site. This resulted in a total of 78 peat samples, 39 each from the bog and fen. We determined that 50 m from the road was undisturbed by comparing vegetation in this area to vegetation throughout the study sites (i.e., several hundred meters around the road). Further, satellite imagery showed little change before and after the road construction at this distance from the road.

In order to ensure stable temperature and hydrological conditions during sampling, the peat samples from each study site were all collected on the same day (i.e., 9th and 10th August at the bog and fen, respectively). Each peat sample consisted of a 5 x 5 cm square extracted to a depth of 10 cm below the peat surface. The same peat samples were later used to measure EC and pH in the laboratory with multimeter probes (Mettler Toledo, USA) by making a slurry of water and peat

in a 2:1 ratio. In addition to peat sampling, WT depth at the time of sampling was recorded from wells constructed of polyvinyl chloride pipe (3 cm internal diameter, 1 m long) installed beside each sampling site. At the time of sampling, soil temperature was also measured from 5 cm below the peat surface resulting in an average value of 14 ± 2 ºC across both sites. Collected samples were stored in air-tight bags and transported in a cooler with ice packs to the University of Waterloo for analysis in the Ecohydrology Biogeochemical Kinetics Laboratory.

3.2.3 Laboratory analyses

In the laboratory, the peat samples were stored at -20 ºC to prevent enzymatic decomposition of peat (Lee, Lorenz, Dick, & Dick, 2007). We assayed hydrolase (glucosidase, sulfatase, xylosidase, glucosaminidase, and phosphatase) and phenol oxidase enzymes because of their importance in carbon and nutrient cycling in peatlands (Dunn et al., 2014; Pinsonneault et al., 2016). The enzyme assays were performed in the third week post-sampling. The details of the enzyme assay methods used are provided in Appendix A1. Before performing enzyme assays, peat samples and prepared substrates were kept in an environmental chamber (CTH-118, Percival Scientific) maintained at the field temperature (14 ºC) for 24 hours. For hydrolase enzyme assays, 400 µM MUF (4-methylumbelliferone) labeled substrates were used except for phosphatase for which 200 µM MUF-phosphate was used. As fluorescence quenching decreases the measured fluorescence intensity when using MUF labelled substrates in environmental samples, a calibration curve was prepared for each sample by mixing the sample with varying concentrations of MUF (Dunn et al., 2014). For phenol oxidase, the model substrate used was a 10 mM solution of L-DOPA (L-3, 4-dihydroxy phenylalanine; Pind et al., 1994).

The loss on ignition of each sample was estimated by following Frogbrook et al. (2009) to calculate the soil organic matter percentage (SOM%). From a separate portion of each sample, the dry

weight of peat was measured and used to normalize enzymatic activities, which were determined from wet samples, per unit dry mass (Saraswati, Dunn, Mitsch, & Freeman, 2016). Phenolic concentrations were measured using Folin-Ciocalteu phenol reagent by following the method of Box (1983).

3.2.4 Statistical analyses

All numeric variables were checked graphically (histogram, Q-Q plots or scatter plots) for the distribution pattern (normality and homogeneity) before performing each statistical analysis (Zuur et al., 2009). Data were analyzed in R (R Core Team, 2017) assuming unequal variances. Therefore, I used the Welch t-test and Welch's one-way ANOVA for comparing two groups/treatments (e.g., bog vs. fen) and more than two groups (e.g., distance from the road), respectively. To investigate the impact (significance level $\alpha = 0.05$) of the three road factors (i.e., side of the road, culvert distance and distance from the road) on each variable (e.g. enzyme activity), general linear models in the nlme package (R Core Team, 2017) were used. Variance structures were added to these models to ensure normality of the residuals as required (Zuur et al. 2009). Post hoc analysis of the significant main and interaction effects was performed by using either Tukey's HSD test or the lsmeans function of the lsmeans package (Lenth, 2016). Residuals were checked visually for normality and homogeneity.

3.3 Results

3.3.1 Site hydrology and chemistry

The average WT depth, pH, and EC (Table 3.1) were significantly lower in the bog compared to the fen (WT: $t = 3.19$, $p = 0.002$; pH: $t = 13.90$; EC: $p < 0.001$; $t = 12.35$; $p < 0.001$). However,

average phenolic concentration (0.11 ± 0.01 mg L^{-1}) in the bog was significantly higher compared to the fen (0.08 ± 0.01 mg L^{-1}; $t = 2.08$; $p = 0.04$).

In the bog, the average WT was significantly shallower on the upstream side of the road compared to the downstream side of the road ($t = 5.99$, $p < 0.001$; Table 3.1; Figure 3.3) and the interaction between the side of the road and the location of culvert was close to significant (Tables 3.1 and 3.2). The pairwise comparison showed that the average WT in the downstream areas not connected by culverts was significantly lower compared to upstream areas not connected by culverts ($t = 5.80$, $p < 0.001$), and upstream areas connected by culverts ($t = 4.58$, $p < 0.001$) in the bog. Culvert distance and distance from the road, were not significant as main effects on WT variation in the bog (Table 3.2).

The three-way interaction including side of the road, culvert distance, and distance from the road explained a significant portion of variation in phenolic concentration in the bog (Table 3.2). Phenolic concentrations in the bog were significantly higher on the upstream side of the road compared to the downstream side of the road ($t = 1.97$, $p = 0.05$), and also varied with distance from the road ($F_{(2,15)} = 3.59$, $p = 0.05$). The highest phenolic concentrations were observed in the upstream areas close to the road (Table 3.1). Culvert distance alone was not explanatory for phenolic concentration variations in the bog (Table 3.2).

Side of the road was a significant explanatory factor for variation in pH in the bog (Table 3.2). Average pH was significantly lower on the downstream side of the road compared to the upstream side ($t = 2.70$, $p = 0.01$, Table 3.1). In the bog, the average pH was slightly lower further away from the road (Table 3.1); however, the effect of distance from the road was not significant (Table 3.2).

Table 3.1 Average (mean ± SE) characteristics of bog and fen sites, Carmon Creek, Peace River, Alberta, 2016.

Treatments	**Levels**	**pH**	**WT** [a] (cm)	**Phenolics** (mg L^{-1})	**SOM%**	**T5** [b] (°C)	**EC** (µS cm^{-1})
			Bog				
Side of the road	Upstream	**5.8 ± 0.2** [c]	**1.9 ± 2.5** [c]	**0.15 ± 0.02** [a]	**78.3 ± 4.9** [c]	18.2 ± 0.6	103.22 ± 16.30
	Downstream	**5.0 ± 0.2** [c]	**-19.6 ± 2.6** [c]	**0.09 ± 0.02** [c]	**90.5 ± 2.2** [c]	17.4 ± 0.8	110.07 ± 11.73
Culvert position	> 20 m	5.3 ± 0.2	-10.6 ± 4.1	0.12 ± 0.02	88.8 ± 3.2	17.6 ±0.9	115.3 ± 14.54
	< 2 m	5.6 ± 0.2	-8.9 ± 3.0	0.13 ± 0.02	80.0 ± 4.6	18.0 ± 0.4	97.98 ± 13.59
Distance from road	2 m	5.8 ± 0.3	-9.1 ± 4.6	0.15 ± 0.02	**75.4 ± 6.1** [c]	18.3 ± 0.5	130.99 ± 23.55
	6 m	5.3 ± 0.3	-10.1 ± 5.5	0.09 ± 0.01	82.5 ± 4.6	18.4 ± 1.1	105.01 ± 13.76
	20 m	5.1 ± 0.2	-10.2 ± 3.8	0.13 ± 0.03	**95.3 ± 1.1** [c]	16.7 ± 0.7	83.93 ± 9.66
Disturbed average		**5.4 ± 0.2** [c]	-9.8 ± 2.6	0.12 ± 0.01	84.4 ± 2.9	**17.8 ± 0.5** [c]	**106.64 ± 9.92** [c]
Undisturbed [d] **average**		**4.6 ± 0.0** [c]	-20.3 ± 8.3	0.07 ± 0.02	91.5 ± 3.9	**21.1 ± 0.6** [c]	**48.12 ± 2.35** [c]
			Fen				
Side of the road	Upstream	7.4 ± 0.1	-3.0 ± 1.4	0.09 ± 0.01	79.3 ± 4.5	**16.4 ± 0.3** [c]	295.41 ± 16.02
	Downstream	7.5 ± 0.1	-1.6 ± 1.5	0.08 ± 0.01	78.3 ± 3.9	**18.3± 0.5** [c]	286.49 ± 17.83
Culvert position	> 20 m	7.5 ± 0.1	-1.8 ± 1.2	0.09 ± 0.01	83.0 ± 3.4	17.5 ± 0.5	292.61 ± 16.02
	< 2 m	7.5 ± 0.1	-2.8 ± 1.6	0.08 ± 0.01	74.6 ± 4.7	17.2 ± 0.4	289.29 ±17.89
Distance from road	2 m	7.5 ± 0.1	-4.2 ± 1.4	0.08 ± 0.01	70.4 ± 7.8	17.5 ± 0.6	**315.76 ± 19.85** [c]
	6 m	7.5 ± 0.1	-1.1 ± 2.0	0.10 ± 0.01	81.2 ± 3.1	17.1 ± 0.6	307.44 ± 21.53
	20 m	7.5 ± 0.1	-1.4 ± 2.0	0.08 ± 0.01	84.8 ± 1.6	17.3 ± 0.5	**249.65 ± 15.62** [c]
Disturbed average		7.5 ± 0.1	-2.3 ± 1.0	0.09 ± 0.01	**78.8 ± 2.9** [c]	17.3 ± 0.3	**290.95 ± 11.84** [c]
Undisturbed average		7.3 ± 0.1	0.8 ± 1.7	0.08 ± 0.01	**93.3 ± 3.6** [c]	17.8 ± 1.5	**216.37 ± 5.61** [c]

[a] Water table position negative refers to the below surface level and positive above the surface
[b] Peat temperature at 5 cm below the surface
[c] Means significantly different between groups ($P < 0.05$)
[d] Plots > 50 m from the road

In contrast, the side of the road, distance to a culvert, and the distance from the road were not significant for explaining variation in WT, phenolics, or pH in the fen (Table 3.2).

Table 3.2 Main and interactive effects of side of the road, culvert distance, and distance from the road on water table, phenolics and pH.

Treatments	Bog site		Fen site	
	F $_{(df1, df2)}$[a]	**P**	**F** $_{(df1, df2)}$	**P**
Water table (WT) depth				
Side[b]	**37.93** $_{(1, 21)}$	**<0.001**	0.40 $_{(1, 20)}$	0.54
Culvert[c]	0.25 $_{(1, 21)}$	0.62	0.22 $_{(1, 20)}$	0.64
Dist[d]	0.03 $_{(2, 21)}$	0.96	0.90 $_{(2, 20)}$	0.42
Side*Culvert	3.30 $_{(1, 21)}$	0.06	0.18 $_{(1, 20)}$	0.68
Side*Dist	1.30 $_{(2, 21)}$	0.29	0.24 $_{(2, 20)}$	0.78
Culvert*Dist	1.18 $_{(2, 21)}$	0.33	1.19 $_{(2, 20)}$	0.32
Side*Culvert*Dist	1.09 $_{(2, 21)}$	0.35	0.21 $_{(2, 20)}$	0.81
Phenolic concentration				
Side	**4.82** $_{(1, 19)}$	**0.04**	0.25 $_{(1, 23)}$	0.74
Culvert	0.19 $_{(1, 19)}$	0.67	0.11 $_{(1, 23)}$	0.50
Dist	2.37 $_{(2, 19)}$	0.12	0.51 $_{(2, 20)}$	0.61
Side*Culvert	0.20 $_{(1, 19)}$	0.66	1.40 $_{(1, 20)}$	0.25
Side*Dist	0.40 $_{(2, 19)}$	0.67	0.16 $_{(2, 20)}$	0.86
Culvert*Dist	0.56 $_{(2, 19)}$	0.58	0.66 $_{(2, 20)}$	0.53
Side*Culvert*Dist	**4.30** $_{(2, 19)}$	**0.02***	0.89 $_{(2, 20)}$	0.42
Peat pH				
Side	**8.09** $_{(1, 24)}$	**<0.00**	0.58 $_{(1, 24)}$	0.45
Culvert	1.08 $_{(1, 24)}$	0.31	0.09 $_{(1, 24)}$	0.76
Dist	2.37 $_{(2,24)}$	0.11	0.12 $_{(2, 24)}$	0.88
Side*Culvert	3.22 $_{(1, 24)}$	0.07	1.26 $_{(1, 24)}$	0.27
Side*Dist	0.60 $_{(2, 24)}$	0.56	0.01 $_{(2, 24)}$	0.99
Culvert*Dist	1.17 $_{(2, 24)}$	0.33	0.65 $_{(2, 24)}$	0.53
Side*Culvert*Dist	0.53 $_{(2, 24)}$	0.59	0.95 $_{(2, 24)}$	0.40

[a] Degrees of freedom between treatments (df1) and within groups (df2).
[b] Side of the road (Upstream and Downstream);
[c] Distance to culvert (< 2 m and > 20 m);
[d] Perpendicular distance from the road (2m, 6m, and 20m);
Bold numbers represent statistically significant values.

3.3.2 Enzyme activities

Average hydrolase activity in the fen (16.88 nmol g^{-1} min^{-1}) was ~ four times higher than in the bog ($t = 5.84$, $p < 0.001$). Specifically, average activities of glucosidase, sulfatase, glucosaminidase

and phosphatase were all significantly higher in the fen ($t = 4.99$, $p < 0.001$; $t = 3.65$, $p < 0.001$; $t = 4.24$, $p < 0.001$; $t = 2.41$, $p = 0.01$) than in the bog. However, average phenol oxidase activity in the bog (0.24 μmol g^{-1} min^{-1}) and the fen were similar (0.17 μmol g^{-1} min^{-1}; $t = 0.90$, $p = 0.36$).

We postulated that there would be significant interactive effects between factors representative of road influence (i.e. distance to a culvert, side of the road, and distance from the road) on enzyme activities, with highest enzyme activities close to the road on the downstream side far from a culvert (Figure 3.1). In the bog, we found significant interactive effects between culvert position and side of the road, culvert position and the distance from the road, and the side of the road and the distance from the road on phenol oxidase activity (Table 3.3; Figure 3.3). Post hoc comparison showed that the downstream side of the road far from culverts had significantly higher phenol oxidase activity compared to the upstream side far from culverts ($t = 3.02$, $p = 0.03$) and the upstream side connected by culverts ($t = 3.161$, $p = 0.02$; Figure 3.3). Even considering these interactions, averaging among samples, phenol oxidase activity was significantly higher on the downstream side compared to the upstream side at 6 m from the road (Figure 3.3).

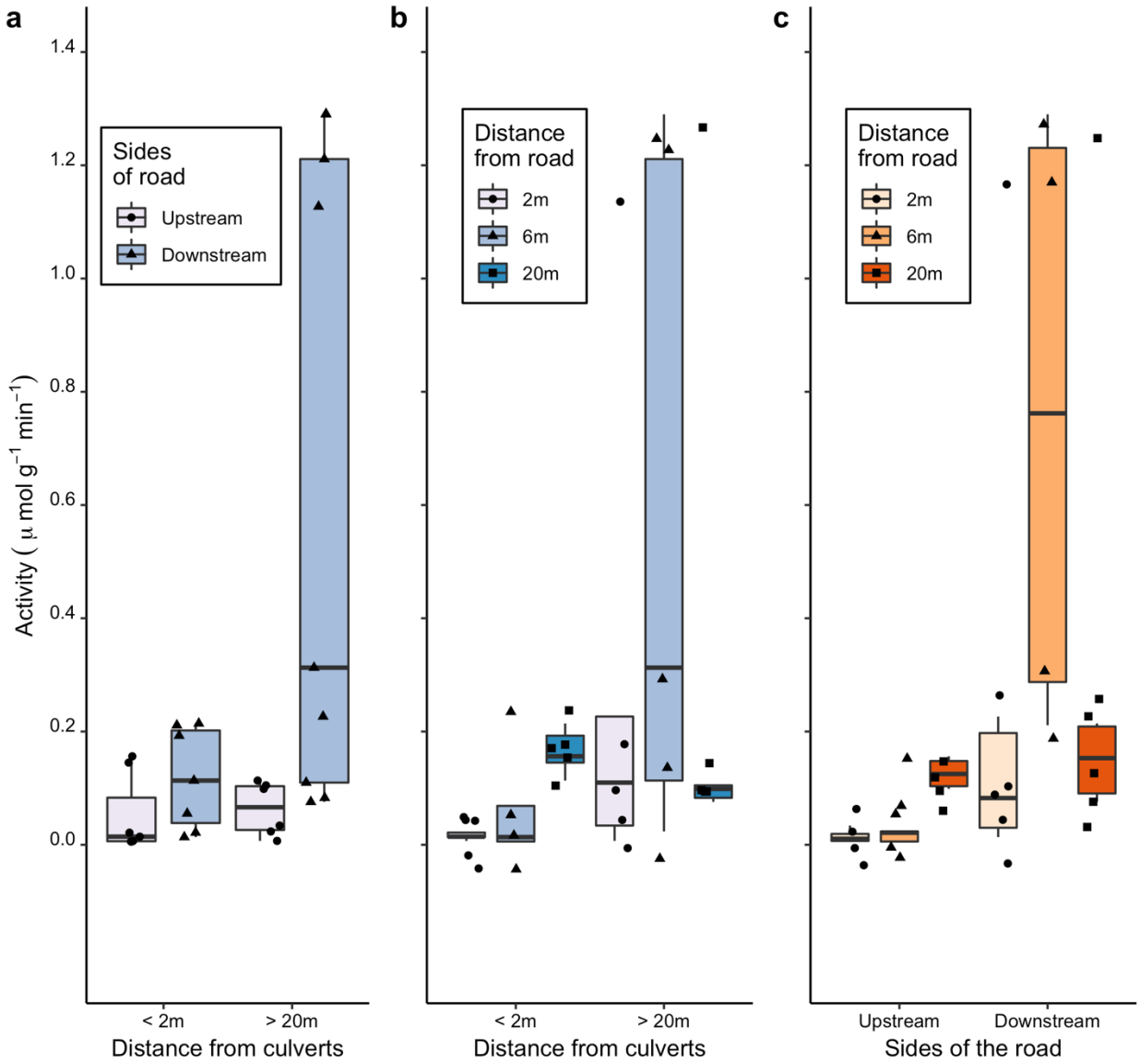

Figure 3.3 Phenol oxidase activities a) between distance to culvert and sides of the road, b) between distance to culvert and distances from the road, and c) between sides of the road and distances from the road in bog, Carmon Creek, Peace River, Alberta.

In the bog, the activity of all hydrolase enzymes, except phosphatase, was significantly higher on the downstream side of the road compared to the upstream side (Table 3.3). Figures 3.4 and 3.5 show data for sulfatase and glucosidase activities, respectively, which are also representative of the trends exhibited in xylosidase and glucosaminidase activities in the bog. There were also significant interactions between distance from the road and culvert position, and the side of the

road and the distance from the road on sulfatase activity in the bog (Table 3.3; Figure 3.4). The sulfatase activity was lowest in samples adjacent to the road on the upstream side and increased away from the road on the transects located near to culverts (Figure 3.4). However, there was no significant interactive effect observed between the side of the road and the distance from the road on glucosidase, xylosidase, glucosaminidase, and xylosidase activities in the bog (Table 3.3).

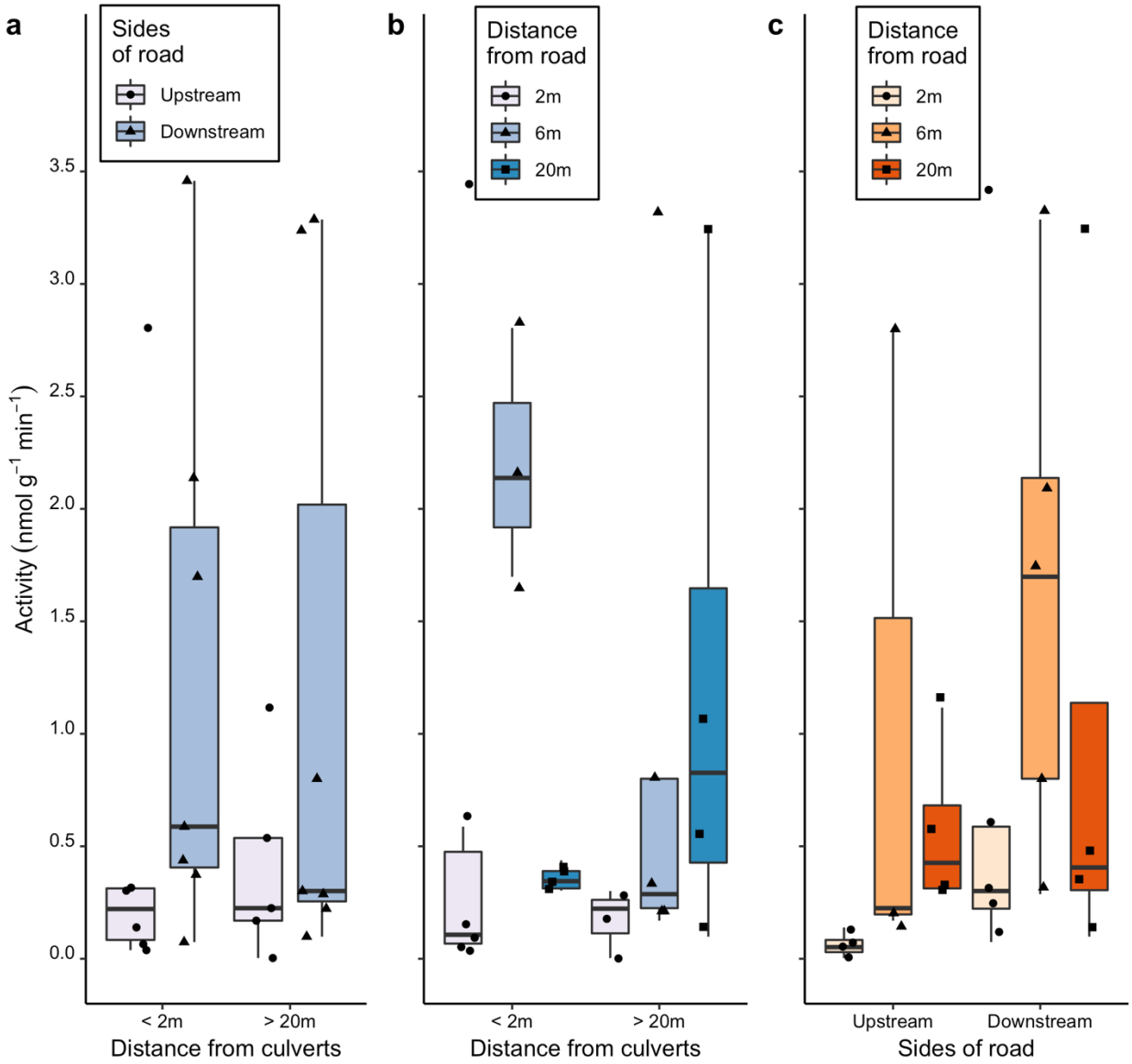

Figure 3.4 Sulfatase activities a) between distance to culvert and sides of the road, b) between distance to culvert and distances from the road, and c) between sides of the road and distances from the road in bog, Carmon Creek, Peace River, Alberta.

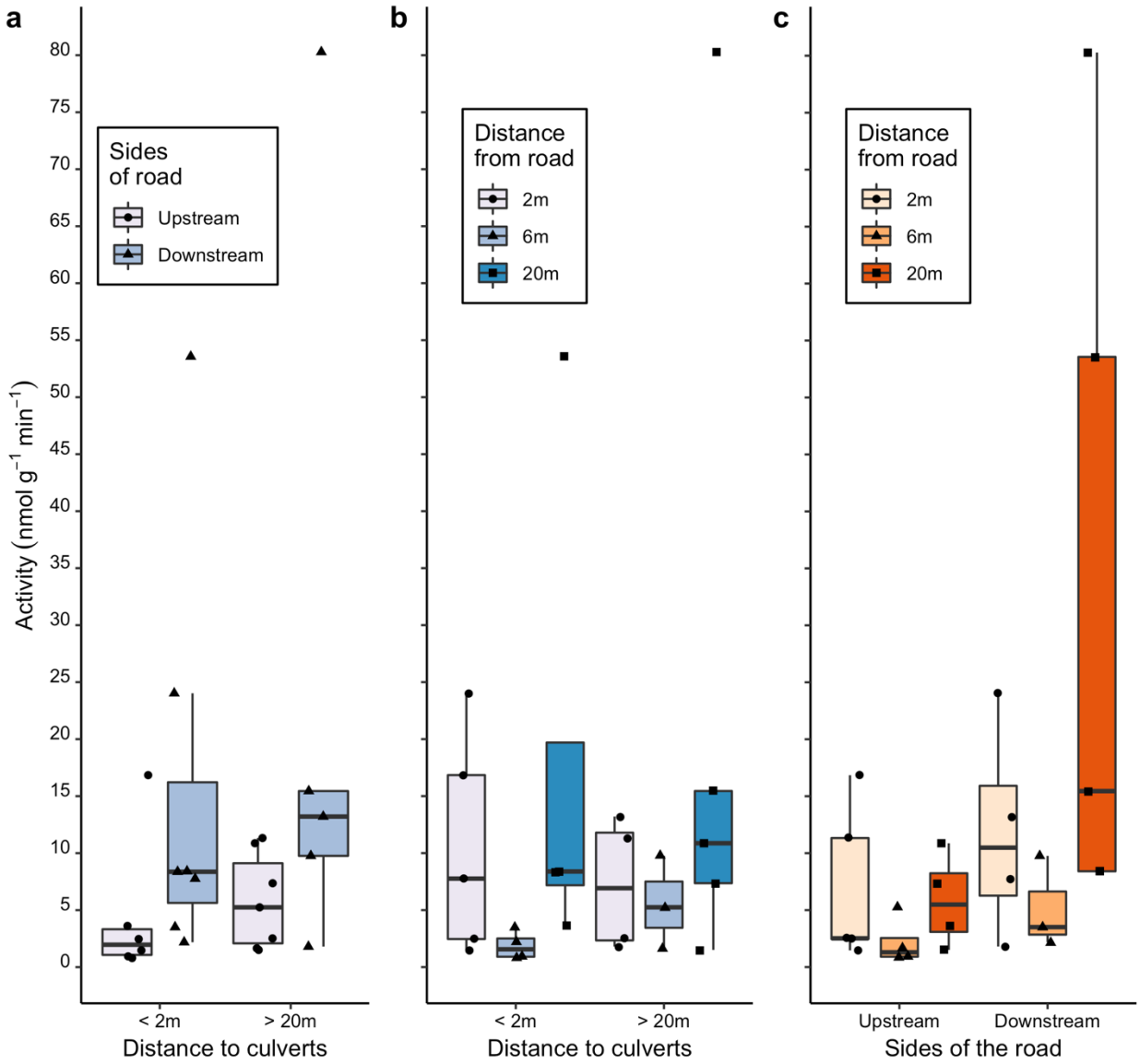

Figure 3.5 Glucosidase activities a) between distance to culvert and sides of the road, b) between distance to culvert and distances from the road, and c) between sides of the road and distances from the road in bog, Carmon Creek, Peace River, Alberta.

We also hypothesized that enzyme activities would be higher in the areas nearest to the road and would decrease further away from the road. This was complicated by the interactions with the side of the road described above. However, we found that in the bog the activities of phenol oxidase, glucosidase, and sulfatase were lower in areas closer to the road compared to the areas further away from the road ($F = 3.59_{(2,15)}$, $p=0.05$; $F = 3.34_{(2,12)}$, $p = 0.05$; $F = 3.85_{(2,14)}$, $p = 0.04$,

respectively; Table 3.3). This was not the case for glucosaminidase, xylosidase or phosphatase activities. In the fen, the activity of xylosidase increased with distance away from the road (Table 3.3).

In the fen a two-way interaction between the side of the road and distance from a culvert, and a three-way interaction between all road associated factors (side of the road, distance from a culvert and distance from the road) were both significant explanatory for xylosidase activity (Table 3.3). Xylosidase activity was significantly higher in the downstream areas adjacent to the road located along transects far from a culvert.

Finally, we expected that enzyme activities would be higher in disturbed areas compared to undisturbed areas. In the bog, we found that the average phosphatase and glucosaminidase activities were significantly higher in disturbed areas compared to undisturbed areas ($t = 2.93$, $p < 0.001$; $t = 4.99$, $p < 0.001$, respectively). The remaining enzyme activities in the bog were not statistically significantly different. In contrast, in the fen, there were no significant differences for any of the enzyme activities between undisturbed and disturbed areas.

Table 3.3 Main and interactive effects of side of the road, culvert distance, and distance from the road on enzyme activities. Significant results i.e. P-values <0.05 are highlighted in bold.

Treatments	**Bog site**		**Fen site**	
	$F_{(df1,df2)}$[a]	**P**	**$F_{(df1,df2)}$**	**P**
Phenol oxidase activities				
Side[b]	**19.90 $_{(1,17)}$**	**<0.001**	0.15 $_{(1,20)}$	0.70
Culvert[c]	0.76 $_{(1,17)}$	0.40	0.49 $_{(1,20)}$	0.49
Dist[d]	**224.11 $_{(2,17)}$**	**<0.00**	0.20 $_{(2,20)}$	0.82
Side*Culvert	**6.70 $_{(1,17)}$**	**0.02**	0.41 $_{(1,20)}$	0.53
Side*Dist	**8.49 $_{(2,17)}$**	**<0.001**	0.06 $_{(2,20)}$	0.94
Culvert*Dist	**3.72 $_{(2,17)}$**	**0.04**	0.18 $_{(2,20)}$	0.83
Side*Culvert*Dist	0.21 $_{(2,17)}$	0.81	0.43 $_{(2,20)}$	0.66
Glucosidase activities				
Side	**7.58 $_{(1,13)}$**	**0.01**	0.01 $_{(1,12)}$	0.96
Culvert	**11.52 $_{(1,13)}$**	**<0.001**	0.07 $_{(1,12)}$	0.80
Dist	**3.57 $_{(2,13)}$**	**0.05**	0.14 $_{(2,12)}$	0.87

Side*Culvert	1.13 $_{(1,13)}$	0.35	2.26 $_{(1,12)}$	0.16
Side*Dist	1.00 $_{(2,13)}$	0.39	0.49 $_{(2,12)}$	0.62
Culvert*Dist	1.13 $_{(2,13)}$	0.35	0.48 $_{(2,12)}$	0.63
Side*Culvert*Dist	0.65 $_{(2,13)}$	0.54	0.81 $_{(2,12)}$	0.47
Xylosidase activities				
Side	**6.18 $_{(1,13)}$**	**0.02**	0.14 $_{(1,19)}$	0.71
Culvert	0.03 $_{(1,13)}$	0.86	0.07$_{(1,19)}$	0.79
Dist	0.32 $_{(2,13)}$	0.73	**3.49 $_{(2,19)}$**	**0.05***
Side*Culvert	0.06 $_{(1,13)}$	0.81	**5.18$_{(1,19)}$**	**0.03***
Side*Dist	0.14 $_{(2,13)}$	0.86	0.30 $_{(2,19)}$	0.74
Culvert*Dist	0.82 $_{(2,13)}$	0.46	0.28 $_{(2,19)}$	0.76
Side*Culvert*Dist	1.38 $_{(2,13)}$	0.28	3.14 $_{(2,19)}$	0.06
Sulfatase activities				
Side	**8.71 $_{(1,13)}$**	**0.01**	1.20 $_{(1,18)}$	0.17
Culvert	2.21 $_{(1,13)}$	0.16	0.40 $_{(1,18)}$	0.53
Dist	**35.37$_{(2,13)}$**	**<0.001**	0.25 $_{(2,18)}$	0.78
Side*Culvert	0.00 $_{(1,13)}$	0.99	0.01 $_{(1,18)}$	0.98
Side*Dist	**28.00 $_{(2,13)}$**	**<0.001**	1.60 $_{(2,18)}$	0.23
Culvert*Dist	**18.39 $_{(2,13)}$**	**<0.001**	0.12 $_{(2,18)}$	0.88
Side*Culvert*Dist	2.15 $_{(2,13)}$	0.16	0.70 $_{(2,18)}$	0.51
Glucosaminidase activities				
Side	**8.46 $_{(1,14)}$**	**0.01**	0.14 $_{(1,18)}$	0.72
Culvert	0.90 $_{(1,14)}$	0.36	0.27 ($_{1,18)}$	0.61
Dist	0.49 $_{(2,14)}$	0.62	0.02 $_{(2,18)}$	0.98
Side*Culvert	1.16 $_{(1,14)}$	0.31	0.03 $_{(1,18)}$	0.86
Side*Dist	0.01 $_{(2,14)}$	0.98	0.78 $_{(2,18)}$	0.47
Culvert*Dist	0.36 $_{(2,14)}$	0.70	0.54 $_{(2,18)}$	0.59
Side*Culvert*Dist	0.25 $_{(2,14)}$	0.61	0.62 $_{(2,18)}$	0.55
Phosphatase activities				
Side	0.19 $_{(1,13)}$	0.67	2.27 $_{(1,18)}$	0.15
Culvert	0.01 $_{(1,13)}$	0.97	0.02 $_{1,18)}$	0.88
Dist	0.33 $_{(2,13)}$	0.72	0.53 $_{(2,18)}$	0.59
Side*Culvert	0.03 $_{(1,13)}$	0.87	1.30 $_{(1,18)}$	0.27
Side*Dist	0.23 $_{(2,13)}$	0.79	0.08 $_{(2,18)}$	0.92
Culvert*Dist	2.47 $_{(2,13)}$	0.12	1.34 $_{(2,18)}$	0.28
Side*Culvert*Dist	0.29 ($_{2,13)}$	0.76	0.40 $_{(2,18)}$	0.67

[a] Degrees of freedom between treatments (df1) and within groups (df2).
[b] Side of the road (Upstream and Downstream);
[c] Distance to culvert (<2m and > 20m);
[d] Perpendicular distance from the road (2m, 6m, and 20m);
Bold numbers represent statistically significant values

Table 3.4 Correlations between enzyme activities and site characteristics in the bog. Only correlations with $p < 0.25$ are listed. Bold numers represent statistically significant values

Variables	Correlation coefficient	P
Phenol oxidase		
Glucosidase	0.40	0.06
Xylosidase	**0.47**	**0.03**
Phenolics	-0.29	0.16
Soil organic matter %	**0.62**	**<0.001**
Water table depth	**-0.57**	**<0.001**
Peat temperature at 5 cm	**-0.44**	**0.02**
Peat pH	**-0.56**	**<0.001**
Average hydrolase activities	0.26	0.18
Glucosidase		
Glucosaminidase	**0.47**	**0.04**
Water table depth	-0.33	0.13
Phenol oxidase	0.40	0.06
Sulfatase		
Glucosaminidase	0.44	0.08
Peat pH	-0.24	0.25
Xylosidase		
Phenol oxidase	**0.47**	**0.03**
Glucosaminidase	**0.51**	**0.03**
Phenolics	**-0.52**	**0.01**
Soil organic matter %	0.27	0.19
Water table depth	**-0.59**	**<0.001**
Peat pH	-0.38	0.06
Glucosaminidase		
Glucosidase	**0.47**	**0.04**
Xylosidase	**0.51**	**0.03**
Sulfatase	0.44	0.08
Phenolics	**-0.43**	**0.04**
Water table depth	**-0.45**	**0.03**
Phosphatase		
Water table depth	0.33	0.12

Overall, many of the observed patterns of enzyme activity mirrored shifts in hydrological and chemical conditions. In fact, we found that phenol oxidase was significantly negatively correlated with WT depth in both the bog (Table 3.4) and the fen ($r = -0.4$, $p = 0.03$), and with pH and phenolics in the bog (Table 3.4) i.e. a shallow WT resulted in lower phenol oxidase activity. Glucosidase, xylosidase and glucosaminidase activities were also negatively correlated with WT depth, pH and phenolic concentrations in the bog (Table 3.4).

3.4 Discussion

3.4.1 Phenol oxidase variations

In undisturbed peatlands, oxygen penetration into peat is limited as the rate of O_2 consumption by aerobic respiration and chemical oxidation exceeds the diffusive flux of O_2 from the atmosphere under saturated conditions (Freeman et al., 2001). Previous studies have shown that phenol oxidase activities fluctuate with peat saturation or WT depth by bringing changes in peat aeration (Bonnett et al., 2017; Fenner et al., 2005; Freeman et al., 2004; Toberman et al., 2008). Our results support this finding as a shallow WT resulted in decreased phenol oxidase activities in both the bog and the fen. The deeper WT in the bog compared to the fen could have contributed to the observed higher phenol oxidase activities in the bog. However, fluctuations in WT depth can also enhance oxygen diffusion into the deeper layers of peat that, in turn, can increase microbial activity and enhance organic matter degradation (Rezanezhad, Couture, Kovac, O'Connell, & Van Cappellen, 2014). More active microbial communities with a high degree of diversity can stimulate the production of phenol oxidase (Freeman et al., 2001) and catabolize phenolics, reducing their concentration (Fenner et al., 2005).

Although a thorough analysis of organic matter quality was beyond the scope of the current study, compared to non-forested peatlands, it is likely that the forested bog site contains higher concentrations of lignin, as the available sources of lignin are diverse (i.e. trees, shrubs, and mosses). Accordingly, it is possible that a larger community of phenol oxidase producing lignin-decomposers is present in the bog, as has been observed in other forested ecosystems (DeAngelis et al., 2011). However, phenol oxidase activity in boreal bogs, independent of the tree cover, is typically constrained by anoxic, acidic conditions, as well as low nutrient availability due to the recalcitrant litter from both the trees (i.e. black spruce) and ground vegetation (mosses).

Consequently, in undisturbed forested bogs, we may observe lower abundance and activity of phenol-oxidase producing lignin decomposers compared to other forested systems, though I did not quantify this in our study.

In a forested bog, we observed higher phenol oxidase activity on the downstream side of the road, far from culverts compared to the upstream side of the road, far from culverts and the upstream side connected by culverts. Therefore, culvert position and side of the road had an interactive influence on phenol oxidase activity in the bog. In addition, there was a significant interaction between the side of the road and the distance from the road on phenol oxidase activity in the bog (Table 3.3). Water ponding on the upstream side of the road at the bog likely decreased oxygen availability and resulted in lower phenol oxidase activities, which ultimately helped to increase the concentration of phenolics in the upstream areas (Table 3.2). In contrast, on the downstream side, particularly in areas not directly connected by culverts, the lower WT position relative to the peat surface would have enhanced the diffusion of oxygen into the deeper layers of peat. This may have helped to trigger phenol oxidase activity, resulting in greater decomposition of organic matter, as reported elsewhere (Fenner & Freeman, 2011; Freeman et al., 2004, 2001). This mechanism for the increased decomposition of organic matter is supported by the negative correlations between both WT position and the concentration of phenolics with phenol oxidase activity in the bog (Table 3.4), a finding consistent with the enzymatic latch hypothesis. Similarly, Bonnett et al. (2017) found enhanced phenol oxidase activity under drought conditions. However, conversely Sun et al. (2010) and Fenner et al. (2005) found enhanced phenol oxidase activity under flooded conditions compared to dry conditions. Although seemingly contradictory, the enhanced phenol oxidase activity in those studies was likely due to short-term flooding following a long-term drought. This change in conditions likely caused priming effects that increased the enzyme activities (Williams

et al., 2000). Since the road blocks water flow in the studied bog, altered WT positions are persistent in the present study, leading to the expected increase in phenol oxidase activity at drier locations.

In contrast to studies that incorporated a wider range of soil types and pH (pH 4 to 10; e.g. Sinsabaugh, 2010 and Sinsabaugh et al., 2008), we observed a negative correlation between phenol oxidase activities and peat pH. It is uncertain whether the minimal pH variations observed in the peat samples in our study (pH ranged between 4.5 - 6.0 and 7.0 - 7.5 in the bog and fen, respectively) contributed to this contrasting result. However, the correlation between phenol oxidase and peat pH can be impacted by many factors including the analytical methods employed (Wiedermann, Kane, Veverica, & Lilleskov, 2017). The phenol oxidase activities determined using L-DOPA represent the oxidative potential of the soil solution, which will vary with pH, while the redox potential of L-DOPA itself declines with increasing pH (Bach et al., 2013). Despite these shortcomings, L-DOPA has been shown to be an appropriate substrate across a broad pH range (Bach et al., 2013). Moreover, the positive correlation of peat pH with WT depth ($r = 0.55$, $p < 0.001$) indicates that collinearity of pH and WT depth likely drives this pattern at our study sites. For instance, in the upstream areas, where pH was higher than in the downstream areas, phenol oxidase activities were suppressed by the higher WT position. The reverse was true in the downstream areas. This is to be expected as biogeochemical oxidation processes, which are more dominant with a deep WT (e.g. ammonia oxidation, equation 1), tend to decrease pH and biogeochemical reduction processes (e.g. sulfate reduction, equation 2), which are more dominant with a high WT, tend to increase pH. This suggests that WT depth could be the main driver of the pH correlation although the dominant oxidation and reduction reactions present at the sites were

not evaluated during this study. Equations 1 and 2 are provided purely as a demonstration of typical oxidation and reduction reactions and their influence on pH.

Equation 1: $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$

Equation 2: $SO_4^{2-} + 2CH_2O + 2H^+ \rightarrow H_2S + 2CO_2 + 2H_2O$

In the fen, there were no significant effects of culvert distance, side of the road or distance from the road on phenol oxidase activity. Although phenol oxidase activities were significantly negatively correlated with the WT depth in the fen, the WT depth varied more according to natural microtopographic variation across the peatland than in relation to the presence of the road. The decreased hydrologic impact of the road at the fen may have resulted from the fact that the dominant flow direction of water is not perpendicular to the road at this site (demonstrated by elevation surveys across the site). As such, there was no significant difference in the average elevation between the upstream and downstream side of the road, a phenomenon also observed by Willier (2017).

3.4.2 Hydrolase activities

At our study sites, the observed variability in hydrolase enzyme activities was associated with variation in either WT depth, phenolics, phenol oxidase activities or a combination of these parameters (Tables 3.1 to 3.4). Though the leaching of minerals from the glacial till filling below the road could have contributed to lower SOM content in areas closer to the road (Partington et al., 2016), the observed hydrolase enzyme activity variations were not likely associated with limitation of substrate or moisture as both SOM (85 ± 10%) and moisture content (80 ± 10%) were high (Allison & Treseder, 2008; Sinsabaugh et al., 2008), irrespective of WT depth. It should be noted, however, that high SOM concentration does not eliminate the possibility that hydrolase

activities may have been limited by the quality of the available SOM, as hydrolase enzymes have been shown to follow resource allocation models (e.g. Pinsonneault et al., 2016). The thorough examination of nutrient chemistry necessary to evaluate this possibility was beyond the scope of this study.

Fenner and Freeman (2011) have previously shown that a deeper WT in peatlands can provide favorable conditions for microbial community abundance and production of phenol oxidase enzymes due to greater oxygen availability and hence more energetically efficient heterotrophic metabolism. The produced phenol oxidase has the capacity to oxidize phenolics which, in turn, promotes increased hydrolytic enzyme activity, resulting in increased nutrient (N, P, S) availability and carbon turnover (Freeman et al., 2012, 2001). Phenolics can decrease glucosidase and xylosidase activities (Kang & Freeman, 1999), and enhanced phenol oxidase activity decreases the phenolic concentration, paving the way for higher hydrolase activities. Similarly, the negative correlation of xylosidase and glucosaminidase with WT depth (Table 3.4) supports that the observed increase in hydrolase activities in the downstream areas in the bog is driven by drying and increased oxygen diffusion. Therefore, the higher glucosidase, xylosidase and glucosaminidase activities downstream of the road in the bog could have been stimulated by enhanced nutrient mineralization due to the deeper WT conditions (Freeman et al., 1996). This likely triggered further bacterial growth, enhancing the decomposition of organic matter and release of greenhouse gases in a biochemical cascade (Fenner & Freeman, 2011). Sun et al. (2010) recorded higher glucosidase activities with a shallower WT because of the temporal dynamics i.e. long-term wet and dry cycles. This suggests that more study on the effect of peatland road crossings on WT fluctuation is needed to fully understand their potential impact on enzyme activities over time. Also, higher sulfatase activities were observed in areas of peatland that were

less waterlogged (Figure 3.4). This finding is in line with previous studies performed by Freeman et al., (1996), Kang and Freeman, (1999) and Pulford and Tabatabai (1988) .

Several studies have previously shown that phosphatase activities decrease under flooded (shallower WT depth) conditions (Kang and Freeman 1999; Ling et al, 2009; Karl et al, 2015). Further, in controlled experiments, Parsons et al. (2017) reported higher phosphatase activities under oxic conditions compared to anoxic conditions, linked to phosphate limitation (increased activities) or abundance (decreased activities), irrespective of water saturation. Parsons et al. (2017) suggest that redox conditions rather than saturation state can control phosphatase activity due to changes to iron hydr(oxide) solubility that in turn influence phosphate bioavailability. However, in the current study we observed no effect of culvert distance, side of the road or distance from the road on phosphatase activities, nor any correlations with WT depth, phenol oxidase activity, phenolic concentration, or pH. This may suggest that WT depth and associated changes to redox conditions did not influence phosphate abundance or limitation in either the bog or the fen. Peatlands are often low in P (Plach et al., 2017) and the insensitivity of phosphatase activities to WT variation may therefore be attributable to sustained P limitation in both study sites, regardless of WT level.

As with phenol oxidase activities in the fen, aside from xylosidase, none of the studied hydrolase enzyme activities were significantly different based on the distance from the road, the side of the road or distance to a culvert. This again could be explained by the non-significant variation of WT depth, phenolic concentration or pH in relation to the road at the fen site.

This study suggests that the differences in the observed impacts of the road between the bog and the fen sites are in response to the road orientation and flow direction at each study site. This is not necessarily a reflection of differing impacts between bog and fen peatlands generally. Bog and

fen are two different types of peatlands exhibiting different vegetation composition, biochemistry, and ecohydrology. Additionally, in this case the two sites also vary with respect to the orientation of the road relative to flow direction. A more extensive study is needed to tease apart all these interacting factors. The present study provides a good baseline demonstrating the impact that resource roads can have on enzyme activities in peatlands. In general, the increased enzyme activities near the bog access road indicates the likelihood of higher decomposition of organic matter in road disturbed areas, potentially enhancing greenhouse gas emissions from peatlands fragmented by roads. Some of the effects of the road observed in the bog could also be due to the short time since road construction, and more research is needed to see how these differences persist over time. Moreover, as observed by Willier (2017) and suggested by Partington et al. (2016), the road associated impacts on peatlands vary based on the peatland type, road orientation and direction, culvert position, and road construction material – together creating hydrological and biogeochemical spatial variation. This variability in hydrological response ultimately impacts enzyme activities and decomposition rates in peatlands resulting in the overall effect of road crossings on peatland carbon stocks.

3.5 Chapter conclusions

We found that an access road had significant impacts on enzyme activities in a forested bog, where the road was perpendicular to water flow, but not in a fen, where water flow was largely parallel to the road. We observed significantly higher phenol oxidase and hydrolase activities in the road disturbed areas compared to undisturbed areas of the peatland. We demonstrate a series of complex and significant interactive effects between factors representative of road impact (distance to a culvert, the side of the road and the distance from the road) and both phenol oxidase and hydrolase enzyme activities. Together the results show an interlinked pattern of variations in enzyme

activities in response to resource roads that bisect peatlands. The phenol oxidase, glucosidase, sulfatase, xylosidase, and glucosaminidase activities were significantly higher in the areas with a deeper WT position (downstream, particularly those close to the road and far from culverts). Lower activities of phenol oxidase, glucosidase and sulfatase were measured in areas with the shallowest WT position (upstream areas close to the road and far from culverts). Similarly, significant variations in terms of culvert presence, distance from the road or the side of the road could be linked with variations in WT depth, phenolic concentration, and pH and their significant correlation with phenol oxidase and hydrolase enzyme activities.

We observed that the lowered WT on the downstream side of access roads, due to blockage of water flow by the road, has the potential to enhance extracellular enzyme activities, likely altering carbon sequestration rates in peatlands. Therefore, it is pivotal for industries and land managers to develop road construction techniques that aim at limiting alteration to WT position. Limiting the alteration of WT position would in turn limit changes to redox conditions, the concentration of phenolic material and enzyme activities. Similarly, there is a need for detailed work on peatland carbon fluxes and peat accumulation in road-affected peatlands to quantify shifts in carbon dynamics.

Chapter 4: Road crossings increase methane emissions from adjacent peatland

4.1 Introduction

Methane (CH_4) is a powerful greenhouse gas (GHG) as it has ~ 28 times higher global warming potential than CO_2 over a 100 years period, and accounts for 20% of global radiative forcing (IPCC, 2013). Of the total annual global CH_4 emission (500 - 600 Tg CH_4 y^{-1}; Bruhwiler, Dlugokencky, & Masarie, 2015; Kirschke et al., 2013), approximately 40% is from natural sources, specifically from wetlands, while grazing, biomass burning, paddy fields, and fossil fuels together account for the other 60% (Denman et al., 2007). Among wetlands, northern peatlands are significant natural CH_4 emitters (Strack et al., 2004 ; Lai, 2009) and contribute nearly 7% to total global annual CH_4 emissions (Wuebbles & Hayhoe, 2002; Zhuang et al., 2006).

In peatlands, CH_4 production occurs under water saturated, highly reduced conditions, where redox potential is below -200 mV, by methanogenic archaea (Bellisario et al., 1999) as a result of anaerobic decomposition of organic matter (Bellisario et al., 1998; Mitsch & Gosselink, 2015). Once produced, CH_4 either diffuses into and remains in peat pores or enters into the atmosphere in the gaseous form by diffusion through the peat, ebullition (bubble release) and/or plant-mediated transport through the aerenchymatous tissues of emergent vascular plants (Bridgham et al., 2013; Chanton et al., 1992; Conrad, 1989; Joabsson et al., 1999; Mitsch & Gosselink, 2015). Methane flux from a peatland depends on environmental factors such as water table position (WT) that determines size of oxic and anoxic zones in the peat profile, peat temperature that controls microbial CH_4 production and oxidation, presence or absence of plant species with aerenchyma, and vegetation cover, soil moisture content, pH, availability of substrate, and atmospheric nitrogen and sulfur deposition (Bergman, 2000; Chanton et al., 1995; Huttunen et al., 2003; Moore et al.,

2011; Turetsky et al., 2008). Therefore, CH_4 emissions from pristine peatlands vary between and within sites in response to variations in local hydrological, biological, and geochemical properties (Juszczak et al., 2013; Mahmood & Strack, 2011; Munir & Strack, 2014; Potter et al., 2001; Strack et al., 2018). For instance, the presence of more labile carbon substrates generally results in higher CH_4 emissions from moderate to rich fens compared to bogs that have less labile forms of organic matter derived from *Sphagnum* spp. and woody plants (Chanton et al., 2008).

Peatland CH_4 emission rate could also be impacted by various anthropogenic disturbances (Strack et al., 2004, 2016; Strack et al., 2017). While previous studies have investigated CH_4 flux from peatlands affected by peat extraction, forestry, agriculture and hydroelectric reservoirs (Elmes & Price, 2019; Strack et al., 2006; Waddington & Warner, 2001), linear disturbances, such as roads, trails, and geological exploration lines, also widely contribute to the modification of northern peatlands (Pasher et al., 2013). The boreal region of Canada has one of the highest densities of peatland coverage and is fragmented by a more than 217,000 km long road network constructed for transportation and to explore and extract natural resources e.g., petroleum and forest products (Pasher et al., 2013). These constructed access roads are raised above the peat surface by the placement of mineral fill and/or geotextile material after clearing the vegetation. This compresses the peat underneath the road, acting as a dam and limiting the hydrological connection between fragmented parts of the peatland (Partington & Clayton, 2012; Saraswati et al., 2019). The resulting hydrological perturbation in the peatland surrounding the access road crossing may lead to altered plant community, peat temperature, substrate availability, microbial activity, and soil biogeochemistry (Bocking et., 2017; Campbell & Bergeron, 2012; Plach et al., 2017; Saraswati et al., 2019; Strack et al., 2018) that could ultimately impact CH_4 emissions from peatlands. However, culvert installation could help to connect the fragmented peatland areas. To our

knowledge, the impact of resource access roads on CH_4 fluxes from peatlands has not been quantified. Therefore, to fill this gap, we conducted a multi-year study investigating the impact of access roads on CH_4 emissions in a forested bog and a shrubby rich fen. The objectives of this study were to determine the extent, magnitude, and direction of the impact of the access roads on two boreal forested peatland CH_4 emissions. Given the observed greater hydrological differences at the bog site than the fen, we expect minimal differences between both sides of the road in the fen. Therefore, at least at the bog,:

1) We expected higher CH_4 flux from areas upstream of the road compared to downstream and undisturbed areas, as the upstream areas would have shallower WT due to blockage of water flow across the road.
2) Furthermore, we anticipated that CH_4 flux would vary with distance from the road, with higher and lower CH_4 emissions from areas close to the road on upstream and downstream sides, respectively, compared to areas further away from the road.
3) However, when culverts are placed underneath the road to connect water flow between fragmented parts of the peatland, we expected no significant difference in CH_4 fluxes between upstream and downstream areas.

4.2 Methods

4.2.1 Site description

Both study sites, a forested bog (56°21’44” N and 116°47’45” W) and a shrubby rich fen (56°22’09” N and 116°46’12” W) were located at Carmon Creek, Peace River, Alberta, Canada. The study peatlands had resource access roads crossing them, and the roads were constructed by

placing geotextile material over the compressed peat in 2013 at fen and 2014 at bog. Detailed information about the road construction is available in Saraswati et al. (2019).

The dominant vegetation types in the bog site included *Picea mariana* (black spruce), *Rhododendron groenlandicum* (bog Labrador tea), *Vaccinium oxycoccos* (bog cranberry), *Rubus chamaemorus* (cloudberry), *Sphagnum* species (largely *Sphagnum fuscum*) and lichens (e.g., *Cladina stellaris, Cladina rangiferina,* and *Cladina mitis*). In the fen, the dominant vegetation included tall shrubs e.g., *Salix* spp. (willow) and *Alnus incana* (grey alder), *Betula papyrifera* (paper birch), sedges (e.g., *Carex utriculata* (beaked sedge), *Carex aquatilis* (water sedge), *Carex canescens* (silvery sedge), and grasses/reeds (e.g., *Calamagrostis canadensis*). The average WT position of the snow free period (May to August), where negative values indicate depth below the surface, recorded at the bog and fen were -11.3 and -2.5 cm in 2016, and -19.2 and -17.9 cm in 2017. The recorded average pH of the bog and fen were 5.4 and 7.5, respectively.

We established six perpendicular transects of 20 m long on both sides of the road. Triplicate transects were aligned either < 2 m from a culvert (referred as low hydrological impact areas, LI) or > 20 m from a culvert (referred as high hydrological impact areas, HI; Figure 4.1). In each transect, we installed six aluminum collars (60 × 60 cm) inserted ~10 cm into the peat, with collars located at 2, 6 and 20 m from the road on both upstream and downstream sides. We also installed three additional collars at least 50 m (referred as reference areas) from the road in both bog and fen. Adjacent to each collar, we installed polyvinyl tubing (wells) of internal diameter 3 cm and a length of 1 m to measure the WT with reference to the surface.

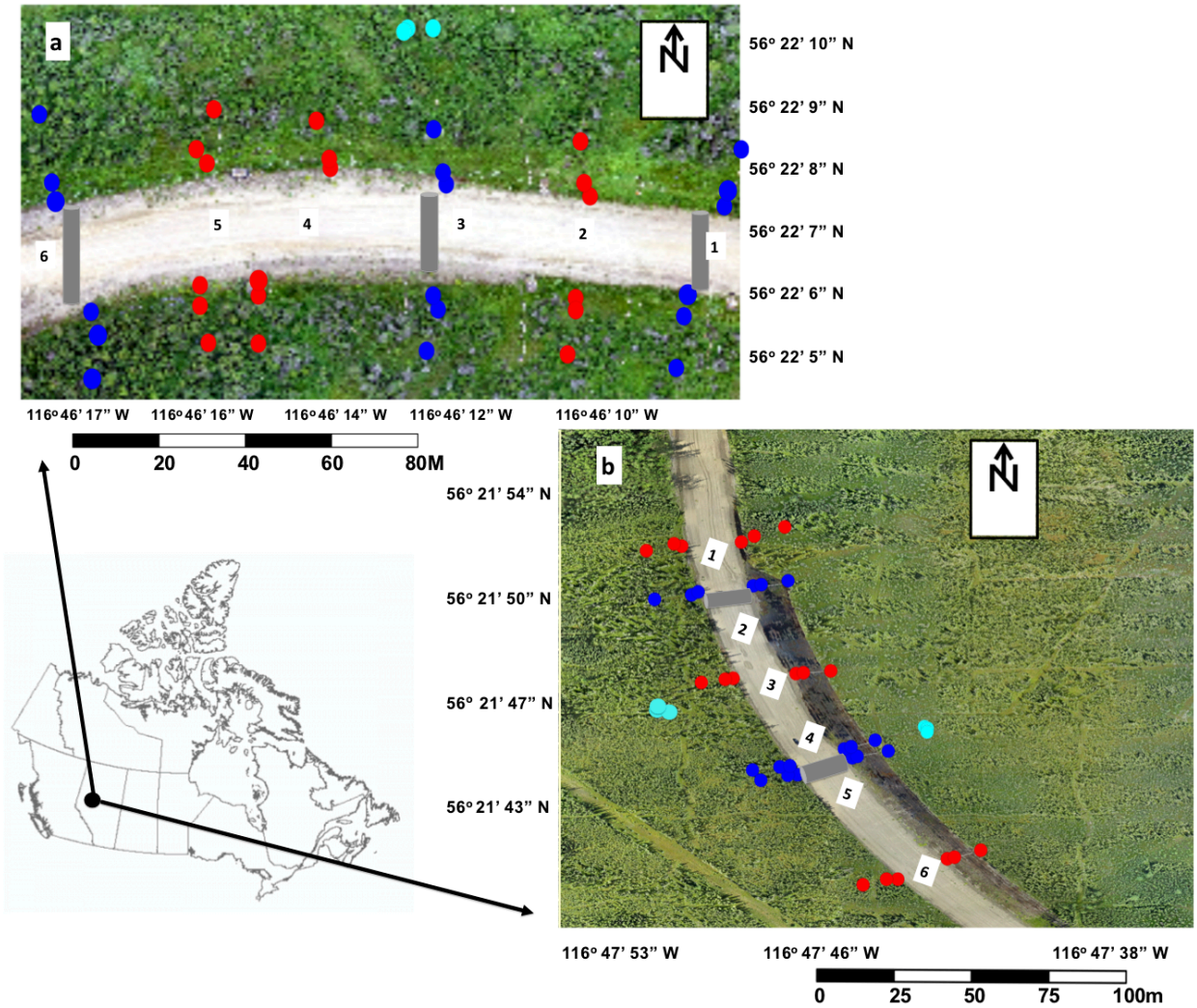

Figure 4.1. Study sites a) a fen and b) a bog, where red dots represent sample plots located along transects > 20 m away from culverts (gray pipes), blue dots represent plots located along transects < 2 m away from culverts, and teal dots represent plots located at reference sites at Carmon Creek, Peace River, Alberta.

4.2.2 Plant cover

Understory vegetation cover survey inside each collar was performed in early August 2017 by visually estimating the plant coverage (both vascular and bryophytes) of each species to the nearest of 5% with the percentage of bare ground also recorded in each collar (Moore et al., 2002). Considering plant communities, in the bog, *Rubus chamaemorus* (34%), *Vaccinium oxycoccos* (28%), and *Rhododendron groenlandicum* (28%) were dominant vascular plants and *Sphagnum*

spp. (86%) was the dominant bryophyte type. Due to the vegetation clearance during road construction, new vegetation (*Eriophorum vaginatum*, *Typha* spp.) started to colonize on the majority of upstream areas of the bog (up to 18 m from the road) where ponding was less severe, but the vegetation cover was <5% in both years. In the fen, the dominant species were *Carex* spp. (45%) and *Calamagrostis canadensis* (35%), and the cover of each species was similar on both sides of the road. However, on the north (downstream) side of the fen, perennial shrubs including *Alnus incan,* and *Betula papyrifera* were almost absent up to 18 m from the road due to clearance during road construction.

4.2.3 CH_4 fluxes

We placed acrylic opaque closed chambers (60 × 60 × 30 cm) on the collars to collect instantaneous measurements of CH_4 fluxes. Each chamber was equipped with a battery-operated fan to mix the air inside chamber. While measuring flux, water was poured on the edges of the collars to seal the chambers. From sealed chambers, air samples were collected at 7, 15, 25 and 35 minutes after closure using 20 mL syringes, and the collected samples were stored in pre-evacuated 12 mL vials (Exetainer, Labco Ltd., UK) as followed by Strack et al (2014). In addition, four ambient air samples were collected on the day of sampling and used for the starting time (i.e., 0 minute) for flux calculations (see below). CH_4 sampling in both study sites was performed bi-weekly from each collar from May to September 2016 and May to August 2017. We collected a total of 23 measurements of flux from each plot throughout the two-year sampling period.

At the time of sampling, chamber air temperatures were recorded using a thermocouple thermometer (Omega Engineering, USA). Peat temperatures at the depth of 2, 5, 10, 15, 20, 25, and 30 cm below the surface were also recorded using thermocouple temperature probes and WT was measured from the well beside each collar.

4.2.4 Laboratory analyses

The collected CH_4 samples were analyzed at the University of Waterloo using a Shimadzu GC-2014 gas chromatograph with a flame ionization detector. The CH_4 flux was estimated from the linear change in concentration over time. Flux measurements, other than near zero fluxes, without a linear relationship ($R^2 < 0.75$) were removed from the dataset as those measurements could be either due to the ebullition events (Christen et al., 2016) or due to the chamber leakage/movements while sampling. As a result, ~ 15% of CH_4 fluxes measurements were lost. Flux with no change in CH_4 concentration (i.e., the change in concentrations within the precision of repeated measurement of standards of 0.5 ppm) were assigned as a zero flux. The accepted flux measurements were corrected for chamber volume and chamber air temperature.

4.2.5 Statistical analyses

Linear mixed-effects models were performed to investigate the variation in CH_4 fluxes (significance level $\alpha = 0.05$) along the road associated factors (i.e., side of the road, culvert distance, and distance from the road). In all models, the plot name was taken as a random effect in order to account for repeated measurements. For the significant factors and interactions, post hoc analyses (Tukey's t-tests) were performed using 'emmeans' package of R (Lenth, 2018). The models were run on log transformed CH_4 fluxes as the CH_4 data was not normally distributed, and the models were separately performed for 2016 and 2017 because of the observed interannual variation in CH_4 fluxes, WT, and peat temperature (T5). Linear mixed-effects models, with years and plots as random effects, were also used to investigate the impact of environmental factors (such as WT, T5, and vegetation cover) on CH_4 fluxes (Korrensalo et al., 2018). Vegetation cover was categorized as moss dominated (vascular <19%), mixed moss and vascular (vascular >19%),

and no vegetation (<5% moss or vascular plants) at the bog and Carex (>50% *Carex* spp.) and grass (>50% *Calamagrostis canadensis*) at the fen.

4.2.6 Annual CH_4 flux estimation

The total growing season (May – August) CH_4 fluxes were estimated by interpolating bi-weekly field measured CH_4 fluxes into a daily flux for each plot following Green and Baird (2017). After that, the annual CH_4 emissions were estimated by adding 15% of the growing season totals to the interpolated value (Saarnio et al., 2007; Strack et al., 2018) since we did not measure CH_4 fluxes representing early spring, fall or winter seasons. Also, to calculate total road related CH_4 flux rates to areas up to 20 m around the road (both upstream and downstream) for each km of the road, in 2016 and 2017 respectively, we calculated the weighted arithmetic mean of interpolated daily CH_4 fluxes at 2, 6, and 20 m plots (equation 1). Road induced emissions were calculated by subtracting CH_4 flux from reference areas from the calculated road adjacent CH_4 flux.

$$Road\ adjacent\ CH_4\ flux_{(x)} = \frac{2CH_{4(x)}*2\ m^2 + 6CH_{4(x)}*4\ m^2 + 20CH_{4(x)}*14\ m^2}{20\ m^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where,

(x) = side of the road (upstream or downstream)

$2CH_4$ = CH_4 fluxes from 2 m areas (g CH_4 m^{-2}),

$6CH_4$ = CH_4 fluxes from 6 m areas (g CH_4 m^{-2}),

$20CH_4$ = CH_4 fluxes from 20 m areas (g CH_4 m^{-2}).

4.3 Results

4.3.1 Environmental conditions

The 30-year normal (1986-2015) growing season weather data recorded at the Peace River Airport Station (~ 40 km from study sites) had daily average temperature and total rainfall of 14.1 ºC and 213.5 mm, respectively (Environment and Climate Change Canada, 2018). Compared to the 30-year normal, the measured growing season rainfall in 2016 (444.2 mm) and 2017 (137.0 mm) indicated that the 2016 growing season was wetter than normal and also a much wetter year compared to the 2017 growing season, which was drier than normal. Based on average daily air temperature at the study sites, 2017 (14.8 °C) was warmer compared to 2016 (13.5 °C). In 2016, WT at bog (-11.29 cm) and fen (-2.51 cm) were significantly shallower compared to 2017 WT positions at bog (-19.16 cm, $z = 7.58$, $p < 0.001$) and fen (-17.91 cm, $z = 13.14$, $p < 0.001$), respectively. The surface elevation between upstream (east, average elevation 622.5 m.a.s.l.) and downstream (west, 621.5 m.a.s.l.) areas at the bog had a moderate decrease in surface elevation with the slope (~1:250) perpendicular to the road. However, at the fen, the surface elevation between upstream (south, 625.4 m.a.s.l.) and downstream (north, 625.1 m.a.s.l.) areas was minimal and the slope was nearly parallel to the road (Saraswati et al., 2019). Consequently, we observed extensive flooding conditions on the upstream areas of the road at the bog, particularly when culverts were > 20 m away from plots. However, in the transects < 2 m from the culverts, the culverts reduced the hydrological impacts of the road by connecting the fragmented parts. Hydrologic impact of the road at the fen was minimal (Saraswati et al., 2019).

The average T5 in 2016 and 2017 in the bog was 14.0 and 15.1 °C, respectively, and in the fen, 10.8 and 12.0 °C, respectively. The linear mixed-effects models, with years and plots as random effects, showed that the T5 varied significantly with culvert position ($F_{1, 24} = 10.69$, $p = 0.003$), but not by side of the road ($F_{1, 24} = 0.46$, $p = 0.51$) and distance from the road ($F_{2, 24} = 0.77$, $p = 0.47$)

at the bog. Post hoc comparison showed that the HI upstream areas of the bog had a significantly higher average T5 (14.4 ± 0.5 °C) compared to the LI downstream areas (9.7 ± 0.4 °C, $t = 2.80$, $p = 0.04$). At the fen, the model showed significant interactive effects of side of the road and culvert position on the T5 variation ($F_{1,48} = 11.33$, $p = 0.001$) with the LI downstream areas having significantly lower T5 (5.1 ± 1.0 °C) compared to LI upstream ($t = -4.31$, $p = 0.001$), HI upstream ($t = -4.43$, $p = 0.001$), and HI downstream areas ($t = 5.20$, $p = 0.001$).

4.3.2 CH_4 flux and controls

The road disturbed areas (plots 2 – 20 m from the road) in the bog were greater sources of CH_4 compared to reference areas in both 2016 ($Z = 3.36$, $p = 0.002$) and 2017 ($Z = 4.41$, $p = 0.001$). In contrast, the fen did not have a significant difference between disturbed and reference areas in either 2016 ($Z = 0.17$, $p = 0.99$) or 2017 ($Z = -1.43$, $p = 0.28$). Average CH_4 flux from reference areas was 6.8 ± 2.5 and 4.6 ± 2.4 mg CH_4 m^{-2} d^{-1} at the bog and fen, respectively.

Overall, within the road disturbed areas, the average CH_4 flux from the bog plots ranged from -0.8 to 470.6 mg CH_4 m^{-2} d^{-1} across the sampling plots in 2016, and from 1.2 to 708.2 mg CH_4 m^{-2} d^{-1} in 2017. At the fen plots, average CH_4 fluxes ranged from 0.8 to 20.7 mg CH_4 m^{-2} d^{-1} in 2016 and from 0.9 to 131.1 mg CH_4 m^{-2} d^{-1} in 2017. The bog was a significantly greater source of CH_4 compared to the fen in both 2016 and 2017 ($p < 0.01$, $Z = -6.77$). The year wise-comparison within the bog site showed a significantly higher CH_4 flux from the bog in 2017 (171.7 mg CH_4 m^{-2} d^{-1}) compared to 2016 (56.4 mg CH_4 m^{-2} d^{-1}, $p < 0.001$, $z = -4.55$). Similarly, the fen site was a significantly greater source of CH_4 in 2017 (14.6 mg CH_4 m^{-2} d^{-1}) compared to 2016 (7.3 mg CH_4 m^{-2} d^{-1}, $p < 0.001$, $z = -2.77$).

In 2016, at the bog, we found significant interactive effects of the side of the road and culvert position, side of the road and the distance from the road, and culvert position and distance from the road on CH_4 flux variations (Table 4.1, Figure 4.2a). Post hoc analyses showed that the plots located at HI upstream areas emitted (124.6 ± 36.2 mg CH_4 m^{-2} d^{-1}) ~16 times more CH_4 than plots located at HI downstream areas ($p < 0.001$, $t = 4.38$). While in general, CH_4 flux was higher in the upstream areas of the road compared to the downstream areas, the difference was greater at HI transects, than LI transects supporting our hypotheses one and three. Moreover, CH_4 flux was highest close to the road, at 2 m, and then declined to 20 m on the upstream side, but CH_4 fluxes were more similar at all distances from the road on the downstream side (Figures 4.2 and 4.3) that supported hypothesis two.

In 2017, we found significant main and interactive effects of side of the road and culvert position, and main effect of the distance from the road on CH_4 fluxes in the bog (Table 4.1, Figure 4.2b). Post hoc analyses showed that the plots located at HI upstream areas emitted (391.2 ± 164.0 mg CH_4 m^{-2} d^{-1}) ~ 49 times more CH_4 than plots located at HI downstream areas ($p = 0.002$, $t = 4.003$), but were not significantly different compared to LI downstream and LI upstream areas ($p = 0.97$, $t = 0.45$; $p = 0.98$, $t = 0.36$, respectively). Also, plots located at 2 m from the road emitted (296.4 ± 95.7mg CH_4 m^{-2} d^{-1}) ~ 6 times, and plots located at 6 m areas emitted (159.8 ± 39.3 mg CH_4 m^{-2} d^{-1}) ~ 3 times more CH_4 than plots located at 20 m ($p = 0.02$, $t = 2.84$; $p = 0.08$, $t = 2.22$, respectively). Results from 2017 CH_4 fluxes in the bog also supported our hypotheses one, two and three.

In contrast, we found no significant road related main and interactive effects for CH_4 fluxes at the fen in either 2016 or 2017 (Table 4.1, Figures 4.3a, 4.3b).

Table 4.1 Main and interactive impacts of side of the road, culvert distance, and distance from the road on methane flux in 2016 and 2017 at bog and fen, Carmon Creek, Peace River, Alberta.

Treatments	**Bog**		**Fen**	
	F values	**P-values**	**F values**	**P- values**
		2016		
Intercept	**848.03** $_{(1, 302)}$	**< 0.01**	**391.07** $_{(1, 213)}$	**<0.01**
Side[a]	**33.60** $_{(1, 24)}$	**< 0.01**	1.05 $_{(1, 24)}$	0.31
Culvert[b]	**13.55** $_{(1, 24)}$	**0.03**	0.09$_{(1, 24)}$	0.76
Dist[c]	**13.49** $_{(2, 24)}$	**< 0.01**	1.14 $_{(2, 24)}$	0.32
Side×Culvert	**4.50** $_{(1, 24)}$	**0.03**	0.34 $_{(1, 24)}$	0.56
Side×Dist	**4.59** $_{(2, 24)}$	**0.01**	0.02 $_{(2, 24)}$	0.31
Culvert×Dist	**3.33** $_{(2, 24)}$	**0.04**	1.18 $_{(2, 24)}$	0.98
Side×Culvert×Dist	0.09 $_{(2, 24)}$	0.91	0.12 $_{(2, 24)}$	0.89
		2017		
Intercept	**283.48** $_{(1, 285)}$	**<0.01**	470.03 $_{(1, 195)}$	**<0.01**
Side[a]	**71.52** $_{(1, 24)}$	**< 0.01**	3.55 $_{(1, 24)}$	0.06
Culvert[b]	**13.65** $_{(1, 24)}$	**0.02**	1.96 $_{(1, 24)}$	0.16
Dist[c]	**19.39** $_{(2, 24)}$	**< 0.01**	0.45 $_{(2, 24)}$	0.64
Side×Culvert	**34.74** $_{(1, 24)}$	**< 0.01**	1.08 $_{(1, 24)}$	0.30
Side×Dist	2.22 $_{(2, 24)}$	0.12	1.51 $_{(2, 24)}$	0.22
Culvert×Dist	1.77 $_{(2, 24)}$	0.17	0.04 $_{(2, 24)}$	0.96
Side×Culvert×Dist	0.27 $_{(2, 24)}$	0.76	1.13 $_{(2, 24)}$	0.33

Bold emphasized F values indicate the significant factors ($p < 0.05$).
[a] Side of the road (upstream or downstream).
[b] Distance from a culvert (<2 m = low impact (LI), > 20 m = high impact (HI).
[c] Distance from the road (2, 6 or 20 m).

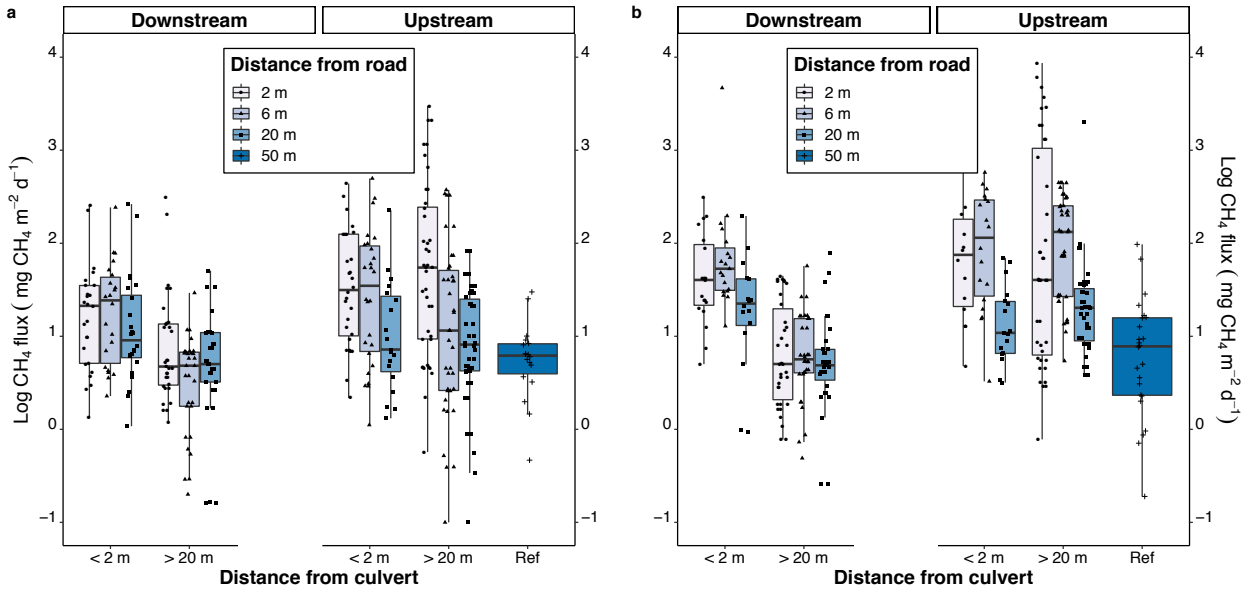

Figure 4.2 Methane fluxes across culvert position and distance from the road in upstream and downstream areas at the bog in a) 2016 and b) 2017. Where > 20 m represent transects aligned at more than 20 m away from culvert position (high impact, HI), < 2 m represent transects located at < 2 m from culvert position (low impact; LI) and Ref represent reference areas (at least 50 m from the road).

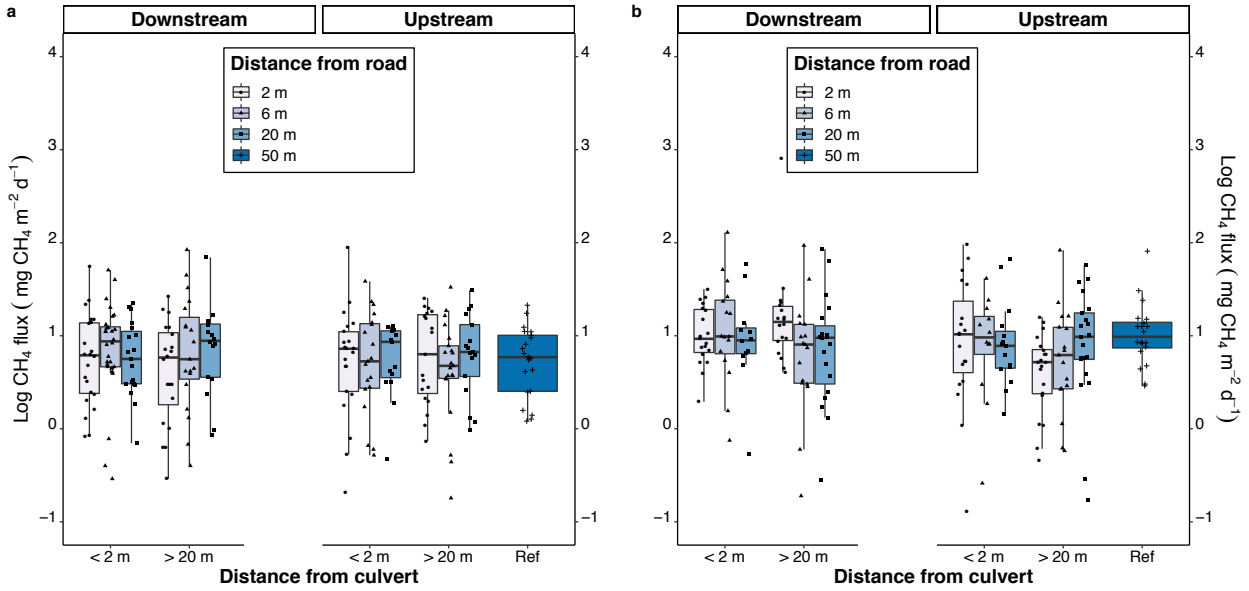

Figure 4.3 Methane fluxes across culvert position and distance from the road in upstream and downstream areas at the fen in a) 2016 and b) 2017. Where > 20 m represent transects aligned at more than 20 m away from culvert position (high impact; HI), < 2 m represent transects located at < 2 m from culvert position (low impact; LI) and Ref represent reference areas (at least 50 m from the road).

The linear mixed-effects model at the bog showed that the interaction of WT and vegetation types and main effects of WT, T5 and vegetation were significant controls on CH_4 fluxes (Table 4.2). Post hoc analyses indicated that the areas with high WT position, but with little vegetation cover had significantly higher CH_4 fluxes ($t = 2.83$, $p = 0.01$). Also, areas with higher temperature emitted more CH_4 ($t = 3.04$, $p = 0.01$). At the fen, WT was the only significant factor in the model where areas with shallower WT had higher CH_4 fluxes (Table 4.2).

Table 4.2 Main and interactive impacts of peat temperature (T5), water table (WT), and vegetation cover on methane flux at bog and fen, Carmon Creek, Peace River, Alberta.

Treatments	**Bog ($R^2 = 0.44$)**		**Fen ($R^2 = 0.1$)**	
	F values	**P-values**	**F values**	**P- values**
Intercept	**464.45** $_{(1, 560)}$	**<0.001**	**697.47** $_{(1, 340)}$	**<0.001**
T5	**26.95** $_{(1, 560)}$	**< 0.001**	1.37 $_{(1, 340)}$	0.24
WT	**34.29** $_{(1, 560)}$	**< 0.001**	**15.08** $_{(1, 340)}$	**0.001**
Vegetation	**5.50** $_{(2, 33)}$	**0.008**	1.79 $_{(2, 33)}$	0.183
T5×WT	**2.99** $_{(1, 560)}$	**0.084**	0.26 $_{(1, 340)}$	0.608
WT×Vegetation	**7.63** $_{(2, 560)}$	**< 0.001**	0.72 $_{(2, 340)}$	0.486

Bold emphasized F values indicate the significant factors ($p < 0.05$).

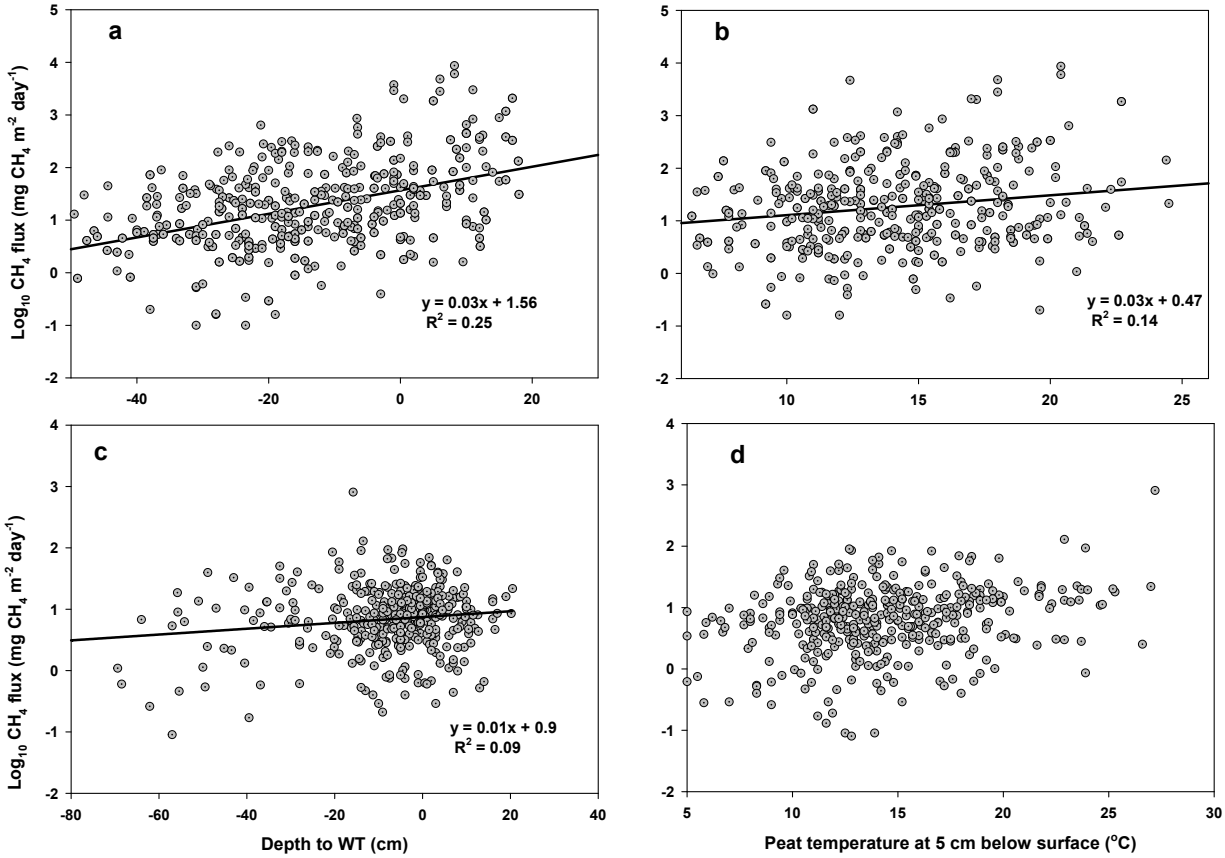

Figure 4.4 Correlation between a) depth to the water table (WT) and $\log_{10}CH_4$ fluxes at bog, b) peat temperature at 5 cm below the surface and $\log_{10}CH_4$ fluxes at bog, c) depth to the water table (WT) and $\log_{10}CH_4$ fluxes at fen, and d) peat temperature at 5 cm below the surface and $\log_{10}CH_4$.

4.3.3 Annual CH_4 emissions

The total 2016 growing season CH_4 fluxes from road disturbed and reference areas were 10.2 ± 2.1 and 0.7 ± 0.05 g CH_4 m^{-2}, respectively in the bog and 0.9 ± 0.08 and 0.5 ± 0.2 g CH_4 m^{-2} in the fen. Similarly, in 2017, growing season CH_4 fluxes from disturbed and reference areas were 17.0 ± 8.8 and 0.5 ± 2.2 g CH_4 m^{-2}, respectively in the bog and 1.7 ± 0.2 and 1.3 ± 0.4 g CH_4 m^{-2} in the fen.

Despite a small CH_4 emission in HI downstream areas of the bog compared to reference plots in 2016, annual CH_4 emissions from all studied transects were substantially greater than reference areas at this site. Moreover, at the bog, annual CH_4 fluxes from HI transects were higher than LI

transects in both 2016 and 2017 (Table 4.2). However, at the fen, the annual CH_4 fluxes from HI transects were higher than LI transects in 2016, but not in 2017 (Table 4.3).

Table 4.3 Annual methane flux (CH_4) ± standard error (SE) at the bog and at the fen in 2016 and 2017, Carmon Creek, Peace River, Alberta.

Sample plots	**Bog CH_4 (mean ± SE) (g CH_4 m^{-2} y^{-1})**	**Fen CH_4 (mean ± SE) (g CH_4 m^{-2} y^{-1})**
	2016	
Upstream areas	9.8 ± 2.7	1.4 ± 0.4
Downstream areas	0.4 ± 0.2	1.0 ± 0.06
HI transects (average)	5.1 ± 2.7	1.2 ± 0.2
Upstream areas	4.2 ± 1.1	0.9 ± 0.3
Downstream areas	2.4 ± 0.7	0.8 ± 0.3
LI transects (average)	3.3 ± 0.7	0.9 ± 0.2
Reference areas	0.8 ± 0.1	0.6 ± 0.1
	2017	
Upstream areas	19.9 ±14.5	2.2 ± 0.4
Downstream areas	1.1 ± 0.3	1.8 ± 0.3
HI transects (average)	10.5 ± 7.7	2.0 ± 0.2
Upstream areas	11.1 ± 4.7	1.5 ± 0.7
Downstream areas	7.4 ± 1.7	1.4 ± 0.2
LI transects (average)	9.3 ± 2.4	1.4 ± 0.3
Reference areas	0.9 ± 0.2	1.1 ± 0.3

4.4 Discussion

In our study, we observed up to an order of magnitude increase in CH_4 emission from road disturbed areas compared to reference areas at the bog. In contrast, the fen exhibited minimal impacts of the road on CH_4 fluxes. The difference between the study sites was largely due to difference in position of the road relative to local slope. Similar to other studies, we observed, the main or combined effects of WT position (Moore & Knowles, 1989; Turetsky et al., 2008), peat temperature (Moore et al., 2011; Turetsky et al., 2008), and vegetation cover (Abdalla et al., 2016; Bubier et al., 1995; Moore et al., 2011) as controls on CH_4 fluxes. I observed greater CH_4 fluxes

from both study sites (bog and fen) in 2017 compared to 2016. Despite receiving less rainfall compared to the 2016 growing season, the measured average WT position at our study sites in 2017 (-19.2 and -17.9 cm at the bog and fen, respectively) indicated that our sites were not moisture limited for CH_4 production (Moosavi et al., 1996), particularly in upstream areas of the bog where the road maintained inundated conditions. Furthermore, we observed higher average T5 and air temperature in 2017, suggesting that warmer soils resulted in higher CH_4 emission despite slightly drier conditions.

As hypothesized, in the bog, we found substantially greater CH_4 fluxes from the HI upstream areas closer to the road. This was primarily linked to the observed shallower WT position and warmer peat temperature. We observed that the WT position was significantly shallower at HI upstream areas compared to the LI upstream areas at the bog site, in both years. This indicates that the flow of water across the peatland was disconnected by the road as culverts were located at > 20 m from HI areas. Consequently, the resulting flooded, anoxic conditions on the upstream HI areas resulted in greater CH_4 emission due to increased anaerobic decomposition (Asada et al., 2005; Turetsky et al., 2008). In contrast, at LI upstream areas, the presence of culverts helped to connect the upstream and downstream areas resulting in similar WT and peat temperature on both sides of the road, leading to less variation in the CH_4 fluxes (Figures 4.3a and 4.3b). Our study is in line with Turetsky et al. (2008), who observed enhanced CH_4 fluxes in a fen when WT position was shallower and peat temperature was increased by ~ 1 °C. Similarly, along with the lower T5 and deeper WT positions, the dominance of trees (e.g. *Picea mariana*) might have suppressed the CH_4 emission from downstream and reference areas of the bog due to shading by trees contributing to lower peat temperature (Bubier et al., 2005; Christensen et al., 2003; Joabsson & Christensen, 2001; Mahmood & Strack, 2011; Malmer et al., 1994).

In general, the presence of aerenchymatous vascular plants (e.g. *Carex rostrata*) enhances CH_4 production by providing more labile litter or root exudates to methanogens (Chanton et al., 2008; Weltzin et al., 2000) and providing a pathway for emission largely bypassing the oxic zone of the peat profile (Crow & Wieder, 2005; Saarinen, 1996; Strack et al., 2006). For instance, Moore et al. (2011) found that areas within an ombrotrophic bog with *Eriophorum vaginatum* (cotton grass) were a greater source of CH_4 than areas without, as this species has ability to increase anaerobic CH_4 production and aerobic consumption. At the bog, we observed greater CH_4 flux from plots with little vegetation cover. This was likely due to the fact that the flooded areas upstream of the road were also largely cleared of vegetation during road construction. At the fen, despite the abundance of sedges and grasses, we did not see a significant impact of the vegetation on CH_4 fluxes at this site because there were no significant variations in the understory vegetation cover as a result of the road. This might also indicate that, after some years at the bog, the impact of understory vegetation cover change brought by the road construction on CH_4 fluxes might be significantly altered depending on the vegetation type that regrows. For instance, the initial colonization by aerenchymatous vascular plants, as observed in the bog, may further stimulate CH_4 emissions on the upstream side of the bog, but this effect could be diminished over time if these plants are replaced by *Sphagnum* moss. Longer term studies are needed to further evaluate how long this shift in plant communities adjacent to peatland road crossings impact CH_4 emission.

We observed elevated CH_4 emissions on downstream LI areas closer to the road of the bog, and that could be because of the water moved across the road by culverts not dispersing well into the surrounding areas. One reason water dispersal was limited is because the area (up to 1 m) surrounding a culvert opening was excavated resulting in lower elevation compared to surrounding downstream areas. Moreover, culverts at the bog were likely spaced too widely to effectively

transmit water across the road resulting in point-sources of water flow downstream of culverts. In contrast, at the fen, we observed few impacts of the road on CH_4 emission, primarily because there was had little effect on water flows between sides of the road as the road was aligned nearly parallel to the flow direction (Saraswati et al., 2019). Therefore, it is important to consider the culvert size, proper installation, and adequate spacing before constructing road crossings across peatlands. Further studies investigating road impacts at fen sites where roads are constructed perpendicular to the dominant water flow direction are also warranted.

We used our estimated road impacts at both the bog and fen (Table 4.3), averaging across both sites for LI and HI transects considering that the road impact extends to 20 m on both sides of the road. Based on this, the estimated road induced CH_4 emissions from two Canadian boreal peatlands is 90.8 and 212.2 kg CH_4 y^{-1} for each km of road using data from 2016 and 2017, respectively. The observed greater emission rates from road disturbed areas indicate that the road construction across peatlands enhances the CH_4 emissions overall, even if emissions decline on downstream sides due to drying. Our calculated totals could have been underestimated because we did not include CH_4 emission as a result of ebullition, which contributes 18 - 64 % of the total CH_4 release pathways from northern peatlands (Christensen et al., 2003;Tokida et al., 2007). Furthermore, the interannual variation, driven largely by warmer temperatures in 2017, may indicate that the road impact on CH_4 emission from peatlands could be significantly increased under predicted global warming and the increased CH_4 emission would again provide a positive feedback to this warming (IPCC, 2013).

4.5 Chapter conclusions

Overall, boreal undisturbed peatlands are natural sources of CH_4. However, the construction of access roads across boreal peatlands has the potential to increase CH_4 flux by creating a shallower WT, warming peat temperature, and altering vegetation cover. We observed up to an order of magnitude increase in CH_4 emission from road disturbed areas compared to reference areas at the bog. In the bog, as hypothesized, we found substantially greater CH_4 fluxes from the high impact (> 20 m from a culvert) upstream areas closer to the road due to observed shallower WT position and warmer peat temperature. In contrast, the fen exhibited minimal impacts of the road on CH_4 fluxes. The difference between the study sites was largely due to difference in position of the road relative to local slope. We estimated that the two Canadian boreal peatland roads are contributing more than 100 kg CH_4 y^{-1} per km road to the atmosphere creating an additional source of anthropogenic GHGs. However, it could be possible that we have underestimated CH_4 emissions by 18 to 60% as we have not included ebullition in this estimate. Results from this study suggest that road associated impacts can be minimized by aligning roads parallel to the water flow direction when and where possible, clearing less vegetation, and placing adequate culverts.

As mentioned above, we observed different responses of peatland CH_4 emissions to road crossings at two study sites, and additional studies at a wider range of peatland types and road orientations are needed to accurately quantify the full extent of roads on boreal peatland GHG exchange. Nonetheless, this study provides a valuable starting point to estimate this potential impact as the study, for the first time, has quantified the impacts of widespread disturbance (roads construction) and provided mitigating strategies to reduce the anthropogenic impacts on forested, northern peatlands. Furthermore, the estimated road induced CH_4 values could be useful to quantify induced CH_4 emissions as a result of wetland road crossings at national to global scales.

Chapter 5: Altered carbon dynamics in boreal peatlands due to access roads

5.1 Introduction

An estimated one-third of the global soil carbon (C) stock, ~550 Pg, is stored in northern peatlands (Gorham, 1991; Tarnocai, 2009). Despite its relatively dry, continental climate (Petrone et al., 2011), peatlands in Canada's western boreal region (WBR) play an important role in the global C cycle as they store ~48 Pg C in their peat (Vitt, Halsey, Bauer, & Campbell, 2000). However, the WBR has been disturbed by networks of road crossings for transportation and exploration and extraction of natural resources including petroleum and forest products (Plach et al., 2017; Timoney, 2003). A more than 217,000 km network of road crossings pass across the boreal region of Canada and >50% pass through peatlands (Pasher et al., 2013) with the potential to bring changes in the C balance in the peatland ecosystem adjacent to the road.

The change in C storage in a peatland is the balance of net ecosystem exchange of carbon dioxide (NEE), methane (CH_4) fluxes, and leaching of carbon in dissolved and particulate forms (Bubier, Bhatia, Moore, Roulet, & Lafleur, 2003; Clymo et al., 1998; Frolking et al., 2010; Potter et al., 2001; Turunen et al., 2002). NEE results from the difference between CO_2 uptake by plants, (i.e., gross ecosystem photosynthesis; GEP) and release of CO_2 during autotrophic and heterotrophic respiration (ecosystem respiration, ER). Stored C may be found in various forms that include peat, vegetation, dissolved organic carbon (DOC), particulate organic carbon (POC), dissolved inorganic carbon (DIC) and CH_4 (Gorham, 1991; Billett et al., 2004).

Peatland ER involves CO_2 efflux from peatlands through living plant-mediated C loss, including plant respiration, microbial respiration in the rhizosphere and root exudate mineralization by microbes, as well as microbial decomposition of litterfall and organic matter in peat (Basiliko et

al., 2013; Beverly & Franklin, 2015; Juszczak et al., 2013; Matteucci et al., 2015; Saraswati et al., 2019; Turetsky et al., 2014). Much of the CO_2 exchange (both GEP and ER) in WBR peatlands occurs during the growing season when all the annual GEP occurs while plant-mediated ER contributes from 35-57 % of the total ER, depending on vegetation/plant functional type and ecosystem properties (Crow & Wieder, 2005).

Environmental factors controlling peatland NEE may include availability of photosynthetically active radiation (PAR), soil temperature, leaf area index (LAI), vegetation type, and soil moisture content (Alm, Schulman, et al., 1999; Chivers et al., 2009; Limpens et al., 2008; Munir et al., 2015). Temperature is a stronger control on ER than GEP (Ow, Griffin, Whitehead, Walcroft, & Turnbull, 2008) and that can reduce CO_2 uptake as NEE by increasing ER with the increased temperature. Similarly, a deeper WT position (dry conditions) in peatlands results in enhanced ER (Gorham, 1991; Hanson et al., 2000). However, dry conditions may also enhance shrub productivity/vascular plant growth that can increase GEP (Cai, Flanagan, & Syed, 2010; Miller, Benscoter, & Turetsky, 2015; Moore & Dalva, 1993; Updegraff, Bridgham, Pastor, Weishampel, & Harth, 2001). Miller et al., (2015) found that peatland drying resulted into enhanced tree biomass (~ 3500 g m^{-2}) in both poor and treed fen. Also, a long-term drainage experiment in Finnish peatlands showed enhanced tree biomass (600 - 6000 g m^{-2}; Laiho & Laine, 1997). In contrast, as dry conditions reduce peat moisture content, this can result in diebacks of bryophytes and reduction of understory CO_2 uptake (Alm et al., 1999; Roulet et al., 2007; Strack et al., 2006). However, this may favor dry-adapted hummock moss and lichens (Miller et al., 2015). A study conducted in the treed bogs of Canada and Finland showed that the lowered WT position can enhance both aboveground and tree fine root biomass (Lohila et al., 2011). When WT is lowered, the root growth is promoted leading to increased autotrophic root respiration by trees/shrubs (Lohila et al.,

2011). Overall, a WT position change in peatlands may have little net effect on NEE if changes in ER and GEP balance out (Sulman et al., 2010).

Along with the natural variability in environmental factors controlling spatial and temporal variation in C exchange in boreal peatlands, anthropogenic disturbances, including peat extraction, drainage, land use change, seismic lines, and roads, can have negative consequences on peatland C uptake and storage (Miller et al., 2015; Plach et al., 2017; Strack et al., 2004; Strack et al., 2019). For example, oil sands mining across three mine sites in Alberta released around 11.4 and 47.3 million metric tons of C (Rooney, Bayley, & Schindler, 2012) and reduced C sequestration by 5,734–7,241 Mg C y^{-1} in peatland ecosystems. Also, seismic lines constructed to explore and extract natural resources in WBR of Canada involve movement of heavy equipment and clearing of vegetation, as a result leaving the ground with reduced vegetation cover and requiring a long time (10- 35 years) to recover woody vegetation (Lee & Boutin, 2005). Wetter conditions on these recovering seismic lines likely also enhance regional CH_4 emissions (Strack et al., 2019).

Road construction normally involves the placement of glacial till on the top of peat and geotextile (Gillies, 2011; Partington & Clayton, 2012) after clearing all vegetation along the road alignment. It has been reported that the placement of glacial till and geotextile material together limit surface and sub-surface water flow across the road crossings as a result of peat compression (Partington et al., 2016; Chapter 2). This compression leads to flooding conditions on the one side (upstream areas) and dry conditions on the other side of the road (downstream areas; Chapter 2; Saraswati, Parsons, & Strack, 2019; Partington, Gillies, Gingras, Smith, & Morissette, 2016). These hydrological alterations may be responsible for short to long- term changes in C uptake and release from adjacent peatlands. Tree removal or dieback due to road construction allow more radiation to reach the peat surface and together with the wetter conditions in upstream areas that enhance

thermal conductivity of peat (Braverman & Quinton, 2016), ultimately speed peat thaw in the early growing season. This could result in higher peat temperatures that increase microbial activity (Braverman & Quinton, 2016) thus enhancing organic matter decomposition. Microbial activity and organic matter mineralization also may be enhanced in downstream areas due to better aeration under dry conditions. The altered hydrology could also change the plant community affecting plant productivity (Miller et al., 2015) and types of litter available, leading to altered decomposition rate (Moore, Trofymow, Siltanen, & Kozak, 2008) and CH_4 production (Strack et al., 2017). However, the level of local topography/slope of peatlands, underlying substrate, and orientation of the road may bring variations in the intensity of road induced impacts on peatlands (Willier, 2017).

Although studies have investigated shifts in peatland WT position, usually in relation to drainage, very few have comprehensively studied the specific impacts of all-season resource access road crossings on boreal forested peatlands C cycling. Available studies are either on the roads' impact on peatland enzymatic activities (Saraswati et al., 2019) or hydrology (Partington et al., 2016; Saraswati et al., submitted), or winter roads' impact on peatland C cycling (Strack, Softa, Bird, & Xu, 2018). Therefore, the objective of our study was to investigate the impacts of access road crossings two boreal forested peatlands' C exchange. Previous research in both study sites has shown that at the bog, the orientation of the road was perpendicular to the water flow direction and this clearly fragmented the bog in upstream and downstream areas (i.e., dry downstream, and wetter upstream of the road). In contrast, minimal hydrologic changes were noticed at the fen site as the road orientation was parallel to the flow direction (Chapter 2). Those hydrological differences had significant impact on the enzymatic activities in the bog, but little effect was observed in the fen (Saraswati et al., 2019). Therefore, it was expected that:

1) The road crossings through a forested bog would have enhanced vascular plant productivity on the dry side of the road that would increase CO_2 uptake as GEP and enhance ER. In contrast, the flooded side of the road would experience tree diebacks and low vegetation cover that would reduce CO_2 uptake. However, no significant changes would be observed in the fen at either side of road in terms of CO_2 and plant productivity.
2) In the presence of culverts, change in C exchange compared to reference areas would be reduced on both sides of the road.
3) In total, net ecosystem carbon balance (NECB) would indicate a reduced C sink in the road adjacent areas compared to reference peatland areas.

5.2 Methods

5.2.1 Study sites

The study was conducted in two boreal forested peatlands: a shrubby rich fen (56°22'09" N and 116°46'12" W) and a forested bog (56°21'44" N and 116°47'45" W) located in the Carmon Creek watershed, near Peace River, Alberta, Canada. Pipelines, low impact seismic lines, inversion pads, and roads were established in the watershed, but were not present in the specific study area used here. As described by Saraswati et al., (2019), the dominant vegetation found in the bog was black spruce (*Picea mariana)*, bog labrador tea (*Rhododendron groenlandicum)*, lingonberry (*Vaccinium vitis-idaea)*, bog craneberry (*Vaccinium oxycoccos)*, *Sphagnum* spp. and lichens (e.g., *Cladina stellaris*, *Cladina rangiferina,* and *Cladina mitis*). In the fen, the dominant plant types were willow (*Salix* spp.), grey alder (*Alnus incana)*, and paper birch (*Betula papyrifera)*, sedges (e.g., *Carex utriculata*, *Carex aquatilis*, *Carex canescens*), and grasses (e.g., *Calamagrostis canadensis*).

Both bog and fen sites have roads crossing them constructed in 2014 and 2013, respectively. The construction of the roads involved clearing of all vegetation from the upstream areas (east side of the road) up to ~18 m from the road in the bog and downstream areas (north side of the road) in the fen. This was done to improve the visibility for vehicles while transporting resources and/or to accommodate utilities such as powerlines. As, the construction of the road in the bog was done in 2014, the upstream areas of the bog was almost devoid of vegetation cover, except in a few areas where herbaceous plants were re-establishing. However, in the fen, while tall shrubs were not present, all cleared areas (north side of the road up to ~18 m from the road) had sedge regrowth. In the bog, the natural slope was decreased in elevation on the downstream (west, average elevation 621.5 m.a.s.l.) areas compared to the upstream (east, 622.5 m.a.s.l.; Saraswati et al., 2019). However, in the fen, the topography was similar on both sides of the road with the slope in the peatland nearly parallel to the flow direction (Saraswati et al., 2019). The average EC and pH at the fen and bog were 285.2 $\mu S\ cm^{-1}$ and 102.1 $\mu S\ cm^{-1}$, and 7.5 and 5.4, respectively.

In each study site, we laid six transects extending 20 m perpendicular to the road on both sides. Half of the transects (n = 3) were located nearby to culverts (< 2 m from culverts), termed low impact transects (LI), and the other half (n = 3) were located far from culverts (> 20 m from culverts), termed as high impact transects (HI). In each transect, to measure understory CO_2 fluxes (see CO_2 flux measurements), aluminium collars (60 x 60 cm) were installed at 2, 6 and 20 m from the edge of the road on both sides of the road (road impacted areas; RI). Also, a set of three collars was installed at reference areas (at least 50 m from the road) on the upstream areas at the bog and on the downstream areas at the fen. A total of 39 collars in each of the bog and fen site were inserted 10 cm below the ground with minimum disturbance to the plant roots. An additional three

collars were added on the downstream reference areas (at least 50 m from the road) in 2017 at the bog.

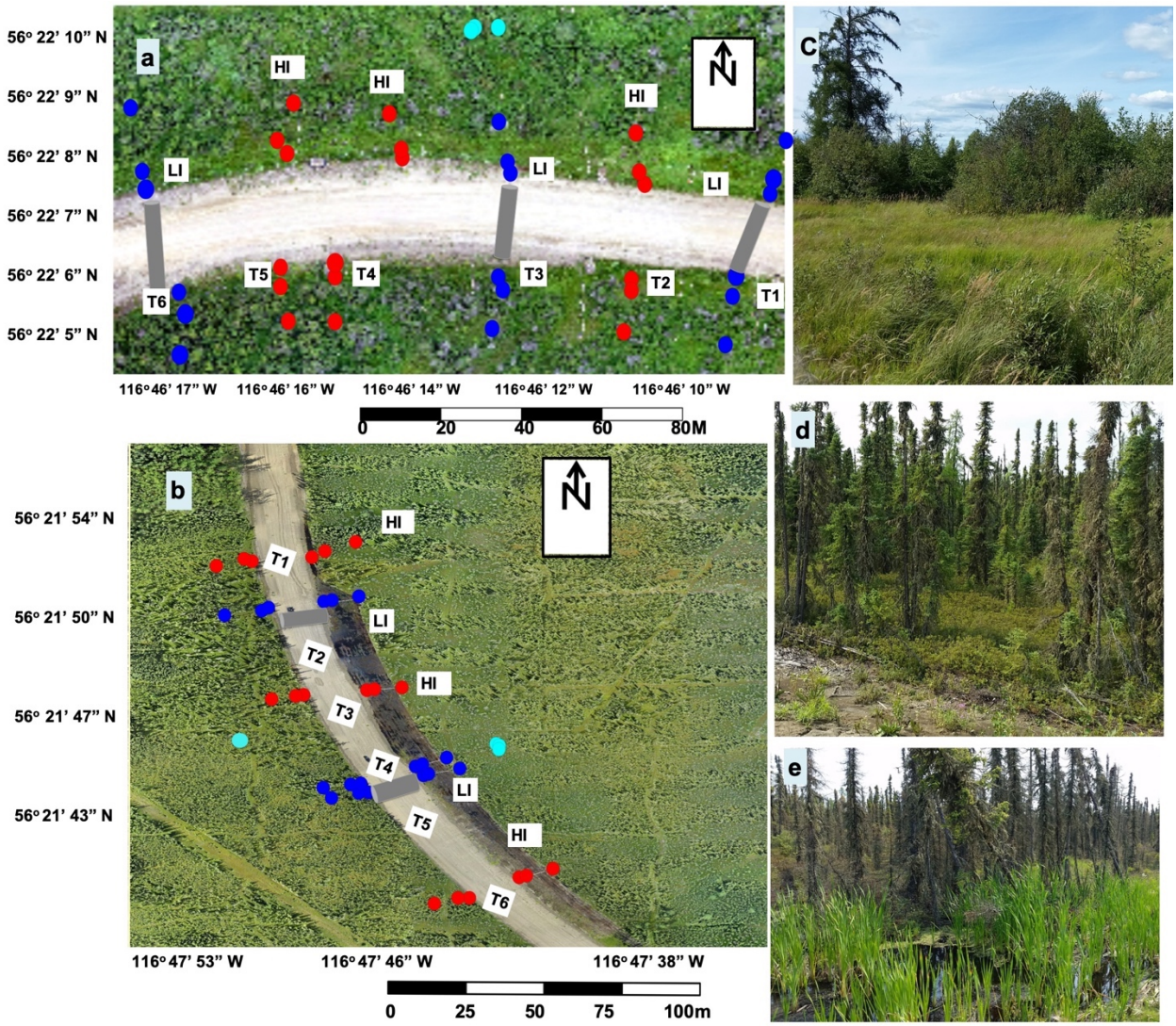

Figure 5.1 Study sites a) a fen and b) a bog, where red circles are sample plots located along transects > 20 m away from culverts (gray pipes, HI), blue circles are plots located along transects < 2 m away from culverts (LI), and teal circles are plots located at reference sites at Carmon Creek, Peace River, Alberta. c) downstream side of the fen site, d) and e) downstream and upstream side of the bog.

5.2.2 CO_2 flux measurements

From collars, we measured bi-weekly CO_2 fluxes (i.e., understory fluxes) from May to September in 2016 and 2017 using closed chamber methods (Strack, Waddington, & Tuittila, 2004). CO_2

fluxes were measured with transparent acrylic chambers (60 × 60 × 30 cm) equipped with two battery operated fans to mix the headspace air. While measuring CO_2 fluxes, the collar's groove was filled with water to create a seal. During each set-up, a total of three sets of NE-CO_2 from the understory (NE_{us}) measurements (each of 1.75 minutes) were performed at each collar using a series of light levels: 1. full sun, 2. single shade (75% transparent) and 3. double shade (50% transparent). Then ecosystem respiration (ER) was measured from fully shaded chambers using an opaque shroud. During each measurement, every 15 seconds, we measured CO_2 concentration change using a portable infrared gas analyzer (PP systems, USA, EGM-4), photon flux density of photosynthetically active radiation (PAR) with a quantum sensor (PP Systems, USA), and air temperature inside the chamber with thermocouple wire and thermometer. In between each measurement, the chambers were vented to flush the air and bring the headspace into equilibrium with the atmospheric conditions. The linear change in CO_2 concentration over time was used to calculate flux, correcting for chamber volume and temperature. The gross understory photosynthesis (GEP_{us}) of measurements was calculated by taking a difference between NE_{us} and ER. The sign convention used was positive for CO_2 released into the atmosphere from the ecosystem and negative for an ecosystem sink. $NE_{max.us}$ and $GEP_{max.us}$ rates were calculated with PAR of at least 1000 μmol m^{-2} s^{-1}(Lafleur et al., 2003). While calculating C balance, the values of CH_4 flux were measured at the same collars, with values here taken from Saraswati and Strack (submitted; Chapter 4).

5.2.3 Environmental variables

With each round of flux measurements, we also measured peat temperatures at depths of 2, 5, 10, 15 and 20 cm, using a thermocouple thermometer, and WT with respect to the surface in wells installed adjacent to each collar. The WT values measured below the surface were negative. By

visually estimating the plant cover (both vascular and bryophytes) of each species, understory vegetation cover was surveyed in August 2017 within each collar.

5.2.4 Above ground tree/shrub biomass and net primary productivity (NPP_{ag})

We used a circular sampling plot (4 m^2), laid within ~ 1.5 m of each collar in both study sites, to conduct surveys of tall shrub and tree biomass and NPP. All trees within the area were measured for basal diameter (BD, to the nearest mm) at 30 cm from the ground (diameter immediately above the butt swell), and height. In the fen, within the plots, the vegetation height, vegetation width, number of stems, and BD of stems were measured for shrubs.

At the bog, stem discs from trees with < 10 cm BD or cores from trees with > 10 cm BD were collected from areas located at 2, 6, and 20 m (a minimum of four discs or cores representing all BD classes from each distance category). In the fen, stem discs from the thickest and tallest five stems from each plant/shrub clump were collected. At the time of disc or core collection, we recorded tree species, diameter at breast height (DBH; if taller than 135 cm; Saraswati et al in preparation), DB and height of the corresponding trees. Tree cores were extracted with an increment borer (Haglof Increment Borer) and collected cores were stored in separate paper straws. In the laboratory, tree cores were glued in groves of lumber frames (60 × 10 cm). Tree discs and cores were manually sanded with sandpaper (80 to 400 grit). Ring widths (precision level 0.001 mm) were measured with the MeasureJ2x software (VoorTech Consulting, Holderness, New Hampshire, USA).

The total aboveground biomass of black spruce was estimated by equation (1) developed by Bond-Lamberty, Wang, & Gower, (2002). Meanwhile, the aboveground biomass of other species (tall

shrubs i.e., shrubs not included in the NE_{us}) found in the fen site (e.g., bog birch, willow and alder) were determined by allometric equation (2) developed by He et al., (2018).

$$log10Y = a + b\,(log10BD) \quad (1)$$

$$Y = aBD^{b} \quad (2)$$

Where, Y is aboveground biomass (g), BD is basal diameter (cm), a and b are the coefficients specific to each species. The annual NPP_{ag} was estimated as the difference between the biomass at the end of subsequent growth year for each species. The BD of each tree/stem for the year 2016 was estimated by subtracting twice the width of the outermost radii (2017 tree ring width) from respective field measured BD. The estimated biomass was converted to C by assuming that 50% of the dry mass was C.

5.2.5 Modelling of CO_2 flux

In order to estimate growing season totals of the understory CO_2 fluxes, understory GEP (GEP_{us}) and ER of early (May and June) and late growing season (July and August) were fitted by following Günther et al. (2015). ER was modelled using equation (3)

$$\mathrm{ER} = \mathrm{ERref} \times e^{\left(\mathrm{Eo}\times\left(\frac{1}{\mathrm{Tref}-\mathrm{To}}-\frac{1}{\mathrm{T}-\mathrm{To}}\right)\right)} \quad (3)$$

where, ER is measured ecosystem respiration, T is the field measured soil temperature (K) at 5 cm below the surface, T_{ref} is a reference temperature 283.5 K, T_o is a temperature constant at the start of biological process (237.48 K), and E_o is the activation energy. Once the growing season total ER flux at each plot was estimated using this model, it was converted into annual flux by adding 15% to growing season ER to account for non-growing season CO_2 emissions (Saarnio et al., 2007).

GEP_{us} was modeled using PAR according to a commonly used rectangular hyperbola, (equation 4).

$$GEPus = \frac{PAR \times \alpha \times GPmax}{PAR \times \alpha + GPmax} \quad (4)$$

where, α is the initial slope of the regression and $GPmax$ (g m^{-2} s^{-1}) is the theoretical maximum rate of GEP and represents the asymptote of the hyperbola.

The errors of modelled understory CO_2 for each plot were estimated using equation 5 (Strack et al., 2018) adapted from Alm et al. (1999).

$$E_{NEus-mod} = \sqrt{\sum_{i=1} n \frac{(NE_{us-obs} - NE_{us-mod})^2}{(n-1) \times 1}} \quad (5)$$

where, $NE_{us\text{-}obs}$ is the field measured NE_{us}, $NE_{us\text{-}mod}$ is the estimated NE_{us} (i.e. sum of modelled ER and modelled GEP for each observation), and n is the number of observations. The model parameters of equation (3) and (4), and the total NE_{us} model errors, the average of which ranged from 47.3 to 95.7 g C m^{-2}, are presented in Appendix (Table A2 and A3).

5.2.6 Annual NECB

Annual NECB from each site was estimated using equation (6) as performed by Strack et al. (2017); and Munir, Perkins, Kaing and Strack (2015).

$$NECB = NE_{us} + NPP_{ag} + NPP_{bg} + L_{tree} + R_r + CH_4 \quad (6)$$

Where, NECB is net ecosystem C balance (g C m^{-2} y^{-1}), NE_{us} is understory net ecosystem exchange of CO_2 (g C m^{-2} y^{-1}), NPP_{ag} is annual aboveground tree (or tall shrub) NPP (g C m^{-2} y^{-1}), NPP_{bg} is annual below ground tree (or tall shrub) NPP (g C m^{-2} y^{-1}), L_{tree} is tree litter fall (g C m^{-2} y^{-1}), R_r is tree (or tall shrubs) root respiration (g C m^{-2} y^{-1}), and CH_4 is CH_4 flux (g C m^{-2} y^{-1}) as measured at the same plots and reported previously in Saraswati and Strack (2019; Chapter 4).

We did not measure R_r, NPP_{bg} and L_{tree} at our sites. Therefore, R_r of plots with trees (or tall shrubs) was estimated as 0.639*NPP + 17.189 (Munir et al., 2015), NPP_{bg} as 22% of the NPP_{ag} for bog and $0.576 \times NPP_{ag}^{0.615}$ for fen (Li, Kurz, Apps, & Beukema, 2003), L_{tree} is 17% of the NPP_{ag} for bog (Munir et al., 2015; Strack et al., 2018). L_{tree} was not added to fen sites as allometric equations used in this study for fen site species included the leaves (He et al., 2018).

5.2.7 Road Induced C calculations

The road induced C emissions were calculated by subtracting reference area NECB from the NECB calculated for the areas within 20 m of the road (Equation 7).

$$Road\ adjacent\ C\ flux_{(x)} = \frac{2C_{(x)}*2\ m^2+6C_{(x)}*4\ m^2+20C_{(x)}*14\ m^2}{20\ m^2} \quad (7)$$

where, (x) is side of the road (upstream or downstream), $2C$ is C fluxes from 2 m areas (g C m^{-2}), $6C$ is the C fluxes from 6 m areas (g C m^{-2}), and $20C$ is C fluxes from 20 m areas (g C m^{-2}).

5.2.8 Statistical analyses

Data analyses were performed in R (R Core Team, 2017). Linear mixed effect models (LMEM), with plots and years as random effects to account for the repeated measurements, were used to investigate differences in the CO_2 flux between the side of the road, culvert position, and distance from the road in each study site. For significant factors, post hoc analyses (Tukey's t- tests) were performed to determine differences between groups. We also used LMEMs to evaluate the control of environmental factors including vascular plant type, WT, and temperature at 5 cm below the peat (T5) and all two-way interactions of these variables on understory CO_2 fluxes with years and plots as random effects. To determine the controls, all factors were included in the LMEM model, then non-significant factors were dropped one at a time from the least significant to a final

significant model. At the bog, vegetation cover was divided into three categories i.e. vascular plant dominated areas (> 19% vascular and mixed moss), moss dominated plots (vascular < 19%) and low vegetation plots (< 5% moss or vascular plants) and at the fen, grass-dominated areas (>50% *Calamagrostis canadensis*) and Carex-dominated areas (>50% *Carex* spp.).

5.3 Results

5.3.1 Environmental factors

The 30-year (1986-2015) growing season (May to August) daily average air temperature and total rainfall recorded at the Peace River Airport Station located at ~ 40 km from study sites were 14.1 ^{o}C and 213.5 mm, respectively (Environment and Climate Change Canada, 2018). The growing season rainfall in 2016 (444.2 mm) and 2017 (137.0 mm) indicated that the 2016 growing season was wetter, and 2017 growing season was drier than average compared to 30-year normal climate data.

At the bog, the average WT position at RI areas was shallower both in 2016 (-11.0 cm) and in 2017 (-18.5 cm) than reference areas (2016: -16.4 cm, $z = 2.86$, $p < 0.01$; 2017: -24.3 cm, $z = 3.27$, $p < 0.001$, respectively). LMEM, with year and plot as random effects, showed that, at the bog, the WT position varied significantly by the interaction of side of the road and culvert position ($F_{1,26} = 4.64$, $p = 0.04$). The average WT position on the downstream areas of HI transects (-28.3 cm) was significantly deeper than upstream areas of both HI (-5.5 cm, $t = 5.36$, $p = 0.001$) and LI transects (-10.9 cm, $t = 4.03$, $p = 0.002$). Similarly, HI upstream areas had significantly shallower WT compared to downstream areas of LI transects (-20.6 cm, $t = 3.5$, $p = 0.01$). In contrast, at the fen, the average WT position did not vary between RI areas in 2016 (-2.5 cm) and in 2017 (-17.5 cm) when compared to reference areas (2016: -2.6 cm, $t = 0.09$, $p = 0.93$; 2017: -22.7 cm, $z = 1.15$, p

=0.26, respectively). The interaction of side of the road and culvert position was close to significant in predicting the variability of the WT ($F_{1,26} = 3.2$, $p = 0.07$), but distance from the road was not a significant factor ($F_{2,26} = 0.20$, $p = 0.82$). Post hoc analysis showed that the downstream HI areas had substantially deeper WT (-14.5 cm) compared to upstream HI areas (-7.3 cm, $t = 2.6$, $p = 0.06$).

At the bog, the average T5 at RI areas (14.3 °C in 2016, and 13.7 °C in 2017) was significantly greater than reference areas in 2016 (13.8°C, $z = 2.02$, $p = 0.04$) and in 2017 (12.0 °C, $z = 3.27$, $p = 0.02$), respectively. However, at the fen, no significantly differences were observed for the average T5 at RI areas (14.0 °C in 2016, and 12.2 °C in 2017) and reference areas in 2016 (14.5 °C, $z = 0.55$, $p = 0.57$) and in 2017 (15.3 °C, $z = 2.32$, $p = 0.09$), respectively.

LMEM with year and plot as random effects showed that, at the bog, T5 varied with the interactions of distance from the road and side of the road ($F_{2,26} = 3.85$, $p = 0.04$) and culvert position and side of the road ($F_{1,26} = 4.02$, $p = 0.03$). The average T5 at the bog was greater at areas close to the road (2 m distance; 15.3 °C) with the lowest average T5 (13.2 °C) at the downstream areas of HI transects. At the fen, the T5 varied significantly by the side of the road ($F_{1,28} = 4.51$, $p = 0.04$) with the significantly greater T5 at the downstream areas (13.6 °C) compared to upstream areas (12.7°C, $t = 2.11$, $p = 0.04$). However, T5 did not vary with the culvert position ($F_{1,28} = 2.48$, $p = 0.12$) and distance from the road ($F_{2,28} = 0.06$, $p = 0.94$).

5.3.2 Understory CO_2 fluxes

In 2016, considering the ground layer flux in full light conditions (i.e., $NE_{max.us}$), RI areas at the bog were sources of CO_2 (1.9 g CO_2 m^{-2} d^{-1}) and significantly different than the reference areas (-1.1 g CO_2 m^{-2} d^{-1}; $z = 2.07$, $p = 0.05$). However, in 2017, both RI areas (-1.4 g CO_2 m^{-2} d^{-1}) and reference areas (-1.1 g CO_2 m^{-2} d^{-1}) were sinks of CO_2 at the bog and not statistically significantly

different ($z = -0.16$, $p = 0.88$). In contrast, at the fen, both RI and reference areas were sinks of CO_2 in both 2016 and 2017 with no significant differences between the zones ($z = 0.32$, $p = 0.77$; $z = -0.71$, $p = 0.49$, respectively).

Considering the ground layer flux at the bog, $NE_{max.us}$, ER and $GEP_{max.us}$ were greater at downstream areas as hypothesized. The LMEM analyses indicated that, at the bog, the interaction of culvert position and side of the road was significant for describing variation in $NE_{max.us}$, ER and $GEP_{max.us}$ ($F_{1,24} = 10.76$, $p = 0.003$ and $F_{1,24} = 20.62$, $p = 0.0001$ and $F_{1,24} = 51.95$, $p < 0.0001$, respectively). Post hoc comparisons of $NE_{max.us}$ showed that the LI downstream areas (6.28 g CO_2 m^{-2} d^{-1}) were significantly larger sources of CO_2 than HI downstream areas and LI upstream areas at the bog ($t = -3.15$, $p = 0.01$; $t = -2.70$, $p = 0.02$, respectively). Also, post hoc comparisons of ER showed that LI downstream areas had significantly higher ER (27.80 g CO_2 m^{-2} d^{-1}) compared to HI downstream (15.32 g CO_2 m^{-2} d^{-1}) and HI upstream areas (9.21 g CO_2 m^{-2} d^{-1}; $t = 7.06$, $p < 0.0001$; $t = 10.01$, $p < 0.0001$, respectively). Post hoc comparisons of $GEP_{max.us}$ showed that the downstream areas of LI transect (1.85 g CO_2 m^{-2} d^{-1}) had significantly greater $GEP_{max.us}$ than upstream areas of LI transects (1.53 g CO_2 m^{-2} d^{-1}, $t = 5.18$, $t = 0.0001$), downstream areas of the HI transects (1.35 g CO_2 m^{-2} d^{-1}, $t = 7.86$, $p < 0.0001$) and upstream areas of HI transect (1.66 g CO_2 m^{-2} d^{-1}, $t = 2.88$, $p = 0.03$).

At the fen, neither main nor interactive effects of culvert position, side of the road and distance from the road were significant for explaining variations in the $NE_{max.us}$ and ER at the fen (Table 5.1). However, $GEP_{max.us}$ varied significantly by culvert position only (Table 5.1). LI areas (-1.7 g CO_2 m^{-2} d^{-1}) had significantly greater $GEP_{max.us}$ than HI areas (-1.47 g CO_2 m^{-2} d^{-1}, $t = 4.73$, $p = 0.0001$).

Table 5.1 Linear mixed effects model results showing the impacts of side of the road, culvert distance, and distance from the road on $NE_{max.us}$ and ER at bog and fen, Carmon Creek, Peace River, Alberta.

Treatments	**Bog**		**Fen**	
	F values	**P-values**	**F values**	**P- values**
		$NE_{max.us}$		
Intercept	0.03 $_{(1, 381)}$	0.86	**29.58 $_{(1, 356)}$**	**< 0.0001**
Side[a]	1.53 $_{(1, 24)}$	0.24	0.41$_{(1, 24)}$	0.53
Culvert[b]	1.49 $_{(1, 24)}$	0.23	1.46 $_{(1, 24)}$	0.24
Dist[c]	1.48 $_{(2, 24)}$	0.24	0.36 $_{(2, 24)}$	0.70
Side×Culvert	**11.19 $_{(1,24)}$**	**0.002**	0.51$_{(1, 24)}$	0.48
Side×Dist	0.69 $_{(2, 24)}$	0.50	0.59 $_{(2, 24)}$	0.56
Culvert×Dist	0.40 $_{(2, 24)}$	0.67	0.70 $_{(2, 24)}$	0.50
Side×Culvert×Dist	0.42 $_{(2, 24)}$	0.66	0.87 $_{(2, 24)}$	0.43
		ER		
Intercept	**629.04 $_{(1, 381)}$**	**< 0.001**	**13.72 $_{(1,356)}$**	**<0.001**
Side[a]	**91.82 $_{(1, 24)}$**	**<0.0001**	0.46 $_{(1, 24)}$	0.50
Culvert[b]	**27.85 $_{(1, 24)}$**	**<0.0001**	0.92 $_{(1, 24)}$	0.35
Dist[c]	2.25 $_{(2, 24)}$	0.13	0.87 $_{(2, 24)}$	0.43
Side×Culvert	**20.62 $_{(1, 24)}$**	**0.0001**	1.10 $_{(1, 24)}$	0.30
Side×Dist	0.23 $_{(2, 24)}$	0.80	0.76 $_{(2, 24)}$	0.48
Culvert×Dist	0.83$_{(2, 24)}$	0.44	1.15 $_{(2, 24)}$	0.33
Side×Culvert×Dist	1.01 $_{(2, 24)}$	0.38	1.06 $_{(1, 24)}$	0.36
		$GEP_{max.us}$		
Intercept	**5356.26 $_{(1,381)}$**	**<0.0001**	**4659.87$_{(1,381)}$**	**<0.0001**
Side[a]	0.21$_{(1,24)}$	0.65	0.50$_{(1, 24)}$	0.48
Culvert[b]	**13.0$_{(1,24)}$**	**0.001**	**18.25$_{(1, 24)}$**	**0.0003**
Dist[c]	3.31$_{(2,24)}$	0.06	1.69$_{(2, 24)}$	0.20
Side×Culvert	**51.95$_{(1,24)}$**	**<0.0001**	0.55$_{(1, 24)}$	0.46
Side×Dist	2.39$_{(2,24)}$	0.11	4.0$_{(2, 24)}$	0.06
Culvert×Dist	2.58$_{(2,24)}$	0.09	1.75$_{(2, 24)}$	0.19
Side×Culvert×Dist	2.19$_{(2,24)}$	0.13	0.96$_{(2, 24)}$	0.39

[a] Side of the road (upstream and downstream of the road); [b] Distance from a culvert (> 20 m from culvert position and < 2 m from culvert position); [c] Distance from the road (2, 6 and 20 m)

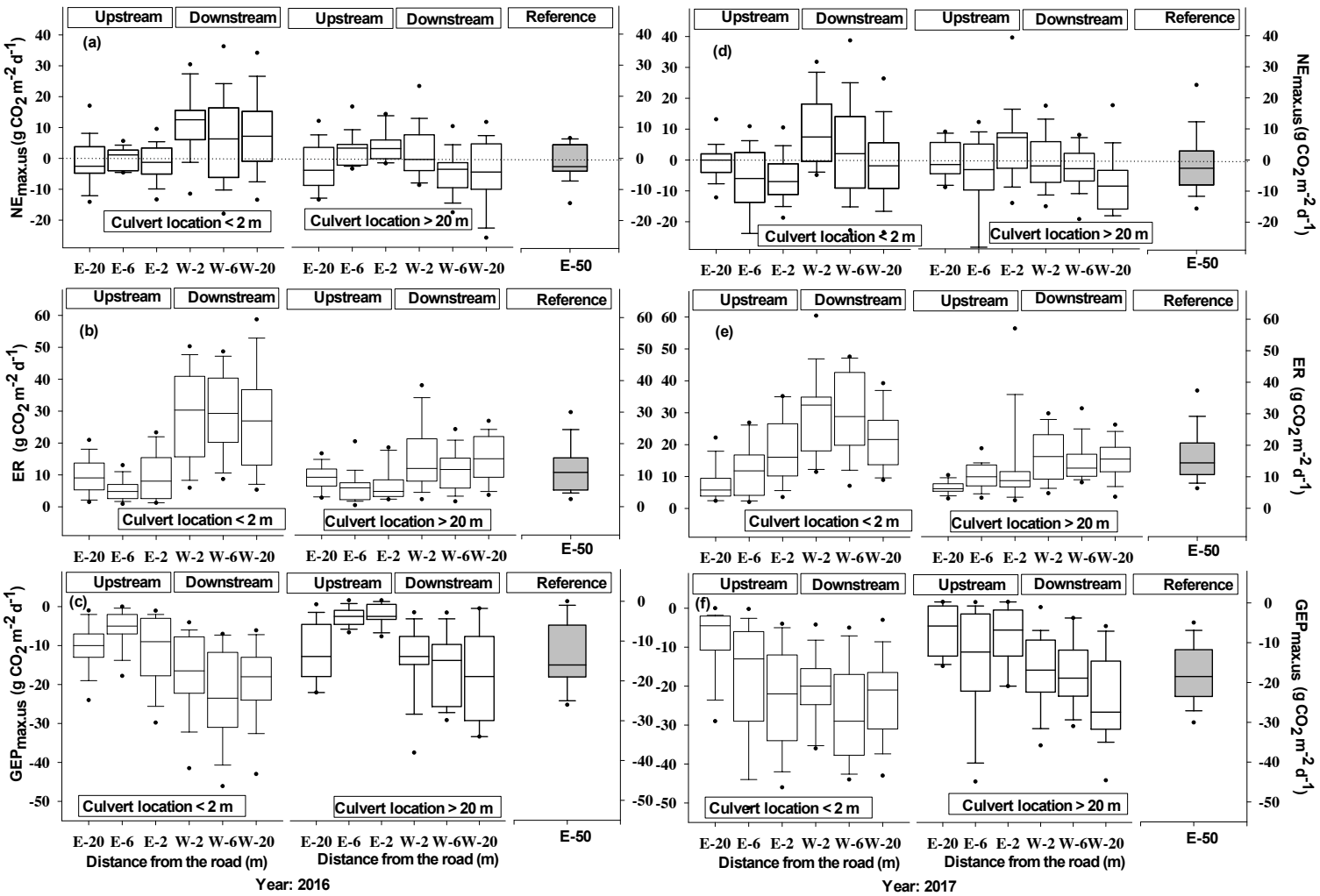

Figure 5.2 (a) Net ecosystem exchange of the understory ($NE_{max.us}$), (b) ecosystem respiration (ER) and (c) understory gross ecosystem photosynthesis ($GEP_{max.us}$) in 2016 and (d) Net ecosystem exchange of the understory ($NE_{max.us}$), (e) ecosystem respiration (ER) and (f) understory gross ecosystem photosynthesis ($GEP_{max.us}$) in 2017 observed across culvert position and distance from the road in the upstream and downstream areas at the bog.

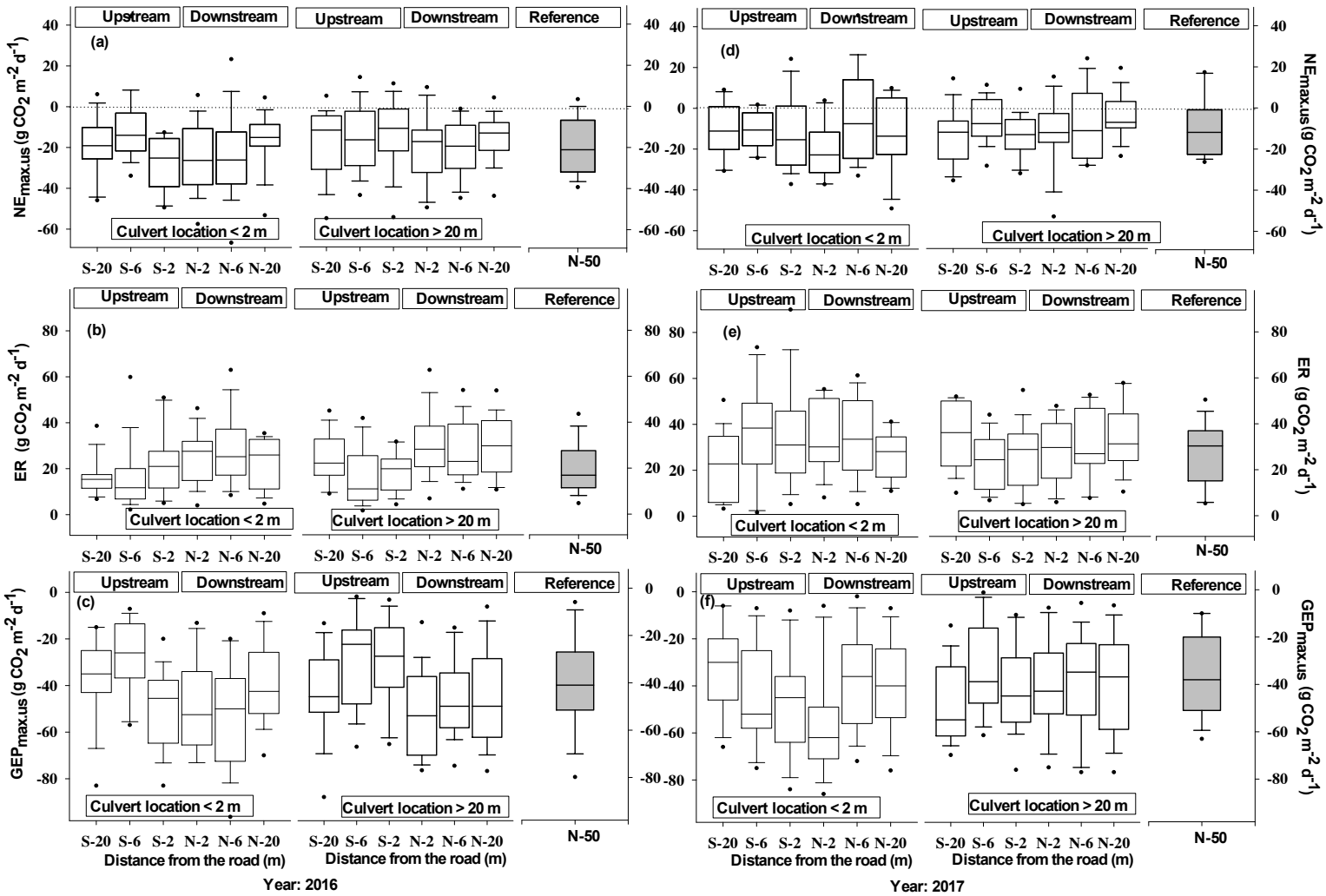

Figure 5.3 (a) Net ecosystem exchange of the understory ($NE_{max.us}$), (b) ecosystem respiration (ER) and (c) understory gross ecosystem photosynthesis ($GEP_{max.us}$) in 2016 and (d) Net ecosystem exchange of the understory ($NE_{max.us}$), (e) ecosystem respiration (ER) and (f) understory gross ecosystem photosynthesis ($GEP_{max.us}$) in 2017 observed across culvert position and distance from the road in the upstream and downstream areas at the fen.

5.3.3 Environmental controls on CO_2 fluxes

At the bog, LMEM with year and plot as random effects showed that both the T5 and vegetation cover were significant for explaining variation in $GEP_{max.us}$, but the $NE_{max.us}$ was significantly controlled by T5 only (Table 5.2). Post hoc comparisons showed that the plots with higher average T5 were significantly greater sources of CO_2 ($t = 3.42$, $p = 0.0007$). In contrast, plots dominated by vascular plants had significantly greater productivity (more negative $GEP_{max.us}$) compared to moss dominated plots ($t = 1.32$, $p = 0.19$) and low vegetation plots ($t = 1.95$, $p = 0.05$). Significant factors controlling ER were the interactions of T5 and vegetation cover, and WT position and vegetation cover (Table 5.3). The plots dominated with vascular plants and with higher T5 had significantly higher ER compared to moss dominated plots ($t = -3.70$, $p = 0.003$) and plots with low vegetation cover ($t = -3.01$, $p = 0.02$). Also, plots dominated with vascular plants with deeper WT emitted more CO_2 as ER compared to moss-dominated plots (12.5, $t = 3.70$, $p = 0.003$).

In contrast, at the fen, vegetation cover, and the interaction of WT and T5 were significant controls on $NE_{max.us}$, while WT and T5 were significant controls on ER. Only T5 was significant for explaining variation in $GEP_{max.us}$ (Table 5.2). The plots dominated with *Carex* with a deeper WT position were significant greater sinks of CO_2 ($NE_{max.us}$) than plots dominated with grass ($t = -3.04$, $p = 0.004$). The plots with higher T5 and deeper WT position ($t = 14.40$, $p = 0.00$) had higher ER.

Table 5.2 Main and interactive impacts of peat temperature (T5), water table (WT), and vegetation cover on $NE_{max.us}$, ER and $GEP_{max.us}$ at bog and fen, Carmon Creek, Peace River, Alberta.

Treatments	F-values	P-values	F-values	P-values
$NE_{max.us}$	**Bog (R^2 = 0.44)**		**Fen (R^2 = 0.33)**	
Intercept	0.19 $_{(1, 363)}$	0.67	192.67 $_{(1, 346)}$	<0.001*
WT[a]	0.04$_{(1, 363)}$	0.85	53.72 $_{(1, 346)}$	<0.001*
T5[b]	11.40 $_{(1, 363)}$	< 0.001*	11.76$_{(1, 346)}$	<0.001*
Vegetation	0.70$_{(3, 38)}$	0.56	9.54$_{(1, 37)}$	0.003*
WT×T5	1.12 $_{(1,363)}$	0.43	5.01$_{(1, 346)}$	0.03*
ER	**Bog (R^2 = 0.55)**		**Fen (R^2 = 0.61)**	
Intercept	245.0 $_{(1, 357)}$	<0.001*	519.15 $_{(1, 349)}$	< 0.001*
WT	2.63 $_{(1, 357)}$	0.11	70.72 $_{(1, 349)}$	< 0.001*
T5	65.42 $_{(1, 357)}$	< 0.001*	213.52 $_{(1, 349)}$	< 0.001*
Vegetation	7.29 $_{(3, 38)}$	0.0006*	1.43 $_{(2, 37)}$	0.24
WT×T5	0.96 $_{(1,357)}$	0.35	11.18 (1, 349)	< 0.001*
T5×Vegetation	4.23 $_{(3, 357)}$	0.006*	1.21(3,349)	0.21
WT×Vegetation	6.67 $_{(3, 357)}$	0.007*	1.36(3,349)	0.23
$GEP_{max.us}$	**Bog (R^2 = 0.45)**		**Fen (R^2 = 0.38)**	
Intercept	294.27 $_{(1, 363)}$	< 0.0001*	774.38 $_{(1, 350)}$	< 0.0001*
WT	3.36 $_{(1, 363)}$	0.07	0.52 $_{(1, 350)}$	0.48
T5	20.61 $_{(1, 363)}$	< 0.0001*	124.22 $_{(1, 346)}$	< 0.0001*
Vegetation	4.43 $_{(3, 38)}$	0.009*	1.77 $_{(1, 37)}$	0.19

[a] water table position; [b] peat temperature at 5 cm below the peat surface.

5.3.4 Tree and tall shrub NPP_{ag}

The average tree NPP_{ag} at the reference areas of the bog was significantly higher in both 2016 (142.7 g C m^{-2} y^{-1}) and 2017 (168.1 g C m^{-2} y^{-1}) compared to RI areas in 2016 (65.9 g C m^{-2} y^{-1}, t = 2.39, p = 0.02) and 2017 (94.5 g C m^{-2} y^{-1} , t = 2.11, p = 0.04, respectively). Similarly, tall shrub NPP_{ag} at the fen reference area was significantly higher in both 2016 (178.2 g C m^{-2} y^{-1}) and 2017 (169.5 g C m^{-2} y^{-1}) compared to RI areas in 2016 (151.8 g C m^{-2} y^{-1}, t = 1.95, p = 0.05) and 2017 (129.7 g C m^{-2} y^{-1}, t = 2.98, p = 0.004), respectively. In both study areas, the plots located at vegetation clearance areas were devoid of tree/tall shrub NPP_{ag}.

At the bog, the NPP_{ag} varied with the side of the road and culvert position (Table 5.4). Plots located at 2 m on the downstream side of the road (190.2 g C m^{-2} y^{-1}) had significantly higher NPP_{ag} than 6 m downstream side of the road (56.9 g C m^{-2} y^{-1}, $t = 8.36$, $p < 0.0001$), 20 m downstream side of the road (65.2 g C m^{-2} y^{-1}, $t = 7.84$, $p < 0.0001$), and 20 m upstream side of the road (40.3 g C m^{-2} y^{-1}, $t = 9.36$, $p < 0.0001$).

The NPP_{ag} also varied at the fen with the side of the road and culvert position (Table 5.4). The average NPP_{ag} at downstream 2 m plots (33.9 g C m^{-2} y^{-1}) was significantly lower than plots located at downstream 20 m areas (197.4 g C m^{-2} y^{-1}, $t = 7.36$, $p < 0.001$), and plots located at 20 m upstream areas (158.1 g C m^{-2} y^{-1}, $t = 5.76$, $p < 0.001$). Upstream LI plots had significantly greater NPP_{ag} (167.1 g C m^{-2} y^{-1}) than upstream HI plots (128.0 g C m^{-2} y^{-1}, $t = 2.74$, $p = 0.03$), downstream HI plots (107.0 g C m^{-2} y^{-1}, $t = 3.74$, $p = 0.002$), and downstream LI plots (111.2 g C m^{-2} y^{-1}, $t = 3.64$, $p = 0.001$).

5.3.5 Annual NECB and road induced C loss

At the bog, as hypothesized, the RI areas were sources of C in both 2016 (89.9 g C m^{-2} y^{-1}) and 2017 (108.9 g C m^{-2} y^{-1}), but reference areas were sinks in both years (Table 5.4). In contrast, at the fen, both RI areas (-744.7, -310.9 g C m^{-2} y^{-1}, in 2016 and 2017, respectively) and reference areas were sinks of C in both years (Table 5.4), but with a substantial reduction in the size of the C sink in 2017, therefore, supporting hypothesis three.

The estimated road induced C losses in 2016 and 2017 were ~ 7.97 and 7.40 Mg C y^{-1} for each km of the road, respectively.

Table 5.3 Annual net ecosystem exchange (NEE), above and below ground net primary productivity (NPP_{ag} and NPP_{bg}), Tree litter (L_{tree}), methane fluxes (CH_4) and net ecosystem carbon balance (NECB) with standard error (SE) on the upstream (up), downstream (down), and reference (ref) areas of high impact (HI) and low impact (LI) transects at the bog in 2016 and 2017, Carmon Creek, Peace River, Alberta

Plots	NEE[a,b]	NPP_{ag}	NPP_{bg}	L_{tree}	CH_4	NECB[b]
			2016			
HI	19 (49)	50 (15)	11(3)	-5(2)	10 (5)	-7 (50)
Down	-53(72)	80 (24)	18(5)	-8(3)	1 (0)	-110 (65)
Up	90 (59)	19 (14)	4(3)	-2 (1)	19 (10)	96 (62)
LI	221(65)	54 (16)	12 (4)	-5.6(2)	5 (1)	187(62)
Down	383(101)	86 (23)	19(5)	-9 (2)	3 (1)	325(102)
Up	59(34)	23 (17)	5(4)	-2(2)	6(1)	49(35)
Ref	-38(25)	162 (0)	36(0)	-17 (0)	1 (0)	-153(25)
			2017			
HI	17(66)	83(34)	24(19)	-8(3)	17(7)	-15(82)
Down	-91(65)	139(58)	1(0)	-14(6)	31(13)	-189(80)
Up	125(108)	21(15)	48(37)	-2(1)	4 (3)	159(119)
LI	267(108)	68(19)	15(4)	-7(2)	15(4)	233(109)
Down	404(127)	116(28)	9(2)	-12(3)	26(6)	330 (136)
Up	129(171)	19(12)	21(8)	-1(1)	4(3)	136(171)
Ref	-45(229)	206(0)	0.89(0.2)	-21 (0)	45(0)	-191(229)

[a]NEE included understory gross ecosystem productivity (GEP), understory ecosystem respiration (ER) and tree root respiration. [b.] negative values indicate uptake by the ecosystem.

Table 5.4 Annual net ecosystem exchange (NEE), above and below ground net primary productivity (NPP_{ag} and NPP_{bg}), methane fluxes (CH_4) and net ecosystem carbon balance (NECB) with standard error (SE) on the upstream (up), downstream (down), and reference (ref) areas of high impact (HI) and low impact (LI) transects at the fen in 2016 and 2017, Carmon Creek, Peace River, Alberta.

Plots	NEE[ab]	NPP_{ag}	NPP_{bg}	CH_4	NECB[b]
			2016		
HI	-78 (25)	92 (21)	19(5)	1(0.2)	-183 (81)
Down	-8 (5)	85(33)	19(7)	1(0)	-111 (43)
Up	-147 (52)	101(26)	20(6)	1 (0)	-255 (95)
LI	-77 (29)	162 (43)	32(9)	1(0)	-252 (102)
Down	-17 (12)	133(70)	26 (14)	1(0)	-161(45)
Up	-137 (56)	191(53)	37(11)	1(0)	-344 (125)
Ref	-179 (89)	178(0)	39(0)	1(0)	-395 (130)
			2017		
HI	-25 (8)	81.9(18.6)	2.2(0.5)	17 (4)	-202 (53)
Down	-15 (5)	77.6(30.9)	1.8(0.3)	17 (7)	-78 (13)
Up	-34 (17)	86.8(21.2)	2.6(1.0)	17 (5)	-126 (36)
LI	-65 (13)	123.2(31.2)	1.7(0.5)	27.1 (6.9)	-189 (56)
Down	-20 (24)	96.8 (47.8)	1.7(0.4)	21.3(10.5)	-87 (19)
Up	-111 (55)	149.7(40.9)	1.7(0.9)	32.9 (9.0)	-292 (85)
Ref	-101 (37)	169.5(0.3)	3.1(1.3)	37.3(0.0)	-305 (105)

Annual net ecosystem exchange of CO_2 (NEE) included understory gross ecosystem productivity (GEP), understory ecosystem respiration (ER) and tree root respiration. [b.] negative values indicate uptake by the ecosystem.

5.4 Discussion

Natural variation in hydrological properties (e.g., WT position, moisture content), vegetation type and cover, and soil temperature drive both local and regional level C dynamics in peatlands (Alm et al., 1999; Chivers et al., 2009; Limpens et al., 2008; Munir et al., 2015). Roads crossing through peatlands bring changes in the peatland ecohydrological conditions and impact the rate of C cycling of the adjacent peatlands. In the current research, we found that the presence of an access road crossing the peatland impacted the RI areas by increasing T5 in areas close to the road (2 m), raising the WT on the upstream side of the road and creating a deeper WT on the downstream side of the road, and reducing vegetation cover, largely through clearance on the one side of the road. Though the road associated impacts were more pronounced in the bog, in line with the hypotheses and previously observed hydrologic impacts, we observed differences in the C cycling between RI and reference areas, and within the RI areas of both bog and fen study sites.

5.4.1 Understory CO_2 fluxes

The construction of an access road in the bog converted the understory in RI areas from sinks (assuming they were similar to reference areas) to sources of CO_2 in 2016, but not in 2017. In contrast, both reference and RI areas in the fen were sinks of CO_2 in both study years. The absence of vegetation cover on the upstream areas (up to 18 m) of the road in 2016 due to recent road construction could be associated with the greater emission of CO_2 from the understory in 2016 in the bog. Although clearing tended to lead to reduced CO_2 uptake in the present study, the construction of roads has potential to enhance GEP in the short-term on the flooded side of the road due to rapid colonization of non-hummock forming moss species (Ballantyne et al., 2014; Sulman et al., 2010) and aerenchymatous plants, although this would need to balance the loss of overstory productivity. In fact, vegetation establishment in 2017 on vegetation cleared areas likely

helped in reducing understory CO_2 emission from RI areas of the bog in 2017. The drier conditions in downstream areas may enhance woody plant growth and thus GEP, as observed by Miller (2011). While there is some indication of this in the present study (Figure 5a), it is difficult to separate the hydrologic impact (and thus the impact of culvert position) on vegetation from clearing near the road. Clearing vegetation adjacent to roads is common (personal observation), but studies on a larger number of peatland road crossings will help to better quantify the specific hydrological impacts on plant productivity. In the fen, since the understory vegetation was fully recovered by 2016, I did not observe a significant difference in the understory CO_2 fluxes between RI and reference areas. The lack of road effect was likely also linked to the limited hydrologic impact of the road at the fen.

As hypothesized, the analyses of understory CO_2 fluxes within RI areas showed that the downstream areas were greater sources of CO_2 (positive $NEE_{max.us}$) than upstream areas at the bog. However, compared to HI downstream areas, the LI downstream side of RI areas at the bog was an even greater source of understory CO_2 emissions. This was primarily influenced by downstream areas (2 and 6 m) of one HI transect (Transect 2), which contributed ~65% to the downstream LI understory CO_2 fluxes and also had the deepest WT position of all measured (-38.9 and -47.6 cm in 2016 and 2017, respectively). The omission of transect 2 from analyses resulted in no significant differences between the understory CO_2 fluxes between LI and HI downstream areas ($t = 1.58$, $p = 0.26$). Since there was no significant difference in terms of Tree NPP between downstream areas of transect 2 (45.72 g C m^{-2} y^{-1}) and other LI downstream areas (41.53 g C m^{-2} y^{-1}; $t = 1.23$, $p = 0.55$), the greater understory CO_2 emissions could be linked with accelerated microbial decomposition in the downstream areas of this transect linked to the particularly dry conditions. Though we did not measure microbial decomposition rate, the downstream sides of the bog may

have experienced an increase in heterotrophic respiration as a result of lower WT position and warmer T5 (Saraswati et al., 2019; Chapter 3), conditions known to stimulate the microbial activity and overall ER (Chivers et al., 2009).

The observed greater positive $NEE_{max.us}$ fluxes from RI downstream areas resulted from the substantially higher ER from those areas. The enhanced ER at the downstream side of the road may be related to the stimulated vascular plant growth in response to the higher T5, and deeper WT position resulting from the road construction. The combined effect of the enhanced T5 and lowered WT resulted in relatively dry conditions at the downstream areas compared to other areas of the peatland – a condition normally favorable to the woody vegetation growth (Miller et al., 2015; Moore & Knowles, 1989; Willier, 2017) and increasing ER due to the stimulated tree root respiration rate (Aurela et al., 2007; Bubier et al., 2003; Cai et al., 2010; Lohila et al., 2011). It is estimated that in treed bogs, tree root respiration could contribute ~24% to the total ER (Hermle, Lavigne, Bernier, Bergeron, & Pare, 2010) at the rate of ~ 3.25 g CO_2 m^{-2} d^{-1} (Munir, Perkins, Kaing, & Strack, 2015). Despite an increase in woody vegetation growth, the substantially dry conditions have the potential to shift the bog from a sink to a source of CO_2 (Munir & Strack, 2014).

At the fen site, higher T5 and deeper WT also contributed positively to the ER and $GEP_{max.us}$, respectively, and all areas were net sinks of the CO_2 (negative $NEE_{max.us}$). However, there was no significant variation in T5 ($F_{8,414}$ = 0.76, p = 0.34), WT position ($F_{8,408}$ = 0.01, p = 0.992), understory ER, $GEP_{max.us}$, and $NEE_{max.us}$ (Table 5.1) as a result of road construction (e.g., impacts of side of the road, culvert distance and distance from the road). Since the road was constructed almost parallel to the water flow direction in the fen, this is likely the reason for the minimum

impacts of the road on hydrological variation (Partington et al., 2016; Chapter 2) and consequently on CO_2 fluxes.

5.4.2 NPP_{ag} variations and annual NECB

Though there was no recovered tree NPP on the vegetation cleared areas (2 and 6 m upstream) of the bog, shrub growth in some areas of the fen resulted in some NPP_{ag} (e.g. downstream 2 m areas; 33.9 g C m^{-2}). However, that NPP at downstream 2 m areas in the fen is substantially lower than reference areas and RI areas where vegetation was not cleared. We measured higher tree NPP_{ag} in the downstream areas and closer to the road because increased T5 and deeper WT on the downstream areas may have favored the tree growth after road construction. In upstream areas, shallow WT position, resulted in a significant reduction in tree NPP_{ag} (even in areas with no vegetation clearance) compared to downstream and reference areas. Tree NPP_{ag} almost ceased in the upstream areas of the bog on transect 1 (HI transect with no vegetation clearance) after road construction. This is linked to the dieback of trees (personal observation) and understory vegetation as a result of long-term ponding conditions after road construction.

The construction of resource access roads induced substantially reduced C uptake from the adjacent study sites in both study years, thereby supporting hypothesis three. At the bog, the annual NECB data showed that the RI areas were sources of C in both 2016 and 2017, but reference areas were sinks in both 2016 and 2017 at the bog and all areas were sinks at the fen. The road induced estimated C losses (i.e., reduced C uptake or conversion to C source) for each km of the road remained almost the same in both years (~ 8 Mg C y^{-1}), despite observed interannual and spatial variability at RI areas in terms of NEE_{us}, ER, GEP, and tree and shrub NPP as a result of road impact on environmental factors (e.g. WT position, vegetation cover, and T5).

This suggests that the construction of all-season access roads has disturbed the function of adjacent peatland areas. However, we could have underestimated the total C loss from RI areas as NECB reported here did not incorporate the carbon loss in the form of DOC and POC, which may contribute up to 10% of C cycling in peatlands (Billett et al., 2004). Also, our estimate does not include the one-time loss of C due to road construction and vegetation clearance and persistent loss of C sink potential along the road. If all of those were also included, our road induced C loss could have substantially increased. This clearly indicates that the construction of roads across these boreal peatlands have increased C emissions from adjacent peatland areas, resulting in additional anthropogenic emissions related to peatland disturbance that are not currently accounted.

In general, the placement of culverts was helpful in connecting the surface and sub-surface water flow (Saraswati et al., submitted; Chapter 2). However, due to the vegetation clearance during road construction, it was difficult to determine the association of culverts and C dynamics in the present study. Overall, culverts did not substantially reduce the road's impact on C exchange in the present study. It is possible that the local ecohydrology, vegetation dynamics, and C cycling in peatlands with relatively old road crossings (e.g. > 5 years old) may be different than what was observed in this study with road crossings < 3 years. Therefore, longer term studies of road impact on peatland function, particularly related to C exchange are recommended.

5.5 Chapter conclusions

The construction of resource access roads has reduced the carbon sink in adjacent peatland areas. At both study sites, the WT position at the reference areas were deeper compared to road impacted (RI) areas in both years. Also, the road resulted into greater T5 in RI areas than reference areas in both sites. Within the RI areas of the bog, the LI downstream areas were greater sources of CO_2 (ER and $GEP_{max.us}$) than other areas, whereas, at the fen, there was no significant differences within

RI areas in term of understory CO_2. Environmental factors such as T5, WT position and vegetation cover controlled the $GEP_{max.us}$, $NE_{max.us}$ and ER in both study sites. However, road associated impacts on understory CO_2 fluxes were pronounced in the bog, but not in the fen because hydrologic impacts were greater in the former due to the orientation of the road nearly perpendicular to the local slope.

The average tree NPP_{ag} at the reference areas of the bog was greater in both study years compared to the RI areas in the bog and fen. In both study areas, the plots located at vegetation clearance areas were devoid of tree NPP_{ag}. Tree NPP_{ag} almost ceased in the upstream areas on the of the bog, even at areas with minimal vegetation clearing, due to dieback of trees as a result of long-term ponding conditions after road construction. Overall, the annual NECB estimates indicated that the RI areas were sources and reference areas were sinks of C in both years at the bog. In contrast, all areas were sinks in the fen. However, when averaged across both study sites, road induced impacts were almost same in both study years (i.e. ~ 8 Mg C km^{-1} y^{-1} considering a 40 m road impacted area). This study, for the first time, provides an estimated road induced C loss from two boreal peatlands and this could be useful in estimating total carbon loss from road fragmented peatlands. The outcomes of the study can also help industries and infrastructure developers in minimizing the impacts of road by aligning the road parallel to the local flow direction when and where possible, improving road construction design (e.g., an adequate number of culverts) and considering hydrogeological setting to reduce hydrologic impacts. Future studies are recommended in multiple peatlands and incorporating C loss as a result of DOC and POC exports in order to comprehensively understand the road associated impacts on peatland C cycling.

Chapter 6: Conclusions and recommendations

6.1 Summary of results

This study showed that the construction of resource access roads disturbed surface and sub-surface water flow at the forested bog, but the effect was minimal at the shrubby fen. The observed hydrological variations in the bog were primarily linked to the road aligned perpendicular to the water flow direction, and the culvert position. Overall at the bog, the shallowest and deepest depth to water table (DTW) positions were measured in transects far from culverts, at the HI (> 20 m away from a culvert) upstream 2 m and HI downstream 2 m plots, respectively. In the bog, the road greatly reduced daily flow (5 mm d^{-1}) in HI areas. Although culverts were shown to improve hydrologic connection across the road, we also observed that culverts provide a point source of water to downstream areas in peatlands, with chances of water not redistributing well.

As a result of altered DTW, the access road had significant impacts on enzyme activities in the bog, where the road was perpendicular to water flow, but not in the fen, where water flow was largely parallel to the road. Significantly higher phenol oxidase and hydrolase activities were observed in the road disturbed areas compared to undisturbed areas. Together the results show an interlinked pattern of variations in enzyme activities in response to resource roads that bisect peatlands. The phenol oxidase, glucosidase, sulfatase, xylosidase, and glucosaminidase activities were significantly higher in the areas with a deep DTW, that is areas downstream of the road, particularly those close to the road and far from culverts. Lower activities of phenol oxidase, glucosidase and sulfatase were measured in areas with the shallowest WT position (upstream areas close to the road and far from culverts). Similarly, significant variations in enzymatic activity in relation to culvert presence, distance from the road, or the side of the road could be linked with variations in WT depth, phenolic concentration, and pH through their significant correlation with

phenol oxidase and hydrolase enzyme activities. The deeper DTW on the downstream side of access roads, due to blockage of water flow by the road, has the potential to enhance extracellular enzyme activities, likely altering carbon sequestration rates in peatlands.

The effects of WT had up to an order of magnitude increase in CH_4 emission from road disturbed areas compared to reference areas at the bog. In the bog, I found substantially greater CH_4 fluxes from the HI upstream areas closer to the road due to the observed shallower DTW and warmer peat temperature. In contrast, the fen exhibited minimal impacts of the road on CH_4 fluxes. The difference between the study sites was largely due to difference in position of the road relative to local slope. I estimated that road crossings in two Canadian boreal peatlands are enhancing atmospheric emissions by more than 100 kg CH_4 per km road, creating an additional source of anthropogenic GHGs. However, results from this study suggest that road associated impacts can be minimized by aligning roads parallel to the water flow direction when and where possible, considering hydrogeological setting before road construction, clearing less vegetation, and placing adequate culverts.

Finally, the presence of roads across peatlands reduced the C sink in adjacent areas. At both study sites, the WT position at the reference areas were deeper compared to the average of road impacted (RI) areas in both years. Within the RI areas of the bog, the LI downstream areas had understories that were greater sources of CO_2 (ER: ecosystem respiration and $GEP_{max.us}$) than other areas, whereas, at the fen, there was no substantial differences within RI areas in terms of understory CO_2 exchange. Environmental factors such as soil temperature at 5 cm depth, DTW, and vegetation cover controlled the $GEP_{max.us}$, $NE_{max.us}$ and ER in both study sites. However, road associated impacts on understory CO_2 fluxes were pronounced in the bog, but not in the fen because hydrologic impacts were greater in the former.

The average tree NPP_{ag} at the reference areas of the bog was greater in both study years compared to the RI areas in the bog. Similar trend was also noticed at the fen. In both study areas, the plots located at vegetation clearance areas close to the road were devoid of tree NPP_{ag}. Tree NPP_{ag} almost ceased in the upstream areas on the of the bog, even at areas with minimal vegetation clearing, due to dieback of trees as a result of long-term ponding conditions after road construction. Overall, the annual NECB estimates indicated that the RI areas were sources and reference areas were sinks of C in both years at the bog, but all areas were sinks in the fen. However, road induced impacts averaged across both study sites were similar in both study years (i.e. ~ 8 Mg C km^{-1} y^{-1} considering a 40 m road impacted area).

6.2 Significance of research

This study provides a valuable starting point to estimate the potential impact of roads on two boreal peatland hydrology and C exchange as it, for the first time, has quantified the impacts of widespread disturbance (roads crossings) and provided mitigating strategies to reduce the anthropogenic impacts on forested, northern peatlands. This is the first study to quantify the extent that culverts improve hydrologic connection across the road, while it was also observed that culverts provide a point source of water to downstream areas in peatlands, with chances of water causing shallow DTW or even flooding in these areas. This work has shown that road effects on peatland hydrology could also be minimized by aligning roads parallel to the water flow direction when and where possible and considering hydrogeological setting before road construction. If water flow is perpendicular to the road, adequate spacing and installation of culverts could help to reduce flow obstruction.

This study, for the first time, provides an estimated road induced CH_4 and C loss from two boreal peatlands and these values could be useful in estimating total CH_4 emissions and C loss from road

fragmented peatlands, and ultimately be used to upscale estimates to larger geographical areas. However, as this study focused on only two peatlands with road alignments and construction specific to these locations, the full range of impacts has likely not been captured. The outcomes of the study also help industries and infrastructure developers in minimizing the impacts of road by aligning the road parallel to the local flow direction when and where possible. Availability of topographic maps during construction along with detailed understanding of underlying substrates' permeability and hydraulic conductivity would guide the general flow direction and can be used to assist with road alignment decisions, but changing orientation may not be possible as roads are constructed to connect the infrastructure. Therefore, permeable road designs and installing adequate number of culverts to reduce hydrologic impacts on adjacent peatlands are likely more feasible options.

6.3 Future research and recommendations

The current study showed impacts of road crossings on the ecohydrology, enzyme activities, CH_4 and C dynamics of the adjacent peatlands. However, this study was conducted in peatlands with relatively recent road crossings (e.g., 2 years at the bog). Therefore, I recommend conducting more studies in peatlands with old road crossings (e.g., > 10 years) because in the longer term, the impacts on adjacent peatland areas can either be intensified or stabilized. It is also recommended to re-conduct a study after some years (e.g., a decade) at the bog and fen sites to compare results and find the trajectory of road associated impacts from the baseline provided by the current research. Also, the pattern of changes over time may depend upon the road orientation as I observed at the fen. Also, determining temporal variations (monthly or seasonally or yearly) of enzymatic activity, plant nutrients and organic matter decomposition rate would lead to better understanding of overall impacts of road on peat accumulation in the adjacent peatlands.

Since, for this study, the impact of road on enzyme activities was measured one time, and hydrology, CH_4 and C dynamics were measured two times, I recommend temporal and spatial expansion of the current study i.e. to conduct multi-year studies with similar objectives (impact of road on hydrology, enzyme activities, CH_4 and C dynamics) in peatlands representing larger geographical coverage. This study was conducted in a forested bog with the road gradient perpendicular to the water flow and a shrubby rich fen with a road gradient parallel to the water flow in Alberta. I recommend additional studies at a wider range of peatland types (e.g., swamps) and road orientations to accurately quantify the full extent of impacts of roads on boreal or other regions' peatland GHG exchange. I recommend that those studies should also incorporate C losses as a result of dissolved organic carbon (DOC) and particulate organic carbon (POC) exports in order to comprehensively understand the road associated impacts on peatland C cycling. Long term studies evaluating effects of shifts in plant communities on CH_4 emission in road constructed peatlands are also recommended.

While considering the impacts of roads on adjacent peatlands, the first option is to avoid the construction of access roads across peatlands; however, if this is not possible, building bridges, using more permeable road designs (permeable fills), building on a corduroy base, or brush mats, and rock fills, aggregate mattress/aggregate seam with geotextile materials would be beneficial (Partington et. al., 2016). Such options will improve hydrologic connections while maintaining the bearing capacity of roads, but studies that specifically quantify their ability to mitigate hydrologic impacts and alterations to C exchange are needed. Minimizing the clearance of vegetation on the sides of the road during road construction is recommended, because the vegetation clearance on one side of the road potentially reduces the C sink and the effectiveness of culverts to mitigate C cycling changes in the adjacent peatland.

In addition, I recommend studying the flow direction and magnitude, and calculating the specific spacing and size of culverts before constructing roads, as road impact on the peatland ultimately depends on expected water flow perpendicular to the road, which will be driven by local slope and hydraulic conductivity. The present culvert spacing practice in peatlands (i.e., 100 to 250 m if ponding conditions are expected) may not be effective in connecting hydrology in road impacted peatlands as the current study found that the culverts may connect water movements up to ~ 15 m distance.

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Appendix 1: Hydrolase and phenol oxidase assay

Hydrolase assay

The MUF-labeled substrates (Table A1.1) were dissolved in methyl cellosolve (2-ethoxyethanol; Sigma Aldrich, UK), without interrupting the activities of the enzymes (Hoppe, 1983). Also, 1000 mL of MUF-labeled substrate solutions were prepared for each enzyme by dissolving (400 µM for all substrates and 200 µM for Phosphatase) in 20 mL of methyl cellosolve (Dunn et al., 2014) and prepared substrates were kept at 4°C. In addition, the stability of solutions was verified by checking the fluorescence intensity. Simultaneously, in a separate beaker, 0.09 g of MUF free acid was dissolved in 20 mL methyl cellosolve and 500 mL ultrapure water to prepare a MUF standard solution of 1000 µm. The prepared standard solution was diluted in 2 mL Eppendorf centrifuge vials (Eppendorf UK Ltd, Stevenage, UK) as per requirements to prepare standard sample solution (described below).

Table A1.1: MUF- labeled substrates used for estimating hydrolase activities.

Substrate	Enzyme	Molecular weight	Enzyme commission number
4-MUF β-D-glucopyranoside	β-D-glucosidase	338.3	3.2.1.21
4-MUF sulfate potassium salt	Arylsulphatase	294.3	3.1.6.1
4-MUF β-D-xylopyranoside	β-D-xylosidase	308.3	3.2.1.37
4-MUF N-acetyl-β-D-glucosaminide	N-acetyl-β-D-glucosaminidase	379.4	3.2.1.96
4-MUF phosphate	Phosphatase	256.2	3.1.3.2

Hydrolase assays were performed in a stomacher bag (Seward, West Sussex, UK). For assays, six stomacher bags (five bags for five substrates and one for standard sample solution) were prepared for each sampling site. In each stomacher bag, approximately 1 g of peat sample was kept and stored at field temperature. Thereafter, 7 mL of the relevant substrate was poured into the

appropriate bag and homogenized for 30 seconds. Then, all bags were incubated at field temperature for 60 minutes, except phosphates that were incubated for 45 minutes when phosphates release maximum fluorescent (MUF) (Freeman, Liska, Ostle, Jones, & Lock, 1995). Five minutes before the end of incubation, 1.5 mL of solution from each bag was transferred into the labeled 1.5 mL centrifuge vials (Fischer Scientific, UK) and centrifuged at 14,000 rpm for 5 minutes. While waiting during the centrifugation, 50 µL of ultrapure water was added to the relevant microplate wells. After centrifugation, 250 µL of the supernatant from each enzyme sample was added to the relevant wells in the microplate (96-wells flat bottom black, Fischer Scientific, UK).

Simultaneously, for the standard sample solution, each standard/control sample was prepared by adding 7 mL of deionized water maintained at field temperature into a stomacher bag with approximately 1 g of peat (Dunn et al., 2014). However, no incubation time was needed for the calibration curve samples. The same centrifugation speed and time were used for the standards as well. Similarly, 50 µL of diluted standards and 250 µL of the supernatant from each standard sample were added to the relevant microplate wells. The Flex station multimode reader (SpectroMax M2e plate reader) was used for the enzymatic activities of hydrolases and phenol oxidase. The MUF labeled substrates of hydrolase can fluorescence at 450 nm emission and 350 nm excitation (Freeman et al., 1995). The obtained fluorescence values from the Flex station were converted into enzymatic activities of phenol oxidase and hydrolase based on Dunn et al. (2014). Enzyme activities were expressed in activity (nmoles) of enzymes released per gram of dry soil per minute.

Phenol oxidase assay

For phenol oxidase, the model substrate used was 10 mM solution of L-DOPA (L-3, 4-dihydroxy phenylalanine; Pind et al., 1994). I followed the same method as the hydrolase assay for the phenol oxidase assay sample preparation. Two stomacher bags of samples were prepared. One bag with an approximately 1 g of peat without substrate labeled as "blank" and another bag with an approximately 1 g of peat with L-DOPA labeled as "substrate". The blank bag was prepared to read the background absorbance of the sample at 475 nm for each sample, which was used to calculate the phenol oxidase activity of the sample. Firstly, 9 mL of ultrapure water was added to both blank and L-DOPA bags followed by 30-second homogenization. Afterwards, 10mL of ultrapure water was added to the blank bag and 10 mL of L-DOPA to the substrate bag. Again, both bags were homogenized for 30 seconds, followed by incubating all bags at field temperature for 10 minutes. The incubation time was predetermined for the samples so that linear oxidation of L-DOPA would occur. It is important to predetermine the incubation time before phenol oxidase assays as it can vary with the soil types.

After incubation, both bags were gently homogenized with hands and poured into three (1.5 mL) centrifuge tubes and centrifuged at 14,000 rpm for 5 minutes. Followed by pipetting 300 µL of the supernatant from each tube into a clear 96 well Sterilin Microplate. The activity of phenol oxidase was expressed as µmol of product formed (dopachrome or 2-carboxy-2,3-dihydroindole- 5,6-quinone) per minute per gram of dry soil.

Appendix 2: Parameters for empirical models

Table A2.1 Parameters for empirical models to estimate gross ecosystem photosynthesis (GEP) and ecosystem respiration (ER) of the understory for each sample plot of the bog site. Error in the estimate net understory carbon dioxide exchange (ENEus) is also given.

					GEP		ER		ENEus
Plot	**SI[a]**	**DT[b]**	**CL[c]**	**Period**	**α**	**GP_{max}**	**R_{ref}**	**E0**	**g C m^{-2}**
				2016					
CC-B1-E2	U	2	HI	May-Jun	-0.0370	-37.7200	10.9900	128.5600	20.6
				Jul-Sep	-0.0370	-7.7200	10.9900	128.5600	
CC-B1-E20	U	20	HI	May-Jun	-0.0308	-8.9132	6.6660	300.0030	34.1
				Jul-Sep	-0.0308	-8.9132	14.6900	40.6200	
CC-B1-E6	U	6	HI	May-Jun	-0.0074	-2.5045	5.2510	573.3340	12.9
				Jul-Sep	-0.0074	-2.5045	5.2510	573.3340	
CC-B1-W2	D	2	HI	May-Jun	0.0491	-1.9391	8.4320	157.8310	53.9
				Jul-Sep	0.0491	-1.9391	26.3350	58.7500	
CC-B1-W20	D	20	HI	May-Jun	-0.0155	-51.9627	13.2180	233.0070	35.0
				Jul-Sep	-0.0476	-44.7781	26.6400	197.0550	
CC-B1-W6	D	6	HI	May-Jun	-0.0274	-77.6856	8.4802	150.0370	44.1
				Jul-Sep	-0.0250	-64.4247	12.9300	54.9900	
CC-B2-E2	U	2	LI	May-Jun	0.0056	-1.0309	3.9550	188.2320	53.8
				Jul-Sep	-0.0361	-37.0190	9.2700	30.7100	
CC-B2-E20	U	20	LI	May-Jun	-0.0183	-18.7755	9.1150	85.4000	30.6
				Jul-Sep	-0.0245	-11.8020	14.3600	64.7300	
CC-B2-E6	U	6	LI	May-Jun	0.0245	-1.5169	6.8690	118.1400	33.2
				Jul-Sep	0.0245	-1.5169	5.0200	148.3400	
CC-B2-W2	D	2	LI	May-Jun	0.0871	-5.7966	26.6200	129.2520	61.0
				Jul-Sep	-0.1601	-18.312	35.235	35.3252	
CC-B2-W20	D	20	LI	May-Jun	-0.2436	-8.9221	10.1325	61.2845	62.9
				Jul-Sep	-0.0387	-82.0451	9.4100	386.4400	
CC-B2-W6	D	6	LI	May-Jun	0.0637	-3.8671	14.4870	260.7870	62.9
				Jul-Sep	0.0637	-3.8671	30.1930	96.8100	
CC-B3-E2	U	2	HI	May-Jun	-0.0058	-0.9933	3.0306	70.5946	52.4
				Jul-Sep	0.0042	-0.5069	3.4800	46.5100	
CC-B3-E20	U	20	HI	May-Jun	-0.0678	-0.0678	8.7010	119.9670	10.5
				Jul-Sep	-0.2963	-20.9440	9.4900	39.6320	
CC-B3-E6	U	6	HI	May-Jun	-0.1139	-19.8600	5.7370	131.8040	35.5
				Jul-Sep	-0.1139	-19.8600	1.2200	408.1700	
CC-B3-W2	D	2	HI	May-Jun	0.0269	-1.8003	3.4890	262.6700	25.6
				Jul-Sep	-0.0802	-15.5058	11.0100	21.2600	

CC-B3-W6	D	20	HI	May-Jun	-0.0737	-8.3116	8.6690	129.8690	43.3
				Jul-Sep	-0.0825	-20.7744	15.9600	37.6400	
CC-B3-W20	D	6	HI	May-Jun	-0.0315	-87.2021	5.4820	231.2430	65.5
				Jul-Sep	-0.1535	-34.3472	18.0500	38.3800	
CC-B4-E2	U	2	LI	May-Jun	-0.1831	-9.6133	13.8700	15.7300	72.4
				Jul-Sep	-0.0302	-29.0998	15.8700	22.9800	
CC-B4-E6	U	20	LI	May-Jun	-0.1831	-9.6133	2.6800	68.2300	37.0
				Jul-Sep	-0.1166	-7.3176	9.0500	5.1100	
CC-B4-E20	U	6	LI	May-Jun	-0.0396	-9.2437	7.5041	176.8350	40.1
				Jul-Sep	0.0555	-2.7264	6.2100	18.6600	
CC-B4-W2	D	2	LI	May-Jun	0.1469	-5.1847	9.9040	200.0400	29.2
				Jul-Sep	-0.0546	-18.3496	18.5300	246.9500	
CC-B4-W6	D	20	LI	May-Jun	-0.3878	-27.9208	15.8200	231.2030	56.8
				Jul-Sep	-0.1732	-41.1154	28.7400	54.9000	
CC-B4-W20	D	6	LI	May-Jun	-0.0285	-102.7063	28.4570	100.3750	67.1
				Jul-Sep	-0.0635	-25.6464	32.7100	84.3400	
CC-B5-E2	U	2	LI	May-Jun	-0.0692	-2.4242	3.8900	21.3100	59.6
				Jul-Sep	-0.1057	-2.0516	0.6500	463.3900	
CC-B5-E6	U	20	LI	May-Jun	-0.0729	-1.9331	5.0100	38.4400	11.6
				Jul-Sep	-0.0409	-1.6321	2.1300	-58.6400	
CC-B5-E20	U	6	LI	May-Jun	-0.0301	-20.0366	7.6140	107.2700	37.1
				Jul-Sep	-0.0640	-16.9922	9.1900	22.9800	
CC-B5-W2	D	2	LI	May-Jun	-0.0138	-72.2512	12.7100	40.5900	41.5
				Jul-Sep	-0.0138	-72.2512	37.4300	65.4100	
CC-B5-W20	D	20	LI	May-Jun	-0.0570	-28.7851	10.0700	328.8700	108.6
				Jul-Sep	-0.0570	-28.7851	37.7400	67.3800	
CC-B5-W6	D	6	LI	May-Jun	-0.0387	-25.6908	18.1800	27.9000	105.6
				Jul-Sep	-0.2744	-35.0349	33.8600	17.1700	
CC-B6-E2	U	2	HI	May-Jun	0.0223	-1.6057	8.0000	241.8320	74.4
				Jul-Sep	-0.0402	-3.1001	5.3500	6.7300	
CC-B6-E20	U	20	HI	May-Jun	-0.0014	0.5948	7.1600	136.3850	18.9
				Jul-Sep	-0.0014	0.5948	10.9600	74.7000	
CC-B6-E6	U	6	HI	May-Jun	0.0184	-1.2109	5.2660	135.6700	19.5
				Jul-Sep	-0.0635	-5.9678	9.0800	15.8700	
CC-B6-W2	D	2	HI	May-Jun	-0.0348	-67.1740	10.3800	150.2200	10.4
				Jul-Sep	-0.0877	-44.7181	31.7900	48.0500	
CC-B6-W20	D	20	HI	May-Jun	-0.0376	-5.4120	6.8800	210.3000	85.0
				Jul-Sep	-0.0499	-54.1090	23.7200	39.2100	
CC-B6-W6	D	6	HI	May-Jun	-0.0175	-24.2934	6.0600	2.2000	84.1
				Jul-Sep	-0.0478	-41.1311	9.8600	38.9400	
CC-B-NA1	N	50	N	May-Jun	0.0309	-1.7215	6.9900	167.9100	55.8

				Jul-Sep	-1.6840	-22.0270	13.0600	151.1600	
CC-B-NA2	N	50	N	May-Jun	0.0010	-0.2819	3.7300	206.0700	44.9
				Jul-Sep	-0.4690	-17.9240	9.5500	233.1300	
CC-B-NA3	N	50	N	May-Jun	0.0007	-0.1073	4.2922	6.4970	55.5
				Jul-Sep	-0.1012	-22.9051	9.3800	195.3100	
				2017					
CC-B1-E2	U	2	HI	May-Jun	-0.0370	-7.7200	8.9489	15.4384	24.0
				Jul-Sep	-0.0370	-7.7200	1.5326	571.4555	
CC-B1-E20	U	20	HI	May-Jun	-0.0045	-0.6468	6.3168	53.7945	15.2
				Jul-Sep	-0.0045	-0.6468	7.0864	33.5153	
CC-B1-E6	U	6	HI	May-Jun	-0.0160	-1.3390	7.8180	23.6050	19.5
				Jul-Sep	-0.0160	-1.3390	2.1900	268.6510	
CC-B1-W2	D	2	HI	May-Jun	-0.0692	-19.4521	12.2590	68.7470	26.8
				Jul-Sep	-0.0692	-19.4521	10.8810	216.6710	
CC-B1-W20	D	20	HI	May-Jun	-0.0366	-78.2956	14.9700	95.4900	39.1
				Jul-Sep	-0.0366	-78.2956	9.5114	369.2079	
CC-B1-W6	D	6	HI	May-Jun	0.0637	-3.8671	13.8280	18.5520	30.7
				Jul-Sep	0.0637	-3.8671	9.4860	202.0470	
CC-B2-E2	U	2	LI	May-Jun	-0.0703	-48.1604	16.9170	73.2210	29.5
				Jul-Sep	-0.1323	-38.2234	16.9170	73.2210	
CC-B2-E20	U	20	LI	May-Jun	-0.0183	-18.7755	3.8397	152.2371	11.7
				Jul-Sep	-0.0183	-18.7755	9.2430	152.0280	
CC-B2-E6	U	6	LI	May-Jun	-0.0676	-26.0386	7.2720	226.8670	35.0
				Jul-Sep	-0.0528	-43.5440	9.4920	168.2740	
CC-B2-W2	D	2	LI	May-Jun	-0.0720	-22.7610	29.7510	73.2620	68.6
				Jul-Sep	-0.1136	-24.8566	50.5920	45.5310	
CC-B2-W20	D	20	LI	May-Jun	-0.0378	-41.4684	16.4950	55.3770	44.2
				Jul-Sep	-0.0378	-41.4684	23.0770	35.0290	
CC-B2-W6	D	6	LI	May-Jun	-0.1945	-16.5570	33.2090	64.3210	74.2
				Jul-Sep	-0.0606	-35.3467	29.6350	76.4010	
CC-B3-E2	U	2	HI	May-Jun	-0.0424	-9.8734	7.1180	9.9180	18.9
				Jul-Sep	-0.1543	-19.4754	10.8770	47.1820	
CC-B3-E20	U	20	HI	May-Jun	-0.1139	-19.8600	6.7862	5.3541	13.9
				Jul-Sep	-0.1139	-19.8600	7.8630	71.1910	
CC-B3-E6	U	6	HI	May-Jun	-0.0731	-14.3441	8.6530	43.5980	81.5
				Jul-Sep	-0.1550	-47.5159	12.4755	17.5743	
CC-B3-W2	D	2	HI	May-Jun	-0.0533	-26.2939	22.2000	259.9190	41.0
				Jul-Sep	-0.0533	-26.2939	30.0233	354.9809	
CC-B3-W6	D	20	HI	May-Jun	-0.1023	-20.7611	19.4770	39.8320	30.6
				Jul-Sep	-0.0761	-29.8342	29.9000	124.3550	
CC-B3-W20	D	6	HI	May-Jun	-0.0583	-47.1043	17.5640	32.8570	89.9

				Jul-Sep	-0.0506	-48.7519	4.8240	522.8370	
CC-B4-E2	U	2	LI	May-Jun	-0.0307	-74.0221	26.9350	31.1870	78.0
				Jul-Sep	-0.1319	-51.0023	26.9350	31.1870	
CC-B4-E6	U	20	LI	May-Jun	-0.1485	-25.7799	15.5700	17.3300	82.2
				Jul-Sep	-0.1850	-52.3617	21.9650	33.6350	
CC-B4-E20	U	6	LI	May-Jun	0.0555	-2.7264	5.2475	391.8665	99.8
				Jul-Sep	0.0555	-2.7264	2.9680	599.0330	
CC-B4-W2	D	2	LI	May-Jun	-0.1040	-30.1610	26.3640	122.9850	105.0
				Jul-Sep	-0.1882	-34.7550	40.0350	62.9280	
CC-B4-W6	D	20	LI	May-Jun	-0.0931	-34.8912	23.3510	20.1850	29.0
				Jul-Sep	-0.1105	-54.6081	30.3420	28.0430	
CC-B4-W20	D	6	LI	May-Jun	-0.1008	-21.8588	38.5490	99.4220	66.0
				Jul-Sep	-0.1008	-21.8588	38.5490	99.4220	
CC-B5-E2	U	2	LI	May-Jun	-0.5700	-17.1000	83.0000	169.0000	174.1
				Jul-Sep	-0.0906	-26.8278	183.3900	1169.9400	
CC-B5-E6	U	20	LI	May-Jun	-0.0905	-2.9668	17.8900	333.6700	206.9
				Jul-Sep	-0.0384	-6.9325	20.3130	262.1320	
CC-B5-E20	U	6	LI	May-Jun	-0.0446	-18.0183	7.2390	116.1610	
				Jul-Sep	-0.0750	-14.7181	3.0334	1089.1717	
CC-B5-W2	D	2	LI	May-Jun	-0.0508	-20.1387	14.2870	281.4510	65.1
				Jul-Sep	-0.0508	-20.1387	21.0600	163.9700	
CC-B5-W20	D	20	LI	May-Jun	-0.0506	-50.6613	17.9030	104.9710	64.5
				Jul-Sep	-0.1105	-33.6514	95.3400	462.5500	
CC-B5-W6	D	6	LI	May-Jun	0.2900	-21.5400	21.7140	189.4760	75.0
				Jul-Sep	-0.2726	-44.4125	41.6060	29.2490	
CC-B6-E2	U	2	HI	May-Jun	-0.5700	-17.1000	16.8860	360.4900	64.6
				Jul-Sep	-0.5700	-17.1000	64.0300	165.5800	
CC-B6-E20	U	20	HI	May-Jun	-0.1054	-6.6010	5.4632	126.0834	112.9
				Jul-Sep	-0.1054	-6.6010	2.4260	449.2070	
CC-B6-E6	U	6	HI	May-Jun	-0.0326	-2.0797	10.5530	76.0220	42.7
				Jul-Sep	-0.0326	-2.0797	13.3660	26.9020	
CC-B6-W2	D	2	HI	May-Jun	-0.0700	-28.0600	14.9400	199.2600	72.6
				Jul-Sep	-0.1142	-31.8610	35.4379	71.6526	
CC-B6-W20	D	20	HI	May-Jun	-0.0670	-21.8429	11.1064	194.3776	48.5
				Jul-Sep	-0.1374	-43.8979	17.9030	104.9710	
CC-B6-W6	D	6	HI	May-Jun	-0.0890	-12.1970	10.3248	22.0478	18.0
				Jul-Sep	-0.0890	-12.1970	19.4520	336.4840	
CC-B-NA1	N	50	N	May-Jun	-0.0880	-18.5300	10.9545	59.6838	21.5
				Jul-Sep	-0.0788	-28.5312	9.1938	140.8166	
CC-B-NA2	N	50	N	May-Jun	-0.1007	-10.1204	26.2840	382.3420	251.8
				Jul-Sep	-0.1007	-10.1204	25.9992	166.8933	

CC-B-NA3	N	50	N	May-Jun	-0.0692	-36.5456	12.4640	105.0170	39.8
				Jul-Sep	-0.0692	-36.5456	15.9870	63.0110	
CC-B-NA4	N	50	N	May-Jun	-0.0389	-48.7173	22.8360	103.4050	57.0
				Jul-Sep	-0.0389	-48.7173	29.6590	123.6500	
CC-B-NA5	N	50	N	May-Jun	-0.0502	-39.2530	7.8590	172.7240	46.0
				Jul-Sep	-0.0502	-39.2530	6.8320	467.5760	
CC-B-NA6	N	50	N	May-Jun	-0.0608	-36.5367	7.8590	172.7240	48.9
				Jul-Sep	-0.0608	-36.5367	7.8590	172.7240	

[a] Side of the road (D – Downstream, U – Upstream, N – Reference), [b] Distance from the road (m), [c] Culvert position (HI – Culvert located at > 20 m from the plots, LI – Culvert located at < 2 m).

Table A2.2 Parameters for empirical models to estimate gross ecosystem photosynthesis (GEP) and ecosystem respiration (ER) of the understory for each sample plot of the fen site. Error in the estimate net understory carbon dioxide exchange (ENEus) is also given.

					GEP		ER		ENEus
Plot	**SI[a]**	**DT[b]**	**CL[c]**	**Period**	**α**	**GP_{max}**	**R_{ref}**	**E0**	**g C m^{-2}**
				2016					
CC-F1-N2	D	2	L	May-Jun	-0.05	-28.52	2.16	1057.04	143.1
				Jul-Sep	-0.06	-22.89	18.34	258.48	
CC-F1-N6	D	6	L	May-Jun	-0.09	-37.96	10.56	659.12	139.6
				Jul-Sep	-0.10	-48.14	54.48	100.32	
CC-F1-N20	D	20	L	May-Jun	-0.06	-30.89	14.62	254.20	152.2
				Jul-Sep	-0.08	-20.63	34.97	301.2400	
CC-F1-S2	U	2	L	May-Jun	-0.1068	-38.4831	19.7600	282.8550	62.0
				Jul-Sep	-0.0975	-46.7156	28.4990	171.5200	
CC-F1-S20	U	20	L	May-Jun	-0.1234	-48.4283	25.3000	190.0370	57.5
				Jul-Sep	-0.1132	-41.2828	21.2140	276.6580	
CC-F1-S6	U	6	L	May-Jun	-0.0343	-28.0297	10.5700	16.6500	90.3
				Jul-Sep	-0.0354	-27.8116	8.6610	327.4010	
CC-F2-N2	D	2	H	May-Jun	-0.0458	-71.7145	16.7700	194.8000	82.1
				Jul-Sep	-0.1063	-45.9277	7.3100	690.5000	
CC-F2-N20	D	20	H	May-Jun	-0.1565	-75.1985	32.8900	105.3400	68.5
				Jul-Sep	-0.1192	-44.3568	14.3090	391.5770	
CC-F2-N6	D	6	H	May-Jun	-0.0903	-52.1386	34.2400	170.4400	62.4
				Jul-Sep	-0.0951	-43.9108	2.8980	1267.8200	
CC-F2-S2	U	2	H	May-Jun	0.0154	-6.3730	9.3730	181.7550	114.1
				Jul-Sep	-0.1046	-48.8799	20.1259	122.0322	
CC-F2-S20	U	20	H	May-Jun	-0.0399	-20.2300	3.6000	651.2600	94.8
				Jul-Sep	-0.0415	-23.2048	10.0900	370.1200	
CC-F2-S6	U	6	H	May-Jun	-0.0959	-47.2727	27.5200	68.4600	58.8
				Jul-Sep	-0.0885	-47.6736	23.4210	167.9070	
CC-F3-N2	D	2	L	May-Jun	-0.0062	-93.4200	19.6650	328.0620	92.3

				Jul-Sep	-0.1163	-40.9277	30.7300	53.9700	
CC-F3-N20	D	20	L	May-Jun	-0.0474	-63.6568	16.8220	444.5320	92.1
				Jul-Sep	-0.0919	-34.3568	28.4630	75.9310	
CC-F3-N6	D	6	L	May-Jun	-0.0712	-50.8610	23.7800	288.1240	150.9
				Jul-Sep	-0.0551	-53.9108	29.3060	7.4410	
CC-F3-S2	U	2	L	May-Jun	-0.0436	-20.8587	9.6200	426.1250	74.3
				Jul-Sep	-0.0638	-21.4323	13.8900	125.7700	
CC-F3-S20	U	20	L	May-Jun	-0.0600	-12.9750	6.4800	103.9200	105.6
				Jul-Sep	-0.0483	-15.3649	8.8330	70.3320	
CC-F3-S6	U	6	L	May-Jun	-0.0523	-23.3908	16.4900	165.2590	50.0
				Jul-Sep	-0.0861	-28.8220	21.1400	68.4600	
CC-F4-N2	D	2	H	May-Jun	-0.0528	-75.7945	27.4900	119.4300	99.0
				Jul-Sep	-0.0813	-50.9682	24.5860	228.9160	
CC-F4-N20	D	20	H	May-Jun	-0.0503	-26.9940	18.3700	41.0500	115.6
				Jul-Sep	-0.0557	-27.4011	20.0680	54.2040	
CC-F4-N6	D	6	H	May-Jun	-0.1200	-46.1300	26.7660	226.3830	109.3
				Jul-Sep	-0.0525	-10.0963	36.2050	10.0460	
CC-F4-S2	U	2	H	May-Jun	-0.0877	-33.9609	19.0140	77.6000	70.0
				Jul-Sep	-0.0692	-48.8477	23.5030	27.5920	
CC-F4-S20	U	20	H	May-Jun	-0.0489	-17.2006	8.3800	358.6400	95.8
				Jul-Sep	-0.0756	-28.6221	9.7210	13.6760	
CC-F4-S6	U	6	H	May-Jun	-0.0909	-28.8155	26.4200	109.1600	75.5
				Jul-Sep	-0.1081	-49.6765	21.0670	213.7310	
CC-F5-N2	D	2	H	May-Jun	-0.0680	-54.8711	12.8500	277.2900	80.5
				Jul-Sep	-0.1042	-42.6453	12.8500	277.2900	
CC-F5-N20	D	20	H	May-Jun	-0.1181	-46.8125	21.7150	202.6900	85.3
				Jul-Sep	-0.1143	-47.2340	40.7200	22.4855	
CC-F5-N6	D	6	H	May-Jun	-0.0706	-45.1949	19.6700	60.6100	79.5
				Jul-Sep	-0.1223	-43.0498	30.2500	80.5380	
CC-F5-S2	U	2	H	May-Jun	0.0091	-3.2637	12.8800	136.6100	52.5
				Jul-Sep	-0.1207	-40.0574	26.3420	2.4350	
CC-F5-S20	U	20	H	May-Jun	-0.0873	-46.0477	21.9010	150.7970	59.1
				Jul-Sep	-0.1250	-44.8267	22.2450	156.8040	
CC-F5-S6	U	6	H	May-Jun	-0.0387	-28.7973	6.3700	64.7900	25.7
				Jul-Sep	-0.0463	-24.0809	7.6490	169.1540	
CC-F6-S2	U	2	L	May-Jun	-0.0310	-22.2276	5.8600	390.3200	130.4
				Jul-Sep	-0.0618	-20.1758	7.6490	169.1540	
CC-F6-S20	U	20	L	May-Jun	-0.0716	-31.0039	9.0800	148.6000	49.4
				Jul-Sep	-0.2279	-33.4397	11.9990	289.9760	
CC-F6-S6	U	6	L	May-Jun	-0.0610	-24.2276	8.8800	175.7800	51.3
				Jul-Sep	-0.1026	-29.4998	18.6750	10.3680	

CC-F6-N2	D	2	L	May-Jun	-0.0536	-41.3972	7.0200	272.0800	86.5
				Jul-Sep	-0.1190	-22.2000	17.8300	90.2300	
CC-F6-N20	D	20	L	May-Jun	-0.0641	-39.6322	19.1200	182.2100	84.3
				Jul-Sep	-0.3476	-24.5296	32.1300	26.9000	
CC-F6-N6	D	6	L	May-Jun	-0.1207	-41.4591	10.7300	245.0700	111.5
				Jul-Sep	-0.2052	-37.4237	29.5300	104.7000	
CC-F-NA1	N	N	N	May-Jun	-0.0526	-26.8333	14.5300	191.1900	105.0
				Jul-Sep	-0.2221	-30.7753	20.7420	10.7800	
CC-F-NA2	N	N	N	May-Jun	-0.0629	-27.8202	16.4900	258.9700	141.8
				Jul-Sep	-0.0969	-27.4448	26.5400	71.6100	
CC-F-NA3	N	N	N	May-Jun	-0.0634	-27.0194	11.0900	347.2000	93.6
				Jul-Sep	-0.1092	-26.7084	16.7300	11.2100	
					2017				
CC-F1-N2	D	2	L	May-Jun	-0.2012	-48.1525	54.6630	241.1750	506.1
				Jul-Sep	-0.8734	-98.7049	53.0170	10.6240	
CC-F1-N6	D	6	L	May-Jun	-0.2754	-37.8844	38.4530	71.9040	87.6
				Jul-Sep	-0.2141	-88.7684	53.3980	10.7220	
CC-F1-N20	D	20	L	May-Jun	-0.2280	-54.4013	21.3220	135.9060	79.4
				Jul-Sep	-0.1913	-25.2025	36.6230	4.6010	
CC-F1-S2	U	2	L	May-Jun	-0.1099	-37.0970	32.4060	139.5910	69.1
				Jul-Sep	-0.3130	-76.0929	21.6820	467.7700	
CC-F1-S20	U	20	L	May-Jun	-0.2030	-36.4201	17.4310	181.5550	100.7
				Jul-Sep	-0.2164	-48.3754	23.3100	374.2800	
CC-F1-S6	U	6	L	May-Jun	-0.1501	-26.8817	23.3100	374.2800	130.7
				Jul-Sep	-0.0755	-41.5419	23.3100	96.7800	
CC-F2-N2	D	2	H	May-Jun	-0.2482	-49.8352	32.2900	190.6200	113.3
				Jul-Sep	-0.2482	-53.8352	55.4500	66.1300	
CC-F2-N20	D	20	H	May-Jun	-0.1676	-46.5646	32.4850	54.0780	95.1
				Jul-Sep	-0.2749	-53.3580	44.9000	14.3800	
CC-F2-N6	D	6	H	May-Jun	-0.1277	-42.0025	38.5100	171.0800	77.3
				Jul-Sep	-0.2909	-58.9394	39.1900	51.3100	
CC-F2-S2	U	2	H	May-Jun	-0.1091	-40.7977	22.3140	165.9890	91.7
				Jul-Sep	-0.1255	-25.0698	24.6000	167.4300	
CC-F2-S20	U	20	H	May-Jun	-0.2092	-39.3178	20.5150	106.0690	66.5
				Jul-Sep	-0.1755	-51.5419	55.9000	307.7000	
CC-F2-S6	U	6	H	May-Jun	-0.1176	-43.8513	27.0920	146.2300	94.2
				Jul-Sep	-0.1176	-43.8513	31.0600	106.6300	
CC-F3-N2	D	2	L	May-Jun	-0.1187	-54.5624	36.8460	46.1980	140.9
				Jul-Sep	-0.3862	-50.2540	65.6900	495.2000	
CC-F3-N20	D	20	L	May-Jun	-0.2328	-40.0257	19.9238	245.6910	61.6
				Jul-Sep	-0.1525	-33.3348	22.7100	192.5700	

CC-F3-N6	D	6	L	May-Jun	-0.1168	-43.1050	24.0510	132.5980	130.4
				Jul-Sep	-0.2409	-30.3986	24.0510	132.5980	
CC-F3-S2	U	2	L	May-Jun	-0.1012	-42.1840	25.8144	198.8302	112.4
				Jul-Sep	-0.1047	-48.5820	32.3200	15.5600	
CC-F3-S20	U	20	L	May-Jun	-0.2116	-19.1387	35.5220	80.9130	92.7
				Jul-Sep	-0.1029	-52.6642	28.3000	142.1200	
CC-F3-S6	U	6	L	May-Jun	-0.1565	-44.3579	18.1230	143.1160	100.4
				Jul-Sep	-0.1092	-53.1092	35.3100	54.0400	
CC-F4-N2	D	2	H	May-Jun	-0.1195	-25.1915	39.3000	226.0560	74.8
				Jul-Sep	-0.4474	-87.6883	39.9200	134.4300	
CC-F4-N20	D	20	H	May-Jun	-0.2664	-36.6284	36.0780	125.2090	182.5
				Jul-Sep	-0.0911	-49.8144	36.0000	143.6500	
CC-F4-N6	D	6	H	May-Jun	-0.1034	-48.7112	28.6840	15.7820	157.4
				Jul-Sep	-0.3344	-60.3111	15.2700	653.1200	
CC-F4-S2	U	2	H	May-Jun	-0.1315	-47.8278	25.8144	198.8302	90.8
				Jul-Sep	-0.0287	-52.8413	22.9900	229.5800	
CC-F4-S20	U	20	H	May-Jun	-0.0986	-32.0865	35.5220	80.9130	119.4
				Jul-Sep	-0.0782	-34.2105	21.4600	49.6900	
CC-F4-S6	U	6	H	May-Jun	-0.1223	-52.6683	18.1230	143.1160	73.0
				Jul-Sep	-0.3729	-60.8226	38.8400	16.3100	
CC-F5-N2	D	2	H	May-Jun	-0.6254	-29.4249	22.6810	133.6320	57.1
				Jul-Sep	-0.0807	-34.2462	42.5100	776.1200	
CC-F5-N20	D	20	H	May-Jun	-0.1863	-44.5584	25.3640	139.9340	50.0
				Jul-Sep	-0.2503	-62.6088	69.3100	462.3900	
CC-F5-N6	D	6	H	May-Jun	-0.1842	-26.3013	38.1200	114.2430	117.8
				Jul-Sep	-0.2490	-44.7280	43.6700	206.6700	
CC-F5-S2	U	2	H	May-Jun	-0.2416	-21.8455	20.2480	240.8200	88.7
				Jul-Sep	-0.2072	-39.0176	28.2600	112.5000	
CC-F5-S20	U	20	H	May-Jun	-0.1609	-23.7486	33.9150	160.5410	89.8
				Jul-Sep	-0.2059	-54.9531	32.9900	137.8900	
CC-F5-S6	U	6	H	May-Jun	-0.1546	-25.3829	8.8260	71.5570	65.5
				Jul-Sep	-0.0311	-22.7443	10.4400	2.1900	
CC-F6-S2	U	2	L	May-Jun	-0.1051	-44.1593	41.1100	147.6800	146.7
				Jul-Sep	-0.6281	-98.7784	99.9700	193.7600	
CC-F6-S20	U	20	L	May-Jun	-0.0950	-42.8900	14.5040	270.8110	70.9
				Jul-Sep	-0.1409	-35.7548	52.2000	153.6900	
CC-F6-S6	U	6	L	May-Jun	-0.1269	-29.3240	8.2822	24.5317	0.0
				Jul-Sep	-0.0880	-29.3240	8.2822	24.5317	
CC-F6-N2	D	2	L	May-Jun	-0.1658	-29.9946	25.2100	171.6300	113.4
				Jul-Sep	-0.1658	-49.9946	25.2100	171.6300	
CC-F6-N20	D	20	L	May-Jun	-0.1954	-39.4166	28.5563	117.2068	41.1

				Jul-Sep	-0.1954	-49.4166	28.5563	117.2068	
CC-F6-N6	D	6	L	May-Jun	-0.1450	-25.0100	26.3600	226.5200	160.6
				Jul-Sep	-0.2270	-41.1737	26.3600	226.5200	
CC-F-NA1	N	N	N	May-Jun	-0.1479	-43.0120	60.8800	489.9000	106.7
				Jul-Sep	-0.1120	-53.5698	60.8800	489.9000	
CC-F-NA2	N	N	N	May-Jun	-0.1583	-39.5325	24.9300	180.3800	97.1
				Jul-Sep	-0.1470	-54.5389	24.9300	180.3800	
CC-F-NA3	N	N	N	May-Jun	-0.1881	-41.1779	30.4600	44.0000	78.5
				Jul-Sep	-0.1255	-54.2452	30.4600	44.0000	

[a] Side of the road (D – Downstream, U – Upstream, N – Reference), [b] Distance from the road (m), [c] Culvert position (HI – Culvert located at > 20 m from the plots, LI – Culvert located at < 2 m).

Appendix 3: Site Pictures

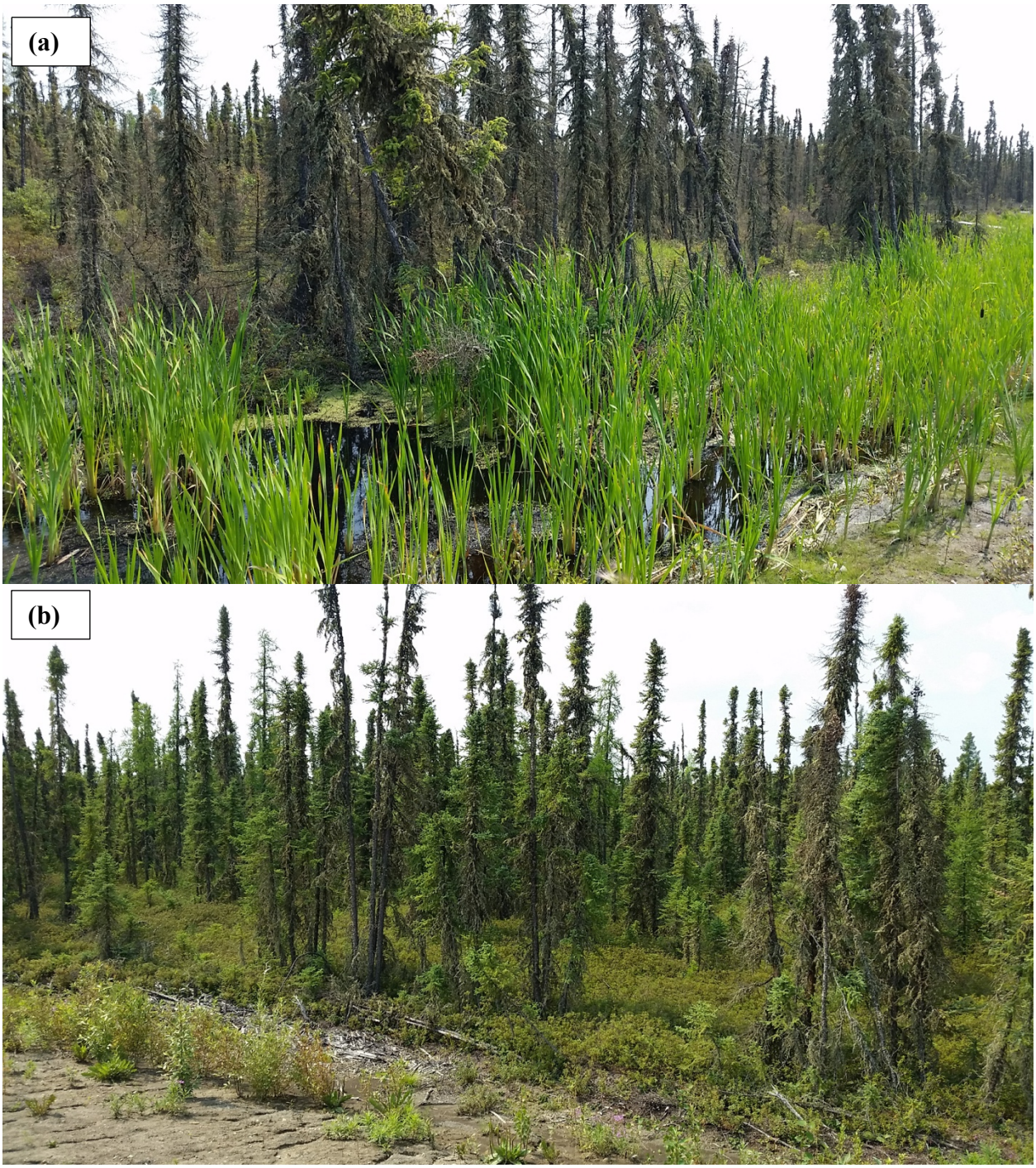

Figure A3.1 Pictures of Bog (a) upstream areas (east side of the road; flooded) showing tree diebacks as a result of long term flooding and (b) downstream areas (west side of the road; dry) showing y conditions leading into more growth of trees at Carmon Creek, Alberta, Canada.

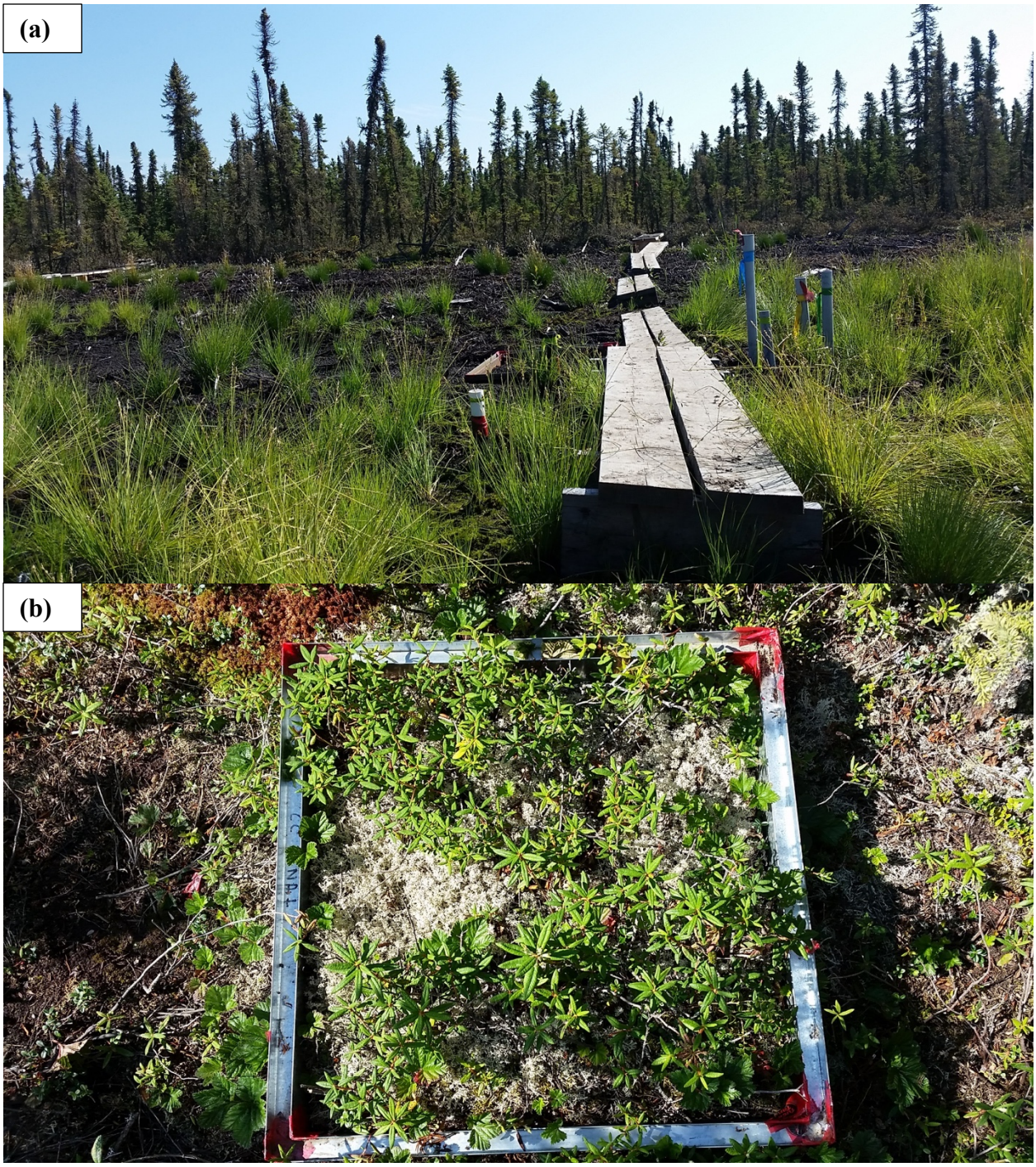

Figure A3.2 Pictures depicting half transect in the bog site with installed water wells, piezometer nest and collars; (b) installed collar can be seen here.

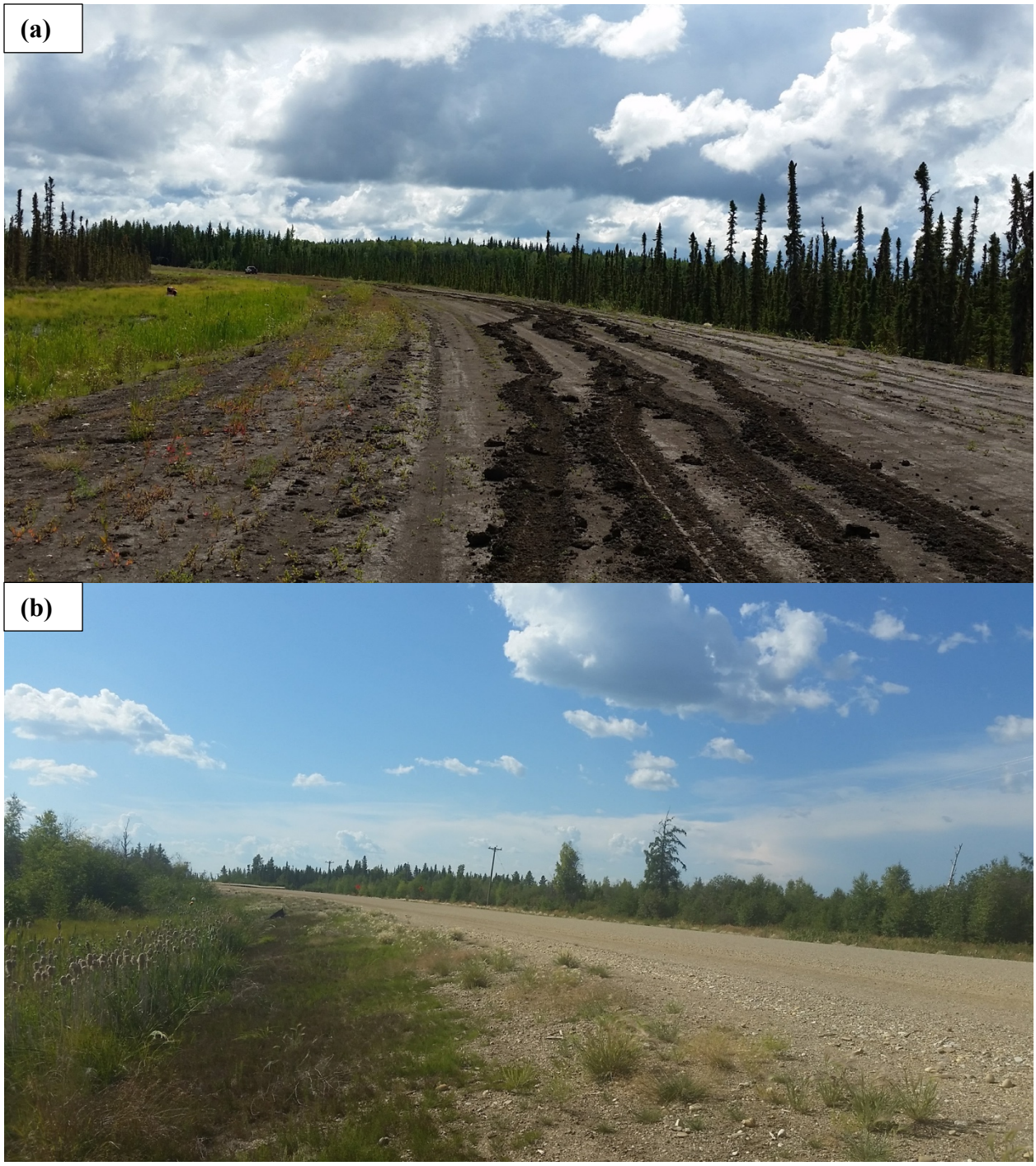

Figure A3.3 Pictures depicting the access road crossings in the (a) a forested bog and (b) a shrubby rich fen, at Carmon Creek, Alberta, Canada.

Figure A3.4 Pictures showing a shrubby rich fen (a) upstream areas (south side of the road) with students taking water samples and measurements and (b) downstream areas (north side of the road).