Cutover Peat Limits Methane Production Causing Low Emission at a Restored Peatland

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Abstract Peatland degradation due to human activities is contributing to rising atmospheric CO₂ levels. Restoring the carbon (C) sink function in degraded peatlands and preventing further stored C losses is a key climate mitigation strategy, given the global scale of peatland disturbance. Active restoration involving a combination of rewetting and vegetation reestablishment at a post-extraction peatland in Canada has been shown to successfully re-establish net CO₂ uptake rates similar to undisturbed peatlands within a decade or two. However, lower than expected CH₄ emissions suggest recovery of belowground C cycling processes may lag behind the recovery of the surface net flux. Using closed chamber measurements over a warm season, we determined that restored Sphagnum, which covers two thirds of the site, was a null source of CH₄. Emissions from the restored site were primarily attributed to vascular plant substrate inputs, measured as acetate, and plant-mediated transport. The C isotopic fractionation factor for CH₄ and CO₂ in the pore water from the restored former peat field suggested reduced hydrogenotrophic CH₄ production deeper in the cutover peat profile (0.8 m depth). In contrast, isotopic fractionation in the former drainage ditches showed a balance of acetoclastic and hydrogenotrophic methanogenesis deeper in the profile, indicative of some bulk peat C turnover. This study suggests that the legacy of substrate quality in the cutover peat can reduce the climate warming impact of newly restored peatlands through a reduction in CH₄ production and thus emission.

Plain Language Summary Restoration of degraded peatlands is increasingly being considered a tool to mitigate the global temperature rise associated with climate change. This study is the first to isolate methane production and emission processes to explain why methane release from post-extraction peatlands restored by a combined rewetting and vegetation reestablishment is lower than that of undisturbed peatlands. Carbon turnover of bulk cutover peat was limited to former ditch areas in the restored peatland, which comprised less than 5% of the site. As a result, methane release from the ecosystem to the atmosphere was low and linked to vascular plant inputs and plant-mediated transport. This study provides evidence that an active restoration method that restores the water table below the surface and aids reestablishment of Sphagnum can be an effective climate mitigation tool by bringing back endemic vegetation more quickly and avoiding large methane release often associated with unmanaged rewetting.

1. Introduction

Peatlands maintain a large reservoir of once atmospheric CO₂ as stored soil organic matter, which has had a long-term cooling impact on the climate (Frolking et al., 2006; Frolking & Roulet, 2007). Long-term C storage in peatlands results from plant production of biomass exceeding decomposition of organic material (Moore et al., 1998). This is primarily related to organic matter decomposition rates which, in northern latitude peatlands (above 45°N), are generally lower than the partial to complete waterlogging, and soil organic matter properties that limit microbial respiration (Moore & Basiliko, 2006). Anoxic conditions caused by shallower water tables also enable CH₄ production that exceeds methanotrophic oxidation, causing peatlands to be a source of atmospheric CH₄ (Vasander & Kettunen, 2006). In contrast, peatlands that are vacuum-harvested for horticultural purposes are heavily disturbed systems no longer capable of sequestering C, and instead become persistent sources of CO₂ and very low CH₄ sources (e.g., Aslan-Sungur et al., 2016; Nugent et al., 2018; Nugent et al., 2019; Rankin et al., 2018). Restoring the hydrology and vegetation to that of an undisturbed peatland is necessary at vacuum-extracted sites to reduce net organic matter mineralization and to return the site to C accumulation (Rankin...
et al., 2018; Waddington & McNeil, 2002). The importance of peatlands as a global C store makes restoring all forms of degraded peatlands a key climate change mitigation strategy (Leifeld & Menichetti, 2018).

Over 25 years of ecological research in Canadian peatlands that have undergone drainage and extraction has led to the development of the moss layer transfer technique restoration approach (Graf & Rochefort, 2016). Large-scale application of the moss layer transfer technique was recently shown to be successful in returning an impacted peatland to a C sink comparable to undisturbed peatlands within 14 years of restoration (Nugent et al., 2018). A persistent inter-annual C sink was achieved in part due to low annual emissions of CH₄ of 4.4 ± 0.2 g C m⁻² yr⁻¹ (Nugent et al., 2018). Former drainage ditches, which dissect the restored peat fields (Figure 1), did not emit a lot of CH₄ because they cover only a small area (Nugent et al., 2018). The CH₄ emitted from the entire restored peatland was lower than that of a reference undisturbed peatland, despite a shallower water table and much higher cover of Eriophorum vaginatum (Nugent et al., 2018). This result led to speculation that slow re-establishment of specialist microbial communities after prolonged drying may be limiting CH₄ production rates, as seen at other restored peatlands (Francez et al., 2000; Juottonen et al., 2012). To address why net emissions were lower at the restored peatland, this study evaluates CH₄ production and oxidation in the restored peat fields and former drainage ditches. We combine surface trace gas flux measurements with dissolved concentrations of CH₄ (dCH₄), CO₂ (dCO₂), and acetate, and stable isotope measurements of CH₄ and co-occurring CO₂. The stable isotope composition (δ¹³C) of CH₄ and CO₂ provides an indirect method to evaluate CH₄ production and oxidation.

In the water saturated zones of undisturbed peatlands, methanogenesis is expected to be the dominant terminal decomposition mechanism, due to anoxic conditions and a general absence of inorganic electron acceptors (Chaser, Chanton, Glaser, & Siegel, 2000; Chaser, Chanton, Glaser, Siegel, & Rivers, 2000; Corbett, Burdige, et al., 2013; Corbett, T файли et al., 2013; Romanowicz et al., 1995). In peatlands, methanogenesis primarily occurs by either H₂/CO₂ reduction (hydrogenotrophic methanogenesis) or acetate fermentation (acetoclastic methanogenesis) and produces a net equimolar amount of CO₂ and CH₄ (Chanton, 2005). With both pathways, δ¹³C of organic matter is fractionated to form a more enriched δ¹³C-CO₂ and a more depleted δ¹³C-CH₄ (Corbett, Burdige, et al., 2013). The degree of fractionation is dependent on the production pathway, with more of a difference between CO₂ and CH₄ with H₂/CO₂ reduction than with acetate fermentation (Chasar, Chanton, Glaser, & Siegel, 2000; Chaser, Chanton, Glaser, Siegel, & Rivers, 2000). This fractionation does not occur in CO₂ production by aerobic respiration or fermentation (Lapham et al., 1999). Transport of CH₄ from the soil to the atmosphere can result in isotopic fractionation, depending on the mode of transport. Aqueous diffusive transport does not result in significant isotopic fractionation, nor does ebullition as bubbles pass quickly through the peat to the atmosphere (Chanton, 2005). Gas transport via plants with aerenchyma likely causes ¹³C enrichment in the rhizosphere, but this process is difficult to differentiate from the effects of rhizospheric CH₄ oxidation (Chanton, 2005).

Methanogenic archaea use a select few small molecules, for example, acetate, H₂, and CO₂, supplied by the metabolic activities of other microbes as substrate. Acetate is considered the most important C intermediate in terrestrial anaerobic systems, rarely accumulating as it is rapidly produced and consumed (Hines et al., 2008). However, Sphagnum-containing peatlands such as bogs and poor fens tend to favor methanogenesis from H₂/CO₂ reduction (Chanton et al., 1995, 2006; Chaser, Chanton, Glaser, & Siegel, 2000; Chaser, Chanton, Glaser, Siegel, & Rivers, 2000; Kelly et al., 1992; Lansdown et al., 1992; Popp et al., 1999); in these systems, acetate has the potential to accumulate in the absence of vascular plants (Hines et al., 2008). Labile C is generally found in the root environment of vascular plants, supplied by root residues and root exudates (Kuzyakov & Domanski, 2000). The plant root system is continuously releasing a wide range of labile C compounds, such as organic acids, amino acids, and carbohydrates that serve as an easily available substrate for microbial decomposition (Joabsson et al., 1999; Proctor & He, 2017). Root release of acetate and precursors to acetate can have a substantial effect on CH₄ production in the soil (Joabsson et al., 1999; Ström & Christensen, 2007; Ström et al., 2003, 2005, 2012). However, acetate concentrations found in the pore water and from Eriophorum scheuchzeri root exudation equate to only a few hours of CH₄ flux, suggesting a continuous input is needed to maintain the acetate fermentation pathway (Ström et al., 2012).

Previous studies in extracted peatlands that have been restored have found low or even insignificant CH₄ emissions (Komulainen et al., 1998; Nugent et al., 2019; Strack et al., 2014, 2016; Strack & Zuback, 2013; Tuittila et al., 2000; Waddington & Day, 2007; Wilson et al., 2009). Lower emissions relative to undisturbed peatlands could be due to greater overall CH₄ oxidation (during diffusion or rhizosphere oxidation) or reduced CH₄ production, or both. Rewetting causes the water table to rise relative to its drained position, but greater seasonal
fluctuations can occur (McCarter & Price, 2015). Some rewetting efforts have resulted in flooded landscapes; however, our focus is on systems with a water table restored to below the surface. In deeper water table systems, CH$_4$ oxidation has the potential to significantly reduce the amount of CH$_4$ emitted (Roulet et al., 1993). Symbiosis among methanotrophic bacteria and Sphagnum has been reported to supply a significant portion of moss C by oxidizing peat CH$_4$, even below the water table (Raghoebarsing et al., 2005). As well, vascular plants such as E. vaginatum can promote a high degree (>90%) of rhizospheric CH$_4$ oxidation (Ström et al., 2005). Drainage is an

Figure 1. A map of the restored site, with collar locations marked by ‘x’s. The restored peat fields (green shading) are separated at 30 m intervals by former drainage ditches (blue lines), which have been infilled but are depressed.
environmental stress that has the potential to adversely affect methanogen populations over decades (Juottonen et al., 2012). Acetoclastic methanogens tend to be physically fragile (Dannenberg et al., 1997), which suggests their population would react more to stress (Hines et al., 2008). The recalcitrant nature of cutover peat (Andersen et al., 2006; Basiliko et al., 2007; Glatzel et al., 2004; Juottonen et al., 2012), overlain by a restored Sphagnum layer, where Sphagnum is known to have antimicrobial compounds (Hines et al., 2008), could be an environment that inhibits methanogenesis. Acetate accumulation in the pore water profile would indicate acetate was a major end product of anaerobic metabolism rather than CH₄ production, suggesting methanogenesis inhibition (Hines et al., 2008).

In Canada, approximately 34,000 ha of vacuum-harvested peatlands are currently, or will soon be, in need of active restoration (ECCC, 2018). Nugent et al. (2018) has shown that surface net C uptake can be restored at former vacuum-harvested peatlands. The aim of this study is to develop a mechanistic understanding of subsurface C cycling to better understand the lower CH₄ emission occurring in these restored peatlands (Komulainen et al., 1998; Nugent et al., 2019; Strack et al., 2014, 2016; Tuittila et al., 2000; Wilson et al., 2009). We hypothesize that the physiochemical nature of underlying cutover peat is acting to inhibit CH₄ production and that CH₄ release from the restored peat fields is occurring primarily via plant-mediated transport.

2. Methods

2.1. Site Description

Our study took place at the restored Bois-des-Bel peatland near Rivière-du-Loup, Québec, Canada (47°58’1.95”N, 69°25’43.10”W). The peatland complex is 210 ha, of which 11.5 ha was vacuum-harvested in the 1970’s. The moss layer transfer technique restoration approach was used to restore 8.1 ha in the autumn of 1999 (Figure 1), in the first ever landscape scale attempt. Graf and Rochefort (2016) provide a detailed description of the restoration process while Nugent et al. (2018) have a more complete description of the geographical history of the Bois-des-Bel complex and its post-restoration biophysical characteristics. The climate of the region is cool-temperate and experiences an average annual temperature of 3.5 ± 2.9°C with 962 mm of precipitation, of which 270 mm is snowfall over November–March (1981–2010 climate normal, St-Arsene, Environment Canada). Precipitation is spread fairly evenly over the months with the coldest month being January (−12.4 ± 2.6°C) and the warmest month July (17.6 ± 1.2°C).

2.2. Flux Measurements

The experimental set up focused on comparing vascular and non-vascular plant communities in the restored peat fields and former drainage ditches at the restored peatland. Six vascular plots and three non-vascular plots were set up in the features (field, ditch), respectively, for a total of 18 plots. Plot selection was based on the dominant vegetation within the respective features, with E. vaginatum (six plots) and Sphagnum spp. (three plots) chosen non-randomly in the restored peat field while plots with Typha latifolia (six plots) and bare ditch (three plots) areas were non-randomly selected in the former ditches. While the bare ditch plots were initially devoid of vegetation, vascular plants did spread through the area over the course of the season as the water table dropped below the surface. Sprouts within the collars were removed on a regular basis. Boardwalks were used to span the former ditches and to traverse the restored peatland. An additional six Sphagnum plots were created in the adjacent undisturbed peatland, located within the same peatland complex, which was used as a reference site.

Net CO₂ and CH₄ flux measurements were carried out using the closed chamber technique on permanently installed collars. A laser gas analyzer (LGR-UGGA, Los Gatos Research, CA, USA) connected to a clear poly carbonate chamber enabled simultaneous measurements of CO₂ and CH₄ (and H₂O) concentration at 1 Hz. A rectangular chamber (60 × 60 × 30 cm; 0.108 m³) and collar combination was used at the restored field plots while a cylindrical chamber (100 cm height × 26 cm diameter; 0.053 m³) and collar combination was deployed in the former ditches, to accommodate vertical growth of T. latifolia. We equipped the chambers with fans to maintain a well-mixed headspace, as well as a cooling system to prevent excessive warming during closure. Instantaneous net ecosystem exchange of CO₂ (NEE) and CH₄ flux (FCH₄) were calculated from the linear change in CO₂ and CH₄ headspace concentration, respectively, over a measurement period of 2 min. A tarp was used to block incoming radiation within the chamber over a successive closure. Gross primary productivity (GPP) was
calculated from the difference between the unshrouded measurement and the fully dark measurement which provided ecosystem CO₂ respiration (ER).

Gas temperature (°C) was measured at 1 Hz by the LGR-UGGA while photosynthetically active radiation (PAR; μmol m⁻² s⁻¹) was recorded every 10 s during chamber closure by a quantum sensor. Following chamber deployment, soil temperature at 2, 5, 10, 15, 20, 25, and 30 cm depth was measured next to each collar using a digital thermocouple temperature probe, while water table depth was manually measured at adjacent wells. Dataloggers (CR5000 and CR23X, Campbell Scientific, AB, CAN) were used to record half hourly air temperature and soil temperature at multiple depths (5, 10, 20, 40, 60, 80 cm) in the restored field and former ditch locations over the measurement season using type T thermocouples (Omega Engineering). Paired Leveloggers and Barologgers (Model 3001, Solinst, Ontario, Canada) determined half hourly water table depth in proximity to the soil temperature profiles.

A total of 600 chamber closures were performed over the snow-free season of 2016; 148 closures occurred on days when pore water samples were collected (Table 1). Standard chamber flux calculations (Bubier et al., 2003) were made for linear changes in headspace CO₂ and CH₄ over time. In the case where CH₄ bubbling was captured with the LGR-UGGA, a piece-wise linear fitting routine modified from Goodrich et al. (2011) was used to separate linear from non-linear CH₄ increase in headspace concentration. CH₄ ebullition occurred repeatedly in the ditch plots and was characterized by a sudden break in the slope of the CH₄ mixing ratio over short durations (generally <20 s). The first difference of the CH₄ mixing ratio time series and standard deviation of the first difference was used to distinguish non-linear events. In total, 78 non-linear events passed the criteria in 2016 and were separated out from the linear dataset. The linear slope before and after the concentration jump was determined in order to quantify jump magnitude as well as baseline magnitude, which theoretically should continue during bubble events (Goodrich et al., 2011). Bubble magnitude was calculated as the difference between the jump magnitude and baseline magnitude and then converted to CH₄ mass released (mg CH₄) using chamber volume, temperature, and pressure. The fraction of total emissions attributed to the ebullition pathway was estimated by calculating the cumulative ebullitive and diffusive flux over the periods where sampling took place.

2.2.1. Pore Water Sample Collection and Analyses

In-situ concentration of dissolved organic C (DOC), dCO₂, and dCH₄ was determined using six replicate sets of pore water samplers installed 0.2 and 0.8 m below the former ditch and restored field surface, respectively, as well as at the reference site. Sampling occurred on DOY 134, 163, 182, 201, 216, 243, and 276. Pore water samplers were made of a 0.2 m length of ABS pipe (25 mm I.D.) closed at both ends, slotted at the middle 0.1 m, and covered in mesh to prevent clogging. Tygon tubing connected to one end was extended above the soil surface to allow for water collection by syringe from a stopcock. Installations occurred 30 days in advance of sampling and temporally representative samples were obtained by removing 60 mL of pore water from each sampler 48 hr before sampling (Strack & Waddington, 2008). The headspace degassing technique (Ioffe and Vitenberg, 1984) was used to acquire gas from the water samples. Ambient air was drawn into the syringe in equal volume to the collected pore water (30 mL) and the sample was degassed by shaking the sample vigorously (Waddington & Day, 2007). A matching ambient air sample was included to correct headspace gas concentrations. Gas samples were then transferred to evacuated 12 mL sealed vials (Exetainers, Labco, UK) and stored in a cooler for transport to McGill University, Montreal, Canada for analysis. Gas concentrations of CH₄ and CO₂ were determined using a gas chromatograph (Mini-2, SRI Instruments, California, USA). The remaining water sample was passed through 0.45 μm glass fiber filters (Macherey-Nagel MN 85/90) and acidified before being analyzed for DOC concentration on a total organic C analyzer (TOC-V, Shimadzu, Maryland, USA).

Pore water sampling to determine δ¹³C and acetate concentration was undertaken on DOY 163, 200–201, 216–217, and 242. The experimental set-up targeted the root zone (0.2 m) and below the root zone in the cutover peat (0.8 m) using “sipper” sets (rhizosphere and deep) permanently installed in the flux collars in both the restored fields and former ditches. Note that δ¹³C and acetate sampling in the field plots was prevented beyond DOY 163 by a water table deeper than 0.2 m and by strong resistance when drawing up pore water from 0.8 m. Extraction was made difficult by the nature of the cutover peat, which had low porosity caused by subsidence after drainage (Waddington & McNeil, 2002). Sippers are 6 mm diameter stainless steel tubes with mesh-covered holes drilled at the base and a length of Tygon tubing with a stopcock. Sippers were flushed with a small amount of soil water prior to slowly drawing 20 mL using a syringe. A 2-hr wait period was followed in the case of same-day sampling for δ¹³C and acetate.
Stable C isotope samples were filtered in the field through 0.1 μm in-line syringe filters (Whatman Grade GF/D glass microfiber) and injected into 11 mL evacuated glass vials sealed with 20 mm-thick butyl rubber septa. Samples were duplicated and acidified in the field with 1 mL of 30% \( \text{H}_3\text{PO}_4 \) and stored upside down on ice before being express shipped to Florida State University, Tallahassee, FL, USA.

Table 1
Summary of Measurements

| Feature  | Vegetation | No. of locations | Depth (m) | Measurement type | No. of measurements |
|----------|------------|------------------|-----------|------------------|---------------------|
| Ditch    | *T. latifolia* | 6                | –         | Chamber flux (NEE, ER, \( \text{FCH}_4 \)) | 44                  |
|          |            |                  |           | Pore water [ ] (DOC, dCO\(_2\), dCH\(_4\)) | 42                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CO\(_2\)            | 23                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CH\(_4\)            | 23                  |
|          |            |                  |           | Pore water acetate [ ] | 21                  |
|          |            |                  | 0.2       | Chamber flux (NEE, ER, \( \text{FCH}_4 \)) | 40                  |
|          |            |                  |           | Pore water [ ] (DOC, dCO\(_2\), dCH\(_4\)) | 40                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CO\(_2\)            | 24                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CH\(_4\)            | 24                  |
|          |            |                  |           | Pore water acetate [ ] | 22                  |
|          |            |                  | 0.8       | Root exudate acetate [ ] | 48                  |
| Bare ditch |            | 3                | –         | Chamber flux (NEE, ER, \( \text{FCH}_4 \)) | 23                  |
|          |            |                  |           | Pore water [ ] (DOC, dCO\(_2\), dCH\(_4\)) | 20                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CO\(_2\)            | 12                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CH\(_4\)            | 12                  |
|          |            |                  |           | Pore water acetate [ ] | 6                   |
|          |            |                  | 0.8       | Chamber flux (NEE, ER, \( \text{FCH}_4 \)) | 20                  |
|          |            |                  |           | Pore water [ ] (DOC, dCO\(_2\), dCH\(_4\)) | 20                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CO\(_2\)            | 12                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CH\(_4\)            | 12                  |
|          |            |                  |           | Pore water acetate [ ] | 9                   |
| Field    | *E. vaginatum* | 6                | –         | Chamber flux (NEE, ER, \( \text{FCH}_4 \)) | 40                  |
|          |            |                  |           | Pore water [ ] (DOC, dCO\(_2\), dCH\(_4\)) | 38                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CO\(_2\)            | 6                   |
|          |            |                  |           | Pore water δ\(^{13}\)C-CH\(_4\)            | 3                   |
|          |            |                  |           | Pore water acetate [ ] | 6                   |
|          |            |                  | 0.8       | Chamber flux (NEE, ER, \( \text{FCH}_4 \)) | 44                  |
|          |            |                  |           | Pore water [ ] (DOC, dCO\(_2\), dCH\(_4\)) | 38                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CO\(_2\)            | 4                   |
|          |            |                  |           | Pore water δ\(^{13}\)C-CH\(_4\)            | 4                   |
|          |            |                  |           | Pore water acetate [ ] | 4                   |
|          |            |                  |           | Root exudate acetate [ ] | 44                  |
| Sphagnum |            | 3                | –         | Chamber flux (NEE, ER, \( \text{FCH}_4 \)) | 21                  |
|          |            |                  |           | Pore water [ ] (DOC, dCO\(_2\), dCH\(_4\)) | 20                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CO\(_2\)            | 3                   |
|          |            |                  |           | Pore water δ\(^{13}\)C-CH\(_4\)            | 3 - Undetectable    |
|          |            |                  |           | Pore water acetate [ ] | 3                   |
|          |            |                  | 0.8       | Pore water [ ] (DOC, dCO\(_2\), dCH\(_4\)) | 20                  |
|          |            |                  |           | Pore water δ\(^{13}\)C-CO\(_2\)            | 3                   |
|          |            |                  |           | Pore water δ\(^{13}\)C-CH\(_4\)            | 3                   |
|          |            |                  |           | Pore water acetate [ ] | 3                   |
Isotope samples were brought to ambient pressure with helium, pressurized to one atmosphere, and shaken to extract gas into the headspace. The gas concentration and isotopic ratio in the headspace were determined by direct injection on a gas chromatograph combustion-interfaced stable isotope mass spectrometer (MAT Delta V, Finnigan, USA). The stable isotope analysis is described in further detail in Corbett et al. (2015). We determined the dominant CH₄ production pathway at the sampling points in the soil profile using two stable isotope abundance metrics. First, acetate fermentation (acetoclastic methanogenesis) yields CH₄ whose δ¹³C values fall within a typical range of −65 and −50‰ whereas CH₄ from H₂/CO₂ reduction (hydrogenotrophic methanogenesis) has δ¹³C values typically between −110 and −60‰ (Hornibrook & Aravena, 2010; Whiticar et al., 1986). Second, the apparent fractionation factor for C (α) in Equation 1 is a measure of the separation between CH₄ and co-occurring CO₂ (Chaser, Chanton, Glaser, & Siegel, 2000; Hornibrook et al., 1997; Whiticar et al., 1986).

\[
\alpha = \frac{\delta^{13}C - \text{DIC} + 1000}{\delta^{13}C - \text{CH}_4 + 1000}
\]

The factor is referred to as apparent because while CO₂ is a precursor for CO₂ reduction, it is not an immediate precursor for CH₄ formed from acetate fermentation (Chanton et al., 2006). Nonetheless, variation in α is interpreted to represent variations in the CH₄ production mechanism. Microbial culture-derived α values for H₂/CO₂ reduction are found to range between 1.031 and 1.077, while α values between 1.007 and 1.027 are characteristic of acetate fermentation (Chaser, Chanton, Glaser, & Siegel, 2000; Conrad et al., 2002; Hornibrook et al., 1997, 2000a, 2000b). In general, values of α > 1.065 and α < 1.055 are characteristic of environments dominated by H₂/CO₂ reduction and acetate fermentation, respectively (Whiticar, 1999; Whiticar et al., 1986). Duplicate acetate samples were filtered in the field through 0.1 μm in-line syringe filters into 5 mL plastic vials and frozen prior to being shipped to Lund University, Lund, Sweden. Acetate concentration was additionally sampled directly from the roots of T. latifolia and E. vaginatum plants. This was undertaken by threading individual roots through a tiny hole in a syringe with attached Tygon tubing and stopcock. Three roots were sampled from six plants of each species (36 roots total), with a blank syringe (root hole included) placed in the vicinity of each sampled plant (12 blanks total). Deionized water was replaced in the root syringes 24 hr prior to sampling in order to have a temporally representative sample.

Organic acid concentrations, for example, acetic acid/acetate, were analyzed using a high-pressure liquid chromatography tandem-ionspray mass spectrometry system. The system consisted of a chromatography system (ICS-2500, Dionex, Sunnyvale, California, USA) and a triple quadrupole mass spectrometer (2000 Q-trap, Applied Biosystems, Foster City, California, USA). Further analysis details and quality controls can be found in Ström et al. (2012). Results are presented in μM of acetate, given that acetate dominates at pH > 4.76. Other organic acids, namely, citric, formic, glycolic, and lactic, were also detected but were present at insufficient amounts to pursue further analysis.

### 2.2.2. Greenness Index

Canopy greenness at the restored peatland was monitored using a digital camera, which took daily JPEG images with red (R), green (G), and blue (B) channels at solar noon. The images were analyzed using the Phenocam GUI application, available as a MATLAB© program (phenocam.sr.unh.edu/webcam/tools), to calculate the green chromatic coordinate (gcc), where:

\[
g_{cc} = \frac{G}{R + G + B}
\]

for a predefined region of interest (ROI) (Sonnetag et al., 2012). We selected two ROIs within each image: an area of restored peat field and a restored drainage ditch with T. latifolia. As a continuous variable, gcc was used to better understand the seasonal pattern of the discrete flux measurements and to establish when leaf out and senescence occurred.

### 2.3. Statistical Analysis

Statistical analyses were performed on the DOY 163 to DOY 243 means of the flux and pore water concentration sample groups (i.e., five vegetation groups, two depths). All data were tested for normal distribution using the Shapiro-Wilk test before further analyses. To test for significant differences between groups, the Mann-Whitney
U test was used to test significance between two non-parametric sample groups, while the Student's t-test was used for parametric data. For three or more groups, the Krusal-Wallis one-way analysis of variance was used to test non-parametric data, while an ANOVA was used to test parametric data. All statistical analyses were done using R (R Core Team, 2017). Results were regarded as significant if p values were ≤0.05.

3. Results

3.1. Surface Fluxes

Net CO₂ exchange at the surface of the restored peatland showed differing seasonal patterns in the former drainage ditches and restored peat fields (Figures 2a and 2b). *T. latifolia* in former ditches were a source of CO₂ prior to leaf-out and after senescence (delimited by the greenness index) but had the highest net CO₂ uptake rate mid-season (July & August), with a mean (±SE) light-saturated NEE of −13.3 ± 1.0 μmol m⁻² s⁻¹, where negative measurements are net uptake by the ecosystem (Figure 2a; Table 2). Bare ditches were a low source of CO₂ with a mid-season mean CO₂ release of 2.5 ± 0.4 μmol m⁻² s⁻¹ (Figure 2a; Table 2). *E. vaginatum* and *Sphagnum* in the restored peat fields actively took up CO₂ throughout the study period (15 May to 1 November 2016), at about
Diffusive CH$_4$ flux measured simultaneously with NEE exhibited seasonal changes in all restored peatland plots except those dominated by Sphagnum (Figures 2c and 2d). Net CH$_4$ emission from bare ditch area increased from 98 $\pm$ 25 nmol m$^{-2}$ s$^{-1}$ during the early season to 1,174 $\pm$ 296 nmol m$^{-2}$ s$^{-1}$ mid-season (Figure 2c; Table 2). Bare ditch CH$_4$ emission declined sharply once the water table dropped below the surface in August and did not recover with a late season rise in the water table (Figure 2c; Table 2). T. latifolia had the second highest mid-season net CH$_4$ emission, of 266 $\pm$ 213 nmol m$^{-2}$ s$^{-1}$ (Table 2), with net CH$_4$ flux peaking in advance of light-saturated NEE, seasonally (Figures 2a and 2c).

Net CH$_4$ emission from T. latifolia also decreased alongside water table lowering in August, but fluxes remained at 129 $\pm$ 22 nmol m$^{-2}$ s$^{-1}$ after senescence, similar to mean emissions (139 $\pm$ 15 nmol m$^{-2}$ s$^{-1}$) prior to leaf out (Table 2). E. vaginatum net CH$_4$ flux increased from 0 to 73 $\pm$ 159 nmol m$^{-2}$ s$^{-1}$ mid-season as the water table position reached ~0.3 m below the surface (Figure 2d; Table 2). Lower fluxes occurred during the water deficit period in August and did not recover alongside late season water table rise, although E. vaginatum remained a source of 34 $\pm$ 11 nmol m$^{-2}$ s$^{-1}$ (Figure 2d; Table 2). Restored Sphagnum was on average a null CH$_4$ source throughout the season, measuring low uptake ($\sim$8 nmol m$^{-2}$ s$^{-1}$) to low release ($<10$ nmol m$^{-2}$ s$^{-1}$) (data not shown). Fluxes from Sphagnum overlain by shrub species were similar to Sphagnum-only collars and were a net zero emission (data not shown). Comparatively, the reference site Sphagnum was a mean source over the season, with emissions peaking at 50 $\pm$ 7 nmol m$^{-2}$ s$^{-1}$ and had a significantly higher flux during the early season compared to restored Sphagnum and E. vaginatum (Table 2).

Methane ebullition occurred most often in bare ditch areas, less frequently in T. latifolia plots, during a single campaign in one E. vaginatum plot, and never in Sphagnum plots. In both ditch type plots, ebullition ceased by early August when the water table dropped below the ditch surface. The frequency of bubble events occurring from the former ditches over the sampling period was estimated to be 1,820 days$^{-1}$ and the mean flux was estimated at 42 nmol m$^{-2}$ s$^{-1}$, corresponding to 9% of total emissions (ebullitive & diffusive) over the sampling period.

### 3.1.1. Pore Water Carbon Concentration

The concentration of dCH$_4$ was significantly higher 0.8 m below the surface (i.e., cutover peat) in the former ditches compared to the restored peat fields (Mann-Whitney U test, p < 0.0001) (Figure 3a; Table 3). Pore water sampling 0.2 m below the surface (i.e., root zone) of the restored peat fields was not possible after the month of June due to seasonal water table drawdown; values are shown in Figures 3a and 3b after DOY 175 were determined from pore air samples. In contrast, the former ditch water table did not drop more than 0.17 m below the surface during the 2016 season (Figure 2c). dCH$_4$ concentration in the root zone and in the cutover peat was statistically different in the bare ditch areas (Mann-Whitney U test, p = 0.011) and in T. latifolia plots (Mann-Whitney U test, p < 0.0001). T. latifolia dCH$_4$ was significantly lower than bare ditch dCH$_4$ at both sampling depths (Mann-Whitney U test, p < 0.0001). Bare ditch dCH$_4$ had an early-season peak, coinciding with a rainy period that preceded the beginning of the seasonal water table drawdown (Figure 2c). Meanwhile, dCH$_4$ at the two depths below T. latifolia showed a synchronized seasonal pattern, with stable or increasing concentration during the early season, a mid-season decrease, and stable concentration during...
the late season. A comparison of June–August measurements in the restored peatland and reference peatland determined that bare ditch, *T. latifolia* and reference *Sphagnum* plots were not significantly different at 0.8 m depth (Kruskal-Wallis test, *p* = 0.078; Table 3). In contrast, the concentration at 0.2 m depth was significantly lower in restored peat fields and *T. latifolia* plots and significantly higher in bare ditch plots compared to the reference plots (Mann-Whitney *U* test, *p* < 0.0001; Table 3). The concentration of dCO$_2$ was significantly higher in the former ditches than in the restored peat fields (Mann-Whitney *U* test, *p* < 0.0001) and in the cutover peat compared to the root zone in all plot types (Mann-Whitney *U* test, *p* < 0.0001) (Figure 3b; Table 3). dCO$_2$ appeared to peak mid-season in the former ditches while the inverse appeared to be the case in the restored peat fields (Figure 3b). dCO$_2$ in June–August was significantly higher in the former ditches compared to the reference peatland at 0.2 m (Mann-Whitney *U* test, *p* = 0.0017) and at 0.8 m (Mann-Whitney *U* test, *p* < 0.001; Table 3). dCO$_2$ in the restored peat fields differed significantly from reference values at 0.8 m depth (Kruskal-Wallis test, *p* < 0.0001; Table 3).

DOC concentration in the cutover peat was not significantly different in the former ditch and restored peat field *E. vaginatum* plots whereas restored *Sphagnum* values were significantly lower when grouped seasonally (Kruskal-Wallis test, *p* = 0.002). DOC at 0.2 m depth was significantly higher in the former ditch than the restored peat field (Kruskal-Wallis test, *p* < 0.001), but not amongst the former ditch plots and restored peat field plots, respectively (Mann-Whitney *U* test, *p* = 0.32, *p* = 0.88). *T. latifolia* root zone DOC appeared to decrease over the season whereas concentrations appeared to increase slightly in the three other former ditch cohorts (Figure 3c). DOC at 0.8 m depth was significantly higher in the reference peatland compared to the restored peatland (Mann-Whitney *U* test, *p* < 0.0001). Meanwhile, DOC at 0.2 m depth was significantly higher in the restored peatland former ditches compared to the reference site (Mann-Whitney *U* test, *p* < 0.001; Table 3).

### 3.1.2. Acetate Concentration

Acetate concentration in the restored peat field plots could not be measured beyond the June campaign (DOY 163) due to the position of the water table and strong resistance to water extraction at 0.8 m depth (Figures 4g–4j). Mean (SE) acetate concentration in the root zone of *E. vaginatum* plots in June was 28.6 ± 6.7 μM compared to
34.8 ± 8.3 μM in the cutover peat (Table 3). Concentration in the root zone and cutover peat of restored *Sphagnum* was 8.6 ± 1.8 μM and 39.1 ± 14.7 μM, respectively (Table 3). Within the former ditches, the *T. latifolia* root zone had the highest mean concentration (14.9 ± 2.3 μM) on DOY 163, compared to 6.2–8.5 μM otherwise (Figures 4b and 4c). *T. latifolia* root zone was the only sampling cohort to exhibit a significant seasonal pattern (Kruskal-Wallis test, *p* = 0.007 Figure 4b). A root isolation experiment found *T. latifolia* root exudation of acetate to peak mid-season (Figure 4a), whereas *E. vaginatum* root exudation did not change over the season (Figure 4f).

### 3.1.3. Carbon Isotopic Composition of Dissolved CH$_4$ and CO$_2$

Pore water δ$^{13}$C-CO$_2$ in samples extracted from the restored peatland ranged from −24‰ to −2‰, with the least enriched signature found in the root zone of restored *Sphagnum* (−22‰) and *E. vaginatum* (−24‰ to −18‰) plots and the most enriched signature in bare ditch cutover peat (−10‰ to −2‰) (Figure 4). The mean δ$^{13}$C-CO$_2$ in the root zone of the above respective communities was −21.2 ± 1.0‰, −22.1 ± 0.5‰, −18.8 ± 0.2‰ and −5.8 ± 0.8‰, respectively (data not shown). The δ$^{13}$C-CO$_2$ signature beneath the bare ditch area (0.2 & 0.8 m depth) did not exhibit seasonal change (*p* = 0.063). Instead, one bare ditch plot had significantly less depleted values than the other two plots (Kruskal-Wallis test, *p* < 0.001). Individual *T. latifolia* plots became less enriched in δ$^{13}$C-CO$_2$ over the course of four sample dates, but, when the plots were analyzed together, no seasonal pattern emerged (Kruskal-Wallis test, *p* = 0.11) (Figure 5). The δ$^{13}$C-CO$_2$ signature in *T. latifolia* cutover peat, on the other hand, did show a seasonal shift toward less enriched values during mid-season (Kruskal-Wallis test, *p* = 0.020).

Pore water δ$^{13}$C-CH$_4$ ranged from least enrichment in the samples taken at 0.8 m depth (−69‰ to −57‰) to most enrichment (max −48‰) in the *T. latifolia* root zone (*T. latifolia* 0.2 m in Figure 5). Mean δ$^{13}$C-CH$_4$ in the

### Table 3

| Feature                  | Vegetation            | Depth  (m) | [DOC] (mM) | [dCH$_4$] (mM) | [dCO$_2$] (mM) | [Acetate] (μM)$^a$ | Root [acetate] (μM) | δ$^{13}$C-CH$_4$ (%)$^a$ | α$^a$ |
|-------------------------|-----------------------|------------|------------|----------------|----------------|-------------------|---------------------|-------------------------|-------|
| **Restored peat field** | *E. vaginatum*        | 0.2        | 6.0 (4.0)  | 0.04 (0.01)   | 0.49 (0.11)    | 28.6 (6.7)        | 9.0 (1.2)           | −62.4 (1.9)             | 1.043 |
|                         |                       |            | [n = 8]    | [n = 38]      | [n = 38]       | [n = 6]           | [n = 48]            | [n = 3]                 | [n = 4] |
|                         |                       | 0.8        | 7.0 (0.5)  | 0.30 (0.14)   | 2.39 (0.21)    | 34.8 (8.3)        | −               | −57.9 (0.4)             | 1.049 |
|                         |                       |            | [n = 40]   | [n = 39]      | [n = 39]       | [n = 4]           | [n = 4]            | [n = 4]                 |       |
|                         | *Sphagnum*            | 0.2        | 4.9 (0.2)  | −            | 0.18 (0.02)    | 8.6 (1.8)         | −               | −               |       |
|                         |                       |            | [n = 5]    |              | [n = 20]       | [n = 3]           | [n = 4]            | [n = 4]                 |       |
|                         |                       | 0.8        | 5.0 (0.2)  | 0.12 (0.02)   | 1.42 (0.32)    | 39.1 (14.7)       | −               | −63.0 (3.3)             | 1.053 |
|                         |                       |            | [n = 16]   | [n = 20]      | [n = 20]       | [n = 3]           | [n = 3]            | [n = 3]                 |       |
| **Former drainage ditch** | *T. latifolia*        | 0.2        | 7.0 (0.1)  | 0.17 (0.02)   | 3.54 (0.21)    | 8.8 (1.2)         | 9.0 (2.0)          | −54.1 (0.4)             | 1.044 |
|                         |                       |            | [n = 42]   | [n = 42]      | [n = 42]       | [n = 21]          | [n = 44]           | [n = 23]                 | [n = 23] |
|                         |                       | 0.8        | 6.4 (0.2)  | 0.40 (0.02)   | 4.75 (0.12)    | 8.3 (0.7)         | −               | −63.7 (0.4)             | 1.059 |
|                         |                       |            | [n = 40]   | [n = 40]      | [n = 40]       | [n = 40]          | [n = 22]           | [n = 24]                 |       |
|                         | *Bare ditch*          | 0.2        | 6.8 (0.3)  | 0.49 (0.05)   | 3.29 (0.21)    | 4.1 (0.5)         | −               | −56.6 (0.5)             | 1.051 |
|                         |                       |            | [n = 21]   | [n = 21]      | [n = 21]       | [n = 6]           | [n = 12]          | [n = 12]                 |       |
|                         |                       | 0.8        | 7.2 (0.2)  | 0.66 (0.05)   | 5.03 (0.21)    | 7.0 (1.3)         | −               | −63.5 (0.5)             | 1.062 |
|                         |                       |            | [n = 21]   | [n = 21]      | [n = 21]       | [n = 9]           | [n = 12]          | [n = 12]                 |       |
| **Reference peatland** | *Sphagnum*            | 0.2        | 4.5 (0.3)  | 0.35 (0.02)   | 2.21 (0.15)    | −               | −               | −               |       |
|                         |                       |            | [n = 17]   | [n = 19]      | [n = 19]       | [n = 12]          | [n = 12]          | [n = 12]                 |       |
|                         |                       | 0.8        | 11.0 (0.8) | 0.50 (0.05)   | 2.84 (0.11)    | −               | −               | −               |       |
|                         |                       |            | [n = 20]   | [n = 20]      | [n = 20]       | [n = 20]          | [n = 20]          | [n = 20]                 |       |

$^a$Restored field averages only include samples collected in June as subsequent sampling was prevented by a water table deeper than 0.2 m and strong resistance when drawing up pore water from 0.8 m.
Figure 4. Pore water acetate concentration (µM) in the former drainage ditches (top panel) and restored peat fields (bottom panel). Concentration was measured under *T. latifolia* in (a) isolated roots, (b) root zone (0.2 m depth), and (c) cutover peat (0.8 m depth); from bare ditch in (d) root zone, and (e) cutover peat; under *E. vaginatum* in (f) isolated roots, (g) root zone, and (h) cutover peat; and under *Sphagnum* in (i) root zone, and (j) cutover peat. Isolated root samples were accumulated in syringes in a deionized water medium over a 24-hr period. Sampling in the restored fields only occurred on DOY 163, with the exception of root sampling. Significant differences between sampling dates were evaluated using the Kruskal-Wallis test, denoted as “a” and “b” above the box and whiskers (alpha = 0.05); a “+” marks an outlier data point (outside 1.5 times the interquartile range above the upper quartile).

Figure 5. Cross-plot showing stable isotope C composition ($^{13}$C) of dissolved CO$_2$ ($\delta^{13}$C-CO$_2$) and dissolved CH$_4$ ($\delta^{13}$C-CH$_4$) in pore water. Results are classified by depth below the surface (0.2 and 0.8 m) and plant community (*T. latifolia* in former ditches, bare ditch, *E. vaginatum* in restored peat fields, and *Sphagnum* in restored peat fields). Dashed diagonal lines show equal fractionation between dissolved CH$_4$ and co-occurring CO$_2$, with the apparent fractionation factor (α) decreasing from top-left to bottom-right. Values of α above 1.065 suggest H$_2$/CO$_2$ reduction is the dominant methanogenesis pathway while values below 1.055 suggest acetate fermentation is dominating (Conrad, 2005).
Cutover peat below *E. vaginatum*, *Sphagnum*, *T. latifolia* and bare ditch plots was $\sim-57.9 \pm 0.4\%e$, $-63.0 \pm 3.3\%e$, $-63.7 \pm 0.4\%e$ and $-63.5 \pm 0.5\%e$, respectively (Table 3). Mean $\delta^{13}$C-$\text{CH}_4$ in the root zone was $-62.4 \pm 1.9\%e$ for *E. vaginatum*, $-54.1 \pm 0.4\%e$ for *T. latifolia* and $-56.6 \pm 0.5\%e$ in bare ditch plots (Table 3); three pore water samples were extracted from beneath *Sphagnum* on DOY 163, but all were below the $^{13}$C-$\text{CH}_4$ detection limit, as was the case in three of six *E. vaginatum* samples. The $\delta^{13}$C-$\text{CH}_4$ signature of bare ditch cutover peat did not significantly change over the season (Kruskal-Wallis test, $p = 0.96$), whereas root zone $\delta^{13}$C-$\text{CH}_4$ became significantly more enriched over the course of the season (Kruskal-Wallis test, $p = 0.033$; Figure 5). The signature of the *T. latifolia* root zone also exhibited significant seasonal enrichment (Kruskal-Wallis test, $p = 0.044$), while the cutover peat beneath *T. latifolia* was least enriched on the two mid-season sampling dates (DOY 201 & 216) (Figure 5).

The apparent fractionation factor for $\text{CO}_2 \rightarrow \text{CH}_4$, $\alpha$, ranged from a mean of $\sim-1.045$ in the root zone (0.2 m) of *T. latifolia* and *E. vaginatum* to a mean of 1.062 in bare ditch cutover peat (0.8 m) (Table 3). Cutover peat samples generally grouped above 1.06, with the exception of *E. vaginatum* (Figure 5). *Sphagnum* cutover peat showed a relatively large range in $\alpha$, but, amongst only the three detectable samples. Caution is needed in interpreting these values given the limited data availability for the restored field plots on DOY 163 and the lack of data during the subsequent three data collection campaigns. Root zone samples (0.2 m) were generally grouped below 1.05, although this was the case only later in the season in bare ditch areas (Figure 5).

## 4. Discussion

### 4.1. Methane surface exchange

Methane emissions measured at a post-extraction peatland restored 16 years prior reveal that *Sphagnum*-dominated areas were a null emission of $\text{CH}_4$ over the warm season. This is a significant finding as approximately two thirds of the restored peatland was pure *Sphagnum* or *Sphagnum* with Ericaceous shrubs (*Chamaedaphne calyculata*, *Rhododendron groenlandicum*, etc.) (Nugent et al., 2018). Our $\text{CH}_4$ measurements are similar to those measured at the same site at 10 years post-restoration, which were linked to a deeper water table (<0.2 m) and expected limitations in substrate quality (Strack & Zuback, 2013). Comparatively, *Sphagnum* plots in the reference peatland surrounding the restored site emitted $\text{CH}_4$ at rates (50 nmol m$^{-2}$ s$^{-1}$ during mid-season) similar to other dry *Sphagnum* peatlands (20–70 nmol m$^{-2}$ s$^{-1}$ mid-season) (Lai et al., 2014; Moore et al., 2011; Strack et al., 2004). *E. vaginatum* tussocks, which occupied roughly a third of the restored site, emitted $\text{CH}_4$ at a rate (73 nmol m$^{-2}$ s$^{-1}$ during mid-season) that was within the range of *Eriophorum* emissions in undisturbed peatlands (20–433 nmol m$^{-2}$ s$^{-1}$) (Green & Baird, 2011; Greenup et al., 2000; Lai et al., 2014; Moore et al., 2011; Öquist & Svensson, 2002; Ström & Christensen, 2007; Ström et al., 2005; Waddington et al., 1996), as well as the range reported in restored peatlands (6–142 nmol m$^{-2}$ s$^{-1}$) (Cooper et al., 2014; Komulainen et al., 1998; Lee et al., 2017; Marinier et al., 2004; Tuittila et al., 2000; Wilson et al., 2009, 2016). Diffusive $\text{CH}_4$ flux from the former ditch, which occupy 4% of the restored site, was high in non-vegetated areas when the water table was at or above the surface. Ebullition was a regular pathway until the water table fell below the surface of the former ditches. We estimate that this criterion was met over 55% of the growing season. The mean magnitude of $\text{CH}_4$ emission from the cutover peat beneath *E. vaginatum* was $\sim 0.26$ mol m$^{-2}$ s$^{-1}$ during bubble events which was similar to events at a temperate poor fen (Goodrich et al., 2011). Although no attempt was made to calculate the daily ebullition rate, as our temporal resolution was too coarse, ebullition was estimated to account for 9% of total emissions from the former ditches. Plant-mediated $\text{CH}_4$ emission from *T. latifolia*, which densely occupied two of seven inner ditches in the restored section (Figure 1), was similar to rates measured at a temperate freshwater *Typha angustifolia*-dominated marsh (Strachan et al., 2015). At our site, *T. latifolia* attenuated former ditch emissions when the water table was above the surface but was a continual source even once the water table dropped below the surface and diffusive fluxes in the bare sections were significantly reduced by oxidation. The former ditches were a $\text{CH}_4$ emission hotspot within the site as was expected owing to the preferential water holding, a pattern also seen at other restored or rewetted peatlands (e.g., Cooper et al., 2014; Vanselow-Algan et al., 2015; Wilson et al., 2013, 2016). Plant-mediated transport was the overall dominant emission pathway, both in the ditches and in the restored fields.

Our flux measurements from the restored peat fields can be compared in more detail to those of the Mer Bleue peatland, which is located within the same climate zone as our study site and has similar water storage (Nugent et al., 2018). At Mer Bleue, *Sphagnum* with *Chamaedaphne calyculata* emitted $\text{CH}_4$ at a rate of 36–72 nmol m$^{-2}$ s$^{-1}$ during mid-summer even as the water table approached 0.5–0.6 m below the surface (Moore et al., 2011). In
comparison, *Sphagnum* at our site fluctuated between being a minimal source and sink for CH₄. Mid-summer peak *E. vaginatum* emission at Mer Bleue was 145–433 nmol m⁻² s⁻¹, whereas only two of six *E. vaginatum* collars at our restored site had fluxes above 100 nmol m⁻² s⁻¹ (data not shown). There appear to be factors reducing surface emission of CH₄ across the restored peat fields, although clearly more so in *Sphagnum*-dominated areas (see discussion: CH₄ production pathways and oxidation).

4.2. Belowground Carbon Cycling

Pore water measurements indicate that areas of restored peatland *Sphagnum* had the lowest concentration of dCH₄, dCO₂, and DOC at the peatland complex. *E. vaginatum* in the restored peat fields also exhibited lower dCH₄ and dCO₂ relative to concentrations in the former ditches. dCH₄ concentration beneath *E. vaginatum* in the restored peatland was substantially lower than that of natural *E. vaginatum*-dominated areas at a palsa peatland in Sweden (Ström & Christensen, 2007) and at Mer Bleue (Beer & Blodau, 2007). On the other hand, dCH₄ beneath *E. vaginatum* in this study exceeded concentrations at a nearby spontaneously recolonized post-extraction peatland (Mahmood & Strack, 2011) and early-restoration values at our own site (1–3 years post-restoration; Waddington & Day, 2007). This suggests a progressive shift in CH₄ pool accumulation over time post-restoration moving toward, although still distinct from, undisturbed peatland conditions. Our restored peat field flux measurements demonstrate that CH₄ precursors (acetate, H₂, and CO₂) were present in great enough concentration to maintain CH₄ production below *E. vaginatum*. However, a zero release of CH₄ early in the season suggests that time was needed to accumulate the CH₄ pool. Comparatively, the reference peatland had relatively high early season pore water CH₄ concentration and net emission. The winter preceding the study period had abnormally warm conditions, causing only the top 0.10 m of the restored peatland to freeze (Nugent et al., 2018). Comparative snow cover measurements at the reference peatland are lacking, but are unlikely to drastically differ given the proximity of their locations. This suggests that winter and/or early spring CH₄ production was higher at the reference portion of the study site, leading to an earlier build up in the pore water.

The reference peatland *Sphagnum* plots exhibited a seasonal increase in DOC in the deeper peat layer commonly seen at undisturbed peatlands (e.g., Blodau et al., 2007; Waddington & Roulet, 1996). In contrast, neither the restored peat fields nor former ditches showed significant seasonal DOC accumulation (Figure 3c). Radiocarbon evidence has shown dissolved organic matter (DOM) up to 3 m depth to be younger than the surrounding bulk peat (Chanton et al., 2008), indicating the importance of vertical C movement in peatlands. Our restored peat field mean DOC concentration was similar to measurements done four years prior (~5.8 mM), during which DOC chemistry was determined to be less labile than at the reference peatland (Strack et al., 2015). The not fully recovered DOC chemistry was attributed to a hydrological disconnect between the cutover peat and new *Sphagnum* growth, caused by the high bulk density of the cutover peat (McCarter & Price, 2013; Strack et al., 2015). In *Sphagnum* peatlands, CO₂ and CH₄ are generally derived from a combination of DOM and bulk peat decomposition whereas labile DOM appears to be the main methanogenesis substrate in sedge-dominated peatlands (Chanton et al., 2008). The greater reliance on bulk peat may be one of several reasons why *Sphagnum* peatlands typically produce low amounts of CH₄ compared to sedge-dominated peatlands (Bridgham et al., 2013). At our restored peatland, the limited contact in the restored peat fields between cutover peat pore water and new litter appears to continue to be a factor reducing labile DOM input to deeper peat. This could in turn be reducing the priming effects of the DOM on the recalcitrant peat (Basiliko et al., 2012). Our results are consistent with other studies which have suggested that increased recalcitrance in old peat may induce a starvation-survival state in microbial communities that reduce overall activity (Andersen et al., 2006; Fisk et al., 2003; Morita, 1990).

In general, dCH₄, dCO₂ and DOC concentrations were higher in the former ditches compared to the reference peatland, with the exception of deeper peat DOC. This shows an active turnover of C in the former ditches. Bare ditch area accumulated the highest dCH₄ concentrations, equal at both sampling depths, and did not exhibit the seasonal drawdown seen beneath *T. latifolia* (Figure 3a). The seasonal pattern in the *T. latifolia* plots illustrates that net CH₄ removal (rhizospheric oxidation and plant-mediated transport) exceeded production during the mid-season months.

Acetate was present in the restored peat fields at a significantly higher concentration (~30 μM) than in the former ditches (~8 μM) on DOY 163 but was at the low end of the range found at undisturbed *Sphagnum* and sedge-dominated peatlands (5 μM to >1,000 μM) (Avery et al., 1999; Blodau et al., 2007; Ström et al., 2003, 2012, 2015). Note that acetate samples could not be taken from under the restored field plots in the
subsequent three data collection campaigns, limiting our ability to interpret the results. Concentrations derived from the restored area *E. vaginatum* root exudates remained about 9 μM over the summer which, while a low concentration, was occurring over approximately one-third of the site. At Mer Bleue, acetate concentration in the pore water ranged from 10 to 50 μM (Blodau et al., 2007), with the lowest acetate levels coinciding with the timing of peak CH₄ emissions from plant communities (Lai et al., 2014; Moore et al., 2011). *T. latifolia* root exudation at our restored site peaked mid-season even as acetate and CH₄ concentration were declining in the pore water toward a minimum at the end of August. In contrast, acetate levels in bare ditch areas did not vary significantly over the season. It seems likely that *T. latifolia* was the primary source of labile C substrate for acetoclastic methanogenesis occurring throughout the former ditches.

### 4.2.1. CH₄ Production Pathways and Oxidation

A δ¹³C-CO₂ value that is closer to the isotopic signature of organic matter (−26‰) in conjunction with CH₄ enrichment is evidence that CH₄ oxidation was occurring in the restored peat fields at 0.2 m depth (Singleton et al., 2018). Stable isotope values in the cutover peat (0.8 m) additionally reveal that hydrogenotrophic methanogenesis was not the dominant methanogenesis pathway, although data are confined to June only. *E. vaginatum* roots at the restored site reached a mean depth of 0.24 m, with no roots extending beyond 0.3 m (data not shown). As the maximum depth reached by the water table was ~0.5 m, 0.8 m depth sampling should have been unaffected by vascular plant rhizospheric inputs and oxidation. Consequently, it is notable that none of the δ¹³C samples from the restored peatland showed H₂/CO₂ reduction dominating (α > 1.065). Comparatively, δ¹³C data of CO₂ and CH₄ at Mer Bleue (α > 1.069) suggested that acetate fermentation was of less importance and that H₂/CO₂ reduction dominated from 0.35 m depth up to a sampling maximum of 3.7 m (Beer & Blodau, 2007). The stable isotopic signature does not define the relative importance of methanogenesis in terms of total respiration, merely the relative importance of the two CH₄ production pathways (Hines et al., 2008). The reduced importance of H₂/CO₂ reduction at depth in the restored peat fields may be a sign of the insignificance of methanogenesis rather than the dominance of acetate fermentation early during the season. A lack of CH₄ concentration build-up in the restored field cutover peat over the season points toward production limitations in the recalcitrant peat. Lower DOM compared to the surrounding reference peatland suggests that bulk peat C turnover was slower, reducing CH₄ production. Substrate supply for CH₄ production instead was from *E. vaginatum* growth, with methanogenesis occurring close to the roots and CH₄ being rapidly consumed or transported to the atmosphere via plant tissues. While pH was not measured in this study, a previous study completed 6 years prior at the restored site found a pH of 5.8 ± 0.5 in the restored fields and 5.7 ± 0.8 in the former ditches (Strack et al., 2010). In a peat slurry experiment from six peatlands, Ye et al. (2012) found that a higher pH (5.5–6.5) enhanced CH₄ production and that acetate pooling in ombrotrophic peats at higher pH suggests CH₄ production is inhibited by other factor(s) in addition to pH. We cannot say conclusively whether acetate pooling occurred in the restored fields later in the season, although it was not the case in the *E. vaginatum* root exudates over the course of the season. Other studies have demonstrated inhibition of methanogenesis by humic substances (e.g., Keller & Takagi, 2013; Minderlein & Blodau, 2010), which may be the case here. Methane production could also be limited by inactive methanogen communities, a legacy of the water level being dropped during extraction and of the highly recalcitrant cutover peat remaining.

It is possible that the importance of oxidation in this system is more than we hypothesize. In a thawing permafrost peatland, the most oxidized CH₄ and highest methanotroph abundances were found to occur in peat that was inundated >90% of the time (Singleton et al., 2018). The same study found that CH₄ concentration, which increased with depth, was the key driver of methanotroph community patterns within the bog section (Singleton et al., 2018). We would argue that the very low concentrations found within the saturated peat profile of the restored fields would not be a hospitable location for methanotrophs, lending toward the interpretation that production is the limiting factor within the cutover peat. The influence of microbial oxidation could be investigated further by pairing ¹³C isotope sampling with stable hydrogen isotopes (Coleman et al., 1981; Happell et al., 1994; Qin et al., 2020), while radiocarbon (¹⁴C) measurements can offer information on whether methanogenesis is fueled by decomposition of residual peat or C recently fixed by vegetation (McNicol et al., 2020).

The relatively enriched δ¹³C-CO₂ signature in the former ditches throughout the season, on the other hand, conclusively demonstrates methanogen activity. *T. latifolia* root zone samples were the most enriched in δ¹³C-CH₄ (−54.1 ± 0.4‰), while δ¹³C α values were ~1.045, illustrating more acetoclastic production or consumption by oxidation. Here, the higher surface flux measurements suggest production better explains the isotopic results.
Although $H_2/CO_2$ reduction did not dominate at 0.8 m depth, an $\alpha$ of 1.055–1.065 shows that recalcitrant peat in the former ditches was supporting CH$_4$ production. In contrast, the acetate, pore water CH$_4$ concentration, and surface flux results are taken together suggest little bulk peat turnover is occurring in the restored peat fields.

5. Conclusion

Our study argues that low bulk peat C turnover is the likely explanation for lower CH$_4$ emissions at post-extraction restored Sphagnum peatlands. Because the saturated zone contributes relatively little to ecosystem respiration (Blodau et al., 2007), a successful ecological restoration can result in net CO$_2$ uptake at rates similar to undisturbed Sphagnum peatlands (Nugent et al., 2018). Net CH$_4$ production and emission in restored peat fields, however, has been shown here to be reduced and to occur only with E. vaginatum substrate input and plant-mediated transport. The lack of a hydrogenotrophic methanogenesis signature in the restored peat fields indicates limited decomposition of the recalcitrant cutover peat. In contrast, a mixed methanogenesis signature deeper in the former ditch profile is evidence that older organic matter decomposition was occurring within the ditch confines. The legacy of cutover peat substrate quality is suggested to be an important mechanism in reducing CH$_4$ production and emission. Greater hydrological connectivity between the Sphagnum layer and underlying cutover peat is expected as new peat continues to develop. On this basis, C turnover of the new peat, with potential DOM priming of the bulk peat, over time would limit the impacts of the cutover peat on the surface flux. However, evidence after 16 years of recovery suggests that cutover peat effects could last for a period (decades) beyond the successful return of a C sink, which is beneficial to reducing the climate warming impact of newly restored extracted peatlands.

Data Availability Statement

The dataset for this research are available as: Strachan, I.B. and K.A. Nugent 2020. Post-extraction restored peatland methane production and emission ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/06bd-777b095f582a950ecfde444c2f10 (Accessed 2020-10-26).

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