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Access roads impact enzyme activities in boreal forested peatlands

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Abstract

We investigated the impacts of resource access roads on soil enzyme activities in contrasting forested boreal peatlands (bog and fen). In August 2016, a total of 72 peat samples were collected from twelve 20 m long transects perpendicular to access roads, with a further six samples collected from undisturbed reference areas. Sampling locations represent a range in three variables associated with roads: 1) side of the road (upstream/downstream), 2) distance to a culvert (longitudinal; <2 and >20 m), and 3) distance from the road (lateral; 2, 6, and 20 m). Phenol oxidase and hydrolase (glucosidase, sulfatase, xylosidase, glucosaminidase, and phosphatase) enzyme activities were determined for each sample, in addition to water table depth, phenolic concentration, pH, and peat temperature. The average hydrolase activities in the fen were ~ four times higher than in the bog. At the bog, the water table depth, phenolic concentration, pH and the activities of phenol oxidase, sulfatase, glucosidase, xylosidase and glucosaminidase were all significantly influenced by one or more road associated factors. The highest enzyme activities in the bog occurred on the downstream side of the road at plots located far from the culvert. In contrast, the flow of water in the fen was not perpendicular to the road. Consequently, no significant variations in water table depth, phenolic concentration, pH or enzyme activity were found with respect to road associated factors. Results indicate that road crossings in boreal peatlands can indirectly alter enzyme activities, likely as part of a causal chain following changes to hydrology and redox conditions. Two of six investigated enzymes had significantly higher activities in the road disturbed areas compared to undisturbed areas, suggesting ultimately that roads may enhance organic matter decomposition rates. However, adequate hydrologic connections through culverts and road construction parallel to the water flow can minimize the road-induced impacts.

Keywords: access road, linear disturbance, hydrolase, phenol oxidase, forested peatland, water table

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 and 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 and 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 and Clymo, 2001; Bhatti et al., 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 and 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 et al., 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, 2010; Sinsabaugh and Moorhead, 1994). 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 et al., 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 et al., 1985). Glucosaminidase breaks down chitin (a derivative of glucose) into amino sugars and plays a significant role in nitrogen and carbon cycling (Kang et al., 2005). Phosphatase helps to liberate phosphate from organic matter by hydrolyzing phosphomonoester bonds present in phosphosugars, mononucleotides and phospholipids (Nannnipieri et al., 1979; Turner et al., 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 and Reddy, 1998). Further, under higher temperatures (> 30 ^{o}C), 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 and Kelly, 1994; Levén et al., 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 and 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 and 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 and 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 boreal forested peatlands. We 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 (Fig. 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 (Fig. 1).

2. Methods

2.1. Study sites

The study sites (Fig. 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.

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. We 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 reestablish 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.

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 (Fig. 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.

2.3. Laboratory analyses

In the laboratory, the peat samples were stored at -20 ºC to prevent enzymatic decomposition of peat (Lee et al., 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

Supplementary material. 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 e 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 et al., 2016). Phenolic concentrations were measured using Folin-Ciocalteu phenol reagent by following the method of Box (1983).

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, we 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 α = 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 (Pinheiro et al., 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. Results

3.1. Site hydrology and chemistry

The average WT depth, pH, and EC (Table 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 1; Fig. 3) and the interaction between the side of the road and the location of culvert was close to significant (Tables 1, 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 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 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 1). Culvert distance alone was not explanatory for phenolic concentration variations in the bog (Table 2).

Side of the road was a significant explanatory factor for variation in pH in the bog (Table 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 1). In the bog, the average pH was slightly lower further away from the road (Table 1); however, the effect of distance from the road was not significant (Table 2).

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 2).

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 (Fig. 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; Fig. 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 ($t = 3.161$, $p = 0.02$; Fig. 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 (Fig. 3).

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). Figs. 4 and 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; Fig. 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 (Fig. 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).

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). 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).

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). 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.

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 4) and the fen (r = -0.4, p = 0.03), and with pH and phenolics in the bog (Table 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 4).

4. Discussion

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 et al., 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 we 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). 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 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 and 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 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 et al., 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).

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 1 to 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 and 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 and 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 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 and 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 (Fig. 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.

Our 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.

5. 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.

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Figure captions

Fig. 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, we also hypothesized that the impact will be minimum in areas nearby culverts (light shaded; upstream (UL) and downstream low impact (DL) areas). Reference (undisturbed by road) areas represented by L.

Fig. 2. Study sites, a) a fen and b) a bog, in Carmon Creek watershed, Peace River, Alberta, Canada. Where red dots represent transects > 20m from culverts position, blue dots represent transects <2m away from culverts position and teal dots represent undisturbed reference areas. Yellow arrow heads show the general water flow direction in each study sites.

Fig. 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. All sample values used to construct the boxplots are also shown with different symbols used to identify the individual treatments within each panel.

Fig. 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. All sample values used to construct the boxplots are also shown with different symbols used to identify the individual treatments within each panel.

Fig. 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. All sample values used to construct the boxplots are also shown with different symbols used to identify the individual treatments within each panel.

Table 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

Table 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 (<2m and > 20m);

[d] Perpendicular distance from the road (2m, 6m, and 20m);

Table 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				

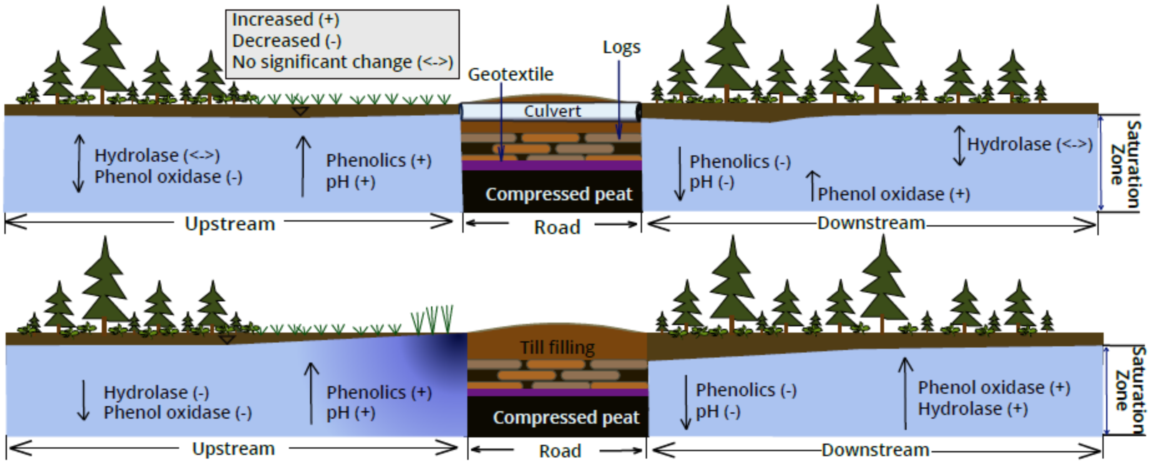
Side	0.19 (1,13)	0.67	2.27 (1,18)	0.15
Culvert	0.01 (1,13)	0.97	0.02 1,18)	0.88
Dist	0.33 (2,13)	0.72	0.53 (2,18)	0.59
Side*Culvert	0.03 (1,13)	0.87	1.30 (1,18)	0.27
Side*Dist	0.23 (2,13)	0.79	0.08 (2,18)	0.92
Culvert*Dist	2.47 (2,13)	0.12	1.34 (2,18)	0.28
Side*Culvert*Dist	0.29 (2,13)	0.76	0.40 (2,18)	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);

Table 4. Correlations between enzyme activities and site characteristics in the bog. Only correlations with $p < 0.25$ are listed.

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



Graphical abstract

Highlights:

- Roads alter local ecohydrology and hydrochemistry that may impact enzyme activity.
- We observed changes in water table and enzyme activity in a road-fragmented bog.
- Water flow parallel to the road in the fen limited road impact on enzyme activity.

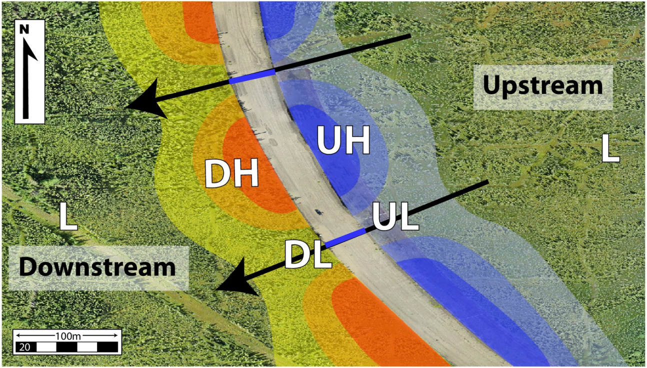


Figure 1

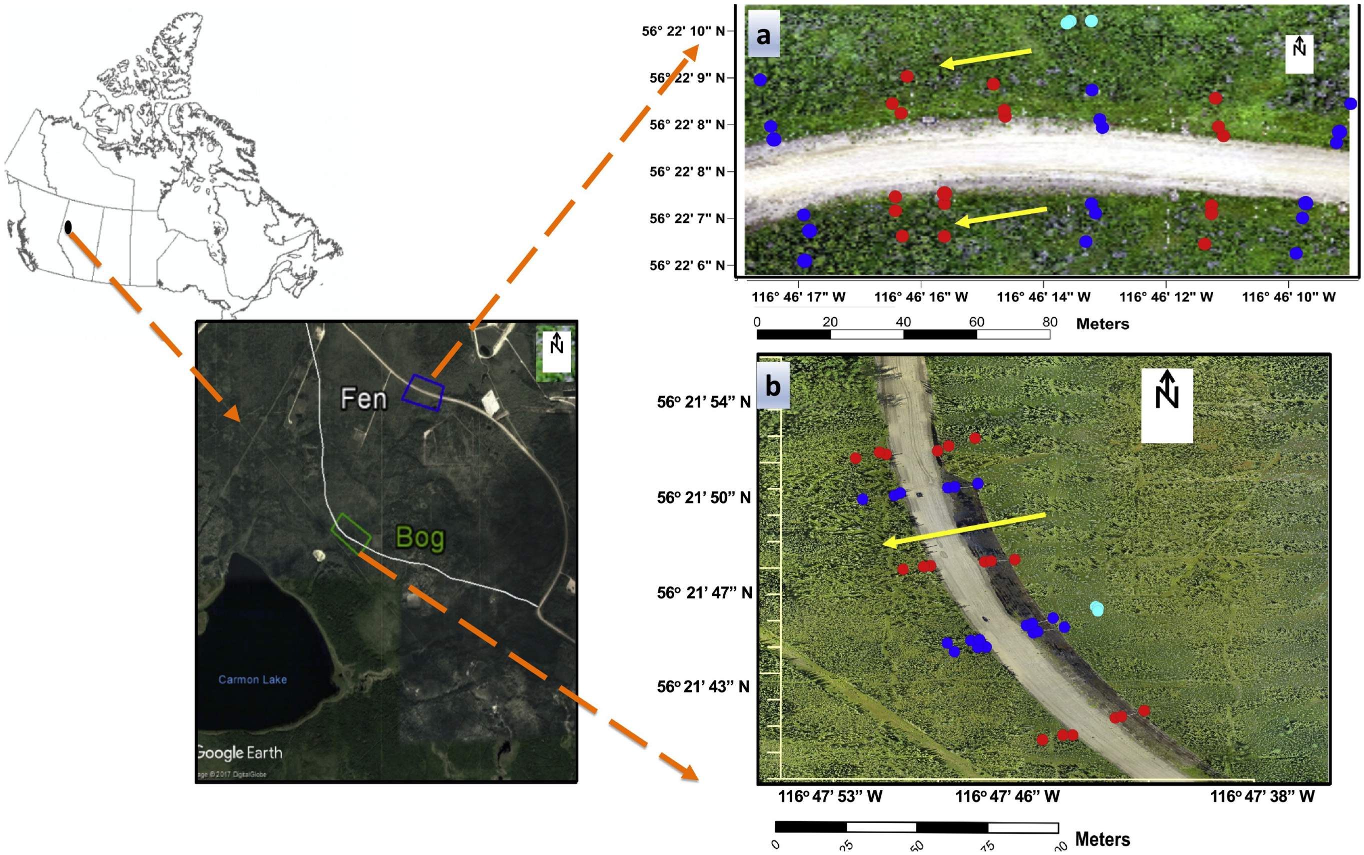

Fen
Bog
Carmon Lake
Google Earth
age ©2017 DigitalGlobe
N
a
56° 22' 10" N
56° 22' 9" N
56° 22' 8" N
56° 22' 8" N
56° 22' 7" N
56° 22' 6" N
116° 46' 17" W
116° 46' 16" W
116° 46' 14" W
116° 46' 12" W
116° 46' 10" W
0
20
40
60
80
Meters
b
56° 21' 54" N
56° 21' 50" N
56° 21' 47" N
56° 21' 43" N
116° 47' 53" W
116° 47' 46" W
116° 47' 38" W
0
25
50
75
Meters

Figure 2

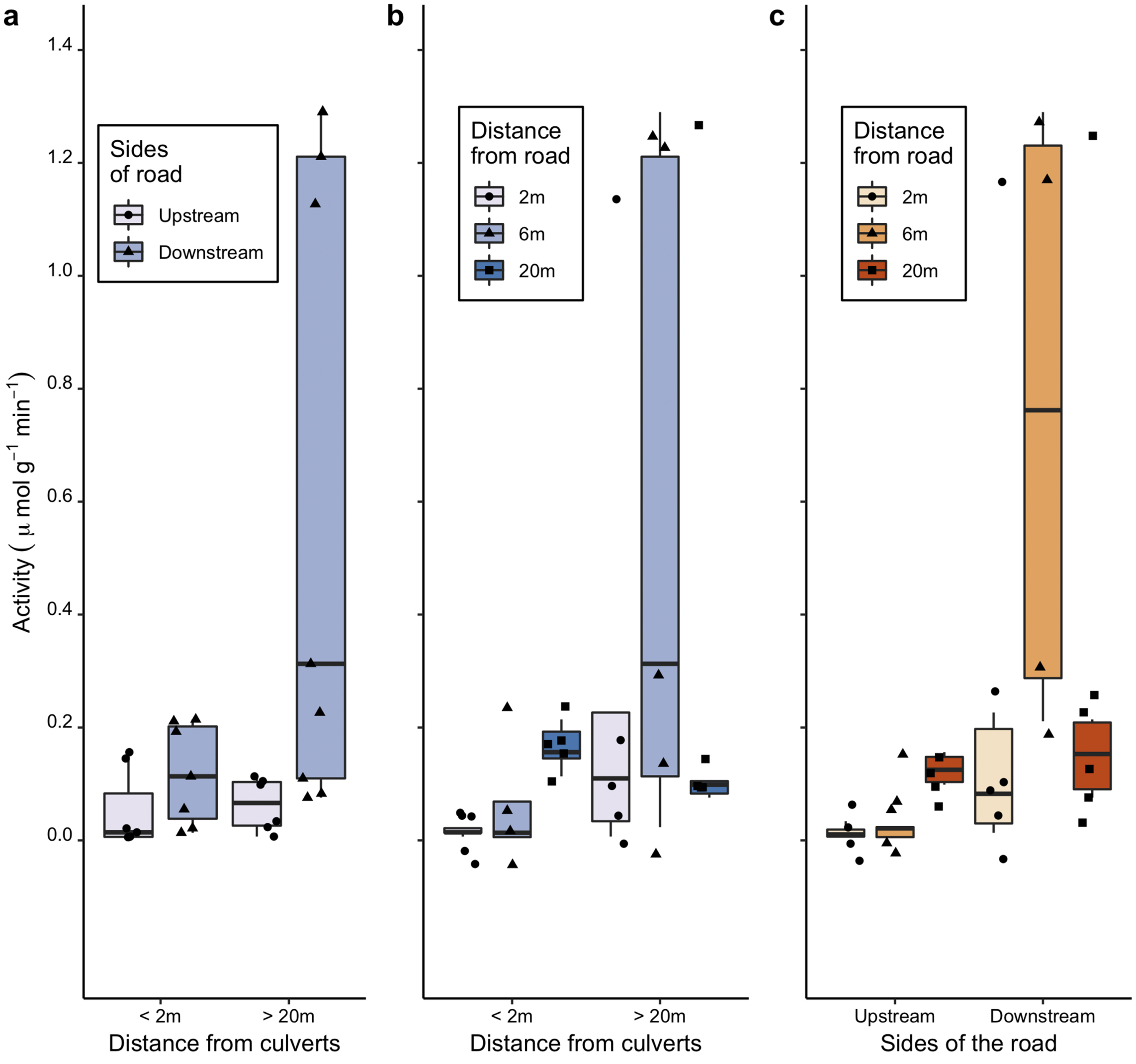

a
b
c
Activity (μ mol g⁻¹ min⁻¹)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Sides of road
Upstream
Downstream
Distance from road
2m
6m
20m
< 2m
> 20m
Distance from culverts
Upstream
Downstream
Sides of the road

Figure 3

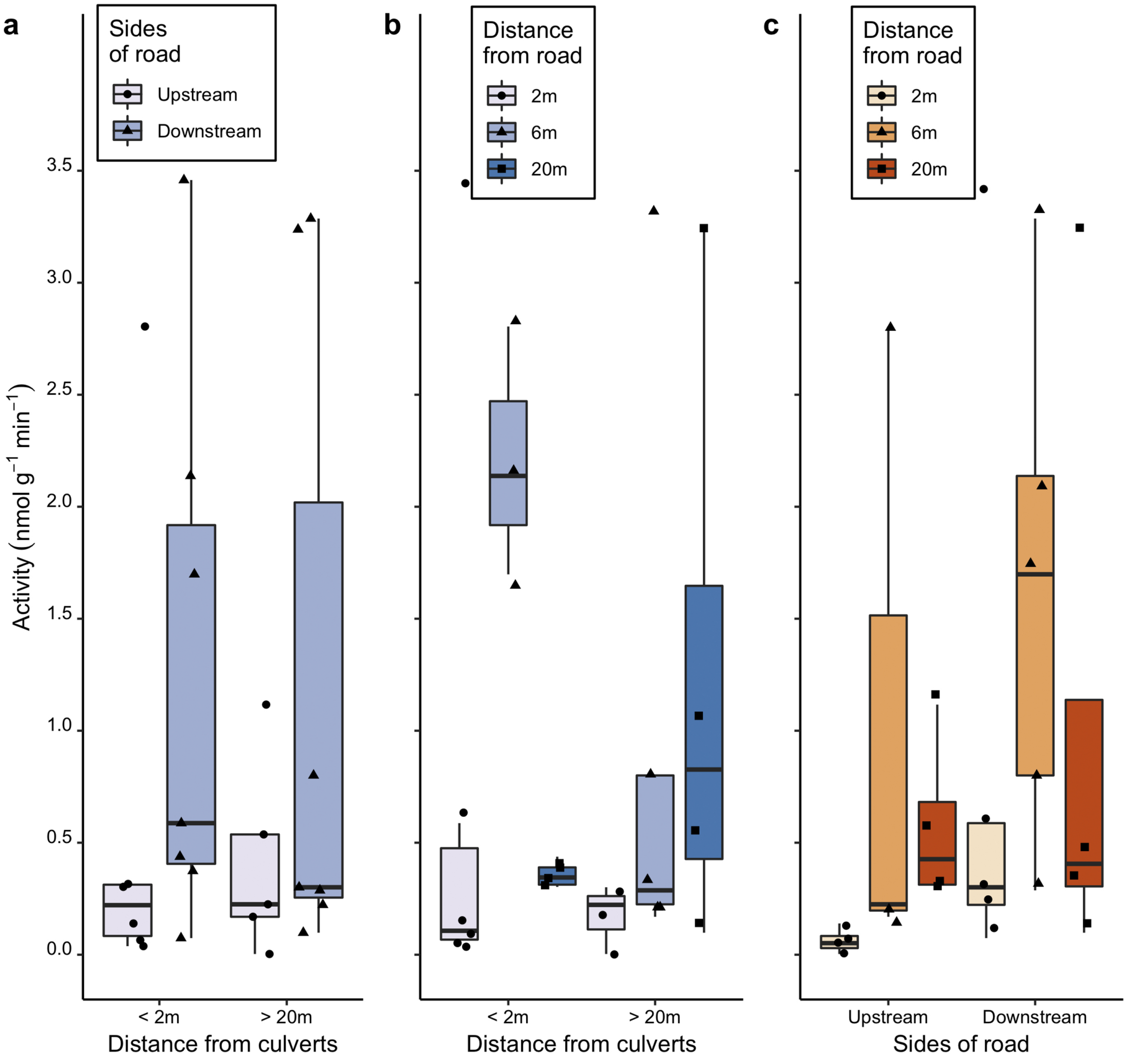

a
Sides of road
Upstream
Downstream
b
Distance from road
2m
6m
20m
c
Distance from road
2m
6m
20m
Activity (nmol g^{-1} min^{-1})
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
< 2m
> 20m
Distance from culverts
< 2m
> 20m
Distance from culverts
Upstream
Downstream
Sides of road

Figure 4

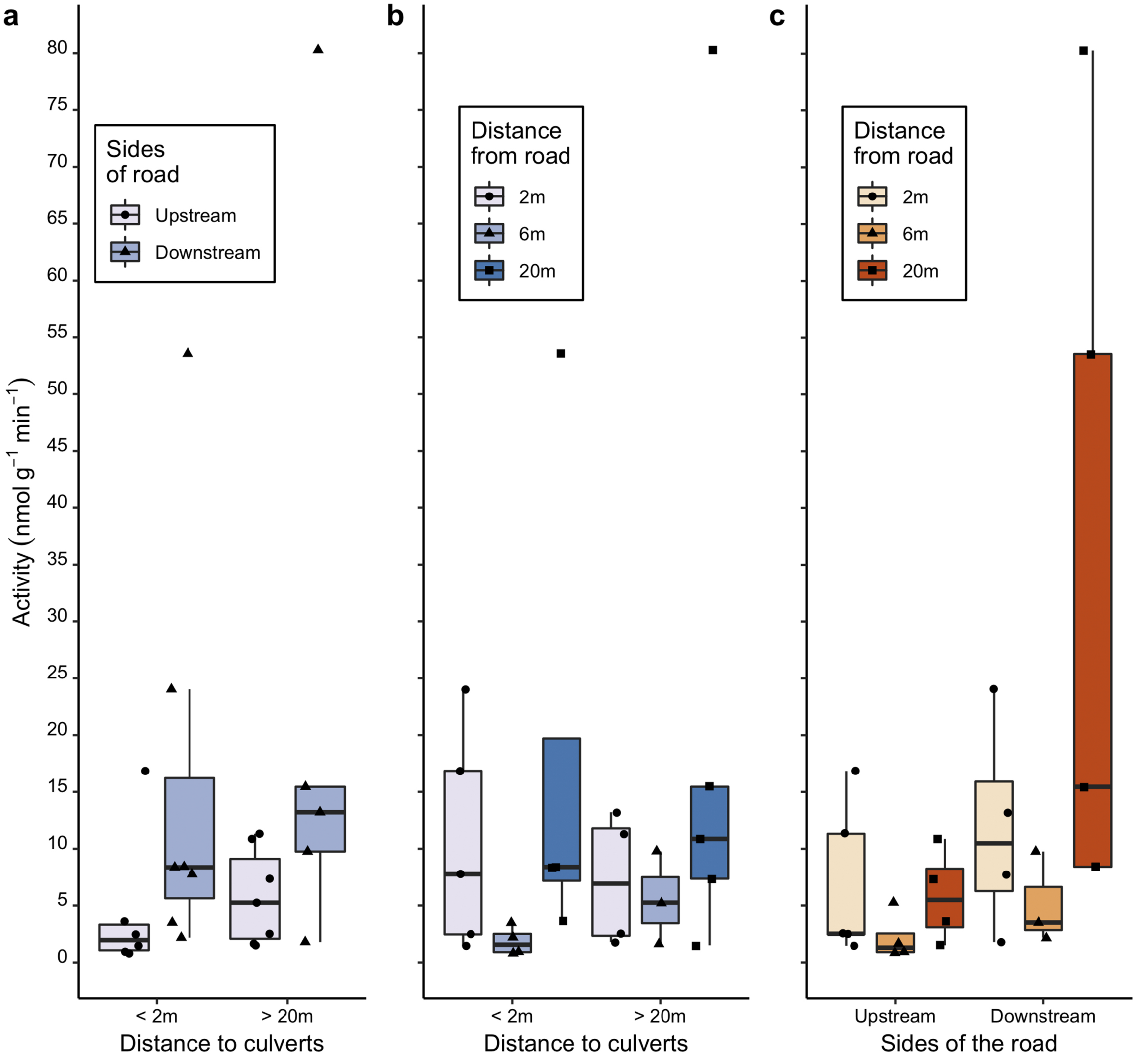

a
b
c
Activity (nmol g^{-1} min^{-1})
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Sides of road
Upstream
Downstream
Distance from road
2m
6m
20m
Distance from road
2m
6m
20m
< 2m
> 20m
Distance to culverts
< 2m
> 20m
Distance to culverts
Upstream
Downstream
Sides of the road

Figure 5