Methane dynamics of a constructed fen in the Athabasca Oil Sands Region, Alberta

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, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

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This thesis is written in manuscript format, and some of the information within the manuscripts may have been previously stated.

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This manuscript reports on data collected at the constructed fen and reference sites near Fort McMurray over the 2015 growing season. M. Strack assisted with the design of the study sites and the development of the manuscript. N. Barlow assisted with biomass processing and analysis. The manuscript, including tables and figures, was written in its entirety by K. Murray and reviewed by the co-authors prior to submission.

Abstract

Oil sands mining activities in the Athabasca Oil Sands Region in northeastern Alberta, Canada have resulted in an extensive amount of land disturbance. The Alberta government requires some reclamation of disturbed land to be to wetland ecosystems, and given the predominance of fen peatlands in the area, fen construction on post-mined landscapes has recently been attempted. Peatlands sequester substantial amounts of carbon over thousands of years due to waterlogged conditions and inefficient decomposition, and on a large time scale provide a cooling effect on the planet's radiative budget. However, peatland conditions are also ideal for production of the strong greenhouse gas methane (CH_4). Natural peatlands emit a significant amount of CH_4 to the atmosphere, particularly following formation when these ecosystems have a net warming effect associated with the large CH_4 flux. Given the knowledge that the conditions that are conducive to CH_4 production and flux in natural peatlands also result in the eventual accumulation of peat and carbon sequestration, understanding the CH_4 dynamics of constructed fens may indicate biogeochemical function, along with the ability of these ecosystems to ultimately accumulate peat, a major goal of reclamation. Further, understanding important controls on CH_4 dynamics from the constructed fen, including vegetation and geochemistry, in comparison to natural sites, is beneficial for the development of recommendation that may result in lower CH_4 flux through vegetation impacts, but appropriate water chemistry for peat accumulation.

For this research CH_4 flux, CH_4 concentration, and variables including vegetation and hydrochemistry were monitored from a constructed fen and two natural reference sites in northeastern Alberta over the 2015 growing season. A factorial greenhouse experiment was also used to understand differences in CH_4 flux, concentration, and oxidation between two vascular plants, *Carex aquatilis* and *Juncus balticus*, planted for fen construction. This greenhouse experiment further considered how water sourced from the reclaimed constructed fen influenced CH_4 dynamics compared to natural rich fen water. Both the field data from 2015 and the greenhouse experiment results found lower CH_4 concentration from constructed fen plots compared to natural fen plots. Differences in hydrochemistry/water chemistry variables were found between constructed fen and natural fen plots in both studies, including evidence of terminal electron acceptors known to influence CH_4 production such as sulfur, iron, manganese, and inorganic forms of nitrogen. While aboveground biomass and productivity in the field was

found to be similar or higher at the constructed fen site compared to the two reference sites, belowground biomass was lower. In the greenhouse experiment, on the other hand, above and belowground biomass and productivity was similar between *Carex aquatilis* and *Juncus balticus* plots. Overall, several vegetation and hydrochemistry/water chemistry variables were found to significantly explain the CH_4 results in the field and greenhouse experiment. For example, in both cases high sulfur at the constructed fen plots decreased CH_4 flux and concentration. Lower CH_4 concentration and higher relative oxidation found from plots including *Juncus balticus* compared to *Carex aquatilis* in the greenhouse experiment suggest that planting *Juncus balticus* in future constructed fen projects may result in lower CH_4 flux. However, CH_4 emissions will likely remain low at constructed fens if water chemistry does not change over time, or if future constructed fen designs are not altered to result in water chemistry more similar to natural sites.

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Chapter 1: Introduction

Peatland ecosystems play a significant role in the global carbon cycle and carbon sequestration (Vitt, 2006). It is projected that between 15-30% of the world's soil carbon is stored as peat in boreal and subarctic peatlands (Limpens et al., 2008). Changes to the carbon stored in undisturbed peatlands depends on (1) net ecosystem exchange of carbon dioxide (CO_2), (2) methane (CH_4) flux resulting from waterlogged conditions, (3) waterborne exchange of dissolved organic, inorganic, and particulate organic carbon (Strack et al., 2008; Lai, 2009). Human activities such as agriculture, drainage, and horticultural peat extraction cause peatlands to change from net sinks to net sources of greenhouse gases (GHGs; Waddington and Price, 2000). In the Athabasca Oil Sands Region (AOSR) near Fort McMurray, Alberta, Canada surface mining associated with oil sands extraction has effectively eliminated a substantial area of peatland ecosystems (Vitt et al., 1996). This has resulted in a tremendous release of carbon to the atmosphere that otherwise would have been stored in peat soil in this region (Rooney et al., 2011). This has led to recent attempts at peatland reclamation through fen construction (Pollard et al., 2012). This study investigates CH_4 dynamics following fen construction.

Restoration of peatlands used for horticulture peat extraction, particularly from cutover bogs, have been studied extensively (e.g. Price et al., 1998; Rochefort et al., 2003; González et al., 2014), and this has led to the development of a North American restoration guide (Quinty and Rochefort, 2003). Procedures for restoring cutover bog sites typically include blocking drainage ditches, introducing vegetation, including *Sphagnum* diaspores, from a nearby natural donor site on the area being restored, covering the vegetation with straw mulch to stabilize the surface and reduce water loss, and possibly applying phosphate rock fertilizer to promote vegetation establishment (Waddington et al., 2010; Strack et al., 2014). This technique has also been tested

for fen restoration (Rochefort et al., 2016). For example, Cobbaert et al. (2004) found successful re-establishment of vascular fen plants when fen donor material was used to restore a minerotrophic surface after horticulture peat harvesting. Research focused on alternative techniques for restoring fen species on harvested or mined peatlands also exists (e.g. Graf and Rochefort, 2008; Cooper and MacDonald, 2000).

Until recently peatland construction on disturbed land with no remnants of a former peatland ecosystem had not been attempted (Price et al., 2010). In the AOSR the Alberta Government requires a portion of reclamation activities to be to wetland ecosystems (OSWWG, 2000); in the past this has resulted in research focused on marsh and open water wetland reclamation (Harris, 2007). Given the prevalence of peatlands, particularly fens, in the AOSR, recent attempts have also been made to construct fen peatlands on post-mined landscapes; two examples of constructed fens are the Nikanotee Fen (Daly et al., 2012) and the Sandhill Fen (Wytrykush et al., 2012). These projects were created using donor peat transferred from a natural peatland (Nwaishi et al., 2015b) and are designed to provide appropriate hydrologic inputs for fen functionality (Price et al., 2010). Specifically, at the Nikanotee constructed fen, techniques used to restore vegetation at cutover peatlands in North America, such as spreading vegetation donor material and mulch on the surface, were tested through the vegetation experimental design (A. Borkenhagen, unpublished). Other methods for establishing fen vegetation were also attempted, including direct seedling plantation and the sowing of seeds.

Peatland restoration results in different GHG emissions compared to natural and unrestored areas (Strack et al., 2014). The measurement of greenhouse gas fluxes at restored peatlands can be used to indicate if these altered ecosystems may eventually sequester carbon as they once did, as this is often a major goal of restoration (Andersen et al., 2010). Further, knowledge of GHG

emissions and controls on emissions from restored, unrestored, and natural sites across Canada can assist in national emission inventory reporting and recommendations to future restoration projects that may minimize GHG fluxes to the atmosphere (Strack et al., 2016a). Methane is a powerful GHG as it effectively traps heat in the atmosphere (IPCC, 2013). Since CH_4 emissions are strongly controlled by water table position (Wilson et al., 2016) and vegetation, especially the presence of vascular species with aerenchymous tissues (Couwenberg and Fritz, 2012), understanding CH_4 flux from restored peatlands can provide information on the hydrologic and ecological functioning of these sites in comparison to natural peatlands. Generally, CH_4 flux has been found to increase following restoration of horticulturally-extracted sites (compared to unrestored areas), that is primarily associated with a rise in water table following re-wetting (Tuittila et al., 2000) and the emergence of vascular vegetation (Waddington and Day, 2007).

Similarly, monitoring GHG emissions including CH_4 from newly constructed fen ecosystems compared to natural sites can indicate hydrological and ecological functioning of these reclaimed ecosystems. Methane dynamics of constructed fens can also be used to understand biogeochemical cycling, as hydrochemistry and the associated redox conditions also influence CH_4 production and emissions (Bridgham et al., 2013). Considering the hydrochemical controls on CH_4 dynamics is important at the Nikanotee constructed fen for several reasons. First, fragmentation occurred to the donor peat used for fen construction, associated with dewatering and transport (Nwaishi et al., 2015b). Further, reclamation materials used for construction, such as tailings sand, are known to influence the water chemistry input of the constructed fen (Pouliot et al., 2012). Finally, elevated sulfur content at the constructed fen may occur due to emissions in the industry-dominated AOSR (Proemse et al., 2012). Nwaishi et al. (2016) reported a low CH_4 flux from the Nikanotee Fen one-year post reclamation compared

to natural sites in the area, associated with differing physical peat properties and hydrochemistry. Continual monitoring at the Nikanotee Fen is valuable to understand how CH_4 dynamics and fen functionality may change over time.

1.1 Objectives

While Nwaishi et al. (2016) reported on CH_4 emissions as well as physical and hydrochemical controls on CH_4 from the Nikanotee constructed fen compared to a nearby rich fen shortly following reclamation across different vegetation cover types, it is beneficial to understand how these results may change over time. Further, understanding how vegetation functional groups, species, and specific plot-scale vegetation parameters influence CH_4 dynamics at a constructed fen compared to natural reference sites can be used to make recommendations to future projects that may result in decreased CH_4 emissions. The objectives of this research were to:

1. Quantify CH_4 emissions and CH_4 pore water concentration across several vegetation cover types at a constructed fen, and between a constructed fen and natural reference sites in the region with similar vegetation.
2. Through a greenhouse experiment, compare CH_4 flux, concentration, and oxidation between two vascular species (*Carex aquatilis* and *Juncus balticus*) used for fen construction and grown in water from a constructed fen or natural rich fen.
3. Determine controls on the CH_4 variables measured in the field or greenhouse experiment to make recommendations to future constructed fen projects from a CH_4 flux perspective.

Chapter 2: Methane emission dynamics from a constructed fen and reference sites in the Athabasca Oil Sands, Alberta

2.1. Introduction

Surface mining activities associated with the extraction of oil sand ore is a common land-use in northeastern Alberta, Canada specifically in the Athabasca Oil Sands Region (AOSR) deposit around Fort McMurray where 896 km^2 of land is affected by oil sands mining (Alberta Government, 2015). Mining in this area disturbs landscapes that were made up of over 40% wetlands, of which 90% were peatlands, pre-disturbance (Vitt et al., 1996). The Alberta Government requires reclamation of mined landscapes after use, a portion of which must be reclaimed to wetland ecosystems (OSWWG, 2000). As peatlands sequester carbon (Loisel et al., 2014) and have a high capacity to store water (Rydin and Jeglum, 2006) it is advantageous to restore disturbed areas to peatlands where possible. Fens are the dominant peatland type in the AOSR (Natural Resources Canada, 2011), an area where potential evapotranspiration exceeds precipitation most years in the sub-humid climate of northeastern Alberta (Devito et al., 2005; Petrone et al., 2007). Recently, the construction of fen peatlands on post-surface mined landscapes has been attempted (Daly et al., 2012; Wytrykush et al., 2012). It has been suggested that the ecosystem function of constructed fen systems may not align with natural sites, since fen creation could result in unique hydrology and water chemistry conditions, causing the eventual development of novel ecosystems (Nwaishi et al., 2015a). Ongoing monitoring to understand how these potential novel ecosystems function should consider carbon dynamics (Wytrykush et al., 2012). Further, it is useful to compare carbon cycling results to natural reference ecosystems, or regional representative peatlands used for the development of reclamation plans, and against which the outcome of constructed fens can be assessed (Daly et al., 2012). Specifically, an enhanced understanding of greenhouse gas (GHG) emissions, including (CH_4), from constructed

fens several years' post-reclamation will provide valuable knowledge about these recently created ecosystems (Nwaishi et al., 2016).

Although natural peatland ecosystems act to sequester carbon through the uptake of carbon dioxide (CO_2), the incomplete decomposition of organic matter in waterlogged soils over thousands of years results in a CH_4 loss from undisturbed peatlands to the atmosphere in the order of 30 Tg CH_4 annually (Frolking et al., 2011). Specifically, CH_4 is produced by microbes known as methanogens that utilize acetate, alcohols, or methylated compounds for anaerobic respiration through the process of methanogenesis, a final step in the stages of complex polymer decomposition in peat (Le Mer and Roger, 2001). Methane can also be consumed in the peat profile through oxidation by methanotrophic bacteria that are aerobic organisms that consume reduced carbon compounds for energy and use formaldehyde as a cellular carbon source for growth (Anthony, 1986). The most recent Intergovernmental Panel on Climate Change (IPCC, 2013) AR5 report indicates that CH_4 as a GHG is 28 times more effective at trapping heat on a 100-year time scale than CO_2. Over the long term (thousands of years), northern peatlands have had a cooling impact on Earth's radiative budget related to persistent CO_2 uptake; however, CH_4 emissions from these ecosystems are significant in the short term, causing net warming for some time following peatland formation (Frolking et al., 2006).

Vascular plant species influence the way CH_4 is produced, transported, and consumed in peat soils (e.g., Ström et al., 2005). The presence of vascular plant species in peatlands increase CH_4 emissions by providing substrate that amplifies methanogenesis through litter and labile carbon from root systems and efficiently transporting CH_4 through aerenchymous tissues to the atmosphere (Whalen, 2005). Vascular plants also introduce oxygen into the rhizosphere through the process of radial oxygen loss (ROL; van Bodegom et al., 2005). Radial oxygen loss can

reduce CH_4 emissions by creating oxic zones in the deeper anoxic peat profile appropriate for oxidation by methanotrophs, and can also cause the re-oxidation of reduced terminal electron acceptors (TEAs) that may subsequently inhibit methanogenesis (Bridgham et al., 2013). Vascular plants were particularly important in a tool developed by Couwenberg et al. (2011) known as greenhouse gas emission site types, where vegetation from rewetted peatlands in Europe was used to indicate long-term water level and GHG emissions, including CH_4. Species-specific effects on CH_4 dynamics between vascular plants in peatlands have been observed (Mahmood and Strack, 2011).

Moss, especially *Sphagnum* species, have also been found to influence CH_4 dynamics in peatlands through a syntrophic relationship between the moss and the methanotrophic bacteria that can consume CH_4 through oxidation (Raghoebarsing et al., 2005). Endophytic methanotrophs living within *Sphagnum* moss may consume CH_4, with the *Sphagnum* subsequently utilizing the carbon produced from the oxidation reaction (Basiliko et al., 2004). *Sphagnum* associated methanotrophy has been found to be highest with increasing temperature, as well as in submerged mosses where water table was at or above the surface (Kip et al., 2010). In a tundra environment, Liebner et al. (2011) found evidence of submerged brown mosses (particularly *Scorpidium scorpioides*) consuming CH_4 through oxidation.

Besides vegetation type, controls on CH_4 dynamics from peatlands include water table position, soil temperature, and plant productivity (Lai, 2009). Peat geochemistry also influences CH_4 flux as the presence of inorganic TEAs including nitrate, iron (III), manganese (III), and sulfate can inhibit methanogenesis due to thermodynamically favoured reduction reactions that utilize fermentation products necessary for CH_4 production (Roden and Wetzel, 1996; Dise and Verry, 2001). Further, alternative TEAs may reduce CH_4 emissions from peatlands associated

with the process of anaerobic CH_4 oxidation (AOM; Smemo and Yavitt, 2007). While AOM in peatlands is not fully understood, it is likely linked to sulfate, nitrate, or iron (III) replacing oxygen as an electron acceptor through various microbial-mediated mechanisms (Smemo and Yavitt, 2011).

Restoration and reclamation efforts of peatland ecosystems has resulted in different rates of CH_4 emissions compared to natural and unrestored areas, varying in relation to restoration technique and time since disturbance (Höper et al., 2008). Studies focused on CH_4 emissions after peatland restoration of horticulturally extracted sites or well-pads that include rewetting have found that emissions increase compared to the pre-restored state, associated with a higher water table and the emergence of vegetation that efficiently transport CH_4 (Strack et al., 2016a; Komulainen et al., 1998). While past studies support lower CH_4 flux after restoration compared to nearby natural peatland sites (Strack and Zuback, 2013; Tuittila et al., 2000), it is important to consider how emissions CH_4 may offset an uptake of carbon as CO_2 following restoration or reclamation (Waddington and Day, 2007). A constructed fen in the AOSR was found to have low CH_4 flux two growing seasons post-reclamation, associated with different hydrochemistry and soil properties (bulk density and organic matter) compared to a natural reference site in the area (Nwaishi et al., 2016). As altered CH_4 emissions from reclaimed peatlands are expected, management objectives with regard to CH_4 dynamics should be created prior to projects such as fen construction. Objectives could focus on reclaimed sites acting as low GHG emitters overall (e.g. through the flux of CO_2, CH_4, and nitrous oxide (N_2O)), or may be associated with similar CH_4 dynamics to natural sites related to comparable hydrochemistry and vegetation. Management objectives would allow CH_4 measurements to be one functional characteristic used to indicate the reclamation outcome from a biogeochemical perspective (Nwaishi et al., 2015a).

Past studies have found evidence of a vegetation species-specific effect on CH_4 dynamics in peatlands, and further research focused on how different vascular and moss species may influence CH_4 cycling is needed. Additional information on CH_4 dynamics at a constructed fen compared to natural reference sites is beneficial in an effort to understand functionality of reclaimed fens. In this study CH_4 emissions, pore water CH_4 concentration, and environmental variables known to control CH_4 flux and concentration were monitored over a growing season at a constructed fen and two peatland reference sites in northeastern Alberta with a focus on two vascular species *(Carex aquatilis* and *Juncus balticus)*, and including a consideration of the presence of moss. Specifically, the objectives were to: 1) evaluate CH_4 flux and CH_4 pools at *Carex aquatilis*, *Juncus balticus*, moss, mixed graminoid and moss, and bare plots in a constructed fen, 2) compare the CH_4 flux and CH_4 pore water pools of a constructed fen to reference sites with similar vegetation including *Carex aquatilis* + moss, *Juncus balticus*, moss, and bare plots, and 3) determine how water table position, soil temperature, geochemistry and vegetation productivity and biomass influence CH_4 flux at the constructed fen and reference sites.

2.2 Study Sites

The study was conducted at three sites within 40 km of Fort McMurray, Alberta, Canada. The constructed fen site was ~30 km north of Fort McMurray (56° 55.8701 N, 111° 25.0166 W). Two natural reference sites, a poor fen located ~40 km south of Fort McMurray (56° 22.610 N, 111°14.164 W) and a saline fen ~10 km south of Fort McMurray (56°34.398 N, 111° 16.518 W) were also used, as *Carex aquatilis* and *Juncus balticus*, the vascular species considered in this study from the constructed fen, occurred at the poor fen and saline fen, respectively. The study sites are within the Central Mixedwood Natural Subregion of Alberta's Boreal Forest Natural

Region (Natural Regions Committee, 2006) that receives on average 419 mm of precipitation per year with an average temperature of 0.96°C, as indicated from the 30-year trend (1980-2010; Government of Canada, 2016).

Constructed fen

The constructed fen site (also referred to as the Nikanotee Fen) is ~3 ha in size within a 32 ha watershed. It includes an upland aquifer constructed using tailings sands with high hydraulic conductivity that allows groundwater to flow towards the fen, that is surrounded by the upland as well as three previously reclaimed hillslopes (Ketcheson and Price, 2016; Price et al., 2010). This results in an alkaline, nutrient-rich water input to the fen from the upland watershed and tailing sand (Kessel, 2016). Two meters of donor peat was placed on a layer of petroleum coke at the base of the slopes. Donor peat was collected from a nearby rich fen peatland that had been dewatered for two years prior to being transported to the reclamation site for construction (Nwaishi et al., 2015b). Vegetation was planted on the site with a randomized split-block, split-split plot design to test vegetation establishment (A. Borkenhagen, unpublished). Specifically, for this study, seedling plantation (Cooper and Macdonald, 2000) of two vascular species (*Juncus balticus, Carex aquatilis*), moss layer transfer (Quinty and Rochefort, 2003), and bare control cover types were considered (Fig 2.1). Seedlings used for the seedling areas were propagated in a commercial nursery (A. Borkenhagen, unpublished). For the moss layer transfer areas, vegetation donor material, including the top 0.05-0.1 m of moss, vascular plants, and peat, was collected from a nearby rich fen and spread at a ratio of 1:10 donor area to reclaimed area, following Quinty and Rochefort (2003). Other vegetation methods not considered in this study included sowing of seeds and community plots with a mix of saline (*Juncus balticus* seedlings,

Calamagrostis inexpansa seedlings, and *Triglochin martima* seedlings) and freshwater (*Carex aquatilis*, *Betula glandulosa*, *Oxycoccus microcarpus*, *Sarricenia purpurea*) species. Vascular plant introduction and the moss transfer were accomplished in early summer 2013.

Metal collars (0.6 m x 0.6 m) inserted ~0.2 m into the peat were used to capture carbon flux measurements at the constructed fen and reference sites. At the constructed fen six vegetation cover types were considered, including: 1) *Carex aquatilis*, 2) *Juncus balticus*, 3) *Carex aquatilis* + moss, 4) *Juncus balticus* + moss, 5) bare, and 6) moss. Each cover type had four plot replicates. The moss species found in collars with moss (*Carex aquatilis* + moss, *Juncus balticus* + moss, moss) were brown mosses including: *Tomenthypnum nitens, Drepanocladus aduncus, Bryum pseudotriquetrum,* and *Leptobryum pyriforme*. The moss cover type had plots located in the southwest section of the constructed fen where peat and donor material was spread in 2014; consequently, these plots included moss without a heavy cover of vascular vegetation (Fig 2.1).

Fig 2.1. Constructed fen study site including vegetation plot types and flux collar locations. Flux collar cover types include four plot replicates of bare (B1-B4), *Carex aquatilis* (C1-C4), *Carex aquatilis* + moss (CM1-CM4), *Juncus balticus* (J1-J4), *Juncus balticus* + moss (JM1-JM4), and moss only (M1-M4). In the Community plots a variety of freshwater or saline species were planted, and sowing of seeds occurred in the Seeds plots. The moss layer transfer method was used in the Moss plots. Community, Seeds, and Moss plots were not considered in this study.

Saline fen

The saline fen reference site is influenced by saline groundwater, associated with Devonian carbonates causing halite deposits within the strata (Wells and Price, 2015). In total the saline fen comprises an area of about 27 ha. Measurements at saline fen were made within a ~0.5 ha area at the northern down-gradient end of the site that had vascular vegetation dominated by *Calamagrostis stricta, Hordeum jubatum, Triglochin maritima*, and *Juncus balticus*. Metal collars to capture carbon fluxes were placed in areas dominated by *Juncus balticus* or in bare areas, with four replicates of each cover type. Late in the growing season it was determined that one of the plots thought to be dominated by *Juncus balticus* was actually *Eleocharis palustris* (common spike rush). Data from this plot were included in the analysis as no difference in CH_4 flux, concentration, vegetation cover, or hydrochemical variables compared to the plots including *Juncus balticus* were found.

Poor fen

The poor fen reference study site (also known as Pauciflora Fen) is an ~8 ha poor fen surrounded by upland coniferous forest. Discharge from the surrounding treed upland as well as bogs within the basin supply groundwater to the site (Khadka et al., 2016). This site includes both a treed poor fen ecosite and a shrubby poor fen ecosite (A. Borkenhagen, unpublished). Measurements were made in the richer shrubby poor fen area dominated by a *Sphagnum fuscum* and *Sphagnum angustifolium* carpet as well as *Carex aquatilis, Andromeda polifolia*, *Chamaedaphne calyculata*, *Betula glandulosa* and *Oxyccoccus microcarpus*. At this site metal collar plots were placed across a ~20 m long transect and included four replicates of moss dominated plots and four replicates of plots made up of primarily *Carex aquatilis* + moss.

2.3 Methods

Methane flux measurements

Flux measurements were made using the closed chamber method (Alm et al., 2007) between May 16-September 3, 2015. The height of each collar from the soil surface was measured in order to correct chamber headspace volume to calculate CH_4 flux. Flux measurements were made nine times at each plot over the growing season at the constructed fen and seven times at the two reference sites.

Methane flux was measured using opaque chambers (0.6 m x 0.6 m x 0.3 m; 0.108 m^3) placed on the collars. Water was poured around the collar edge to seal the system, a battery-powered fan mixed the chamber headspace, and a thermocouple and thermometer measured chamber temperature at the time of CH_4 sampling. The chamber had a plug with a tube equipped with a three-way valve that was inserted into a hole drilled into the top of the chamber. Using a syringe, 20 mL gas samples were taken from the chamber at intervals of 7, 15, 25, and 35 minutes and injected into evacuated Exetainers (Labco, UK). A gas chromatograph (GC; Shimadzu GC2014, Mandel Scientific, Canada) with a flame ionization detector was used to determine CH_4 concentration of the gas samples collected in the field, and the flux was determined from the linear change in concentration over time including corrections for temperature and volume of the chamber. Small negative or positive flux values, where the change in concentration was within the variance of the GC (±20%), were assigned a value of 0 for flux. Larger negative flux values (≤ -5.5 mg CH_4 m^2 d^{-1}) with concentrations that varied by more than 20% were removed from the data set as these fluxes were likely associated with ebullition followed by uptake inadvertently caused by chamber placement. In instances where

flux values appeared to capture true ebullition events, only values with an $R^2 > 0.80$ were kept in the data set. These procedures resulted in loss of 8.6% of the data across all sites.

Pore water CH_4 concentration

Pore water CH_4 samples were collected adjacent to the metal flux collars at all sites over similar time periods and frequencies as the flux measurements. All samples were collected at both 0.2 m and 0.7 m depth with the intent of understanding CH_4 pools in and below the rooting zone (A. Borkenhagen, unpublished). Pore water samplers were made using 0.2 m long, 0.025 m inner diameter PVC pipe slotted at the middle 0.1 m, with stoppers inserted at both ends. Nitex screening (250 μm mesh size) covers were sewed and placed around the slotted intake to prevent clogging, and tygon tubing was attached to the top stopper and extended above the surface (Strack et al., 2004). To collect pore water samples a three-way valve on the sampler was used to attach the tygon tubing to a 60 mL syringe, and 60 mL of water was flushed first before a 20 mL sample was collected. After sample collection 20 mL of ambient air was added to the syringe. The sample was subsequently shaken for 5 minutes to equilibrate dissolved gases into the syringe headspace before the air in the syringe was transferred to an evacuated Exetainer (Labco, UK). The time of equilibration was used to obtain the equilibration temperature from a meteorological station installed at each site. Pore water CH_4 concentration at depth was calculated following the analysis of the headspace samples for CH_4 concentration on the GC (Strack et al., 2016b; Mahmood and Strack, 2011; Kampbell and Vandegrift, 1998).

Vegetation variables

Aboveground and belowground vegetation biomass sampling occurred from August 7-17, 2015 across the three study sites. For this study above and belowground biomass refers to only vegetation (aboveground plant parts, roots, rhizomes) and no other living organisms (insects, microbes). A quadrat (0.2 m x 0.6 m) was placed adjacent to all collars used for flux measurements, and all aboveground vegetation was clipped from the soil surface. Litter was separated from live biomass in the field. Aboveground biomass samples were bagged and transported back to the University of Waterloo, Ontario, where they were sorted to species, dried for 72 hours at 60˚C, and weighed to determine aboveground dry biomass. *Sphagnum magellanicum* and *Sphagnum angustifolium* dominated the aboveground biomass samples from the poor fen. The *Sphagnum* was clipped at the capitulum base and the capitulum, deemed to be the photosynthetically active portion of the moss (Clymo, 1970), was dried to determine dry biomass.

Belowground biomass cores within the clipped aboveground biomass quadrat were sampled. All sites were sampled to at least 0.2 m depth, but deeper cores were taken at randomly selected plots. At the constructed fen belowground biomass was sampled to a maximum depth of 0.7 m. Due to difficult site access, cores at the reference sites (poor fen, saline fen) were only sampled to 0.4 m depth. At the constructed fen a Wardenaar peat profile sampler (Eijkelkamp Soil & Water, The Netherlands) was used to sample adjacent to two of the plots from each vascular vegetation cover type (*Carex aquatilis, Carex aquatilis* + moss, *Juncus balticus,* and *Juncus balticus* + moss) and one moss plot to 0.7 m (cores 0.10 m x 0.12 m). Compression of the soil cores occurred while using the Wardenaar profile sampler and soil core depth was adjusted accordingly in the field. Compression ranged from 22-45% of the soil core. For the remaining

collars at the constructed fen not sampled to 0.7 m depth, belowground biomass was sampled to 0.2 m depth using a saw (0.10 m x 0.12 m). A saw was also used to sample belowground biomass adjacent to two plots of each cover type at the reference sites to either 0.2 m (poor fen: moss; saline fen: bare) or 0.4 m (poor fen: *Carex aquatilis* + moss; saline fen: *Juncus balticus*) depth. Belowground biomass cores were cut to 0.2 m increments in the field and bagged. Extracted cores were transported to the University of Waterloo, Ontario, where they were frozen prior to analysis. In the laboratory root biomass was sorted from the peat using tweezers and grouped into woody or herbaceous categories prior to being oven dried at 60°C for 72 hr and weighed to estimate belowground dry biomass (Moore et al., 2002).

To understand vegetation productivity of plots, net ecosystem exchange (NEE) of CO_2 was measured at similar CH_4 flux measurement periods and frequencies to generate gross ecosystem productivity (GEP) values also using the chamber method (see *Methane flux measurements*). A clear chamber (0.108 m^3) with battery-powered fans was placed on the water-filled collars. A portable infrared gas analyzer (EGM-4, PP Systems, Massachusetts, USA) connected to the chamber using tubing measured CO_2 concentration in parts per million (ppm). An integrated temperature and photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}) sensor connected to the EGM-4 and software logged CO_2, PAR, and temperature in the chamber at 10-second intervals from 0 to 120 seconds after the system had equilibrated and CO_2 concentrations were stable. Net ecosystem exchange was determined from the linear change in the CO_2 concentration over time, with corrections for temperature and chamber volume. For each flux measurement date, NEE was measured in full light as well as in the dark using an opaque tarp to determine ecosystem respiration (ER). Gross ecosystem productivity was calculated as the difference between the NEE and ER. Only NEE fluxes with a PAR photon flux density greater

than 1000 µmol m^{-2} s^{-1} were used, resulting in maximum GEP values (GEP_{max} *sensu* Bubier et al., 2003). Negative GEP_{max} values in this study indicate uptake of carbon by the ecosystem (i.e. more negative values correspond to higher productivity).

A vegetation survey to determine cover was conducted at the study sites from July 29-August 2, 2015 in the metal collars used for carbon flux measurements. Each live plant was identified to species and percent canopy cover including litter was visually estimated for each species to the nearest 1%. Litter was not included in total vegetation cover values.

Hydrochemical variables

Water table position and soil temperature were measured adjacent to collars to understand controls on CH_4 dynamics across the study sites. Water table position was measured with ~1 m long PVC standpipe (diameter 0.05 m) that had holes drilled for its full length and a mesh covering. Soil temperature at 0.2 m and 0.7 m depth were measured with a thermocouple probe inserted into the peat and thermometer.

In order to understand hydrochemistry across the constructed fen and reference sites, plant root simulator (PRS)^{TM} probes (Western Ag Innovations Inc., Saskatoon, Canada) were used. The PRS probes included a 10 cm^2 resin membrane that mimicked the plant surface and measured *in situ* ion supply in the soil solution (Qian and Schoenau, 2002). Cation probes were chemically treated with HCO_3^- while anion probes were saturated with Na^+ prior to use, enabling counter-ions to adsorb onto the PRS probe following burial. The PRS probes estimated a supply rate of nutrients over the resin area in the soil for the length of burial based on physical, chemical, and biological properties (Nwaishi et al., 2016; Wood et al., 2015; Wang et al., 2016).

However, as all forms of sulfur, iron, and manganese adsorbed to the PRS probes and were measured during analysis, these results were unable to quantify the presence of specific ionic species for these compounds, but rather indicated the supply rate of any mobile species of sulfur, iron, and manganese in the soil (Eric Bremmer, personal communication). Hence, the PRS probes were used in this study to understand large differences between study sites that could imply the presence of TEAs that are known to limit methanogenesis.

The PRS probes were buried directly outside metal collars at 0.2 m and 0.7 m depths for 14 days from July 6-July 20, 2015. Four replicates each of anion and cation probes were buried around each collar. After the probes were removed they were cleaned with deionized water, placed in Ziploc® bags and transported to Western Ag Innovations in a cool box. Upon arrival at Western Ag Innovations 0.5 M HCl was used to wash the probes, and analytical analysis occurred on the resultant eluate (Hangs et al., 2004). Analysis for ammonium ions (NH_4^+) occurred via colorimetrically with an automated flow injection analysis system, while sulfur, iron, and manganese were analyzed via inductively-coupled plasma spectrometry (PerkinElmer Optima 3000-DV, PerkinElmer Inc., Shelton, CT).

Data Analysis

A one-way ANOVA with repeated measures that accounted for date was used to compare differences in CH_4 flux, CH_4 pore water concentration at 0.2 m and 0.7 m depth, GEP_{max}, water table position, and pH and EC at 0.2 m and 0.7 m depth, over the growing season across cover types and between sites (Kravchenko and Robertson, 2015). The one-way ANOVA was further used to compare above and belowground biomass, percent cover of total vegetation, moss, shrub, graminoid, and litter, and sulfur, iron, ammonium, and manganese supply rates at 0.2 and 0.7 m

depth, across vegetation cover types and between sites. The Levene test was applied to ensure data met equality of variance assumptions, and the Shapiro-Wilks test was used to test data residuals for normality. Data that did not meet normality and equal variance conditions were log transformed. A value of 1 was added to CH_4 flux and environmental variable values prior to log transformation as data included values of 0. In some instances, data were still not normally distributed after the log transformation was applied. In these instances, the ANOVA was still used because sample sizes were moderate and balanced, and ANOVA tests are robust to deviations from normality under these conditions (Whitlock and Schluter, 2009). A pairwise t-test with adjusted p-values using the Bonferroni method was applied to determine which sites were different in the case of a significant ANOVA result.

To understand environmental controls on CH_4 dynamics across all plots at the three study sites the non-parametric Spearman's rank correlation test was applied to seasonal averages of CH_4 flux and CH_4 pore water concentration at 0.2 m and 0.7 m depth and environmental variables at each plot. Seasonal averages were used in the correlation tests for GEP_{max}, water table position, soil temperature, pH, and EC. Results from the vegetation survey (percent cover of total vegetation, moss, shrub, graminoid, and litter) and biomass sampling conducted at the end of the growing season were used for the correlation analysis. The PRS results obtained from the middle of the growing season were used for the measurement of redox reactive ions in soil water. In order to understand variance in data across study sites, principle component analysis (PCA) was applied to seasonal average CH_4 flux and pore water concentration (0.2 m and 0.7 m depth) data along with vegetation, physical, and chemistry environmental variables that had the highest correlation coefficient (Spearman's rho) of alike variables with a similar and significant relationship with CH_4 flux and CH_4 concentration at 0.2 m and 0.7 m. Principle component

analysis was conducted on the correlation matrix, and the variables were shifted to be zero centred, log transformed, and scaled to account for unit variance before analysis to standardize the data. The statistical program R 3.2.5. (R Core Team, 2016) was used for all statistical analysis, and a significance of $\alpha = 0.05$ was applied.

2.4 Results

Methane flux and concentration

Across all cover types at the constructed fen the *Juncus balticus* plots specifically had significantly higher flux compared to bare and moss cover types ($F_{5,74} = 2.7$, $p = 0.03$; Fig 2.2I; Appendix 1). Considering all plots measured at each site, the poor fen had higher seasonal average CH_4 flux compared to the constructed fen and saline fen ($F_{2,33} = 28.8$, $p < 0.001$; Fig 2.2II) sites. Comparing similar cover types at the constructed fen and reference sites revealed that the bare sites at the saline fen had higher average CH_4 flux compared to the constructed fen ($F_{1,21} = 4.4$, $p = 0.04$), while the *Juncus balticus* cover type at the two sites had similar flux ($F_{1,23} = 2.5$, $p = 0.126$; Fig 2.2III). Both poor fen cover types had higher average CH_4 flux compared to similar cover types at the constructed fen (*Carex aquatilis* + moss: $F_{1,25} = 57.7$, $p < 0.001$; moss: $F_{1,16} = 13.9$, $p < 0.01$; Fig 2.2IV). At the poor fen, seasonal CH_4 flux values were influenced by high fluxes likely associated with ebullition events (five fluxes ranging from 45.6-173.0 mg CH_4 m^{-2} d^{-1} where the linear change in concentration over time had an $R^2 > 0.80$). At the constructed fen and saline fen no high flux values indicating ebullition were measured.

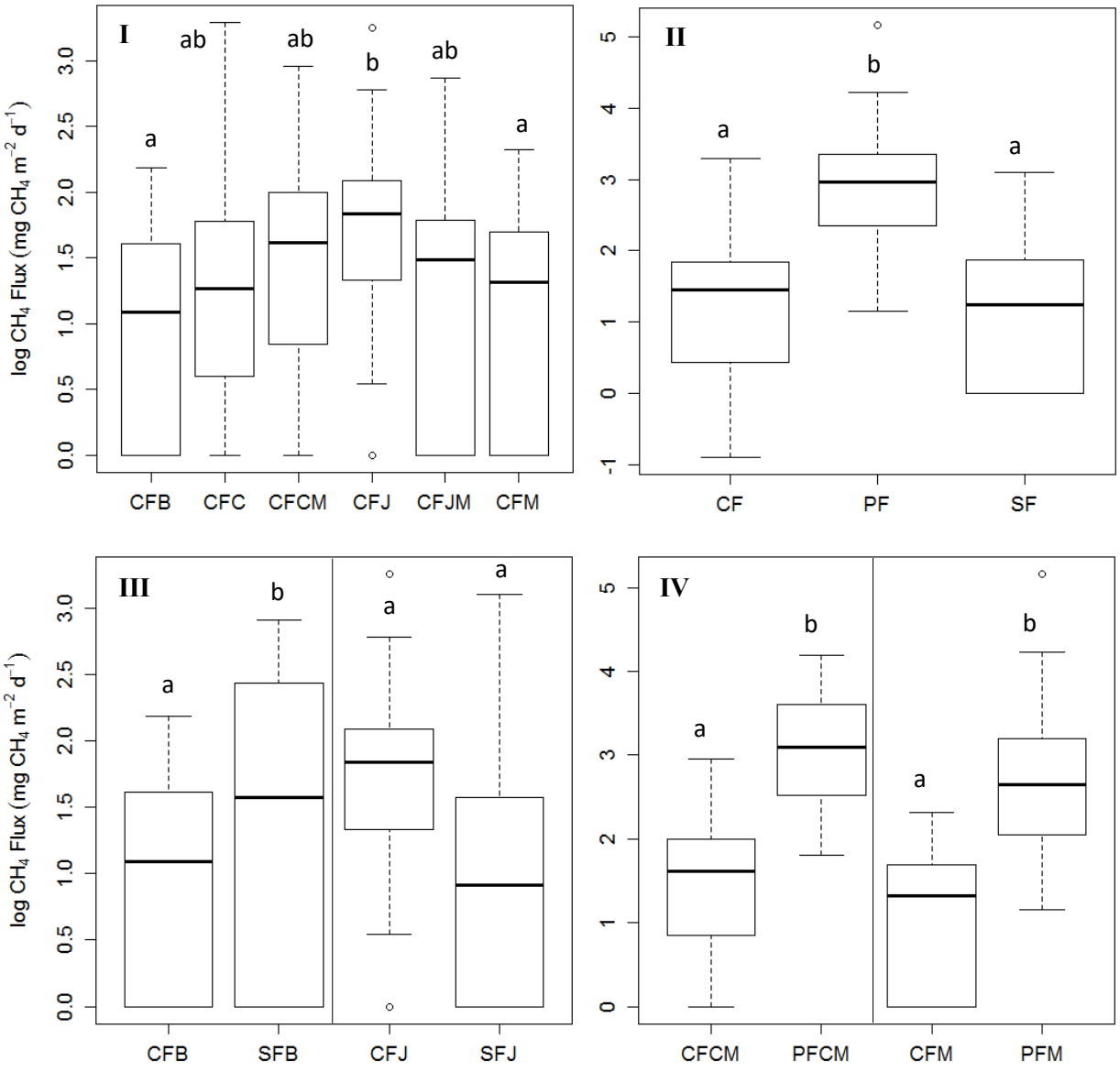

Fig 2.2. Methane (CH_4) flux (log transformed +1) across cover types and sites. I: cover types at the constructed fen (CF) including bare (CFB), *Carex aquatilis* (CFC), *Carex aquatilis* + moss (CFCM), *Juncus balticus* (CFJ), *Juncus balticus* + moss (CFJM), and moss (CFM). II: Flux between CF, poor fen (PF), and saline fen (SF) sites. III: Flux between similar cover types at the CF and SF (bare: CFB, SFB; *Juncus balticus*: CFJ and SFJ). IV: CH_4 flux from similar cover types at the CF and PF (*Carex aquatilis* + moss: CFCM, PFCM; moss: CFM, PFM). Each cover types included four plot replicates. Letters indicate significant differences between cover types (I, III, IV) or sites (II). In III and IV, letters indicate significant differences between cover types in a similar column only (eg. CFB, SFB).

The average seasonal CH_4 pore water concentration also varied between cover types at the constructed fen (Fig 2.3Ia; Appendix 1). At 0.2 m depth the moss cover type had significantly higher concentration compared to the *Carex aquatilis* + moss cover type, *Juncus balticus* + moss cover type, and *Juncus balticus* cover type ($F_{5,54} = 3.7$, $p < 0.01$). Deeper in the peat profile at 0.7 m the bare and moss plots at the constructed fen had higher average CH_4 concentration compared to all the cover types with vascular plants (*Carex aquatilis, Carex aquatilis* + moss, *Juncus balticus,* and *Juncus balticus* + moss; $F_{5,56} = 17.6$, $p < 0.001$). Further, the *Carex aquatilis* and *Juncus balticus* + moss cover type had higher CH_4 concentration compared to *Carex aquatilis* + moss that had the lowest concentration of all cover types.

Considering seasonal averages of all of the plots at each study site, the poor fen had the highest CH_4 pore water concentration at 0.2 m, while the constructed fen had a lower belowground CH_4 concentration compared to both reference sites ($F_{2,24} = 114.2$, $p < 0.001$; Fig 2.3IIa). At 0.7 m, the constructed fen still had the lowest CH_4 concentration, but the poor fen and saline fen had similar concentrations ($F_{2,21} = 29.8$, $p < 0.001$). At 0.2 m depth, both saline fen cover types had higher average CH_4 concentrations compared to similar cover types at the constructed fen (bare: $F_{1,15} = 86.8$, $p < 0.001$; *Juncus balticus*: $F_{1,17} = 27.5$, $p < 0.001$; Fig 2.3IIIa). Similarly, at 0.7 m, the saline fen cover types had higher average concentrations (bare: $F_{1,15} = 13.3$, $p < 0.01$; *Juncus balticus*: $F_{1,15} = 34.8$, $p < 0.001$). Consistent with the CH_4 flux results, the poor fen had higher CH_4 concentrations when considering similar cover types between the constructed fen and poor fen at 0.2 m (moss: $F_{1,13} = 27.7$, $p < 0.001$; *Carex aquatilis* + moss: $F_{1,17} = 289.7$, $p < 0.001$; Fig 3IVa) and 0.7 m (moss: $F_{1,13} = 5.3$, $p = 0.04$; *Carex aquatilis* + moss: $F_{1,16} = 198.1$, $p < 0.001$).

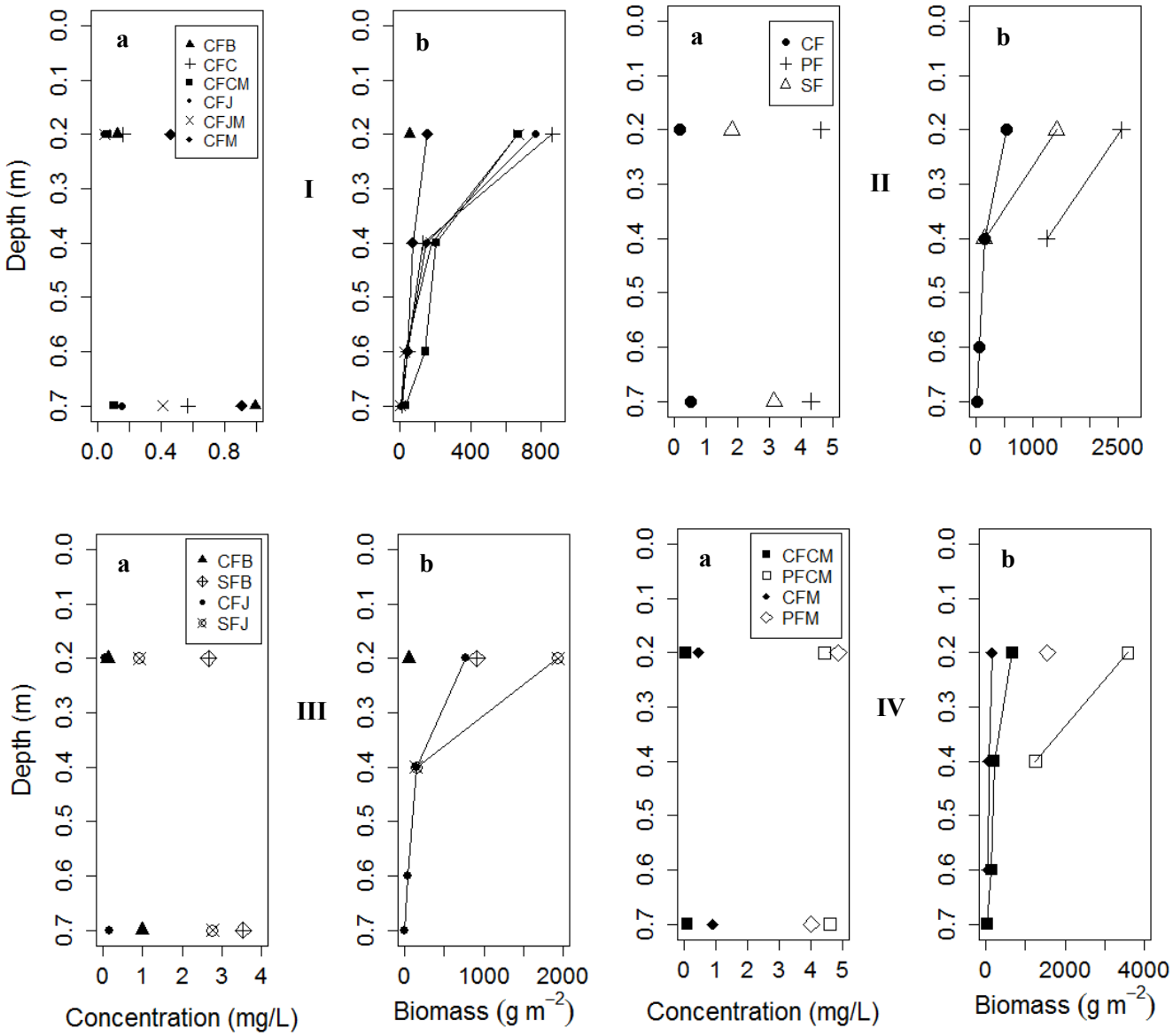

Fig 2.3. Methane (CH_4) concentration (left; a) and total vegetation biomass (right; b) at depth. Refer to Fig 2.2 for a description of cover types and sites. Each cover types represents four plot replicates for concentration data, and two to four replicates for biomass data. To increase clarity, error bars were excluded, and concentration data was not displayed log transformed.

Vegetation

Aboveground biomass was similar at the constructed fen cover types *Carex aquatilis, Carex aquatilis* + moss, *Juncus balticus, Juncus balticus* + moss, and moss, while these cover types had higher biomass compared to the bare cover types at this site (Table 2.1; $F_{5,18} = 7.1$, $p < 0.001$). A study focused on biomass at the constructed fen that had a more rigorous sampling design than the present study found total live aboveground biomass to be in a similar range as the values reported here for *Carex aquatilis*, *Juncus balticus,* and *Juncus balticus* + moss cover types (L. Messner, unpublished). However, the biomass study found a higher value for *Carex aquatilis* + moss (530.9 ± 39.5 (standard error of the mean (SEM)) g m^{-2} than the average value determined for this study (384.7 ± 40.6 (SEM) g m^{-2}).

The cover types *Carex aquatilis* + moss at the constructed fen and poor fen had similar aboveground biomass, while the moss cover types had higher aboveground biomass at the poor fen compared to the constructed fen ($F_{9,30} = 10.4$, $p < 0.001$). The *Juncus balticus* cover types at the constructed fen produced significantly higher biomass compared to the saline fen, with bare plots at these sites having significantly similar biomass ($F_{9,30} = 10.4$, $p < 0.001$). Aboveground biomass was dominated by herbaceous vascular plants (95.1% of total biomass weight) across all plots at the constructed fen, while moss made up 4.9% (results not shown). Litter was not included in the total aboveground biomass result; however, the proportion of litter weight to total live biomass weight was 31.9% at the constructed fen. The poor fen biomass included a much higher portion of moss across plots (31.6%), with herbaceous vascular vegetation accounting for 41.7% of the biomass, and woody vascular vegetation (woody shrub tissues) making up 26.7% of biomass. Litter was 14.5% of the total live biomass at the poor fen. Finally, at the saline fen the biomass mostly included herbaceous vascular plants (98.3%). Moss was only found in one of

the *Juncus balticus* biomass plots and made up just 1.7% of total aboveground biomass. Average litter biomass at the *Juncus balticus* plots at the saline fen was higher than litter found at either the constructed fen or the poor fen, and in total litter had a mass equal to 94.9% of the total live biomass across saline fen plots.

Belowground biomass from 0-0.2 m across the constructed fen was higher at cover types including vascular species (*Carex aquatilis, Carex aquatilis* + moss, *Juncus balticus,* and *Juncus balticus* + moss) compared to the bare and moss cover type that had similar belowground biomass (Fig 2.3Ib; $F_{5,18} = 7.2$, $p < 0.001$). Deeper in the peat profile, from 0.2-0.4 m, cover types sampled (not including the bare cover type) at the constructed fen did not have significantly different biomass ($F_{4,4} = 2.2$, $p = 0.23$). The belowground biomass at a depth of 0.4-0.6 m for all the plots across the constructed fen (with the exception of the bare cover type) had an average biomass of 64.4 ± 26.7 (SEM) g m^{-2}. From 0.6-0.7 m depth belowground biomass was not present in either the bare or the moss cover type, while the rest of the cover types that included vascular species had belowground biomass averaging just 13.1 ± 6.9 (SEM) g m^{-2}. Results from the intensive biomass study at the constructed fen found higher total belowground biomass compared to the values found in this study (L. Messner, unpublished). The more thorough biomass study found belowground biomass values from 0-0.5 m depth for *Carex aquatilis, Carex aquatilis* + moss, *Juncus balticus,* and *Juncus balticus* + moss cover types of 1402.0 ± 85.0, 1802.3 ± 150.5, 1537.0 ±111.0, and 1673.9 ± 106.1 (SEM) g m^{-2}, respectively.

Of the three study sites, belowground biomass across all cover types was significantly highest at the poor fen compared to both the constructed fen and saline fen that had similar biomass from 0-0.2 m and 0.2-0.4 m depth intervals (Fig 2.3IIb; 0-0.2 m: $F_{2,29} = 5.7$, $p < 0.01$; 0.2-0.4 m: $F_{2,10} = 12.1$, $p < 0.01$). However, comparing similar cover types at the saline fen to the

constructed fen revealed higher belowground biomass at the saline fen *Juncus balticus* plots compared to the constructed fen from 0-0.2 m (Fig 2.3IIIb; $F_{3,7} = 10.7$, $p < 0.01$). Roots that made up the belowground biomass at the constructed fen and saline fen were from herbaceous vascular vegetation (results not shown). At the poor fen, woody shrub roots accounted for, on average, ~29% of all belowground biomass.

Average growing season productivity, as measured through GEP_{max}, was highest and similar at cover types including vascular species compared to the bare and moss cover types across the constructed fen (Table 2.1; $F_{5,181} = 3.3$, $p < 0.01$). A similar pattern was found when considering total cover ($F_{5,18} = 27.6$, $p < 0.001$), graminoid cover ($F_{5,18} = 21.7$, $p < 0.001$), and litter cover ($F_{5,18} = 463.5$, $p < 0.001$), with the vascular cover types having higher percent cover compared to the bare and moss plots at the constructed fen. While shrub cover was similar across all cover types at the constructed fen site ($F_{5,18} = 0.4$, $p = 0.81$), a higher cover of moss was found in plots including *Juncus balticus* + moss compared to the bare, *Carex aquatilis* and *Juncus balticus* only cover types ($F_{5,18} = 6.1$, $p < 0.01$). Across all sites average growing season productivity was higher at the constructed fen and poor fen cover types compared to the saline fen ($F_{9,307} = 4.2$, $p < 0.001$). Vegetation survey results showed that the poor fen had highest total ($F_{2,37} = 4.3$, $p = 0.02$), shrub ($F_{2,37} = 19.5$, $p < 0.001$), and moss cover compared to the other sites, with significantly higher moss cover also found across cover types at the constructed fen compared to the saline fen ($F_{2,37} = 25.7$, $p < 0.001$). The constructed fen had higher graminoid percent cover than the poor fen and similar cover compared to the saline fen ($F_{2,37} = 6.0$, $p < 0.01$). Litter was highest overall at the saline fen compared to the other sites, associated with high litter cover found in the *Juncus balticus* plots ($F_{2,37} = 3.5$, $p = 0.04$).

Table 2.1. Vegetation parameters ± standard error of the mean measured at the constructed fen (CF), poor fen (PF), and saline fen (SF) flux collars. *

Cover types	AG ($g\ m^{-2}$)	GEP_{max} ($g\ CO_2\ m^{-2}\ d^{-1}$)	Moss (%)	Shrub (%)	Graminoid (%)	Litter (%)	Total (%)
Constructed Fen							
CFB	45.0 ± 30.7^{ac}	-7.1 ± 3.3^{a}	2 ± 0.01^{b}	2 ± 0.01^{a}	9 ± 0.03^{ac}	0 ± 0^{a}	14 ± 0.03^{a}
CFC	554.7 ± 92.1^{b}	-32.3 ± 2.9^{b}	3 ± 0.01^{b}	2 ± 0.01^{a}	60 ± 0.0^{b}	11 ± 0.01^{b}	65 ± 0.04^{b}
CFCM	384.7 ± 40.6^{bc}	-31.6 ± 1.3^{b}	21 ± 0.0^{ab}	2 ± 0.01^{a}	68 ± 0.03^{b}	9 ± 0.02^{b}	91 ± 0.07^{bc}
CFJ	425.2 ± 48.3^{b}	-31.0 ± 5.0^{b}	8 ± 0.05^{b}	3 ± 0.02^{a}	47 ± 0.08^{b}	11 ± 0.02^{b}	58 ± 0.10^{b}
CFJM	431.4 ± 67.4^{b}	-29.0 ± 2.6^{bc}	39 ± 0.1^{a}	4 ± 0.02^{a}	60 ± 0.08^{b}	9 ± 0.02^{b}	102 ± 0.04^{bc}
CFM	387.2 ± 83.1^{bc}	-5.5 ± 1.9^{a}	12 ± 0.04^{ab}	3 ± 0.0^{a}	5 ± 0.04^{a}	0 ± 0^{b}	20 ± 0.05^{a}
Poor Fen							
PFCM	323.6 ± 23.9^{bc}	-26.0 ± 3.0^{bc}	93 ± 0.03^{c}	15 ± 0.05^{b}	15 ± 0.03^{ac}	11 ± 0.07^{b}	122 ± 0.02^{c}
PFM	325.1 ± 60.6^{bc}	-15.4 ± 3.1^{c}	101 ± 0.01^{c}	21 ± 0.05^{b}	0 ± 0^{a}	0 ± 0^{a}	121 ± 0.05^{c}
Saline Fen							
SFB	23.0 ± 6.9^{ac}	-13.4 ± 3.1^{a}	0 ± 0^{b}	1 ± 0.01^{a}	12 ± 0.04^{ac}	0 ± 0^{a}	13 ± 0.04^{a}
SFJ	154.5 ± 17.8^{c}	-21.2 ± 2.4^{c}	11 ± 0.11^{b}	8 ± 0.04^{ab}	39 ± 0.16^{c}	53 ± 0.24^{c}	58 ± 0.12^{b}

*Cover types at the CF included bare (CFB), *Carex aquatilis* (CFC), *Carex aquatilis* + moss (CFCM), *Juncus balticus* (CFJ), *Juncus balticus* + moss (CFJM), and moss (CFM). At the PF cover types were *Carex aquatilis* + moss (PFCM) and moss only (PFM), and the SF had a bare cover type (SFB) and *Juncus balticus* cover types (SFJ). All cover types were made up of measurements from four plot replicates. Aboveground biomass (AG biomass) was sampled in August 2015. Gross ecosystem productivity (GEP) values used were measurements made in full light conditions (photon flux density greater than 1000 $\mu mol\ m^{-2}\ s^{-1}$) to determine GEP_{max}. GEP_{max} was averaged over the growing season. Percent cover of vegetation functional groups (moss, shrub, graminoid, litter) was determined from a vegetation survey from July-August, 2015. Letters indicate significant differences between all cover types across the study sites (CFB, PFCM, SFB, etc.) for one variable only (AG, GEP_{max}, etc.).

Hydrochemistry

Seasonal mean water table position across cover types at the constructed fen over the growing season did not differ significantly (Table 2.2; $F_{5,178} = 0.8$, $p = 0.57$). Mean water table during the study period across the constructed fen was -6.9 cm. Average water table of all plots across this site was shallowest early in the growing season in May, averaging 2.7 cm, and deepest in August with a mean water table position of -12.9 cm (results not shown). Mean water table position at the saline fen was deeper seasonally compared to the constructed fen (-10.9 cm) and similar to the water table found at the poor fen (-9.6 cm; $F_{2,274} = 26.9$, $p < 0.001$). The constructed fen and poor fen also had statistically similar average water table positions. The saline fen was driest in June and wettest in July, whereas the poor fen experienced its deepest water table positions in August and its shallowest levels in May.

Differences in pH among cover types at the constructed fen were not significant at both 0.2 m ($F_{5,66} = 1.0$, $p = 0.45$) and 0.7 m depth ($F_{5,67} = 0.1$, $p = 0.99$), while there were distinct differences in pH between the three study sites at both depths (Table 2.2; 0.2 m: $F_{2,126} = 20.6$, $p < 0.001$; 0.7 m: $F_{2,137} = 48.0$, $p < 0.001$). A range of EC values were found across the constructed fen, although no statistical differences emerged based on vegetation cover types (0.2 m: $F_{5,66} = 0.7$, $p = 0.65$; 0.7 m: $F_{5,67} = 0.3$, $p = 0.93$). The saline fen cover types had much higher EC values than the constructed fen, and they were especially higher when compared to EC at the poor fen (0.2 m: $F_{2,126} = 64.6$, $p < 0.001$; 0.7 m: $F_{2,137} = 106.0$, $p < 0.001$).

Table 2.2. Physical and chemical parameters (water table (WT), soil temperature (Soil Temp.), pH, and electrical conductivity (EC)) across cover types and sites at the constructed fen (CF), poor fen (PF), and saline fen (SF). *

Site/Cover type	WT (cm)**	Depth (m)	Soil Temp. (˚C)	pH	EC (µS/cm)
Constructed Fen	-6.9±0.53[a]	0.2	13.7±0.3[a]	7.3±0.08[a]	2912.0±79.5[a]
		0.7	9.3±0.2[a]	7.4±0.06[a]	2297.0±69.2[a]
CFB	-4.6±2.8	0.2	14.7±0.8	7.2±0.04	2859.1±606.9
		0.7	9.8±0.5	7.5±0.2	1885.2±233.8
CFC	-10.0±3.7	0.2	13.5±0.4	7.4±0.1	2811.0±232.8
		0.7	9.1±0.4	7.5±0.04	2035.6±218.5
CFCM	-6.7±1.5	0.2	13.5±0.7	7.4±0.04	2871.0±237.3
		0.7	9.1±0.5	7.4±0.03	2416.0±203.9
CFJ	-10.0±3.5	0.2	13.5±0.4	7.3±0.04	2918.4±349.5
		0.7	9.3±0.3	7.5±0.07	2196.4±186.7
CFJM	-5.9±1.9	0.2	13.2±0.5	7.5±0.03	2930.1±343.8
		0.7	9.1±0.5	7.5±0.06	2628.9±436.7
CFM	-4.8±1.4	0.2	13.7±0.5	7.3±0.04	2683.1±204.9
		0.7	9.1±0.3	7.4±0.02	2306.1±151.1
Poor Fen	-9.6±1.02[ab]	0.2	12.9±0.4[a]	5.5±0.16[b]	42.9±4.4[b]
		0.7	9.3±0.1[a]	5.7±0.21[b]	61.5±3.3[b]
PFCM	-8.9±0.9	0.2	13.1±0.3	5.6±0.14	45.0±3.1
		0.7	9.2±0.2	5.9±0.06	68.4±4.9
PFM	-10.8±0.4	0.2	12.3±0.7	5.3±0.12	53.8±11.8
		0.7	9.3±0.3	5.6±0.15	55.3±3.8
Saline Fen	-10.9±1.8[b]	0.2	14.52±0.4[a]	6.1±0.07[c]	11410.0±549.5[c]
		0.7	11.4±0.3[b]	6.2±0.09[c]	17440.0±567.1[c]
SFB	-5.4±2.7	0.2	14.7±0.2	6.0±0.05	12140.2±934.4
		0.7	11.5±0.2	6.3±0.10	17342.1±450.0
SFJ	-16.6±1.8	0.2	14.3±0.3	6.1±0.13	10559.6±1168.2
		0.7	11.3±0.2	6.1±0.04	17543.4±1385.4

*Refer to Table 2.1 for a description of cover types. Each cover type includes the growing season average of four plot replicates. Letters indicate significant differences in variables (WTD, Soil Temp., pH., EC) across sites; for Soil Temp., pH, and EC differences between sites are compared at similar depths (0.2 or 0.7 m).

Plant root simulator results did not reveal major differences in the supply rate of sulfur (0.2 m: $F_{5,18} = 0.6$, $p = 0.71$; 0.7 m: $F_{5,18} = 1.1$, $p = 0.42$), ammonium (0.2 m: $F_{5,18} = 2.3$, $p = 0.09$; 0.7 m: $F_{5,18} = 2.2$, $p = 0.10$), iron (0.2 m: $F_{5,18} = 1.1$, $p = 0.38$; 0.7 m: $F_{5,18} = 0.4$, $p = 0.87$), or manganese (0.2 m: $F_{5,18} = 1.2$, $p = 0.34$; 0.7 m: $F_{5,18} = 0.4$, $p = 0.82$) across distinct cover types at the constructed fen study site (Fig 2.4). The constructed fen had a much larger supply rate of sulfur compared to both reference sites, with an ~134% higher supply rate compared to the poor fen and ~20% higher rate than the saline fen at 0.2 m depth (0.2 m: $F_{2,37} = 964.4$, $p < 0.001$; 0.7 m: $F_{2,37} = 626.3$, $p < 0.001$). The poor fen had a higher supply rate of ammonium compared to the two other sites at both depths (0.2 m: $F_{2,37} = 67.9$, $p < 0.001$; 0.7 m: $F_{2,37} = 41.8$, $p < 0.001$), and iron was highest at the poor fen and lowest at the saline fen, with the constructed fen having an iron supply rate in between the two reference sites (0.2 m: $F_{2,37} = 58.6$, $p < 0.001$; 0.7 m: $F_{2,37} = 50.8$, $p < 0.001$). The manganese supply rate was higher and similar at the poor fen and constructed fen compared to the saline fen (0.2 m: $F_{2,37} = 20.3$, $p < 0.001$; 0.7 m: $F_{2,37} = 10.7$, $p < 0.001$).

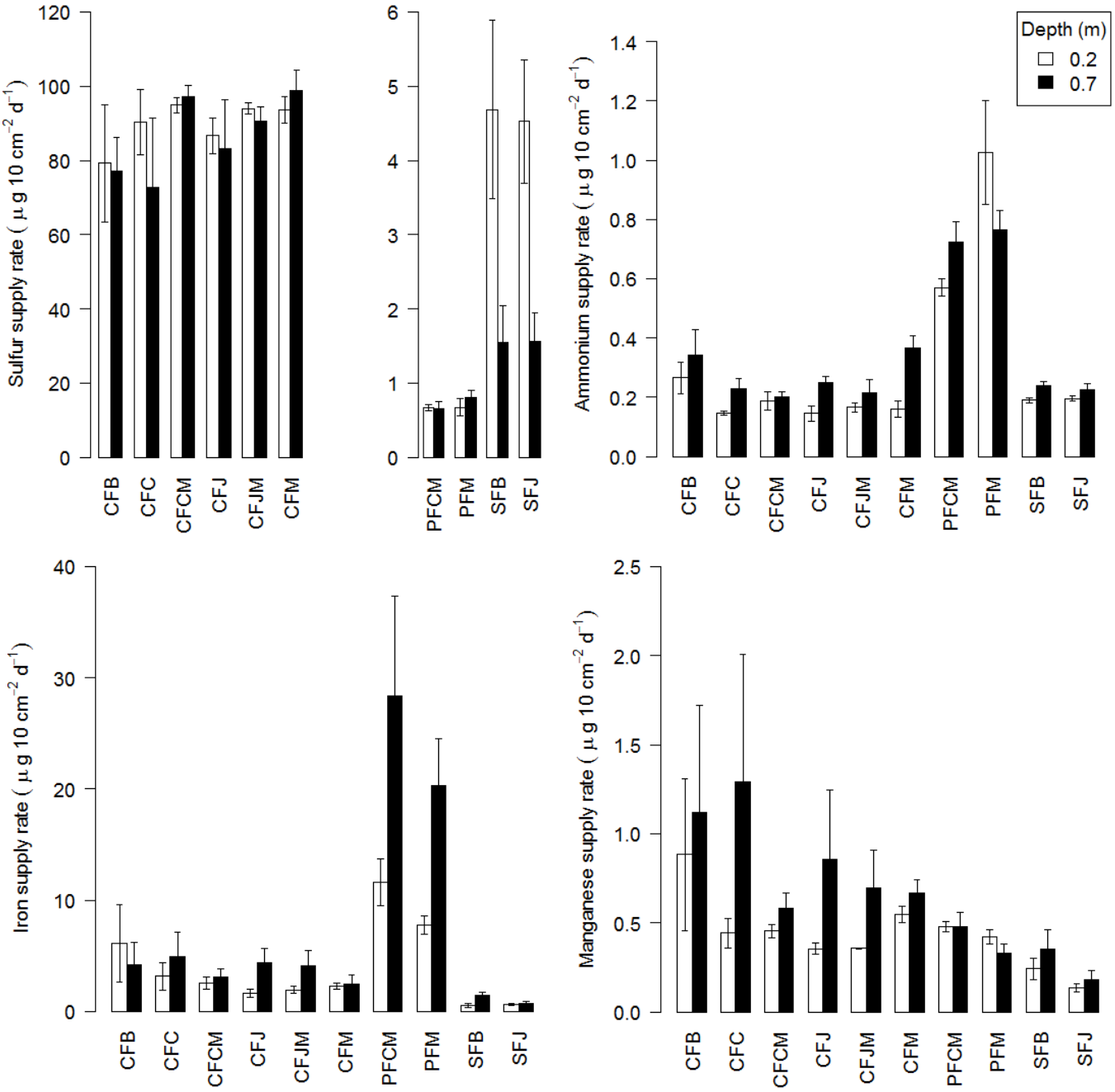

Fig 2.4. Plant root simulator (PRS) probe supply rates of sulfur, ammonium, iron, and manganese from cover types at the constructed fen (CF), poor fen (PF), and saline fen (SF). Refer to Fig 2.2 for a description of cover types. Each cover type was made up of four plot replicates. At each plot four of each anion and cation PRS probes were buried beside the flux collar on four sides. PRS probes were buried for 14 days from July 6-July 20, 2015. Notice the different scale used in the sulfur figure (top left) for the different sites (CF vs. PF and SF).

Controls on CH_4 dynamics

Several vegetation and chemical controls on CH_4 flux and concentration at depth were found across plots at the three study sites (Table 2.3). Higher CH_4 flux and pore water concentration was found to be correlated to higher belowground biomass, moss, shrub, and total plant percent cover, soil temperature and iron and ammonium supply rate. Negative correlations implied that lower CH_4 flux and concentrations were associated with higher values of aboveground biomass, percent cover of graminoid and litter, pH, EC, and sulfur supply rate. The positive correlation between CH_4 concentration at both depths and GEP_{max} suggested lower CH_4 was related to higher productivity. Considering CH_4 flux, more variables associated with pore water chemistry (pH, EC, and sulfur, iron, and ammonium ion supply rate) were found to be significantly correlated compared to vegetation variables. Conversely, more vegetation related controls on CH_4 pore water concentration at 0.2 m and 0.7 m depth were observed (total belowground biomass, aboveground biomass, GEP_{max}, and percent cover variables).

Table 2.3. Spearman correlation results of methane (CH_4) flux and CH_4 concentration at 0.2 m and 0.7 m with environmental variables. *

	CH_4 flux		CH_4 Conc. 0.2 m		CH_4 Conc. 0.7 m	
Variable	rho	p-value	rho	p-value	rho	p-value
AG	0.04	0.828	-0.33	**0.041**	-0.47	**0.002**
$BG_{0\text{-}0.2}$	0.42	**0.006**	0.54	**<0.001**	0.48	**0.002**
$BG_{0.2\text{-}0.4}$	0.59	**0.036**	0.20	0.517	0.09	0.765
GEP_{max}	0.05	0.753	0.43	**0.006**	0.42	**0.007**
Moss	0.48	**0.002**	0.29	0.071	0.17	0.306
Shrub	0.49	**0.001**	0.59	**<0.001**	0.48	**0.002**
Graminoid	-0.19	0.235	-0.57	**<0.001**	-0.54	**<0.001**
Litter	0.01	0.884	-0.31	**0.048**	-0.34	**0.032**
Total	0.55	**<0.001**	0.27	0.094	0.17	0.300
WT	-0.16	0.329	-0.27	0.094	-0.31	0.054
Soil Temp.	-0.18	0.253	-0.04	0.797	0.36	**0.022**
pH	-0.44	**<0.01**	-0.76	**<0.001**	-0.70	**<0.001**
EC	-0.51	**<0.001**	-0.21	0.189	-0.21	0.187
Sulfur	-0.60	**<0.001**	-0.78	**<0.001**	-0.79	**<0.001**
Iron	0.40	**0.011**	-0.21	0.189	0.32	**0.048**
Ammonium	0.42	**0.006**	0.61	**<0.001**	0.66	**<0.001**
Manganese	0.14	0.381	-0.04	0.794	-0.23	0.150

*Environmental variables include aboveground biomass (AG), belowground biomass (BG; 0-0.2 m depth and 0.2-0.4 m depth), maximum gross ecosystem productivity (GEP_{max}), percent cover of moss, shrub, graminoid, litter, and total cover, water table (WT), soil temperature (Soil Temp.), pH, electrical conductivity (EC), and sulfur, iron, ammonium, and manganese supply rate. Average seasonal plot values were used for CH_4 Flux, CH_4 Conc. 0.2 m, CH_4 Conc. 0.7 m, GEP_{max}, WTD, Soil Temp., pH, and EC. For CH_4 Flux and CH_4 Conc. 0.2 m, soil temperature at 0.2 m depth was used; for CH_4 Conc. 0.7 m, soil temperature at 0.7m depth was used. Bold indicates a significant result ($p < 0.05$).

Differences were found between the PCAs that included CH_4 flux and CH_4 pore water concentration at 0.2 m and 0.7 m depth (Fig 2.5). The first two principal components (PCs) were important (eigenvalues > 1, accounting for 81.1% of data variance) in the PCA that included CH_4 flux and correlated environmental variables (Fig 2.5I). Methane flux, followed by ammonium supply rate contributed the most to PC1, while belowground biomass and total vegetation cover had the highest loadings on PC2 (Appendix 2). The PCA indicated strong clustering based on sites, with the constructed fen and saline fen plots overlapping and the poor fen plots located on the opposite side of PC1. The first two PCs were also important, with eigenvalues greater than one, for the PCA that included CH_4 pore water concentration at 0.2 m and highly correlated environmental variables. These PCs accounted for 84.3% of data variance, with CH_4 concentration at 0.2 m and pH contributing the most to PC1, and belowground biomass and graminoid percent cover contributing most highly to PC2 (Fig 2.5II; Appendix 2). In this PCA strong clustering based on site also occurred, with plots from each site clustering in distinct locations on the PCA. Finally, the PCA including CH_4 pore water concentration at 0.7 m depth and environmental controls had three important principle components that accounted for 87.7% of data variance (Fig 2.5III). The first two variables with the highest loadings for PC1, PC2, and PC3 were: CH_4 concentration at 0.7 m and pH, soil temperature at 0.7 m and ammonium supply rate, and graminoid and shrub vegetation cover, respectively (Appendix 2). In this PCA plots from each of the three study sites also clustered in different locations when examining the first two PCs.

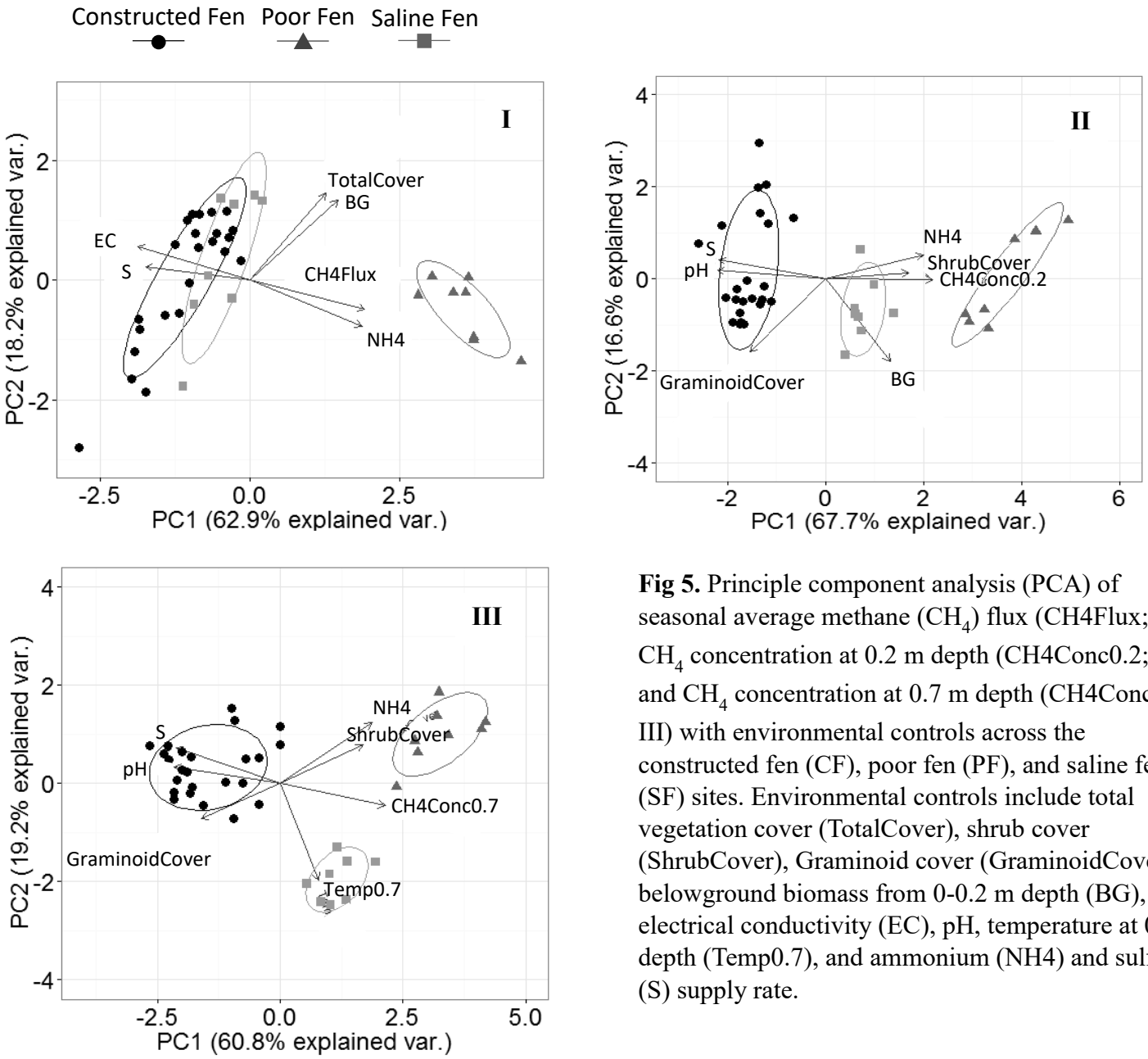

Fig 5. Principle component analysis (PCA) of seasonal average methane (CH_4) flux (CH4Flux; I), CH_4 concentration at 0.2 m depth (CH4Conc0.2; II), and CH_4 concentration at 0.7 m depth (CH4Conc0.7; III) with environmental controls across the constructed fen (CF), poor fen (PF), and saline fen (SF) sites. Environmental controls include total vegetation cover (TotalCover), shrub cover (ShrubCover), Graminoid cover (GraminoidCover), belowground biomass from 0-0.2 m depth (BG), electrical conductivity (EC), pH, temperature at 0.7 m depth (Temp0.7), and ammonium (NH4) and sulfur (S) supply rate.

2.5 Discussion

Due to waterlogged and reducing conditions, CH_4 is produced and emitted from most natural peatlands (Gorham, 1991), driven by microbial processes that regulate CH_4 production and oxidation (Edwards et al., 1998). Fen construction in the AOSR in northeastern Alberta has recently been attempted. An understanding of carbon cycling including the CH_4 dynamics of these systems for some time post-reclamation is advantageous to provide knowledge on biogeochemical functionality of these ecosystems compared to natural peatlands. In this study different CH_4 flux and pore water pools were observed at a constructed fen in its third growing season post-reclamation compared with natural reference fens that had similar vegetation cover types as at the constructed fen. These differences were correlated with distinct hydrochemistry and differences in vegetation type and productivity.

Average 2015 CH_4 flux values from the constructed fen (4.0 mg CH_4 m^{-2} d^{-1}) and saline fen (4.4 mg CH_4 m^{-2} d^{-1}) in this study were substantially lower compared to values measured from the poor fen in this study (23.9 mg CH_4 m^{-2} d^{-1}) as well as from other fen sites in the region. For instance, Long et al. (2010) reported average emissions of 25.6 mg CH_4 m^{-2} d^{-1} over a growing season at a moderately-rich treed fen in Alberta using eddy covariance. More broadly, in a synthesis of CH_4 emissions from wetlands at a range of latitudes, Turetsky et al. (2014) report a growing season mean flux from boreal undisturbed wetlands including all types (bog, fen, etc.) of 72.7 mg CH_4 m^{-2} d^{-1}. Monitoring GHG fluxes from the constructed fen considered in this study by Nwaishi et al. (2016) in 2013 directly after vegetation was planted, and in 2014 one-year post reclamation, also revealed low seasonal CH_4 flux (<2.5 mg CH_4 m^{-2} d^{-1}), with no difference found across a range of vegetation cover types or between the two years. Subsurface CH_4 pore water concentration at the constructed fen (0.16-0.52 mg/L at 0.2-0.7 m depth) was

also low compared to both the poor fen (4.63-4.30 mg/L at 0.2-0.7 m depth) and saline fen (1.81-3.14 mg/L at 0.2-0.7 m depth) in this study. Strack et al. (2004) also found higher average subsurface CH_4 concentrations in a pool-ridge complex of a poor fen in southern Quebec, where CH_4 pore water concentration ranged from 1.3-5.8 mg/L from 0.2-1 m depth. Further sampling over time will be required to determine when the CH_4 pool at the constructed fen may increase enough to result in a similar concentration to natural sites in the AOSR such as the poor fen analyzed in this study.

Vegetation controls on CH_4 dynamics

At the constructed fen, bare and moss plots were found to have significantly lower flux compared to *Juncus balticus* plots (Fig 2.2I). Lower pore water CH_4 concentration at the *Juncus balticus* plots compared to the bare and moss plots (Fig 2.3Ia) despite higher flux indicates that the *Juncus balticus* plants transported CH_4 to the atmosphere through aerenchyma (Whalen, 2005). Evidence of CH_4 transport from the other cover types with vascular plants was also found, given lower concentrations at depth despite similar fluxes compared to the moss and bare cover types. While no evidence of differences in CH_4 emissions between *Carex aquatilis* and *Juncus balticus* were found in the present study, low flux values at the constructed fen made it challenging to parse out the vegetation influence on the flux.

Similar patterns across sites emerged when all vegetation variables were considered. The constructed fen had similar or higher aboveground biomass, productivity through GEP_{max}, and graminoid cover compared to the reference sites in this study (Table 2.1). This indicates that the direct planting of vegetation at the constructed fen was beneficial for biomass accumulation and

for the CO_2 sink to establish (Nwaishi et al., 2016). The vegetation results revealed that planted graminoid species at the constructed fen were thriving two years' post-restoration, supporting past laboratory studies that found graminoids to reproduce quickly and be highly resistant to the influence of oil sands process-affected water with high salinity and naphthenic acid concentrations (Pouliot et al., 2012; Rezanezhad et al., 2012). Methane emissions have been reported to increase with higher vascular vegetation productivity (Joabsson and Christensen, 2001), cover (Bubier et al., 1995a), and biomass (Bellisario et al., 1999). The present study did not support these findings, as even though vegetation biomass, productivity, and total cover at the constructed fen was higher or similar to the reference sites, CH_4 flux remained low. Moreover, the correlation results across all study sites indicated lower CH_4 pore water concentration with increasing aboveground biomass, percent cover of graminoid, and GEP_{max} (Table 2.3). Methane pore water concentration at 0.7 m was found to be lower at plots with higher litter cover, despite the expectation that more litter would increase the CH_4 pool due to the presence of highly decomposable labile substrate (Shannon et al., 1996). While the *Juncus balticus* cover types at the saline fen had highest litter cover across all sites, more replicates at the constructed fen with high litter cover apparently influenced the negative correlation results (Table 2.1). Overall, the vegetation correlation results were related to low CH_4 concentration at the constructed fen despite high vegetation cover and productivity, signifying that the hydrochemical conditions masked the vegetation effect. This was supported by the three PCA results, where vegetation variables such as total, graminoid, and shrub cover, did not fall along PC1 that correlated strongly with CH_4 flux and pore water concentration, but rather in between PC1 and PC2 (Fig 2.5).

The species composition across the three study sites differed, with the percent cover of shrub, moss, and total vegetation highest at the poor fen (Table 2.1). As the poor fen had the highest CH_4 flux and concentration, the correlations that included these vegetation variables had positive relationships (Table 2.3). Moss was especially common in the vegetation survey at the poor fen, causing the total cover to be highest across sites. Due to the symbiotic relationship between methanotrophic bacteria (CH_4 consumers) and moss, it may have been expected that the higher cover of moss found at the poor fen could result in low CH_4 flux (Larmola et al., 2010). However, the result of highest CH_4 emissions from the poor fen where moss cover was highest supports a predictive model for CH_4 by Bubier et al. (1995b) using bryophyte species cover. This model was based on a negative relationship between CH_4 flux and height above the mean water table, suggesting that bryophyte cover can indicate wetness, with bryophyte species found in wetter pools where CH_4 flux is higher. The poor fen also had higher shrub cover compared to the other sites, and the shrub cover included woody tissues. Generally, woody shrub species have been known to result in lower CH_4 flux compared to other vascular species, related to low transport through stems and leaves of woody species (Shannon and White, 1994). The relationship of higher CH_4 flux and concentration with higher shrub cover in this study was associated with the low flux values from the constructed fen and saline fen.

Belowground biomass was sampled to understand how roots that differentially affect CH_4 production and consumption (Segers, 1998) may be influencing CH_4 dynamics across cover types and sites. Higher belowground biomass from 0-0.2 m was found at cover types with graminoids at the constructed fen compared to the bare and moss cover types (Fig 2.2Ib). Lower CH_4 concentration at the cover types with higher biomass supports vascular species at the constructed fen acting as gas conduits for CH_4 transport (Joabsson and Christensen, 2001). Moss

and *Carex aquatilis* + moss cover types at the poor fen, and at the saline fen *Juncus balticus* cover type from 0-0.2 m, had significantly higher belowground biomass compared to the constructed fen (Fig 2.3III-IVb), and correlation results revealed that the total biomass at these depths was related to higher CH_4 emissions or concentration (Table 2.3). Greater belowground biomass was possibly one reason for a greater CH_4 pool at the saline fen compared to the constructed fen, as evidenced by PCA results (Fig 2.5II-III), that may have been associated with labile root exudates increasing CH_4 production (Megonigal et al., 1999; Whiting and Chanton, 1993).

A similar and low CH_4 flux was found between the saline fen *Juncus balticus* plots and the constructed fen *Juncus balticus* plots, despite a higher CH_4 concentration at the saline fen. It is possible that this was related to ROL from *Juncus balticus* causing CH_4 consumption at the saline fen, especially from 0-0.2 depth where belowground biomass, particularly fine root biomass, was abundant (results not shown). This was further evidenced by the higher concentration of CH_4 at the saline fen bare cover type, with less belowground biomass compared to the *Juncus balticus* cover type (Fig 2.2III). Further research on the role of *Juncus balticus* on peatland CH_4 dynamics is required to better understand its role in rhizospheric oxidation. Likely aerobic oxidation above the 0.2 m pore water sampler also contributed to the low CH_4 flux at the saline fen, given the relatively deep water table position across this site (-10.9 cm on average seasonally), particularly in areas dominated by *Juncus balticus* (Table 3.2).

Hydrochemical controls on CH_4 dynamics

Several studies have reported that water table acts as a dominant control on CH_4 flux (e.g. Bubier et al. 1993; Pelletier et al., 2007; Couwenberg and Fritz, 2012) with a shallower water table corresponding to greater flux, associated with a smaller oxic zone where CH_4 consumption could occur. This relationship is so dominant in undisturbed peatlands that water table has been used as a forcing in CH_4 emission models (Walter and Heimann, 2000). Couwenberg and Fritz (2012) argue that water table position can be used as a proxy for CH_4 emissions in boreal and temperate peatlands, with a water table position of 20 cm or shallower resulting in a significant increase in flux. Water table position is a known determinant of redox conditions (Belyea, 1999). The geochemistry implied an availability of alternative TEAs across sites in this study (Fig 2.4). This was related to reduced CH_4 concentration and emissions (Table 2.3; Fig 2.5), particularly at the constructed fen, and suggests that water table position cannot be used to indicate CH_4 emissions from reclaimed fen systems in the AOSR.

Chemical parameters varied across sites (Table 2.2). Distinct pH at each study site (constructed fen>saline fen>poor fen) largely explained CH_4 flux and concentration. The constructed fen and saline fen sites had pH values within the optimal range for CH_4 production (pH 6-7; Blodau, 2002), while the poor fen had a slightly lower pH (5.3-5.9). Higher CH_4 emissions and concentration from the poor fen regardless of lower pH levels indicate an adaption by methanogen communities to tolerate the more acidic conditions at this site (Updegraff et al., 1996). Electrical conductivity was especially high at the saline fen, associated with high concentrations of chloride and sodium ions influenced by the unique groundwater (Wells and Price, 2015). Higher EC found at the constructed fen than the poor fen is likely related to higher concentration of ions in the water that moved from the upland slopes to the reclaimed fen, or

could be associated with a high ion concentration in the donor peat used for reclamation. Strong correlations found between pH and CH_4 flux and concentration, and EC and CH_4 flux (Table 2.3, Fig 2.5), highlight the distinct hydrochemistry across the three study sites.

Differences in ammonium, as well as sulfur, iron, and manganese supply rate measured with the PRS probes implied that different TEAs and redox conditions occurred across the study sites (Fig 2.4). High sulfur supply rate at the constructed fen was highly correlated with the low CH_4 emissions and concentration (Table 2.3, Fig 2.5). Sulfate is known to reduce CH_4 release as sulfate-reducing bacteria efficiently compete for substrates necessary for CH_4 production (Dise and Verry, 2001). Although the PRS probes did not specifically measure sulfate ions, it is highly likely that a portion of the measured sulfur supply rate was sulfate, particularly at the constructed fen where a circumneutral pH occurred (Table 2.2) and the anion binding capacity of sulfate was likely low (Lamers et al., 1998). Even if the PRS probes had adsorbed other mobile forms of sulfur, such as organic sulfur, sulfide, or disulfide, the eventual recycling of these forms to sulfate was possible (Pester et al., 2012). Regardless of the sulfur form, the PRS probes effectively revealed that the drastic differences in sulfur cycling across study sites influenced methane emissions and pore water concentration. High sulfur found at the constructed fen could be associated with the influence of tailing sand used for upland construction, or a high concentration of sulfur ions present in the donor peat (discussed above), or due to deposition of reactive sulfur associated with industrial activity in the oil sands area (Proemse et al., 2012).

Methanogenic bacteria in wetlands have also been found to be supressed by inorganic compounds such as nitrate (Balderston and Payne, 1976), or by iron III oxide (Roden and Wetzel, 1996). Higher ammonium at the poor fen suggests a larger inorganic nitrogen pool at this site compared to the saline fen or constructed fen. Wood et al. (2015) found that elevated

total inorganic nitrogen supply rate at natural sites in the AOSR was associated with an external supply, such as from groundwater, as opposed to from internal processes including mineralization and decomposition. Hence, the higher ammonium supply rate at the poor fen in this study does not necessarily indicate less reduced conditions that may be non-ideal for methanogenesis. The iron ions adsorbed on the PRS probes were likely the more soluble iron (II) ions (Kirk, 2004), and the higher iron supply rate found at the poor fen corresponding to higher CH_4 emissions suggests that the redox state at the poor fen was actually more reduced compared to the other site, causing less CH_4 suppression.

2.6 Conclusions

In this study differences in CH_4 flux, CH_4 pore water concentration, and environmental variables, particularly geochemistry, were found between a constructed fen, poor fen, and saline fen in the AOSR. This indicates that constructed fens do not function similarly to natural reference fens in the area shortly after reclamation, although given the extent of disturbance to the pre-reclaimed landscape where fen construction occurred, this finding is not surprising. Controls on CH_4 flux and concentration suggest that explaining CH_4 emissions from a reclaimed fen requires in-depth knowledge of plot-scale ecohydrological and chemical conditions. As CH_4 is a strong GHG, low CH_4 flux from a constructed fen may actually be seen as beneficial in future fen creation projects by reducing GHG emissions. However, CH_4 production and flux indicate highly reduced conditions that inhibit organic matter decomposition resulting in its accumulation in the soil, and lack of these conditions are a concern for long term peat accumulation rates. Ultimately a clear statement of reclamation goals (e.g., greenhouse gas sink

vs. similar biogeochemical function as natural fens) will be required to determine how CH_4 flux and its controls relate to the success of constructed fen projects, particularly over the long-term.

Chapter 3: The influence of *Carex aquatilis* and *Juncus balticus* on methane dynamics: a comparison with water sourced from natural and constructed fens

3.1 Introduction

Peatlands play a significant role in carbon sequestration related to the long-term carbon dioxide (CO_2) sink of these ecosystems, associated with the incomplete decomposition of organic matter over thousands of years (Vitt, 2006). Methane (CH_4) and nitrous oxide (N_2O) are strong greenhouse gases (GHGs) that can also be emitted from peatlands, and may partially offset the CO_2 sink (Strack et al., 2008). This is especially true for CH_4, as it is estimated that natural peatlands globally release approximately 30 Tg CH_4 on an annual basis; in comparison, annual N_2O emissions have been found to be in the order of 0.02 Tg, while the CO_2 sink of natural peatlands is around 100 Tg carbon per year (Frolking et al., 2011).

In the Athabasca Oil Sands Region (AOSR) near Fort McMurray, Alberta, Canada surface mining has impacted large expanses of fen peatlands (Rooney et al., 2011). Consequently, fen construction in this area has been attempted (Pollard et al., 2012). Constructed fens have the potential to minimize the GHG footprint of mining companies by acting as an overall sink of CO_2, CH_4, and N_2O (Nwaishi et al., 2016). It is valuable to understand rates of carbon cycling and GHG emissions from these reclamation projects (Daly et al., 2012). Understanding differences in variables that influence carbon cycling and GHG emissions, such as water chemistry and vegetation, between constructed fens and natural fens will provide information on the biogeochemical functioning of constructed fens (Nwaishi et al., 2015). For instance, constructed fen designs can result in alkaline ground water discharge that contains soluble organic chemicals (Pouliot et al., 2012) that can influence water chemistry and affect GHG emissions of reclaimed peatlands (Nwaishi et al., 2016). A variety of vegetation techniques have been attempted at constructed fens in order to test vegetation establishment (Vitt et al.,

2016; A. Borkenhagen, unpublished), and these different vegetation cover types have been previously found to result in different GHG dynamics (Nwaishi et al., 2016). Recommendations for fen construction that could result in reduced GHG fluxes, including CH_4, are beneficial to support the creation of these reclaimed ecosystems.

Methane flux from peatlands is dependent on how the GHG is produced, consumed, and transported in the peat profile (Lai, 2009). Two types of methanogens produce CH_4 under different conditions. Acetotrophic methanogens produce CH_4 and CO_2 from acetate, while hydrogenotrophic methanogens produce CH_4 through the reduction of CO_2 while using hydrogen as an electron donor (Whalen, 2005). Methanotrophic bacteria can also consume CH_4 in the peat profile through oxidation (Anthony, 1986). Three main processes result in CH_4 release from peatlands to the atmosphere including diffusion through the peat matrix, transport through vascular plants, and ebullition (bubbling; Bridgham et al., 2013). The diffusion of CH_4 through peat, associated with a concentration gradient between the soil and atmosphere, is slower compared to release through plant-mediated transport and ebulltion events (Lai, 2009).

As methanogenesis is strongly related to soil water chemistry, this is an important control regulating CH_4 emissions from peatlands (Blodau, 2002). Methanogens can withstand a range of environmental conditions (Dise and Verry, 2001) when a redox potential lower than -300 mV exists, and thrive when electron acceptors including oxygen, nitrate, ferric iron (iron III), manganese (manganese III, manganese IV) and sulfate are not present (Kamal and Varma, 2008). The presence of oxygen and nitrate can prevent methanogenesis due to aerobic and denitrifying bacteria, respectively, directly eliminating labile carbon from the system (Le Mer and Roger, 2001). The anaerobic decomposition process utilizing iron III, and manganese III and IV reduction can supress CH_4 production, particularly in mineral wetlands (Roden and Wetzel,

1996; Lovely and Phillips, 1988). Sulfate-reducing bacteria are more efficient at competing for substrates necessary for methanogenesis (labile carbon, hydrogen) and sulfate availability also limits CH_4 production (Lovely and Klug, 1983). Soil acidity should be considered in relation to methanogenesis as methanogen growth is favoured in a pH range between 6-7 (Dunfield et al., 1993); however, field studies have indicated that adaptions to acidic conditions have allowed CH_4 production to also occur outside this range (Updegraff et al. 1996).

Soil water chemistry can also affect CH_4 emissions by influencing CH_4 oxidation. Given that CH_4 is a necessary substrate for methanotrophy, oxidation is positively correlated to CH_4 production, and consequently soil water chemistry controls on methanogenesis discussed above will also indirectly control CH_4 oxidation rates (Le Mer and Roger, 2001). Aerobic CH_4 oxidation has been estimated to consume between 40-70% of the CH_4 created by methanogenesis in wetlands (Megonigal et al., 2004). However, CH_4 can also be oxidized via anaerobic oxidation of CH_4 (AOM) that is largely dependent on soil water chemistry (Smemo and Yavitt, 2007). Globally, AOM alleviates a large portion of the CH_4 flux to the atmosphere in marine environments (Reeburgh, 2007). Anaerobic oxidation of CH_4 is possible when oxygen is replaced by alternative electron acceptors and is understood to involve a process known as "reverse methanogenesis", where methanogens act as oxidizers when hydrogen is in low supply, and the oxidation equation of CH_4 involves H_2O as an electron acceptor while releasing hydrogen and CO_2 as products (Hoehler et al., 1994). A syntrophic relationship between sulfate-reducing bacteria and the methanogens maintain the low hydrogen concentration necessary for reverse methanogenesis as the sulfate-reducers use the hydrogen produced by the CH_4 oxidation as an electron donor to reduce sulfate (Valentine and Reeburgh, 2000). Recently, Gupta et al. (2013) found evidence for AOM in a range of North American peatlands using ^{13}C tracers,

although it was unclear which electron acceptor(s) was (were) driving this process. In contrast, previous reviews had found little evidence of AOM in peatlands (Smemo and Yavitt, 2011). This was thought to be the case mostly because sulfate, an important alternative electron acceptor in marine and salt water AOM, is not usually observed in high quantities in freshwater environments (Smemo and Yavitt, 2011). It is accepted that nitrate, iron, manganese and organic alternative electron acceptors may also play a role in freshwater AOM (Bridgham et al., 2013). Nitrate as an electron acceptor could provide adequate free energy for AOM (Raghoebarsing et al., 2006). While metal concentrations including iron and manganese are lower in peat soils compared to mineral soils, the ability of these metals to be oxidized and reduced could also drive AOM in peatlands (Beal et al., 2009). Further research regarding controls on AOM in peatlands is necessary to understand how this process may limit CH_4 emissions across natural and reclaimed ecosystems with a range of biogeochemical and environmental conditions (Gupta et al., 2013).

The presence of vascular plants also acts as a strong control on the CH_4 dynamics in peatlands (Joabsson et al., 1999). Plant-mediated transport of CH_4 occurs through vascular plant aerenchyma that allow for gas movement, and consequently can distribute oxygen to the root zone (Couwenberg and Fritz, 2012). Plant-mediated diffusion occurs when oxygen consumption caused by plant respiration results in a concentration gradient causing oxygen flow to the roots and rhizomes in the anoxic peat layer (Whalen, 2005). This outflow of oxygen from the plant tissue corresponds to an influx of CH_4 that can diffuse up the aerenchyma of the plant and be released to the atmosphere (Lai, 2009). Besides increasing CH_4 transport in peatlands, vascular plants can also amplify CH_4 flux to the atmosphere by providing organic matter from decomposing biomass that methanogens can utilize for production (Nilsson and Bohlin, 1993).

Vascular plants release several labile carbon compounds (organic acids, sugars, phenolics, amino acids, etc.) through root systems (Joabsson et al., 1999). These fresh compounds can increase CH_4 emissions as they cause greater soil microbial activity that produces end products in the peat soil, such as acetate, important for CH_4 production (Ström et al., 2003). Finally, vascular plant species can reduce CH_4 emissions in peat soils influenced by radial oxygen loss (ROL), providing the necessary conditions for methanotrophs to consume CH_4, even in soils that are largely anoxic (Wießner et al., 2002). Radial oxygen loss can also drive the oxidation of reduced terminal electron acceptors (TEAs) that may both decrease CH_4 production and increase the potential for AOM (Laanbroek, 2010).

Variation in vascular plant characteristics results in species-specific effects on CH_4 dynamics across peatlands (Ström et al., 2005). For instance, previous studies have found woody plants to have lower CH_4 emissions compared to herbaceous species, associated with less transport through aerenchymous roots (Grosse et al., 1992), more lignin and suberin in root cells causing reduced ROL (Armstrong and Armstrong, 2001), slower decomposition of woody tissues, and a smaller labile belowground carbon pool related to decreased root exudates (Vann and Megonigal, 2003). As biomass, productivity, and vegetation cover are known to be positively correlated to CH_4 emissions in natural peatlands, dissimilarities in these variables, even within similar herbaceous plant groups, can result in differential CH_4 dynamics (Joabsson and Christensen, 2001; Bellisario et al., 1999; Bubier et al., 1995a). Differences in the efficiencies of vascular plants in contributing to CH_4 oxidation have also been found: Wießner et al. (2002) measured higher rates of ROL from *Typha latifolia* compared to *Juncus effusus* in a hydroponic experiment, while Ström et al. (2005) found evidence that oxidation in the

rhizosphere decreased CH_4 emissions by 20-40% for *Carex rostrata* but >90% for *Eriophorum vaginatum* and *Juncus effusus*.

Production of CH_4 in peatlands signifies anoxic conditions as well as slow, inefficient decomposition necessary for peat accumulation (Moore and Basiliko, 2006). The occurrence of CH_4 production and flux similar to natural peatlands may indicate the ability of constructed fen ecosystems to ultimately accumulate peat, a goal of fen creation (Daly et al., 2012). Hence, a thorough understanding of controls on CH_4 production and flux from constructed fen peatlands, including soil water chemistry and vegetation, is required to predict if appropriate chemical conditions exist to promote the eventual accumulation of peat. Given the evidence of species-specific effects of vascular plants on CH_4 emissions, it is further valuable to understand how vascular species used in reclamation projects differentially influence CH_4 dynamics to make recommendations for future projects that could result in a decreased flux of this GHG. A factorial greenhouse experiment was conducted to determine the effects of two vascular plant species used for reclamation, *Juncus balticus* and *Carex aquatilis,* on CH_4 dynamics. The goal of the greenhouse experiment was to understand how water sourced from a constructed fen influenced the CH_4 dynamics of *Carex aquatilis* and *Juncus balticus* compared to water from a natural fen. Specific objectives were: 1) to evaluate CH_4 emissions, pore water CH_4 concentration through the peat profile, and CH_4 oxidation for *Carex aquatilis* and *Juncus balticus* grown in water from a constructed fen or natural fen and 2) determine controls on CH_4 emissions, pore water CH_4 concentration, and oxidation of the different plant species and water types. Based on literature results (eg. Ström et al., 2005), it was hypothesized that *Juncus balticus* would have lower CH_4 emissions and concentration related to higher ROL causing more CH_4 oxidation compared to *Carex aquatilis*. It was further hypothesized that plots with water

from the constructed fen would have lower CH_4 emissions and concentration associated with a pre-existing knowledge of high sulfur in soil water and low CH_4 flux observed at the constructed fen site (Chapter 2; Nwaishi et al., 2016).

3.2 Methods

Greenhouse experiment set-up

A factorial greenhouse experimental design with two factors (cover and water type) was used for this study (Fig 3.1). This design included two vegetation species (*Carex aquatilis* and *Juncus balticus*) and a bare control, and water with two different chemical compositions (collected from a constructed fen and rich fen). Measurements were made in a light and temperature regulated greenhouse located at the University of Waterloo, Ontario, Canada, from January until July 2016. *Carex aquatilis* and *Juncus balticus* were collected from a constructed fen located in northeastern Alberta (56° 55.8701 N, 111° 25.0166 W; Chapter 2; Ketcheson and Price, 2016; Price et al., 2010) and transported back to the university in early September, 2015. Rhizomes and roots of the *Carex aquatilis* and *Juncus balticus* vegetation were rinsed thoroughly upon arrival at the university, after which they were kept damp and refrigerated (<4˚C) prior to planting. Approximately 80 L of water was also collected for use in the experiment from a ponded area at the constructed fen. A similar quantity of water was collected from a stream running through a rich-fen located in southern Ontario within the Fletcher Creek Ecological Preserve (43° 41.5671 N 80° 11.7077 W; see Duval et al., (2011) for full site description).

In November 2015 three PVC pipes (diameter x height = 0.2 m x 0.3 m) were placed in each of six large bins (length x width x height = 0.8 m x 0.5 m x 0.4 m) in the greenhouse (Fig 3.1). Four pore water samplers (see *Water Chemistry* description below) were placed in the middle of each PVC pipe, with tygon tubing extending out of 0.012 m diameter holes drilled in the side of the PVC. Mesh screening was attached to the bottom of the open pipe. Next, ~1700 g of milled peat (Premier Sphagnum Peat Moss) was weighed and placed in each PVC pipe. This resulted in a bulk density in the cores of 0.18 g/cm^3, similar to the near-surface bulk density found at the constructed fen site in 2013 (0.19-0.36 g/cm^3; Nwaishi et al., 2015b). A visually similar biomass of senesced *Carex aquatilis* or *Juncus balticus* individuals were planted in the peat of two of the PVC pipes within a bin, while a bare control was used for the third PVC pipe (PVC pipes are referred to as peat cores for the remainder of this study).

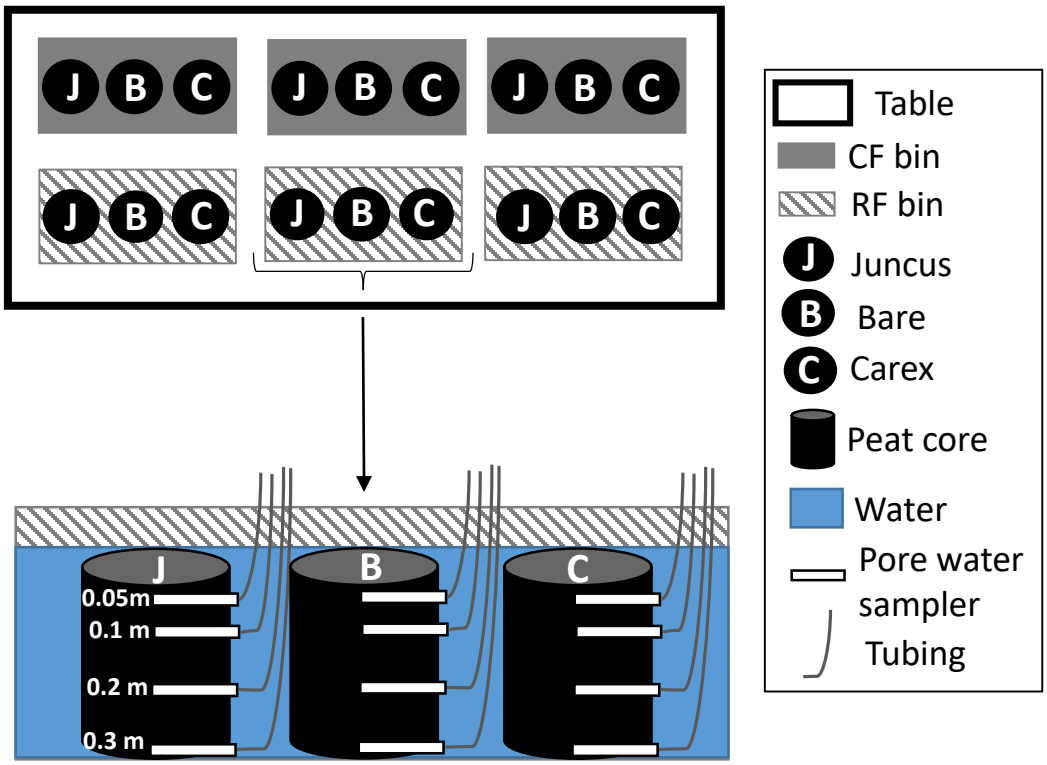

Fig 3.1. Schematic of the greenhouse experimental design (not to scale). Grey boxes represent bins that held peat cores and had water from the constructed fen (CF), while patterned boxes were bins that contained water from the rich fen (RF). Black circles and cylinders represent peat cores that had plants including *Juncus balticus* (J), *Carex aquatilis* (C), or bare controls (B).

The plants were left to establish for 70 days (Rezanezhad et al., 2012) before the water collected from the fen sites was introduced. Over this time ~50 L of untreated well water was added to the peat cores and bins as necessary to keep the peat wet and allow for plant growth. In January, 2016 the well water from the bins was drained and 25 L of water from the constructed fen was added to three of the bins, with 25 L of rich fen water added to the remaining three bins. The water was poured directly over the peat cores to allow infiltration. A further 17 L of well water was added the following day. The next week 12 L of well water was added, resulting in the soil cores being waterlogged within the bins. Measurements from the peat cores began on January 28, 2016 (day of experiment (DOE) 1), once the bins were waterlogged. Over the following weeks of the experiment ~8 L of well water per week was added as necessary to each bin to maintain the waterlogged peat and provide appropriate conditions for anoxia within the peat core profiles.

At the time of planting on November 13, 2015 until March 23, 2016 (DOE 55) an artificial high intensity discharge (HID) light fixture above the experiment bench was used, resulting in photosynthetically active radiation (PAR) of over 1000 μmol m^{-2} s^{-1} throughout each 24-hour day. Around DOE 60 the lights in the greenhouse were turned off as natural lighting was sufficient for plant growth and survival. From approximately DOE 78 until the end of the experiment (DOE 189) shades on the greenhouse windows were used as necessary to regulate greenhouse temperature. This resulted in a range of PAR values (27-1254 μmol m^{-2} s^{-1}) during measurements from about DOE 60 until the end of the experiment. Over the experiment the temperature in the greenhouse was maintained between 20 and 25 °C, with an average soil temperature at 2 cm of 21.3 ˚C.

Water chemistry

Water sampling occurred throughout the experiment to understand how constructed and natural fen water influenced CH_4 production and oxidation. Water samples were taken from pore water samplers at each peat core, as well as from the bins that contained the peat cores. The pore water samplers were constructed using 0.1 m long, 0.012 m inner diameter polyethylene piping (SharkBite, United States) slotted the length of the pipe, with screening attached around the pipe to prevent clogging, and tygon tubing attached to barbed fitting (Spaenaur, Canada) at the end of the sampler that extended out of the peat core. Pore water from the specific depths was sampled using a 20 mL syringe attached to the three-way valve. Electrical conductivity (EC) and pH were sampled monthly at a depth of 0.2 m from the peat cores. Anion sampling including chloride, nitrate, and sulfate occurred twice over the experiment, on DOE 29 and DOE 147, from 0.1 m and 0.3 m depths from the peat cores, however water from the bins was only sampled on DOE 29. Anion concentrations were determined via a Capillary Ion Chromatograph (IC) system (Dionex ICS-5000, ThermoFisher Scientific, United States) following filtration and preservation with chromate added approximately one hour after sampling (Ecohydrology Research Group Analysis Laboratory, University of Waterloo). On DOE 96 major cations including calcium, iron, manganese, sodium, and magnesium were sampled at a depth of 0.2 m from the peat cores, with analysis occurring by means of an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; iCAP 6300, Thermo Scientific, Unites States) after filtration and preservation with ultrapure nitric acid that occurred approximately one hour following sampling (Ecohydrology Research Group Analysis Laboratory, University of Waterloo).

Plant monitoring

The plants in peat cores with either *Carex aquatilis* or *Juncus balticus* were monitored over the experiment. Canopy cover was estimated three times on DOE 1, DOE 67, and DOE 173. Percent cover was visually estimated to the nearest 1% inside the peat core, including living tissue and litter of *Carex aquatilis* or *Juncus balticus*.

To understand productivity of the peat cores, a measurement of net ecosystem exchange (NEE) of CO_2 was determined using the closed chamber method (Alm et al., 2007). The closed chamber method was also used to measure CH_4 flux (see below) with a plastic collar (diameter x height = 0.19 m x 0.15 m) that fit on the peat cores and a clear chamber (diameter x height = 0.20 m x 0.41 m). The height of each collar from the peat surface was measured in order to correct chamber headspace volume for flux calculation. A battery-powered fan at the top of the chamber mixed the headspace. A plug was placed in a hole drilled in the side of the chamber that had a thermocouple to measure chamber temperature during flux measurements, as well as tubes equipped with three-way valves to facilitate measurements. From DOE 1 – DOE 78, CO_2 was logged on an Ultraportable Greenhouse Gas Analyzer (UGGA; Model 915-0011, Los Gatos Research, United States) in part per million (ppm) at the same time as CH_4 flux measurements. Upon chamber placement on the collar, the flux measurement was only started after the system had equilibrated and the GHG concentrations on the UGGA were stable. The CO_2 concentration that was logged every 10 seconds for the first 120 seconds after concentrations had stabilized were considered to calculate NEE after correcting for temperature and chamber volume, from the linear change in the CO_2 concentration over time. From DOE 102 - DOE 132, a portable infrared gas analyzer (IRGA; EGM-4, PP Systems, Massachusetts, USA) connected to the chamber using

tubing measured CO_2 concentration in ppm at similar periods as the CH_4 flux measurements. An integrated temperature and photosynthetically active radiation (PAR; μmol m^{-2} s^{-1}) sensor connected to the EGM-4 measured PAR and temperature in the chamber. To obtain NEE the CO_2 concentration, PAR, and chamber temperature were manually recorded at 15-second intervals from 0 to 105 seconds after the system had equilibrated and CO_2 concentrations were stable. In order to understand ecosystem respiration (ER) of the peat cores, CO_2 was measured with the IRGA under dark conditions using an opaque tarp that fit over the chamber twice over the measurement period (DOE 109 and DOE 138). Gross ecosystem productivity (GEP) could then be determined by calculating the difference between the NEE and ER values. In this study negative values for CO_2 exchange denote an uptake of CO_2 by the peat core.

Aboveground biomass, including all plant parts, was sampled from the peat cores at the end of the experiment from DOE 175 - DOE 189. All *Juncus balticus* or *Carex aquatilis* individuals were clipped from the peat surface of the cores. Litter was separated from live biomass after which the vegetation was dried for 72 hours at 60 °C, and weighed to determine dry biomass. Belowground biomass (roots, rhizomes) from the peat cores was also determined. Following the completion of measurements on DOE 188, peat cores were removed from the greenhouse and frozen. Frozen peat cores were then cut into 0.075 m increments with a saw (0-0.075 m; 0.075-0.15m; 0.15-0.225 m; 0.225-0.30 m). Upon thawing, belowground biomass, including both roots and rhizomes, was sorted from the peat using tweezers into coarse (>2 mm) and fine (<2 mm) biomass. The belowground biomass was then oven dried at 60°C for 72 hr and weighed to estimate dry biomass (Moore et al., 2002).

Methane emissions

Methane flux was monitored approximately biweekly from DOE 1 – DOE 177. From DOE 1- DOE 78, measurements were made on the UGGA (see *Plant monitoring* above). The CH_4 concentration in the chamber in parts per million (ppm) was logged on the analyzer every 10 seconds for 20 minutes, and the chamber temperature was recorded at intervals of 5, 10, 15, and 20 minutes over the flux measurement. The chamber flux was determined from the linear change in concentration over time including corrections for temperature and volume of the chamber. From DOE 102 – DOE 132, CH_4 emissions from the peat cores were determined from gas samples. At intervals of 5, 10, 15, and 20 minutes, air temperature in the chamber was recorded and 20 mL gas samples were taken from the chamber using a syringe. Gas samples were subsequently injected into evacuated Exetainers (Labco, UK). A gas chromatograph (GC; Shimadzu GC2014, Mandel Scientific, Canada) with a flame ionization detector was used to determine CH_4 concentrations of the gas samples, and the flux was determined from the linear change in CH_4 concentration, as above. Small negative or positive flux values where the change in concentration was within the variance of the GC, as determined from control samples, were assigned a value of 0 for flux (variance = ±5.82%). In instances where flux values appeared to capture ebullition events, only values with an $R^2 > 0.80$ were kept in the data set. This resulted in loss of 3.7% of the data analyzed via gas chromatography. Flux measurements were made from DOE 145 to DOE 188, using a Trace Gas Analyzer (TGA; LGR-DLT100, Los Gatos Research, United States). Methane flux was determined with the TGA in a similar manner to the UGGA (see above), with CH_4 concentration (ppm) logged every second for 20 minutes, and the chamber temperature recorded at 5 minute intervals over the flux period. Cross calibration between the

three methods used to determine CH_4 flux revealed that the variance in CH_4 concentration determined by the GC was 1.0% and 5.9% compared to the TGA and UGGA, respectively.

Methane concentration

Pore water CH_4 samples were collected at the same time as CH_4 flux measurements from DOE 1 - DOE 132, from the pore water samplers in the peat cores (see *Water chemistry* above) at four depths: 0.05 m, 0.1 m, 0.2 m, and 0.3 m. Pore water from the four depths was sampled using the three-way valve attached to the tygon tubing and a 60 mL syringe, with 20 mL of water flushed before a 20 mL sample was collected (Strack et al., 2004). Ambient air (20 mL) was next added to the syringe, and the sample shaken for 5 min to equilibrate dissolved gases into the syringe headspace, after which air was transferred to an evacuated Exetainer (Labco, UK). Air temperature in the greenhouse was recorded using a thermometer and thermocouple at the time of pore water sampling. Pore water CH_4 concentration at depth was calculated following the analysis of the headspace samples for CH_4 concentration on the GC (Kampbell and Vandegrift, 1998).

Methane oxidation

At the end of the experiment, from DOE 147 – DOE 188, CH_4 oxidation was determined by comparing CH_4 fluxes taken in both oxic and anoxic conditions (Denier van der Gon and Neue, 1996). Fluxes for the oxic conditions were determined as above using the TGA (see *Methane emissions* above) in both light and dark conditions. Dark conditions were obtained by covering the flux chamber with an opaque tarp. Following the oxic flux measurements at a peat

core, the chamber was flushed with 99% compressed nitrogen (4.8 PP, Praxair) for one hour at a rate of approximately ten times the volume of the chamber to obtain anoxic conditions. During flushing dark conditions were maintained to prevent oxygen formation via plant photosynthesis. Oxygen in the chamber after the flush was determined from air samples stored in evacuated Exetainers and analyzed on the GC. When oxygen in these samples were compared to oxygen free standards ran on the GC, an average variance of 20.3% was calculated, with an average oxygen content of 3.2%. After nitrogen flushing the chamber was left sealed for 18 hours (van der Nat and Middelburg, 1998) before being flushed for a further hour at approximately ten times the volume of the chamber. Following the second nitrogen flush oxygen in the chamber had a variance of 23.3% compared to an oxygen free standard, with an average oxygen content of 3.3%. After ~5 minutes following the second flush a CH_4 flux was taken with the TGA for 40 minutes in dark conditions, and then again in light conditions (without the shade) for 10 minutes. Finally, pore water CH_4 samples at the four depths were taken before the chamber was removed in order to understand changes in pore water CH_4 pools associated with anoxia. Absolute oxidation was determined as the difference between the anoxic and oxic CH_4 fluxes under similar light conditions. Relative oxidation was calculated using the following formula:

$$1 - (abs \frac{CH_4\ Flux_{oxic}}{CH_4\ Flux_{anoxic}}) \quad (1)$$

where abs was the absolute value (necessary for cases when the oxic flux had a negative value) and the CH_4 flux in oxic and anoxic conditions from similar light levels (light or dark) was used.

Data Analysis

The program R 3.2.5. (R Core Team, 2016) was used for all statistical analysis, and a significance of $\alpha = 0.05$ was applied. To understand differences in variables that were measured continuously over the experiment (NEE, pH, and EC) between plots with different water types (rich fen, constructed fen) and between cover types (*Carex aquatilis, Juncus balticus* and bare controls), a two-way ANOVA with repeated measures that accounted for date was used. A pairwise t-test with adjusted p-values using the Bonferroni method was applied to determine differences between water or cover types for each significant factor. Anion concentration data measured twice over the experiment was analysed in a similar way, except an additional factor (depth) was added and a three-way ANOVA was used. To understand differences in variables that were not measured continuously over the experiment (above and belowground biomass and cations) data were analysed similar to above with a two-way ANOVA, except the repeated measures term (date) was not included in the model and the Bonferroni method was not used to adjust p-values. For belowground biomass a three-way ANOVA with depth as a factor was used. Bare plots were not included in the analysis of above and belowground biomass as these peat cores contained no vegetation at the end of the experiment. A one-way ANOVA with repeated measures followed by a pairwise t-test with adjusted p-values using the Bonferroni method was used to determine differences in pH and EC of water in the bins containing the peat cores. Cation and anion data from the bins were analyzed similarly, except repeated measures did not need to be accounted for. In order to understand specific differences between both water and cover types grouped together (ex. rich fen *Carex aquatilis*, constructed fen bare, etc.), one-way ANOVAs with the combination groups were used for analysis as well (with repeated measures as necessary), followed by a pairwise t-test (with the Bonferroni correction as necessary; results

found in tables only). In instances where significant interactions between factors were found in three-way ANOVAs, a one-way ANOVA (with repeated measures as necessary) was applied with each significant factor in the interaction grouped together (e.g. if an interaction was found between depth x cover type, groups would include bare at 0.1 m, *Juncus balticus* at 0.3 m, etc.), followed by a pairwise t-test (with adjusted p-values using the Bonferroni method as necessary). The Levene test and the Shapiro-Wilks test was used to test data for residual normality and equality of variance, respectively. Data that did not meet normality and equal variance conditions were log transformed to meet conditions.

Methane flux and pore water concentration were analyzed in two separate periods, Period 1 (DOE 1-78) and Period 2 (DOE 102-177), because of a significantly higher flux and concentration in Period 2 ($p<0.01$). The Levene and Shapiro-Wilks tests revealed that CH_4 flux and concentration residuals did not meet assumptions of ANOVA tests. As sample sizes were small (<30), the non-parametric two factor Scheirer-Ray-Hare tests were used to determine differences in flux and concentration data (Dytham, 2011). For CH_4 flux in Period 1 and 2, the Scheirer-Ray-Hare test was used with water and cover type included as factors in the model, followed by two separate post-hoc Dunn tests to determine differences in CH_4 flux within water or cover type factors as necessary (Dunn, 1964). For CH_4 flux data Scheirer-Ray-Hare tests were followed by one-way Kruskal-Wallis tests applied to data joined into water and cover type combination groups (eg. constructed fen bare, rich fen *Carex aquatilis*, etc.), followed by post-hoc Dunn tests to understand interactions and differences between combination groups. Methane pore water concentration data in Period 1 and 2 were analysed with three separate Scheirer-Ray-Hare tests, with combinations including two of the water type, cover type or depth factors in each model to determine any interactions between factors, followed by separate post-hoc Dunn tests to

understand differences in CH_4 concentrations within the water type, cover type, and depth factors when necessary. The Bonferroni correction was applied to the CH_4 flux and concentration non-parametric statistics to account for multiple testing. Methane concentration data under anoxic conditions measured at the end of the experiment were analyzed similarly to CH_4 concentration data averaged over Period 1 and 2.

As absolute and relative oxidation determined by flux measurements in light and dark conditions did meet the assumptions of parametric tests, a two-way ANOVA to determine differences across water type and cover types was used, followed by pairwise t-tests if individual water type or cover type factors were found to be significant. Separate one-way ANOVAs were also applied with water type and cover type grouped together (eg. constructed fen bare, rich fen *Carex aquatilis*, etc.) to understand significant interactions and if differences between combination groups occurred, and these ANOVAs were also followed by a pairwise t-test.

Correlation analysis was used to understand controls on CH_4 flux, concentration, and absolute and relative oxidation. For this analysis, CH_4 flux and concentration data from Period 2 were averaged for each peat core plot, and oxidation values determined from DOE 147 – DOE 188 were used. Plot averages from Period 2 for NEE, pH, and EC data were used in correlation tests, along with the above and belowground biomass data sampled at the end of the experiment at each plot. Values of zero were used for the bare plots for above and belowground biomass in correlation tests, as no vegetation grew in these peat cores over the experiment. Finally, the cation and anion data that was sampled on DOE 96 and DOE 147, respectively, from peat pore water were used to understand water chemistry controls on CH_4 flux and concentration. The non-parametric Spearman's rank correlation test was applied to determine significant correlations between CH_4 flux, concentration, or oxidation and the plant and water chemistry variables. In the

correlation between CH_4 flux and oxidation and belowground biomass (coarse, fine, total), belowground biomass at all depths sampled (0-0.075 m, 0.075-0.15 m, 0.15-0.225 m, 0.225-0.30 m) were added together. Pore water CH_4 concentration data from Period 2 at a depth of 0.3 m were correlated against belowground biomass sampled from a similar depth (0.225-0.30 m). The CH_4 concentration from Period 2 at 0.3 m depth was also used for the correlation analysis with the other plant data, because preliminary analysis showed that concentration at this depth had the most significant correlation relationships with the variables compared to average concentration at the shallower depths. Methane concentration and water chemistry at similar depths was used to understand water chemistry controls on CH_4 concentration.

3.3 Results

Water chemistry

Differences in water chemistry were found across the peat cores as well as between water from either the constructed or rich fen bins (Table 3.1; Fig 3.2). The water chemistry of the well water used throughout the experiment to keep the peat cores waterlogged contributed to the overall peat core pore water chemistry. The well water had a higher pH, nitrate concentration (15.8 mg/L) and chloride concentration (294.8 mg/L) compared to the water sampled from the constructed fen pond and rich fen stream. Further, the well water had a higher EC, and a concentration of calcium, sodium, magnesium, and sulfate (30.6 mg/L) compared to the rich fen. Overall, higher pH values were measured from the water sources (rich fen, constructed fen, and well water) and bin water compared to the pore water sampled from the peat cores.

Specifically, there was no water type or cover type effect on EC (water: $F_{1,91} = 1.1$, $p = 0.29$; vegetation: $F_{2,91} = 0.9$, $p = 0.40$) or pH (water: $F_{1,89} = 0.3$, $p = 0.59$; vegetation: $F_{2,89} = 3.0$, $p = 0.057$; Table 1) in pore water extracted from the cores. However, EC was higher in the bins with constructed fen water compared to rich fen water ($F_{1,38} = 13.4$, $p < 0.01$). Considering the cation results, water sampled from all peat cores and bins had higher concentrations of calcium, sodium, and magnesium compared to iron and manganese (Table 3.2). All cations were found in significantly higher concentrations at peat cores including constructed fen water compared to the cores with rich fen water including: calcium ($F_{1,14} = 87.1$, $p < 0.001$), sodium ($F_{1,14} = 8.9$, $p < 0.001$), magnesium ($F_{1,14} = 96.8$, $p < 0.001$), iron ($F_{1,14} = 11.2$, $p < 0.01$), and manganese ($F_{1,14} = 15.8$, $p < 0.01$). Similar results to the peat core pore water were found for cation concentrations across bin water with different water types, except the manganese concentration was similar between bins with constructed fen and rich fen water ($F_{1,5} = 1.2$, $p = 0.33$). Cover type did not influence iron ($F_{2,14} = 0.1$, $p = 0.88$) or manganese ($F_{2,14} = 3.5$, $p = 0.058$) across peat cores. Plots with *Juncus balticus* had higher magnesium compared to *Carex aquatilis* and bare peat cores ($F_{2,14} = 12.4$, $p < 0.01$). Further, sodium and calcium concentrations were higher at *Juncus balticus* plots compared to bare plots, but similar between *Juncus balticus* and *Carex aquatilis* plots (sodium: $F_{2,14} = 21.3$, $p < 0.01$; calcium: $F_{2,15} = 12.7$, $p < 0.001$).

Table 3.1. Water chemistry results ± standard error of the mean measured from the constructed fen (CF) and rich fen (RF) pond and stream, as well as in greenhouse experiment bins and across plots. *

Water Cover	pH	EC (µS/cm)	Ca (mg/L)	Fe (mg/L)	Mn (mg/L)	Na (mg/L)	Mg (mg/L)
RF Bare	5.31±0.1 a	2936.6±225.0 a	42.43±4.7 a	6.62±0.2 a	0.15±0.0 a	474.30±30.7 a	73.74±6.1 a
RF Carex	5.20±0.2 a	3713.0±346.3 a	70.76±18.5 a	7.18±2.5 a	0.20±0.0 a	615.09±27.3 ab	90.15±7.7 a
RF Juncus	5.20±0.2 a	4236.7±448.6 a	91.65±12.5 b	9.76±1.6 a	0.36±0.1 a	693.07±51.8 b	122.16±12.0 b
CF Bare	5.05±0.2 a	4089.1±334.6 a	127.75±5.2 c	16.62±1.8 b	0.40±0.0 b	558.99±11.1 a`	157.33±4.7 b
CF Carex	4.98±0.2 a	4417.9±308.5 a	145.52±13.2 c	13.16±1.4 b	0.41±0.1 b	680.10±3.8 b	163.01±5.3 b
CF Juncus	5.41±0.2 a	5392.9±530.7 a	231.61±17.0 c	11.95±4.2 b	0.56±0.0 b	783.98±33.1 b	231.28±20.7 c
RF_{bin}	7.84±0.1 a	3066.6±133.5 a	120.47±6.7 a	0.03±0.0 a	<0.01±0.0 a	445.31±23.3 a	66.02±3.7 a
CF_{bin}	7.81±0.1 a	3864.1±122.9 b	213.81±2.3 b	0.02±0.0 b	<0.01±0.0 a	542.10±19.8 b	126.08±4.7 b
RF_{stream}	7.35	1017.0	50.68	0.05	<0.01	27.06	22.34
CF_{pond}	7.30	1670.0	412.74	0.02	<0.01	342.98	165.22
Well	7.47	1590.0	96.55	<0.01	<0.01	194.58	27.46

*Peat core plots included rich fen (RF) or constructed fen (CF) water and bare (Bare), *Carex aquatilis* (Carex), or *Juncus balticus* (Juncus) cover. Each water type and cover type combination (RF Bare, etc.) is an average of three plot replicates. Water samples from peat cores were collected from a depth of 0.2 m. Water chemistry results from the bins that contained the peat cores (CF_{bin}, RF_{bin}) is shown, along with data from the RF stream (RF_{stream}), CF pond (CF_{pond}), and untreated greenhouse well water (Well). Electrical conductivity (EC) and pH measurements were taken monthly over the experiment. Cation including calcium (Ca), iron (Fe), manganese (Mn), sodium (Na), and magnesium (Mg) were sampled on DOE 96. Letters indicate significant differences in one variables (pH, EC, etc.) between peat cores (RF Bare, RF Carex, etc.) or bins (RF_{bin}, CF_{bin}).

Considering anion concentrations across the peat cores, there was no water effect for chloride ($F_{1,48} = 1.0$, $p = 0.33$) or nitrate ($F_{1,48} = 1.0$, $p = 0.33$), while significantly higher sulfate was found at peat cores with constructed fen compared to rich fen water ($F_{1,48} = 38.5$, $p < 0.001$; Fig 3.2). Similar results were found with respect to the chemistry of the bin water. A depth ($F_{1,48} = 29.0$, $p < 0.001$) and cover type effect ($F_{2,48} = 13.0$, $p < 0.001$) was found for the chloride concentration sampled from the peat cores, including a significant interaction ($F_{2,48} = 5.3$, $p < 0.01$). The one-way ANOVA with depth and cover type exclusively considered indicated that bare plots at 0.1 and 0.3 m had lower chloride values compared to *Juncus balticus* plots at 0.1 m. Considering nitrate only an effect of depth was found across peat cores ($F_{1,48} = 29.0$, $p < 0.001$), with pore water sampled in the peat cores at 0.3 m having higher nitrate compared to the pore water sampled at 0.1 m ($F_{2,48} = 2.3$, $p = 0.11$). No cover type ($F_{2,48} = 0.1$, $p = 0.9$) or depth ($F_{1,48} = 0.01$, $p = 0.922$) effect on sulfate concentrations in pore water sampled from the peat cores was observed.

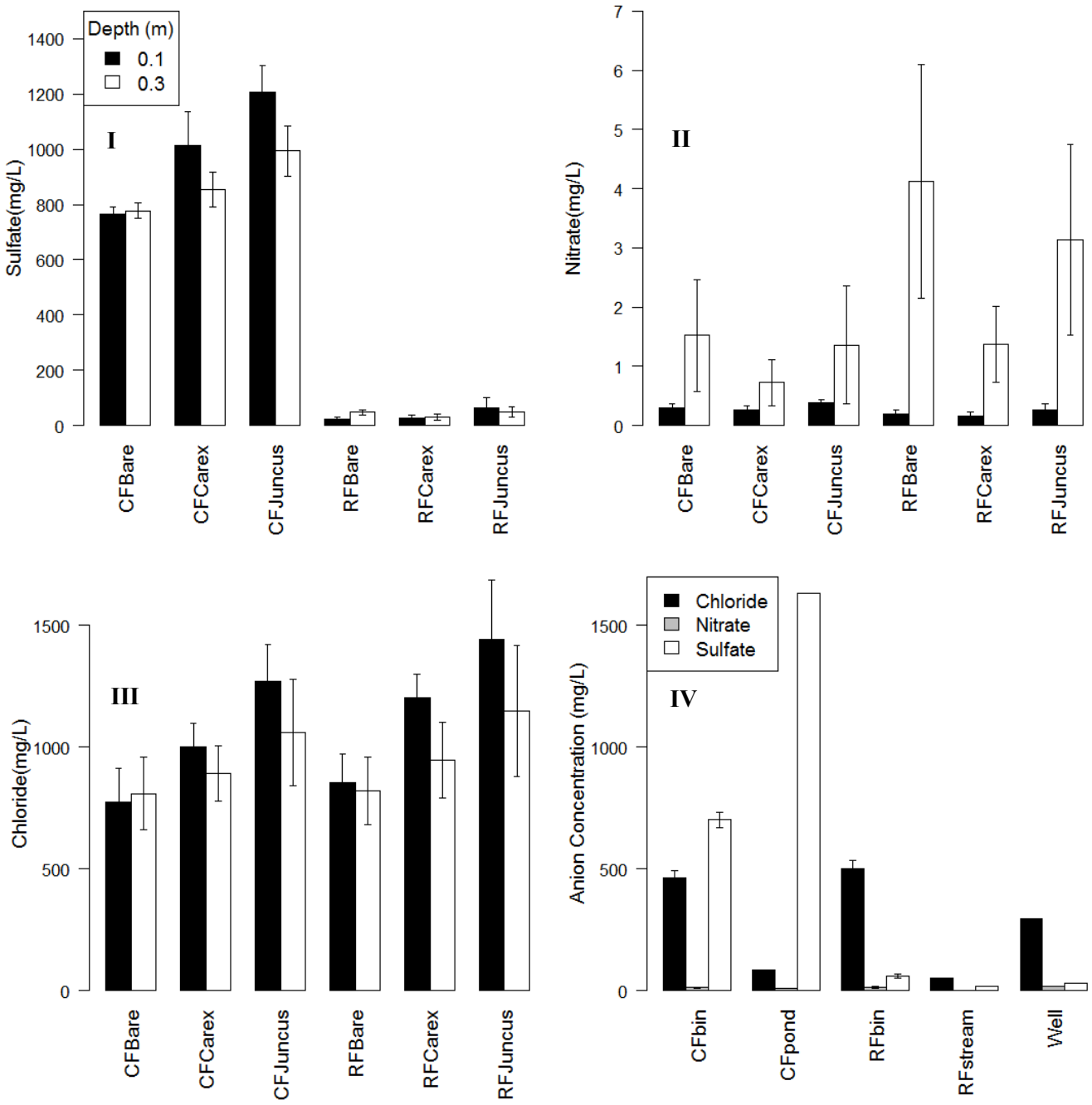

Fig 3.2. Anion water chemistry results. Averages from peat cores include data from two sampling periods (DOE 29 and DOE 147) and at 0.1 and 0.3 m depth (I-III) with water from the constructed fen (CF) and rich fen (RF) and cover types including bare peat (Bare), *Juncus balticus* (Juncus), and *Carex aquatilis* (Carex). Note the different y-axis values from I-III. Anion concentrations CF_{bin} and RF_{bin} in IV were measured on DOE 29 from bins that contained the peat cores. Water was sampled from the CF pond in August, 2015, from the RF stream in October 2015, and from the untreated well water in the greenhouse January, 2016 (IV).

Plant variables

Water from the rich fen and constructed fen did not affect NEE differently across the peat cores from DOE 1 – DOE 177 ($F_{1,179} = 0.1$, $p = 0.79$), although NEE was significantly higher at plots including *Carex aquatilis* and *Juncus balticus* compared to bare plots ($F_{2,179} = 4.8$, $p < 0.01$; Table 3.2). Values of GEP calculated twice over the experiment were highly correlated to NEE values determined at the same time (Spearman rho = 0.98, $p < 0.001$). Consequently, NEE, which was measured more consistently throughout the sampling period was used in regression analysis as a proxy for plant productivity. Plant survey results throughout the experiment showed that litter cover decreased from DOE 1 to DOE 67, but increased again near the end of the experiment on DOE 173 (Appendix 3). Total live plant cover increased from DOE 1 to DOE 67, but decreased to DOE 173 with increasing litter cover. No clear pattern indicating greater CO_2 uptake through NEE over time was observed, associated with varying light levels in the greenhouse (results not shown).

No effect of the water type or cover type on aboveground biomass of *Carex aquatilis* or *Juncus balticus* plots were found (water type: $F_{1,8} = 0.2$, $p = 0.70$; cover type: $F_{1,8} = 2.0$, $p = 0.19$), and while the constructed fen and rich fen water also did not affect litter biomass ($F_{1,8} = 0.8$, $p = 0.40$), plots containing *Carex aquatilis* had higher litter compared to plots with *Juncus balticus* ($F_{1,8} = 25.0$, $p < 0.01$; Table 3.2). Belowground fine root biomass from 0-0.3 m was also not affected by different water type ($F_{1,8} = 5.2$, $p = 0.053$) nor did it differ between *Carex aquatilis* and *Juncus balticus* cores ($F_{1,8} = 2.1$, $p = 0.18$). However, *Juncus balticus* cores had higher coarse belowground biomass throughout the entire peat core than *Carex aquatilis* plots ($F_{1,8} = 6.7$, $p = 0.03$) at plots with both water type ($F_{1,8} = 1.0$, $p = 0.35$). Total belowground biomass (coarse + fine) was affected by depth and was higher at 0-0.075 m and 0.075-0.15 m

compared to 0.15-0.225 and 0.225-0.30 m ($F_{3,40} = 73.9$, $p < 0.001$) while similar across water type ($F_{1,40} = 0.004$, $p = 0.95$) and cover type ($F_{1,40} = 1.7$, $p = 0.20$) plots (Fig 3.5IV).

Table 3.2. Plant parameters ± standard error of the mean measured across plots with different water types and cover types. *

Water type	Cover type	NEE ($g\ CO_2\ m^{-2}\ d^{-1}$)	AG ($g\ m^{-2}$)	AG Litter ($g\ m^{-2}$)	BG_{coarse} ($g\ m^{-2}$)	BG_{fine} ($g\ m^{-2}$)
RF	Bare	1.45±0.96[a]	n.a.	n.a.	n.a.	n.a.
RF	Carex	-17.72±2.72[b]	98.55±6.14[a]	120.38±6.47[a]	238.50±5.74[a]	105.23±11.85[a]
RF	Juncus	-19.70±3.51[b]	93.77±10.90[a]	53.99±13.01[b]	387.83±44.35[b]	59.42±3.58[a]
CF	Bare	2.11±0.45[a]	n.a.	n.a.	n.a.	n.a.
CF	Carex	-23.45±3.02[b]	111.83±11.22[a]	166.66±31.26[a]	258.84±27.72[a]	112.76±9.27[a]
CF	Juncus	-18.07±4.46[b]	88.34±10.44[a]	42.32±16.03[b]	295.58±48.98[b]	116.96±24.11[a]

*Water was from a rich fen (RF) or constructed fen (CF) with bare (Bare), *Carex aquatilis* (Carex), or *Juncus balticus* (Juncus) cover. Each water type and cover type combination group (RF Bare, etc.) was an average of three plot replicates. Live aboveground biomass (AG) and aboveground litter (AG Litter) were separated at the time of sampling. Belowground biomass was separated into coarse (BG_{coarse}; >2mm) and fine (BG_{fine}; <2mm) biomass. Results shown here include belowground biomass of the entire peat core (0-0.3 m). Samples were taken from DOE 175 – DOE 189. Bare plots were not measured for biomass.

Methane flux, concentration, and oxidation

Methane flux across peat core plots increased over time (Fig 3.3). Flux in Period 1 from DOE 1 – DOE 78 was not influenced by the water type ($\chi^2 = 1.0$, $p = 0.31$, df = 1); however, cover type did affect Period 1 flux ($\chi^2 = 43.6$, $p < 0.001$, df = 2; Fig 3.4I). Methane flux was lower from bare plots compared to the *Juncus balticus* and *Carex aquatilis* cover types. In Period 2 (DOE 102 – DOE 177) the two-way ANOVA results indicated that the rich fen water plots had significantly higher flux compared to constructed fen water plots ($\chi^2 = 6.4$, $p = 0.01$, df = 1; Fig 3.4II), with no significant difference between the three cover types ($\chi^2 = 4.5$, $p = 0.1$, df = 2). Although there was no significant interaction between water and cover type, the one-way ANOVA indicated a significantly higher flux from rich fen *Carex aquatilis* plots compared to constructed fen *Juncus balticus* plots.

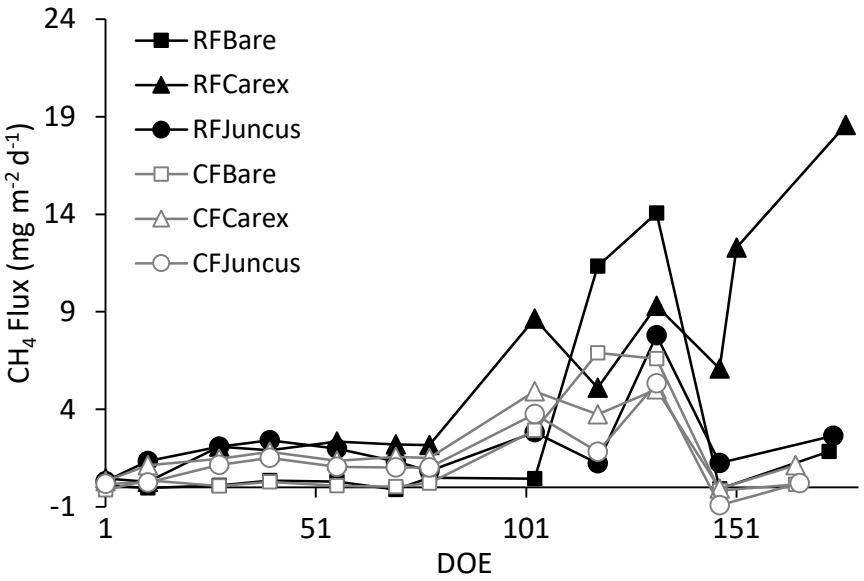

Fig 3.3. Methane (CH_4) flux over the greenhouse experiment from DOE 1 to DOE 188 at peat cores with water from either the constructed fen (CF) or rich fen (RF) and cover types including *Juncus balticus* (Juncus)*, Carex aquatilis* (Carex), or bare (Bare) controls. Each point is an average of three plot replicates.

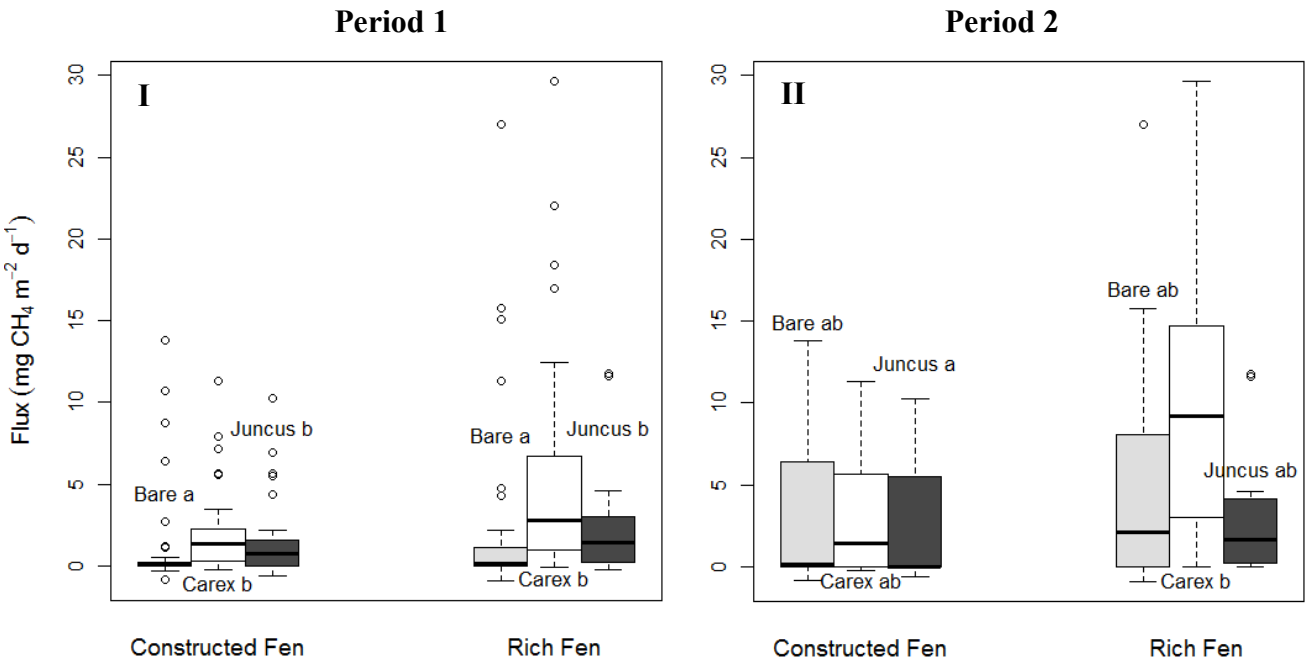

Fig 3.4. Average methane (CH_4) flux across plots with either constructed fen (CF) or rich fen (RF) water types and cover types including bare controls (Bare), *Juncus balticus* (Juncus), or *Carex aquatilis* (Carex) over Period 1 (DOE 1-78; I) or Period 2 (DOE 102-177; II). Each water type and cover type combination group (RF Bare, etc.) represents averages from three plot replicates over the period measurements. Letters indicate significant differences between cover types and water types grouped together (eg. constructed fen bare vs. rich fen bare, etc.).

While water type did not influence the pore water CH_4 concentration in Period 1 ($\chi^2 = 2.2$, $p = 0.1$, df = 1), the bare plots had significantly higher pore water CH_4 compared to *Carex aquatilis* and *Juncus balticus* plots ($\chi^2 = 39.3$, $p < 0.001$, df = 2), and CH_4 concentration increased with depth ($\chi^2 = 122.07$, $p < 0.001$, df = 3; Fig 3.5I). In Period 2 the water type did affect CH_4 concentration, and rich fen water plots had higher CH_4 concentration compared to constructed fen water plots ($\chi^2 = 39.1$, $p < 0.001$, df = 1; Fig 3.5II). Bare plots had a significantly higher CH_4 concentration compared to the plots with vascular species, while *Juncus balticus* had a lower concentration compared to plots with *Carex aquatilis* in Period 2 ($\chi^2 = 53.0$, $p < 0.001$, df = 2). Finally, CH_4 concentration in Period 2 was highest at 0.3 m depth, and similar between depths 0.1 and 0.2 m and 0.1 and 0.05 m, with 0.2 m having a higher concentration than 0.05 m ($\chi^2 = 38.4$, $p < 0.001$, df = 3).

There was a cover type ($\chi^2 = 11.1$, $p < 0.01$, df = 2) and water type ($\chi^2 = 26.1$, $p < 0.001$, df = 1) effect on the CH_4 pore water concentration under anoxic conditions; however, depth was not found to significantly influence CH_4 concentration after nitrogen flushing ($\chi^2 = 2.3$, $p = 0.51$, df = 3; Fig 3.5III). Higher pore water CH_4 concentration under anoxic conditions was observed at rich fen water plots compared to constructed fen water plots, and bare and *Carex aquatilis* plots had higher CH_4 concentrations under anoxic conditions compared to plots with *Juncus balticus.*

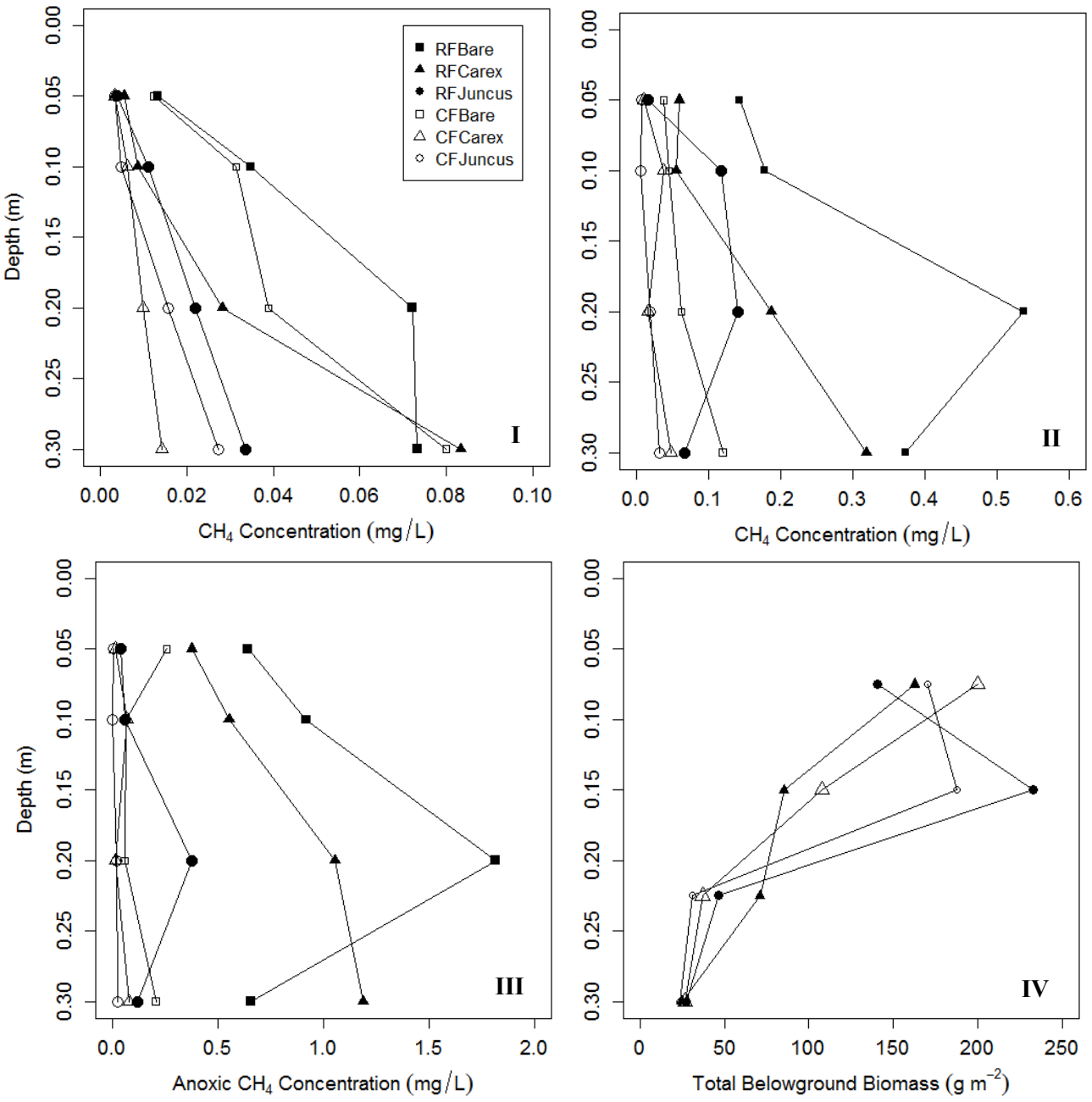

Fig 3.5. Average methane (CH_4) concentration from Period 1 (DOE 1-78; I), Period 2 (DOE 102-177; II), and in anoxic conditions after nitrogen flushing (III), as well as total belowground plant biomass (IV). Plots had water from either the constructed fen (CF) or rich fen (RF) with either *Carex aquatilis* (Carex), *Juncus balticus* (Juncus) or bare control cover. Methane concentration in anoxic conditions was measured from DOE 147- DOE 188. Total belowground biomass was measured at the end of the greenhouse experiment (DOE 175-189) and includes coarse + fine biomass. Each water type and cover type combination is made up of three plot replicates**.** Error bars were excluded for clarity. Note the difference in x-axis scale between CH_4 concentration plots (I-III).

Considering absolute CH_4 oxidation in dark conditions, there was a significant interaction between water type and cover type ($F_{2,12} = 12.4$, $p < 0.01$; Table 3.3). There was also a significant effect of both water type ($F_{1,12} = 10.1$, $p = 0.01$) and cover type ($F_{2,12} = 14.3$, $p < 0.01$) on oxidation. The subsequent one-way ANOVA with water type and cover type grouped together revealed that the *Carex aquatilis* growing in rich fen water had significantly higher absolute oxidation compared to all other plots ($F_{5,12} = 12.7$, $p < 0.001$). A significant interaction between water type and cover type was also found for the absolute CH_4 oxidation determined through flux in light conditions ($F_{2,11} = 5.4$, $p = 0.02$), with the one-way ANOVA revealing that the rich fen *Carex aquatilis* plots had significantly higher CH_4 oxidation compared to the constructed fen *Carex aquatilis* plots ($F_{5,11} = 3.6$, $p = 0.04$). However, no individual significant effect of water type ($F_{1,11} = 3.4$, $p = 0.09$) or cover type ($F_{2,11} = 1.8$, $p = 0.22$) was found, suggesting that the interaction between factors was masking the main effects, and implying that the *Carex aquatilis* was particularly impacted by the different water types.

Water type did affect relative oxidation in dark conditions ($F_{1,12} = 14.4$, $p < 0.01$), with peat cores in bins including constructed fen water having higher relative oxidation compared to rich fen water, but the two-way ANOVA did not reveal significant differences across cover types ($F_{2,12} = 2.3$, $p = 0.15$; Table 3.3). While there was no significant interaction between water type and cover type for the relative oxidation in dark conditions, the one-way ANOVA indicated that higher relative oxidation occurred specifically at constructed fen bare and *Juncus balticus* plots compared to rich fen *Carex aquatilis* plots ($F_{5,12} = 4.1$, $p = 0.02$). In light conditions, relative oxidation was affected by both water type ($F_{1,12} = 44.1$, $p < 0.001$) and cover type ($F_{2,12} = 11.0$, $p < 0.01$), with constructed fen water plots having higher relative oxidation compared to rich fen plots, and *Carex aquatilis* having lower relative oxidation compared to bare and *Juncus balticus*

plots. Specifically, the one-way ANOVA results revealed that bare and *Carex aquatilis* plots with rich fen water had lower relative oxidation in light conditions compared to all plots with constructed fen water, and the *Juncus balticus* rich fen plots had similar oxidation to bare and *Carex aquatilis* plots with constructed fen water, but lower oxidation compared to *Juncus balticus* constructed fen water plots ($F_{1,12} = 13.2$, $p < 0.01$).

Table 3.3. Average absolute and relative methane (CH_4) oxidation results ± standard error of the mean across cover types with constructed fen (CF) or rich fen (RF) water. *

Water type	Cover type	Light condition	Oxic CH_4 Flux (mg m^{-2} d^{-1})	Anoxic CH_4 Flux (mg m^{-2} d^{-1})	Absolute oxidation (mg m^{-2} d^{-1})	Relative oxidation (%)
RF	Bare	Dark	0.41±0.95	4.34±0.43	$3.93±1.38^{a}$	$70.7±18.4^{ab}$
RF	Carex	Dark	16.51±6.38	27.41±6.96	$10.89±0.58^{b}$	$44.0±8.6^{a}$
RF	Juncus	Dark	2.34±0.86	7.57±1.41	$5.23±0.60^{a}$	$71.4±7.0^{ab}$
CF	Bare	Dark	0.24±0.28	4.74±0.19	$4.50±0.34^{a}$	$93.3±5.1^{b}$
CF	Carex	Dark	0.77±0.27	5.69±0.26	$4.92±0.49^{a}$	$86.0±5.3^{ab}$
CF	Juncus	Dark	0.17±0.24	5.21±0.23	$5.04±0.40^{a}$	$93.1±2.0^{b}$
RF	Bare	Light	0.62±1.23	2.69±0.62	$2.07±0.62^{ab}$	$46.7±16.1^{a}$
RF	Carex	Light	18.57±5.99	26.99±8.42	$8.43±2.62^{a}$	$30.8±3.3^{a}$
RF	Juncus	Light	2.36±1.02	5.42±1.43	$3.05±0.60^{ab}$	$59.3±7.6^{ab}$
CF	Bare	Light	0.15±0.55	3.48±0.13	$3.33±0.44^{ab}$	$80.8±7.4^{bc}$
CF	Carex	Light	1.13±0.30	3.06±0.72	$1.94±0.42^{b}$	$64.0±1.6^{bc}$
CF	Juncus	Light	0.20±0.32	3.57±0.81	$3.36±0.85^{ab}$	$90.7±5.3^{c}$

*Cover types included bare (bare), *Carex aquatilis* (Carex), and *Juncus balticus* (Juncus). Oxidation was measured from DOE 147 - DOE 188. Oxic CH_4 flux was determined before nitrogen flushing, after which anoxic CH_4 flux was determined. Each water type and cover type combination is made up of an average of three plot replicates**.** Letters indicate significant differences between water type and cover type grouped together (RF Bare, CF Juncus, etc.) only between one light condition (dark or light).

Controls on CH_4 flux, concentration, and oxidation

Several water chemistry variables were found to correlate to CH_4 flux in Period 2, while both plant and water chemistry parameters were significantly related to CH_4 concentration at the four depths measured (Table 3.4). Methane flux was lower when higher values of EC, calcium, iron, manganese, magnesium, sulfate, and nitrate were measured in the peat pore water. Net ecosystem exchange was positively correlated to CH_4 concentration at 0.3 m, indicating less CH_4 concentration with higher productivity. Similarly, aboveground biomass, and total and fine belowground biomass had a negative relationship with CH_4 concentration at 0.3 m. Similar to flux, pore water CH_4 concentration decreased with higher concentrations of EC, calcium, manganese, sodium, magnesium, and sulfate.

No water chemistry or plant variables were found to correlate significantly with absolute CH_4 oxidation in dark (Table 3.4) or light conditions (results not shown). Relative oxidation in dark conditions increased with higher calcium, magnesium, sulfate, and nitrate. In light conditions relative oxidation increased with higher calcium, magnesium, and nitrate at 0.1 m depth (results not shown).

Table 3.4. Spearman correlation results of methane (CH_4) flux, CH_4 concentration, and absolute and relative oxidation with water chemistry and plant variables across plots with different water types and cover type. *

Variable	Depth (m)	CH_4 Flux		CH_4 Concentration ***		CH_4 Oxidation (Absolute)		CH_4 Oxidation (Relative)	
		rho	p-value	rho	p-value	rho	p-value	rho	p-value
AG		0.45	0.15	-0.52	**0.03**	0.45	0.06	-0.23	0.36
Litter		0.43	0.16	-0.28	0.26	0.46	0.06	-0.34	0.17
BG	**	-0.08	0.82	-0.55	**0.02**	0.36	0.14	-0.04	0.87
BG_{coarse}	**	-0.10	0.75	-0.33	0.18	0.35	0.15	-0.09	0.71
BG_{fine}	**	-0.03	0.94	-0.60	**<0.01**	0.34	0.17	-0.06	0.82
NEE		0.27	0.27	0.68	**<0.01**	-0.15	0.54	0.05	0.86
pH	0.2	-0.06	0.82	-0.06	0.81	-0.28	0.26	-0.36	0.14
EC	0.2	-0.68	**<0.01**	-0.59	**0.01**	-0.23	0.35	0.33	0.18
Ca	0.2	-0.63	**<0.01**	-0.81	**<0.001**	-0.14	0.57	0.58	**0.01**
Fe	0.2	-0.57	**0.01**	-0.52	0.03	-0.26	0.30	0.41	0.09
Mn	0.2	-0.60	**0.01**	-0.65	**<0.01**	-0.21	0.39	0.42	0.09
Na	0.2	-0.42	0.08	-0.61	**<0.01**	0.17	0.50	0.19	0.44
Mg	0.2	-0.62	**<0.01**	-0.79	**<0.001**	-0.14	0.57	0.62	**<0.01**
SO_4	0.1	-0.55	**0.02**	-0.78	**<0.001**	-0.18	0.48	0.63	**0.01**
	0.3	-0.49	**0.04**	-0.66	**<0.01**	-0.30	0.23	0.63	**0.01**
NO_3	0.1	-0.58	**0.01**	-0.44	0.07	-0.40	0.10	0.65	**<0.01**
	0.3	-0.61	**<0.01**	-0.45	0.06	-0.25	0.32	0.51	**0.03**
Cl	0.1	-0.16	0.52	-0.44	0.07	0.35	0.16	-0.23	0.35
	0.3	-0.40	0.17	-0.41	0.09	0.18	0.48	-0.08	0.75

*Averages from Period 2 (DOE 102 – DOE 177) were used in correlation for CH_4 flux and concentration, as well as for net ecosystem exchange (NEE), pH, and electrical conductivity (EC). Aboveground biomass (AG), litter biomass (Litter), and belowground biomass (total: BG; coarse (>2mm): BG_{coarse}; fine (<2mm): BG_{fine}) were sampled at the end of the experiment. Cations, including calcium (Ca), iron (Fe), manganese (Mn), sodium (Na), and magnesium (Mg), were sampled on DOE 96 and anions sulfate (SO_4), nitrate (NO_3), and chloride (Cl) were sampled on DOE 147. Bold indicates significant correlation ($p<0.05$). Plots included water from either the constructed fen or rich fen and cover types with *Carex aquatilis*, *Juncus balticus*, or bare controls. For the bare controls vegetation variables (AG, Litter and BG) were assigned a value of zero.

**For CH_4 flux and oxidation belowground biomass (BG, BG_{coarse}, BG_{fine}) from the total core (0-0.3 m) was used, while belowground biomass from 0.225-0.3 m was used in the correlation with CH_4 concentration at 0.3 m.

***CH_4 concentration at 0.3 m depth was correlated with AG, Litter, and NEE. For correlations including CH_4 concentration and water chemistry variables, the CH_4 concentration used was at the same depth as the water chemistry sample.

3.4 Discussion

Constructed fen reclamation projects in the AOSR near Fort McMurray, Alberta have recently been attempted to convert landscapes disturbed by surface mining into ecosystems that can support hydrological and ecological conditions similar to natural fen peatlands (Price et al., 2010). As undisturbed peatlands function as natural carbon sinks over thousands of years (Loisel et al., 2014), developing constructed fens that also may accumulate carbon is beneficial, especially given the extensive release of carbon associated with peatland loss due to surface mining in the AOSR (Rooney et al., 2011). Carbon accumulation in peatlands is dependent on hydrophilic vegetation that can survive anaerobic conditions, resulting in highly productive ecosystems with low decomposition rates (Taylor and Smith, 1980). Emissions of the strong GHG CH_4 also should be considered when discussing peatland carbon budgets, as a substantial amount of carbon is released as CH_4 from these ecosystems (Roulet, 2000). On the other hand, high CH_4 emissions coincide with poorly decomposed peat and may indicate the ability of reclaimed peatland to eventually accumulate carbon (Limpens et al., 2008). A greenhouse experiment was conducted to understand how CH_4 dynamics are influenced by *Juncus balticus* and *Carex aquatilis*, that are clonal, native peatland plants, and were used in the reclamation of a constructed fen in the AOSR of Alberta. A goal of this study was to make recommendations to future projects that could reduce the release of the GHG to the atmosphere, resulting in a greater sink of carbon overall in constructed fens. In order to replicate conditions at the constructed fen in question, pond water from the reclaimed site was added to half of the peat cores in this experiment. Since natural fens, including rich fens, are common in northern Alberta (Chee and Vitt, 1989), the experiment also included adding rich fen water to the remaining peat cores in

order to understand CH_4 dynamics from *Carex aquatilis* and *Juncus balticus* under conditions more similar to natural peatlands in the AOSR.

Water chemistry impacts – CH_4 flux and concentration

Methane flux and concentration was significantly higher in Period 2 (DOE 102 – DOE 177), after which the peat cores had been fully saturated with constructed fen or rich fen water for more than 12 weeks, compared to Period 1 (DOE 1 – DOE 78; Fig 3.4; Fig 3.5). This indicates that CH_4 production in the peat cores did not ensue quickly following water saturation, and supports a study by Blodau and Moore (2003) who found that weeks to months were required for dissolved CH_4 pools to accumulate following flooding in a mesocosm experiment. In Period 2, average CH_4 flux from peat cores in the bins with constructed fen water (2.8 mg CH_4 m^{-2} d^{-1}) were lower than values found from the constructed fen site in the AOSR in 2015 (4.0 mg CH_4 m^{-2} d^{-1}; Chapter 2), three-years post reclamation. Similarly, Nwaishi et al. (2016) found higher CH_4 flux from a rich fen in the AOSR near the constructed fen site (>20 mg CH_4 m^{-2} d^{-1}) in 2014 compared to average CH_4 flux values from Period 2 from the peat cores in the bins with rich fen water (6.13 mg CH_4 m^{-2} d^{-1}). Average CH_4 concentration from Period 2 from peat cores in bins with constructed fen water at 0.2 m depth (0.03 mg/L) was also lower compared to values found in the field at the constructed fen site in 2015 at this depth (0.16 mg/L; Chapter 2). Further, the mesocosm study by Blodau and Moore (2003) found a CH_4 concentration in peat cores from a natural oligotrophic peatland with a constant water table of 8.02 mg/L at a depth of 0.4 m. This indicates a CH_4 pool that was orders of magnitude higher compared to the CH_4 pool found at depth from peat cores with rich fen water in this study, with an average concentration of 0.25 mg/L at 0.3 m over Period 2.

Given that the water table in the bins was maintained above the peat surface in the cores over the experiment to promote CH_4 production in anoxic conditions and limit aerobic CH_4 oxidation (Couwenberg and Fritz, 2012), higher CH_4 flux from the peat cores, especially from the plots including rich fen water, were expected. The lower CH_4 flux and concentration results may have been associated with the quality of the milled peat used for the experiment. Basiliko et al. (2007) found very low anaerobic CH_4 production potential rates in a horticulturally harvested site in Quebec (~0.005-0.010 μg CH_4 g^{-1} peat d^{-1}, 0.1-0.4 m depth), associated with low nutrient and substrate availability resulting in limited microbial activity. This result was related to the horticulture peat extraction process that involves draining and removing the top layer of vegetation from the peatland surface (Quinty and Rochefort, 2003). It is possible that few microbes, including methanogens capable of CH_4 production, were available in the peat that could become active following saturation of the peat core. Acidity may have also limited CH_4 production in the peat cores, as pH values (ranging from 5-5.4 across all plots) were lower than pH values ideal for methanogenesis (Table 3.1; Dunfield et al., 1993). The milled peat used for the experiment was likely acidic and influenced the low pH values, as the water sourced from the constructed fen pond water, rich fen stream water, and well water had pH values of 7.3, 7.4, and 7.5, respectively that were higher than those measured in the peat cores. Methane production may also have been limited by nitrate (Balderston and Payne, 1976) that likely was sourced from the well water used for the experiment (Fig 3.2). Nitrate was higher in the pore water at 0.3 m depth across all plots, averaging 2.9 and 1.2 mg/L at the rich fen and constructed fen plots, respectively. An ongoing investigating of CH_4 production potential at depth within the peat cores used in the present study will assist in understanding the CH_4 flux and concentrations observed.

In Period 2 higher CH_4 flux and concentration was observed in bins with rich fen water compared to constructed fen water (Fig 3.4II; Fig 3.5II). Correlation results revealed that average CH_4 flux from Period 2 was correlated with water chemistry, as opposed to plant cover or biomass, and higher EC, calcium, iron, manganese, magnesium, sulfate, and nitrate resulting in lower flux (Table 3.3). Negative correlations with pore water CH_4 concentration at the depths examined and similar water chemistry variables were also found. These water chemistry parameters predominantly had higher values from water samples taken in the peat cores and bins that contained constructed fen water (Table 3.1; Fig 3.2), indicating that by Period 2 the influence of constructed fen water was limiting methanogenesis, as was hypothesized. Specifically, reduction processes of manganese, iron, and sulfate, found in higher concentrations at the cores with constructed fen water, are known to supress CH_4 production (Blodau, 2002). Higher pore water concentrations of other elements, including calcium and sodium, at the constructed fen peat cores compared to rich fen peat cores correlated to lower CH_4 flux or concentration (Table 3.1; Table 3.4). This indicates that higher salinity levels decreased CH_4 flux, supporting previous studies in natural peatlands (Bartlett et al., 1987). Overall, evidence for the higher concentration of TEAs found at the peat core plots with constructed fen water indicated a greater oxidative capacity compared to plots with rich fen water, suggesting a higher availability of oxidants for organic matter respiration and quicker decomposition rates (Limpens et al., 2008). This finding suggests that the water chemistry at constructed fens may cause peat accumulation to ensue at a slow rate.

Water chemistry impacts – CH_4 oxidation

Considering higher CH_4 concentrations found in Period 2 and under anoxic conditions between the plots with rich fen and constructed fen water (Fig 3.5II; Fig 3.5III) it was surprising that the absolute oxidation was similar across plots with constructed fen water compared to plots with rich fen water in light conditions, and that relative oxidation values were higher at plots with constructed fen water in both light and dark conditions (Table 3.3). Correlation results indicate higher calcium, magnesium, and sulfate found at plots with constructed fen water resulted in higher relative oxidation (Table 3.4). This suggests that methanotrophy may be stimulated by a greater concentration of TEAs in the peat, although no previous studies exist that focus on the effect of nutrient deposition on methanotroph structure and diversity in peatlands that may support this speculation (Andersen et al., 2013). It is also possible that AOM was occurring across plots with constructed fen water due to higher concentrations of TEAs known to be important for AOM in freshwater systems (Gupta et al., 2013). Measurements of oxidation made in this study focused on aerobic methanotrophy associated with ROL and potential AOM rates were not determined.

Plant impacts – CH_4 flux and concentration

Methane flux in Period 1 was lower in bare peat cores compared to cores including either *Carex aquatilis* or *Juncus balticus* (Fig 3.4I). A higher pore water concentration of CH_4 observed in Period 1 at the bare plots compared to the plots with *Carex aquatilis* or *Juncus balticus* indicates that the aerenchymous plants were transporting CH_4 from peat to the atmosphere (King et al., 1998). In Period 2 no prominent pattern based on cover type was found to relate to CH_4

flux, although pore water CH_4 concentration at bare peat cores remained highest, while *Carex aquatilis* plots had higher concentration compared to *Juncus balticus* (Fig 3.5II). These results indicate that the plants were still transporting CH_4 through tissues, and also suggest that bare plots had increased release of CH_4 through diffusion and ebullition processes (Lai, 2009), compared to in Period 1. Higher CH_4 concentration found at *Carex aquatilis* plots compared to *Juncus balticus* plots given similar productivity, aboveground biomass, and total belowground biomass (Table 3.2, Fig 3.5IV) could indicate a species-specific influence on CH_4 production between these graminoids. Ström et al. (2005) found higher CH_4 emissions from *Carex rostrata* compared to *Juncus effusus* and *Eriophorum vaginatum* possibly due to different CH_4 production pathways. In this study ^{14}C-labelled acetate was predominantly emitted as $^{14}CH_4$ from *Carex* plots suggesting acetoclastic production, but as $^{14}CO_2$ from the *Juncus* and *Eriophorum* plots, possibly indicating that the CH_4 emitted from these plots was produced via hydrogenotrophic methanogenesis instead (Zinder, 1993). Data collected from the current study were not able to imply CH_4 production pathways or substrate dynamics within the peat profile. However, flux and concentration results suggest that *Carex aquatilis* and *Juncus balticus* have differential influences on belowground processes that drive CH_4 production or oxidation (see discussion below).

Plant parameters were correlated to CH_4 concentration at the four depths examined in this study (Table 3.3). Higher above and belowground biomass resulted in lower pore water CH_4 concentration in the peat profile, as did higher productivity through NEE. This is further support for CH_4 transport through aerenchyma that could explain the depletion of the CH_4 pool in the peat cores. These results also support higher CH_4 oxidation through ROL from the cores including vascular species (Armstrong and Armstrong, 1988). No plant controls on CH_4 flux

across the peat cores were found, despite past studies that show higher CH_4 flux with more aboveground biomass (Bellisario et al., 1999) and productivity (Joabsson and Christensen, 2001). Peat chemistry has previously been found to be the main control on CH_4 production (Valentine et al., 1994), and it is therefore possible that the plant effect on CH_4 flux was masked by the overwhelming influence of water chemistry on the flux across all peat cores.

Plant impacts - oxidation

Methane oxidation was determined in both dark and light conditions as previous research supports diurnal cycles influencing subsurface microbial activities and CH_4 concentrations in the presence of wetland plants (Thomas et al., 1996). This suggests that light availability could potentially cause changes to methanotrophy. Measurements of relative and absolute oxidation in both light and dark conditions (Table 3.3) showed similar values of oxidation across the different cover types in this study (p=0.08). This result supports the findings of van der Nat and Middelburg (1998) who did not find diurnal differences in rhizospheric oxidation rates of two peatland species. Previous research has provided evidence for seasonality controlling CH_4 oxidation (e.g. Lombardi et al., 1997), and it would be beneficial for future studies to measure CH_4 oxidation more than once over a study period to understand if oxidation associated with the vascular species considered for this study may vary depending on growth cycle stage. van der Nat and Middelburg (1998) found higher CH_4 oxidation rates during plant growth, and as oxidation was measured at the end of the experiment in this study, when plants appeared to be entering a senescence stage (Appendix 3), it is possible that oxidation was lower than if measurements had been made at an earlier date.

Given the expectation that oxidation in this study would be dominated by plant processes, particularly ROL from the vascular species causing rhizosphere oxidation, values of oxidation measured at the bare plots in this study were expected to be much lower than cores with plants. As the water table was maintained at the top of the peat core to prevent aerobic oxidation that could occur in a potential oxic peat zone (Whalen, 2005), zero oxidation was predicted from bare peat cores. However, absolute and relative oxidation from bare plots was often found to be similar to or greater than vegetated plots regardless of water type (Table 3.4). Absolute and relative oxidation from bare peat cores does suggest that some oxygen diffused from the surface into the cores despite the attempt at total peat saturation, possibly due to evapotranspiration at the plots in the greenhouse, as ~8L of water was added consistently each week to the peat cores in the bins. Some oxygen likely occurred in the peat cores in instances when water was not added directly following evapotranspiration, for instance on hot summer days when peat and air temperature, as well as PAR in the greenhouse, may have increased and caused greater evapotranspiration (Brown et al., 2010). It is possible that air bubbles may also have been trapped in the peat core when water was added to the bins. It is argued that the results of this study that focused on the species-specific effects on oxidation by *Carex aquatilis* and *Juncus balticus* are still valid, given that the peat cores including the vascular plants would have been impacted similarly by oxygen in the peat core that was not associated with ROL.

Results of this study provided evidence for species-specific effects on oxidation (Table 3.3). The absolute oxidation calculated in dark conditions indicated that *Carex aquatilis* from the rich fen water plots had higher oxidation compared to all other plots. The result of higher absolute oxidation likely associated with ROL from *Carex aquatilis* at the rich fen water plots was not expected, as past studies have found *Carex aquatilis* to be inefficient at reducing CH_4

emissions through rhizosphere oxidation (Nielson et al., 2016), while certain species of *Juncus* have been found to efficiently limit CH_4 flux due to oxidation associated with ROL (Ström et al., 2005). The higher absolute oxidation from rich fen *Carex aquatilis* plots compared to *Juncus balticus* could relate to the greater CH_4 pool (Fig 3.5II) and consequent source of CH_4 for methanotrophy across *Carex aquatilis* plots compared to *Juncus balticus* plots (Le Mer and Roger, 2001). However, no significant correlations between absolute oxidation and CH_4 concentration were found (results not shown). It is possible that the higher absolute oxidation calculated at the *Carex aquatilis* rich fen plots with higher CH_4 concentration may not solely represent ROL associated with *Carex* cover. A greater concentration gradient between the peat core and atmosphere may have resulted in the much higher flux in anoxic conditions across *Carex aquatilis* rich fen plots, given that the anoxic CH_4 concentration was higher than under the typical oxic conditions (Lai, 2009; Fig 3.5II, III).

While higher absolute values of oxidation were found at *Carex aquatilis* plots, the percent relative oxidation was higher on average at *Juncus balticus* plots compared to *Carex aquatilis* plots across both water types. Therefore, the relative oxidation results do overall support the hypothesis that *Juncus balticus* would have greater ROL and methanotrophy compared to *Carex aquatilis*, and rhizospheric oxidation likely influenced the low CH_4 flux and concentration found across *Juncus balticus* plots (Fig 3.4II; Fig 3.5II).

3.5 Conclusions

In this study differences in CH_4 flux, concentration, and oxidation were found across greenhouse peat core water type (constructed fen and natural rich fen water) and cover type (bare, *Carex aquatilis* and *Juncus balticus*) plots. Consistent with results from field studies at the

constructed fen site focused on GHG emissions including CH_4 post-reclamation (Chapter 2; Nwaishi et al., 2016), peat cores in this study with water from the constructed fen had lower CH_4 emissions compared to peat cores with natural rich fen water. This was associated with water chemistry results that indicated a high concentration of ions in the peat pore water that are known to limit methanogenesis including manganese, iron, and sulfate. While plant productivity measured through net ecosystem exchange, as well as above and belowground biomass, were similar between *Carex aquatilis* and *Juncus balticus* plots regardless of water type, evidence for species-specific effects on CH_4 production and oxidation were found. Higher pore water CH_4 concentration coincided with higher absolute values of oxidation at *Carex aquatilis* plots compared to *Juncus balticus* plots, while *Juncus balticus* had a smaller CH_4 pool in pore water but higher relative oxidation. From a GHG perspective, *Juncus balticus* may be beneficial to plant at future constructed fen projects to keep CH_4 emissions low. However, data from this study suggests that CH_4 emissions from constructed fens will remain lower than emissions from natural fens, regardless of plant species, associated with the water chemistry at the constructed fen site. Future monitoring of the constructed fen will be required to understand if the concentration of ions that inhibit CH_4 production, such as sulfate, decrease over time in order to better predict the ability of constructed fens to eventually accumulate peat. Future constructed fen projects may follow recommendations made by Nwaishi et al. (2016) who advised that reclamation materials be assessed prior to construction in order to decrease the impact of water chemistry on GHG fluxes. In these instances, planting *Juncus balticus* will likely result in lower CH_4 emissions compared to *Carex aquatilis* and, given similar productivity between these two species, could increase the overall carbon sink of these reclaimed ecosystems.

Chapter 4: Recommendations and Implications for Fen Construction

Results from the field data and greenhouse experiment considered for this research indicated that understanding the methane (CH_4) dynamics of a constructed fen site compared to natural sites can provide important information about constructed fen ecological and biogeochemical functioning. Future monitoring at the Nikanotee Fen is recommended to understand when CH_4 production and emissions may increase to become more similar to natural reference fens in the Athabasca Oil Sands Region (AOSR), as the current differences suggest a distinct functionality at the constructed fen. Continual monitoring of CH_4 from the constructed fen can indicate biogeochemical cycling, including redox conditions and the concentration of terminal electron acceptors (TEAs) that supress CH_4 flux, such as sulfate. Overall, geochemistry or water chemistry effects on CH_4 flux and concentration were found to be more dominant than vegetation or plant effects in this research, and it is therefore suggested that future studies of CH_4 emissions from constructed fens include a consideration of geochemical controls, particularly while the concentration of TEAs such as sulfate remains high. Further pore water sampling using different methods besides PRS probes at the constructed fen would be helpful to verify the form of the ions measured. More research focused on anaerobic oxidation of CH_4 (AOM) at the constructed fen would also be beneficial, given the high concentration of TEAs found at this site that are seemingly necessary for this process to occur. It is further recommended that future studies on CH_4 dynamics at the constructed fen include microbial data on the methanogen and methanotroph communities and function in order to better understand CH_4 production and oxidation rates.

Planting *Juncus balticus* at future constructed fens could be beneficial as this species will likely limit CH_4 flux due to high relative rhizospheric oxidation observed from *Juncus* in this and

previous studies. However, this suggestion is exclusively made from a greenhouse gas (GHG) standpoint. Ecological recommendations for other potential constructed fen projects should also be based on the results of current and future ecological monitoring at the Nikanotee Fen to determine which species or vegetation will be most successful at establishment and long-term survival given the biogeochemical conditions at the constructed fen. Fluxes of CO_2 and evapotranspiration should also be considered when making ecological recommendations for fen construction in order to take into account the carbon accumulating and water use efficiency function of different species and vegetation.

The low CH_4 flux and concentration at the constructed fen in the AOSR and greenhouse plots influenced by constructed fen water found in this research may indicate that peat and carbon accumulation at the constructed fen will not occur at a similar rate to natural sites. Therefore, it would be beneficial to more clearly understand the source of the TEAs, such as sulfate, that are in the constructed fen pore water and inhibiting CH_4 production. These water chemistry conditions may also supress peat accumulation through increased microbial respiration and organic matter decomposition. A better understanding of the effects of donor peat compared to the water influx from the upland slopes of the constructed fen on the water chemistry within the fen could allow for valuable recommendations to future projects that could result in biogeochemistry, including CH_4 dynamics, being more similar to natural fens. However, given the similar or higher productivity and biomass of vascular species found at the constructed fen site and greenhouse plots influenced by constructed fen water, if future constructed fen designs are altered to achieve similar biogeochemical cycling to natural sites, it is likely that CH_4 emissions will be high, especially in the short-term. This may be disadvantageous for companies involved in constructed fen projects who may be interested in decreasing their overall GHG

footprint through reclamation. Consequently, management goals that incorporate a consideration of GHG emissions including CH_4 will be required for future fen construction projects in order to make effective recommendations.

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Appendix 1. Absolute values for average seasonal methane (CH_4) flux and concentration ± standard error of the mean across the constructed fen (CF), saline fen (SF), poor fen (PF). *

Site/Cover type	CH_4 Flux (mg m^{-2} d^{-1})	CH_4 Conc. 0.2m (mg/L)	CH_4 Conc. 0.7m (mg/L)
Constructed Fen	3.95±0.31	0.15±0.04	0.52±0.07
CFB	2.52±0.46	0.13±0.08	0.99±0.24
CFC	4.08±1.04	0.16±0.13	0.57±0.21
CFCM	4.72±0.89	0.06±0.03	0.10±0.07
CFJ	5.85±0.82	0.04±0.02	0.15±0.07
CFJM	3.74±0.70	0.05±0.01	0.41±0.14
CFM	2.86±0.45	0.46±0.18	0.90±0.16
Poor Fen	23.90±3.98	4.63±0.35	4.31±0.39
PFCM	23.93±3.14	4.43±0.50	4.61±0.57
PFM	23.86±8.27	4.85±0.53	4.00±0.56
Saline Fen	4.40±0.82	1.81±0.23	3.14±0.35
SFB	5.63±1.27	2.66±0.25	3.53±0.52
SFJ	3.22±1.04	0.92±0.26	2.76±0.47

*Cover types at the CF included bare (CFB), *Carex aquatilis* (CFC), *Carex aquatilis* + moss (CFCM), *Juncus balticus* (CFJ), *Juncus balticus* + moss (CFJM), and moss (CFM). Cover types at the PF included *Carex aquatilis* + moss (PFCM) and moss (PFM), and at the SF cover types were bare (SFB) and *Juncus balticus* (SFJ).

Appendix 2. Principle component analysis (PCA) loadings for significant principle components in analysis with methane (CH_4) flux and concentration at 0.2 m and 0.7 m depth and correlated environmental variables. *

	CH_4 Flux		CH_4 Conc. 0.2 m		CH_4 Conc. 0.7 m		
Variables	PC1	PC2	PC1	PC2	PC1	PC2	PC3
CH_4**	0.46	-0.21	0.43	-0.01	0.44	-0.17	0.06
Sulfur	-0.42	0.10	-0.43	0.16	-0.44	0.26	-0.23
Ammonium	0.45	-0.34	0.40	0.21	0.39	0.45	0.01
pH	-	-	-0.44	0.08	-0.45	0.12	-0.20
EC	-0.45	0.25	-	-	-	-	-
Belowground	0.35	0.59	0.26	-0.72	-	-	-
TotalCover	0.30	0.64	-	-	-	-	-
GraminoidCover	-	-	-0.30	-0.64	-0.33	-0.27	0.84
ShrubCover	-	-	0.34	0.05	0.35	0.29	0.32
Temp 0.7 m	-	-	-	-	0.16	-0.73	-0.31

* Environmental controls included total vegetation cover (TotalCover), shrub cover (ShrubCover), Graminoid cover (GraminoidCover), belowground biomass from 0-0.2 m depth (Belowground), electrical conductivity (EC), pH, temperature at 0.7 m depth (Temp0.7), and ammonium and sulfur supply rate.

**Refers to either CH_4 Flux (first column) or CH_4 concentration at 0.2 m (second column) or 0.7 m (third column) depth.

Appendix 3. Vegetation survey results ± standard error of the mean at plots with water from a rich fen (RF) or constructed fen (CF) and including *Carex aquatilis* or *Juncus balticus* plants.

DOE	Water	Vegetation	Total (%)	Litter (%)
1	RF	Carex	21.0±4.0	9.3±1.8
67			28.7±3.7	4.7±0.7
173			20.0±2.0	12.0±1.2
1	RF	Juncus	7.3±0.9	7.7±1.3
67			21.0±0.6	4.0±1.2
173			19.7±2.8	7.3±1.8
1	CF	Carex	19.3±2.3	10.7±0.7
67			29.7±1.7	4.7±0.9
173			22.3±0.7	17.0±1.2
1	CF	Juncus	10.0±2.0	7.5±0.5
67			19.0±3.2	2.7±0.7
173			16.7±1.7	6.7±2.9