Methane cycling in horticultural extracted, restored, and unrestored peatlands in central Alberta, Canada

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.

Statement of Contributions

The thesis comprises of three manuscripts that are intended for three separate publications; thus, the study site description and some methods are repeated in more than one chapter.

Tentative titles of the future publications:

Manuscript 1: Methane cycling microorganisms and CH_4 production and oxidation rates in horticultural peatlands: Comparing natural, currently extracted, unrestored, and different ages of restored sites. Authors: Bieniada, A., Hug., L. A., Parsons, C., Van Cappellen, P., Strack, M.

Manuscript 2: Steady and ebullitive methane fluxes from active, restored and unrestored horticultural peatlands. Authors: Bieniada, A., Strack, M.

Manuscript 3: Subsurface free-phase gas content in natural, extracted, unrestored, and restored horticulture peatlands. Authors: Bieniada, A., Mwakanyamale, K. E., Moorman, B., Strack, M.

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Dr. Christopher Parsons mentored the analytical part of the research included in Chapter 2 of the thesis, advised and consulted on methodology and laboratory analyses.

Dr. Philippe Van Cappellen provided funding for the analytical part of the research included in Chapter 2, as well as laboratory access and resources to complete the analyses.

Dr. Kisa Edson Mwakanyamale provided training in ground-penetrating radar use and data analysis, and calibrated the soil moisture content probes.

Dr. Brian Moorman provided the ground-penetrating radar, and revised Chapter 4 of the thesis.

Abstract

Horticulture peat extraction drastically changes peatland ecosystems and their carbon and greenhouse gas balance. Comprehensive study on the combined response of methane (CH_4) cycling (*i.e.*, CH_4 production, oxidation, subsurface storage and release) to peat extraction, abandonment and restoration is lacking. It is still unknown how much CH_4 is released through abrupt episodic ebullition events and whether they occur at unrestored sites, and how the subsurface free-phase gas, potentially comprised up to 50 % of CH_4, recovers post-extraction. To date, there are no studies on ebullition and free-phase gas development with the age of restoration. There are few studies focused on methanogenic and methanotrophic members of the microbial community, its structure, abundance, and activity in these sites. Here, I address these research gaps to better understand the role of peatland restoration in greenhouse gas balance recovery and to support informed decision making on peatland management. My objectives were to determine ebullition contribution to CH_4 emission from currently extracted and unrestored sites and from sites restored at different times in the past; to quantify the subsurface free-phase gas content and dissolved CH_4 concentration to determine if there is a progression in subsurface CH_4 pool recovery at both restored and unrestored sites; and to identify the CH_4-cycling microorganisms, their community structure, abundance, diversity, and the potential rates of CH_4 production and oxidation. The outcome of the research is discussed in the context of peat physicochemical properties.

The study site was located west of Edmonton, Alberta (53° 33' N, 114° 44' W) at a horticulture extraction peatland complex managed by Sun Gro Horticulture and comprised of several sites of different management stages from current extraction (the Active site), through Unrestored, to restored at different times in the past: in 1991, 2009, and 2012 (RES-1991, RES-2009, and RES-2012, respectively). A natural bog (Natural) within the complex served as a reference site.

The community composition and abundance of methanogens and methanotrophs was determined with Illumina Tag 16S rRNA gene sequencing and linked to physicochemical conditions across the sites and the dominant type of peat surface cover. Potential CH_4 production and oxidation rates (MP and MO, respectively) were determined in microcosms

with peat collected above and below the water table at the Natural, Unrestored and RES-2009 sites. I observed more diverse and abundant CH_4-cycling communities in the oldest and the youngest restored sites (RES-1991 and RES-2009), while RES-2012 showed similar microbial characteristics to that in the Unrestored and Natural sites and generally low MP and MO likely affected by peat chemistry. In conclusion, restoration promotes development of CH_4-cycling microorganisms more abundant and diverse than in the Natural site, while lack of restoration leads to poorly developed community of methanogens and methanotrophs and low potential MP and MO rates.

I also measured pore water CH_4 concentrations in summer 2017 and steady and ebullitive CH_4 fluxes from all study sites across two growing seasons using a closed chamber method with a portable greenhouse gas analyzer. Fluxes and dissolved CH_4 were evaluated in relation to the dominant type of surface cover, and other potential controls. Ebullition occurred only at RES-1991 and RES-2012, where the highest steady fluxes were measured, but no ebullition and low fluxes were found at RES-2009, despite similar wetness and dominance of graminoids. Both steady and ebullitive emissions were related to the water table, soil temperature, and gross ecosystem production, but only steady fluxes depended on the cover of vascular plants. The magnitude of ebullition was positively correlated with pore water CH_4 concentration and the magnitude of the steady flux. The concentration of dissolved CH_4 recovered to the natural level in restored sites but remained low and did not increase over the season in the Unrestored site. Flooded, sedge-dominated restored sites can emit more CH_4 than natural bogs, including ebullition, but steady fluxes contribute > 90 % of total CH_4 emission.

Free-phase gas content was measured with soil moisture content probes and ground penetrating radar (GPR). It did not follow the CH_4 emission pattern, but large amounts, close to that at the Natural site, were found in the Unrestored and RES-2009 (sites with low CH_4 emission and production rates), likely due to peat structure that promotes gas accumulation. This was also observed in the Active site.

Methane emission across the sites generally followed the pattern of the abundance and diversity of CH_4-cycling microbes, with flooded, sedge-dominated restored sites showing the highest values and Unrestored and Active sites the lowest. Local environmental conditions appear to have more impact on these aspects of CH_4 cycling in restored peatlands than the age

of restoration. Methane fluxes cannot predict free-phase gas content, which was observed to be independent of restoration, and can be high if peat is compacted.

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Dr. Kisa Mwakanyamale taught me how to operate the GPR and process the radargrams. She flew from the US to meet me at the site and showed extraordinary hospitality when I came to Champaign, IL to be trained in data processing. Kisa, thank you for your time and patience.

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Dedication

I dedicate this thesis to my Heavenly Father who opens doors that no one can shut.

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List of Abbreviations

A (in primers) .....Adenine

ACT .....The Active site

AGC .....Automatic Gain Control (function)

ANME .....Archaeal anaerobic methane oxidizers

AOM .....Anaerobic oxidation of methane

c .....speed of the light in vacuum (0.3 m ns^{-1})

C (in primers) .....Cytosine

CCA .....Canonical correspondence analysis

CH_4 .....Methane

CO .....Common offset

CO_2 .....Carbon dioxide

CMP .....Common midpoint

C:N .....Total carbon to nitrogen ratio

CS probes .....Campbell Scientific water content reflectometer probes

DOC .....Dissolved organic carbon

DOE .....Department of Energy

DOM .....Dissolved organic matter

DOY .....Day of year

EC .....Electrical conductivity

EM wave .....Electromagnetic wave

ESVs .....Exact sequence variants

f .....Antenna frequency

Fe^{2+} .....Ferrous iron

Fe^{3+} .....Ferric iron

Fe(II) .....Iron in its +2 oxidation state

Fe(III) ..... Iron in its +3 oxidation state

$FeCl_2 * 4H_2O$ .....Iron II chloride tetrahydrate

$FeSO_4 * 4H_2O$ .....Iron II sulfate tetrahydrate

FOR .....Forbs

FW .....Forward primer

G (in primers).....Guanine

GC .....Gas chromatograph

GEP .....Gross ecosystem production

GHG .....Greenhouse gases

GPR .....Ground-penetrating radar

GRA .....Graminoinds

IRGA .....Infrared gas analyzer

JGI .....Joint Genome Institute

LME.....Linear mixed effect (model)

MO .....Potential rates of methane oxidation

MOS .....Moss

MP.....Potential rates of methane production

M, H, V, T, W .....Standard abbreviations for ambiguity in primers (*e.g.*, W – adenine or thymine)

N_2 .....Nitrogen

NaOH .....Sodium hydroxide

NAT ..... The Natural site

NEE .....Net ecosystem exchange

NO_3^- .....Nitrate

NO_3^{2-} .....Nitrite

O_2 .....Oxygen

PC .....Principal component

PCA .....Principal component analysis

PCoA .....Principal coordinate analysis

POM .....Particulate organic matter

RES-2009 .....The site restored in 2009

RES-2012 .....The site restored in 2012

RES-1991 .....The site restored in 1991

PO_4^{3-} .....Phosphate

PVC .....Polyvinyl chloride

RW .....Reversed primer

PW25[CH_4] in tables only.....Methane concentration in pore water at 25 cm depth

PW50[CH_4] in tables only .....Methane concentration in pore water at 50 cm depth

SHR .....Shrubs

SO_4^{2-} .....Sulphate

T (in primers) .....Thymine

TEA .....Terminal electron acceptors

TDR ..... Time domain reflectometry

T1 – T6 …….....Transects 1 to 6

T2 – T30.....Temperature at depths 2 – 30 cm

UNR .....The Unrestored site

V .....Velocity

VGC .....Volumetric gas content

WT .....Water table

WTFZ .....Water table fluctuation zone

λ .....Electromagnetic wave length

$\varepsilon_{r(s)}$ …….....Dielectric permittivity of soil

$\varepsilon_{r(w)}$ …..……Dielectric permittivity of water

$\varepsilon_{r(a)}$ ...…….Dielectric permittivity of air

$\varepsilon_{r(b)}$ ...……...Dielectric permittivity of bulk peat

η ...…….....Porosity

Θ ....……Volumetric water content

[ ] .....Concentration

CHAPTER 1: Introduction and Literature Review

1.0. INTRODUCTION

Conditions prevailing in natural peatlands, mainly waterlogged, anoxic soil, and the presence of plants that are typical for peatlands, allow for only slow mineralization of organic matter that accumulates over time creating the peat deposit (Frolking *et al*., 2001). Peat extraction severely disturbs these conditions so that the ecosystem is no longer functional. The natural balance between carbon dioxide (CO_2) sequestration from the atmosphere and methane (CH_4) emission is disrupted (Sundh *et al*., 2000, Turetsky *et al*., 2002, Waddington *et al*., 2002, Abdalla *et al*., 2016, Strack *et al*., 2016). Peatland restoration aims to recover the natural functions of the ecosystem, re-establish peatland vegetation and primary productivity, return hydrological conditions and greenhouse gas balance (Quinty and Rochefort, 2003). Methane cycling in extracted and post-extraction unrestored and restored peatlands is not yet fully understood. Methane is produced by methanogens and oxidized by methanotrophs. Since their highest abundance and activity has been observed in the water table fluctuation zone (Sundh *et al*., 1994, 1995, Martí *et al*., 2015) in shallow peat that is removed during extraction, the community of these organisms and their required environmental conditions are likely severely disturbed. Nevertheless, research on the CH_4-cycling community in managed peatlands is limited to a few studies (Juottonen *et al*., 2012, Putkinen *et al*., 2018, Reumer *et al*., 2018). Methane emissions in post-extracted sites are small from unrestored sites but can be higher than natural in restored sites (*e.g*., Strack *et al*., 2014); however, abrupt ebullition events, venting large amounts of CH_4 directly to the atmosphere, are not well understood in post-extraction sites. Furthermore, natural peatlands have the ability to store free-phase gas (*e.g*., Parsekian *et al*., 2010, 2011), but there is limited knowledge on the post-extraction and post-restoration subsurface pool of gaseous CH_4, the main component of free-phase gas. Complex research linking all aspects of CH_4 cycling in managed peatlands is required to understand the processes occurring post-extraction in unrestored and restored sites. This thesis investigates CH_4-cycling microbial community, potential rates of CH_4 production and oxidation, quantifies CH_4 emission, including abrupt ebullition, and subsurface pool of free-phase gas and dissolved

CH_4 in restored horticultural peatland sites compared to unrestored, currently extracted, and natural sites.

1.1. RELEVANT LITERATURE

1.1.1. Peatland occurrence, carbon storage and methane emission

According to the Canadian Wetland Classification System, peatlands are wetland ecosystems with at least 40 cm of peat (National Wetlands Working Group, 1997), but some geological definitions accept the thickness of minimum 30 cm of peat (*e.g.*, Joosten and Clarke, 2002, Frolking *et al*., 2011). A biological definition classifies peatlands as wetlands with potentially peat-forming vegetation (Laine and Vasander, 1996). Peat is classified as organic soil (histosol) that contains ≥ 35 % of organic matter (RSPO Peatland Working Group 2, 2014). Three zones can be identified in the vertical stratification of the peat deposit: acrotelm, the surface peat, usually poorly decomposed and partially unsaturated (Ingram, 1978), mesotelm, the zone of the water table fluctuation (Clymo and Bryant, 2008), and catotelm, the deepest zone of permanently saturated peat (Ingram, 1978).

Globally, peatlands cover 4.23 million km^2, which is 2.84 % of the land (Xu *et al*., 2018). About 67.5 % of them (2,853,955 km^2) have developed in high and mid latitudes of the northern hemisphere and are referred to as the northern peatlands (Xu *et al*., 2018). Approximately 113 million ha of peatlands (26.8 % of all peatlands worldwide) are located in Canada, and cover ~ 13 % of the country's surface area (Xu *et al*., 2018).

According to previous estimates, northern peatlands store almost one third of the global soil carbon stock (455 – 547 Gt, Gorham, 1991, Yu *et al*., 2010, Yu, 2012), while the newest estimates suggest storage of 1000 Gt (Amesbury *et al*., 2019). About 70 % of the total carbon pool in northern peatlands was accumulated in early Holocene, when the climate was warm (MacDonald *et al*., 2006, Yu *et al*., 2010, Yu, 2012). Then, the accumulation rates decreased (from 38 g C m^{-2} y^{-1} 8000 – 9000 BP to 5.6 g C m^{-2} y^{-1} 2000 – 3000 BP) as a result of Neoglacial climate cooling and permafrost development (Yu, 2012).

Wetlands, including peatlands, play an important role in global carbon cycling. For example, wetland CH_4 emission (mainly in tropical regions) explained 70 % of interannual variations in atmospheric CH_4 concentration in years 1984 – 2003 (Bosquet *et al*., 2006). Their contribution to total CH_4 emission from all sources is estimated to be at least 20 % (Shindel *et al*., 2004, Bridgham *et al*., 2013), and that of tropical wetlands will increase as a result of climate warming (Zhang *et al*., 2017). Methane emitted from northern terrestrial ecosystems (> 50˚N) originates mainly from wetlands and accounts for 36 Tg of CH_4-C y^{-1} (Zhuang *et al*., 2006).

Although CH_4 cycling (production, oxidation, subsurface storage, and emission) in natural peatlands has been studied in the past, the understanding of these processes in post-extraction restored and unrestored sites remains limited.

1.1.2. Peat extraction

Peatland disturbance of any kind (draining for forestry and agriculture, mining, linear disturbances, and peat harvesting for horticulture and fuel) involves altering local hydrological conditions, often including lowering the water table (Waddington and Price, 2000). This, in turn, changes redox conditions and accelerates mineralization of organic matter, which results in CO_2 emission, while CH_4 production either ceases or decreases and most CH_4 is oxidized to CO_2 in the drained peat profile before reaching the atmosphere (Sundh *et al*., 2000, Turetsky *et al*., 2002, Glatzel *et al*., 2004, Bonn *et at*., 2014). The drainage ditches remain a source of CH_4 (Sundh *et al*., 2000, Waddington and Day, 2007, Cooper *et al*., 2014, Nugent *et al*., 2018).

Peat in Canada is extracted mainly for horticultural use and not as a fuel (ECCC, 2018), while peat in Europe is utilized for both combustion and horticultural purposes (*e.g*., Vasander *et al*., 2003). In Canada, only bogs larger than 50 ha and at least 2 m deep are profitable for horticultural peat extraction (Keys, 1992, ECCC, 2018). Kivinen and Pakarinen (1981) estimated that 4.4×10^6 ha of northern peatlands were subjected to extraction for horticulture and fuel. This number has likely increased over the last decades. Only 34,000 ha have been disturbed for peat extraction in Canada (ECCC, 2018), which is considered a local environmental disturbance (Price *et al*., 2003). The horticulture industry requires peat of a

certain quality (*i.e*., poorly decomposed, *Sphagnum* peat), thus a layer of low quality peat is usually left behind, while the whole peat deposit can be harvested from peatlands exploited for energy (Tuittila *et al*., 2000a; Wind-Mulder and Vitt, 2000).

In Canada, vacuum harvesting of peat replaced the peat cutting method in the 1980s (Rochefort, 2000, Wind-Mulder and Vitt, 2000, Environment Canada, 2006, 2015). Vacuum harvesting of peat starts with lowering the water table by installation of drainage ditches across and around the peatland. This is followed by vegetation removal, further draining of the site by milling until the moisture content decreases to ~ 45 %, harvesting using heavy machinery and stocking in piles for up to six months (Cleary *et al*., 2005, Basiliko *et al*., 2007, Waddington *et al*., 2009b, ECCC, 2015). The greenhouse gas emission from peat extraction in Canada increased from 0.9 Mt CO_2 equivalent (CO_2-e) in 1990 to 2.7 Mt CO_2-e in 2000, then decreased to 2.3 Mt CO_2-e in 2013 and 1.5 Mt CO_2-e in 2016 (Environment Canada, 2015, ECCC, 2018). These emissions originated mainly from peat decay and peatland drainage (ECCC, 2018).

Vacuum harvesting leaves the peat surface stripped of all vegetation and acrotelm, with a relatively flat surface of exposed old catotelm that developed thousands of years ago. The remaining old peat is highly decomposed, has low porosity and undergoes subsidence that additionally lowers specific yield and saturated hydraulic conductivity (Van Seters and Price, 2002). Thus, fluctuations of the water table at post-extracted sites are of a magnitude not observed at natural peatlands (Price and Whitehead, 2001, Van Seters and Price, 2002, McNeil and Waddington, 2003).

1.1.3. Peatland restoration

Peat extraction in Canada is centered mainly in Alberta, Quebec, New Brunswick, and Manitoba (ECCC, 2018). In all these provinces, post-extraction restoration is required, *e.g*, the Mining Act (Government of Quebec, 2019) and Alberta Public Land Administrative Regulation, Conservation and Reclamation Regulation, and Environmental Protection and Enhancement Act that obligates the industry to reclaim the harvested sites, while the Alberta Government Directive on Allocation and Sustainable Management of Peat Resources on Public

Land specifies that post-extracted peatlands are required to be reclaimed back to an early successional peatland (Government of Alberta, 2016). Although the term reclamation is used in some contexts, actions required are best defined as restoration rather than reclamation according to the definition of restoration as assisted recovery of a damaged ecosystem (Quinty and Rochefort, 2003). Post vacuum-harvested sites represent extremely harsh conditions that exclude spontaneous revegetation as an efficient method of peatland restoration as the seed bank has been removed (Quinty and Rochefort, 2003) and the remaining peat has been compacted by heavy machinery.

The restoration goal is to return the ecosystem to a naturally functioning and self-sustaining state with established peatland vegetation dominated by *Sphagnum* or true moss depending on the peatland type (bog or fen) and the level of primary productivity that enables peat accumulation (Rochefort, 2000). This includes a well developed acrotelm and biochemical cycles of carbon and other nutrients at levels characteristic for natural peatlands (Rochefort, 2000). Andersen *et al*. (2010) defined restoration as successful when decaying plant litter reaches the anoxic zone (catotelm) without being entirely decomposed in the acrotelm. According to Price *et al*. (2003), full restoration is rather unlikely in a short period of time and rehabilitation is a more realistic goal in a short timescale. However, modelling shows that the restoration of carbon accumulation and a functional acrotelm should be achieved within < 20 years (Lucchese *et al*., 2010). Cleary *et al*. (2005) claim that peatlands restored after horticultural extraction, that became a sink for CO_2, would need 2000 years to rebuild the carbon pool removed during extraction.

Generally, the methods of restoration differ between regions and countries, *e.g*., first restoration of harvested peatlands in Europe relied on natural revegetation after peatland rewetting by filling or blocking the drainage ditches (Lavoie and Rochefort, 1996). However, the response of a post-extraction peatland to restoration depends on the type of peatland and methods of peat extraction. In Canada, the reestablishment of vegetation and increase of water table are essential steps of peatland restoration (Basiliko *et al*., 2007). Plant reintroduction (moss layer transfer technique) is a common method to assist in recolonization of extracted peatlands with bog-specific species (Quinty and Rochefort 2003, Rochefort *et al*., 2003). First attempts of peatland restoration using moss layer transfer in Canada date back to the early 1990s (Rochefort, 2000), thus the evaluation of restoration is limited to less than 30 years.

Prior to moss transfer, the peatland surface is scraped to expose the under layer and levelled for better development of the vegetation (Rochefort, 2000). Shredded *Sphagnum* diaspores and other bog vegetation are collected from a donor site and spread onto the peat surface in ratio 1:10, then covered with straw mulch (1,500 kg ha^{-1}, Rochefort, 2000). When heavy machinery involved in the restoration process are no longer needed, the drainage ditches are blocked to raise the water table (Quinty and Rochefort, 2003). The mulch allows adequate light supply for *Sphagnum* by decreasing the incoming PAR by 40 – 50 % (Rochefort, 2000), keeps the surface moist by decreasing evaporation and increasing relative humidity, lowers daily temperature amplitude of the peat surface, and prevents frost heaving common at extracted sites, that can destroy developing *Sphagnum* (Price, 1997). Additionally, the presence of nurse or companion plants more resistant to frost heaving than *Sphagnum*, *e.g.*, *Polytrichum strictum* and *Eriophorum* spp. support successful *Sphagnum* regeneration by stabilizing the peat surface to accelerate the regeneration of the ecosystem to carbon sink (Rochefort, 2000, McNail and Waddington, 2003,Tuittila *et al*., 2004). Thus, additional phosphorus fertilizer is often used to enhance germination of nurse plants (Quinty and Rochefort, 2003).

The post-restoration peat accumulation rates are higher than at natural peatlands, *e.g.*, the natural rates vary from 12 – 26 cm per 100 years (Government of Alberta, 2016) to 10 cm per 100 years (0.1 cm yr^{-1}; Glaser *et al*., 2004), while Lucchese *et al*. (2010) observed 2.3 cm accumulated 4 years and 13.6 cm 8 years post-restoration in a Canadian Bois-des-Bel peatland in Quebec. Interestingly, the unrestored post-extraction part of the site also showed organic matter accumulation, but at a slower rate of 0.2 and 0.8 cm at 24 and 28 years post-extraction, respectively (Lucchese *et al*., 2010). The layer of fresh peat developing on top of that old peat as the restoration progresses is hydrologically disconnected from the old peat layer due to capillary barrier which poses an additional challenge in restoration progress (McCarter and Price, 2015).

The newest study of Nugent *et al*. (2019) shows that restoration of post-extraction peatlands contributes to overall greenhouse gas removal from the atmosphere. Thus, peatland restoration mitigates climate change and is achievable if proper restoration techniques are applied promptly after peat extraction ceases (Nugent *et al*., 2019).

1.1.4. Methane cycling in peatland ecosystems

The aspects of CH_4 cycling in peatlands (CH_4 production, oxidation, subsurface storage and release) have been studied mainly in natural ecosystems, but less is known about how extraction and different extents of post-extraction management shapes these elements of peatland function.

1.1.4.1. Methane production and oxidation in peatlands

Methane is produced by methanogenic *Archaea*, which are obligate anaerobes, in the process of methanogenesis (Garcia *et al*., 2000, Rosenberry *et al*., 2006), the last stage of anaerobic degradation of organic matter (*e.g*., Lai, 2009, Couwenberg and Fritz, 2012, Andersen *et al*., 2013a). There are several pathways of CH_4 production, *e.g*. acetoclastic, when acetate is converted to CH_4 and CO_2, and hydrogenotrophic, when H_2 oxidation is coupled with CO_2 reduction to create CH_4 and water; H_2 can be replaced by formate in this reaction (Conrad, 1999, Galand *et al*., 2002, Horn *et al*., 2003, Lai, 2009, Bridgham *et al*., 2013). Some methanogens can use alcohols, carbon monoxide or simple methylated compounds (Conrad, 1999, Lai, 2009, Deppenmeier, 2002, Ye *et al*., 2012, Bridgham *et al*., 2013, Schmidt *et al*., 2016, Lyu *et al*., 2018). Acetate is the primary product of anaerobic degradation of organic matter in northern bogs (Duddleston *et al*., 2002) and a product of fermentation or homoacetogenesis (reaction of CO_2 with H_2) or incomplete oxidation of organic carbon by heterotrophic microbes that use non-oxygen terminal electron acceptors (TEAs) for respiration (Ye *et al*., 2012, 2016, Bridgham *et al*., 2013). Although methanogens are mostly hydrogenotrophic, the acetoclastic pathway coupled to acetogenesis is the most energetically efficient and should theoretically account for the majority of CH_4 produced in anoxic sediments (Conrad, 1999). Yet, in acidic ombrotrophic peatlands, the hydrogenotrophic pathway dominates, while the acetoclastic pathway prevails in fens (Duddleston *et al*., 2002, Galand *et al*., 2002, 2005, Horn *et al*., 2003, Bridgham *et al*., 2013, Schmidt *et al*. 2018, Evans *et al*., 2019). Only two methanogenic genera known to date are capable of acetoclastic methanogenesis: *Methanosaeta* and *Methanosarcina* (Schmidt *et al*., 2016).

Regardless of the pathway of CH_4 production, methanogenesis is less thermodynamically favourable than other anaerobic pathways of organic matter degradation suggesting that methanogens can be outcompeted by non-oxygen terminal electron acceptor (TEA) reducers (Conrad, 1999, Hausmann *et al*., 2016). Nitrite (NO_2^-), nitrate (NO_3^-), sulphate (SO_4^{2-}), ferric iron (Fe^{3+}), and manganic manganese (Mn^{4+}) are the main potential inorganic TEAs (Megonigal *et al*., 2003) that can regenerate in peat when the water table decreases exposing peat to atmospheric O_2 (Küsel *et al*., 2008). However, the abundance of substrate can overcome the competition of non-oxygen terminal electron acceptor reducers (Wieder *et al*., 1990). Dissolved organic matter (DOM) rich in humus, as well as particulate organic matter (POM, *i.e.*, peat itself), also serve as TEAs and have the potential to reduce CH_4 production rates (Lovely *et al*., 1996, Minderlein and Blodau, 2010, Miller *et al*., 2015, Gao *et al*., 2019). This humic reduction is thermodynamically less favourable than reduction of Fe^{3+} and NO_3^-, but more favourable than that of SO_4^{2-} (Cervantes *et al*., 2000).

Methane produced in anoxic peat diffuses through the oxic layer where it becomes partially oxidized by methanotrophic *Bacteria* (Roslev and King, 1996, Popp *et al*., 2000, Esson *et al*., 2016). Methanotrophs belong to *Alphaproteobacteria*, *Gammaproteobacteria*, *Verrucomicrobia*, and *Methylomirabilota*, formerly candidate phylum NC10 (Dedysh *et al*., 2005, Dunfield *et al*., 2007, Dedysh, 2009, Ho *et al*., 2013). In acidic boreal wetlands, type II methanotrophs (*Alphaproteobacteria*) prevail, while at higher pH (5.0 – 6.0), type I methanotrophs (*Gammaproteobacteria*) become active and both types metabolize CH_4 (Dedysh, 2009, Ho *et al*., 2013). Methanotrophs appear to be more resistant than methanogens to changing environmental conditions and more adapted to bounce back once the adverse conditions cease (*e.g*., Blodau and Moore, 2003). Some methanotrophs switch to use alternative TEAs in the absence of oxygen, while some can use short chain fatty acids (products of fermentation; Min and Zinder, 1990) when deprived of CH_4 (Dedysh *et al*., 2005).

The proportion of oxidized CH_4 depends on the thickness of oxic zone in the peat profile (*i.e*., the water table level), the condition of the methanotrophic and methanogenic community, and the presence of deep roots supplying oxygen to waterlogged peat. Popp *et al*. (1999) reported up to 34 % attenuation of CH_4 emission due to rhizospheric oxidation at a fen dominated by *Carex* spp. This proportion can be lower when temperatures increase followed

by an exponential increase in methanogenesis when more CH_4 is produced than methanotrophs can oxidized. For example, Van Winden *et al*. (2012) observed that at 5 – 15 ˚C almost the entire pool of diffusing CH_4 was oxidized by methanotrophs living in symbiosis with *Sphagnum* in hyaline cells (Raghoebarsing *et al*., 2005), but only 50 % was oxidized at 25 ˚C.

Given the extent of disturbance in extracted and post-extracted peatlands, especially drainage, disruption of oxic and anoxic conditions and removal of the shallow peat where the majority of methanogens and methanotrophs are known to occur, it is justified to presume that the abundance and diversity of these microorganisms and their activity would be severely altered. To date, few studies have focused on methanogenic and methanotrophic population and their activity in extracted, restored and unrestored peatlands, while some research, (*e.g*., Artz *et al*., 2008, Basiliko *et al*., 2003, 2013, Andersen *et al*., 2006, 2010, 2013 a, b) has been dedicated to the entire microbial community. Differences in CH_4-cycling microorganism abundance, community structure and activity in restored, unrestored and natural sites and the link between CH_4-microorganisms' recovery and development of *Sphagnum* at restored sites were found by Putkinen *et al*. (2018) and Reumer *et al*. (2018). Moderate differences in methanogenic and methanotrophic community composition in natural and restored sites were observed by Juottonen *et al*. (2012). Potential rates of CH_4 production and oxidation at managed peatlands have received more attention, including the above publications that showed low CH_4 production rates in actively extracted and unrestored peatlands (Reumer *et al*, 2018, Putkinen *et al*., 2018) and the highest rates in old restored and natural sites (Putkinen *et al*., 2018). However, the methodology, incubation conditions, and time of the experiment have varied between studies. Basiliko *et al*. (2007) observed that CH_4 production declined at actively extracted and post-extraction unrestored sites compared to natural and restored sites, while the latter showed higher rates of CH_4 production and oxidation than the natural site. Similarly, Glatzel *et al*. (2004) obtained lower CH_4 production rates for extracted sites and large values for restored sites with shallow water table. Waddington and Day (2007) reported the highest CH_4 production rates at a restored site, compared to unrestored and natural.

The highest potential rates of CH_4 production and the greatest abundance of methanogens have been found in the mesotelm below the water table, where the conditions are anoxic but there is still abundance of plant exudates that serve as a labile carbon source for

methanogens (Sundh *et al*., 1994, Martí *el al*., 2015). The highest potential for CH_4 oxidation was found immediately above and below the water table, where O_2 and CH_4 are readily available (Sundh *et al*., 1995, Segers, 1998, Clymo and Bryant, 2008). Interestingly, Frances *et al*., (2000) observed an increase in CH_4 production rates from 0 to 4 µg C g^{-1} d^{-1} with depth down to 160 cm eight years post-restoration. Glatzel *et al*. (2004) observed the highest CH_4 production rates in the surface peat layer above the water table in restored and unrestored sites. Apparently, methanogens can stay active above the water table in anoxic microsites (Glatzel *et al*., 2004, Juottonen *et al*., 2012), while rising water table can trap bubbles of air in otherwise saturated peat (Baird *et al*., 2004) potentially creating short-term oxic microsites for methanotrophs; however, I am unaware of any studies published on methanotrophic activity in these microsites.

Considering the limited number of studies on methanogenic and methanotrophic communities and their activity across peatlands of different management, as well as rapid development and increasing availability of molecular methods, further research would benefit from associating the presence of CH_4-cycling microorganisms with their function, and from complex studies that could link the CH_4-cycling community and activity to other aspects of CH_4 turnover in restored and unrestored peatlands. The study presented in this thesis shows the outcome of high-throughput Illumina Tag 16S rRNA gene sequencing on a large number of samples from active, post-extraction unrestored and restored sites linked to peat physicochemical properties and other aspects of CH_4 cycling.

1.1.4.2. Methane emission from natural, and post-extracted restored and unrestored peatlands

There are three pathways of CH_4 release from peatlands to the atmosphere: diffusion from the peat surface, plant-mediated transport, and ebullition (Chanton, 2005, Coulthard *et al*., 2009, Couwenberg and Fritz, 2009, Green and Baird, 2013, Stamp *et al*., 2013). Diffusion of CH_4 occurs along a concentration gradient between peat and the atmosphere (Tuittila *et al*., 2000a, Tokida *et al*., 2007a). Plant-mediated transport varies from 30 to 100 % of total CH_4 flux (Whiting and Chanton, 1992, Bridgham *et al*., 2013 and the references therein) and is considered the major mode of CH_4 emission from peatlands. Waddington *et al*. (1996)

measured 55 – 85 % lower seasonal fluxes after clipping *Eriophorum vaginatum* and 30 % lower after clipping *Carex rostrata* in Scandinavian peatlands. In practice, plant mediated transport is difficult to separate from diffusion through the peat matrix, thus both pathways are usually captured in CH_4 flux measurements.

The contribution of ebullition to the total CH_4 flux has been measured at natural peatlands and only one study has been completed on ebullition from a restored site (Nugent, 2019). Ebullition at natural sites varies from just a few percent of total CH_4 emission (*e.g.*, Green and Baird, 2013) to 90 % (Landsdown *et al*., 1992). This wide range may occur not only as an individual peatland characteristic, but also due to different methodology and research approaches, although overall, ebullition is considered an important mode of CH_4 release (*e.g.*, Romanowicz *et al*., 1995, Baird *et al*., 2004; Glaser *et al*., 2004, Tokida *et al*., 2005, 2007a,b, Rosenberry *et al*. 2006, Coulthard *et al*., 2009, Waddington *et al*., 2009a, Parsekian *et al*., 2010, Stamp *et a*l., 2013). Large amounts of CH_4 can be emitted via ebullition directly to the atmosphere bypassing the peat oxidation zone (*e.g*., Rosenberry *et al*., 2006, Comas *et al*., 2008, Bridgham *et al*., 2013, Stamp *et al*., 2013). For example, Comas *et al*. (2008) measured 39 – 74 g of CH_4 m^{-2} emitted via ebullition in less than 3.5 h. Glaser *et al*. (2004) observed up to 136 g CH_4 m^{-2} released in only three ebullition events from an undisturbed bog. Strack *et al*. (2005) measured ~ 600 mg m^{-2} d^{-1} of CH_4 emitted via ebullition in a peat monolith collected form a fen. Tokida *at al*. (2007a) obtained up to almost 30 mg CH_4 m^{-2} h^{-1} released in single ebullition events from a Japanese ombrotrophic peatland. Pelletier *et al.* (2007) reported up to 117 mg CH_4 m^{-2} d^{-1} emitted in individual ebullition events from three Canadian peatlands. Goodrich *et al*. (2011) calculated mean ebullition magnitude of 0.18 mg CH_4 at a fen and seasonal mean ebullition frequency 272.1 – 403.5 events m^{-2} d^{-1} with the largest number of events in spring and the lowest in autumn. Nugent (2019) observed similar amounts of CH_4 released through ebullition at a restored Canadian bog, accounting for 9 % of total CH_4 emission. Ebullition events can release more CH_4 within hours than daily (Comas and Wright, 2012) or even annual average fluxes via other emission pathways (Glaser *et al*., 2004). Spatial and temporal variability of ebullition is technically challenging to quantify (Tokida *et al*., 2005, Comas *et al*., 2011), but Goodrich *et al*. (2011) observed a clear temporal pattern of ebullition, contradicting the concept of ebullition as unpredictable, random events.

A compilation of CH_4 flux data from boreal and subarctic peatlands showed a mean of 83 mg CH_4 m^{-2} d^{-1} (Turetsky *et al*., 2014). Average CH_4 flux from northern fens is higher (56.36 mg CH_4 m^{-2} d^{-1}) than from bogs (25.98 mg CH_4 m^{-2} d^{-1}), with restored peatlands having a higher flux than unrestored sites (15.37 and 10.98 mg CH_4 m^{-2} d^{-1}, respectively; Abdalla *et al*., 2016). Individual studies have shown CH_4 uptake from the atmosphere at unrestored peatlands, *e.g*., -4 mg CH_4 m^{-2} d^{-1} obtained from a boreal extracted peatland in Canada (Strack *et al*., 2014), and -7 mg CH_4 m^{-2} d^{-1} (Waddington and Day, 2007), while fluxes from restored sites have large variability, *e.g*., from -1.77 to 394.68 mg CH_4 m^{-2} d^{-1} (Strack *et al*., 2014) and a mean of 0.1 mg CH_4 m^{-2} d^{-1} (Waddington and Day, 2007). Ebullition is rarely included in measured fluxes.

Methane fluxes are, to date, the main source of information on CH_4-cycling post restoration. With only one study that quantified the ebullition contribution to total CH_4 emission at a restored site (Nugent, 2019), there is an undeniable necessity for further research to understand how much CH_4 is emitted from restored sites via abrupt ebullition events, what environmental conditions drive ebullition at these sites, and if there are ebullition patterns across sites of different age post-restoration and geographical locations. Also, it is unknown if any ebullition occurs at currently extracted and unrestored sites.

1.1.4.3. Methods of methane emission measurements

While methods of flux measurements (either chamber method or eddy covariance towers) are well established (*e.g*., Goodrich *et al*., 2015, Strack *et al*., 2017, 2018, Rinne *et al*., 2018, Rankin *et al*., 2018), the methods of ebullition capture and quantification vary between studies. Some research has been conducted on peat cores in the laboratory (*e.g.*, Baird and Waldron, 2003, Baird *et al.*, 2004, Tokida *et al.*, 2005, Kellner *et al.*, 2006, Green and Baird, 2012, 2013), and others *in situ* (*e.g.*, Rosenberry *et al.*, 2003, Tokida *et al.*, 2007a, b, Strack and Waddington, 2008, Gogo *et al.*, 2011, Comas and Wright, 2012). Klapstein *et al*. (2014), Pelletier *et al*. (2007) and Stamp *et al*. (2013) used funnels to catch ebullitive gas, while Strack *et al.* (2005, 2006a) combined funnels with time domain reflectometry (TDR) probes. Comas and Wright (2012) equipped gas traps with time-lapse cameras. Rosenberry *et al.* (2003) used

hydraulic head to measure ebullition and Glaser *et al*. (2004) used GPS to calculate ebullition from peat surface elevation changes.

Ebullition is difficult to separate from diffusion and plant mediated transport emission of CH_4. All these emission pathways can contribute to fluxes measured with static chambers. The measurements that contain discrepancies in CH_4 concentration change over time from linear regression, that may be due to episodic ebullition, are usually rejected. Windsor *et al.* (1992), and Tokida *et al.* (2007a) used the chamber method and included ebullition in CH_4 emission. They reported rapid changes in CH_4 emission by two orders of magnitude over minutes or hours that contributed 50 – 64 % of total CH_4 flux (Tokida *et al*., 2007a). Large ebullition events during spring ice thaw and snowmelt have been observed using static chambers, *e.g.*, > 10 mg CH_4 m^{-2} h^{-1} (> 240 mg CH_4 m^{-2} d^{-1}) (Tokida *et al.*, 2007b) and > 200 mg CH_4 m^{-2} d^{-1} (Windsor *et al.*, 1992).

Static chambers are likely to capture ebullition and steady CH_4 emission from shallow layers of peat. Isotopic studies showed that CH_4 emissions measured with the chamber method was produced in shallow peat from recently deposited plant tissues (Chanton *et al.*, 1995, Glaser *et al.*, 2004). According to Parsekian *et al*. (2011), episodic ebullition from below 1 m depth is difficult to detect using chamber methods due to spatial and temporal variability of these events. Other studies have combined the chamber method with high-resolution detectors that measured CH_4 concentration continuously and enabled better detection of ebullition (Goodrich *et al.*, 2011, Gogo *et al*., 2011).

1.1.4.4. Factors controlling methane production and emission

Methanogenic activity is sensitive to changing temperature, pH, the availability of substrate, fluctuating water table, presence of TEAs and their reducers, and the accumulation of short chain fatty acids (Segers, 1998, Coles and Yavitt, 2002, Horn *et al*., 2003, Andersen *et al*., 2010, Ye *et al*., 2012). Low pH of peat is not optimal for methanogens and enhanced methanogenesis has been observed at increasing pH (Garcia *et al*., 2000, Ye *et al*., 2012). Methanogenesis can be slowed down at pH < 6.0 as acetate turns into acetic acid and becomes toxic for methanogens at concentrations higher than 5-10 mM (Bräuer *et al*., 2004, Russell,

1991). Nevertheless, methanogens occur and are active in the harsh environment of acidic peatlands in cold regions far from their environmental optima and are classified as extremophiles together with other *Archaea* that can thrive in places inhabitable for many other microorganisms (*e.g.*, Reed *et al.*, 2013).

Methane fluxes are driven mainly by factors affecting CH_4 production (Waddington *et al.*, 1996, MacDonald *et al.*, 1998, Pelletier *et al.*, 2007, Turetsky *et al.*, 2008, 2014, Abdalla *et al.*, 2016, Strack *et al.*, 2017). Methanogenesis generally increases exponentially with temperature (Dunfield *et al.*, 1993, Davidson and Janssens, 2006, Lai, 2009, Andersen *et al.*, 2010), but the amount of CH_4 emitted from peatlands depends on the water table level, with deeper water table enhancing CH_4 oxidation and lowering emission. The combined effect of the water table (WT) and soil temperature explained 40 % in CH_4 flux variability at natural and flooded sites (Turetsky *et al.*, 2014). An exponential relationship of WT and CH_4 flux was observed at natural, drained and flooded peatlands in temperate and subtropical climate zones (Turetsky *et al.*, 2014). Peatland drainage lowered CH_4 flux considerably, even by 84 % (Abdalla *et al.*, 2016, Turetsky *et al.*, 2014). However, Goodrich *et al.* (2011) captured the largest peak of ebullition events when the water table level decreased and temperature increased.

Vascular plants increase CH_4 flux (*e.g.*, Chanton *et al.*, 2005, Nugent, 2019). A synthesis of CH_4 emission from peatlands in different regions, including northern peatlands, showed that when the dominant plant functional type was vascular plants, there was significantly higher CH_4 flux, with the largest flux being at sedge-dominated locations, while no effect of non-vascular plants on the CH_4 flux was found (Turetsky *et al.*, 2014). Also, the importance of vascular plants in controlling CH_4 flux depends on the WT depth, *e.g.*, at low water table when the roots were above the waterlogged zone, the presence of vascular plants increased the flux to a lesser extent than at shallow water table when roots were in the saturated zone (Waddington *et al.*, 1996). Higher CH_4 emission and lower CH_4 concentration in pore water were measured from locations dominated by graminoids than from those dominated by *Sphagnum*, due to sedge-mediated CH_4 transport that reduced the subsurface pool of dissolved CH_4 (Gogo *et al.*, 2011, Murray *et al.*, 2017). The role of vascular plants is important post-restoration, since restored peatlands in Canada tend to return to an early peatland succession

stage with fen-like type of vegetation dominated by sedges, that in combination with wet or flooded conditions (also characteristic for some of these sites), may result in high CH_4 emission (Strack *et al*., 2014).

Vascular plants produce root exudates that supply labile carbon for microbial processes including methanogenesis (Ström *et al*., 2005, Bridgham *et al*., 2013). In contrast, low quality carbon substrate causes disturbance in CH_4 production in extracted and restored peatlands (Waddington and Day, 2007) and becomes the main control on CH_4 fluxes when water table is stabilized (Strack *et al*., 2016). Strack *et al*. (2016) measured higher CH_4 production at a restored peatland where graminoids and shrubs were present. Also, CH_4 oxidation (Reumer *et al*., 2018) and the abundance of methanotrophs (Juottonen *et al*., 2018) depends largely on the availability of CH_4. The reestablishment of vegetation is essential for improving carbon quality, but a 2-year delay caused by low carbon quality was observed between recovery of peat forming vegetation and that of peat microbial community (Andersen *et al*., 2006, 2013a). Methane emission at restored sites can remain low for a long time even if the new vegetation is well developed (Tuittila *et al*., 2000a). A further lag was observed in recovery of CH_4 production and oxidation and the recovery of CH_4-cycling microorganisms in restored peatlands (Reumer *et al*., 2018). Roots promote CH_4 production, but also oxidation by supplying oxygen to the rhizosphere (Ström *et al*., 2005, Bridgham *et al*., 2013). Some vascular plants (*e.g*., *Carex* spp.) grow their roots to great depths (Saarinen, 1996, Saarnio and Silvola, 1999) extending the zone of CH_4-cycling microorganisms' activity. Frenzel and Rudolf (1998) showed that no rhizospheric oxidation was associated with *Eriophorum* spp. although these species were responsible for the majority of CH_4 emission.

1.1.4.5. Biogenic free-phase gas formation and storage

Natural peatland ecosystems have a unique function of storing subsurface biogenic free-phase gas, but little is known if this ability is maintained in actively extracted, unrestored and restored sites and if restoration promotes the recovery of this function. Only part of the CH_4 that is produced in peatlands reaches the atmosphere. Although, as described above, the majority can be oxidized, some CH_4 also remains in the peat. The subsurface CH_4 pool occurs

in a gaseous form or dissolved in pore water. Methane is the major component of biogenic free-phase gas, accounting for up to half of the gas volume (Glaser *et al*., 2004, Tokida *et al*., 2007a, Stamp *et al*., 2013); however, Strack *et al*. (2005) reported up to 84 % of CH_4 in gas collected at < 1 m depth. The amount of CH_4 in the gaseous state can be three times greater than in the dissolved phase, as observed in a floating mat by Fechner-Levy and Hemond (1996). Free-phase gas constitutes up to 19 % of the volume of the peat deposit (Rosenberry *et al*., 2006 and references therein).

Bubbles of biogenic gas form when dissolved gas concentration exceeds a certain threshold (Beckwith and Baird, 2001, Baird *et al*., 2004, Gogo *et al*., 2011) or when the partial pressures of dissolved gases exceed the hydrostatic pressure (Fechner-Levy and Hemond, 1996). Methane molecules are non-polar and therefore CH_4 solubility in water is low (*e.g*., only 3.122×10^{-5} mol fraction will be dissolved at 15 ˚C and pressure of 1 atmosphere; Gevantman, 1992). As for any gas, CH_4 solubility decreases as the temperature increases and is directly proportional to the pressure of the gas above the solvent (Henry's law: $Cg = k \cdot Pg$, where Cg is solubility of gas, Pg is partial pressure of gas, k is a proportionality constant that depends on the identity of gas, the solvent and the temperature of the solution; Henry, 1832). The volume of gas (V) is directly proportional to the temperature (T) and the number of gas moles (n) and inversely proportional to the pressure (P), which is expressed in the ideal gas law: PV = nRT where R is the gas constant (Oxford World Encyclopedia, 2014). Thus, increasing temperature and decreasing atmospheric pressure (P_{atm}) cause bubble expansion (exsolution of gases from pore water) and can trigger ebullition (Fechner-Levy and Hemond, 1996, Baird *et al*., 2004, Kellner *et al*., 2006, Strack *et al*., 2006a, Tokida *et al*., 2007a), while decreasing temperature and increasing P_{atm} shrink bubbles, increase CH_4 solubility and immobilize bubbles that were stuck in peat pores, which also may cause ebullition events (Fechner-Levy and Hemond, 1996, Baird *et al*., 2004).

Peat structure, depending on the type of peat and its degree of decomposition, has been recognized as one of the major controls on the distribution, storage and release of free-phase gas (Baird *et al*., 2004, Kellner *et al*., 2005, Strack *et al*., 2005, 2006a, Comas *et al*., 2011, Chen and Slater, 2015). Peat porosity accounts for 65 % of peat's ability to store gas, while the spatial distribution of peat components account for the remaining 35 % (Kettridge and Binley,

2011). Kellner *et al*. (2005) found a relation between spatial variation in peatland vegetation and spatial variation in peat structure that drove different biogenic gas storage ability of peat. Parsekian *et al*. (2011) found higher gas content under woody vegetation and lower under lawns and open water. Dense sedge root system can act as a barrier preventing bubble release (Strack *et al*., 2006a, Coulthard *et al*., 2009, Kettridge *et al*., 2011).

The subsurface movement of gas and water creates a dynamic system with spatial variability of free-phase gas distribution (Comas *et al*., 2005, 2014) and rapid changes in its concentration (Comas *et al*., 2007). The free-phase gas content depends on CH_4 production and increases as the CH_4 concentration in pore water increases (Strack and Mierau, 2010). It is also driven by hydrological conditions, that can be altered by low porosity and already accumulated free-phase gas. These conditions slow down lateral groundwater drainage by lowering saturated hydraulic conductivity, thereby promoting further gas accumulation (Kettridge *et al*., 2013, Waddington *et al*., 2015).

The amount of free-phase gas resident in the peat matrix and changes in its content can be measured at a plot scale with TDR probes and moisture probes (*e.g*., Baird *et al*., 2004, Kellner *et al*., 2004, Strack *et al*., 2005, Tokida *et al*., 2005, see section 1.1.4.3). Rosenberry *et al*. (2003) used hydraulic head and Kellner *et al*. (2004), hydraulic head with pressure transducers buried at certain depths to calculate gas content in peat. Ground-penetrating radar (GPR) has been used in a few studies to assess the free-phase gas content non-invasively on a scale of several meter long transects down to the bottom of the peat deposit. The method is based on common offset (CO) and common midpoint (CMP) surveys with low frequency antenna (100 – 200 MHz) combined with the Dix equation for interval electromagnetic wave velocity calculation (Parsekian *et al*., 2010; 2011 after Dix, 1955) and the complex refractive index model (CRIM) for volumetric water content determination (Θ); then, the volumetric gas content is calculated from porosity and Θ (*e.g*., Parsekian *et al*., 2010, Strack and Mierau, 2010, Comas *et al*., 2014). Almost all previous studies using GPR for gas content estimation have been conducted on natural peatlands (Comas *et al*., 2005, 2007, 2008, 2011, Parsekian *et al*., 2010, 2011, Strack and Mierau, 2010). Only one study (Mwakanyamale *et al*., submitted) quantified free-phase gas in post-extracted restored and unrestored sites. This element of CH_4

cycling requires further research in managed peatlands to assess if the subsurface CH_4 pool recovers with restoration and whether progress can be observed with the age of restoration.

1.2. RESEARCH GAPS

Peat extraction drastically changes peatland ecosystems and alters hydrological and geochemical conditions, while restoration returns harvested sites to early stages of peatland succession that resemble fen-like conditions, often with high water table and sedge-dominated vegetation. As highlighted throughout the literature review, there are several gaps in knowledge on post-extraction peatlands' CH_4 cycling. Specifically, the following questions remain: 1) Does ebullition occur at unrestored and actively harvested sites and if so, what is its contribution to total CH_4 flux? 2) Does ebullition and the pool of subsurface dissolved and free-phase CH_4 increase following restoration and as the restored site ages? 3) Are the patterns of seasonal changes in ebullition, free-phase gas content and dissolved CH_4 concentrations similar to those at natural peatlands? 4) How do methanogenic and methanotrophic community members and their activity in post-extracted restored and unrestored sites vary from these at natural peatlands? Even when some data are available to contribute to answering these questions, to my knowledge, there are no studies that combine understanding of the return of CH_4 cycling in restored peatland across all these processes within one study site.

1.3. OBJECTIVES

In order to address the knowledge gaps I have identified, the objectives of the research presented in this thesis were:

1. To characterize methanogenic and methanotrophic community composition and abundance in an actively extracted site, post-extraction unrestored and restored sites with regards to different age of restoration and physicochemical characteristics of peat, and to quantify potential rates of CH_4 production and oxidation (Chapter 2).

2. To quantify the amount of CH_4 emitted from these sites through abrupt ebullition and steady CH_4 flux and determine factors affecting both pathways of CH_4 emission (Chapter 3).
3. To quantify free-phase gas content in these sites and its changes over summer and determine factors that govern its accumulation and release (Chapter 4).

CHAPTER 2: Methane cycling microorganisms and CH_4 production and oxidation rates in horticultural peatlands: Comparing natural, currently extracted, unrestored, and different ages of restored sites.

2.1. ABSTRACT

Horticultural peat extraction removes the top peat layer, where the majority of microbial activity occurs. The peat microbial community is largely responsible for organic matter turnover and greenhouse gas emissions, but our understanding of its response through extraction and restoration remains limited. We determined how physicochemical conditions in natural, restored, unrestored, and actively extracted peatlands influence the methanogenic and methanotrophic community members and the rate of potential methane (CH_4) production (MP) and methane oxidation (MO). Methane cycling microorganisms comprised < 0.1 % of the 16S rRNA gene amplicon sequence data. Methane cycling communities were similar in sites restored in 1991 and 2009 (25 and 7 years prior to our research). A different, shared pattern of microbial membership was observed at sites restored in 2012, Unrestored, Natural, and Active. Methanotrophs generally reached their highest abundances close to the water table (WT), at high and moderate concentrations of phosphate, propionate, and citrate and low concentrations of formate. In contrast, most methanogens were associated with the opposite side of these gradients. The abundance of methanogens increased with depth, while methanotrophs were more evenly distributed in both oxic and anoxic zones. MP was highest in the Natural site, with hot spots at the site restored in 2009, and lowest at the Unrestored site. MP was significantly higher at depths immediately below the WT compared to depths 10 – 20 cm above the WT and was affected by the concentration of several short chain fatty acid ions, Fe^{3+}, and electrical conductivity. MO was not significantly influenced by physicochemical factors, and did not vary between depth zones, but was highest in the Natural site. The presence of dense vascular plants and high WT at restored sites seemed to drive the structure of CH_4-cycling communities more strongly than the age of restoration of the peatlands. However, while spontaneous re-vegetation at parts of the Unrestored site increased the abundance of CH_4-cycling

microorganisms compared to that at bare peat locations, MP and MO remained close to zero, suggesting that CH_4 cycling function is slower to return without active restoration.

2.2. INTRODUCTION

Wetlands are the largest natural methane (CH_4) emitters, contributing at least 20 % of global CH_4 emission from all sources (Shindel *et al*., 2004, Bridgham *et al*., 2013). The amount of CH_4 released from these ecosystems is determined by the microbial processes of CH_4 production by methanogenic *Archaea* in waterlogged anoxic conditions and CH_4 oxidation by methanotrophic *Bacteria* in oxic conditions (Horn *et al*., 2003, Andersen *et al*., 2013a, Esson *et al*., 2016). Microorganisms phylogenetically close to archaeal anaerobic methane oxidizers (ANME) have been sporadically found in peatlands (Raghoebarsing *et al*., 2006, Etto *et al*., 2012); however, the anaerobic oxidation of CH_4 (AOM) is not well understood, but can potentially be as important in the gas budget as aerobic oxidation of CH_4 (Smemo and Yavitt, 2007, Zhu *et al*., 2012).

Peatlands in the Canadian boreal region selected for horticulture peat extraction are usually well developed bogs with peat deposits that are > 2 m meters deep (ECCC, 2018). These peats naturally form vertical zonation: acrotelm, catotelm (Ingram, 1978) and mesotelm (Clymo and Bryant, 2008). Catotelm makes up the major bulk of peat, and is saturated with pore water (anoxic conditions). Acrotelm forms on top of catotelm, and is therefore younger, usually less decomposed, partially unsaturated, and contains abundant substrates for microbial processes. Mesotelm describes a zone of water table fluctuation, situated between the acrotelm and catotelm (Clymo and Bryant, 2008). The presence of the poorly decomposed acrotelm with large pore sizes prevents excessive fluctuation of the water table (WT; Waddington *et al*., 2015), keeping the oxic and anoxic zones relatively stable for methanogens and methanotrophs. An undisturbed acrotelm is a habitat for peat forming plants. Methanogens rely on the presence and productivity of vascular plants to release root exudates and produce litter, both sources of labile carbon for methanogens (Tuittila *et al*., 2000a, Bridgham *et al*., 2013). Some plants (*e.g*., *Carex* spp.) can grow their roots down to the depth of 230 cm (Saarinen, 1996, Saarnio and Silvola, 1999), supplying exudates to the zones otherwise poor in labile

substrates (Tuittila *et al*., 2000a, Bridgham *et al*., 2013). These roots also supply oxygen (O_2) to anoxic peat, supporting CH_4 oxidation (Ström *et al*., 2005, Bridgham *et al*., 2013) and regeneration of terminal electron acceptors (TEA), *e.g.*, formation of Fe^{3+} at high concentrations, which can potentially suppress methanogenesis even in waterlogged conditions (Metje and Frenzel, 2005). The highest potential rates of CH_4 production and the highest abundance of methanogens have been found below the WT (shallow peat, mesotelm), where methanogens have access to a fresh carbon source from decaying litter and root exudates and where conditions are anoxic (Sundh *et al*., 1994, Martí *el al*., 2015), while CH_4 oxidation was observed around the oxic-anoxic boundary where O_2 and CH_4 are readily available (Sundh *et al*., 1995, Segers, 1998, Clymo and Bryant, 2008). Once appropriate redox conditions are established, the availability of the carbon substrate is the most important factor controlling CH_4 production and oxidation (Couwenberg 2009, Ho *et al*., 2013, Reumer *et al*., 2018) and the abundance of methanogens (Sun *et al*., 2012) and methanotrophs (Juottonen *et al*., 2012).

Methanogenesis is the terminal stage of the degradation of organic matter and depends on syntrophic bacteria (Conrad, 1999, Bridgham *et al*., 2013). Short chain fatty acid ions (*e.g*., lactate, acetate, succinate, butyrate, pyruvate, and propionate) are products of fermentation of organic matter and serve as electron donors in anaerobic reactions of organic matter turnover (Min and Zinder, 1990). Their accumulation in protonated forms inhibits methanogenesis (Horn *et al*., 2003). Acetate is used as an electron donor in the acetoclastic pathway of CH_4 production by members of the *Methanosaeta* and *Methanosarcina* genera (Schmidt *et al*., 2016), but at pH < 6, it turns into acetic acid that is toxic to methanogens (Russell, 1991, Horn *et al*., 2003, Bräuer *et al*., 2004). The majority of known methanogens utilize the hydrogenotrophic pathway of CH_4 production that dominates in ombrotrophic peatlands, where formate can replace H_2 (Galand *et al*., 2002, Horn *et al*., 2003, Bridgham *et al*., 2013). Methanogenesis is thermodynamically less favourable compared to other decomposition pathways (*e.g.*, sulfate and nitrate reduction; Conrad, 1999, Hausmann *et al*., 2016) and can be suppressed in the presence of non-oxygen TEAs and their reducers. Large WT fluctuations at extracted peatlands promote the regeneration of TEAs, including nitrite (NO_2^-), nitrate (NO_3^-), sulphate (SO_4^{2-}), and ferric iron (Fe^{3+}) (Küsel *et al*., 2008). Dissolved and particulate organic matter also serve as TEAs (Lovely *et al*., 1996, Gao *et al*., 2019).

Some proportion of the CH_4 produced in the anoxic zone can be oxidized by methanotrophs while diffusing through the oxic zone up to the atmosphere (*e.g.*, Roslev and King, 1996, Popp *et al*., 2000, Esson *et al*., 2016). There are four bacterial groups that contain known methanotrophs: *Alphaproteobacteria*, *Gammaproteobacteria*, *Verrucomicrobia*, and *Methylomirabilota* (formerly candidate phylum NC10, whose members conduct anaerobic CH_4 oxidation coupled to nitrification, Ho *et al*., 2013). In acidic boreal wetlands, type II methanotrophs (*Alphaproteobacteria*) from the genera *Methylocella*, *Methylocystis*, and *Methylocapsa* predominate and actively assimilate CH_4 (Dedysh, 2009). These genera are classified as stress tolerating (*i.e.*, tolerant of constant high acidity, low temperatures, but stable conditions; Ho *et al*., 2013, Putkinen *et al*., 2018). At higher pH (5.0 – 6.0), type I methanotrophs (*Gammaproteobacteria*), classified as competitor-ruderal (*i.e.*, associated with disturbed peatlands and unstable conditions; Ho *et al*., 2013, Putkinen *et al*., 2018), become active. At these higher pH values, typically both type I and II metabolize CH_4 (Dedysh, 2009, Ho *et al*., 2013). Some methanotrophs can use short chain fatty acid ions for growth (Dedysh *et al*., 2005).

The natural hydrological conditions are disturbed in extracted peatlands and so is the stability of the oxic and anoxic zones. The WT is lowered by the installation of drainage ditches and fluctuates extensively due to peat subsidence (increased bulk density and decreased specific yield) in the exposed catotelmic zone (*e.g.*, Price, 1996, 1997). The top layers of peat are removed together with the primary production and seedbank (Quinty and Rochefort, 2003); thus, no source of labile carbon is available, and the chance for self-recovery of peatland vegetation is slim (Poulin *et al*., 2005). The remaining lower quality peat of various thicknesses is left behind and is poorly colonized by methanogens and methanotrophs (*e.g.*, Waddington and Day, 2007, Basiliko *et al*., 2013, Reumer *et al*., 2018).

Peat extraction is a drastic and initially abrupt alteration to the ecosystem, but once the extraction starts, it lasts 20 – 30 years (Wind-Mulder and Vitt, 2000). During this interval, the resilience of soil microorganisms (*e.g.*, fast growth rate, physiological flexibility, rapid evolution; Allison and Martiny, 2008) and their ability to move (*e.g.*, chemotaxis; Ebrahimi and Or, 2017) may promote the establishment of new microbial community structure. Additionally, post-extraction peat shows high water retention due to its small pore size

(Waddington and Price, 2000), meaning it can form anoxic microsites above the WT (Estop-Aragonez *et al*., 2013) that could potentially sustain anaerobic microbial metabolisms. There is no published research on methanogenic activity specifically in these microsites, but studies on CH_4 production in freshwater wetlands revealed a possibility of oxic CH_4 production (Angle *et al*., 2017).

Peatland restoration aims to re-establish the natural hydrological conditions and peatland vegetation, with the primary goal of recovering a fully self-sustaining, functional ecosystem able to accumulate peat (Rochefort *et al*., 2003). Although the microbial component cannot be directly restored, and microbial community regeneration is beyond the scope of regular site monitoring, research shows that restoration efforts improve the microbial characteristics of post-extracted peatlands (Andersen *et al*., 2006, 2010, 2013a,b, Bossio *et al*., 2006, Reumer *et al*., 2018). Since peatland restoration in Canada started only about 30 years ago, the available data are limited by the age of the restored sites (Strack *et al*., 2016). Young restored peatlands vary in ecohydrological and functional features from their original natural form of a bog. The vegetation is usually dominated by graminoids and the vascular plant cover is higher than at natural sites (Tuitilla *et al*., 2000b, Gonzalez and Rochefort, 2014, Putkinen *et al*., 2018). The WT is generally shallow, and the site's chemical features can be closer to those of fens (Wind-Mulder *et al*., 1996, Wind-Mulder and Vitt, 2000). The microhabitat, geochemistry and the type of vegetation are the strongest determinants for the microbial community composition (Jaatinen *et al*., 2007, Lin *et al*., 2014, Robroek *et al*., 2015, Putkinen *et al*., 2018). Thus, lack of vegetation at abandoned sites or shifts in vegetation at restored sites, in combination with altered hydrological conditions, will shape the microbial community characteristics differently than at unextracted peatlands.

To date, there is little published research that assesses methanogenic and methanotrophic communities in post-extracted, restored, and unrestored peatlands. Putkinen *et al*. (2018) found a link between *Sphagnum* recovery at restored peatlands and the methanogenic and methanotrophic abundance, community structure, and activity. They observed differences in the methanogenic community between young (2 years) and old (17 – 63 years) restored sites and increasing potential rates of CH_4 production from the youngest restored, through the older restored, to the natural, and large potential rates of CH_4 oxidation in

hummocks (Putkinen *et al*., 2018). Juottonen *et al*. (2012) found only moderate differences in methanogenic community and no difference in methanotrophic community composition between natural and 10 – 12 year old restored sites, but the restoration was limited to filling in the drainage ditches with peat. In contrast, the abundance of CH_4-cycling microorganisms recovered well in a restored site 15 years post-restoration (ditch damming only) with the return of *Sphagnum* (Reumer *et al*. 2018). The authors observed distinct methanogenic community composition at natural and restored sites, different from those in active and unrestored peatlands; however, they suggested more than another 15 years would be required to fully reverse the impact of peat extraction on microbial communities (Reumer *et al*., 2018). Nevertheless, most of the microbiological studies on actively extracted, unrestored, and restored peatlands are focused on the entire microbial community (*e.g*., Galand *et al*., 2005, Artz *et al*., 2008, Basiliko *et al*., 2003, 2013, Andersen *et al*., 2006, 2010, 2013a, b). Basiliko *et al*. (2013) reported the archaeal community being site specific. Restoration resulted in an archaeal community similar as in the natural peatland, with all restored sites having similar community composition (Basiliko *et al*., 2013).

The goal of this study was to determine how different physicochemical conditions influence the methanogenic and methanotrophic community characteristics and their potential for CH_4 production and oxidation in actively extracted (Active), post-extraction unrestored (Unrestored), restored, and undisturbed sites (hereafter referred to as Natural). We hypothesize that:

1) Both the abundance and diversity metrics of methanogens and methanotrophs will be lower at the Active and Unrestored sites compared to the restored and Natural sites. The restored and the Natural sites will have similar methanogenic community composition that will vary from that at Unrestored and Active sites.
2) The abundance and diversity metrics of methanogens will be higher in deep peat where stable anoxic conditions prevail, while methanotrophs will occur mainly in shallow peat where oxic conditions prevail.
3) The abundance of methanotrophs and potential rates of CH_4 oxidation (MO) will not be affected by the concentration of inorganic ions and short chain fatty acid ions, assuming that O_2 is available.

4) Given the recurring nature of potential TEAs, *i.e.*, quickly changing concentration as the WT fluctuates, they will not affect the abundance of methanogens but rather their activity. The accumulation of short chain fatty acid ions will be associated with lower activity of methanogens, except for formate and acetate that are essential compounds in hydrogenotrophic and acetoclastic methanogenesis, respectively.

2.3. STUDY SITE

The study was conducted at a horticultural peat extraction complex of peatlands near Seba Beach, Alberta (53° 33' N, 114° 44' W). Prior to peat extraction, the original peatlands were boreal bogs. Six sites from Active, through Unrestored, restored at different times, to Natural were included in the research. We selected three sites restored with moss layer transfer technique (Quinty and Rochefort, 2003): restored in 1991 (RES-1991), 2009 (RES-2009), and 2012 (RES-2012) (Fig. A.1.1). The depth of the peatlands was assessed by ground penetrating radar (GPR) surveys (Chapter 4). The Natural site was a treed bog with over 5 m thick peat deposit, characteristic hummocks and hollows and poorly decomposed *Sphagnum* peat (Fig. A.1.3A). RES-1991 was one of the oldest peatlands restored in Canada. The peat deposit was 3 – 4 m deep, depending on the location. The site was flooded with a partially floating mat. After prolonged drought, the WT remained at or slightly above the peat surface, but parts of the peatland were still inaccessible due to wet conditions. The drainage ditches were blocked but not filled in. A mosaic of dense hummocks of *Sphagnum* and sedges dominated the peatland vegetation (Fig. A.1.3B). Peat at RES-2009 was 1.5 – 2.5 m deep, largely covered with sedges, grasses and shrubs with overgrowing *Sphagnum* and true moss (Fig. A.1.3C). RES-2012 was relatively large (~ 40 ha) but only 7 ha were selected for intensive study (Fig. A.1.3D). The shallow (~ 1.5 m) and dry east part of RES-2012 gradually transitioned to deeper (> 3 m) peat with wet conditions in the west part. The wet part was severely flooded following heavy rain events. We sampled in a moderately wet middle part. The Unrestored site was a part of a larger extracted site left unrestored for the research purpose in 2012 at which time the drainage ditches were filled up with peat and the surface levelled. No other restoration effort was taken, and natural peatland vegetation had not recovered. The great majority of the site remained bare, though birch and sedges progressively colonized the west part of the site and peatland

margins (Fig. A.1.3E). The peat was moderately decomposed (H5 – H7 in the Von Post scale) at vegetated parts and poorly decomposed (H3 – H4) at the bare part. The peat deposit exceeded 2.5 m depth. The Active site was < 2 m deep at the sampling location, with poorly decomposed *Sphagnum* peat (H3 – H4) compacted by heavy machinery and active drainage ditches. The peat surface was stripped of vegetation (Fig. A.1.3F). More information on vegetation at the sites can be found in Chapter 4.

2.4. METHODS

2.4.1. Sampling

Samples for molecular analyses were collected in August 2016 at high WT levels. At each site except the Active, two cores were sampled targeting major peat surface cover types: sedgy and mossy at the restored sites, bare peat and sedgy at the Unrestored site, and hummocks and hollows that were both covered with dense moss at the Natural site. One core representing bare peat was sampled from the Active site. A total of 11 cores (119 samples with depth sectioning) were collected for molecular analyses. Eleven cores for paired physicochemical analyses were taken within a 20 cm radius of the molecular cores. Peat was sampled with a Russian corer to the depth of 1 m. Additionally, one 10 cm long sample per core was taken from the greatest depth possible to sample in the given conditions. Peat segments of 10 cm in length were packed into sterile plastic bags and immediately flash frozen in liquid nitrogen, transported the same day in dry ice and stored at -80 °C. Corresponding segments of peat for physicochemical analyses were packed in Ziploc bags, immediately placed in coolers with ice, transported to the lab and stored at -20 °C. During sampling, minimal exposure to ambient air and aseptic conditions were ensured. All equipment was thoroughly washed with 70 % ethanol before and between sampling.

Cores for microcosms were sampled at the Natural, RES-2009 and Unrestored sites in August 2017 when the WT was ~20 cm deeper than during the 2016 sampling. Triplicate cores were collected from hummocks and hollows at the Natural site, from sedge- and moss-dominated locations at RES-2009 and bare peat at the Unrestored site. We used PVC pipes, sharpened at one end, to minimize peat compaction during sampling. We used 70 % ethanol to

sanitize the equipment. The depth of sampling was calculated with respect to the actual WT level at each sampling plot. Three depths were targeted: a 10 cm thick peat layer immediately below the WT (depth zone A), 0 – 10 cm above the WT (depth zone B), and 10 – 20 cm above the WT (depth zone C). The pipes were immediately sealed and placed in a cooler with ice. Corresponding peat for physicochemical analysis was sampled into plastic Ziploc bags and placed in a cooler with icepacks. Cores were stored at 4 °C and physicochemical samples at -20 °C. Sampling procedures ensured minimal exposure of samples to O_2. A total of 15 cores were collected (44 samples). Due to a shallow WT position, one core from RES-2009 comprised of only two peat samples immediately below and above the WT.

2.4.2. Microcosms

We followed the protocol of Daté (2016) to prepare the microcosms. Cores were processed in a glove box in a nitrogen (N_2) atmosphere with up to 3 % hydrogen added to bond trace oxygen (O_2) and form H_2O for removal by filters. The O_2 concentration was constantly monitored and an anoxic atmosphere maintained. Ethanol 70 % was used to clean all equipment during peat core processing. Cores were cut into segments representing depths A, B, and C. Each sample was placed in a Ziploc bag and thoroughly homogenized by hand. For each sample, a 10 g subsample was placed in a 250 mL sterile jar and inundated in MilliQ water purged with N_2 to ensure anoxic conditions during incubation for CH_4 production potential assessment. Another 10 g of peat was placed in a separate sterile jar for oxic microcosms to quantify CH_4 oxidation potential. Oxic and anoxic blank microcosms were prepared for each batch of samples separately. An oxic blank was an empty sterilized jar, while an anoxic blank was a sterilized jar with distilled water. Anoxic microcosms were tightly closed in the glovebox with lids and Teflon tape wrapped around the jar thread to isolate the microcosms from atmospheric O_2 and prevent gas leaks. Oxic microcosms were exposed to ambient air and then closed with lids and Teflon tape. No inhibitors of CH_4 production or oxidation were added to the microcosms as we designed this experiment to resemble natural conditions. All microcosms were placed in a growth chamber at 10 °C for a 24-hour preincubation (temperature equilibration). The next day, the anoxic microcosms were flushed with N_2 and oxic microcosms with ambient air. A

total of 44 oxic and 44 anoxic microcosms were incubated at 10 ˚C in darkness for six weeks for anoxic conditions and at least two weeks for oxic. Gas from the headspace of anoxic microcosms was sampled every day during the first week of incubation, then every three days. Gas from the headspace of oxic microcosms was sampled every day until it reached the ambient level (~ 2 ppm), which could span from 7 to 9 days, then a mixture of CH_4 and ambient air was added to obtain [CH_4] ~100 ppm in the microcosm headspace. Then, gas was sampled every day for at least seven days (hence, the total incubation time was at least 2 weeks). Methane concentrations were measured using a Shimadzu GC-2014 gas chromatograph equipped with a flame ionization detector and injected with EST Flex automatic sampler. Potential CH_4 production (MP) and CH_4 oxidation (MO) rates were calculated from the slope of CH_4 concentration increase (in anoxic microcosms) or decrease (in oxic microcosms), respectively, corrected with the slope for the blank samples.

The incubation temperature was chosen based on 30-minute temperature averages for peat at the RES-2009 site at 20 cm depth, measured with a HOBO logger from August 8th to 25th, 2017, during core collection. The average peat temperature for that time window was 11.0 °C. Thermocouple wires attached to the CS1000 water content reflectometry data loggers (Campbell Scientific) installed at sites restored in 2012 and 1991 (the youngest and the oldest restored sites) showed that soil temperature was lower by about 2 °C at 50 cm depth compared to 25 cm depth. In the majority of cases, the water table level at our sampling plots was within the 25 – 50 cm depth zone, therefore the incubation temperature was set for 1 °C less than the 11 °C measured at 20 cm depth. The temperature of incubation was below the optimum (25 °C), but still within the acceptable range for methanogenesis (Metje and Frenzel, 2005).

2.4.3. Physicochemical and environmental conditions

The Von Post scale of peat humification was used to assess the degree of decomposition of peat (Government of Canada, 2013). The WT was measured in water wells installed near each sampling location. Peat porosity (S_t) was calculated from bulk density (D_b) and particle density (D_p) as follows: $S_t = 1 – (D_b\ D_p^{-1})$, (Hao *et al*., 2008) in 10 cm increments down to 1 m depth. Peat cubes of known volume (V_t) were collected at each depth. The cubes were weighed and

dried at 70 °C (Andersen *et al*., 2013a) until constant mass (m_s). Soil bulk density was calculated using $D_b = m_s V_t^{-1}$ (Hao *et al*., 2008). Dried samples were ground and sieved through 500 µm mesh. A known mass of the sample (m_s) was placed in a volumetric cylinder with a known volume of kerosene (fluid displacement method). The difference between the volume of kerosene with peat and the initial volume of kerosene gives the volume of soil particles (V_s). Particle density was calculated from $D_p = m_s V_s^{-1}$ (Hao *et al*., 2008). The ratio of carbon to nitrogen (C:N) was calculated from total carbon (C) and total nitrogen (N) percentage concentration measured on ground, sieved, and freeze-dried peat. Total C and total N were measured at the Agriculture and Food Laboratory at the University of Guelph, Ontario using the Elementar Vario Macro Cube. Conductivity and pH of peat was measured in dried, ground samples inundated in deionized water in 1:15 ratio (w/w) using Hanna conductivity/pH meter.

For DOC and anion concentration, 2 g of flash frozen peat (liquid nitrogen, stored at -80 °C) was transferred into centrifuge tubes and shaken at 450 rpm with 10 g of MilliQ water for 1 hour. Tubes were centrifuged at 5000 rpm for at least 10 minutes. We followed standard operating procedures of the Ecohydrological Lab for dissolved organic carbon (DOC), and organic and inorganic ion concentration analyses in water. Briefly, the supernatant was filtered through 0.45 µm filters and diluted 1:1 with MilliQ water in test tubes. DOC was measured using the non-purgeable organic carbon method on a Shimadzu TOC-LCPH/CPN equipped with a non-dispersive infrared (NDIR) gas analyzer. The calibration was performed using certified standards diluted within the expected range of DOC concentration in triplicate for each concentration. For the analysis of organic acid anions and inorganic anions, a part of the initial supernatant was filtered through a 0.2 µm polypropylene filter and stored at -20 °C prior to analysis. We targeted acetate, lactate, succinate, butyrate, pyruvate, propionate, citrate, nitrite (NO_2^-), nitrate (NO_3^{2-}), sulphate (SO_4^{2-}), and phosphate (PO_4^{3-}). Samples were analyzed with a Dionex (Thermo Fisher) ICS-5000 Capillary Ion Chromatograph. The calibration was made in external mode using triplicate of at least five different standard concentrations.

To determine the concentration of ferric iron (Fe^{3+}) in peat, the ferrozine method was used (Stookey, 1970 modified according to Lovley and Phillips, 1986 and Viollier *et al*., 2000). The ferric iron concentration was calculated from the concentration of Fe^{2+} subtracted from the total concentration of Fe^{2+} and Fe^{3+}. Ferrous ions form a colour complex with ferrozine

(monosodium salt hydrate of 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid) that absorbs visible light with a peak at 562 nm wave length. The absorption was measured with a UV-visible spectrophotometer (Thermo Scientific, Evolution 260 Bio). For the analysis of Fe^{2+}, 0.5000 g of dried and sieved (500 µm, plastic mesh) peat was freeze dried, digested on a shaker with 5 mL 0.5 M HCl for 1 hour to mobilize Fe(II) and centrifuged at 4300 rpm for 10 minutes. 50 µL of the peat solution was added to 2.45 mL of ferrozine solution (1 g L^{-1} of ferrozine in 50 mM HEPES buffer adjusted to pH 7 using HCl and NaOH). To measure the concentration of total Fe^{2+} and Fe^{3+}, 200 µL of 6.25N hydroxylamine hydrochloride (2.17 g in 5 mL of MilliQ water) was added to the remaining peat solution and digested for 1 hour on a shaker to mobilize Fe(III) and reduce it to Fe(II). 50 µL of the peat solution was added to 2.45 mL of ferrozine solution. All samples were prepared in triplicate and measured immediately after preparation. All steps of the analysis (except the initial peat weighing) as well as reagent preparation were performed in a glovebox (similar conditions as used in microcosm preparation) using an analytical balance accurate to 0.00001g. All liquids used in the glovebox were purged with N_2 to remove O_2. Sample digestion was performed at room temperature. In this method, Fe(II) and Fe(III) adsorbed on mineral particles and Fe(II) immobilised in minerals are mobilized and measured. We used MilliQ water of 18.2MΩ cm^{-1} resistance. The initial standards were prepared in the glovebox from a solution of $FeCl_2.4H_2O$ in 0.5 M HCl in concentrations 50 mM, 75 mM, 150 mM, and 300 mM and stored in tightly sealed amber bottles in the glovebox. The dilutions of the initial standards were made to obtain the calibration curve ($R^2 > 0.99$) covering the range of Fe^{2+} concentration in our peat samples (at least seven different concentrations prepared in triplicates). The dilutions were stored for no longer than a month in tightly sealed amber jars at 4°C. Fe^{2+} salts are easily oxidized, therefore the quality control on the calibration curve was made using standards of $FeSO_4.4H_2O$ in 0.5 M HCl prepared in identical conditions and concentrations as the $FeCl_2.4H_2O$ standards. The concentration of Fe^{2+} measured in the quality control samples was within 10 % of the concentration calculated from the same absorbance using the calibration curve.

Descriptive statistics for physicochemical variables (n, mean, sd) were calculated using the R package Rmisc (Hope, 2013) and converted to csv files using tables package (Murdoch, 2019). Kruskal-Wallis one-way analysis of variance was used to determine if the physicochemical factors varied between sites.

2.4.4. Microbial community

DNA was isolated using the MoBio PowerSoil® DNA Isolation Kit for 0.25 g of soil sample following the procedure of the manufacturer, with 50 µL of Solution C6 used instead of 100 µL for elution. DNA was kept at -20 °C until analysis. Quality control on DNA extracts was performed following the DOE Joint Genome Institute (JGI) standard operational procedure (SOP) "iTag sample amplification QC" prior to analysis (Daum, 2016). Targeted Illumina sequencing was performed at JGI laboratories following "iTag Sample Preparation for Illumina Sequencing, v.1.0" SOP (Daum, 2017). Briefly, the amplicon libraries (iTags) were generated and sequenced using the Illumina MiSeq platform. A total of 119 samples were processed and 2x301 base pair amplicons were obtained spanning the V4 region of the 16S rRNA gene to determine the presence of *Bacteria* and *Archaea*. Primers used were: 16S rRNA V4 region primers: FW (515F): GTGCCAGCMGCCGCGGTAA, RV (805R): GGACTACHVGGGTWTCTAAT. The reads were demultiplexed, contaminants removed, and adapters trimmed using BBDuk version 37.90 (Bushnell, n.d.). Forward and reverse reads were split using khmer version 2.1.1 (Crusoe *et al*., n.d.) and screed version 1.0 (Crusoe *et al*., n.d.). Further analysis was carried out in QIIME 2 v. 2018.11 and 2019.11 (Bolyen *et al*., 2018). Metadata files were checked for validity using Keemei (Rideout, 2016). The sequences were denoised and corrected using DADA2 (q2-dada2 plugin, Callahan *et al*., 2016). A feature classifier was trained for 16S rRNA using q2-feature-classifier plugin (Bokulich *et al*., 2018) based on the 132 release of SILVA database with a threshold of 99 %+ identity for taxonomic assignments (Quast *et al*., 2013). A total of 56 samples with 16S rRNA sequence count lower than 20,000 were filtered out, resulting in 63 samples remaining in the database (Tab. A.2.1). The methanogenic and methanotrophic communities were analysed based on exact sequence variants (ESVs) tables rarefied to the depth of 28,000 based on the lowest number of sequence counts. The raw reads are available at: http://genome.jgi-psf.org/pages/dynamicOrganismDownload.jsf?organism=Lanleaitagspl1 and http://genome.jgi-psf.org/pages/dynamicOrganismDownload.jsf?organism=Lanleaitagspl2.

Alpha diversity and richness were calculated in QIIME 2 (Bolyen *et al*., 2018). Since DADA2 output was free of singletons, we did not use abundance-based coverage estimator (ACE) or Chao1 and Chao2 metrics, as these rely on the abundance of low frequency features

to estimate the number of under sampled species (Gotelli and Chao, 2013). Richness and diversity plots and taxa barplots were visualized in R (R Core Team, 2019) using the ggplot2 package (Wickham, 2016). We applied the number of observed ESVs, Faith's index (PD, stands for 'Faith's diversity'; Faith, 1992) and Shannon's index (H; Shannon and Weaver, 1949**)**. The non-parametric Kruskal-Wallis test was used to determine significant differences between grouped alpha diversity metrics. We grouped them according to the location of the sample in relation to the WT fluctuation zone (WTFZ; within, or below).

The principal coordinates analysis (PCoA) of weighted and unweighted UniFrac (Lozupone and Knight 2005, Lozupone *et al*., 2007) was used to determine the methanogenic and methanotrophic community's similarity between samples and sites. Beta diversity was calculated in R using the phyloseq package (McMurdie and Holmes, 2013) with the tidyverse package (Wickham, 2017) and qiime2R to read QIIME 2 output files (Bisanz, 2018).

Heatmaps of the absolute abundance of methanogens and methanotrophs on the normalized dataset were made in QIIME 2 on the rarefied dataset with taxa collapsed at the genus level. Abundances were normalized by adding a pseudocount 1 followed by a log10 transformation in QIIME 2.

Canonical correspondence analysis (CCA) was performed using the vegan R package (Oksanen *et al*., 2019) to determine the relationship between physicochemical variables and the absolute normalized abundance of methanogens and methanotrophs in the rarefied dataset. CCA models were validated according to Oksanen (2012) using an ordistep function. CCA graphs were made using ggplot2 (Wickham, 2016), ggvegan (Simpson, 2019), and ggrepel (Slowikowski, 2019). The WT variable used for CCA analysis was the depth of peat in relation to the WT at the time of sampling, *e.g.*, when the actual WT level was at -10 cm below the ground, the sample from 10 – 20 cm depth was assigned 0 cm, and the sample from 0 – 10 cm depth was +10 cm.

2.4.5. Physicochemical drivers of potential rates of CH_4 production and oxidation

Principal component analysis (PCA) in R was used to ordinate the physicochemical variables of peat in the microcosms. Linear mixed effect (LME) models were built for MP and MO with

principal components (PCs) as the explanatory variables and the peat core location as a random variable. One-way ANOVA calculated using the nlme R package (Pinheiro *et al*., 2019), was applied to the models to identify which PCs significantly explained the variability in MO and MP. All LME models were validated for distribution of residuals. R^2 was calculated using the MuMIn package in R (Barton, 2019). Kruskal-Wallis one-way analysis of variance with Dunn post-hoc analysis and p-values adjusted with the Benjamini-Hochberg method (R packages: dplyr (Wickham *et al*., 2019), and FSA (Ogle *et al*., 2019)) were used to determine the spatial variability of MP and MO among sites, depths, and surface cover types. Additional R packages used for calculation and visualization of MP and MO analysis output included factoexta (Kassambara and Mundt, 2017) and dplyr (Wickham *et al*., 2019). Shapiro-Wilk test in R (R Core Team, 2019) was used to test if data were normally distributed. Additionally, skewness and kurtosis were calculated and histogram of frequencies was used to identify data distribution.

2.5. RESULTS

2.5.1. Physicochemical and hydrological conditions

The water table fluctuation zone (WTFZ) was calculated from the lowest and highest levels of the WT (Fig. 2.1). Over 1,300 WT measurements were collected in years 2016 and 2017. The WT fluctuated from -5.5 cm to -79 cm at the Natural site (with larger fluctuations at the hummock site than at the hollow), from +20.5 cm to -60 cm at RES-1991, from +10 cm to -87 cm at the RES-2009, from +20 cm to -79 cm at RES-2012, from +6.5 cm to -79 cm at the Unrestored, and from -3 cm to -67 cm at the Active site. The Unrestored site had the lowest porosity and the highest WT fluctuations, while RES-1991 and the Natural site had the highest porosity and relatively stable WT, aside from the hummock locations at the Natural site.

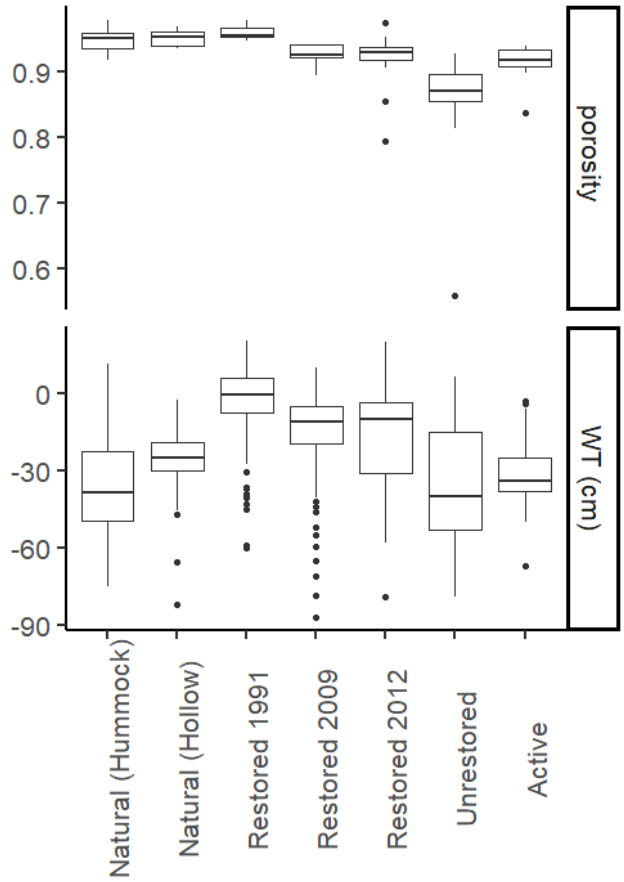

Figure 2. 1. Water table fluctuation and porosity at studied sites.

Almost all physicochemical factors, except [lactate] and [pyruvate], varied significantly between sites (Kruskal-Wallis one-way analysis of variance; Tab. A.2.4). The depth profiles of peat physicochemical properties showed individual patterns for each site in most cases, but the highest [succinate] was found in shallow peat of the Natural site and RES-1991 compared to greater depths and other sites (Tab. A.2.1). Also, [PO_4^{3-}] and [pyruvate] was the highest in surface peat of the Natural site and all restored sites but not at Unrestored and Active sites (Tab. A.2.1.).

Among the 63 samples with adequate sequence counts for inclusion in the molecular analysis, the only sample from the Natural hummock (20 – 30 cm depth) showed unique chemical balance (the highest [DOC], C:N, EC, [succinate], [pyruvate], [PO_4^{3-}], and one order of magnitude higher [citrate] than in other sites, but the lowest pH, [formate], and very low

[Fe^{3+}], but relatively high [SO_4^{2-}]). We did not detect any methanogens or methanotrophs in this sample. The mean [DOC] was comparable for the remaining sites and surface cover types aside from a relatively low values found in the Active site and Unrestored sedgy location (mean 1.4 and 1.2 mg g^{-1}, respectively) (Tab. 2.1, Tab. A.2.1). The lowest C:N ratios were in sedgy cores of the Unrestored, RES-2009, RES-1991 and in the Active site (15.5, 19.4, 21.7, and 20.2, respectively) with higher values for the Unrestored bare peat, Natural hollow, and RES-2012 (32.3, 34.4, and 30.8 – 37.3, respectively). The mean EC was comparable between sites and surface cover types, in most cases > 200 µS cm^{-1}, with the lowest value for the Active site (140.4 µS cm^{-1}). Fe^{3+} was the most abundant TEA except for at the Active and bare location of the Unrestored site, where SO_4^{2-} was observed in higher concentrations than Fe^{3+}. [Fe^{3+}] exceeding 1000 µg g^{-1} of dry peat was found in the sedgy core from RES-2009. Butyrate, pyruvate, and lactate were the least, and acetate and formate the most abundant short chain fatty acid ions. The Unrestored and Active sites were relatively poor in short chain fatty acid ions, while the restored sites comparatively rich.

The samples for the microcosm experiment were collected in 2017 at the WT about 20 cm deeper than during 2016 core collection, and had different physicochemical characteristics than samples for the microbial analysis (Tab. 2.2, Tab. A.2.2). We analysed peat physicochemical characteristics for both molecular samples and microcosm samples to determine if there is an impact of peat properties on the abundance of methane cycling microorganisms and methane production and oxidation rates. Here we compare peat collected for microcosms (2017) with corresponding depths from 2016. [DOC], C:N, and pH did not vary considerably between samples from 2016 and 2017, but EC was notably higher in 2017. At the RES-2009 site, [acetate] increased over twofold in 2017 compare to 2016. [Formate] remained below 10 µg g^{-1} of dry peat at the Natural site in both years and increased from <10 (2016) to over 18 µg g^{-1} of dry peat (2017) at RES-2009. [Succinate] and [citrate] were higher in 2017 compared to 2016 at all sites. Core #2 collected in 2017 at RES-2009 had the highest [acetate], [propionate], [succinate], [butyrate], and [pyruvate], but relatively low [formate] compared to other cores from both years. The [Fe^{3+}] were similar within each peatland in 2016 and 2017, *e.g.*, the Natural site showed consistently the lowest [Fe^{3+}] and [SO_4^{2-}] in both years and [NO_2^-] and [NO_3^-] were the lowest of all TEAs. [PO_4^{3-}] from 2017 was higher than in 2016 across all sites.

Table 2. 1. Mean values and standard deviations of physicochemical variables in 63 samples with sequence count above the rarefication threshold. DOC – dissolved organic carbon (mg g^{-1}), EC – electrical conductivity (µS cm^{-1}), WT – water table (cm); short chain fatty acids (µg g^{-1} of dry peat): ACE – acetate, BUT – butyrate, CIT – citrate, FOR – formate, LAC – lactate, PYR – pyruvate, PRO – propionate, SUC – succinate; ions (µg g^{-1} of dry peat).

Site	Surface	DOC			C:N			pH			EC			Von Post			Porosity			WT			WT at the time of sampling
		n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	
Natural Hollow	mossy	4	2.2	0.6	4	34.4	5.4	4	4.1	0.2	4	184.1	47.5	4	4	0	15	0.95	0.02	139	-26	12	-30
Natural Hummock	mossy	1	4.4		1	51.9		1	3.7		1	286.1		1	3					132	-36	18	-17
Restored 1991	mossy	6	2.5	0.9	0			0			0			7	4	2	11	0.96	0.01	242	-3	13	0
	sedgy	5	2.6	0.7	5	21.7	3.1	5	5.1	0.1	5	225.9	138.8	5	3	1							0
Restored 2009	mossy	10	2.3	1.0	0			0			0			10	4	1	11	0.92	0.02	269	-14	14	0
	sedgy	9	1.2	0.4	9	19.4	9.3	8	5.1	0.4	8	248.1	55.9	10	4	1							0
Restored 2012	mossy	8	2.0	0.4	8	37.3	12.1	8	4.4	0.3	8	233.4	52.8	9	3	0	17	0.92	0.04	218	-16	19	-7
	sedgy	5	1.9	0.2	5	30.8	14.4	5	4.4	0.3	5	219.5	17.8	6	4	1							-7
Unrestored	bare peat	5	1.9	0.5	5	32.3	9.4	5	4.0	0.2	5	244.0	36.3	6	4	1	12	0.85	0.10	292	-36	22	-30
	sedgy	3	1.2	0.3	3	15.5	1.4	3	4.6	0.3	3	182.3	13.9	4	5	0							-30
Active	bare peat	1	1.4		1	20.2		1	4.6	NA	1	140.4		1	3		10	0.91	0.03	35	-31	16	-3

Site	Surface	LAC			ACE			FOR			SUC			BUT			PYR			PRO			CIT		
		n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
Natural Hummock	mossy	4	0.0	0.0	4	65.8	47.9	4	8.3	3.3	4	1.5	3.0	4	0.0	0.0	4	0.0	0.0	4	4.9	3.5	4	1.9	3.9
Natural Hollow	mossy	1	0.0		1	41.0		1	5.7		1	227.5		1	0.0		1	8.0		1	7.1		1	696.3	
Restored 1991	mossy	6	1.9	4.7	6	29.7	22.1	6	12.6	7.8	6	122.0	170.5	6	0.0	0.0	6	1.4	3.4	6	7.1	3.3	6	57.5	80.9
	sedgy	5	2.9	6.4	5	29.0	19.0	5	13.0	6.6	5	70.4	111.1	5	1.4	3.2	5	0.0	0.0	5	8.5	6.7	5	91.1	114.7
Restored 2009	mossy	10	0.0	0.0	10	19.7	9.6	10	22.1	13.1	10	1.3	2.9	10	0.0	0.0	10	0.0	0.0	10	1.9	2.1	10	0.2	0.8
	sedgy	9	3.5	5.7	9	22.8	8.0	9	18.0	19.8	9	10.0	19.4	9	0.0	0.0	9	1.4	4.3	9	6.1	4.1	9	3.3	8.5
Restored 2012	mossy	8	12.1	18.4	8	24.1	7.4	8	43.8	24.0	8	15.2	16.9	8	0.0	0.0	8	0.0	0.0	8	4.4	6.7	8	3.3	7.4
	sedgy	5	0.8	1.8	5	28.4	7.1	5	38.6	19.2	5	71.8	48.2	5	0.0	0.0	5	0.4	0.9	5	11.9	8.0	5	11.3	10.9
Unrestored	bare peat	5	0.5	1.2	5	12.5	7.9	5	10.7	12.7	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	4.3	5.0	5	6.2	9.5
	sedgy	3	0.0	0.0	3	16.6	4.3	3	50.3	17.0	3	4.1	1.3	3	0.0	0.0	3	0.0	0.0	3	3.1	2.1	3	0.0	0.0
Active	bare peat	1	0.0		1	36.0		1	31.1		1	0.0		1	0.0		1	0.0		1	44.2		1	14.1	

Site	Surface	NO_2^-			NO_3^{2-}			SO_4^{2-}			PO_4^{3-}			Fe^{3+}		
		n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
Natural Hummock	mossy	4	0.0	0.0	4	3.7	1.2	4	25.9	4.2	4	21.6	20.2	4	47.6	39.4
Natural Hollow	mossy	1	0.0		1	3.3		1	100.8		1	169.9		1	18.4	
Restored 1991	mossy	6	0.0	0.0	6	3.0	2.5	6	50.5	30.5	6	23.7	30.7	5	444.5	335.8
	sedgy	5	0.0	0.0	5	1.5	2.1	5	50.1	23.0	5	20.9	30.2	5	334.3	142.2
Restored 2009	mossy	10	1.2	2.2	10	2.7	1.4	10	59.3	47.6	10	1.0	2.1	9	802.4	616.2
	sedgy	9	0.6	1.0	9	5.4	5.7	9	213.9	129.1	9	17.9	50.0	9	1626.5	762.5
Restored 2012	mossy	8	4.4	3.1	8	14.4	21.4	8	36.8	34.5	8	35.7	82.9	9	269.6	229.5
	sedgy	5	1.1	1.1	5	3.3	0.6	5	25.2	2.8	5	28.7	57.1	6	302.8	276.3
Unrestored	bare peat	5	1.6	1.6	5	5.9	4.1	5	321.6	84.2	5	8.9	6.6	5	257.0	91.5
	sedgy	3	0.5	0.8	3	1.7	0.4	3	77.1	15.0	3	1.1	1.9	3	678.9	545.5
Active	bare peat	1	0.0		1	7.7		1	29.7		1	0.0		1	11.3	

Table 2. 2. Mean values and standard deviations of physicochemical variables of peat samples collected for the microcosm experiment. DOC – dissolved organic carbon (mg g^{-1}), EC – electrical conductivity (µS cm^{-1}), WT – water table (cm); short chain fatty acids (µg g^{-1} of dry peat): ACE – acetate, BUT – butyrate, CIT – citrate, FOR – formate, PYR – pyruvate, PRO – propionate, SUC – succinate; ions (µg g^{-1} of dry peat). Lactate concentration in all samples was 0 µg g^{-1} dry peat.

Peatland	Surface type	DOC			C:N			pH			EC			Von Post			WT		
		n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
Natural (Hollow)	mossy	9	2.4	0.7	9.0	32.1	4.4	9.0	4.1	0.1	9	308	31	9	4	0	3	-45	8
Natural (Hummock)	mossy	9	3.0	1.1	9.0	30.7	2.9	9.0	4.1	0.1	9	236	53	9	3	1	3	-27	3
Restored 2009	mossy	9	2.9	1.4	9.0	29.6	9.0	9.0	4.7	0.3	9	276	47	9	5	1	3	-22	8
Restored 2009	sedgy	8	2.8	0.9	8.0	23.7	7.3	8.0	4.7	0.2	8	297	45	8	4	1	3	-22	8
Unrestored	bare peat	9	1.5	0.7	9.0	17.1	3.3	9.0	4.7	0.2	9	230	53	9	6	2	3	-59	14

Peatland	Surface type	ACE			FOR			SUC			BUT			PYR			PRO			CIT		
		n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
Natural (Hollow)	mossy	9	98.7	47.9	9.0	5.5	2.0	9.0	4.6	13.8	9.0	0.0	0.0	9.0	0.0	0.0	9.0	0.9	1.7	9.0	39.5	42.7
Natural (Hummock)	mossy	9	54.1	42.2	9.0	5.2	1.3	9.0	3.6	10.8	9.0	0.0	0.0	9.0	0.0	0.0	9.0	0.0	0.0	9.0	28.7	31.6
Restored 2009	mossy	9	59.0	25.1	9.0	3.1	1.1	9.0	33.7	35.9	9.0	0.0	0.0	9.0	0.6	1.2	9.0	1.8	4.0	9.0	10.8	11.2
Restored 2009	sedgy	8	97.0	90.9	8.0	4.1	4.5	8.0	60.2	68.6	8.0	4.5	11.4	8.0	3.8	7.6	8.0	15.3	34.5	8.0	14.2	7.5
Unrestored	bare peat	9	13.2	6.6	9.0	18.4	26.1	9.0	0.7	1.4	9.0	0.0	0.0	9.0	0.0	0.0	9.0	0.0	0.0	9.0	2.8	2.5

Peatland	Surface type	NO_2^-			NO_3^{2-}			PO_4^{3-}			SO_4^{2-}			Fe^{3+}		
		n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
Natural (Hollow)	mossy	9	1.1	2.2	9.0	3.9	1.8	9.0	67.2	57.6	9.0	57.2	15.1	8.0	59.7	58.6
Natural (Hummock)	mossy	9	0.0	0.0	9.0	4.1	1.0	9.0	77.4	68.1	9.0	48.6	10.8	8.0	96.9	126.6
Restored 2009	mossy	9	0.5	0.8	9.0	2.2	1.1	9.0	24.7	32.8	9.0	259.5	116.4	9.0	815.1	434.6
Restored 2009	sedgy	8	0.4	0.7	8.0	1.6	1.1	8.0	29.8	55.7	8.0	211.1	148.6	8.0	819.0	321.0
Unrestored	bare peat	9	1.1	1.3	9.0	2.6	1.5	9.0	1.6	1.5	9.0	298.2	307.2	9.0	553.4	311.4

2.5.2. Abundance of methanogens and methanotrophs

Methanogens were found in 60 of 63 samples, missing only from surface peat (0 – 10 cm) of the Unrestored bare peat core, the RES-2009 sedgy core (0 – 10 cm), and the sample from Natural hummock (20 – 30 cm, Tab. A.2.3). Methanotrophs were found in 61 of 63 samples, missing from Unrestored bare peat at 30 – 40 cm depth and from the Natural hummock sample (Tab. A.2.3). Based on 16S rRNA amplicon sequencing, the sum of both methanotrophs and methanogens accounted for < 0.1 % of the total bacterial and archaeal community (Fig. 2.2). Lower relative abundance of both groups was found in RES-2012, and the Unrestored site, while RES-1991 and RES-2009 showed the highest abundance. At Natural hollow, the abundance of methanogens noticeably increased at 60 – 70 cm (below the WTFZ). At RES-1991 mossy location the abundance of methanotrophs decreased with depth and that of methanogens increased, while in the sedgy core a sudden large increase in the abundance of both groups at 10 – 20 cm depth was observed. Methanogens dominated over methanotrophs in the mossy core from RES-2012 and in RES-2009 below the WTFZ. The sedgy core at the Unrestored site showed more methanogens than the bare peat core. Only at the lower boundary of the WTFZ did the abundance of methanogens increase. The only existing sample from the Active site had more methanogens than methanotrophs.

2.5.3. Methanogenic community

Methanogens were distributed along depth patterns characteristic for each site and similar group composition was present at RES-2012, the Unrestored and Active sites (Tab. A.2.3 and Fig. 2.3A). The majority of methanogens belonged to the classes *Methanobacteria*, *Methanomicrobia*, *Thermococci*, and *Thermoplasmata* within the phylum *Euryarchaeota* (32,139 of 40,122 sequence counts; 19 taxa of 21 identified methanogens). The codes for methanogenic taxa follow the alphabetic order of the taxa names in Tab. 2.3. *Methanobacterium* (MG3) was the only genus observed from the *Methanobacteria* class (Thauer *et al*., 2008, Liu, 2010). Three methanogenic orders of the class *Methanomicrobia* were found: *Methanosarcinales* (Garcia *et al*., 2000, Liu, 2010, Lackner *et al*., 2018), *Methanomicrobiales* (Garcia *et al*., 2000, Thauer *et al*., 2008, Liu, 2010), and *Methanocellales*

(Sakai *et al*., 2008). Two genera of *Methanosarcinales*, *Methanosaeta* (MG13) and *Methanosarcina* (MG14), are related to the only known acetoclastic methanogens (Lackner *et al*., 2018). The class *Methanocellales* was represented by three genera: *Methanocella* (MG4; Sakai *et al*., 2008), Rice Cluster I (MG5, Sakai *et al*., 2008) and an uncultured genus within an uncultured family (MG6). All three genera were present mainly in RES-1991. Six genera of *Methanomicrobiales* were detected: *Methanoregula* (MG7, Sakai *et al*., 2012), *Methanospirillum* (MG8, Ferry *et al*., 1974), *Methanosarcina sp.* (MG9; Rice Cluster II; *e.g*., De Vrieze *et al*., 2012), an uncultured archaeon MG10; Cluster II family), and two other uncultured methanogenic *Archaea* (MG11, and MG12) that were present only in RES-1991. Three uncultured methanogenic genera (MG18 and MG19, MG20) of the order *Methanomassiliicoccales* (Borrel *et al*., 2014; class *Thermoplasmata*) are related to H_2-depended methylotrophic methanogens (which reduce methanol or methylamines using H_2; Evans *et al*., 2015, Lang *et al*., 2015) but are also the only methanogenic order without the capacity for hydrogenotrophic methanogenesis (Vanwonterghem *et al*., 2016). MG20 was present only in the deepest peat (340 – 350 cm) of RES-1991, while MG18 was present in the whole profile of RES-1991 and RES-2009, but was found only at greater depths at RES-2012 and the Unrestored site (Fig. 2.3A, Tab. A.2.3). The greatest abundance of MG19 was found below 50 cm at the RES-2009. An uncultured methanogen from the class *Thermoplasmata* (MG21) was present only at the Natural site and was the only *Thermoplasmata* found in cores from that peatland. Three uncultured members of the methanogenic order *Methanofastidiosales* (Verwonterghem *et al*., 2016, Lyu *et al*., 2018), class *Thermococci* (MG15, MG16, MG17) occurred only in very small numbers in deep peat from RES-1991 and RES-2009. Two other methanogens were classified as *Candidatus* Methanomethylicus (MG2, Verwonterghem *et al*., 2016) of the phyla *Crenarchaeota* (class *Verstraetearchaeia*) and an uncultured methanogenic archaeon of the class *Bathyarchaeia* (MG1; Lyu *et al*., 2018). These two organisms are related to methylotrophic methanogens (Evans *et al*., 2015, Vanwonterghem *et al*., 2016). *Cand.* Methanomethylicus was found in deep peat of RES-2012 and RES-1991. MG1 thrived in all sites regardless of the surface type cover, but only below the depth of 20 – 30 cm.

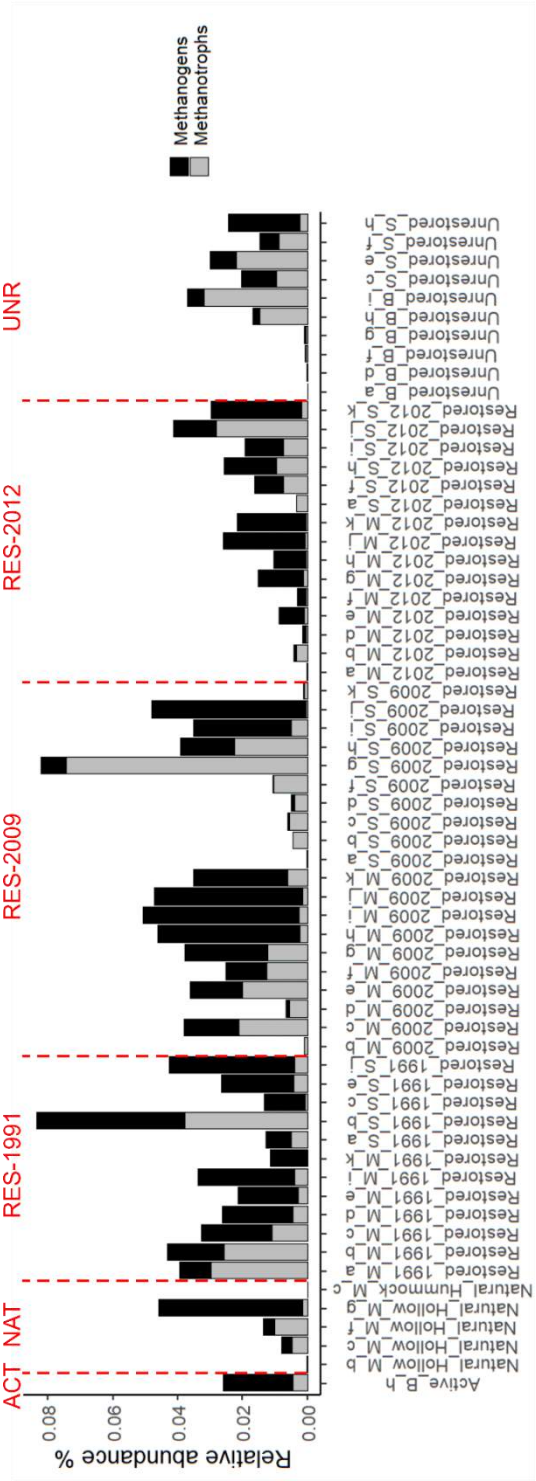

Figure 2. 2. Relative abundance of methanogens and methanotrophs in the 16S rRNA amplicon sequencing. Letters a – k denote depths: a (0 – 10 cm), b (10 – 20 cm), c (20 – 30 cm), d (30 – 40 cm), e (40 – 50 cm), f (50 – 60 cm), g (60 – 70 cm), h (70 – 80 cm), i (80 – 90 cm), j (90 – 100 cm), k is the deepest sample collected from the site (340 – 350 cm at RES-1991, 150 – 160 cm at RES-2009 mossy, 113 – 123 cm at RES-2009 sedgy, 140 – 150 cm at RES-2012 mossy, 130 – 140 cm at RES-2012 sedgy). ACT – Active, NAT – Natural, UNR – Unrestored, M – mossy, S – sedgy, B – bare peat.

Overall, few methanogenic taxa were observed in the Natural site down to 70 cm depth, with peat at 60 – 70 cm containing their largest abundance dominated by *Methanosarcina sp*. (MG9). RES-1991 showed the largest diversity of methanogens among all peatlands, mainly uncultured *Bathyarchaeia* (MG1), *Cand*. Methylomethylicus (MG2), *Methanobacterium* (MG3), *Methanocella* (MG4), *Methanoregula* (MG7), the uncultured member of Rice Cluster II (MG10), *Methanosaeta* (MG13), and uncultured *Methanomassiliicoccales* (MG18). RES-2009 was dominated by an uncultured *Thermoplasmatales* archaeon (MG19) at high abundance levels not seen at other sites. RES-2012 and the Unrestored sites (especially the sedgy cores) were very similar to each other in methanogenic community composition and relative abundances. These sites contained mainly *Cand*. *Methanomethylicus* (MG2), *Methanobacterium* (MG3), the uncultured archaeon from Rice Cluster II (MG10), and the uncultured member of *Methanomassiliicoccaceae* (MG18). Peat from the Active site contained uncultured *Bathyarchaeia* (MG1), *Cand*. Methylomethylicus (MG2), *Methanoregula* (MG7), and the uncultured member of Rice Cluster II (MG10).

Alpha diversity and richness of methanogens generally increased with depth, and were the highest in RES-1991, but at the remaining sites, they showed similar values (Fig. 2.4A). Other restored sites showed similar values and patterns as the Unrestored site. The values for the sample from the Active site were equivalent to the highest diversity at the Natural site. The Kruskal-Wallis pairwise comparison showed that observed ESVs ($n_B = 14$, $n_W = 46$, $p = 0.004$, $H = 8.05$), Faith's ($p = 0.004$, $H = 7.92$), and Shannon ($p = 0.021$, $H = 5.34$) diversity indices were significantly higher in samples from below (B) than from within (W) the WTFZ.

The weighted UniFrac beta diversity PCoA showed clustering by site but not depth and not the location in relation to the WTFZ (Fig. 2.5A). The methanogenic communities from RES-2009 and RES-1991 clustered close to each other but were grouped by site. The communities in the Natural site were similar to those in the Unrestored and RES-2012. In the unweighted UniFrac PCoA (Fig. A.2.2A), some samples from RES-1991 and RES-2009 at 0 – 40 cm depth clustered relatively close together while the majority of the remaining samples formed another cluster with site transition from RES-1991 through RES-2009 (the older restored site) to RES-2012, the Unrestored, Active and Natural sites which showed similar diversity.

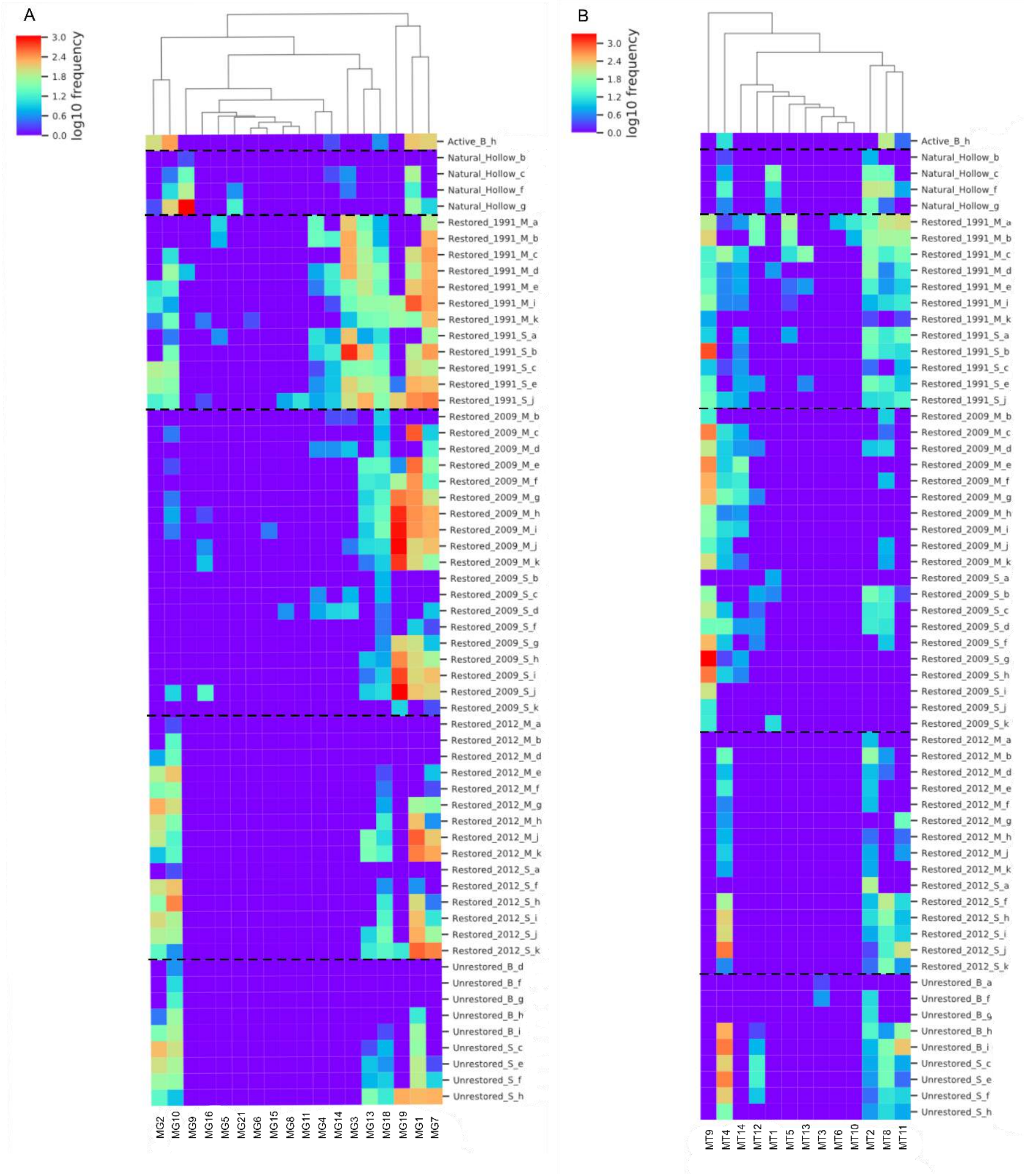

Figure 2. 3. Heatmaps of absolute abundance of methanogens (A) and methanotrophs (B) in the normalized dataset. Taxa are clustered by abundance. Three taxa (uncultured *Methanomicrobiales*, *Methanofastidiosales*, and *Methanomassiliicoccles*) were identified only to the level of order are not shown. Letters a to k denote depths: a (0 – 10 cm), b (10 – 20 cm), c (20 – 30 cm), d (30 – 40 cm), e (40 – 50 cm), f (50 – 60 cm), g (60 – 70 cm), h (70 – 80 cm), i (80 – 90 cm), j (90 – 100 cm), k is the deepest sample collected from the site (340 – 350 cm at RES-1991, 150 – 160 cm at RES-2009 mossy, 113 – 123 cm at RES-2009 sedgy, 140 – 150 cm at RES-2012 mossy, 130 – 140 cm at RES-2012 sedgy). M – mossy, S – sedgy, B – bare peat. See table 2.3 for the explanation of codes for identified methanogens and methanotrophs.

A)

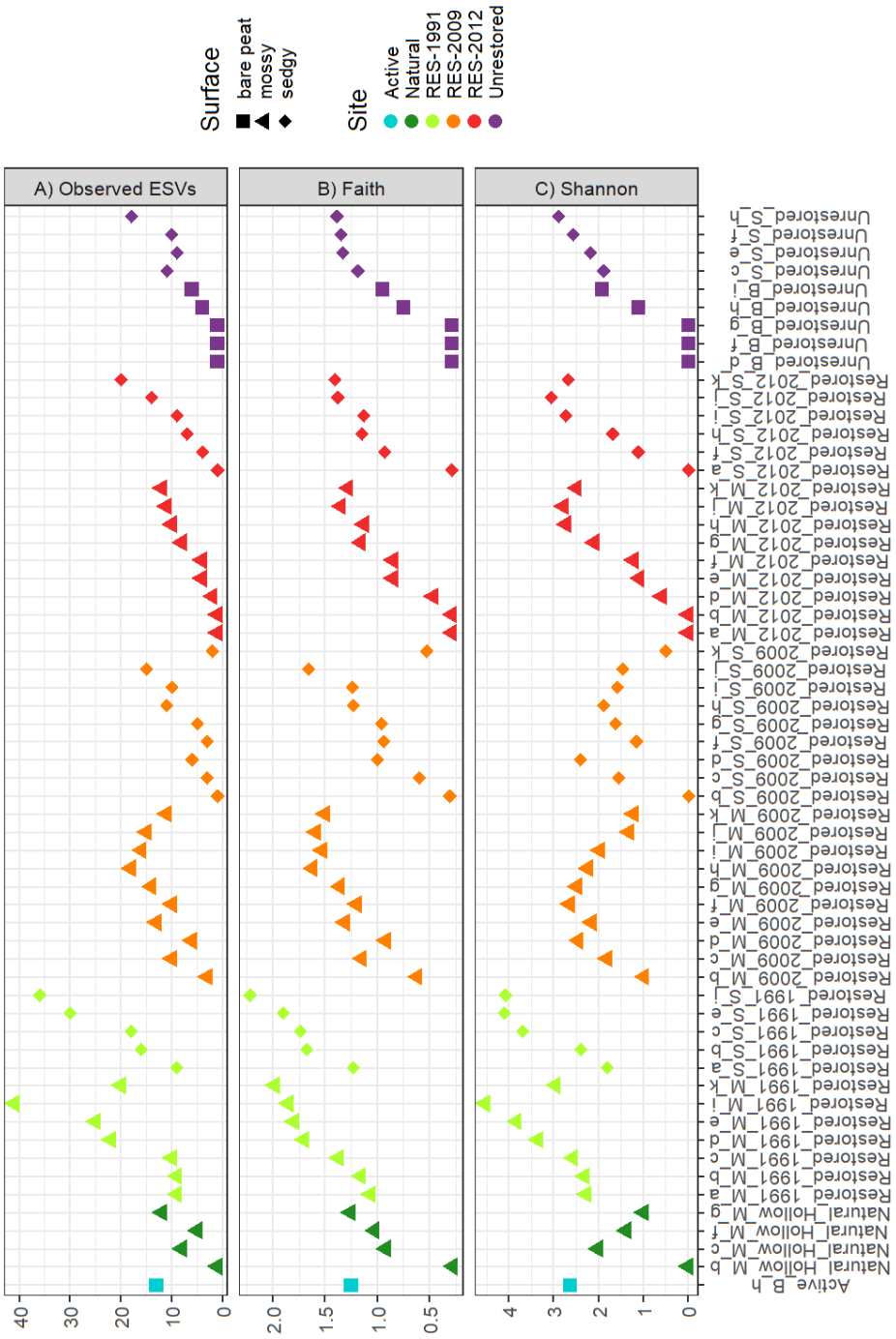

Figure 2. 4. Alpha richness and diversity metrics of CH_4-cycling organisms: A) methanogens, B) methanotrophs in the Active site, Unrestored, restored in 1991 (RES-1991), in 2009 (RES-2009), in 2012 (RES-2012), and the Natural site. Letters a – k denote depths: a (0 – 10 cm), b (10 – 20 cm), c (20 – 30 cm), d (30 – 40 cm), e (40 – 50 cm), f (50 – 60 cm), g (60 – 70 cm), h (70 – 80 cm), i (80 – 90 cm), j (90 – 100 cm), k is the deepest sample collected from the site (340 – 350 cm at RES-1991, 150 – 160 cm at RES-2009 mossy, 113 – 123 cm at RES-2009 sedgy, 140 – 150 cm at RES-2012 mossy, 130 – 140 cm at RES-2012 sedgy). M – mossy, S – sedgy, B – bare peat.

B)

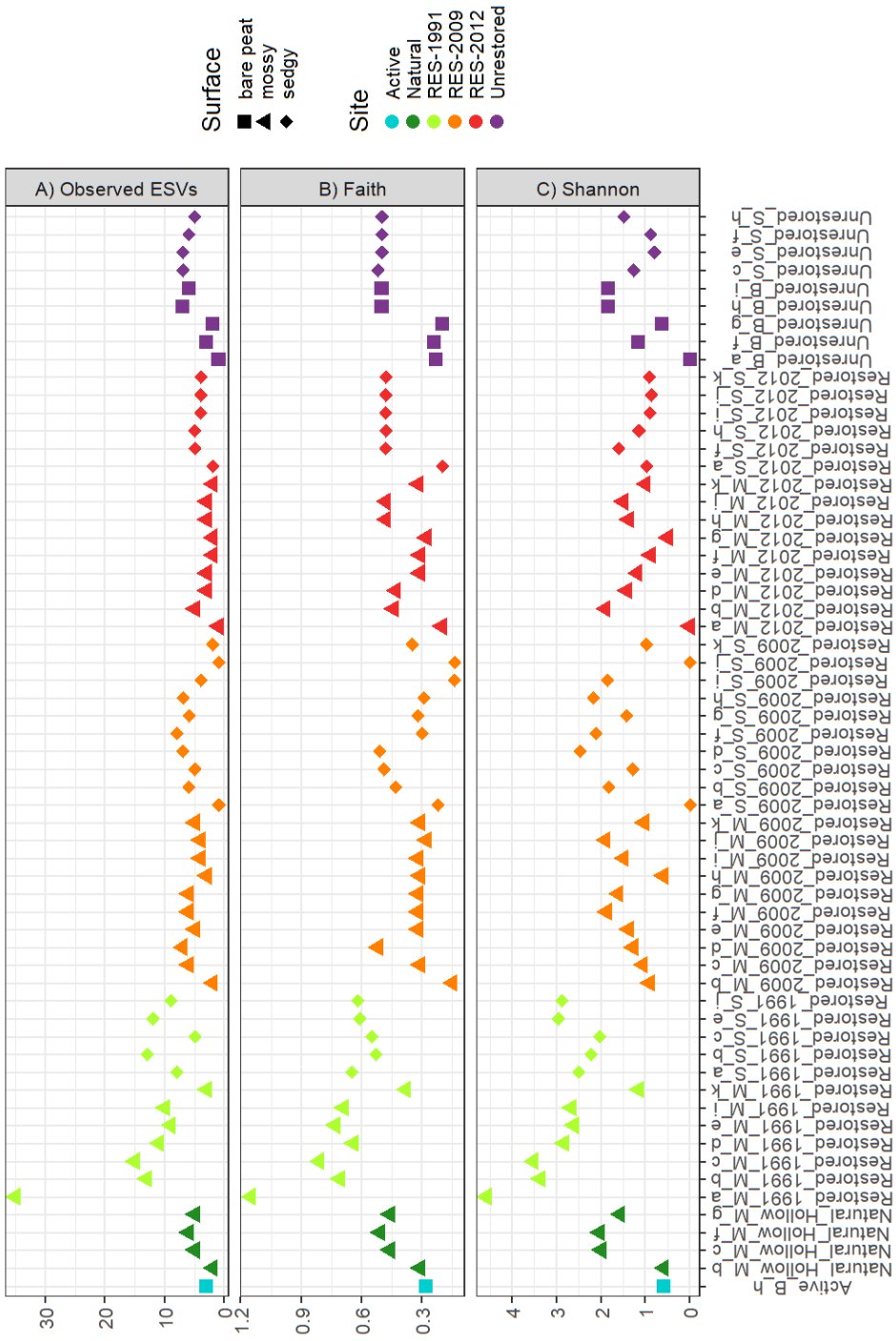

Figure 2. 4. [Continuation]. Note different scale on the y-axis is for methanotrophs and methanogens.

A)

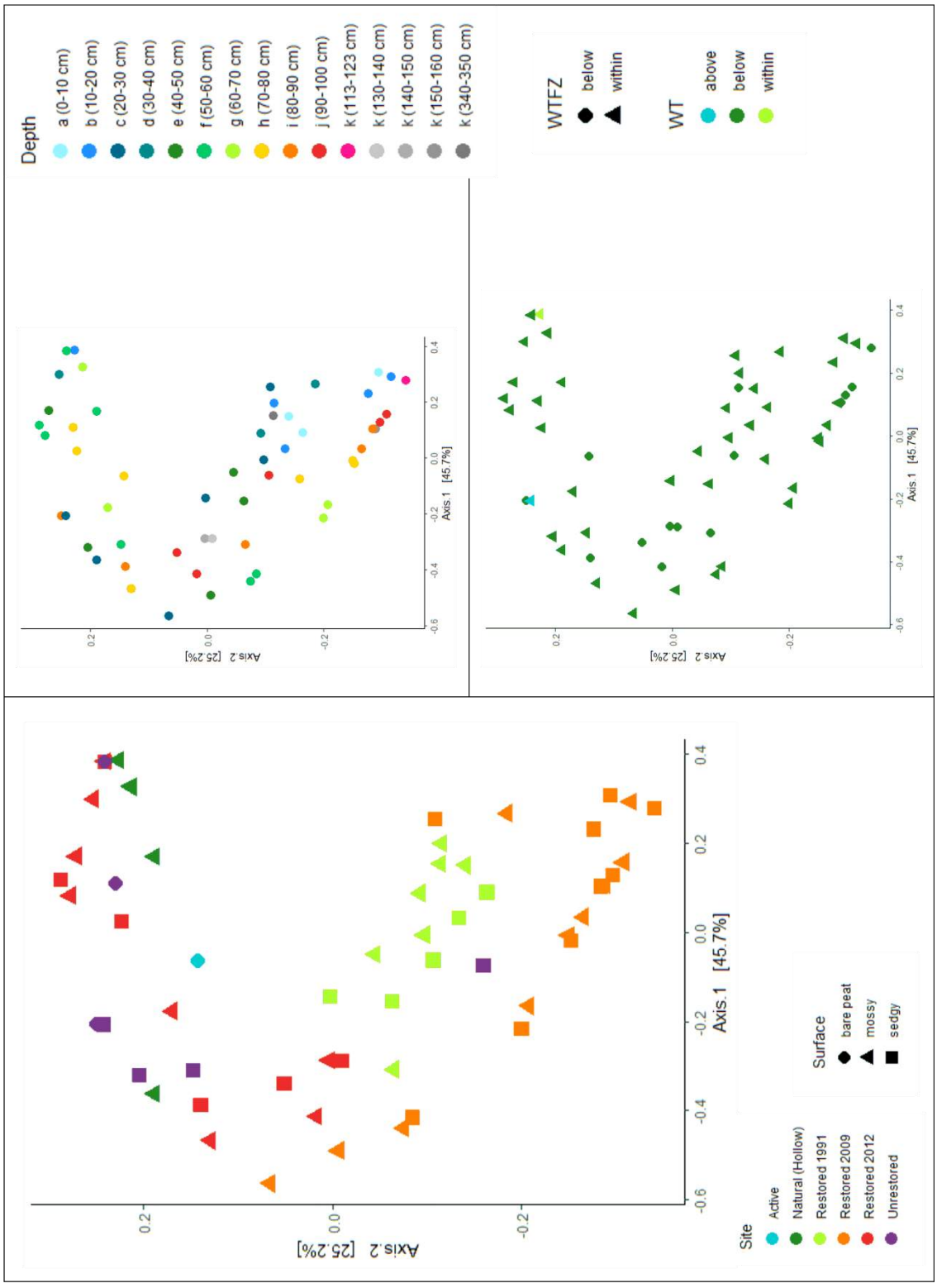

Figure 2. 5. Weighted UniFrac Beta diversity measures for CH_4-cycling organisms in the Natural, Active, Unrestored, and restored sites. A) methanogens, B) methanotrophs. WT – water table, WTFZ – water table fluctuation zone.

B)

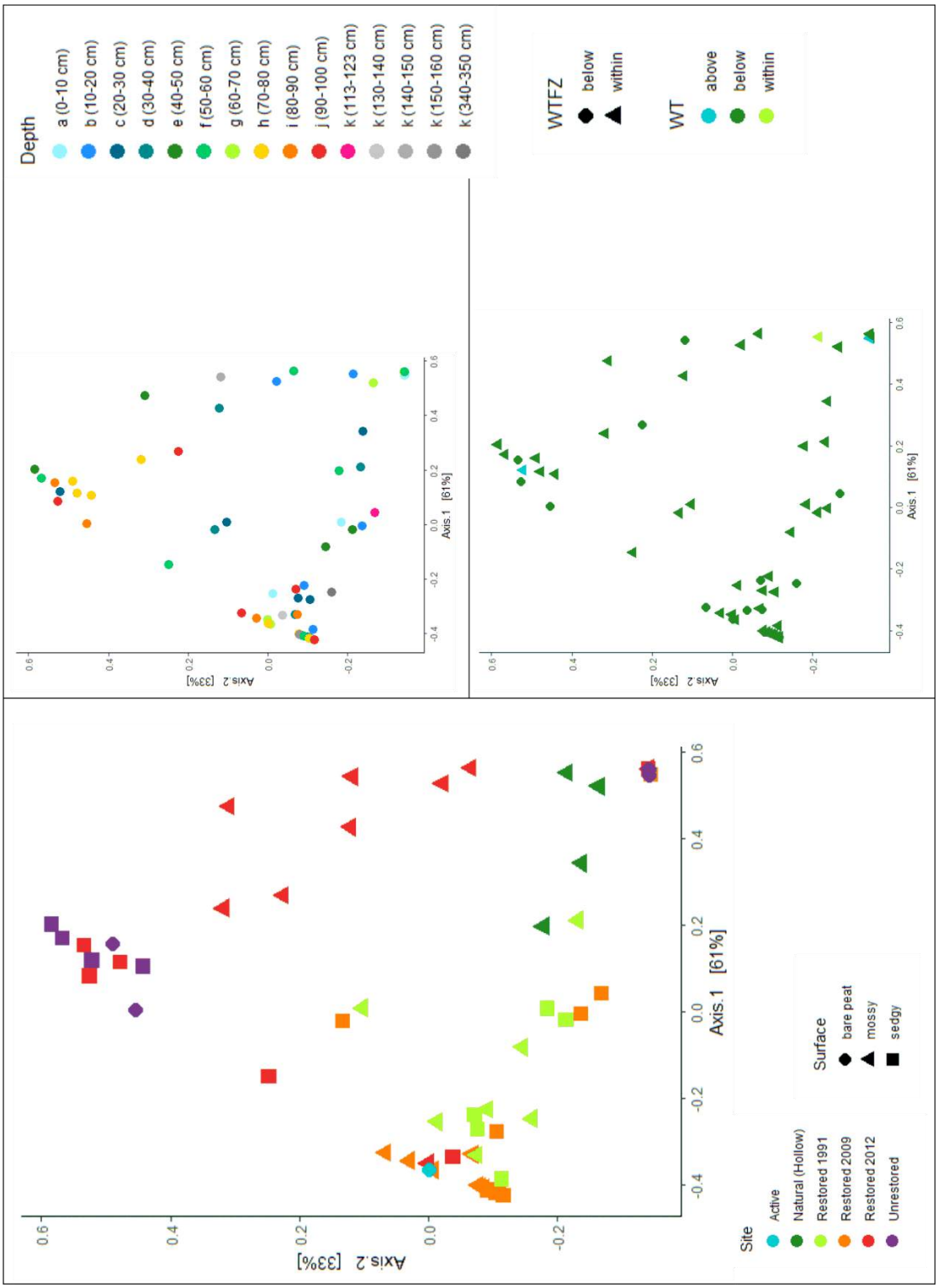

Figure 2. 5. [Continuation]

2.5.4. Methanotrophic community

The codes for methanotrophic taxa follow the alphabetic order of the taxa in Tab. 2.3. Fifteen ESVs related to methanotrophic genera from phyla *Proteobacteria* were found (Tab. 2.3). *Methylocella* (MT1, Dedysh *et al*., 2005), *Methylocystis* (MT2, Larmola *et al*., 2010), and *Methyloferula* (MT3, Dedysh *et al*., 2015) belong to class *Alphaproteobacteria*. The remaining methanotrophs were *Gammaproteobacteria* of a methanotrophic class *Methylococcales* (Orata *et al*., 2018) and family *Methylomonaceae*; genera: *Candidatus* Methylospira (MT4, Danilova *et al*., 2016), *Methylocaldum* (MT5; Bodrossy *et al*., 1997), *Methylomagnum* (MT6, Khalifa *et al*., 2015), *Crenothrix* (MT8, Oswald *et al*., 2017), *Methylobacter* (MT9; Smith *et al*., 2018), *Methyloglobulus* (MT10, Deutzmann *et al*., 2014, Schink and Deutzmann, 2015), *Methylomonas* (MT11, Bowman *et al*., 1990, Kalyuzhnaya *et al*., 1999), *Methylovulum* (MT12; Mateos-Rivera *et al*., 2018), pLW-20 (MT13, Nercessian *et al*., 2005), one uncultured (MT14) and two unidentified genera (MT7, and MT15).

Each site had their unique composition of methanotrophs distributed more uniformly than methanogens; however, similarities between sites were observed, *e.g*., between RES-2012 and the Unrestored site or between RES-1991 and RES-2009 (Tab. A.2.3 and Fig. 2.3B). We found methanotrophs even at depths where peat was waterlogged. RES-1991 contained 14 of 15 identified methanotrophs, excepting *Methyloferula* (MT3). RES-2009 was dominated mainly by *Methylobacter* (MT9), *Cand*. Methylospira (MT4), and an uncultured *Methylomonaceae* (MT14) with some *Methylocystis* (MT2) and *Crenothrix* (MT8). RES-2012 was colonized mainly by *Cand*. Methylospira (MT4) and *Methylocystis* (MT2), with the addition of *Crenothrix* (MT8) and *Methylomonas* (MT11) at depths below 50 cm. Similar methanotrophic composition was found in the Unrestored site (except the surface peat of the bare peat core, which contained *Methyloferula* only) and in the Natural site, where additionally *Methylocella* (MT1) was present. The only sample from the Active site, from depth 70 – 80 cm, showed low abundance of *Methylocystis* (MT2) and *Crenothrix* (MT8). The most abundant methanotroph at RES-1991 and RES-2009 was *Methylobacter* (MT9). It was not observed at the Natural, Unrestored, and RES-2012, possibly not captured due to its low abundance (Tab. A.2.3).

The alpha richness and diversity metrics for the methanotrophic communities did not show a clear pattern with depth (Fig. 2.4B). The highest values were observed at RES-1991. The values for other sites were similar and relatively consistent across the depths in the peat profiles. Faith's index (PD) was similar in most of the sedgy RES-2012, sedgy Unrestored, and mossy RES-2009 profiles.

The weighted UniFrac PCoA of methanotrophic communities showed similarities between RES-2009 and RES-1991, but not RES-2012 (Fig. 2.5B). The sample from the Active site was similar to the ones at restored sites. The communities in the Natural site were relatively similar to each other but not separated from other sites in the ordination. Communities from the Unrestored site were closely grouped with RES-2012 in both weighted and unweighted UniFrac PCoA. The unweighted UniFrac (Fig. A.2.2B) grouped all samples from RES-1991 along one axis. Other samples accompanied the RES-1991 on this line, but there was no site or depth pattern within this cluster aside from the Natural, Unrestored, and RES-2012 samples being close together. The communities from the medium depths of the RES-2009 were similar to each other and formed two separate clusters.

The Kruskal-Wallis pairwise comparison of observed ESVs, Faith, and Shannon for the methanotrophic community showed no significant difference ($p > 0.05$) between samples from within and below the WTFZ.

2.5.5. Taxa abundance relationships with the physicochemical and environmental conditions

The CCA model validation excluded some explanatory variables as redundant leaving C:N, WT, formate, propionate, citrate, PO_4^{3-} and Fe^{3+} in the model (Fig. 2.6). [PO_4^{3-}], [Fe^{3+}], [formate], and C:N were the dominant factors controlling the abundance of targeted taxa. Most methanotrophs grouped close to the WT, at high and moderate [PO_4^{3-}], [propionate], and [citrate] and low [formate], while most methanogens were on the opposite site of these gradients. *Alphaproteobacteria* were likely to reach their highest abundance at high [propionate], C:N, [PO_4^{3-}], and WT, and low [Fe^{3+}]. Two uncultured methanogens from orders *Methanomassiliicoccales* (MG20) and *Methanocellales* (MG6) were the most abundant in

Table 2. 3. Methanogenic *Archaea* and methanotrophic *Bacteria* identified in peat samples with sequence count above the rarefication threshold and their codes used in the canonical correspondence analysis.

Taxon	Code
Archaea; Crenarchaeota; Bathyarchaeia; uncultured methanogenic archaeon	MG1
Archaea; Crenarchaeota; Verstraetearchaeia; Methanomethyliales; Methanomethyliaceae; Candidatus Methanomethylicus	MG2
Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanobacterium	MG3
Archaea; Euryarchaeota; Methanomicrobia; Methanocellales; Methanocellaceae; Methanocella	MG4
Archaea; Euryarchaeota; Methanomicrobia; Methanocellales; Methanocellaceae; Rice Cluster I	MG5
Archaea; Euryarchaeota; Methanomicrobia; Methanocellales; uncultured; uncultured archaeon	MG6
Archaea; Euryarchaeota; Methanomicrobia; Methanomicrobiales; Methanoregulaceae; Methanoregula	MG7
Archaea; Euryarchaeota; Methanomicrobia; Methanomicrobiales; Methanospirillaceae; Methanospirillum	MG8
Archaea; Euryarchaeota; Methanomicrobia; Methanomicrobiales; Rice Cluster II; Methanosarcina sp.	MG9
Archaea; Euryarchaeota; Methanomicrobia; Methanomicrobiales; Rice Cluster II; uncultured archaeon	MG10
Archaea; Euryarchaeota; Methanomicrobia; Methanomicrobiales; uncultured; uncultured Methanomicrobiales archaeon	MG11
Archaea; Euryarchaeota; Methanomicrobia; Methanomicrobiales; uncultured	MG12
Archaea; Euryarchaeota; Methanomicrobia; Methanosarcinales; Methanosaetaceae; Methanosaeta	MG13
Archaea; Euryarchaeota; Methanomicrobia; Methanosarcinales; Methanosarcinaceae; Methanosarcina	MG14
Archaea; Euryarchaeota; Thermococci; Methanofastidiosales; uncultured; uncultured archaeon	MG15
Archaea; Euryarchaeota; Thermococci; Methanofastidiosales; uncultured; uncultured bacterium	MG16
Archaea; Euryarchaeota; Thermococci; Methanofastidiosales; uncultured	MG17
Archaea; Euryarchaeota; Thermoplasmata; Methanomassiliicoccales; Methanomassiliicoccaceae; uncultured	MG18
Archaea; Euryarchaeota; Thermoplasmata; Methanomassiliicoccales; uncultured; uncultured Thermoplasmatales archaeon	MG19
Archaea; Euryarchaeota; Thermoplasmata; Methanomassiliicoccales; uncultured	MG20
Archaea; Euryarchaeota; Thermoplasmata; uncultured; uncultured methanogenic archaeon	MG21
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Beijerinckiaceae; Methylocella	MT1
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Beijerinckiaceae; Methylocystis	MT2
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Beijerinckiaceae; Methyloferula	MT3
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylococcaceae; Candidatus Methylospira	MT4
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylococcaceae; Methylocaldum	MT5
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylococcaceae; Methylomagnum	MT6
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylococcaceae;__	MT7
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylomonaceae; Crenothrix	MT8
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylomonaceae; Methylobacter	MT9
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylomonaceae; Methyloglobulus	MT10
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylomonaceae; Methylomonas	MT11
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylomonaceae; Methylovulum	MT12
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylomonaceae; pLW-20	MT13
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylomonaceae; uncultured	MT14
Bacteria; Proteobacteria; Gammaproteobacteria; Methylococcales; Methylomonaceae	MT15

deep peat with high [formate]. *Methylobacter* (MT9) and *Methanospirillum* (MG8) preferred moderate to high [Fe^{3+}] and low C:N observed at RES-2009.

2.5.6. Potential rates of CH_4 production and oxidation

The potential rates of CH_4 production (MP) and CH_4 oxidation (MO) measured to assess the activity of methanogens and methanotrophs around the WT at sites RES-2009, the Natural, and Unrestored, showed different range of values for each site with similar patterns in MO at the restored and Unrestored sites. A total of 11 of 44 MP and 16 of 44 MO microcosms were rejected due to r^2 <0.75 for the slope of CH_4 concentration change over time or when positive MO was observed, meaning possible CH_4 production was still occurring in the samples. Low MO was found in the Unrestored site at bare peat locations (0.36 – 0.89 µmol g^{-1} d^{-1}), mossy and sedgy locations of RES-2009 (< 2 µmol g^{-1} d^{-1}), and 10 – 20 cm above the WT at the Natural site hollow (1.02 µmol g^{-1} d^{-1}; Fig. A.2.3, Tab. A.2.2). The highest MO was observed in the hummock directly below the WT (7.11 CH_4 µmol g^{-1} d^{-1}). Kruskal-Wallis for MO by site and type of surface cover (H(4) = 14.616, p = 0.005568) followed by Dunn test with p values adjusted with the Benjamini-Hochberg method showed that MO at Natural hummocks varied significantly from RES-2009 mossy (p = 0.01814), RES-2009 sedgy (p = 0.01614) and Unrestored bare peat (p = 0.01968, Fig. 2.7). When MO was compared between sites without specified cover type (Kruskal Wallis H(2) = 14.198, p = 0.0008261), it was significantly higher at the Natural site than at RES-2009 (p = 0.00112) and Unrestored (p = 0.0058). MO did not differ significantly between the depth zones. No significant differences in either MP or MO were found between types of surface cover without specifying management status.

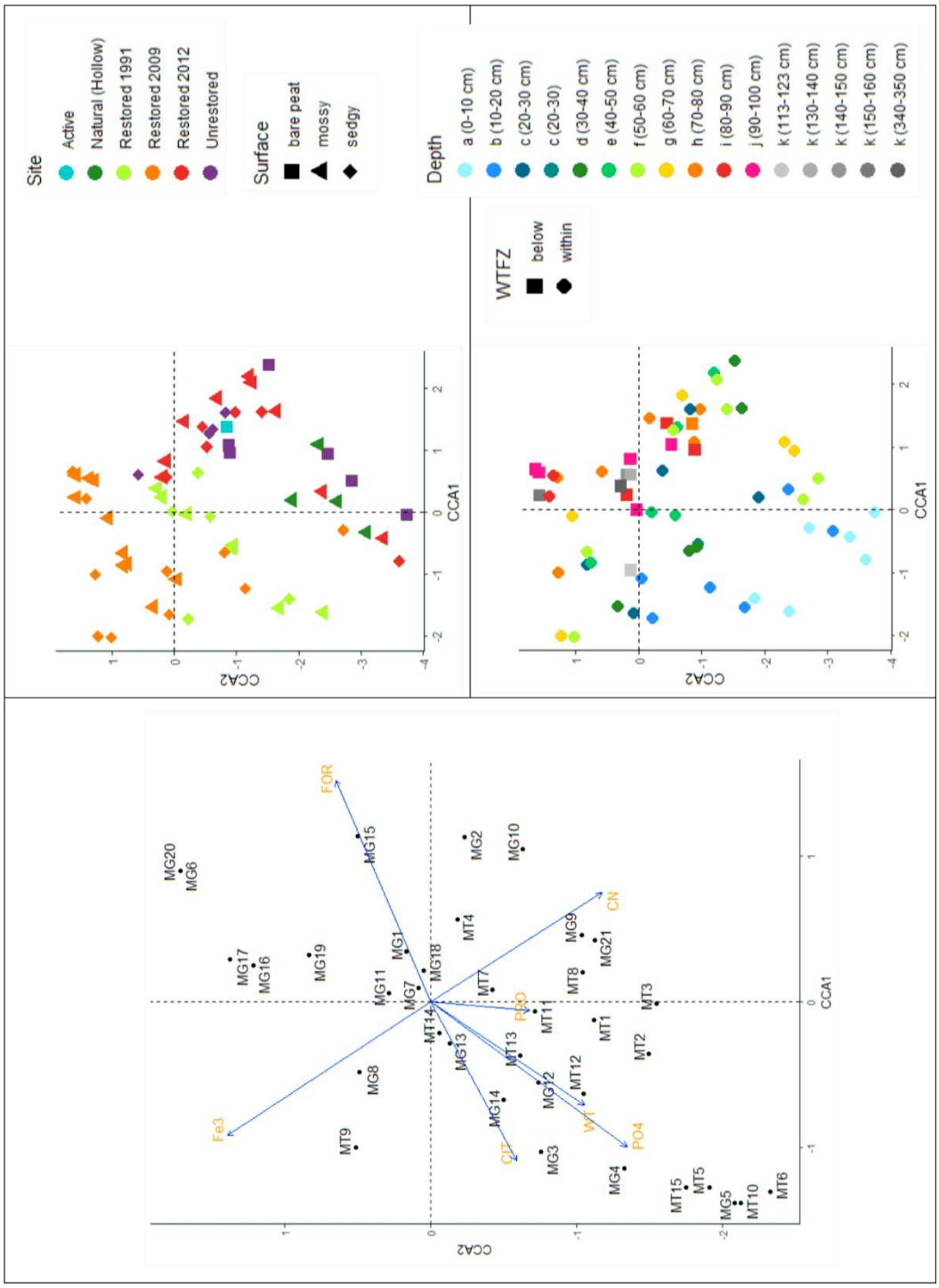

Figure 2. 6. Canonical correspondence analysis (CCA) of physicochemical conditions and their relationship with the absolute normalized abundance of methanogens and methanotrophs. Fe3 – ferric iron, PO4 – phosphate, CIT – citrate, PRO – propionate, FOR – formate, CN – total C:N ratio, WT – depth of peat in relation to the water table level at the time of sampling, WTFZ – water table fluctuation zone. See Tab. 2.3. for the explanation of codes for methanogens and methanotrophs.

MP did not vary significantly between sites and surface cover (Fig. 2.7). MP in RES-2009 and the Unrestored site was below 0.09 µmol g^{-1} d^{-1} except for high MP in core #2 (4.36 and 2.52 µmol g^{-1} d^{-1}) and one sample from below the WT in a mossy core (3.71 µmol g^{-1} d^{-1}). MP in the Natural site was 0.01 – 1.53 µmol g^{-1} d^{-1} (Tab. A.2.2). Higher MP was observed in peat below the WT compared to above it, aside from at the Unrestored site and Natural hollow (Fig. A.2.4) but only at 10 – 20 cm above the WT was it significantly lower than below the WT (Kruskal-Wallis for MP by depth zone $H(2) = 10.393$, $p = 0.00554$; Dunn post-hoc $p = 0.00416$, Fig. 2.7).

Three principal components (PC1, PC2, PC3, Fig. 2.8 and Fig. 2.9) of the physicochemical variables were included in the LME models as the explanatory variables for MP and MO. A significant positive relationship between MP and PC2 ($F_{1,17} = 31.88$, $p < 0.0001$) was found. PC2 consisted of [propionate], [acetate], [butyrate], [succinate], [pyruvate], [Fe^{3+}], and EC (Tab. 2.4A). MO did not show significant relationships with the physicochemical properties of peat. The ordination was influenced by a few samples (RES-2009-2A and 2B, NAT-2C and 6C, and UNR-4A) that showed unique physicochemical characteristics.

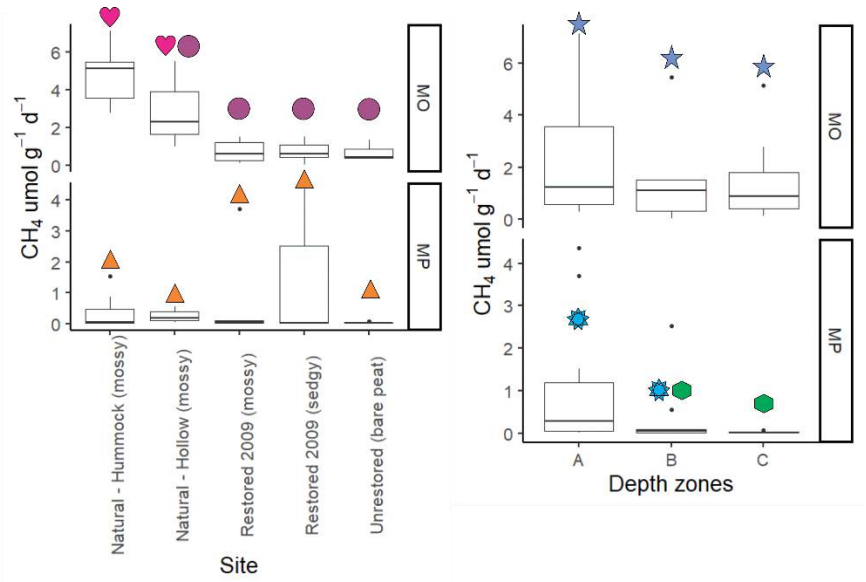

Figure 2. 7. Mean potential CH_4 production rates (MP) and mean potential CH_4 oxidation rates (MO) for each site and depth zone. Zone A is 0 – 10 cm below the water table (WT), zone B is 0 – 10 cm above the WT, and zone C 10 – 20 cm above the WT. MO and MP that vary significantly between sites or depth zones are labelled with no symbol in common. Symbols should be compared within a graph only, not between graphs.

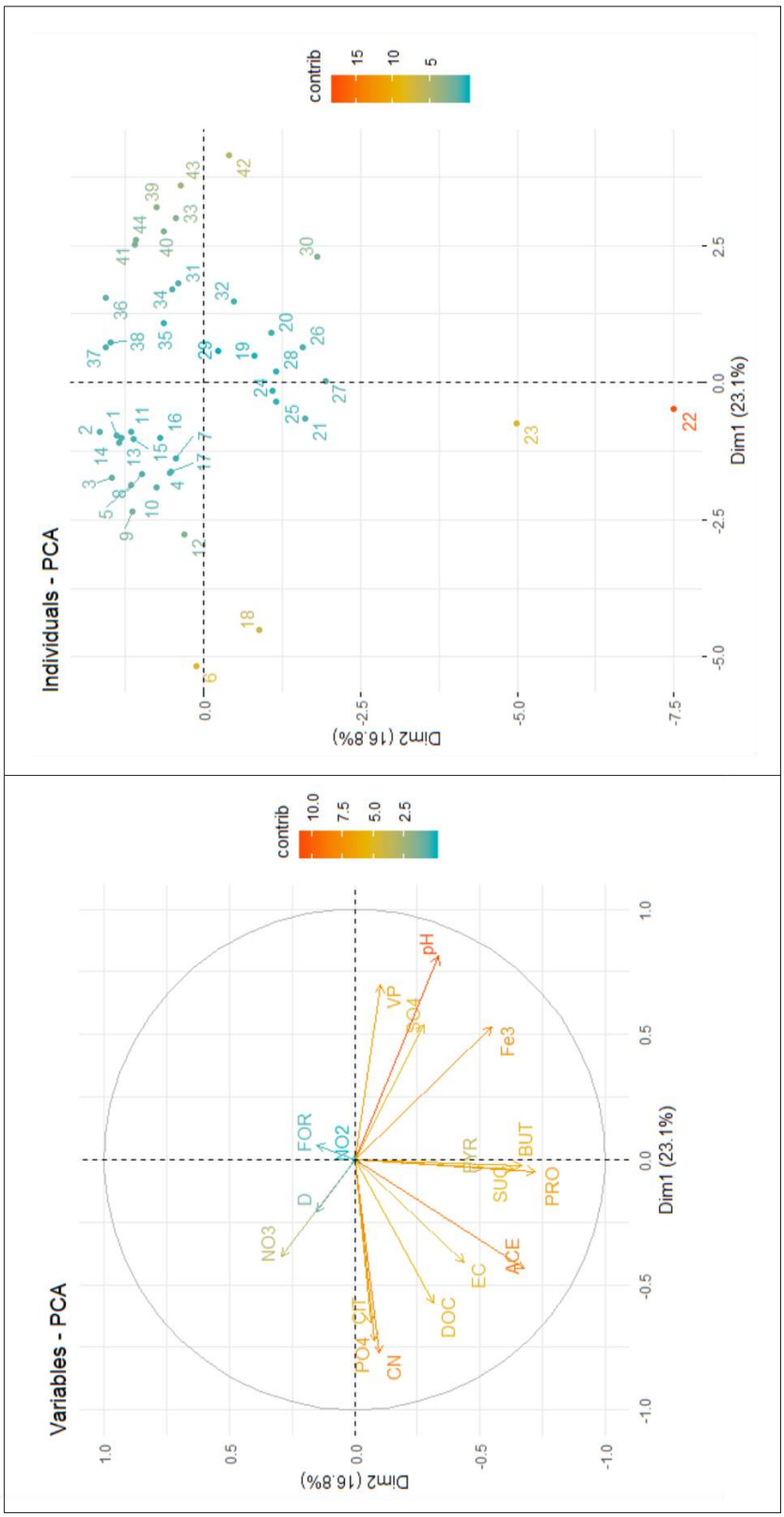

Figure 2. 8. Principal component analysis (PCA) showing variable (left) and sample (right) ordination of PC1 and PC2. See Tab. 2.4A, B (below) for explanation of variable and sample codes.

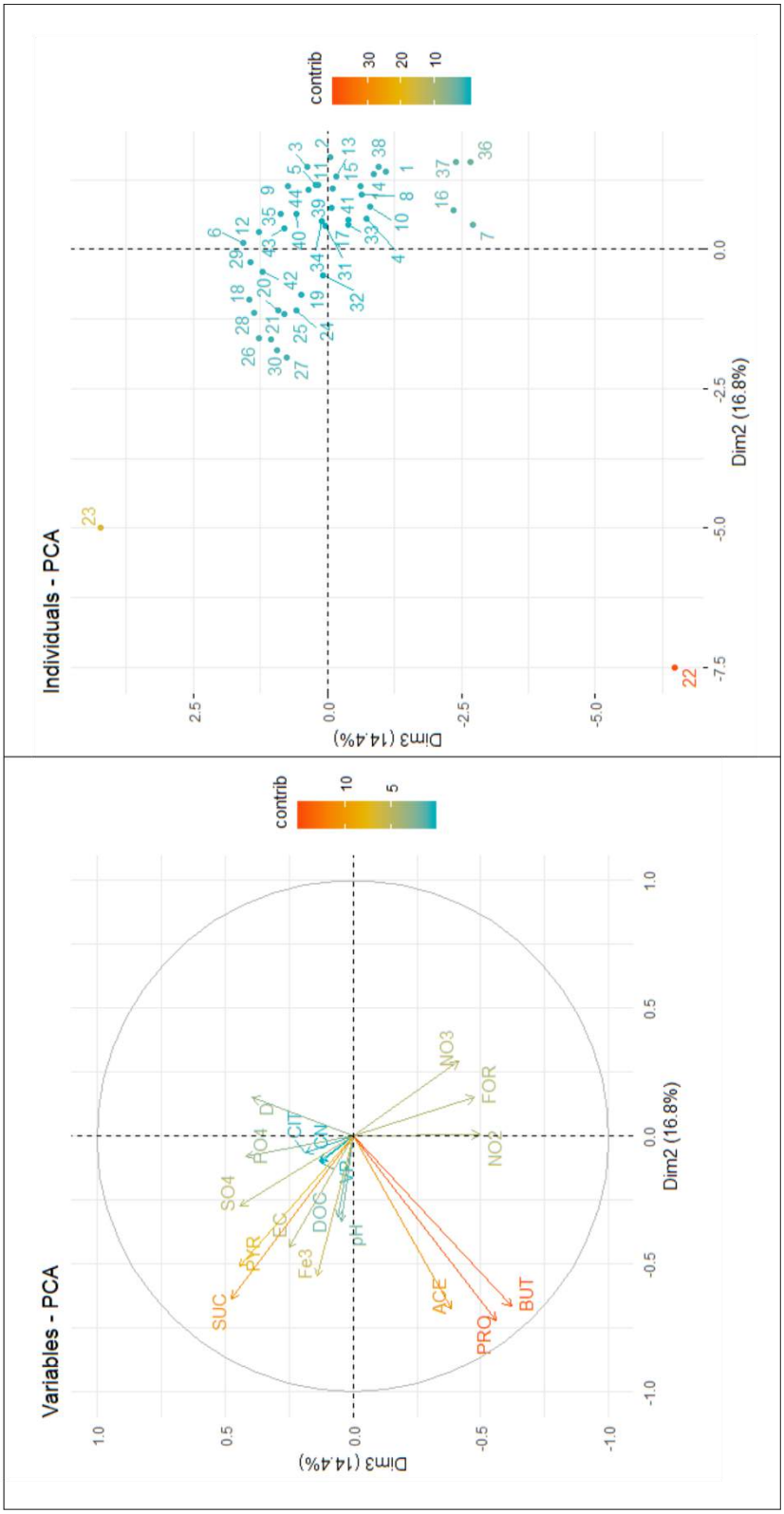

Figure 2. 9. Principal component analysis (PCA) showing variable (left) and sample (right) ordination of PC2 and PC3. See Tab. 2.4 A, B (below) for explanation of variable and sample codes.

Table 2. 4. Contribution of physicochemical variables to PC1, PC2, and PC3 (A) and codes used in the PCA sample ordination in Fig. 2.8 and 2.9 (B). RES-2009 - site restored in 2009, NAT - Natural site, UNR – Unrestored site, [ACE] – acetate, [BUT] – butyrate, [CIT] – citrate, [FOR] – formate, [PRO] – propionate, [PY R] – pyruvate, [SUC] – succinate.

A)

CODE	VARIABLE	PC1 (%)
pH	pH	15.90
CN	C:N	14.42
PO4	$[PO_4^{3-}]$	12.56
VP	Von Post	11.75
CIT	[CIT]	10.25
DOC	DOC	7.89
SO4	$[SO_4^{2-}]$	7.04
Fe3	$[Fe^{3+}]$	6.78

CODE	VARIABLE	PC2 (%)
PRO	[PRO]	17.20
ACE	[ACE]	15.11
BUT	[BUT]	14.65
SUC	[SUC]	13.30
DE3	$[Fe^{3+}]$	9.96
PYR	[PYR]	8.61
EC	EC	6.28

CODE	VARIABLE	PC3 (%)
BUT	[BUT]	14.81
PRO	[PRO]	12.07
NO2	$[NO_2^{-}]$	9.56
SUC	[SUC]	8.74
FOR	[FOR]	8.68
PYR	[PYR]	7.62
SO4	$[SO_4^{2-}]$	7.55
PO4	$[PO_4^{3-}]$	6.68
NO3	$[NO_3^{2-}]$	6.61
D	depth	5.99

B)

CODE	SAMPLE
1	NAT-1A
2	NAT-1B
3	NAT-1C
4	NAT-2A
5	NAT-2B
6	NAT-2C
7	NAT-3A
8	NAT-3B
9	NAT-3C
10	NAT-4A
11	NAT-4B
12	NAT-4C
13	NAT-5A
14	NAT-5B
15	NAT-5C
16	NAT-6A
17	NAT-6B
18	NAT-6C
19	RES-2009-1A
20	RES-2009-1B
21	RES-2009-1C
22	RES-2009-2A
23	RES-2009-2B
24	RES-2009-3A
25	RES-2009-3B
26	RES-2009-3C
27	RES-2009-4A
28	RES-2009-4B
29	RES-2009-4C
30	RES-2009-5A
31	RES-2009-5B
32	RES-2009-5C
33	RES-2009-6A
34	RES-2009-6B
35	RES-2009-6C
36	UNR-2A
37	UNR-2B
38	UNR-2C
39	UNR-3A
40	UNR-3B
41	UNR-3C
42	UNR-4A
43	UNR-4B
44	UNR-4C

2.6. DISCUSSION

2.6.1. Methanogenic and methanotrophic communities

We hypothesized that the abundance and diversity of CH_4-cycling microorganisms would be the highest in all restored sites and in the Natural site, and the lowest in the Unrestored and Active sites. However, we observed similar abundance of methanogens and methanotrophs averaged for each site in RES-2012, the Unrestored, and Natural sites (< 500 sequence counts), while it was higher in RES-1991 (929 sequence counts) and in RES-2009 (788 sequence counts). It should be noted that the Natural profile was limited to only a few depths and the Active profile to one depth. Both methanogenic and methanotrophic alpha diversity was the highest in RES-1991 and then comparable across the other sites. We did not observe a clear pattern of methanogenic and methanotrophic community composition, abundance and diversity with the age of restoration. The ratio of the sums of methanogenic to methanotrophic sequence counts also did not show any pattern with the age of restoration. The methanogenic ESV numbers were comparable with OTU numbers reported for peatlands by Yavitt *et al*., 2012, and so was Shannon diversity except for the values in RES-1991 that were twice as high as reported for a range of peatlands in North America (Yavitt *et al*., 2012). The general increase of methanogenic alpha diversity metrics with depth, and significantly higher values for communities below the WTFZ compared to the ones above the WTFZ, were expected. The ratio of the sums of methanogenic to methanotrophic sequence count followed the general pattern of increase with depth. Shallow peat is usually exposed to WT fluctuation but at greater depths it is constantly waterlogged, thus providing unchanged anoxic conditions required by methanogens prevail. However, beta diversity did not show clear dissimilarities between the methanogenic communities within the WTFZ and below the WTFZ. No significant diversity variation with depth was found for the methanotrophic community. Methanotrophs were more ubiquitous and often uniformly distributed in the peat profile as they are better adapted to life at various depths and redox conditions; *e.g*., Blodau and Moore (2003) determined that methanotrophs responded to changes in WT fluctuation within days while methanogens required months. The presence of methanotrophs in waterlogged peat can be explained with oxic microsites in the bulk anoxic peat, which could be related to the WT fluctuations and

trapping air bubbles (Baird *et al*., 2004) and O_2 supply by the root system (Strack *et al*., 2006b).

Following Reumer *et al.* (2018), who obtained similar CH_4 turnover trends for all restored sites, we expected all restored sites to be similar in their CH_4 cycling community, but only RES-1991 and RES-2009 had similar communities. In contrast, the youngest restored site, RES-2012, had a distinct community structure that was similar to the one in Unrestored, Active, and Natural sites. Beta diversity did not clearly separate any of the sites from each other, likely due to their location in the same peatland complex. Yavitt *et al*. (2012) found similar methanogenic community composition (UniFrac) in sites located close to each other. We expect that the vegetation (*i.e*., abundance of graminoids) combined with hydrological conditions can supersede the age of restoration, and hence result in the observed similarities between RES-1991 and RES-2009. We sampled at a moderately wet, less sedgy part of RES-2012, which may explain why its microbial community resembled those of the Unrestored and Natural sites. Andersen *et al*. (2013b) reported more similarities in microbial functions in Natural and vegetated Unrestored sites compared to a restored one, finding a greater importance of vegetation than restoration-related physicochemical features of peat for the microbial processes. In contrast, Reumer *et al*. (2018) found the methanogenic community of unrestored sites similar to each other but not to these at restored and natural sites.

A fairly large portion of the methanogenic community in the Seba Beach peatlands were organisms with no cultured relatives. We detected taxa that were only recently identified as methanogenic: *Bathyarchaeia*, *Verstraetearchaeia* and *Methanofastidiosales* (Lyu *et al*., 2018). *Methanomicrobiales* dominated the methanogens in Seba Beach sites, similar to other bogs and fens (Horn *et al*., 2003, Cadillo-Quiroz *et al*., 2006, Hoj *et al*., 2008, Godin *et al*., 2012). *Methanoregula* (MG7, one of the *Methanomicrobiales*) known for its abundance in acidic bogs (Sun *et al*., 2012, Reumer *et al*., 2018) and *Bathyarchaeia* (MG1) were the most abundant methanogens and were found across all peatlands. *Methanomicrobiales* and *Bathyarchaeia* (previously *Bathyarchaeota*) formed the majority of the archaeal community in terrestrial ecosystems and are potentially symbiotic with each other (Xiang *et al*., 2017). The methanogenic community at the Natural site was dominated by *Cand*. Methanomethylicus and *Methanosarcina sp.* of Rice Cluster II that has been detected in northern bogs (*e.g*., Cadillo-

Quiroz *et al*., 2006) and fens (*e.g*., Godin *et al*., 2012). RES-1991 contained almost all methanogenic taxa identified in our study including acetoclastic *Methanosaeta* and *Methanosarcina* that were not observed in the Natural site. Other methanogens that were found in RES-1991, have been observed at natural bogs and fens. *Methanomassiliicoccales* was widely distributed in European bogs and fens (Söllinger *et al*., 2015), *Methanosaetaceae* (MG13) was abundant in fens (Galand *et al*., 2005, Godin *et al*., 2012) and *Methanobacterium* (MG3) was found in Canadian natural, restored and unrestored post-extracted peatlands by Basiliko *et al*. (2013) and in natural acidic northern Finnish peatlands by Metje and Frenzel (2005). We detected Rice Cluster I at low abundance only in the surface peat of RES-1991, while it was abundant in a natural Canadian peatland (Basiliko *et al*., 2013). It appeared that lack of vegetation at the Unrestored site determined the absence of *Methanoregula* and *Methanosaeta*. Likely the encroachment of non-wetland plant species in the Unrestored sites shifted the methanogenic community, at least partially, towards the ones observed at the youngest restored site, but it did not translate directly into higher methanogenic activity.

Our results indicate that there might be a link between the abundance of vascular plants in restored sites and the CH_4-cycling community structure, but we did not find significant differences between methanogens in the mossy and sedgy locations. Cadillo-Quiroz *et al*. (2009) found that the archaeal community structure varied in peat depending on the species of plant and the presence of roots. Putkinen *et al*. (2018) reported that *Methanosaetaceae* were associated with shrubs, *Methanobacteriaceae* with sedges and *Methanoregulaceae* with *Sphagnum* moss. These associations were not observed in Seba Beach peatlands.

The structure of the methanotrophic communities in Seba Beach sites was fairly similar to that observed in other peatlands. *Gammaproteobacteria* of the order *Methylococcales* dominated the methanotrophs. Similar community composition with low abundance of type II methanotrophs and high abundance of *Methylococcales* was reported by Narrowe *et al*. (2017) in wetland soils. The largest abundance of *Alphaproteobacteria* was found in the Natural site, which is in accordance with their identification as stress tolerant in natural peatlands (Ho *et al*., 2013). *Alphaproteobacteria* have been found in boreal fens and acidic bogs (Yrjälä *et al*., 2011, Juottonen *et al*., 2012, Esson *et al*., 2016). *Methylocella* (MT1) is a facultative anaerobe that can utilize CH_4, acetate, succinate, and pyruvate, but prefers acetate over CH_4 and shuts

down methanotrophy in the presence of acetate (Dedysh *et al*., 2005). *Methylocystis* (MT2) can also use acetate instead of CH_4 (Belova *et al*., 2011). *Methylomonas* (MT11) and *Methylovulum* (MT12) found mainly in RES-1991 (mean pH 5.1) were the active type I methanotrophs in an acidic bog (Esson *et al*., 2016). *Methylobacter* was one of the most abundant methanotrophic taxa detected in our samples and was found only in the older restored sites (RES-1991 and RES-2009). *Methylobacter* is ubiquitous in various environments, found in both oxic and anoxic conditions, can use various C1 substrates with denitrification potential that could sustain respiration when O_2 is depleted, and has been reported as the most abundant key taxon for CH_4 oxidation in wetlands (Smith *et al*., 2018). The versatility of this genus likely explains its abundance in disturbed peatlands; however, the abundance of this and other methanotrophs in anoxic peat could be due to the presence of inactive organisms.

We expected the Unrestored site to vary in its CH_4-cycling community from the restored and the Natural sites and indeed, the methanotrophic profile of the bare peat location at the Unrestored site was unique; four out of six methanotrophic taxa present in the bare peat core (*Cand*. Methylospira, *Crenothrix*, *Methylomonas*, and *Methylovulum*) were not found in the top 70 cm of the core – their largest abundances were detected below the WTFZ. Intense WT fluctuations could have forced these microorganisms down into deep peat. Both methanogens and methanotrophs can be shifted in the peat profile by fluctuating water table in disturbed peatlands (Andersen *et al*., 2013a, b). The diversity and abundance of methanotrophic *Bacteria* was higher in older restored sites than in Unrestored or young restored sites showing that restoration promotes regeneration of the methanotrophic community in post-extracted peatlands.

2.6.2. Potential rates of CH_4 production and oxidation

We hypothesized that MP would be highest in the sedgy restored site followed by the Natural and Unrestored, but MP was low at the restored site and comparable to MP in the Unrestored. Rates of MP at RES-2009 were mostly < 0.1 μg g^{-1} of dry peat, similar to values reported by Yrjälä *et al*. (2011) and Reumer *et al*. (2018), with only a few CH_4 production hot spots at RES-2009 likely related to the presence of sedgy roots and their exudates (*e.g*., Knorr *et al*.,

2008a, Robroek *et al*., 2015) and/or the presence of acetoclastic methanogens. The overall MP at the Natural and RES-2009 was higher than at the Unrestored as observed in another study (Basiliko *et al*., 2007). MP in the Natural site showed a broader range of values (0.01 – 1.53 μmol g^{-1} d^{-1}) than a natural Finnish mire at a similar temperature of incubation (0.25 – 0.5 μmol g^{-1} d^{-1}, Metje and Frenzel, 2005), and was comparable with MP reported by Putkinen *et al*. (2018) for a natural fen at 15 ˚C (0.48 – 1.92 μmol g^{-1} d^{-1}).

As hypothesized, MP was significantly higher below than above the WT (Sundh *et al*., 1994, Waddington and Day, 2007, Knorr *et al*., 2008b). MP that occurred above the WT in our study showed that CH_4 production was not limited to permanently waterlogged peat (Glatzel *et al*., 2004, Juottonen *et al*., 2012), contrary to the observation of Andersen *et al*. (2013a).

We expected high MO in the Unrestored site, but it was considerably lower than at the Natural site. This supports the importance of substrate availability in CH_4 oxidation (Juottonen *et al*., 2012). No significant differences in MO among depth zones reflected the depth-independent distribution of methanotrophs at the Seba Beach sites, while other studies showed the WT level dependence of MO (*e.g*., Whalen and Reeburgh, 2000). The zone above the WT is predominantly unsaturated and thicker at hummocks than at hollows. MO at hummocks in our study was higher (1.02 – 7.11 μmol g^{-1} d^{-1}) than in the remaining sites (< 2 μmol g^{-1} d^{-1}, similar to rates < 2.4 μmol g^{-1} d^{-1} in restored and natural fens (Putkinen *et al*., 2018)). These rates were comparable with MO at 25 ˚C in unrestored and active sites reported by Reumer *et al*. (2018) and in a fen at 15 ˚C (Yrjälä *et al*., 2011). MO in the Natural site was at the level of MO at hummocks and hollows in a Tibetan peatland (Deng *et al*., 2013). The Seba Beach Natural site consisted mainly of poorly decomposed *Sphagnum* and MO is known to increase with *Sphagnum* cover (Putkinen *et al*., 2018), which is likely linked to symbiotic methanotrophs that colonize *Sphagnum* (*e.g*., Raghoebarsing *et al*., 2005, Larmola *et al*., 2010, Kip *et al*., 2011).

2.6.3. The effect of inorganic ions and short chain fatty acid ions on the methanogenic and methanotrophic community structure and potential rates of CH_4 production and oxidation.

Since the Seba Beach sites were likely one peatland before peat extraction, we expected that physicochemical characteristics would be similar in all restored sites; however, the two oldest restored sites had moderate to high [Fe^{3+}] and low C:N while the other sites had low [Fe^{3+}], high C:N and, aside from the Active site, high [propionate]. These features were reflected in the similarities between methanogenic and methanotrophic communities in RES-1991 and RES-2009 and between RES-2012, Unrestored, Natural, and Active sites. [Fe^{3+}] was the only potential TEA significantly linked to MP (Linear mixed effect models with MP and PCA output as explanatory variables; the significance of PC2 composed of short chain fatty acid ions and Fe^{3+}) and to the abundance of CH_4-cycling microorganisms (CCA analysis). High [Fe^{3+}] in RES-2009 could have had a negative impact on CH_4 production, hence the generally low MP observed at this site, but it did not seem to decrease methanogenic population. We suppose that high [Fe^{3+}] in RES-2009 was supplied from clay. The organic-mineral soil interface was at 150 – 160 cm depth, with the WT fluctuation down to 90 cm depth. High [Fe^{3+}] (2187.5 µg g^{-1} of dry peat) was found in the bottom peat that was mixed with clay. Jeffrey *et al*. (2019) observed a relationship between CH_4 emission from wetlands and the geochemistry of underlying sediment that was expressed as a significant negative relationship between CH_4 flux and the concentration of Fe^{3+} and SO_4^{3-}.

With SO_4^{2-} present only in the first 30 cm of peat, and with very low [NO_2^-] and [NO_3^-], high [Fe^{3+}] was the most abundant TEA, and thus most likely the main factor supressing methanogenesis at RES-2009. Iron is also required by methanogens in physiological processes (Basiliko and Yavitt, 2001), which additionally enhances the importance of Fe^{3+}. All Seba Beach restored sites showed higher [Fe^{3+}] than the Natural site, similar to the higher [Fe] observed in restored fens than in natural ones by Aggenbach *et al*. (2013). High [Fe] hinders restoration progress as Fe^{2+} is toxic to plants. A reliable O_2 supply via roots to deplete the [Fe^{2+}] is required, and the dominance of vascular plants like sedges in restored sites is likely due to their resistance to high [Fe^{2+}] (Begg *et al*., 2004, Aggenbach *et al*., 2013).

Although microorganisms require PO_4^{3-} for physiological processes, PO_4^{3-} inhibits acetoclastic methanogenesis (Conrad *et al*., 2000, Paulo *et al*., 2005); however, the highest abundance of acetoclastic *Methanosaeta* and *Methanosarcina* at the Seba Beach sites were close to the WT (likely due to root exudates supply) where also moderate to high [PO_4^{3-}] was found. The highest abundance of microorganisms in peatlands was previously found in the mesotelm, where they mobilize PO_4^{3-}, reducing its limitation (Lin *et al*., 2014). Many hydrogenotrophic methanogens and methanotrophs were found at moderate to high [PO_4^{3-}] in the mesotelm at the Seba Beach sites which underlines its importance for microbial growth and CH_4 cycling.

Contrary to our hypothesis that some TEA and short chain fatty acids would affect MO, we found no significant relationships. Acetate (together with propionate, butyrate, and succinate) was one of the main factors that significantly affected MP, but there were only a few samples (*e.g*., sedgy core #2. RES-2009 from 2017) that contained large amounts of these protonated acids and thus drove the importance of these variables in our analyses. We did not find the relationship posited by Horn *et al*. (2003), where formate, acetate, propionate, and butyrate inhibit the production of CH_4. Since the availability of substrate is one of the most important factors controlling methanogenesis (*e.g*., Couwenberg, 2009), the link between the high abundance of short chain fatty acids and high MP we observed was likely related to the abundance of labile carbon from root exudates in the dense rhizosphere at RES-2009.

2.7. CONCLUSIONS

RES-1991 and RES-2009 showed similar methanogenic and methanotrophic community characteristics, which were different from the shared profiles seen in RES-2012, the Unrestored, Natural, and Active sites. Methanogen counts, abundance, and evenness increased with depth, whereas methanotrophs were more evenly distributed in peat profile. The highest MP was found immediately below the WT. Fe^{3+} was the most important TEA affecting CH_4 community characteristics and MP. The highest [Fe^{3+}] were found in the restored sites. The shallow peat at RES-2009 was likely supplied with [Fe^{3+}] from the mineral soil under the peat deposit, which could have led to the observed methanogenesis suppression, even though the

CH_4-cycling community was relatively well developed. MP hot spots were found RES-2009, some of them linked to the presence of dense sedges and high concentration of short chain fatty acid ions. The Natural site showed relatively uniform and high MP and the highest MO, especially at hummocks. MO was similar below and above the WT, but MP was significantly higher immediately below the WT compared to 10 – 20 cm above it for all locations. MO was not driven by any of our measured physicochemical factors, while MP was affected by the concentration of acetate, propionate, butyrate, succinate, and pyruvate. The abundance of methanogens and methanotrophs was associated with the concentration of formate, citrate, propionate and phosphate. Restoration promoted the development of active CH_4-cycling microorganisms while post-extraction abandonment resulted in largely inactive and less diverse communities. The spontaneous re-vegetation of the Unrestored site increased the abundance of CH_4-cycling organisms compared to the bare peat, but not MP and MO, which remained close to zero. Restoration assists the recovery of methanogenic and methanotrophic community; however, the presence of dense vascular plants, mainly sedges, at early stages of restoration, in combination with high WT, is likely to form different community structure than at the Natural site and may overshadow the contribution of the site's restoration age to the microbial community development.

CHAPTER 3: Steady and ebullitive methane fluxes from active, restored and unrestored horticultural peatlands

3.1. ABSTRACT

Peatlands used for horticultural peat extraction lose their ecological function of carbon accumulation. While their restoration has been shown to increase methane (CH_4) flux compared to unrestored sites, the time required for the greenhouse gas (GHG) balance to recover and the factors affecting the recovery remain unclear. We quantified CH_4 emission from several ages of restored sites, as well as unrestored post-extraction (Unrestored) and actively extracted (Active) sites, and compared them to CH_4 emission from a natural boreal bog (Natural). All study sites were located within one horticulture peatland complex in central Alberta, Canada. Both steady (diffusive and steady ebullitive) fluxes and abrupt ebullitive events were determined using manual chambers and a portable greenhouse gas analyzer. Abrupt ebullition occurred only at two restored sites that showed the highest steady flux, were flooded/wet and dominated by vascular plants. Ebullition accounted for 7 % of total CH_4 emission at the site restored in 1991 (25 – 26 years post-restoration), and 6 % at the site restored in 2012 (4 – 5 years post-restoration). The third restored site (7 – 8 years post-restoration), showed no abrupt ebullition and mean steady flux lower than at the Natural site, likely caused by geochemistry of peat overriding shallow water table and dense sedge cover. The lowest CH_4 emission was found at the Unrestored and Active sites. At sites where it occurred, ebullition was significantly but weakly correlated with CH_4 flux, [CH_4] in pore water, soil temperature, water table (WT), gross ecosystem production (GEP), and percentage cover of moss. Steady CH_4 fluxes were driven by soil temperature at 20 cm depth, the WT, GEP, and the cover of shrubs and graminoids. The physicochemical peat characteristics can supersede these environmental factors and suppress CH_4 emission even in restored, wet, and sedge-dominated sites. Restoration enhanced CH_4 emission compared to the Unrestored site. Restored sites with more fen-like conditions (wet and sedgy) are likely to show abrupt ebullition events and higher CH_4 fluxes than undisturbed bogs, with local controls seemingly more important than time since restoration on resulting CH_4 emission.

3.2. INTRODUCTION

Approximately 67 % of peatlands are located in the northern hemisphere in temperate, boreal and subarctic regions above 45°N and are referred to as the northern peatlands (Limpens *et al*., 2008, Serkebaeva *et al*., 2013, Xu *et al*., 2018). They began to develop after the last glacial period, and continued accumulating organic matter during the Holocene, acting as a sink for carbon dioxide (CO_2) and cooling the atmosphere (Harden *et al*., 1992). These peatlands store up to 1000 Pg (Pg = 10^{15} g) of carbon (Gorham, 1991, Yu, 2012, Amesbury *et al*., 2019). Natural peatlands, thanks to their low pH, waterlogged peat, the presence of vegetation with decay resistant litter, and the nutrient poor environment for microorganisms, combined with low temperatures of northern latitudes, promote organic matter accumulation (*e.g*., Frolking *et al*., 2011), with current accumulation rates up to 10 cm per 100 years (0.1 cm yr^{-1}, Glaser *et al*., 2004).

About 26.8 % of the world's peatlands (113 million ha) is located in Canada (Xu *et al*., 2018), but only 34,000 ha have been disturbed for horticultural peat extraction (ECCC, 2018), while according to Canadian Sphagnum Peat Moss Association, this number is overestimated, since ECCC add 1000 ha to the estimation each year, and was 31,676 ha in 2017 (CSPMA, 2018). Thus, horticulture use of peatlands constitutes more of a local environmental disturbance in Canada (Price *et al*., 2003). Meanwhile, peat extraction in Europe represents a proportionally larger contribution (~ 6 %, Vasander *et al*., 2003). Peat in Canada is extracted solely for horticultural purposes with the vacuum-harvesting method, which has been widely used since 1980s as an alternative to the peat cutting method (Environment Canada, 2006, 2015). Horticulture peat extraction proceeds until it reaches peat of undesirable quality for the industry, usually for 20 – 30 years (Tuittila *et al*., 2000a, Wind-Mulder and Vitt, 2000, Waddington *et al*., 2009b); however, the industry ensures that peat of sufficient thickness for restoration is left after peat extraction. Peatland restoration in Canada started in 1990s and thus Canadian restored sites are relatively young (Rochefort, 2000). However, measurable changes occur at restored peatlands even within the first decades after restoration, *e.g*., the recovery of peatland vegetation following the moss layer transfer technique of restoration (Graf and Rochefort, 2016), improved hydrological conditions (higher and more stable water table,

McCarter and Price, 2015), and subsequently the return of the carbon accumulation function of peatland (*e.g.*, Nugent *et al.*, 2018).

Currently, natural northern peatlands represent a weak net sink of carbon dioxide (CO_2), and a source of methane (CH_4; Roulet *et al.*, 1994, Baird *et al.*, 2009, Frolking *et al.*, 2011). Wetlands are the largest natural source of CH_4 with peatlands alone accounting for 5 – 10 % of the global CH_4 emission to the atmosphere (Blodau, 2002, McKenzie *et al.*, 2009). Peat extraction disrupts the natural gas balance as peat fields become CO_2 emitters and sinks for CH_4 (Sundh *et al.*, 2000, Turetsky *et al.*, 2002, Waddington *et al.*, 2002); however, if left open, drainage ditches remain a source of CH_4 (Sundh *et al.*, 2000, Waddington and Day, 2007, Strack and Zuback, 2013, Nugent, 2019).

Methane is produced in waterlogged anoxic conditions by methanogenic *Archaea* in the last stage of anaerobic degradation of organic matter and it is utilized by methanotrophic *Bacteria* (Garcia *et al.*, 2000, Rosenberry *et al.*, 2006, Lai, 2009, Couwenberg and Fritz, 2012, Andersen *et al.*, 2013a). Produced CH_4 can be released from peatlands to the atmosphere via diffusion through the peat matrix, plant-mediated transport through aerenchyma (diffusion and pressurized flow), and ebullition of free-phase gas bubbles (Chanton, 2005, Coulthard *et al.*, 2009, Couwenberg 2009, Green and Baird, 2013, Stamp *et al.*, 2013). A portion of CH_4 diffusing through the oxic peat zone becomes oxidized to CO_2 (Fechner-Levy and Hemond, 1992). Plant-mediated transport accounts for 30 – 100 % of total CH_4 emission (Whiting *et al.*, 1992, Bridgham *et al.*, 2013 and the references therein). The contribution of ebullition to the total CH_4 emission varies, although it is considered an important mode of CH_4 release (*e.g.* Glaser *et al.*, 2004, Tokida *et al.*, 2005, Strack and Waddington, 2008, Coulthard *et al.*, 2009, Parsekian *et al.*, 2010). Steady ebullition occurs when bubbles are released in a steady stream, while episodic ebullition refers to a single abrupt release of large amounts of free-phase gas (Green and Baird, 2012). In this study, we refer to a linear increase in CH_4 concentration ($[CH_4]$) in the chamber headspace as steady flux and this can possibly contain both diffusive emission and steady ebullition, while ebullition is used to describe the episodic ebullition events only.

Increasing temperature intensifies CH_4 production (Dunfield *et al.*, 1993, Lai, 2009) and enhances both diffusion and ebullition rates (Fechner-Levy and Hemond, 1996,

Waddington et al., 2009a, Gogo *et al*., 2011, Jeffrey *et al*., 2019). The water table (WT) level, together with the capillary fringe, set the boundaries between oxic and anoxic zones in the peat deposit and affects largely CH_4 production and emission (Guertin *et al*., 1987, Niedermeier and Robinson, 2007, Lai, 2009, Couwenberg and Fritz, 2012, Martí *el al*., 2015). Shallow WT is associated with higher CH_4 fluxes (Strack *et al*., 2017), while a falling WT (decreasing hydrostatic pressure) triggers ebullition (Fechner-Levy and Hemond, 1996). Rising WT can entrap atmospheric air in surface peat, forming air pockets and promoting CH_4 exsolution from pore water and bubble growth (Baird *et al*., 2004). Following peat extraction, fields lack an acrotelm, known to regulate hydrological conditions in peatlands. The remnant cutover peat has lower porosity and specific yield resulting in a WT that fluctuates more than in natural peatlands (Price *et al*., 2003, Basiliko *et al*., 2007) and induces instability of the anoxic zone for methanogens. In contrast, some restored sites can become flooded, resemble a fen-like ecosystem, and emit more CH_4 than natural bogs (Strack *et al*., 2014, Jordan *et al*., 2016, Putkinen *et al*., 2018).

The presence of vascular plants increases the steady CH_4 flux due to CH_4 venting through aerenchymatic tissue (*e.g*., Ström *et al*., 2005, Koelbener *et al*., 2010, Green and Baird, 2012). Plant-mediated emission and rhizospheric CH_4 oxidation lowers gaseous and dissolved [CH_4] (Ström *et al*., 2005, Coulthard *et al*., 2009, Kettridge *et al*. 2011). Root exudates provide labile carbon for methanogenesis and increase CH_4 flux, which is linked to the magnitude of primary production (Waddington *et al*., 1996, Joabsson *et al*., 1999, Marinier *et al*., 2004). Vegetation is removed from extracted peatlands and restoration at its early stages often leads to recovery of peat forming vegetation different than at natural bogs (*e.g*., higher cover of sedges and other vascular plants, Gonzalez and Rochefort, 2019). Thus, CH_4 emission patterns are likely to vary from those at natural sites. The role of plants in ebullition is not fully understood, but the presence of sedges (*e.g*., *C. rostata* and *E. vaginatum*) has been linked to higher CH_4 ebullition in some studies (Christensen *et al*. 2003; Ström *et al*., 2005, Strack *et al*., 2006a, Gogo *et al*., 2011, Klapstein *et al*., 2014). In contrast, dense roots are known to trap gas bubbles preventing their release (Strack *et al*., 2006a, Coulthard *et al*., 2009).

Several studies report CH_4 fluxes from restored peatlands (*e.g*., Komulainen *et al*., 1998, Tuittila *et al*., 2000a, Waddington and Day, 2007, Wilson *et al*., 2009, Mahmood and

Strack, 2011, Juottonen *et al*., 2012, Strack and Zuback, 2013, Strack *et al*., 2014, 2016, 2017), but it is unknown how the flux and CH_4 dissolved in pore water develops over time post-restoration. To the best of our knowledge among ebullition studies on peatlands (*e.g*., Strack *et al*., 2005, 2006a, Tokida *et al*., 2007a,b, Gogo *et al*., 2011, Goodrich *et al*., 2011, Stamp *et al*., 2013, Klapstein *et al*., 2014), there is only one that involves ebullition quantification at a restored site (Nugent, 2019) and no such studies are available for a chronosequence of restored sites, or for unrestored and currently extracted sites. We do not know whether the ebullitive contribution to total CH_4 emission from these sites is substantial or what factors contribute to ebullition post-extraction.

The objectives of the study were to quantify CH_4 emission from sites restored following horticultural peat extraction, including ebullition contribution, evaluate how these fluxes vary over the growing season, determine the development of CH_4 flux and pore water concentration over time since restoration, and compare results with currently extracted (Active), unrestored post-extraction (Unrestored) and natural bog (Natural) sites. We also investigated the environmental factors that controlled CH_4 stocks and fluxes. We hypothesized that:

1) CH_4 emission would be higher at restored sites than at the Natural site and will be the lowest at the Unrestored and Active sites. The older restored sites would have a higher CH_4 emission than the younger ones. Ebullition will occur at the Natural and restored sites only.
2) The [CH_4] in pore water, steady flux, and ebullition will increase over the growing season. The [CH_4] in pore water will reflect the emission pattern; it will increase from the Active and Unrestored sites, through young restored to the oldest restored and Natural sites.
3) Ebullition will be driven by the same environmental factors as steady emission including the percentage cover of vascular plants (shrubs and graminoids), soil temperature, water table, and the [CH_4] in pore water.

3.3. STUDY SITE

The Seba Beach peatland complex is located in central Alberta, Canada (53° 33' N, 114° 44' W) west of the city of Edmonton. The site has been used for peat extraction since 1975 (Wind-Mulder and Vitt, 2000). The complex comprises closely neighboring currently extracted, natural, and post-extraction restored, and unrestored sites (Fig. A.1.1). Most likely they were a part of the same peatland that became divided into extraction sites and separated by access roads. We chose a currently extracted (Active) site, an unrestored (Unrestored), three sites restored in 2012, 2009 and 1991 (RES-2012, RES-2009, RES-1991, respectively) and a natural peatland (Natural) within the peatland complex. The natural site was a treed boreal bog with well-developed hummocks and hollows and constituted a reference site (Fig. A.1.3A). The moss layer transfer technique was used to restore RES-1991, RES-2009 and RES-2012 (see Rochefort *et al*., 2003 for details of the method). The ditches in RES-1991 (Fig. A.1.3 B) have been blocked but not filled with peat (both are standard methods, Quinty and Rochefort, 2003). The site was flooded, with the presence of a partially floating mat, dense *Sphagnum* forming mats and hummocks, and dense sedges. A thick layer (> 20 cm) of newly developed fresh peat suggests that the peat accumulation exceeded the regular rates of 0.1 cm per year. RES-2009 (Fig. A.1.3C) was a shallow site (< 150 cm to 240 cm) with wet and moderately wet to dry parts. The latter was covered with a *Sphagnum* carpet overgrown with dense sedges and shrubs, while the wet part had a mix of shrubs, cattail, and true moss. The study site at the RES-2012 (Fig. A.1.3D) was set up in the southern part of the peatland. The east side of the study site was flooded and overgrown with graminoids but with little to no *Sphagnum* coverage, while the west part was dry, partially bare, with cottongrass tussocks and patches of true moss (largely *Polytrichum strictum*). The middle part was moderately wet with a drainage ditch running across the borderline between the wet and dry part, filled with peat but still saturated and covered with dense moss. The wet and moderately wet parts were flooded in 2017. The Unrestored site (Fig. A.1.3E) was left unrestored in 2012 for research purposes after the ditches were filled with peat and the surface levelled. A part of the site was vegetated with *Betula* spp. and *Carex* spp., but the majority of the peat surface was bare. The Active site was completely bare peat with drainage ditches located ~ 30 m apart (Fig. A.1.3F). The study was conducted in the northern part of the site close to the access road. Access boardwalks were

installed at all sites except the Active (due to active peat extraction) to minimize peat disturbance during data collection. More information on vegetation at these sites is presented in Chapter 4.

3.4. METHODS

3.4.1. CH_4 flux measurements

Plots were established in May and June 2016 at all study sites but the Active. Two main types of surface cover were targeted for the plot locations at each site in triplicate: sedgy and mossy at RES-1991, RES-2009, and RES-2012, sedgy and bare peat at the Unrestored, and mossy hummocks and hollows at the Natural. Altogether, six metal collars (60 × 60 cm) were installed up to 20 cm into the ground at each site (30 collars in total) and left in place for the remainder of the study (See Fig. A.1.2 for detailed location of the collars). Three collars were placed in the ground at the Active site only for the time of measurement and removed immediately afterwards due to heavy machinery operating at the site.

A transparent chamber, 60 × 60 × 30 cm, was placed on the collar and a portable gas analyzer (Los Gatos Research, GGA-30p ultraportable greenhouse gas analyzer) was used to measure [CH_4] and [CO_2] in the chamber headspace, corrected for moisture content, simultaneously every second. The chamber was equipped with two battery-powered fans that mixed the air inside the chamber and a cooling system to prevent overheating. The cooling system was a closed circuit of cold water constantly pumped through a copper tubing installed inside the chamber just in front of the fans.

Long, 30 – 45-minute, measurements were conducted under full light and used for calculating CH_4 flux. Methane exchange was measured from May 17th to July 5th, 2016 with the portable analyzer. Due to equipment malfunction, CH_4 flux was measured using gas samples collected in vials between July 11th to August 31st, 2016. Almost all flux measurements in 2017 were performed with the portable analyzer, except when the access to the plots was limited due to severely flooded conditions and the vial method was used instead. Both methods give comparable results (Murray, 2017). Gas exchange from each plot was measured biweekly from May 17th to August 31st in 2016 and every week from May 8th to

August 28th in 2017. Fluxes at the Active site were measured once a month from May to August 2017. For CH_4 flux measurement with vials, we used a syringe to collect 20 mL of gas from the chamber headspace into evacuated 12 mL exetainers (Labco Ltd. UK). In 2016, samples were collected at 5, 15, 25, and 35 minutes after chamber closure, while in 2017 samples were collected at 5, 10, 15, and 20 minutes as data from in field measurements of [CH_4] with the portable analyzer indicated that accumulation of CH_4 in the chamber reduced measured flux over longer closure times. In both cases, ambient air was collected to vials at least once for each set of measurements at a site. Methane concentration in vial samples was measured using a gas chromatograph (GC; Shimadzu GC2014 with EST Flex automatic sampler) at the Wetland Soil and Greenhouse Gas Exchange Lab at the University of Waterloo. Measurements were collected at different times of day during the growing season at each collar to avoid bias. Steady CH_4 fluxes were calculated based on the linear increase in [CH_4] in the chamber over time, corrected for actual volume of the chamber and air temperature. For flux measurements using vials, fluxes were considered acceptable when the R^2 of the CH_4 concentration increase or decrease in the chamber headspace was ≥ 0.75 (Strack *et al*., 2018). When the concentration was < 5 ppm and varied less than 0.5 ppm (precision of the GC, determined from repeated analysis of standards) over the closure period, the flux was considered below detection and set to zero. In cases where the initial concentration was > 5 ppm followed by a decline in concentration over time, the measurement was discarded as this likely indicates ebullition caused by disturbance during the measurement. In the case of measurements with the portable analyzer (precision 0.25 ppb; Los Gatos Research, n.d.), concentration change for each measurement was visually inspected and the linear portion used for steady flux calculation. When no increasing or decreasing trend in [CH_4] was detected, or flux was within the precision of either instrument, the value was set to zero.

Altogether, 98 CH_4 measurements using vials (including 16 rejected; 16.33 %) were taken during May – August 2016 and 2017; 486 measurements using the portable analyzer were taken including 8 rejected (1.65 %). Among the accepted ones, ebullition was quantified in 41 (8.44 %) of the portable analyzer measurements. Two high resolution measurements at RES-2012-2 (sedgy, flooded collar) and two at RES-2012-3 (mossy, moderately wet collar) that captured intensive bubbling were rejected as the steady flux and baseline slope were unidentifiable (see details of ebullition calculations below).

3.4.2. Ebullition calculation

Ebullition identification and quantification was performed following Goodrich *et al*. (2011) and Nugent (2019). Ebullition was defined as an abrupt change in the slope of the CH_4 concentration over time during the chamber closure. The first difference and the standard deviation of the first difference (> 0.01) were used to identify ebullition events during measurements made with the portable GHG analyzer. Since steady emission likely occurred simultaneously with ebullition, the magnitude of ebullition was determined from the difference between the base slope and the ebullition slope and converted to units of mg CH_4 m^{-2} d^{-1} using the same method as for the steady flux calculation. Lack of ebullition was expressed as 0 mg CH_4 m^{-2} d^{-1} of ebullitive flux.

3.4.3. CH_4 concentration in pore water

Pore water samplers were made of 30 cm long, 2.5 cm inner diameter PVC pipes, perforated in the middle 10 cm of the pipe length, blocked on both ends, covered with synthetic nylon (Nitex®) and equipped with tubing sufficiently long to reach from the bottom of the sampler to the peat surface, where a valve was installed to close and open the system as needed (*e.g*., Strack *et al*., 2004). A pair of samplers was installed close to each collar at all sites, except the Active, with one at 25 cm and one at 50 cm depth. Methane in pore water and subsurface free-phase gas were sampled from June 12th until the end of August 2017 at the time of gas flux measurements. Gas accumulated at the top of the tubing was collected first, which could possibly cause the exsolution of CH_4 from pore water to the gaseous phase (*i.e*., due to negative pressure from the syringe) and therefore we used the [CH_4] in the gaseous phase to correct the total [CH_4] in pore water. We aimed for 20 mL of subsurface gas to be sampled into an evacuated exetainer. If < 20 mL was available, a known volume of gas was taken and topped up with the ambient air to 20 mL to maintain similar pressure in all vials. Ambient air samples were also collected to account for ambient [CH_4].

Similarly, 20 mL of pore water was collected in a 60 mL syringe, mixed with 20 mL of ambient air and shaken vigorously for 5 minutes. To prevent cross-contamination with other

pore water samples, the syringe was flushed with small amount of pore water from the current sampler prior to each sampling. If < 20 mL of pore water was available, a known amount was collected and analyzed as above. The gas in the headspace of the syringe was collected in an evacuated vial and the [CH_4] determined on the GC. Methane concentration in pore water was calculated after Kampbell and Vandergrift (1998).

3.4.4. Environmental conditions

The temperature in the chamber during flux measurement was recorded every minute using a HOBO logger (ONSET, HOBO U23 Pro v.2 external temperature data logger). For each flux measurement, the mean temperature value was calculated and used for individual correction of molar volume in the chamber. Also during each flux measurement, the soil temperature was measured adjacent to the collar using an Omega HH200A temperature probe at depths: 2, 5, 10, 15, 20, 25, 30 cm. Additionally, HOBO loggers (HOBO U23 Pro v.2) were installed at each site except RES-2012 in 2016 and the Active site in both years. They recorded average air temperature and soil temperature at 20 cm depth every 30 minutes.

Carbon dioxide exchange was measured from May 17th to July 5th, 2016 with the portable analyzer. Due to equipment malfunction, CO_2 exchange was measured using an infrared gas analyzer (IRGA EGM-4 PPSystems) from July 11th to August 31st, 2016. Almost all gas measurements in 2017 were performed with the portable analyzer except when the access to the plots was limited due to severely flooded conditions and the IRGA was used instead. Measurements were taken with the same frequency as for CH_4 fluxes. An opaque tarp was placed on the chamber to obtain short, 2-minute, measurements in dark conditions that were used to calculate respiration (R) (Strack *et al*., 2016). Then, the tarp was removed and the chamber vented until the ambient [CH_4] in the headspace was reached. Changes in [CO_2] over the first two minutes of the longer closures used for CH_4 flux determination were used to calculate net ecosystem exchange (NEE). Gross ecosystem productivity (GEP) was calculated from a difference between NEE and R.

Water wells were installed adjacent to the collars. When collars were close to each other, often only one well was installed between the two collars. The elevation difference

between wells and ground surface in the collar was measured to account for differences in the peatland microtopography and used for the WT correction. A levelogger (Solinst) was installed at each site in one of the wells to monitor the WT level every 30 minutes over the growing season in 2016, but only two loggers were installed in 2017 (at the Natural site and RES-2009). Barologgers (Solinst) were installed at RES-2012 in 2016 and at RES-1991 in 2017 and used to correct the levelogger data. All missing values for the atmospheric pressure in the 2017 growing season (May 8th – July 17th) were taken from the nearest meteorological station where hourly average pressure data were available (Edmonton Stony Plain CS, about 60 km east from Seba Beach peatlands complex, coordinates: 53°32'50.080" N, 114°06'30.070" W, elevation 766.30 m, Government Canada, 2018. The atmospheric pressure recorded at Seba Beach sites and the Stony Plain CS station for the period of time when both sets of data were available showed a strong correlation ($y = 0.878x + 11926$; $r^2 = 0.82$). Corrected levelogger values were used to calculate WT using a regression equation obtained from manual WT measurements. The levelogger at RES-1991 sank in 2016 and we were unable to retrieve the data. Monthly total precipitation and average air temperatures were taken from the Tomahawk AGDM meteorological station located about 11.5 km SSW from Seba Beach peatland complex (coordinates: 53°26'22.000" N, 114°43'06.000" W, elevation 814.00 m, Government Canada, 2018) that was the closest station to our study site. Long term average mean temperature and precipitation (1981 – 2010) were taken from the nearest possible meteorological station Edmonton Stony Plain (53°32'51.006" N , 114°06'30.090" W, elevation 766.30 m, Government Canada, 2018) that stored long term data. The long term averages were not available at the Tomahawk station.

3.4.5. Statistical analysis

All statistical analyses were completed in R (R Core Team, 2019). Descriptive statistics for geochemical variables (n, mean, sd) were calculated in Rmisc package (Hope, 2013) and converted to csv files using pdftables package (Persson, 2016). Linear mixed effect (LME) models were built in nlme package (Pinheiro *et al*., 2019) and marginal ANOVA with Tukey pairwise comparison applied to determine how the steady CH_4 flux and [CH_4] in pore water at 25 cm and 50 cm depth varied between sites and over time. Separate models were built to

determine the significance of environmental variables for explaining variation in CH_4 flux, and $[CH_4]$ in pore water at both depths. As ebullition was only observed at RES-1991 and RES-2012, differences between sites were clear and no further statistical analysis was conducted, except of correlations between ebullition and environmental variables, CH_4 flux and $[CH_4]$ in pore water calculated using Spearman rank correlation in ggpubr package in R (Kassambara, 2018). In all LME models, plot (collar) was specified as a random variable to account for repeated measurements at the same plot. Final models with environmental controls were obtained by subsequent removal of variables with the highest p value until only variables with p value > 0.05 remained in the model. LME models were validated by inspecting the normality and homogeneity of residuals and patterns of residuals versus fitted values. The R^2 of the models was calculated in MuMIn package in R (Barton, 2019). Package lsmeans (Lenth, 2016) and function lsmeans with Tukey pairwise comparison was used to check at which sites CH_4 flux, and $[CH_4]$ in pore water varied significantly over time during the summer season. The same procedure was applied when significant relationship between dependent variable and the type of vegetation cover (*e.g.*, mossy, sedgy, bare peat) occurred. Graphs were made in ggplot2 package (Wickham, 2016).

3.5. RESULTS

All data included in this study is compiled in Tab. A.3.1. deposited at Scholars Portal Dataverse.

3.5.1. Environmental conditions

The air temperature was higher in May 2016 and 2017 than the long term average, but similar to the average in the rest of the season (Tab. A.3.2). May and June 2016 were dry, but the rest of the season was wet, with August precipitation over 100 mm higher than the long-term average. The 2017 summer season started with regular precipitation in May, followed by wet conditions in June where most of the precipitation occurred in the second half of the month. The precipitation in July and August 2017 was lower than in 1981 – 2010 (by half in August),

which was reflected in decreasing WT at the end of the season. But, even at lower than normal precipitation, the WT remained higher than in 2016 and the restored sites remained partially flooded.

Patterns of WT fluctuation in 2016 were similar at all sites. In 2017, WT fluctuations were synchronized at the Natural and restored sites, except RES-1991; whereas at the Unrestored and Active sites, WT showed an overall decrease over time. The largest fluctuations of the WT were observed at the Unrestored site in both years (Fig. 3.1).

Peat temperatures were generally highest in RES-2012 and the Unrestored sites, lower in the other two restored sites (Fig. 3.2) and lowest in the Natural site. The pattern of temperature varied between the study years. In 2016, the temperature had an increasing tendency until the end of July (~ day 210) and then decreased in August. In 2017, the initial rapid increase of temperature continued until the second week of July (~ day 190), stabilized for the rest of the summer season and slightly decreased at RES-2012 and the Unrestored (the warmest sites) and RES-1991 (the flooded site) by the end of the study period (Fig. 3.2).

3.5.2. Gross ecosystem production (GEP)

The GEP at the restored sites was the largest over the summer 2016 and 2017 (from 26.3 ± 21.4 g of CO_2 m^{-2} d^{-1} at RES-1991 to 33.1 ± 20.3 g of CO_2 m^{-2} d^{-1} at RES-2009). Restored sites' GEP was at least twice as high as at the Natural (from 12.3 ± 9.4 at the hummock to 14.0 ± 9.3 at the hollow; Tab. A.3.3), while at the Unrestored it was lower than at the Natural (9.0 ± 8.4 g of CO_2 m^{-2} d^{-1}). The lowest GEP was observed at the Active site (0.6 ± 0.4 g of CO_2 m^{-2} d^{-1}).

3.5.3. Steady CH_4 fluxes

Steady fluxes accounted for the majority of CH_4 emission at RES-2012 and RES-1991, where ebullition also occurred (Tab. 3.1). Fluxes were not significantly different at the Natural, RES-2009, Unrestored and Active sites, but were the lowest at the Unrestored and Active (Tab. 3.1).

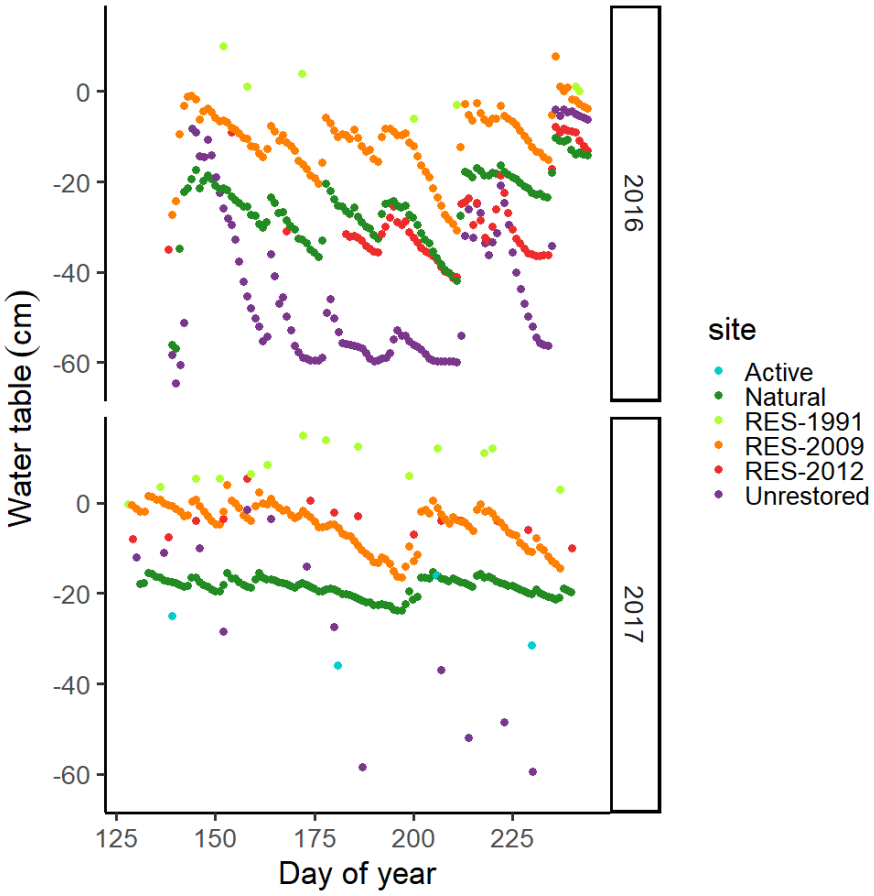

Figure 3. 1. Water table (WT) fluctuation over time in summer 2016 and 2017 at the Natural, Active, Unrestored and three restored sites: RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012. The WT was measured continuously with leveloggers. Missing data were replaced with manual measurements.

Fluxes at RES-1991 and RES-2012 were significantly higher than those at other sites (195.2 ± 181.1 and 178.2 ± 536.5 mg CH_4 m^{-2} d^{-1}, respectively; Tab. 3.1). The temporal (day of year; DOY) pattern of steady log-transformed CH_4 flux over the growing season varied significantly between sites (DOY x site; Tab. 3.2), and between years 2016 and 2017 ($F_{1, 523} = 18.4$, $p < 0.0001$, not included in tables). In 2016, only flux at RES-2012 and RES-1991 increased over the season, while in 2017 the tendency occurred at all sites except the Unrestored and Active (Fig. 3.3). The flux at the Natural site and RES-2009 followed the same temporal pattern in both years.

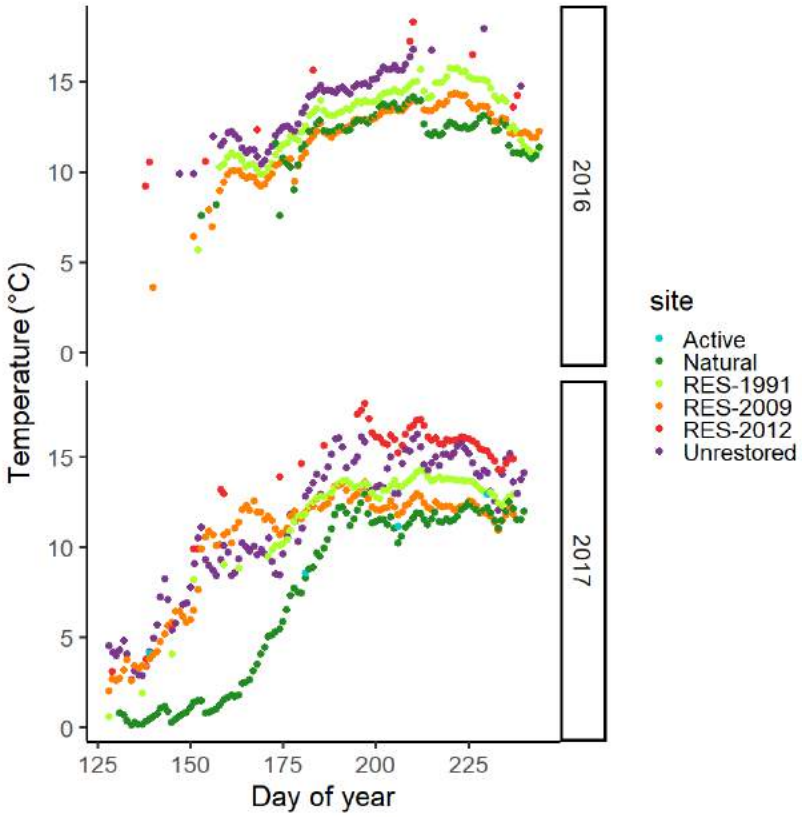

Figure 3. 2. Mean daily peat temperature (˚C) at 20 cm depth over time measured continuously with HOBO loggers in summer 2016 and 2017 at the Natural, Unrestored and three restored sites: RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012. Missing values were replaced with manual temperature measurements at the same depth. No logger was installed at the Active site.

The type of peat surface cover significantly affected the steady CH_4 flux (*i.e.*, significant difference among collars grouped by site and surface cover, $F_{10, 516} = 15.8$, $p < 0.0001$), but the differences were largely driven by the site itself. Thus, no significant differences in CH_4 flux were found between the surface covers within any given site. Only RES-1991 sedgy plots had significantly higher flux than all other surface types, except RES-1991 mossy (Tab. 3.1).

The steady CH_4 flux was significantly explained mainly by the peat temperature at 20 cm depth, GEP, WT, and the percentage cover of shrubs and graminoids (Tab. 3.3). The steady flux was not significantly affected by the $[CH_4]$ in pore water or ebullition.

Table 3. 1. Mean, and standard deviation of CH_4 steady flux, ebullition, and total CH_4 emission (mg m^{-2} d^{-1}) over the summer 2016 and 2017. RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012. The Tukey pairwise comparison was done for steady CH_4 flux. Letters in common indicate values that do not vary significantly from each other.

Site	Surface cover	Steady CH_4 flux		Steady CH_4 flux - site	
		n	mean (sd)	n	mean (sd)
Natural	Hollow - mossy	54	8.6 (15.9)^{ab}	113	6.8 (13.7)
	Hummock - mossy	59	5.2 (11.2)^{ab}		
RES-1991	mossy	52	166.9 (167.2)^{cd}	99	195.2 (181.1)
	sedgy	47	226.4 (192.2)^{d}		
RES-2009	mossy	58	4.1 (6.2)^{a}	120	7.7 (14.5)
	sedgy	62	11.0 (18.7)^{a}		
RES-2012	mossy	57	197.9 (635.1)^{ab}	101	178.2 (536.5)
	sedgy	44	152.7 (378.0)^{bc}		
Unrestored	bare peat	57	0.3 (2.4)^{a}	115	0.2 (1.8)
	sedgy	58	0.0 (0.9)^{a}		
Active	bare peat	12	0.4 (1.4)^{a}		

Site	Surface cover	Ebullition		Ebullition - site	
		n	mean (sd)	n	mean (sd)
Natural	Hollow - mossy	0	0	0	0
	Hummock - mossy	0	0	0	0
RES-1991	mossy	47	20.4 (38.2)	87	16.2 (33.1)
	sedgy	40	11.3 (25.5)		
RES-2009	mossy	0	0	0	0
	sedgy	0	0	0	0
RES-2012	mossy	51	37.9 (248.5)	82	97.0 (627.6)
	sedgy	31	194.1 (972.1)		
Unrestored	bare peat	0	0	0	0
	sedgy	0	0	0	0
Active	bare peat	0	0		

Site	Surface cover	Total CH_4 emission		Total CH_4 emission - site	
		n	mean (sd)	n	mean (sd)
Natural	Hollow - mossy	54	8.6 (15.9)	113	6.8 (13.7)
	Hummock - mossy	59	5.2 (11.2)		
RES-1991	mossy	52	185.3 (189.4)	99	209.4 (195.0)
	sedgy	47	236.0 (199.7)		
RES-2009	mossy	58	4.1 (6.2)	120	7.7 (14.5)
	sedgy	62	11.0 (18.7)		
RES-2012	mossy	57	231.8 (675.6)	101	256.9 (770.7)
	sedgy	44	289.5 (886.0)		
Unrestored	bare peat	57	0.3 (2.4)	115	0.2 (1.8)
	sedgy	58	0.0 (0.9)		
Active	bare peat	12	0.4 (1.4)		

Table 3. 2. The linear mixed effect models' output. Models were built for the spatial and temporal variability of the dependent variable with Site, DOY (day of year), and Site x DOY as fixed explanatory variables and the plot (collar) as a random variable to avoid pseudo-replication. R^2m – marginal R^2, variance explained with fixed variables; R^2c – conditional R2, variance explained by both fixed and random factors (Barton, 2019). PW25[CH_4] and PW50[CH_4] are concentrations of CH_4 in pore water at 25 cm and 50 cm depth, respectively. The model for PW25[CH_4] showed no significant patterns when the interaction DOY x Site was included, therefore a separate model with no interaction was built and the output included in the table.

Dependent variable	Explanatory variable	F statistics	p-value	R^2c	R^2m
Log CH_4 flux	DOY	(1,519) = 0.087679	0.7673	0.71	0.63
	Site	(6,27) = 3.045292	0.0209		
	DOY x Site	(6,519) = 6.539903	< 0.0001		
PW25 [CH_4]	DOY	(1,169) = 14.024421	0.0002	0.75	0.35
	Site	(5,24) = 4.219118	0.0068		
PW50 [CH_4]	DOY	(1,181) = 6.724743	0.0103	0.67	0.47
	Site	(5,25) = 1.651210	0.1833		
	DOY x Site	(5,181) = 3.095982	0.0105		

Table 3. 3. The linear mixed effect models' output. Models were built with fixed explanatory variables: GEP (gross ecosystem production, g CH_4 m^{-2} d^{-1}), SHR (percentage shrub cover), FOR (percentage forb cover), GRA (percentage graminoid cover), MOS (percentage moss cover), T (temperature ˚C) at a significant depth (*e.g.*, T25 means T at 25 cm depth), WT (water table, cm). The significant depth was found by building a separate model for temperatures at depths: 2, 5, 10, 15, 20, 25, and 30 cm. All significant environmental variables were used to build models with paired interactions between them. The output of the latter is given in the table. Plot (collar) was used as a random variable. R^2m – marginal R^2, variance explained with fixed variables; R^2c – conditional R^2, variance explained by both fixed and random factors (Barton, 2019). PW25[CH_4] and PW50[CH_4] are concentrations of CH_4 in pore water at 25 cm and 50 cm depth, respectively.

Dependent variable	Explanatory variable	F statistics	p-value	R^2c	R^2m
Log CH_4 flux	GEP	(1,422) = 18.06489	< 0.0001	0.73	0.29
	SHR	(1,422) = 5.42193	0.0204		
	GRA	(1,422) = 7.60589	0.0061		
	T20	(1,422) = 47.57682	< 0.0001		
	WT	(1,422) = 17.67577	< 0.0001		
PW25 [CH_4]	T25	(85,84) = 1.706004	0.0075	0.84	0.32
	WT	(1,85) = 6.900298	0.0102		
PW50 [CH_4]	T25	(86,101) =1.654089	0.0076	0.75	0.21

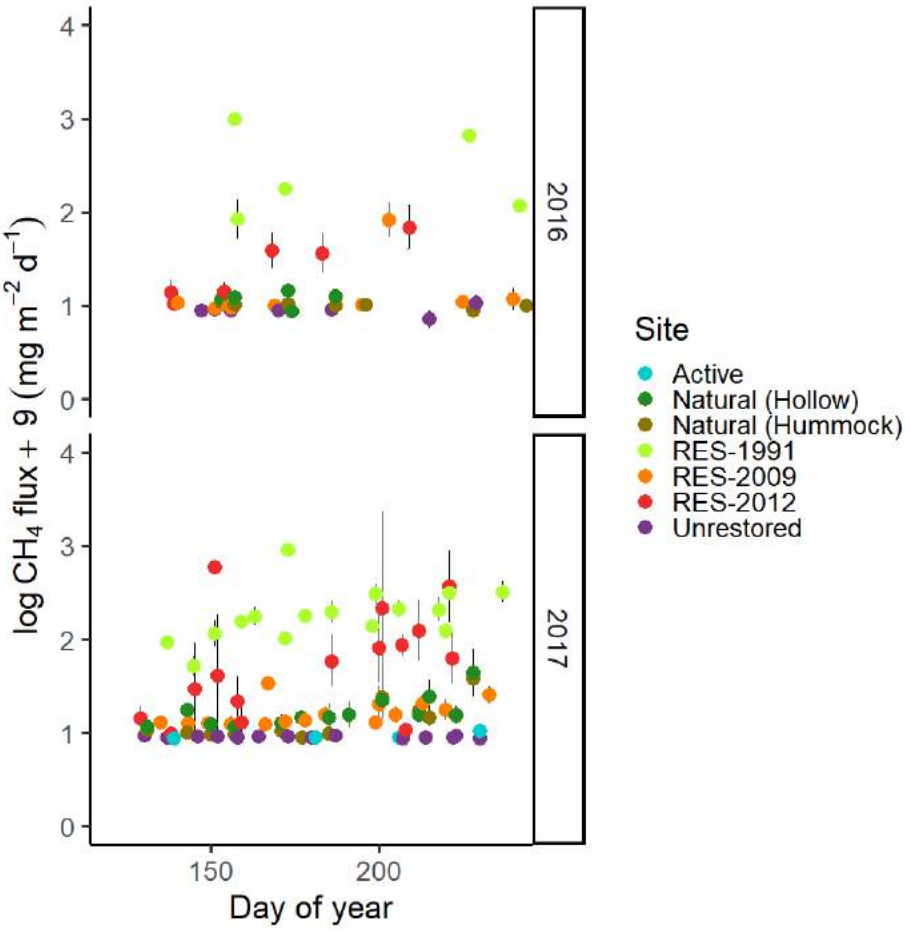

Figure 3. 3. Mean and standard error of log-transformed steady CH_4 fluxes over time in May – August 2016 and 2017. RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012.

3.5.4. Ebullition

Ebullition occurred only at two sites, flooded RES-1991 and wet/moderately wet parts of RES-2012 (Tab. 3.1), regardless of the vascular plant cover (Tab. 3.4, Tab. A.3.4). Considering all CH_4 measurements at RES-1991 and RES-2012, including the ones that did not contain ebullition events, the contribution of ebullition to total CH_4 emission was 6.7 % at RES-1991 and 5.9 % at RES-2012 (percentage contribution calculated from Tab. 3.1). The mean ebullitive flux from RES-2012 was higher than at RES-1991 but occurred in rare spontaneous

events of large CH_4 emission. At RES-1991, ebullition was more regular and emitted smaller amounts of CH_4 (Tab. A.3.4). There was a significant positive correlation with the percentage cover of moss and ebullition, but no such correlation with vascular plant cover (Tab. 3.4). At RES-2012, more CH_4 was emitted via ebullition at sedgy than at mossy locations (1203.6 ± 2359.9 and 967.2 ± 1135.3 mg CH_4 m^{-2} d^{-1}, respectively), but at RES-1991, ebullition was similar at sedgy and mossy plots (Tab. A.3.4). Ebullition was also significantly positively correlated with soil temperature, WT, GEP, steady CH_4 flux and [CH_4] in pore water at both depths of 25 and 50 cm (Tab. 3.4).

Table 3. 4. Spearman rank correlation between ebullition and CH_4 flux, [CH_4] in pore water at depths 25 cm and 50 cm (PW25[CH_4], PW50[CH_4]), and environmental variables: T10 – temperature (˚C) at 10 cm, Taverage - average temperature (˚C) at 2 – 30 cm, WT – water table (cm), GEP – gross ecosystem production (g CO2 m^{-2} d^{-1}), MOS – percentage cover of moss.

Factor	p-value	Correlation coefficient
T10	0.006802	0.21
T15	0.000779	0.26
T20	0.000483	0.27
T30	0.009643	0.20
WT	2.53E-11	0.48
GEP	0.04454	0.16
MOS	0.009459	0.20
CH_4 flux	0.000224	0.28
PW50[CH_4]	0.008033	0.20
PW25[CH_4]	0.02221	0.27

The mean log-transformed ebullition over time (day of year) is shown in Fig. 3.4. Ebullition started at the end of May 2017 at RES-1991 and over a month later (beginning of July) at RES-2012. It increased over time at RES-1991 but did not show a pattern at RES-2012. The equipment malfunction on July 5th, 2016 prevented further collection of ebullition data that year, but the measurements taken by that time showed lack of ebullition at RES-2012 (consistent with year 2017) and the beginning of ebullition on June 6th at RES-1991.

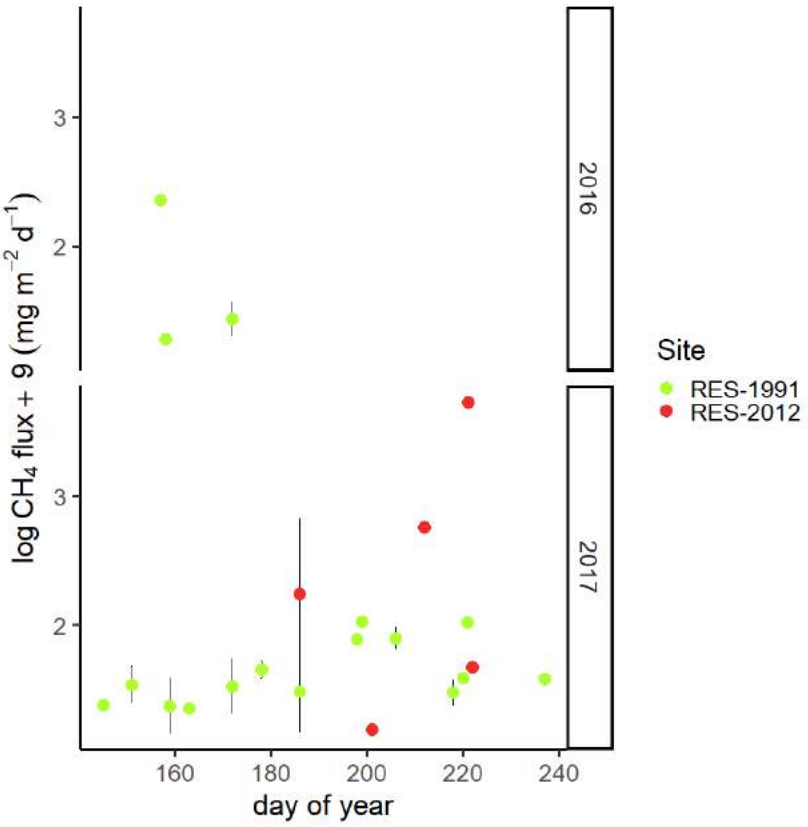

Figure 3. 4. Mean and standard error of log-transformed ebullitive fluxes over time in May – August 2016 and 2017. RES-1991 – site restored in 1991, RES-2012 – site restored in 2012.

3.5.5. CH_4 concentration in pore water

The [CH_4] in pore water at 50 cm was higher than at 25 cm depth at all sites (Fig. 3.5). The latter varied significantly between sites (Tab. 3.5) with higher values at RES-1991 than at RES-2009 (Tukey pairwise comparison, $p = 0.0266$) and at the Unrestored site ($p = 0.0102$). The [CH_4] at 25 and 50 cm depth was significantly affected by the type of peat surface cover ($F_{9, 20} = 3.4$, $p = 0.0106$ and $F_{9, 21} = 16.3$, $p < 0.0001$, respectively; see Tukey pairwise comparison for which types of peat surface cover the [CH_4] in pore water was significantly different, Tab. 3.5) and significantly increased over the summer (significant p-values for day of year (DOY) in Tab. 3.2); however, at 50 cm depth, the [CH_4] pattern in time significantly depended on the site (DOY x Site, $p = 0.0105$ in Tab. 3.5). The Natural hummock and hollow,

RES-1991, and RES-2012 showed the highest increase of [CH_4] in pore water at 50 cm, while RES-2009 had lower mean values that only slightly increased over the season. The values for the Unrestored site oscillated around zero and did not increase in time at either depth (Fig. 3.5). The [CH_4] in pore water at both depths was the highest at sedgy RES-1991 and RES-2012 but not always significantly different from other sites and types of vegetation cover (Tab. 3.5). Also, pore water [CH_4] was usually higher at restored sedgy than at mossy locations within a site except RES-2009 that had more [CH_4] in pore water under moss (Tab. 3.5). The pore water [CH_4] at both depths significantly depended on the temperature at 25 cm depth (T25), and the WT was a factor significantly affecting [CH_4] in pore water at 25 cm depth (Tab. 3.3).

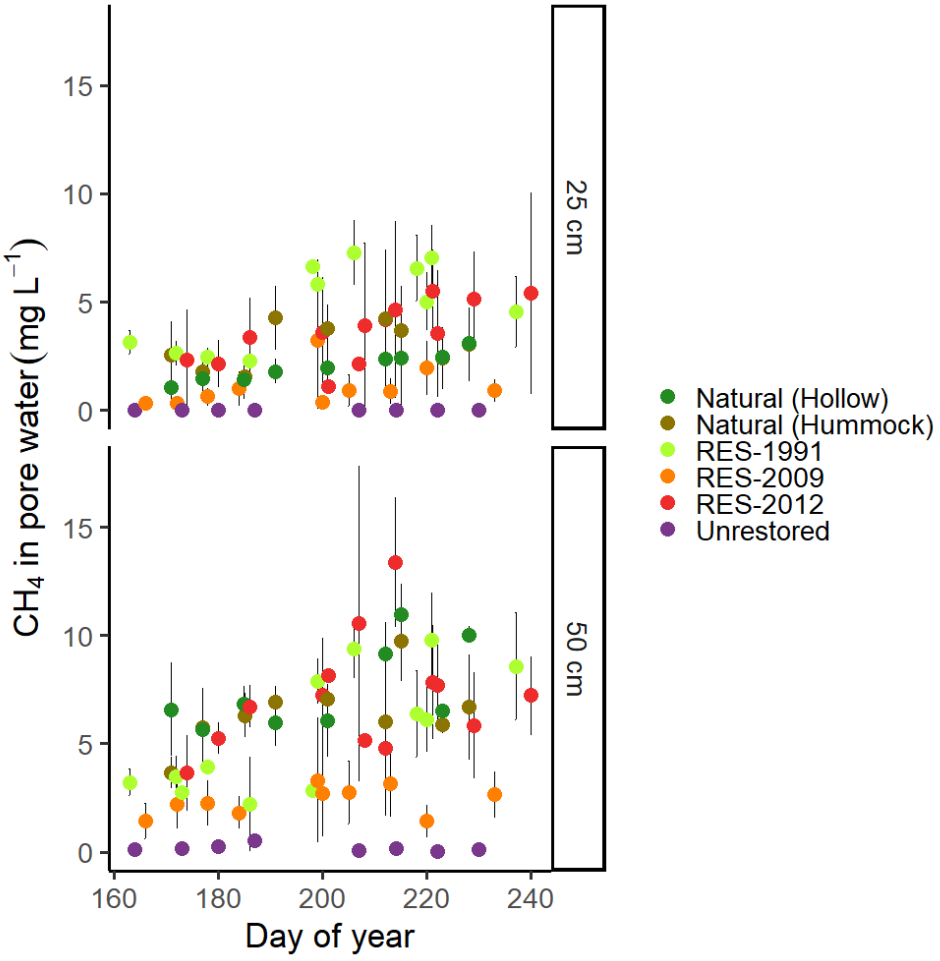

Figure 3. 5. Mean and standard error of [CH_4] in pore water over time in June – August 2017 in the Natural, Unrestored and three restored sites: RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012.

Table 3. 5. Mean, and standard deviation of [CH_4] in pore water (mg L^{-1}) at 25 cm and 50 cm depth during summer 2017. RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012. Letters in common indicate values that do not vary significantly from each other. They should be interpreted within a depth and not between depths. Pore water was not collected at the Active site.

Site	Surface cover	PW25[CH_4]		PW25[CH_4] - site	
		n	mean (sd)	n	mean (sd)
Natural	Hollow - mossy	23	2.0 (1.6)^{ab}	43	2.5 (1.9)
	Hummock - mossy	20	3.1 (2.1)^{ab}		
RES-1991	mossy	25	3.6 (1.6)^{ab}	43	5.1 (3.2)
	sedgy	18	7.2 (3.6)^{b}		
RES-2009	mossy	26	1.5 (2.2)^{ab}	50	1.0 (1.7)
	sedgy	24	0.4 (0.5)^{ab}		
RES-2012	mossy	24	3.0 (3.2)^{ab}	40	3.9 (4.3)
	sedgy	16	5.1 (5.4)^{c}		
Unrestored	bare peat	8	0.0 (0.0)^{ab}	24	0.0 (0.0)
	sedgy	16	0.0 (0.0)^{a}		

Site	Surface cover	PW50[CH_4]		PW50[CH_4] - site	
		n	mean (sd)	n	mean (sd)
Natural	Hollow - mossy	24	7.5 (2.7)^{cd}	44	7.0 (2.5)
	Hummock - mossy	20	6.5 (2.2)^{cd}		
RES-1991	mossy	24	4.7 (2.0)^{bcd}	44	6.5 (4.2)
	sedgy	20	8.6 (5.2)^{de}		
RES-2009	mossy	26	3.9 (3.0)^{abc}	51	2.3 (2.7)
	sedgy	25	0.7 (0.7)^{ab}		
RES-2012	mossy	22	4.7 (2.5)^{cd}	41	7.2 (4.6)
	sedgy	19	10.0 (5.0)^{e}		
Unrestored	bare peat	18	0.1 (0.1)^{a}	38	0.2 (0.2)
	sedgy	20	0.2 (0.3)^{a}		

3.6. DISCUSSION

3.6.1. Methane emission and pore water concentration

Steady fluxes accounted for the majority of the total CH_4 emission. The mean steady flux at the Natural peatland (6.8 mg m^{-2} d^{-1}) was lower than the average for pristine northern bogs, which is 26 mg m^{-2} d^{-1} (Abdalla *et al*., 2016). Steady fluxes at our restored sites were either lower than the average for northern restored sites of 15 mg m^{-2} d^{-1} reported by Abdalla *et al*., (2016) as observed at site RES-2009 (7.7 mg m^{-2} d^{-1}) or exceeded that average over tenfold at RES-1991 and RES-2012 (average fluxes 195.2 and 178.2 mg m^{-2} d^{-1}, respectively). High CH_4 emission was also observed by Vanselow-Algan *et al*. (2015) 30 years after rewetting. Steady mean flux at the Natural site was tenfold lower than at natural subarctic and boreal peatlands (mean 83 mg CH_4 m^{-2} d^{-1}, Turetsky *et al*., 2014); however, that mean was exceeded at RES-1991 and RES-2012. Steady fluxes at RES-1991 and RES-2012 were comparable to those reported by Nugent (2019) for bare drainage ditches at a post-extracted site at the beginning of the summer season (~135 mg m^{-2} d^{-1}, converted from 98 nmol m^{-2} s^{-1}), to CH_4 flux from a recolonized cutover peatland (0.11 – 232.60 mg m^{-2} d^{-1}, Mahmood and Strack, 2011), to CH_4 emission from natural flooded wetlands, e.g., 6.2 – 3165 mg m^{-2} d^{-1} (Pelletier *et al*., 2007), and to fluxes obtained at wetlands in northeastern Ontario (91 – 350 mg m^{-2} d^{-1}; Bubier *et al*. (1993) where much lower fluxes were measured at non-flooded parts of the sites). Our steady fluxes from the restored sites were within the range of fluxes obtained at a Canadian boreal post-extracted restored site by Strack *et al*. (2014) (-1.77 to 394.68 mg CH_4 m^{-2} d^{-1}). As expected, the mean steady flux remained very low at the Unrestored and Active sites, as observed in other studies (Waddington and Day, 2007, Strack *et al*., 2014) and was similar to flux at the unrestored part of Bois-del-Bel peatland in Quebec, Canada (Strack and Zuback, 2013).

We hypothesized that ebullition would occur at all restored and Natural sites, but it was observed only at two restored sites where also high steady CH_4 emission was measured. These restored sites were the oldest and the youngest, which indicates that at this very early stage of restoration, environmental conditions play more important role in CH_4 dynamics than the age of restoration itself. The ebullition contribution to CH_4 emission in our study was comparable to rates measured in former drainage ditches at the restored part of Bois-del-Bel, where the

same method of ebullition quantification was used (9 %, Nugent, 2019). In contrast, the contribution of ebullition in other studies varied, *e.g.*, 1.5 – 3.3 % (Green and Baird, 2013), 20 % (Strack and Waddington, 2008), 17 – 50 % (Christensen *et al*., 2003), 50 – 64 % (Tokida *et al*., 2007a) and 89 % of the total CH_4 flux (Lansdown *et al*., 1992). Even a few percent contribution of ebullition should not be neglected in post-extraction and post-restoration monitoring of affected sites, as the amount of CH_4 released to the atmosphere can be substantial (Glaser *et al*., 2004, Goodrich *et al*., 2011). Based on our research, we recommend including the quantification of CH_4 emission through ebullition in regular monitoring of flooded, wet, and moderately wet restored sites, particularly at revegetated locations. Including this type of data would improve the quality, reliability and credibility of the GHG inventories from post-extracted horticultural peatlands. We also recommend using portable gas analysers. This method minimized the number of rejected measurements in our research by tenfold compared to the manual method, captured ebullition events that would be rejected in manual measurements, and increased the rate of acceptable linear measurements due to frequent concentration recording.

As we had hypothesized, we did not observe ebullition or high CH_4 fluxes at the Unrestored and Active sites, and expect this to be the case even if the sites become temporarily wet after prolonged rain events. The main CH_4 emission driver, CH_4 production, was close to zero at the Unrestored site, linked to lower methanogen abundance (Chapter 2) and so was the measured steady CH_4 flux. It is unlikely that the microbial community would recover fast enough during wet periods to result in substantial CH_4 emissions (*e.g.*, Blodau and Moore, 2003, Knorr and Blodau, 2009), particularly given the recalcitrant nature of the peat exposed at these sites (Basiliko *et al*., 2007).

To a certain degree, $[CH_4]$ in pore water reflected the emission pattern as hypothesized, but since the emission did not show the expected pattern of increase from the Active through Unrestored, RES-2012, RES-2009, RES-1991, to Natural, our hypothesis was supported only partially. Low $[CH_4]$ in pore water in the Unrestored site indicates that the pool of dissolved CH_4 is likely not to recover without active peatland restoration. However, $[CH_4]$ in pore water can be low even in restored sites (*e.g.*, RES-2009) where CH_4 production is suppressed by peat chemical conditions (Chapter 2).

We converted the ebullition magnitude to the unit of flux (mg m^{-2} d^{-1}) to assess the total CH_4 emission from the sites. However, the extrapolation of ebullition to nighttime can produce inaccurate results since increased ebullition and peat surface displacement have been observed at night compared to day time (Goodrich *et al*., 2011, Gogo *et al*., 2011, Glaser *et al*., 2004). Higher night CH_4 fluxes and [CH_4] in pore water have been observed as well (Waddington *et al*., 1996), but higher daytime fluxes have been reported from an Australian seasonal wetland (Jeffrey *et al*., 2019). Future research on nighttime CH_4 fluxes at post-extracted sites is required to clarify the diurnal pattern of CH_4 and CO_2 emission and to quantify the contribution of ebullition to emission more accurately.

3.6.2. Environmental factors controlling CH_4 emission

Ebullition quantification can be technically challenging due to equipment requirements, spontaneous nature, and large spatial variability of ebullition events (*e.g*., Goodrich *et al*., 2011; Parsekian *et al*., 2011). However, our study showed that ebullition increased in time during the summer season at RES-1991. Similar temporal patterns were observed by Goodrich *et al.* (2011) at a natural fen. Goodrich (2010) linked the peat structure (less compact, less decomposed, more porous peat characteristic for natural bogs) to more constant ebullition events that show temporal patterns, both diel and seasonal with increasing ebullition in summer, and were likely driven by CH_4 production. No seasonal pattern was found at RES-2012 but rather spontaneous events emitting large amounts of CH_4. This happened at both mossy and sedgy locations of RES-2012. Irregular, spontaneous and fairly large ebullition events are associated with small pore sizes, more compact and decomposed layers in peat that trap accumulating gas until its pressure is large enough to overcome the trapping forces (Glaser *et al*., 2004, Kellner *et al*., 2006). Peat in RES-2012 was in fact less porous than in RES-1991 (0.92 and 0.96, respectively; Chapter 4), although peat decomposition was similar in both sites (Chapter 2). Peat structure likely drove rare and large ebullition events at RES-2012. These events occurred mainly at severely flooded, sedgy locations, but also at mossy parts of RES-2012 that were not flooded or extensively wet. It is possible that at RES-2012, the positive significant correlation between ebullition and percentage moss cover was at least partially due to the peat structure rather than the presence of moss itself (*e.g*., low porosity of the first 10 cm

of surface peat (Chapter 4) that could cause short-term accumulation of gas close to the peat surface and its abrupt release once the gas pressure was sufficient to burst through the thin layer of peat. The lack of correlation between ebullition and the percentage cover of sedge could have been caused by the complex interaction of multiple processes including increased CH_4 production due to root exudation (Joabsson *et al*., 1999), more efficient CH_4 emissions via aerenchyma (Ström *et al*., 2005) and trapping gas bubbles in dense sedge roots (Klapstein *et al*., 2014).

The percentage cover of vascular plants (shrubs and graminoids) significantly affected steady CH_4 flux (*e.g*., Mahmood and Strack, 2011), but not ebullition and $[CH_4]$ in pore water. This was in contrast to the findings of Murray *et al*. (2017) that showed a significant correlation between percentage cover of different plant functional groups and $[CH_4]$ in pore water. Also, at all restored sites except RES-2009, pore water $[CH_4]$ was higher at sedgy than at mossy locations (*e.g*., Waddington *et al*., 1996), but other studies have reported lower $[CH_4]$ in pore water and higher CH_4 fluxes under sedges than under moss, which was associated with plant mediated transport of CH_4 (Green and Baird, 2012, Murray *et al*., 2017, Strack *et al*., 2017). Productivity of the vegetation likely increased CH_4 production due to supply of root exudates (Zhai *et al*., 2013), an observation also supported by the significant correlation between GEP and both steady and ebullition fluxes.

The increasing soil temperature over summer in shallow peat and the WT were likely the main drivers of the mean steady flux, ebullition, and $[CH_4]$ in pore water increase in time (Strack and Waddington, 2008, Strack *et al*., 2017). While we had hypothesized this seasonal pattern over time for all sites (except ebullition only at restored and Natural sites), it did not occur in the Unrestored site. The WT was generally lower and fluctuating more intensively in 2016 compared to 2017, likely causing different patterns of CH_4 flux increase in time at the same peatland in these two years (*e.g*., no increase in 2016 at the Natural site and RES-2009 that occurred in 2017). We also observed a correlation between ebullition and pore water $[CH_4]$ as previously reported by Strack and Waddington (2008). These factors are directly linked to optimum conditions for CH_4 production (anoxia and relatively high temperatures) and have previously been identified as major drivers of CH_4 flux (MacDonald *et al*., 1998, Pelletier *et al*., 2007, Turetsky *et al*., 2008, Strack *et al*., 2017).

RES-2009 was the only restored site where ebullition did not occur and CH_4 fluxes were considerably lower than at other restored sites, despite wet conditions (at least in 2017) and high cover of sedges. This contradicted our hypothesis that the CH_4 flux would be the highest at all restored sites. The [CH_4] in pore water was also the lowest at RES-2009 of all restored sites. Microcosm experiment (Chapter 2) showed that CH_4 production was suppressed at RES-2009 and that this was likely caused by large concentration of ferric iron due to shallow peat and close vicinity of clay to the lower boundaries of the WT fluctuation zone. Peat chemical properties can be as important for CH_4 production and emission as hydrological conditions (*e.g.*, Westermann and Ahring, 1987, Kellner *et al*., 2007, Reiche *et al*., 2008, Ye *et al*., 2016, Jeffrey *et al*., 2019) and may have increasing importance at restored peatlands where peat extraction reduces the thickness of the peat layer, potentially allowing greater influence of the underlying substrate. Since non-oxygen terminal electron acceptors (TEAs) can affect CH_4 production and emission in restored sites, it is recommended to include these analyses in studies on CH_4 processes in post-extraction peatlands. Also, more research on the impact of these interactions on the overall outcome of peatland restoration would benefit making informed decisions on restoration planning. The research of Emsens *et al*. (2016) showed that rewetting fens rich in iron negatively impacts peatland restoration success by inducing organic matter break down, which was not observed when the [Fe] was low. This shows a possibility that the effect of high [TEAs] can reach beyond CH_4 processes in managed peatlands.

3.7. CONCLUSIONS

Restoration increased steady CH_4 fluxes that were driven mainly by soil temperature, water table and GEP, with the percentage cover of shrubs and graminoids being of lesser importance. Steady CH_4 fluxes comprised the majority of CH_4 emission, while ebullition contributed only 6 – 7 % of total CH_4 emission and occurred only on flooded/wet and sedge-dominated restored sites, while no ebullition was recorded at the Natural site. No ebullition was observed at the Unrestored and Active sites. Sites where ebullition occurred had the highest steady CH_4 flux, larger than the Natural bog that was dominated by *Sphagnum* and had a deeper water table than the restored sites. Ebullition was correlated with soil temperature, water table, GEP, and percentage cover of moss, but not sedges and shrubs. Ebullition also depended on the

concentration of CH_4 in pore water and steady CH_4 flux. Restoration recovered the subsurface dissolved pool of CH_4. In contrast, no clear evidence of CH_4 emission or pore water concentration recovery was found at the Active and Unrestored sites, where bare peat and low, frequently fluctuating water table prevailed. Methane emission was suppressed at the site restored eight years prior to our research even though the site was wet and dominated by vascular plants. We attribute this to unique geochemistry of the site with considerable concentration of ferric iron, likely originating from the clay underlaying the shallow peat deposit with highly fluctuating water table. Therefore, local environmental factors were more important for driving recovery of post-restoration CH_4 stock and flux than the age of the restored site.

CHAPTER 4: Subsurface free-phase gas content in natural, extracted, unrestored, and restored horticulture peatlands

4.1. ABSTRACT

Little is known about free-phase gas accumulation in post-extraction horticultural peatlands. In this study, we assessed the subsurface pool of free-phase gas and possible factors affecting its dynamics. Ground-penetrating radar (GPR) surveys were conducted at a horticulture peatland complex in May, June, July, and August 2017, at a currently extracted site (Active), post-extraction unrestored site (Unrestored), three sites restored 5, 8, and 26 years prior to our research (in 2012, 2009, and 1991: RES-2012, RES-2009, and RES-1991, respectively), and at a natural bog (Natural) within one horticulture peatland complex. The radargrams were used to assess the volumetric gas content (VGC) in these sites at a monthly temporal resolution. The VGC was the highest in Unrestored (11.8 %) and Natural (10.1 %), lower in Active (9.6 %), RES-1991 (9.5 %) and RES-2009 (9.9 %), and the lowest in RES-2012 (7.2 %). Additionally, water content reflectometry probes and thermocouples were installed at sites restored in 2012 and 1991 to record hourly fluctuation of volumetric gas content (VGC) and soil temperature at 25, 50, and 75 cm depth. Results from the water content probes indicated that the hourly changes in VGC over time mimicked temperature fluctuation. Gas release was sometimes associated with decreases in atmospheric pressure and sharp increases of water table (WT) after longer period of WT drawdown. Generally, the VGC increased with depth and occasionally reached > 20 % close to the bottom of the peat deposits. We did not find clear evidence of free-phase gas recovery progress in post-extraction peatlands with the age of restoration, but rather local conditions driving its accumulation. Presumably, compacted peat in the Unrestored and Active sites acted as a semi-confining layer and was responsible for high VGC, where CH_4 (one of the main components of the free-phase gas) production and emission was close to zero. The VGC alone cannot be interpreted as the restoration of the CH_4 balance in post-extracted sites and CH_4 flux is not a good predictor of subsurface VGC. Results indicate that biogenic gas accumulation in peat recovers at restored sites and is also maintained to a certain degree at Unrestored and Active sites, but the mechanisms determining the amount

of accumulated free-phase gas at restored and Unrestored sites appear to vary in relation to site management and local conditions.

4.2. INTRODUCTION

Peatlands are terrestrial freshwater ecosystems with at least a partially waterlogged acidic organic matter deposit > 40 cm thick, and characteristic peat forming vegetation, *e.g.*, *Sphagnum* moss and sedges (Laine and Vasander, 1996, National Wetlands Working Group, 1997). Undisturbed peatlands sequester carbon dioxide (CO_2) but also emit considerable amounts of methane (CH_4) due to the presence and activity of methanogenic *Archaea* that are responsible for the last stage of organic matter decomposition in anoxic conditions (*e.g.*, Horn *et al*., 2003). Not all produced CH_4 is emitted; in fact, biogenic gas can be stored in peatlands and constitute up to one fifth of peat volume (Rosenberry *et al*., 2006 and references therein). A part of the subsurface CH_4 pool is dissolved in pore water, but since CH_4 has low solubility in water (Gevantman, 1992), the majority occurs in a gaseous state (Fechner-Levy and Hemond, 1996) as one of the main components of biogenic free-phase gas (20 – 54 % of total gas volume; Shannon *et al*., 1996, Glaser *et al*., 2004 and references therein, Tokida *et al*., 2007b, Stamp *et al*., 2013). Shannon *et al*., (1996) observed the volumetric gas content (VGC, defined as percent of pore space that is not occupied by water) increase in September compared to December of the previous year but this increase was site-specific. Given that the peat matrix is deformable and its buoyancy increases with the free-phase gas content, the compressibility of gas is of limited issue (*e.g.*, Strack *et al*., 2006a). The remaining components of the free-phase gas below the water table are nitrogen (N_2) and small amounts of CO_2 (Comas and Wright, 2012). To the best of our knowledge, the studies on biogenic gas accumulation and release are limited to natural peatlands (Comas *et al*., 2005, 2007, 2008, 2011, Parsekian *et al*., 2010, 2011, Strack and Mierau, 2010), with only one study on restored and unrestored sites to date (Mwakanyamale *et al*., submitted). Further research is required to understand the accumulation of free-phase gas in managed peatlands.

One of the main controls on the free-phase gas distribution, movement, trapping, and release is the peat structure, which is controlled by peat type and degree of decomposition in undisturbed peatlands (Baird *et al*., 2004, Kellner *et al*., 2005, Strack *et al*., 2005, 2006a,

Comas *et al*., 2011, Kettridge and Binley, 2011, Chen and Slater, 2015, Ramirez *et al*., 2016). Peatland vegetation contributes to variation in peat structure and free-phase gas storage, *e.g*., free-phase gas content has been found to be higher under woody vegetation and lower under lawns and open water (Kellner *et al*., 2005, Parsekian *et al*., 2011). The amount of stored CH_4 depends on the balance between the amount of produced CH_4 and the amount of CH_4 lost to oxidation and emission. The majority of oxidation occurs in the presence of oxygen (O_2) when CH_4 diffuses through the zone of peat above the WT or below the WT when plant roots supply O_2 (Ström *et al*., 2005, Bridgham *et al*., 2013). Some studies suggest anaerobic CH_4 oxidation (AOM) occurs in peatlands, although the mechanisms of AOM governed by *Archaea* and coupled to non-oxygen terminal electron acceptor reduction are still not fully understood in peatlands (*e.g*., Smemo and Yavitt, 2007, 2011). Methane production depends on anoxia, the availability of substrate (Coles and Yavitt, 2002), the presence of active methanogens, the presence and activity of competitor microorganisms, peat geochemistry (*e.g*., Segers, 1998), pH (Ye *et al*., 2012) and temperature (Andersen *et al*., 2010). Methane production slows down as the temperature decreases but can also increase over winter likely due to substrate accumulation (Juottonen *et al*., 2008, Couwenberg and Fritz, 2012). Large amounts of biogenic gas are accumulated under ice and snow and released during the spring thaw, accounting for almost a half of the annual CH_4 emission (Windsor *et al*., 1992, Huttunen *et al*., 2003, Tokida *et al*., 2007b, Slater and Comas, 2009).

Biogenic free-phase gas forms when dissolved gas concentration exceeds a certain threshold (Beckwith and Baird, 2001, Baird *et al*., 2004, Gogo *et al*., 2011) or when the partial pressures of dissolved gases exceed the hydrostatic pressure (Fechner-Levy and Hemond, 1996). The formation of free-phase gas and the balance between gaseous and dissolved phase are governed by the ideal gas law and Henry's law. Increasing temperature and decreasing atmospheric pressure (P_{atm}) raise free-phase gas volume due to gas expansion and exsolution of gases from pore water (Fechner-Levy and Hemond, 1996, Baird *et al*., 2004, Kellner *et al*., 2006, Strack *et al*., 2006a, Tokida *et al*., 2007a), while decreasing temperature increases CH_4 solubility, and increasing P_{atm} shrinks and mobilizes free-phase gas bubbles (Fechner-Levy and Hemond, 1996, Baird *et al*., 2004).

Once present, bubbles can move, merge, grow, shrink and become trapped in peat, creating a lag between gas production and emission (Kellner *et al*., 2005, Waddington *et al*., 2009a, Kettridge and Binley, 2011). They move until they encounter an obstacle (*e.g*., pores of smaller diameter), aggregate or increase their volume due to CH_4 production or pressure and temperature changes, and become immobilized (Kellner *et al*., 2005, Waddington *et al*., 2009a, Kettridge and Binley, 2011). Gas accumulation creates overpressurized areas in peat, leading to peat deformation and subsequent rupture of peat layers, causing spontaneous gas release (Rosenberry *et al*., 2006, Waddington *et al*., 2009a). Two conceptual models of free-phase gas storage (a part of larger models of CH_4 cycling) can be applied to specific sites (Baird *et al*., 2004, Parsekian *et al*., 2011, Comas *et al*., 2011, 2014). In the model of Coulthard *et al*. (2009), shallow peat (up to 1 m deep) is the major source of bubbles and ebullition (abrupt or steady release of subsurface free-phase gas through the peat matrix to the atmosphere) in peatlands, due to the presence of trapped air acting as nuclei for bubble growth and the presence of labile substrate for methanogens. Confining layers that accumulate small amounts of free-phase gas can form when bubbles clog the peat pores preventing subsurface gas from upward movement (Romanowicz *et al*., 1995, Kellner *et al*., 2004, Strack *et al*., 2005, 2006a). Glaser's *et al*. (2004) model is based on the presence of confined woody peat layers acting like traps for biogenic gas produced mainly in deep peat (below 2 m depth). The presence of confining peat layers that have limited permeability and flexibility promotes formation of large entrapped gas deposits (Rosenberry *et al*., 2003, Glaser *et al*., 2004, Strack *et al*., 2006a).

The entrapped free-phase gas plays an important role in regulating peatland hydrology. It reduces hydraulic conductivity by blocking the peat pores (Beckwith and Baird, 2001, Kettridge *et al*., 2013, Waddington *et al*., 2015), affects the pressure gradients thereby changing patterns of water and solute movements (Kellner *et al*., 2004), and increases peat buoyancy that leads to surface level oscillations (Glaser *et al*., 2004, Strack *et al*., 2006a). The recovery of free-phase gas in restored sites can be an important factor returning this peatland function post-extraction.

Peat extraction involves procedures that drastically alter natural peatland ecosystems (*e.g.*, installation of drainage ditches, removal of all vegetation, and decades of peat harvesting with heavy machinery) and potentially releases subsurface biogenic gas accumulated in the

peat deposit. Furthermore, lowering the WT increases the thickness of the unsaturated zone and can enhance CH_4 oxidation. Once peat extraction is completed, it is unknown to which degree the pool of free-phase gas and CH_4 is able to recover in unrestored site and if the recovery of subsurface CH_4 progresses with the age of restoration. Our objectives were to quantify subsurface free-phase gas in a currently extracted (Active) site, a site left unrestored in 2012 (Unrestored), in sites restored at different times (1991, 2009, and 2012: RES-1991, RES-2009, and RES-2012, respectively) and in a natural site (Natural) and identify potential relationships between the VGC and environmental variables. We hypothesized that:

1) The largest amounts of free-phase gas will be found in RES-1991 and RES-2012 where ebullition, the largest steady CH_4 fluxes, and the highest [CH_4] in pore water were detected (Chapter 3). RES-2009, where relatively low CH_4 production and emission were observed, will likely have lower free-phase gas content than other restored sites but will be comparable to that at the Natural peatland. Both Unrestored and Active sites will have the lowest content of free-phase gas.
2) As the air and soil temperatures increase, the VGC will increase over the summer in Natural and restored sites. Since potential CH_4 production rates were very low in the Unrestored site (Chapter 2) we do not expect such increase in seasonal VGC in Unrestored and Active sites.

4.3. STUDY SITE

The study site was located in Seba Beach horticulture peatland in central Alberta, Canada (53° 33' N, 114° 44' W; Fig. A.1.1 and enlarged sites in Fig. A.1.2) and managed by Sun Gro Horticulture using the peat vacuum harvesting method. The complex consists of sites at various stages of peat extraction and post-extraction restoration. For our study, we chose a natural bog (Natural, Fig. A.1.3A) as a reference site, sites restored at different times: in 1991 (RES-1991; Fig. A.1.3B), in 2009 (RES-2009; Fig. A.1.3C), and in 2012 (RES-2012; Fig. A.1.3D), an unrestored (Unrestored, Fig. A.1.3E), and a currently extracted site (Active, Fig. A.1.3F).

The Natural site was a treed bog with well-developed hummocks and hollows. The vegetation was dominated by *Sphagnum*, but true mosses were also present in hollows, *Picea*

mariana (Mill.) B.S.P. (black spruce), *Rhododendron groenlandicum* (Oeder) Kron & Judd (Labrador tea), *Andromeda polifolia* L. var. *glaucophylla* (Link) DC. (bog rosemary), *Vaccinium vitis-idaea* L. (lingonberry), *Vaccinium oxycoccos* L. (bog cranberry), *Rubus chamaemorus* L. (cloudberry), *Maianthemum canadense* Desf. (Canada mayflower). The depth of the peat deposit reached 500 – 540 cm at the study location with poorly decomposed and highly porous *Sphagnum* peat (Tab. 4.1).

Peat in RES-1991 was 360 – 470 cm deep at the main study location where the CH_4 emission and molecular studies were conducted (Chapter 2 and 3) but the ground-penetrating radar (GPR) surveys were carried out in 2017 when much of the peatland was severely flooded, which made that part of the site inaccessible. Instead, we used access from a boardwalk installed in 2016 away from metal collars used for flux measurements, about 150 m east from the main study location where the peat deposit was 360 – 398 cm deep (see the map of RES-1991 in Fig. A.1.2). The moss-dominated locations were covered mainly with dense *Sphagnum* forming a partially floating mat where also *Drosera* spp. was found, while at sedge-dominated locations, true mosses were present instead of *Sphagnum*. Sedges were dominated by *Carex aquatilis* Wahlenb. (water sedge) with other graminoids, *e.g.*, *Carex canescens* L. (silvery sedge) and *Scirpus cyperinus* L. (Kunth) (wool grass), found in lower abundance. Occasionally shrubs (*Betula* spp. and *Salix* spp.) were present. Peat was poorly decomposed and highly porous (Tab. 4.1). The water table was constantly above the peat surface.

The composition of graminoids at RES-2009 was similar to those at RES-1991. Both *Sphagnum* and true mosses were abundant at RES-2009, except for the spots where dense sedge tussocks and *Salix* spp. shrubs covered 100 % of the surface. *Eriophorum vaginatum* L. (cottongrass) was also found. Dense *Typha* spp. (cattail) was abundant in the west (wet) part of the site closer to the access road but our transects were not located in this zone. Peat at the GPR transect locations was 192 – 240 cm deep but was even shallower (< 150 cm) at other spots (Chapter 2).

The thickness of moderately decomposed peat in RES-2012 (Tab. 4.1) at the GPR locations increased from 190 cm at the east part to 300 cm at the west part of the site. The vegetation was dominated by *Eriophorum vaginatum* (Fig. A.1.3D), *Carex canescens* and small amounts of *Agrostis scabra* Willd. (ticklegrass). *Polytrichum* spp. was the most abundant

moss, with *Sphagnum* constituting only a small fraction of moss cover. Dwarf *Salix* spp. and *Betula* spp. were the main shrubs found at the site.

The Unrestored site was a small part of a post-extraction site where the peat deposit did not exceed 265 cm depth, and ditches were filled with peat. The surface was leveled in 2012, but for the purpose of research, no further restoration tasks were conducted. A part of the site became spontaneously colonized by *Betula* spp. and *Carex canescens*, but bare peat dominated at the site. The mean porosity of the first 1 m peat layer was 0.85, lower than at the currently extracted site (0.91; Tab. 4.1). Sedgy peat with compressed horizontally oriented debris was found below 60 – 70 cm depth underlaying well-decomposed *Sphagnum* peat.

The surface of the Active site was entirely bare peat. The peat deposit was 190 – 220 cm deep where our study was conducted. Down to 1 m depth, peat consisted of moderately decomposed *Sphagnum*, but an increase in porosity from 0.84 to 0.94 in the first 60 cm indicated peat compaction at the surface (Fig. 4.1, Tab. A.4.1).

4.4. METHODS

4.4.1. Ground-penetrating radar (GPR)

The pulse EKKO ground penetrating radar with antenna 200 MHz was used to assess the subsurface volumetric gas content (VGC) in peatlands (Doolittle and Butnor, 2009, Slater and Comas, 2009). The choice of the antenna frequency is a compromise between penetration depth and resolution. Lower frequencies (*e.g.*, 100 MHz) penetrate soil deeper but with lower resolution, while higher frequencies (*e.g.*, 200 MHz) reach shallower depths at higher resolution (Cassidy, 2009, Parsekian *et al*., 2010). Peatlands in our study were relatively shallow; thus a better resolution was chosen for the GPR surveys. The vertical resolution is equal to ½ - 1 of the EM wavelength calculated as $\lambda = V f^{-1}$ where V is the velocity of the EM wave and f is the antenna frequency (Møller and Vosgerau 2006). The velocity of 0.036 m ns^{-1} frequently obtained for peat gives the vertical resolution of 0.09 – 0.18 m.

During GPR surveys, an electromagnetic (EM) wave pulse is sent by the transmitter into the ground, where it propagates until it is reflected by underground reflectors (depths at which the dielectric permittivity changes) and returns to the peat surface where it is registered

by the receiver (Comas *et al*., 2008, Parsekian *et al*., 2010). Since the signal is attenuated in mineral soil, the depth of the peat deposit can be assessed using common offset (CO) with both the transmitter and receiver being moved along the transect in equal distance increments (*e.g*., every 5 – 10 cm) while the distance between them remains unchanged (Fig. 4.1). The depth of the peat deposit is then used in common-midpoint (CMP) image processing (Parsekian *et al*., 2010). The CMP method (Fig. 4.1) gives a one-dimensional vertical velocity profile that enables volumetric water content (Θ) and VGC calculation from vertical variations in EM wave velocity (Lunt *et al*., 2005, Slater and Comas, 2009, Parsekian *et al*., 2010, Strack and Mierau, 2010). In this technique, the transmitter and receiver are placed parallel to each other at the middle of the transect (the common midpoint) and are subsequently moved apart in equal steps (5 – 10 cm) while the EM wave pulse is sent and received at each position. The GPR registers the time that it takes for the EM wave to travel from the transmitter to the reflector and then back to the receiver, hence two-way travel time in Fig. 4.1. The CMP approach can be used when subsurface dip angle is smaller than 15° (Neal, 2004). Peat layers oriented horizontally and parallel to each other, which was assessed in CO radargrams at the study sites (Fig. 4.1, Fig. A.4.1), meet this technical requirement for the CMP approach (Parsekian *et al*., 2010). Each site was surveyed once a month along 2 – 6 transects at each site; only two transects were set up at RES-1991 due to severely flooded conditions that limited access with the GPR equipment and three transects at the Natural site due to dense vegetation and uneven terrain. The beginning and the end of each transect was marked in the field at the beginning of the season and remained there until the end of the August GPR survey to ensure data collection at the same transects each month. Altogether, 110 surveys were conducted (CMP and CO) in May – August 2017 and 10 rejected due to low quality radargrams.

The ground-penetrating radar (GPR) is sensitive to changes in the dielectric properties of soil caused by changes in soil moisture content (Warner *et al*., 1990). Other factors (*e.g*., humification, bulk density) can also affect bulk dielectric properties (Doolittle and Butnor, 2009), but change in soil water content is the strongest control that causes the signal attenuation (Parsekian *et al*., 2010). Dielectric permittivity is related to the propagation velocity of the EM wave in peat (Cassidy, 2009) according to $V = c\ (\varepsilon_{r(b)}^{0.5})^{-1}$, where V is the EM wave velocity, c is the speed of the light in vacuum (0.3 m ns^{-1}), and $\varepsilon_{r(b)}$ is the dielectric permittivity of bulk peat (Huisman *et al*., 2003).

We applied the complex refractive index model (CRIM) that has been previously used for VGC calculations in peatlands (*e.g*., Parsekian *et al*., 2010, Strack and Mierau, 2010, Comas *et al*., 2014, Mwakanyamale *et al*., submitted). It allows for calculation of the volumetric water content (Θ) from dielectric and volumetric properties of water, soil and air:

$$\varepsilon_{r(b)}{}^{\alpha} = \Theta\, \varepsilon_{r(w)}{}^{\alpha} + (1-\eta)\, \varepsilon_{r(s)}{}^{\alpha} + (\eta-\Theta)\, \varepsilon_{r(a)}{}^{\alpha}$$

where α accounts for the orientation of the electrical field and the arrangement of peat layers and depends on peat humification (0.35; Kellner and Lundin, 2001, Parsekian *et al*., 2012); $\varepsilon_{r(s)}$; $\varepsilon_{r(w)}$; $\varepsilon_{r(a)}$ are the dielectric permittivity of soil particles (2 for peat), water (80 at 21 °C, 84.1 at 10 °C, and 86.1 at 4.1 °C), and air (1) respectively, and η is porosity (Comas *et al*., 2008, 2011, 2014). Since the temperature of peat at 75 cm depth was between 5 and 10 ˚C, we used $\varepsilon_{r(w)} = 86$ (Malmberg and Maryott, 1956) for VGC calculations in the entire peat deposits as the temperature at greater depths was likely around 5 ˚C. The VGC was calculated from the difference between porosity and Θ (*e.g*., Strack and Mierau, 2010). Before CRIM was applied, interval velocities (the EM wave velocities for peat intervals between two reflectors) were calculated from the Dix relation (Parsekian *et al*., 2010; 2011 after Dix, 1955).

GPR images were processed in REFLEXW version 9.0 (Sandmeier, n.d. a). Static correction of start time was applied to correct the zero time to peat surface. Dewow filter was applied to eliminate low frequency component from GPR data. Due to attenuation and spherical electromagnetic wave spreading of the signal, the gain was applied to the dewowed data. We used Automatic Gain Control (AGC) to amplify the attenuated signal (Sandmeier, n.d. b). Semblance analysis was used to identify most likely true reflectors. The semblance output gives an assessment of EM velocities at the depths of these reflectors (Parsekian *et al*., 2010). Based on the semblance analysis, the arrival time of the signal at the chosen reflectors was picked manually (Comas *et al*. 2007, Parsekian *et al*., 2010, Mwakanyamale *et al*., submitted).

We calculated the VGC in the whole peat profiles from the surface down to the bottom of the peat deposit including the zone of potentially unsaturated peat (above the WT). The WT reflectors could not be identified due to noise from the air wave. Because of high spatial variability of the VGC and the reflector depths on different transects of the same

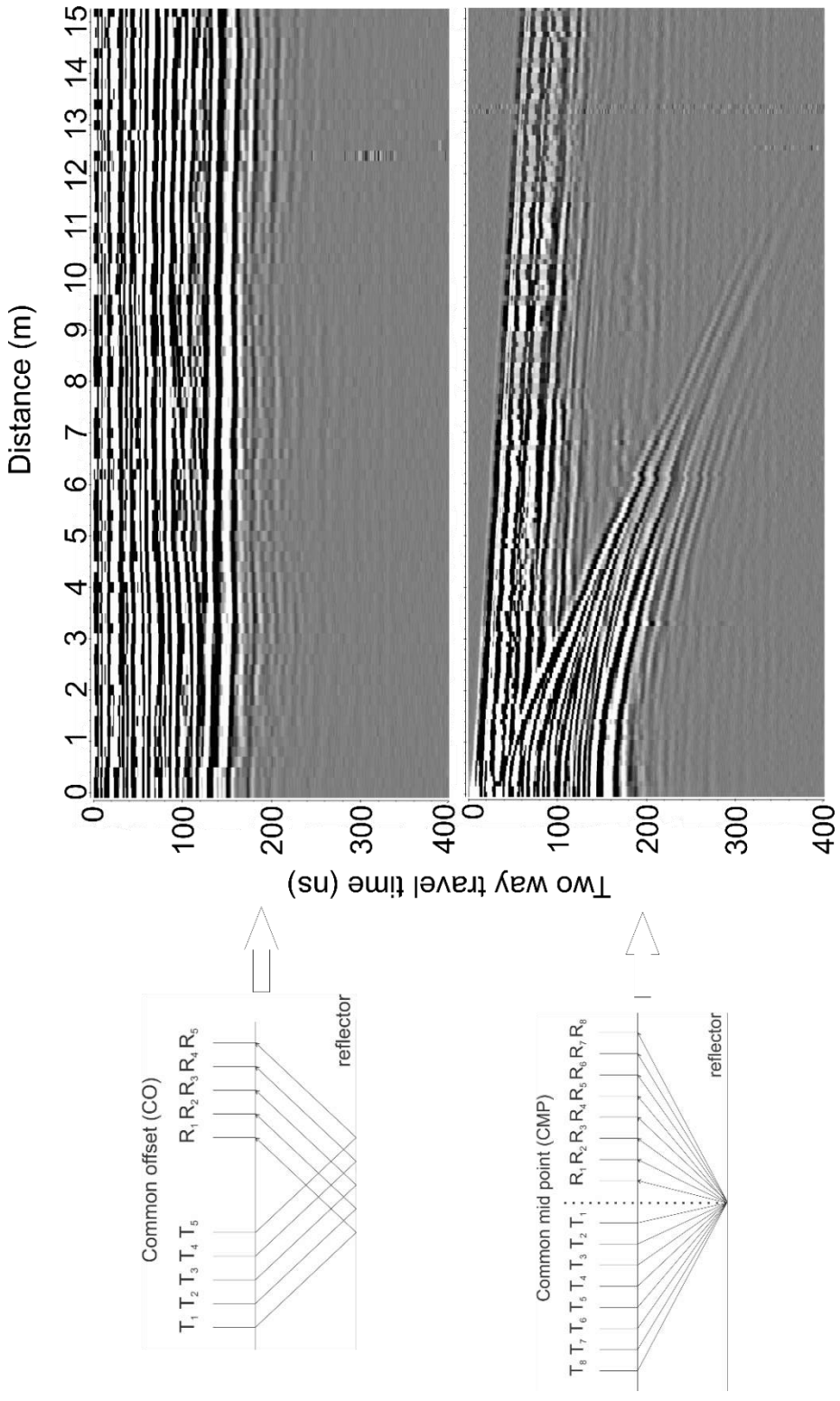

Figure 4. 1. Examples of ground-penetrating radar (GPR) common offset (CO) and common midpoint (CMP) radargrams. GPR survey was conducted at the Active site on June 19th, 2017.

peatland, fixed depth zones were established across all transects (0 – 25 cm, 25 – 50 cm, 50 – 100 cm, 100 – 150 cm, 150 – 200 cm, 200 – 250 cm *etc*. down to the bottom of the peat deposit, as outlined in Fig. 4.4). Then, vertical weighted VGC was calculated for each depth zone within a transect. For example, if the first reflector was at 60 cm, the VGC above this reflector was used for depth zones 0 – 25 cm, and 25 – 50 cm, while the VGC for the 50 – 100 cm depth zone was calculated as a weighted average of the VGC above the reflector (50 – 60 cm) and below the reflector (60 – 100 cm). Finally, a mean for a given depth zone was calculated from the values obtained in this depth zone at each transect within the site and month. These monthly means were used to calculate the average VGC for each site over the whole season. The potentially unsaturated zone could increase the VGC down to the first reflector; thus, we also calculated the VGC for the fixed depth zones that were unaffected by unsaturated peat (see Fig. 4.4 for these zones marked with asterisk).

4.4.2. Volumetric gas content change over time in restored peatlands

CS616 (Campbell Scientific) water content reflectometer probes (referred to as CS probes hereafter) were installed at the end of May 2016 at depths 25 cm, 50 cm, and 75 cm at RES-2012 and RES-1991, at both moss- and sedge-dominated parts of each site. The probes were positioned vertically with the depth of interest at the upper end of the metal rods in the probes (the position of the targeted depth at the probes are indicated with blue arrow in the top left picture in Fig. A.1.5). A cut ~ 10 cm wide was made in peat above the depth of insertion so that the probe could be installed. Care was taken to avoid disturbance to any peat layers that the probes were measuring. The probes registered variations in the time that it takes for an electromagnetic wave to travel the distance along the transmitter length, through the soil and back to the receiver and the period from the wave of the recorded signal is related to volumetric water content (Campbell Scientific Inc., 2012). The period was measured every hour along with soil temperature that was measured by adjacent thermocouple wires installed at corresponding depths. The period was corrected for the temperature following manufacturer calibrations (Campbell Scientific Inc., 2012, Mwakanyamale *et al*., submitted). The VGC was calculated by subtracting Θ from the porosity (Baird *et al*., 2004). We present the VGC change in relation to the first recorded measurement in May 17th (RES-2012) and May 25th (RES-

1991), because the differences between values measured by different probes can result in the difference in Θ up to 1.5 % while the precision of the measurement is 0.1 % (Campbell Scientific Inc., 2012). Therefore, the change in the VGC over time in relation to the first measurement allows the evaluation of small changes of gas content, useful for exploring gas accumulation and release.

4.4.3. Porosity

Eleven peat cores were collected from 0 – 100 cm depth in 10 cm increments by cutting out shallow peat (0 – 50 cm) and using a Russian sampler for deeper layers of peat. If possible, a sample from below 100 cm was collected (Fig. 4.1, Tab. A.4.1). We cored the same depths that were analysed for microbial community and geochemistry (Chapter 2). At each site, except the Active site, two cores were collected targeting: hummocks and hollows at the Natural, bare and sedgy surface at the Unrestored, and mossy and sedgy locations at RES-1991, RES-2009 and RES-2012. Only one core was taken at the Active site (bare peat). Peat segments of known volume were dried in the oven at 70 ˚C until the mass of peat was constant (all moisture removed; Basiliko *et al*., 2007, Andersen *et al*., 2013a) to determine bulk density. Particle density was measured using the displacement of kerosene by a known mass of peat with kerosene used instead of water due to low particle density of peat relative to mineral soils. Bulk density and porosity of peat was calculated according to Hao *et al*. (2008) for each 10-cm peat increment. Humification of all collected peat samples was determined prior to drying, using the von Post scale (Government of Canada, 2013).

The actual peat porosity at a given depth was used to calculate the VGC between reflectors down to 100 cm. For depths below 100 cm, porosity at 90 – 100 cm was used (Strack and Mierau, 2010). However, if porosity was available for 90 – 100 cm and then for a short segment from deeper in peat profile (*e.g*., 130 – 140), we applied the porosity at 90 – 100 cm down to 130 cm depth and then the porosity at 130 – 140 cm for the rest of the profile. If porosity was missing for the surface peat, the value from the depth immediately below was used. In cases of missing porosity for any depth intervals in the top 100 cm, the mean porosity was calculated from measured values immediately above and below that depth (all extrapolated

and mean values are highlighted in grey in Tab. A.4.1). Peat below the cored depths was difficult to obtain without squishing the samples, and especially challenging to obtain at sites where peat was compacted and/or contained large amounts of wooden debris. Even though the peat deposit in RES-1991 was relatively more accessible, we applied the same sampling plan as at other peatlands for consistency (down to 100 cm depth and then the deepest possible sample, which was at 340 – 350 cm). If peat below the sampling depths was in real life more porous than the applied value, the VGC presented here would be underestimated and if less porous, the VGC would be overestimated. The mean porosity in Tab. 4.1 was calculated only from values obtained from the field, excluding values for depths where porosity was extrapolated. All values used in calculations are given in Tab. A.4.1.

4.4.4. Environmental conditions

The water table (WT) was measured manually in water wells close to GPR transects at the time of GPR data collection and additionally by leveloggers (Solinst) installed at RES-1991 and RES-2012 in 2016 that recorded data hourly. The levelogger at RES-1991 sank and we were unable to retrieve the data. The atmospheric pressure was recorded in summer 2016 every hour at RES-2012 with a barologger (Solinst).

4.5. RESULTS

Peat in the Unrestored site was the most decomposed and had the lowest porosity compared to other sites (Tab. 4.1) with considerably low porosity at the peat surface (0.56, Tab. A.4.1, Fig. 4.2e). RES-2009, the Unrestored and Active sites had lower porosity in shallow peat than in the rest of the profiles (Fig. 4.2d, e, f) and the highest mean bulk density of all sites (0.06 – 0.07 g cm^{-3}, Tab. 4.1). The highest particle density was found at the restored sites (1.31 – 1.37 g cm^{-3}, Tab. 4.1). The highest porosity was in RES-1991 (0.96) and in the Natural site (0.95, Tab. 4.1).

The WT was consistently deep in the Active site during the GPR surveys. At other sites, it decreased over the season with the lowest levels in July and August, except RES-1991

where the WT was always above the ground surface (Tab. 4.2). The soil temperature down to 75 cm depth increased until mid-August and then decreased (Fig. 4.3E). Temperature fluctuation at 25 cm depth was more pronounced than at 50 and 75 cm. The change in the VGC at these depths followed the soil temperature fluctuations where increasing temperature was reflected in increasing VGC (Fig. 4.3A, B, and E). Several abrupt gas release (ebullition) events were identified based on the CS probe record at both RES-1991 and RES-2012 at the same time (black arrows in Fig. 4.3A that apply to all panels below the arrows). They corresponded to an atmospheric pressure decrease and a steep increase of the WT level (Fig. 4.3C, D).

The output of CS probes gave an estimate of a change in VGC (Fig. 4.3A, B). The highest increase in VGC, reaching up to 20 – 23 % at RES-2012 and RES-1991, respectively, was observed at 25 cm at moss-dominated locations. At other depths, both sedgy and mossy, the VGC increased up to 18 %. The highest VGC at 25 cm was observed at the end of July, while at greater depths it occurred around August 20th. While the general pattern of VGC change over time was similar at both sites, the estimated VGC varied between depths at each site (Fig. 4.3A, B). The highest VGC at RES-2012 was measured at 25 cm depth, was lower at 50 cm and was the lowest at 75 cm. At all three depths, it was higher at sedgy than at mossy locations. At RES-1991, the VGC was the highest at 25 cm under moss and the lowest at 25 cm under sedges. Also, higher VGC was found at 75 cm than at 25 cm at sedge microsites. Due to equipment malfunction, we were unable to record data from depths 50 cm and 75 cm at moss-dominated microsites. Since peat was saturated over the summer season at RES-1991 the VGC at 25 cm was not affected by atmospheric air. In contrast, WT did drop below 25 cm at RES-2012 (Fig. 4.3D). Thus, VGC at this depth could have been affected by atmospheric air and drops in VGC may be due to wetting up of the peat. The WT in wells close to the CS probes showed highly fluctuating WT dropping down below 25 cm for most of the summer at RES-2012, but rising above 25 cm and remaining above this level in the second half of August 2016.

Table 4. 1. Mean (sd) and range of bulk density, porosity, and peat humification in Von Post scale: 1 – 4 is fibric peat, 5 – 6 mesic peat, and 7 – 10 humic peat (Croft *et al*., 2001). Only values obtained from the field samples are considered, without extrapolated porosity values that were used for the VGC calculation when porosity at a depth was missing. Data were partially included in Chapter 2 of the thesis.

Site	Bulk density (g cm^{-3})					Particle density (g cm^{-3})				
	n	min	max	mean	sd	n	min	max	mean	sd
Natural	23	0.02	0.27	0.08	0.05	15	0.88	2.45	1.26	0.40
RES-1991	22	0.02	0.18	0.06	0.03	11	0.90	1.96	1.36	0.30
RES-2009	22	0.06	0.15	0.09	0.02	11	0.78	1.80	1.37	0.27
RES-2012	15	0.02	0.53	0.12	0.12	17	0.74	2.40	1.31	0.34
Unrestored	18	0.06	0.49	0.15	0.09	12	0.82	1.83	1.19	0.25
Active	10	0.07	0.12	0.09	0.02	10	0.74	1.39	1.05	0.21

Site	Porosity					Von Post humification index (1 - 10)				
	n	min	max	mean	sd	n	min	max	mean	sd
Natural	15	0.92	0.98	0.95	0.02	22	2	4	3	0
RES-1991	11	0.95	0.98	0.96	0.01	20	2	9	4	1
RES-2009	11	0.89	0.94	0.92	0.02	24	3	6	4	1
RES-2012	17	0.79	0.97	0.92	0.04	22	3	5	3	1
Unrestored	12	0.56	0.93	0.85	0.10	18	3	7	5	1
Active	10	0.84	0.94	0.91	0.03	10	3	4	3	0

Using the GPR surveys, we were able to investigate differences in the VGC across a wider range of sites and locations within each site, but at a much lower temporal resolution than CS probes (*i.e.*, monthly). The highest VGC weighted over fixed depths zones within a peatland (depth zones shown in Fig. 4.4) and averaged over all months and transects was found in Unrestored (11.8 %) and in Natural (10.1 %) sites, was slightly lower in RES-2009 (9.9 %), RES-1991 (9.5 %), and Active (9.6 %), and the lowest in RES-2012 (7.2 %). Lack of the VGC data from RES-1991 in May likely caused an underestimation of the mean VGC in this site. When compared to only June – August means, the mean for RES-1991 showed the highest VGC of all restored sites, while RES-2012 the lowest (4.4 %). The Natural site had one of the highest VGC, but the mean calculated from June through August was 8.9 %, close to 9.5 % found in RES-1991, and 9.0 % in RES-2009. These means do include the peat zone above the WT (unsaturated zone) that can affect the VGC down to the first reflector (the depth zones potentially affected by the unsaturated zones are indicated in Fig. 4.4 with asterisk). The mean VGC calculated as above but excluding the potentially unsaturated surface zones were higher: 14.4 % (Natural), 9.1 % (RES-2009), 7.8 % (RES-2012), 13.5 % (Unrestored) and 13.8 %

(Active). The WT at RES-1991 was always above the peat surface, and thus the estimated VGC in saturated conditions remained 9.5 %.

The pattern of mean VGC fluctuation between months over the summer varied between sites but some common trends and potential for highly dynamic VGC at fixed peat depth intervals was observed (Tab. 4.3, Fig. 4.4). When the VGC in the entire peat profile was considered (including the potential influence of the unsaturated peat zone), in Natural, RES-2009, and Active sites, the highest VGC was observed in May, decreased in June, and increased in July followed by another decrease in August. In RES-1991, the VGC increased consistently over the whole summer (data for May not available), with the highest

Table 4. 2. Mean (sd) water table during GPR surveys at the Natural, Unrestored, Active, and three restored sites: RES-1991 – restored in 1991, RES-2009 – restored in 2009, RES-2012 – restored in 2012. No GPR was conducted in May at RES-1991.

Site	Month	n	mean (cm)	sd
Natural	May	3	-17	3
	June	3	-19	0
	July	3	-26	6
	August	3	-25	0
RES-1991	June	2	4	2
	July	2	9	0
	August	2	2	0
RES-2009	May	6	-6	2
	June	9	-2	3
	July	6	-15	7
	August	6	-18	8
RES-2012	May	5	-8	10
	June	5	-1	4
	July	11	-20	13
	August	0	NA	NA
Unrestored	May	7	-21	4
	June	8	-3	7
	July	6	-58	13
	August	6	-51	10
Active	May	0	NA	NA
	June	6	-44	18
	July	6	-37	2
	August	6	-41	7

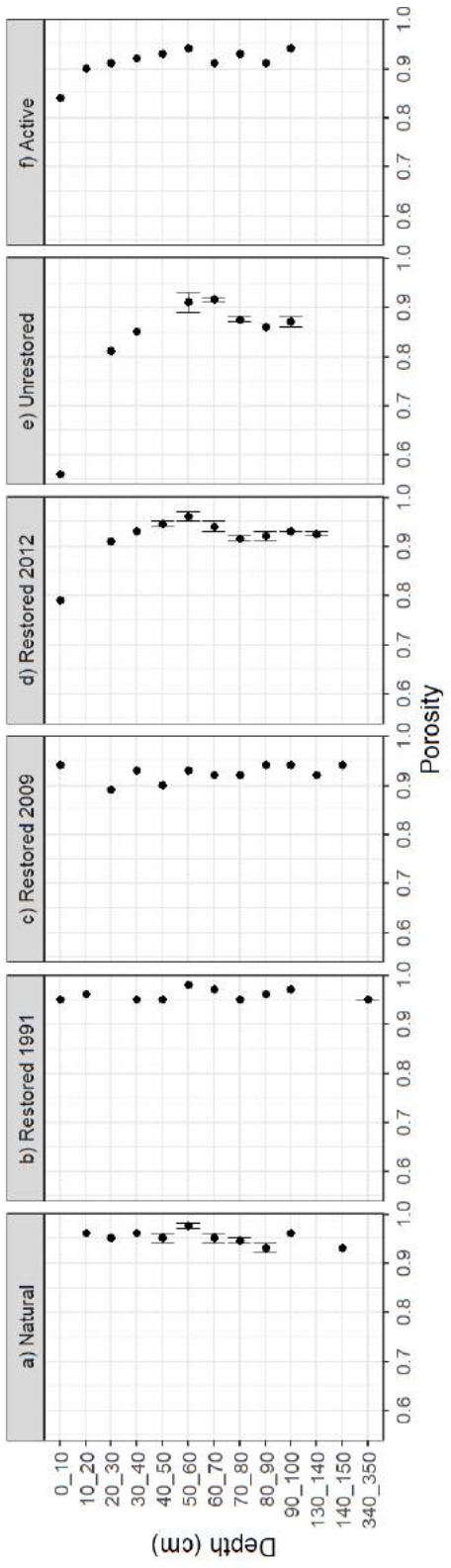

Figure 4. 2. Change in peat porosity with depth. When standard error is given, the value is a mean from two peat samples.

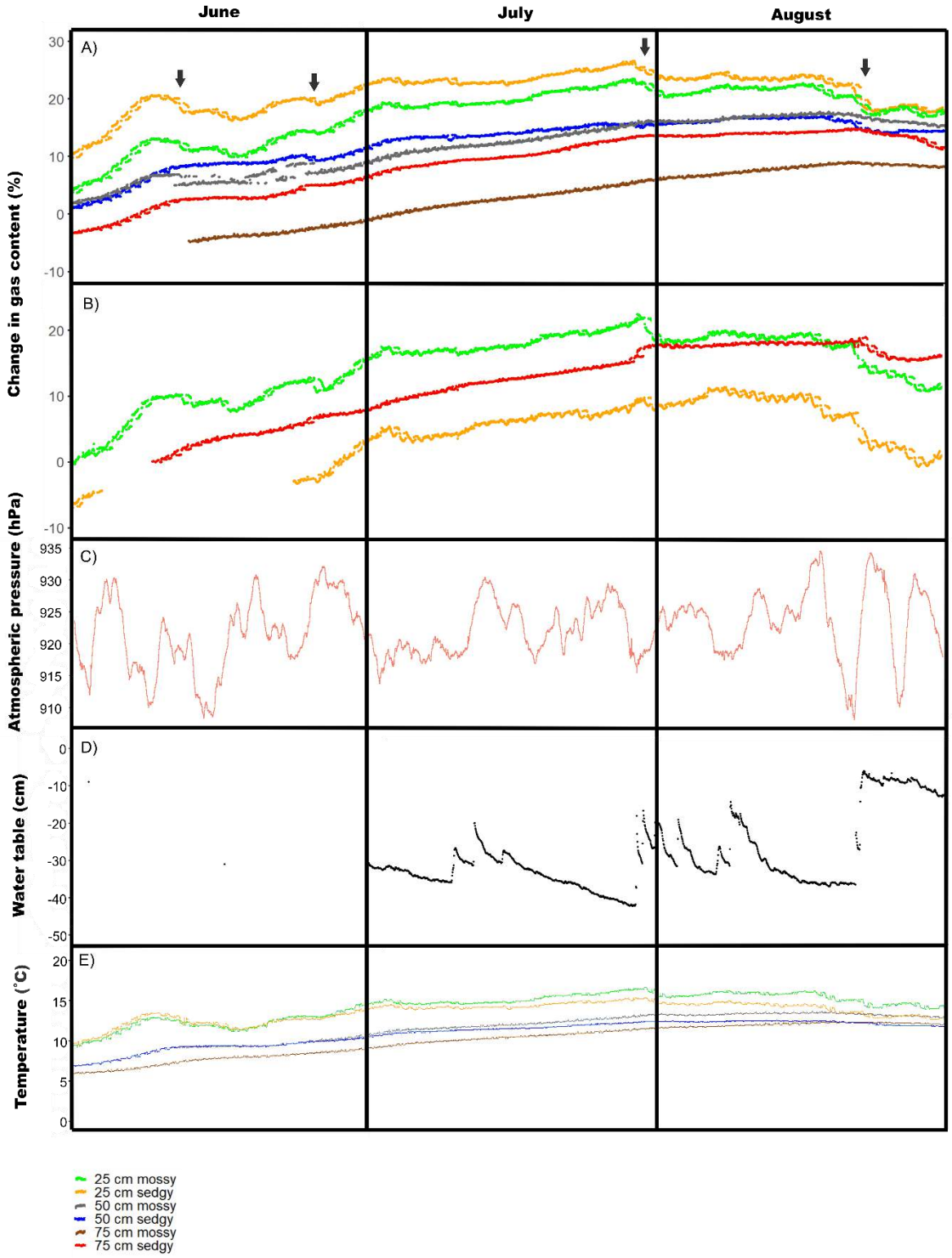

Figure 4. 3. Subsurface free-phase gas content change over time in two restored peatlands: A) RES-2012 and B) RES-1991 (restored in 2012 and 1991, respectively) at depths 25, 50, and 75 cm at mossy and sedgy locations in summer 2016. The gas content was calculated from the output of water content reflectometry probes in relation to the first recorded value in May 17th at RES-2012 and May 25th at

RES-1991; C) atmospheric pressure record with a barologger ; D) water table record with a levelogger at RES-2012; E) Soil temperature at RES-2012. Black arrows indicate VGC decrease events.

Table 4. 3. Monthly mean (sd) volumetric gas content (VGC %) obtained from VGC weighted over fixed depth zones (the zones are outlined in Fig. 4.4) in the Natural, Unrestored, Active and three restored peatlands: RES-1991 – restored in 1991, RES-2009 – restored in 2009, RES-2012 – restored in 2012. No GPR was conducted in May at RES-1991. In the column with potentially unsaturated zone excluded, when the WT was above –18 cm (within the minimum vertical resolution of GPR), we assumed that the impact of the unsaturated zone was negligible, hence the same values as when the unsaturated zone was included in calculations (RES-2009 in May and June, RES-2012 in June and August, and the Unrestored in June).

Site	Month	Potentially unsaturated zone included		Potentially unsaturated zone excluded	
		Mean VGC (%)	sd	Mean VGC (%)	sd
Natural	May	13.6	5.3	12.8	10.9
	June	7.0	7.4	15.1	15.1
	July	12.1	1.3	18.7	3.2
	August	7.7	4.4	11.0	3.9
RES-1991	May	NA	NA	WT above the peat surface	
	June	3.9	2.0		
	July	9.6	1.5		
	August	14.9	8.2		
RES-2009	May	12.5	6.5	12.5	6.5
	June	9.2	4.8	9.2	4.8
	July	13.2	5.3	10.1	5.3
	August	4.6	5.2	4.6	6.9
RES-2012	May	15.5	6.9	16.3	7.3
	June	5.2	2.3	5.2	2.3
	July	4.3	2.8	6.1	5.0
	August	3.6	1.6	3.6	1.6
Unrestored	May	12.4	6.6	13.2	8.0
	June	14.4	3.9	14.4	3.9
	July	11.4	8.1	12.5	10.8
	August	8.9	5.8	13.9	7.3
Active	May	14.5	4.5	WT not available	
	June	5.5	3.4	18.7	NA*
	July	11.1	2.9	13.8	3.4
	August	7.2	1.7	8.9	2.9

* Only one depth at one transect not affected by unsaturated peat

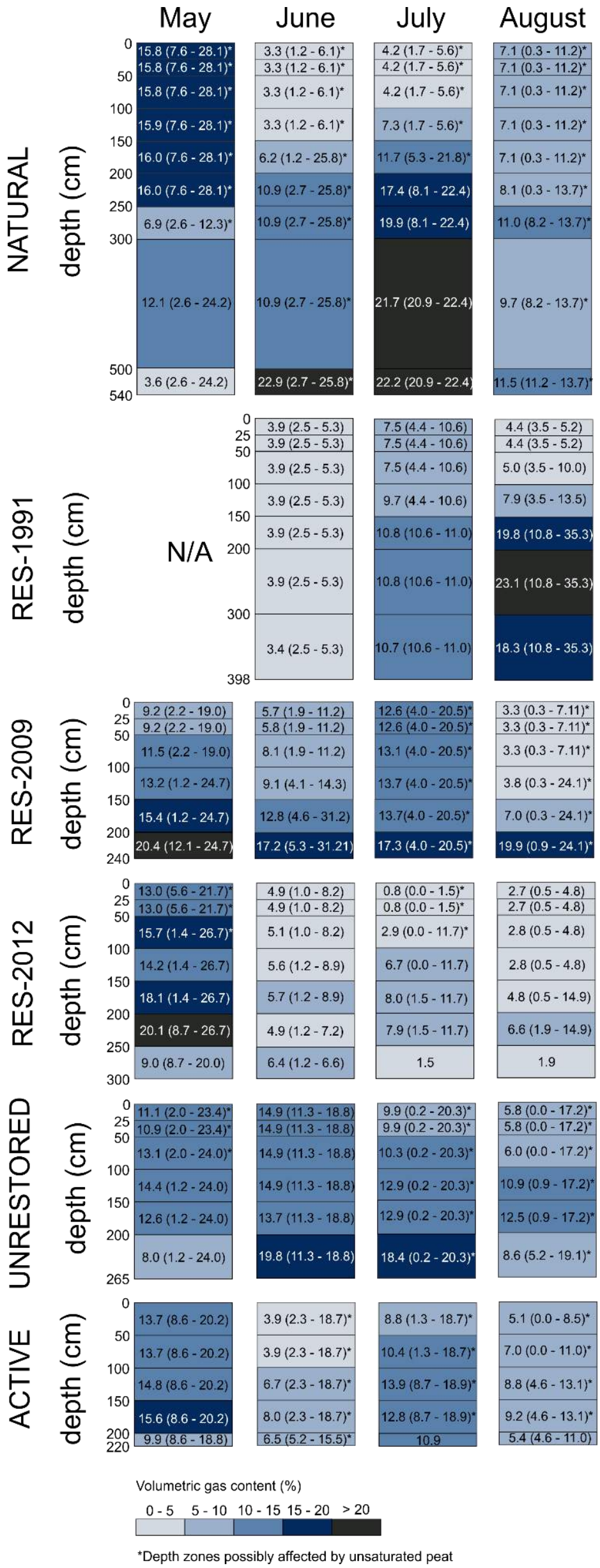

Figure 4. 4. Weighted mean volumetric gas content (VGC %) and the range of values (in the brackets) in Natural, Unrestored, Active and three restored sites: RES-1991 – restored in 1991, RES-2009 – restored in 2009, RES-2012 – restored in 2012. Data for May at the RES-1991 are missing due to extensive flooding at that site. Asterisk denotes the depth zones that were potentially affected by unsaturated peat (down to the first reflector). When the WT was above -18 cm (within the minimum vertical resolution of GPR at 200 MHz, we assumed that the impact of the unsaturated zone was negligible (RES-2009 in May and June, RES-2012 and in the Unrestored site in June) and this profiles do not have asterisk. The water table for the Active site in May was not available. Ground-penetrating radar (GPR) surveys were conducted in summer 2017.

values in August. The trend was opposite in RES-2012, with the VGC decreasing from May to only 3.6 % in August. The Unrestored site showed another unique pattern of VGC change between months with increase from May to June as opposed to a decrease observed at other sites. Then, in July and August, the VGC generally decreased.

When the VGC in peat depth zones that could have been affected by the unsaturated zone were excluded, the pattern of free-phase gas accumulation over time was similar. This however, disappeared in the Active site (Tab. 4.3) where almost the entire profile was potentially affected by the unsaturated zone due to deep first reflector (Fig. 4.4). The first well-defined reflectors in the semblance analysis and reflector pick-up were often found at greater depths than the WT, *e.g.*, WT at -19 cm and the first reflector was at 270 cm depth in the Natural site in June, which means that a large part of the peat profile was not accounted for in these calculations. In many cases, the VGC calculated only for peat below the potential influence of the unsaturated zone was not representative for the whole peat profile.

We found high spatial variability of VGC between transects at the same site and with time within the same transect (Fig. 4.5). The variability of the VGC follows the spatial variability of reflectors between transects at a site, temporal changes in the water content and dynamic nature of the spatial distribution of water and gas in peat matrix (Fig. A.4.1). Sometimes an elevated mean weighted VGC at a certain depth was a result of high VGC in one transect only at this depth; *e.g.*, 6.6 % at RES-2012 in August at 200 – 250 cm (Fig. 4.4) was driven by 15 % VGC at 165 – 250 cm in one transect while in other transects, the free-phase gas constituted only 2 – 5 % of the peat volume. Similarly, the weighted mean VGC > 20 % at 200 – 300 cm in RES-1991 (Fig. 4.4) was determined by 35 % VGC in one of the transects at this depth, while in the other one the VGC was 10 – 11 %. A noticeably high VGC at the bottom of RES-2009 that persisted over the whole summer season, was caused by elevated VGC (14 – 31 %) in three transects in June (Fig. 4.5 shows two transects T3 and T5), rather evenly distributed free-phase gas in July (except one transect that always showed low VGC) and again elevated VGC only in one transect (24 %) in August. The VGC in the Active site below 100 cm depth in June was also not spatially consistent; 15 – 19 % VGC was found at these depths in two transects out of six. However, the mean VGC > 20 % in the Natural site in July was determined by consistently high VGC in all transects at these depths (Fig. 4.5 shows

two transects T1 and T2). Also, the mean weighted VGC at the bottom of the peat sediment in the Unrestored site in June and July was rather consistent spatially. Only two transects in July showed > 5 % of gas, which was considerably lower than in other transects at this depth (15 – 20 %).

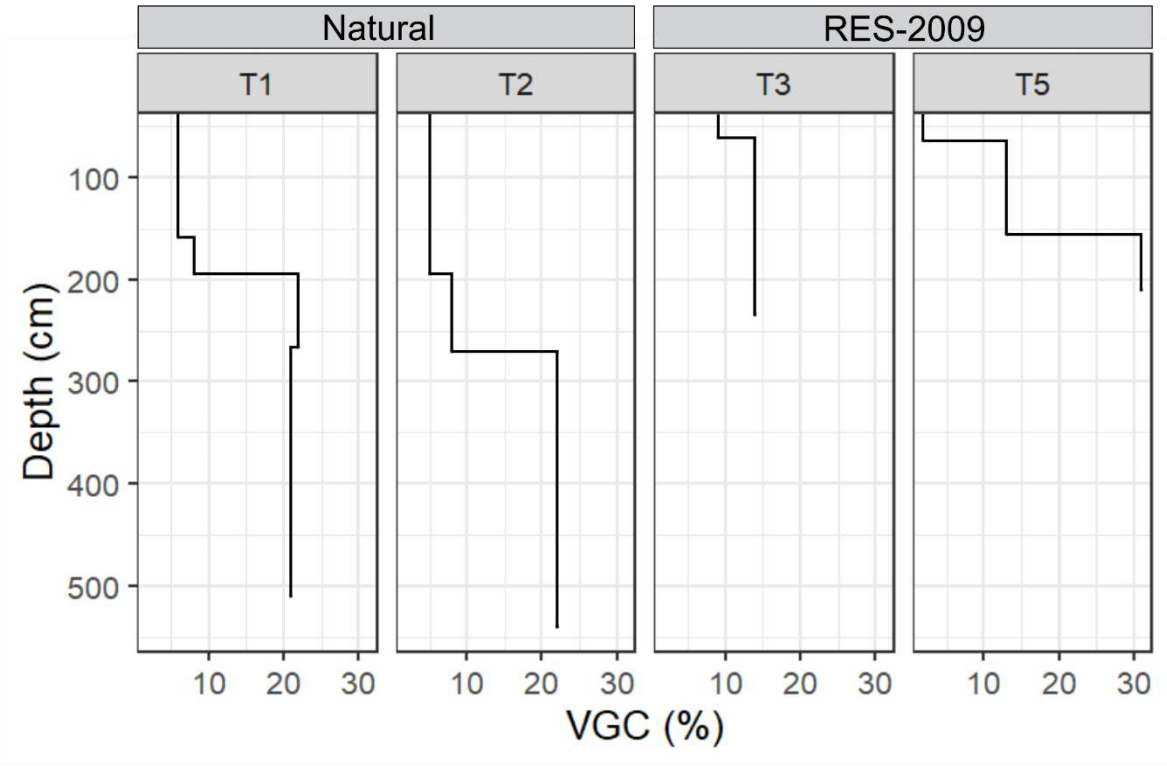

Figure 4. 5. Examples of the volumetric gas content (VGC) depth profiles at two chosen transects from summer 2017 ground-penetrating radar (GPR) surveys at the Natural site and the site restored in 2009 (RES-2009). The graphs show spatial variability of the VGC between transects surveyed the same day. T1, T2, T3, and T5 are transect numbers at these sites.

4.6. DISCUSSION

4.6.1. Subsurface free-phase gas content in peat

The VGC in the studied peatlands was comparable to the ones reported in previous research on the VGC in natural peatlands (up to 20 %, *e.g.*, Rosenberry *et al.*, 2003, Comas *et al.*, 2007, 2008, 2011, Strack and Mierau, 2010, Parsekian *et al.*, 2010, 2011, 2012, Comas *et al.*, 2014).

The VGC > 20 % in deep peat at the Natural and restored sites was similar to the VGC of 24 % previously reported by Parsekian *et al.* (2010) at similar depths. The average VGC in our study was very similar to the ones obtained by Mwakanyamale *et al*. (submitted), where authors measured VGC at the Seba Beach complex in 2014 (three years prior to our research), and obtained values of 7 % VGC at RES-2012 and 13.4 % at the Unrestored site, including the unsaturated zone. Considering high horizontal and vertical variability of the VGC in peat at Seba Beach sites, and reported elsewhere (Comas *et al*., 2005, 2014), the differences between sites, although present, are relatively small and would likely vary if the transects were chosen at other locations within the sites.

The VGC calculated including, and separate calculations that excluded the zones of potential influence of unsaturated peat, are presented in this research to clarify that atmospheric air could potentially change the VGC down to the first reflector. However, when the first reflector is considerably deeper than the WT, the calculations excluding peat between the surface and the first reflector can be not representative for the peatland. Thus, here we discuss the VGC that accounts for the entire peat profile, including the potentially unsaturated zone. The WT gives only an estimate of a depth where the atmospheric air does not disturb the biogenic gas content, but the capillary fringe that keeps peat partially saturated above the WT also sets up the boundaries between saturated and unsaturated zone (Niedermeier and Robinson, 2007). Additionally, highly fluctuating WT that is characteristic for disturbed peatland ecosystems (Price, 1996), can trap atmospheric air and cause an overestimation of the biogenic gas content in subsurface peat (Baird *et al*., 2004).

The VGC in Seba Beach peatlands seemed to be independent of the post-extraction management (*i.e*., large amounts of gas in the Unrestored and restored sites, and the increase in the VGC in the Active and restored sites except RES-2012). Despite a clear increase of mean VGC calculated for June – August from RES-2012 through RES-2009 to RES-1991 (4.4 %, 9.0 %, and 9.5 %, respectively), there was no clear evidence of the VGC recovery progress with time post-restoration due to many possible factors governing free-phase gas accumulation. Important factors include CH_4 production (Chapter 2) that clearly occurred in the Active site even during extraction, hydrology, peat structure that promotes either gas trapping or release, vegetation cover that also affects peat structure (*e.g*., Kellner *et al*., 2005), the amount of emitted CH_4 (Chapter 3), and the amount of CH_4 that becomes oxidized (Sundh *et al*., 1995).

Overall, none of these components alone would be a good predictor of the VGC and the VGC alone would not be a good predictor of CH_4 balance recovery progress; however, the age of restoration could have contributed to the VGC in these sites by providing time for gas to accumulate post-restoration. However, this was not observed. Additionally, high spatial variability in free-phase gas distribution in the peat deposit can overshadow the time post-restoration by elevating the mean VGC at some depths and/or locations within a given site.

The direct relationship between VGC and CH_4 emission was not observed either, even though other research has shown the average VGC and CH_4 flux synchronized in time (Comas *et al*., 2007). Sites RES-2009, Unrestored and Active with low CH_4 emission and potential production rates (*e.g*., 0.2 µmol g^{-1} d^{-1} of CH_4 produced and 0.2 mg m^{-2} d^{-1} of CH_4 emitted at the Unrestored site; Chapter 2 and 3) accumulated considerable amounts of gas over time. A few CH_4 production hot spots at RES-2009 (Chapter 2) could at least partially explain CH_4 accumulation. Although the presence of free-phase gas could be related to air entry in the unsaturated zone, large volumes of gas at depth were also frequently measured at all of these sites. The Natural site appeared to follow this pattern as well; high VGC was paired with moderate CH_4 emission (mean 9 mg m^{-2} d^{-1}; Chapter 3). In contrast, in RES-2012, where CH_4 emission was large (257 mg m^{-2} d^{-1} including ebullition), the subsurface VGC was low and decreased over summer as the flux increased (Chapter 3). Only at the oldest restored site, RES-1991, both gas accumulation and CH_4 emission (209 mg m^{-2} d^{-1}; Chapter 3) were high, which was probably linked to high CH_4 production and low oxidation rates in the waterlogged peat deposit. Methane fluxes, that are to date the main source of information about CH_4 cycling in post-extracted peatlands, therefore appear to be not the best proxy for the subsurface VGC. Flooded restored sites like RES-1991 can show a different pattern of VGC accumulation in relation to CH_4 emission than non-flooded sites. Thus, our first hypothesis based on proportional relation between CH_4 emission and VGC was not supported.

In spite of no clear relationship between the VGC and the CH_4 emission, the latter was probably partially responsible for the VGC change with depth within the profile. The decreasing VGC toward the surface of the peat deposit in Seba Beach sites was likely due to CH_4 emission from the shallower peat zones (Glaser *et al*., 2004, Coulthard *et al*., 2009) that created a CH_4 concentration gradient stimulating the upward diffusion of CH_4 from deeper peat

layers. The upward gas movement was also detected by Comas *et al*. (2011). However, it is not known whether ebullition is mainly sustained by a deep or shallow pool of free-phase gas (Parsekian *et al*., 2011, Comas *et al*., 2014). Since the most intensive CH_4 production occurs in shallow peat immediately under the WT due to the availability of substrate and anoxic conditions (Chapter 2, Chanton *et al*., 1995, Glaser *et al*., 2004, Couwenberg and Fritz, 2012, Bridgham *et al*., 2013, Klapstein *et al*., 2014), high VGC in deep peat in Seba Beach sites was likely derived from low CH_4 production rates over a longer period of time and/or free-phase gas lateral and upward movements. To raise the VGC from 0 % to 22 % in 1 m^2 of a layer 269 cm thick (*i.e*., the VGC in July at 271 – 540 cm shown in Fig. 4.5, transect 2 of the Natural site) from June 1st to August the 31st (92 days), assuming 50 % contribution of CH_4 to the VGC, the daily CH_4 production rate of 0.6 nmol CH_4 g^{-1} (dry peat) d^{-1} would be required to account for such an amount of gas to accumulate (197 g of CH_4 in 2.69 m^3 peat bulk). This rate is three orders of magnitude lower compared to that around the water table in shallow peat (Chapter 2). Since the residence time of CH_4 in peat can be 28 – 120 days in the first 1 m depth (Strack and Waddington, 2008), this production rate could support the 22 % of VGC even if it was produced only in one season at steady rates. However, Comas *et al*. (2007) reported that the subsurface free-phase gas does not accumulate steadily and rapid changes in its accumulation can be detected in weekly measurements. Also, Charman *et al*. (1994) dated gaseous CH_4 at depths < 150 cm at a natural peatland for 2,400 radiocarbon years which means that at least a part of free-phase gas can be thousands of years old in the Seba Beach sites. It is not known if the old pool of CH_4 was released during the extraction or if some of it remained in the peat and new CH_4 production being added to the pool of old CH_4. Since VGC was often still high at depth at Active and Unrestored sites, there is no strong evidence that gas is lost throughout the profile during extraction. Further research, including radiocarbon dating of gaseous CH_4 are recommended to better understand the processes that govern free-phase gas accumulation, its age, depth of origin, movement and release in post-extracted and restored peatlands.

Likely, the peat structure was largely responsible for free-phase gas accumulation at these study sites. Kettridge and Binley (2011) assessed that the variation in peat porosity accounted for 65 % of trapping abilities of peat, and the spatial distribution of peat components for the remaining 35 %. Ramirez *et al*. (2016) reported that peat of low porosity can store free-

phase gas for a longer period of time than peat of high porosity. This explains unexpectedly high amounts of free-phase gas in the Unrestored site and its accumulation over the summer in the Active site. The low peat porosity in these sites was likely caused by peat subsidence due to exploitation, drying, enhanced decomposition (peat oxidation), and highly fluctuating WT (*e.g.*, Price, 1997, Waddington *et al.*, 2015) and constituted a semi-confining layer trapping a slowly increasing volume of free-phase gas (Kellner *et al.*, 2004, Strack *et al.*, 2005, 2006a). Low porosity decreases the permeability of peat, saturated hydraulic conductivity, and lateral drainage and as such promotes gas accumulation (Kettridge *et al.*, 2013, Waddington *et al.*, 2015). Similar mechanisms caused by already accumulated free-phase gas in highly porous sites (*e.g.*, the Natural and RES-1991) would drive further gas accumulation. Low peat porosity of the surface peat at RES-2012 could act as a semi-confining layer for short-term accumulation of free-phase gas followed by abrupt and steady CH_4 ebullition (Chapter 3). This could result from recurring discontinuous gas pockets or zones of high gas pressure (Kellner *et al.*, 2004), with ebullition events hence resulting in low VGC at this site. We presume that peat structure and possible lateral gas movements at depths > 200 cm at RES-2009 were responsible for high VGC at these depths. A fairly continuous reflector was present at 200 cm depth, clearly visible in RES-2009 T4 transect radargrams where the peat deposit reached maximum depth of 240 cm for the site (Fig. A.4.1, transect T4). Additionally, dense sedge roots can enhance rhizospheric oxidation of CH_4 (*e.g.*, Watson *et al.*, 1997, Popp *et al.*, 2000) lowering CH_4 flux at the surface, while the CH_4 pool at greater depths could remain relatively large.

4.6.2. Environmental conditions driving the VGC in peat

The accumulation of CH_4 over winter under the ice and snow (Slater and Comas, 2009) was reflected in high VGC in May. The ice was still thawing in May leaving patches of frozen peat that disappeared by June 6th releasing the accumulated free-phase gas (*e.g.*, Tokida *et al.*, 2007b, Comas *et al.*, 2008, Juottonen *et al.*, 2008), observed as a declination in VGC, except for the Unrestored site. A subsequent increase in VGC in July was observed as the temperatures increased (Dunfield *et al.*, 1993, Lai, 2009).

Similar free-phase gas response to atmospheric and hydraulic pressure changes that has been observed in natural peatlands (Fechner-Levy and Hemond, 1996, Rosenberry *et al*., 2003, Glaser *et al*., 2004, Strack *et al*., 2005, Tokida *et al*. 2005, Tokida *et al*., 2007a, Comas *et al*., 2008, Waddington *et al*., 2009a) was also recorded at our restored sites. Sudden VGC decline was associated with an atmospheric pressure drop and sharp increase in the WT after a longer period of WT drawdown. During these events, the WT dropped to almost -40 cm at RES-2012 (Fig. 4.3D), below the depth of the CS probes installed at 25 cm depth. Likely, the VGC records at this depth were affected by the presence of atmospheric air. When the WT raised, the VGC dropped as a result of replacing air with water which could be falsely interpreted as ebullition. However, sudden VGC decrease was observed at the same time at flooded RES-1991, where rising WT would shrink the gas bubbles or prevent their growth and increase their mobility in peat pores as peat expands with rehydration (Strack *et al*., 2006a). As bubbles move upwards, the low atmospheric pressure promotes their release from near surface peat (Comas *et al*., 2011). Also, with peat rewetting, its volume expands making the VGC appear lower than it is, which could contribute to the VGC decrease at sharp WT increase.

4.7. CONCLUSIONS

The processes of free-phase gas accumulation and release appear to be similar at restored and Natural peatlands. This peatland function can be recovered quickly post-restoration, but also can be sustained at the Unrestored and Active sites likely due to free-phase gas accumulation in compacted peat of low porosity. The age of restoration likely contributes to a complex network of factors that determine VGC accumulation, *i.e*., peat properties and hydrological conditions that govern subsurface water and free-phase gas movement, but no clear evidence was found that the time post-restoration was the main or only factor. The VGC was highly variable horizontally and vertically in peat deposits, but also varied greatly between months, and this could overshadow the effect of the restoration age. High amounts of free-phase gas (> 20 %) were often found at greater depths, but the place of origin, its age and movement directions are unknown, and further research is recommended to understand the free-phase gas dynamics at these depths. Methane emission does not always reflect the VGC and should not be used as a proxy for the VGC assessment in post-extracted peatlands, even though a proportional

relationship was found in previous research on natural bogs. Considerable free-phase gas accumulation in the Unrestored and Active peatlands indicated that the VGC alone does not determine the return of the CH_4 dynamics in post-extraction peatlands.

CHAPTER 5: Conclusions

5.1. SUMMARY OF RESULTS

According to results obtained in my study, post-extraction peatland restoration stimulates the recovery of all elements of CH_4 cycling from CH_4 production to storage, oxidation and release. Despite generally low abundance of CH_4-cycling microorganisms compared to the abundance of all *Bacteria* and *Archaea*, we observed significant differences in CH_4 cycling between restored and unrestored sites. First, the abundance and diversity of CH_4-cycling microorganisms was considerably higher in the oldest restored site than in other sites and the community was similar to that in the site restored in 2009. These sites were restored 25 and 7 years prior to our research but shared similar vegetation (mostly vascular plants dominated by graminoids) and both were wet (the oldest restored site was often flooded), thus we attribute the microbial similarities between these sites to local environmental conditions rather than the age of restoration. Interestingly, the site restored in 2012 (the youngest restored site, with peat sampled at the drier part of the site), the Natural, Unrestored and Active sites had similar CH_4-cycling community, but these similarities did not translate directly to the methanogenic and methanotrophic activity. Potential rates of CH_4 production and oxidation were measured only at three sites: Natural, Unrestored and RES-2009. The highest potential rates of CH_4 production and oxidation were found in the Natural site, but the rates of CH_4 production at the Unrestored were close to zero, similar to the ones in RES-2009 with that difference that a few CH_4 production hot spots were found at the restored site. These hot spots were likely driven by the presence of dense sedge roots and their exudates. We attributed this generally low rate of CH_4 production in RES-2009 to high concentration of Fe^{3+} that could inhibit methanogenesis and was linked to a shallow peat deposit and underlaying clay rich in Fe^{3+}. Highly fluctuating water table could promote upward movement of Fe^{3+} in the peat profile.

Study results also indicate that the occurrence and activity of methanogens and methanotrophs was largely driven by the oxic – anoxic boundary in peat that is roughly determined by the water table position. Thus stable hydrological conditions are essential for these microorganisms to thrive. However, methanotrophs appeared to be more ubiquitous than

methanogens. They were more evenly distributed in peat profiles, although their highest abundance was found close to the water table, but their activity did not vary below and above the water table. Also, the highest abundance of *Alphaproteobacteria* associated with undisturbed but harsh conditions of natural peatlands, was found in the Natural and the oldest restored sites, with *Methylocystis* moderately abundant in other sites as well. In contrast, methanogens preferred deeper peat where anoxic conditions were undisturbed. Their activity was significantly higher immediately below the water table than 10 – 20 cm above the water table. Methanotrophic activity appeared to be indifferent to physicochemical factors; however, the highest abundance of methanotrophs close to the water table coincided with high phosphate, propionate, and citrate concentrations. Methanogenesis was sensitive to the concentration of Fe^{3+}, several short chain fatty acids and EC (the concentration of dissolved solids ionized in pore water). Also, the abundance of methanogens in deep peat coincided with a high concentration of formate. Indeed, the great majority of identified methanogens was hydrogenotrophic. Acetoclastic methanogens were present mainly in the oldest restored site.

We found that CH_4 emission from flooded/wet and sedge-dominated locations at the restored sites (the oldest and the youngest) was two orders of magnitude higher than that at the Natural site. This included both high steady fluxes and abrupt ebullition that occurred only at these sites. Steady fluxes contributed the majority of CH_4 emissions with ebullition accounting for only 6 – 7 %. Surprisingly low CH_4 emissions and low CH_4 concentration in pore water, which barely increased over the summer, were observed in the third restored site, RES-2009. This indicates that chemical conditions in restored sites can suppress not only CH_4 production, but subsequently CH_4 emission and the subsurface pool of dissolved CH_4. Peat chemistry can also supersede the effect of hydrological conditions and vegetation on CH_4 cycling. Despite the possibility of initially high CH_4 emissions from restored sites, restoration is necessary as far as the recovery of CH_4 cycling processes is considered. Without restoration, not only CH_4 production, as shown in Chapter 2, but also emissions remain close to zero, as observed at the Seba Beach Unrestored and Active sites. Also, the pool of CH_4 dissolved in pore water at the Unrestored site did not recover and did not increase over the summer like in restored and Natural sites.

Methane production, emission, and the concertation in pore water appear to be linked to each other, but the subsurface free-phase gas content and its patterns over the summer do not always follow other components of CH_4 cycling in post-extraction restored and unrestored peatlands. We observed almost as high volumetric gas content (VGC) in the Unrestored and Active sites as in the Natural and restored; however the mechanism of free-phase gas accumulation was likely driven by low porosity of compacted and mineralized peat in the Unrestored and Active sites. However, we do not know if some of the free-phase gas remained in peat in spite of extraction or if the entire gas volume observed was produced at low rates during extraction and post-extraction. Nevertheless, even slow production rates can add up to a measurable increase of the VGC, even in severely disturbed peatlands, thereby maintaining the free-phase gas storage.

The VGC in Seba Beach sites proved to be highly dynamic with high spatial (vertical and horizontal) and temporal variability. The most pronounced changes in VGC were observed in shallow peat following changes in atmospheric pressure and fluctuating water table. High VGC was often found in deep peat layers. Its high values in May suggested free-phase gas accumulation in wintertime and CH_4 production occurring under ice and snow in all sites; however, the free-phase gas release observed as a decrease in VGC in June compared to May did not occur in the Unrestored site likely due to peat structure acting as a semi-confining layer that trapped the gas in the peat.

Overall, CH_4 fluxes, often used to assess the recovery of CH_4 cycling mechanisms in restored sites, cannot predict the subsurface pool of free-phase gas. In fact, in some peatlands, like in RES-2012, high CH_4 emission can be coupled to low VGC indicating that the majority of CH_4 is emitted and not stored. Also, given high VGC in the Unrestored and Active sites, I advise that the VGC alone should not be used as a proxy for CH_4 cycling recovery post-extraction but rather considered together with other components of CH_4 cycling.

5.2. SIGNIFICANCE OF THE RESEARCH

My research is the first to show how peatland extraction and further management (either lack of restoration or restoration) changes all major components of CH_4 cycling in Canadian peat

extraction sites: production, oxidation, storage, and emission. It contributes to overall understanding of the role of peatland restoration in returning ecosystem function and ultimately leads to a conclusion that peatland restoration is necessary to re-establish mechanisms of CH_4 cycling as a part of returning the carbon balance in disturbed peatlands. It also provides more detailed information for the industry regarding the improvement of processes associated with peatland restoration. My study is an encouragement for researchers to merge different disciplines and methodologies to answer complex research questions in the field of environmental management. Presented research can potentially contribute to more accurate assessment of CH_4 emission from extracted and restored sites. It highlights the necessity of including other potential sources of CH_4 emission from managed peatlands, *e.g.*, ebullition post-restoration and potential release of free-phase gas in early stages of peatland extraction. Our research can also serve as a baseline to similar studies on CH_4 cycling in peatlands disturbed by other forms of management, *e.g.*, peatland drying for agriculture and disturbance caused by the mining industry.

5.3. RECOMMENDATION FOR FUTURE RESEARCH

With rapidly developing molecular methodologies and increasing availability of metagenomics and metatranscriptomics, future functional analyses of microbial genomes from post-extraction restored and unrestored sites are highly recommended to identify the processes and interactions between the microorganisms that could affect the abundance, diversity, and activity of CH_4-cycling microorganisms. Since the presence and activity of microorganisms depend on peat chemistry (*e.g.*, the presence of potential terminal electron acceptors and other compounds that can inhibit or stimulate methanogenesis) and environmental conditions (*e.g.*, peatland hydrology, the quality of carbon substrate and the presence of vascular plants supplying root exudates, temperature, and pH), I recognize the importance of conducting microbiological studies in parallel to chemical analyses to enable linking the presence and function of microbes to the local conditions prevailing in disturbed peatlands.

Molecular analyses are relatively demanding in terms of methodology, cost, and effort and can be too complicated and time-consuming for the industry to implement in their regular

post-restoration peatland monitoring; thus, the recommended studies would have more of an academic implication and, connected to other elements of CH_4 cycling, could potentially support decision making about peatland management.

Also, future long-term research that would track changes in CH_4 cycling post-restoration occurring at the same site over time would help eliminate the local site-specific conditions and focus on the progress of CH_4 cycling processes' recovery. I recommend close geochemical monitoring of shallow restored peatlands, since considerable concentrations of ions can occur in these sites, depending on the water table fluctuation and the chemistry of the mineral soil underlaying the peat deposit. If these ions are potential TEAs, they can possibly inhibit methanogenesis.

I also acknowledge the need for diurnal and wintertime measurements of CH_4 emission at both undisturbed and disturbed peatlands. Methane emission is often measured over the growing season and then extrapolated to the entire year assuming certain contribution of winter fluxes to the annual CH_4 emission, but this contribution is highly variable in natural peatlands (*e.g.*, Melloh and Crill, 1996, Pelletier *et al*., 2007, Saarnio *et al*., 2007) and is unknown in post-extracted and restored sites. Also, the daily CH_4 fluxes are often extrapolated to diurnal fluxes, but higher night emissions (both steady flux and ebullition) have been observed in northern peatlands (Waddington *et al*., 1996, Glaser *et al*., 2004, Gogo *et al*., 2011, Goodrich *et al*., 2011). More studies on night and wintertime fluxes at post-extracted sites could improve the assessment of the annual CH_4 emission, most importantly from restored sites that can emit more CH_4 than natural peatlands.

I strongly recommend a series of GPR surveys prior to peat extraction and then immediately after water table lowering and removal of the vegetation to assess how much gas has been released to the atmosphere either as CH_4 or CO_2. Later surveys to assess the emission can be challenging to interpret since my study showed that the free-phase gas content can quickly increase even due to peat compaction. The assessed emission should be included in national reports on greenhouse gas emissions from peat extraction. Given results from my study and the previous study of Mwakanyamale *et al*. (submitted) that both unrestored and restored peatlands can accumulate considerable amounts of free-phase gas over a short period of several years, and high VGC is often present in deep peat, future studies on the age of the

residing gas with respect to depth, including temporal variability in the percentage of gas of different age, combined with hydrological studies would greatly contribute to our understanding of free-phase gas storage and dynamics in post-extraction peatlands.

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Appendix 1

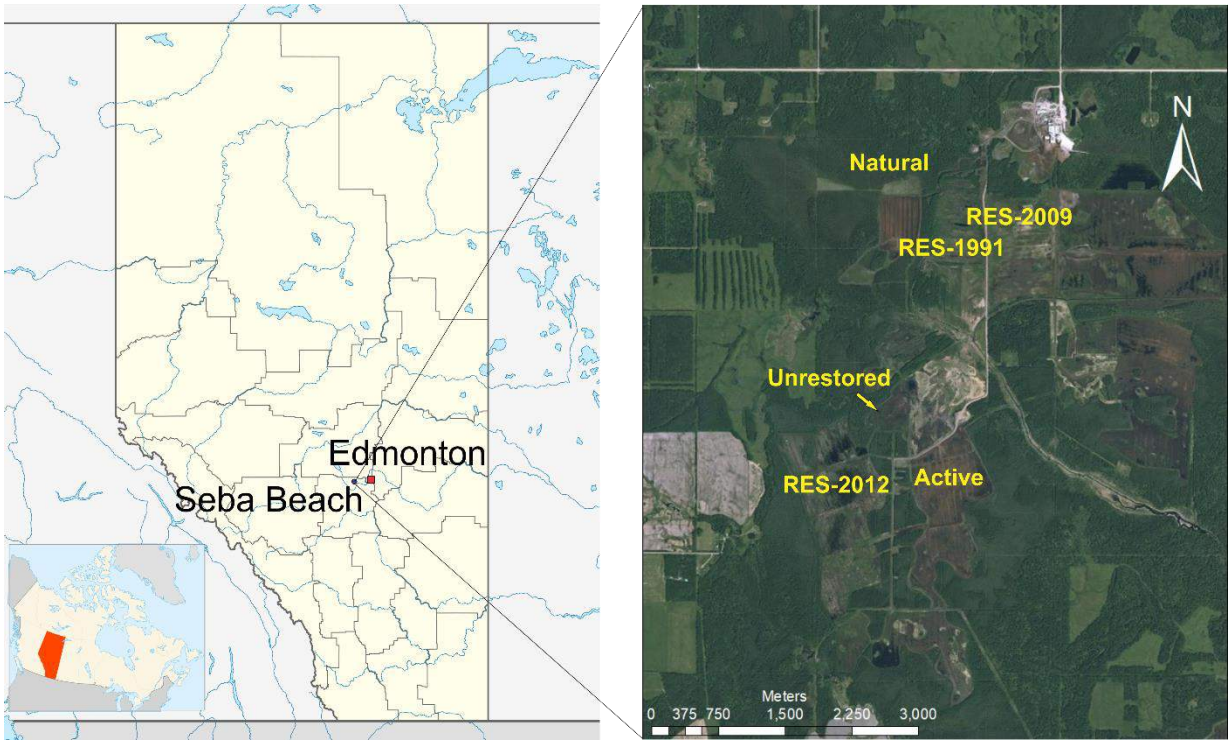


Figure A. 1. 1. Location of the Seba Beach horticulture peatland complex. RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012. Sources: Esri, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community. Source of Alberta map: https://en.m.wikipedia.org/wiki/File:Canada_Alberta_location_map_2.svg

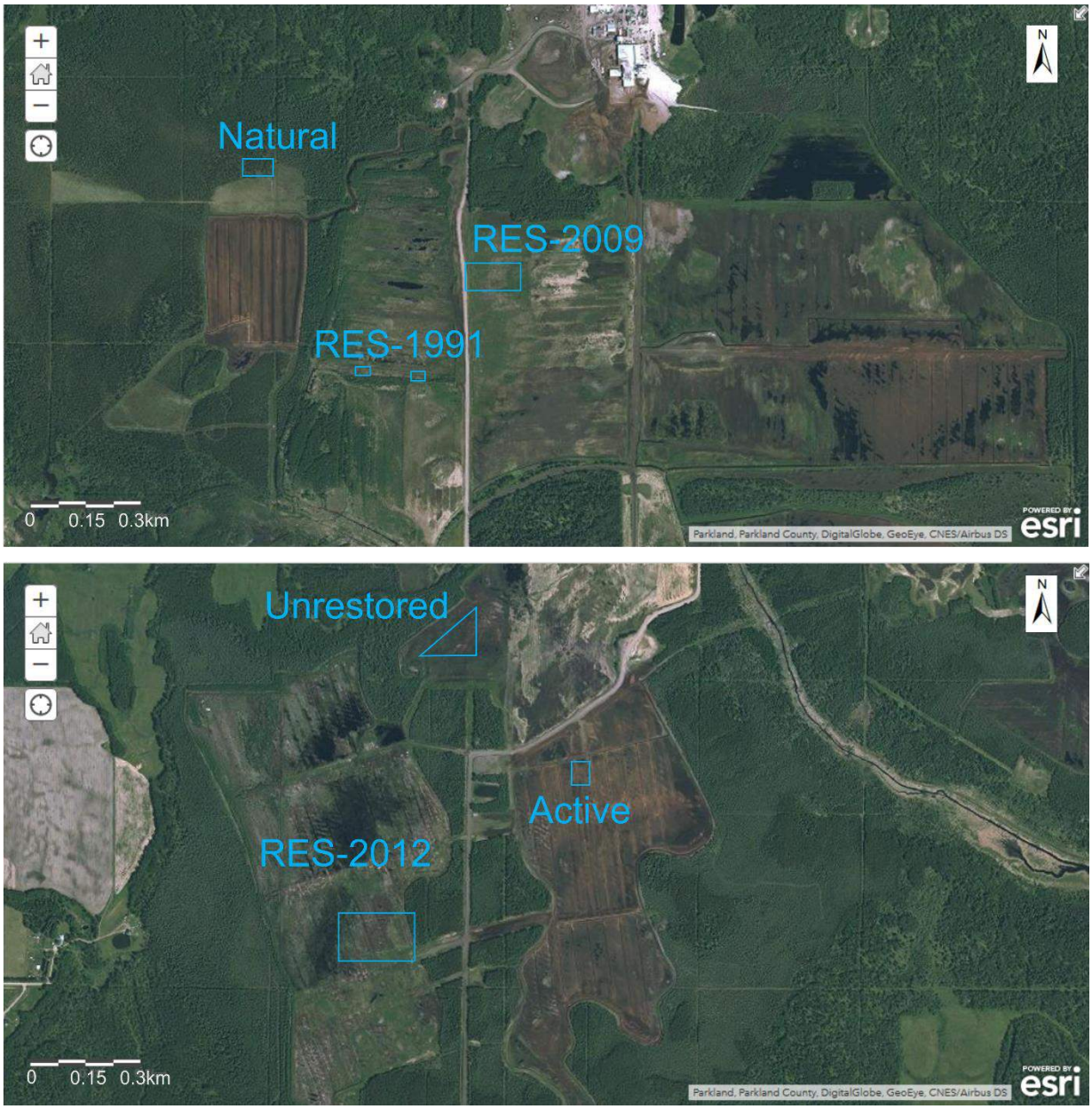


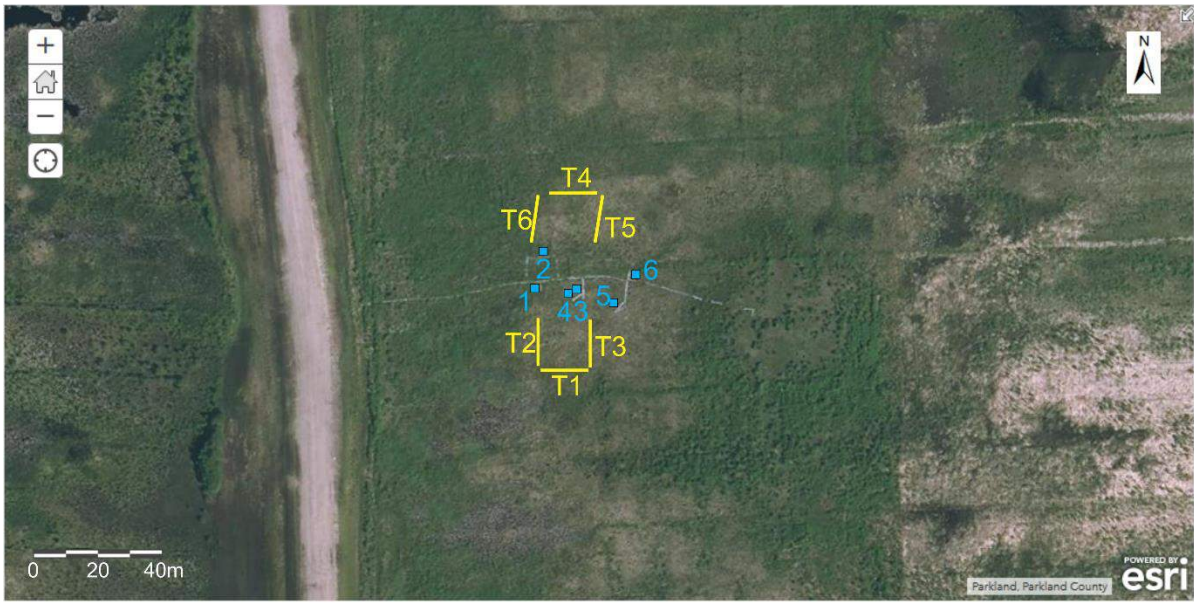
Figure A. 1. 2. Study sites at Seba Beach peatland complex. RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012. Source: Parkland, Parkland County, Digital Globe, Geo-Eye, CNES/Airbus DS. Blue frames indicate the location of the study.

Natural

RES-1991

Figure A. 1. 2. [Continuation]. Water wells were installed adjacent to collars. The legend is available at the end of Fig. A.1.2. (page 159).

RES-2009



RES-2012

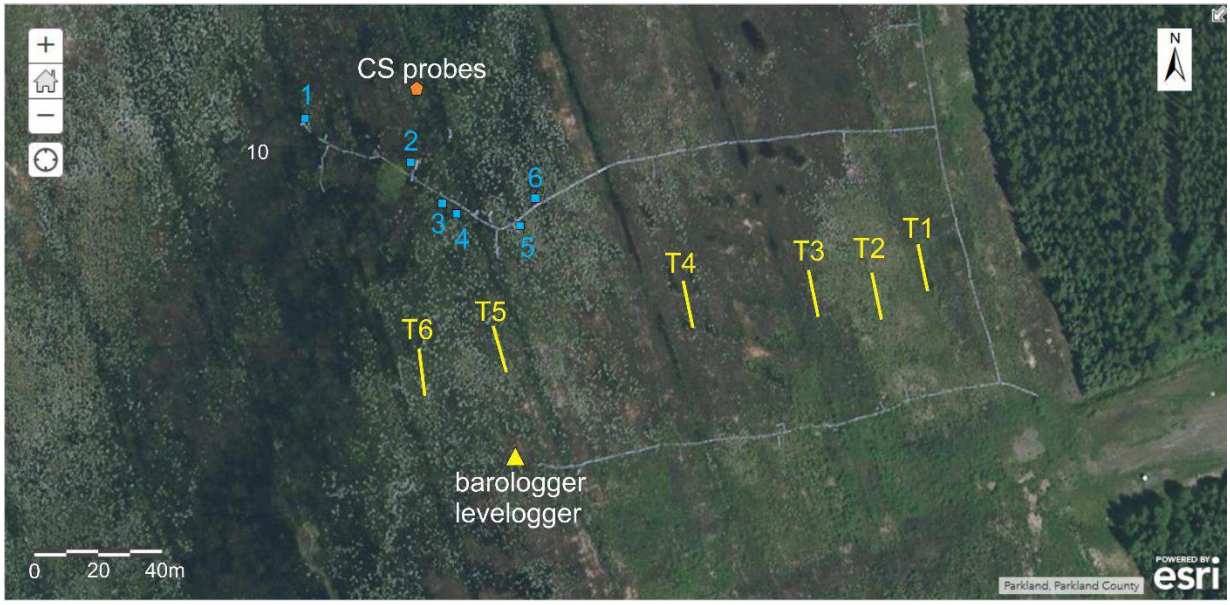
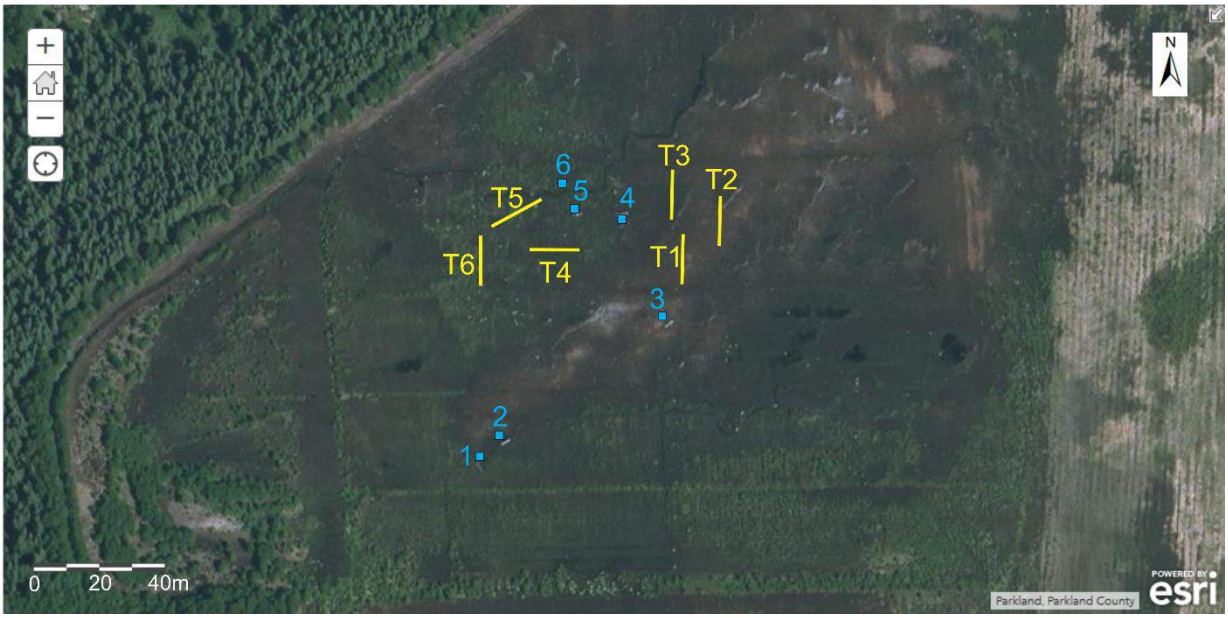
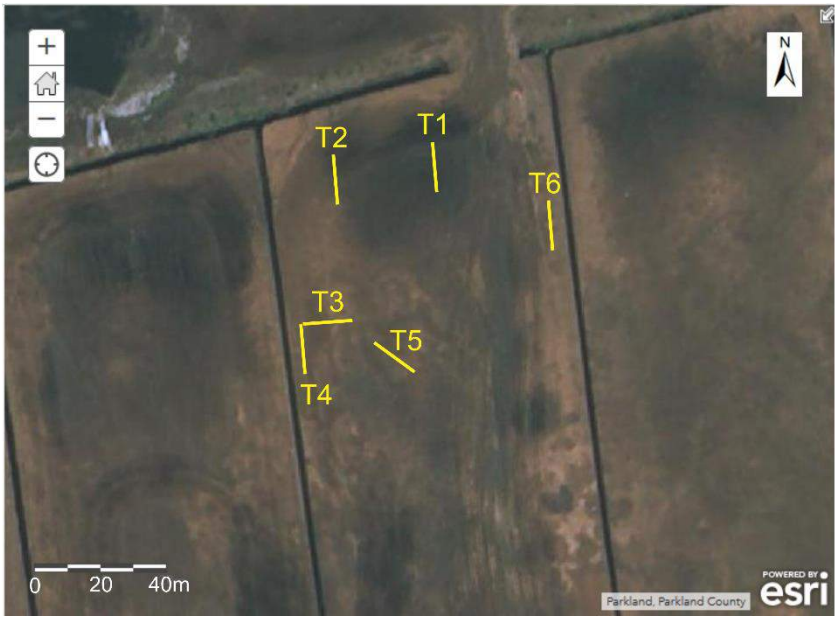


Figure A. 1. 2. [Continuation]. Water wells were installed adjacent to collars. The legend is available at the end of Fig. A.1.2. (page 159).

Unrestored



Active



Legend to all site maps
Meteorological station
T3 GPR transects
6 Collars
CS probes

Fig. A. 1. 2. [Continuation]. Water wells were installed adjacent to collars. No permanent installation was allowed at the Active site.

Figure A. 1. 3. Pictures of the study site in Seba Beach horticulture complex: the Natural site (A), restored in 1991 (RES-1991; B), restored in 2009 (RES-2009; C), restored in 2012 (RES-2012; D), Unrestored (E), and currently extracted site (Active; F). Pictures A and C were taken during the ground-penetrating radar (GPR) surveys. Photo A, and C credit: Martin Brummell.

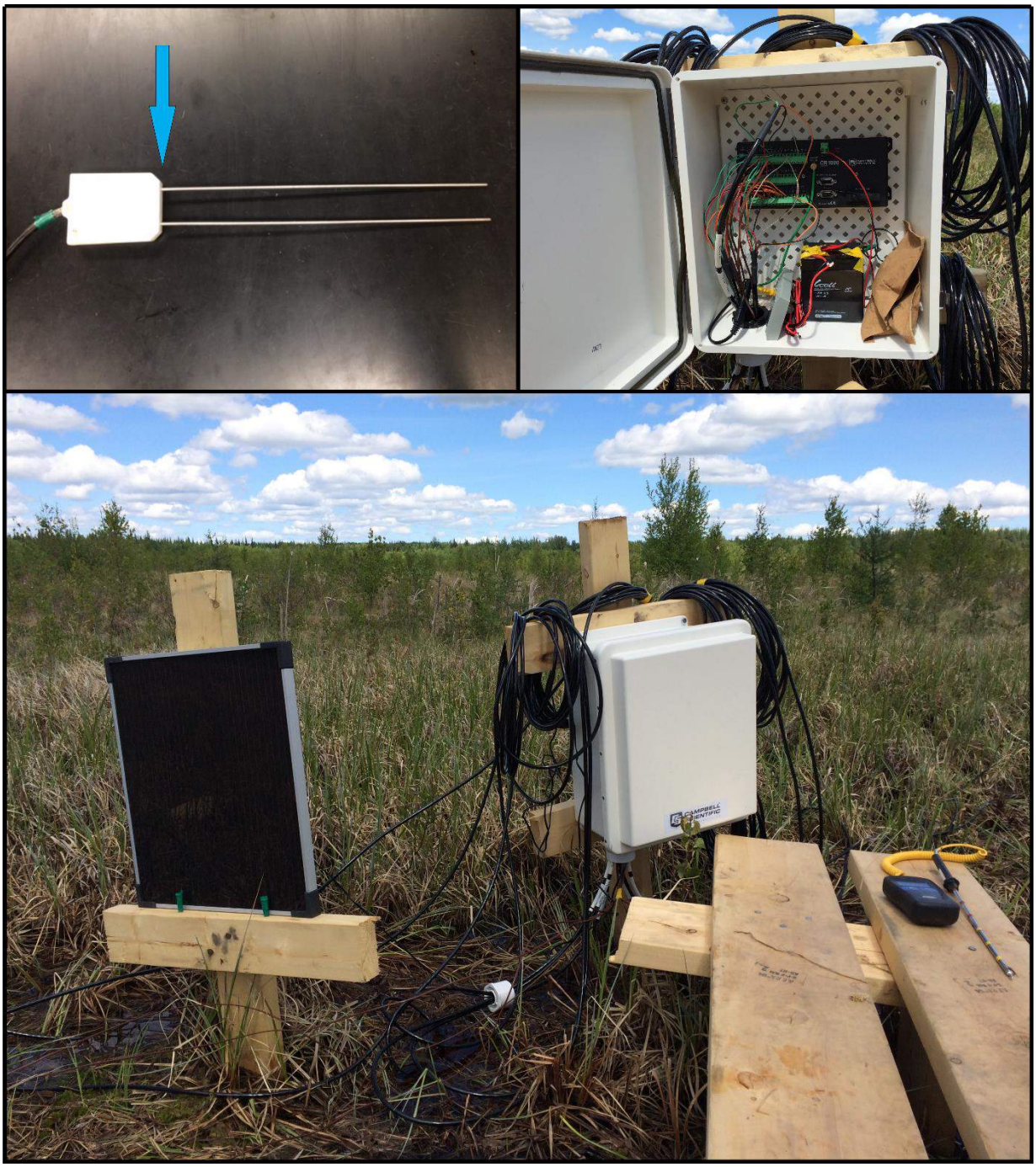


Figure A. 1. 4. A Campbell Scientific (CS) probe (left top picture) with a CS1000 logger (right top picture) and the installation setup at the site restored in 1991 (bottom picture). The blue arrow shows the position of the targeted depth (upper end of the metal rods).

Appendix 2

Table A. 2. 1. Physicochemical properties of 63 peat samples with methanogenic and methanotrophic sequence count above the rarefication threshold. DOC – dissolved organic carbon (mg g^{-1}), EC – electrical conductivity (µS cm^{-1}), WT – water table (cm); short chain fatty acid ions (µg g^{-1} of dry peat): ACE – acetate, BUT – butyrate, CIT – citrate, FOR – formate, LAC – lactate, PYR – pyruvate, PRO – propionate, SUC – succinate; inorganic ions (µg g^{-1} of dry peat).

Sample	Peatland	Depth	Surface	Von Post index	WT	WTFZ	DOC	C:N	pH	EC	Lactate	Acetate	Formate	Succite	Butyrate	Pyruvate	Propiote	Citrate	NO_2^-	NO_3^{2-}	SO_4^{2-}	PO_4^{3-}	Fe^{3+}
NAT_Hummock_M_c	Natural (Hummock)	c (20-30 cm)	mossy	H3	above	within	4.37	51.92	3.68	286.10	0.00	40.98	5.68	227.48	0.00	8.02	7.12	696.28	0.00	3.27	100.81	169.94	18.38
NAT_Hollow_M_b	Natural (Hollow)	b (10-20 cm)	mossy	H3 / H4	within	within	2.76	37.60	3.83	254.90	0.00	114.77	8.58	6.04	0.00	0.00	8.22	0.00	0.00	5.40	31.00	49.64	60.32
NAT_Hollow_M_c	Natural (Hollow)	c (20-30 cm)	mossy	H4	below	within	2.11	26.76	4.10	166.80	0.00	34.93	7.27	0.00	0.00	0.00	0.00	0.00	0.00	3.59	27.01	10.93	16.17
NAT_Hollow_M_f	Natural (Hollow)	f (50-60 cm)	mossy	H3	below	within	2.57	38.83	4.17	153.50	0.00	97.94	4.73	0.00	0.00	0.00	5.28	7.73	0.00	2.74	21.14	22.36	97.84
NAT_Hollow_M_g	Natural (Hollow)	g (60-70 cm)	mossy	H3 / H4	below	within	1.45	34.53	4.17	161.30	0.00	15.75	12.57	0.00	0.00	0.00	6.17	0.00	0.00	2.87	24.26	3.55	16.13
RES-1991_M_a	Restored in 1991	a (0-10 cm)	mossy	H2	below	within	1.89	NA	NA	NA	0.00	69.77	4.95	161.82	0.00	8.43	7.22	84.70	0.00	0.00	84.58	67.18	272.08
RES-1991_M_b	Restored in 1991	b (10-20 cm)	mossy	H3	below	within	3.87	NA	NA	NA	0.00	39.98	8.73	450.46	0.00	0.00	13.37	210.81	0.00	0.00	94.29	58.84	NA
RES-1991_M_c	Restored in 1991	c (20-30)	mossy	H3 / H4	below	within	2.18	NA	NA	NA	11.43	23.72	5.07	62.74	0.00	0.00	4.78	19.95	0.00	4.60	35.10	8.72	366.09
RES-1991_M_d	Restored in 1991	d (30-40 cm)	mossy	H4	below	within	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
RES-1991_M_e	Restored in 1991	e (40-50 cm)	mossy	H4	below	within	1.84	NA	NA	NA	0.00	19.56	13.15	27.24	0.00	0.00	5.03	21.56	0.00	2.87	32.26	3.55	238.49
RES-1991_M_i	Restored in 1991	i (80-90 cm)	mossy	H4	below	below	1.73	NA	NA	NA	0.00	14.06	19.93	29.88	0.00	0.00	7.15	8.07	0.00	5.94	26.27	0.00	306.49
RES-1991_M_k	Restored in 1991	k (340-350 cm)	mossy	H9	below	below	3.25	NA	NA	NA	0.00	10.93	23.67	0.00	0.00	0.00	4.90	0.00	0.00	4.46	30.36	3.89	1039.12
RES-1991_S_a	Restored in 1991	a (0-10 cm)	sedgy	H2	below	within	2.19	20.23	5.21	3.63	0.00	12.68	8.86	13.07	0.00	0.00	5.95	26.25	0.00	4.02	43.35	69.93	564.66
RES-1991_S_b	Restored in 1991	b (10-20 cm)	sedgy	H3 / H4	below	within	3.72	18.81	5.19	319.80	0.00	53.77	7.15	263.20	7.08	0.00	18.42	289.16	0.00	0.00	48.58	30.47	267.46
RES-1991_S_c	Restored in 1991	c (20-30 cm)	sedgy	H3	below	within	2.76	19.33	5.07	363.00	0.00	17.27	22.77	0.00	0.00	0.00	0.00	49.24	0.00	0.00	37.49	0.00	217.43
RES-1991_S_e	Restored in 1991	e (40-50 cm)	sedgy	H4	below	within	1.97	25.38	5.08	218.10	0.00	45.02	9.46	7.88	0.00	0.00	9.71	5.26	0.00	3.48	31.24	3.98	376.41
RES-1991_S_j	Restored in 1991	j (90-100 cm)	sedgy	H3	below	below	2.54	24.65	5.14	225.00	14.30	16.18	16.94	67.62	0.00	0.00	8.33	85.75	0.00	0.00	89.63	0.00	245.44
RES-2009_M_b	Restored in 2009	b (10-20 cm)	mossy	H4	below	within	2.19	NA	NA	NA	0.00	23.49	5.72	0.00	0.00	0.00	5.14	0.00	0.00	3.26	106.63	4.29	399.28
RES-2009_M_c	Restored in 2009	c (20-30 cm)	mossy	H3 / H4	below	within	2.09	NA	NA	NA	0.00	18.76	25.63	0.00	0.00	0.00	3.77	0.00	3.81	3.66	119.56	0.00	628.23
RES-2009_M_d	Restored in 2009	d (30-40 cm)	mossy	H4	below	within	2.35	NA	NA	NA	0.00	20.42	15.12	8.56	0.00	0.00	0.00	0.00	0.00	3.96	147.84	5.62	1111.13
RES-2009_M_e	Restored in 2009	e (40-50 cm)	mossy	H4 / H5	below	within	1.29	NA	NA	NA	0.00	5.46	11.39	0.00	0.00	0.00	0.00	0.00	0.00	2.07	48.76	0.00	251.79
RES-2009_M_f	Restored in 2009	f (50-60 cm)	mossy	H5	below	within	3.80	NA	NA	NA	0.00	12.13	7.34	4.11	0.00	0.00	1.79	0.00	0.00	0.00	39.49	0.00	556.34
RES-2009_M_g	Restored in 2009	g (60-70 cm)	mossy	H4	below	within	3.21	NA	NA	NA	0.00	14.73	18.52	0.00	0.00	0.00	0.00	2.45	0.00	3.14	23.36	0.00	1019.17
RES-2009_M_h	Restored in 2009	h (70-80 cm)	mossy	H4	below	within	3.52	NA	NA	NA	0.00	23.56	30.04	0.00	0.00	0.00	0.00	0.00	2.13	2.26	35.58	0.00	906.14
RES-2009_M_i	Restored in 2009	i (80-90 cm)	mossy	H5 / H6	below	within	1.49	NA	NA	NA	0.00	28.79	48.00	0.00	0.00	0.00	0.00	0.00	0.00	2.62	20.38	0.00	NA
RES-2009_M_j	Restored in 2009	j (90-100 cm)	mossy	H4	below	below	2.52	NA	NA	NA	0.00	38.87	33.72	0.00	0.00	0.00	4.02	0.00	6.36	4.46	42.02	0.00	162.37
RES-2009_M_k	Restored in 2009	k (150-160 cm)	mossy	H6	below	below	0.47	NA	NA	NA	0.00	11.21	25.36	0.00	0.00	0.00	3.94	0.00	0.00	1.08	9.26	0.00	2187.54
RES-2009_S_a	Restored in 2009	a (0-10 cm)	sedgy	H3	below	within	1.90	30.97	4.55	282.00	15.32	22.70	5.24	59.91	0.00	12.97	14.79	25.78	0.00	2.90	469.37	151.19	281.79
RES-2009_S_b	Restored in 2009	b (10-20 cm)	sedgy	H3 / H4	below	within	1.44	32.64	4.62	310.00	0.00	23.77	5.38	10.49	0.00	0.00	2.54	3.64	0.00	18.27	225.09	6.68	1187.95
RES-2009_S_c	Restored in 2009	c (20-30 cm)	sedgy	H5 / H6	below	within	1.66	31.70	4.73	326.00	6.82	38.34	6.44	12.88	0.00	0.00	4.60	0.00	0.00	8.09	279.45	2.95	1307.08
RES-2009_S_d	Restored in 2009	d (30-40 cm)	sedgy	H5	below	within	1.35	16.12	5.00	230.10	0.00	18.59	5.11	6.61	0.00	0.00	0.00	0.00	0.00	0.00	155.82	0.00	1540.51
RES-2009_S_f	Restored in 2009	f (50-60 cm)	sedgy	H5 / H6	below	within	0.54	13.45	5.30	185.10	0.00	23.85	3.18	0.00	0.00	0.00	8.58	0.00	0.00	1.45	33.98	0.00	2195.73
RES-2009_S_g	Restored in 2009	g (60-70 cm)	sedgy	H3 / H4	below	within	0.87	12.55	5.49	184.90	9.78	31.67	4.89	0.00	0.00	0.00	7.30	0.00	1.56	9.00	58.05	0.00	3063.08
RES-2009_S_h	Restored in 2009	h (70-80 cm)	sedgy	H4	below	within	0.71	12.24	5.65	201.10	0.00	12.83	35.00	0.00	0.00	0.00	5.91	0.00	0.00	3.27	194.95	0.00	1992.53
RES-2009_S_i	Restored in 2009	i (80-90 cm)	sedgy	H4	below	within	1.29	12.39	NA	NA	0.00	15.92	49.06	0.00	0.00	0.00	5.33	0.00	2.28	2.59	260.18	0.00	1493.98
RES-2009_S_j	Restored in 2009	j (90-100 cm)	sedgy	H4	below	below	1.29	12.92	5.52	265.30	0.00	17.44	47.50	0.00	0.00	0.00	6.25	0.00	1.91	2.58	248.27	0.00	1576.13
RES-2009_S_k	Restored in 2009	k (113-123 cm)	sedgy	H4	below	below	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
RES-2012_M_a	Restored in 2012	a (0-10 cm)	mossy	H3	within	within	1.58	38.20	4.07	188.40	14.91	30.70	8.18	0.00	0.00	0.00	0.00	0.00	1.85	9.27	33.62	240.75	155.19
RES-2012_M_b	Restored in 2012	b (10-20 cm)	mossy	H3	below	within	1.38	42.54	4.11	159.10	11.41	19.54	7.02	0.00	0.00	0.00	6.43	0.00	0.00	9.29	20.32	10.58	86.13
RES-2012_M_d	Restored in 2012	d (30-40 cm)	mossy	H3	below	within	2.12	54.39	4.21	257.90	6.25	19.74	63.86	12.55	0.00	0.00	0.00	0.00	8.23	4.04	18.19	11.15	0.00
RES-2012_M_e	Restored in 2012	e (40-50 cm)	mossy	H3	below	within	2.13	49.93	4.33	224.50	0.00	12.08	42.65	12.83	0.00	0.00	0.00	0.00	5.40	4.11	18.80	4.77	242.37
RES-2012_M_f	Restored in 2012	f (50-60 cm)	mossy	H3	below	within	2.56	41.04	4.35	253.50	55.75	31.59	57.20	26.60	0.00	0.00	3.58	20.99	2.82	66.51	121.22	8.88	182.05
RES-2012_M_g	Restored in 2012	g (60-70 cm)	mossy	H3	below	within	2.25	26.46	4.42	333.00	0.00	20.68	70.30	9.77	0.00	0.00	0.00	0.00	8.63	4.02	28.60	2.81	370.95
RES-2012_M_h	Restored in 2012	h (70-80 cm)	mossy	H3	below	within	1.98	23.40	4.49	246.10	3.22	33.57	47.62	51.51	0.00	0.00	19.51	5.44	2.69	2.92	25.24	3.74	261.57
RES-2012_M_j	Restored in 2012	j (90-100 cm)	mossy	H3 / H4	below	below	2.11	22.26	4.89	204.40	5.57	24.84	53.96	8.09	0.00	0.00	5.75	0.00	5.89	14.65	28.73	3.10	329.89
RES-2012_M_k	Restored in 2012	k (140-150 cm)	mossy	H3 / H4	below	below	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	798.17
RES-2012_S_a	Restored in 2012	a (0-10 cm)	sedgy	H4 / H5	within	within	2.08	36.12	4.03	227.90	4.03	39.89	5.63	81.80	0.00	2.02	3.77	24.35	1.33	3.78	27.40	130.74	35.16
RES-2012_S_f	Restored in 2012	f (50-60 cm)	sedgy	H3	below	within	1.57	53.83	4.21	232.90	0.00	28.10	49.88	29.89	0.00	0.00	10.39	0.00	0.00	3.06	25.64	0.00	78.68
RES-2012_S_h	Restored in 2012	h (70-80 cm)	sedgy	H4	below	within	1.90	21.50	4.47	236.20	0.00	25.05	42.54	118.46	0.00	0.00	24.81	15.28	1.79	3.13	21.78	4.27	175.09
RES-2012_S_i	Restored in 2012	i (80-90 cm)	sedgy	H4	below	below	1.75	20.49	4.62	200.70	0.00	20.79	53.80	13.56	0.00	0.00	7.60	0.00	2.49	4.06	22.87	3.89	311.74
RES-2012_S_j	Restored in 2012	j (90-100 cm)	sedgy	H3	below	below	1.95	21.91	4.72	200.00	0.00	28.21	41.20	115.17	0.00	0.00	13.08	16.97	0.00	2.53	28.21	4.77	439.39
RES-2012_S_k	Restored in 2012	k (130-140 cm)	sedgy	H3	below	below	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	776.93
UNR_B_a	Unrestored	a (0-10 cm)	bare peat	H3	above	within	1.86	44.13	3.82	186.50	0.00	2.72	4.65	0.00	0.00	0.00	0.00	0.00	0.00	10.54	350.77	2.94	115.89
UNR_B_d	Unrestored	d (30-40 cm)	bare peat	H3	below	within	1.87	37.70	4.00	251.40	2.64	7.99	5.54	0.00	0.00	0.00	0.00	21.39	2.85	3.62	331.43	17.12	231.14
UNR_B_f	Unrestored	f (50-60 cm)	bare peat	H4	below	within	2.54	31.52	3.90	284.50	0.00	23.86	5.70	0.00	0.00	0.00	11.82	0.00	3.30	10.13	393.82	13.59	316.03
UNR_B_g	Unrestored	g (60-70 cm)	bare peat	H5	below	within	1.80	29.11	4.04	238.10	0.00	14.05	4.23	0.00	0.00	0.00	6.76	9.74	1.85	3.56	355.40	8.85	268.47
UNR_B_h	Unrestored	h (70-80 cm)	bare peat	H4	below	within	1.20	19.23	4.23	259.50	0.00	13.84	33.36	0.00	0.00	0.00	3.09	0.00	0.00	1.63	176.52	2.01	353.49
UNR_B_i	Unrestored	i (80-90 cm)	bare peat	H5 / H6	below	below	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
UNR_S_c	Unrestored	c (20-30 cm)	sedgy	H5 / H6	above	within	1.43	17.05	4.24	172.00	0.00	20.75	68.65	4.82	0.00	0.00	5.51	0.00	1.41	1.66	61.18	3.26	187.15
UNR_S_e	Unrestored	e (40-50 cm)	sedgy	H5	below	within	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
UNR_S_f	Unrestored	f (50-60 cm)	sedgy	H5	below	within	1.36	14.89	4.63	176.70	0.00	12.24	35.04	4.88	0.00	0.00	2.08	0.00	0.00	2.11	90.88	0.00	584.03
UNR_S_h	Unrestored	h (70-80 cm)	sedgy	H5	below	within	0.86	14.53	4.92	198.10	0.00	16.72	47.13	2.57	0.00	0.00	1.82	0.00	0.00	1.38	79.27	0.00	1265.65
ACT_B_h	Active	h (70-80 cm)	bare peat	H3	below	within	1.41	20.21	4.62	140.40	0.00	36.04	31.05	0.00	0.00	0.00	44.17	14.06	0.00	7.74	29.74	0.00	11.25

Table A. 2. 2. Physicochemical properties of peat samples collected for microcosm experiment. DOC – dissolved organic carbon (mg g^{-1}), EC – electrical conductivity (µS cm^{-1}), WT – water table (cm); short chain fatty acid ions (µg g^{-1} of dry peat): ACE – acetate, BUT – butyrate, CIT – citrate, FOR – formate, LAC – lactate, PYR – pyruvate, PRO – propionate, SUC – succinate; inorganic ions (µg g^{-1} of dry peat).

Sample	Peatland	Core number	Surface	Depth zone	MP	MO	Von Post index	DOC	C:N	pH	EC	Acetate	Formate	Succinate	Butyrate	Pyruvate	Propionate	Citrate	NO_2^-	NO_3^{2-}	SO_4^{2-}	PO_4^{2-}	Fe^{3+}
Natural_Hummock_1A	Natural (Hummock)	1	mossy	A	0.87	NA	H3	2.23	30.09	4.01	220.60	73.57	5.57	0.00	0.00	0.00	0.00	0.00	0.00	4.47	48.19	28.51	46.42
Natural_Hummock_1B	Natural (Hummock)	1	mossy	B	NA	5.46	H3	2.81	28.77	4.15	175.50	17.04	4.37	0.00	0.00	0.00	0.00	4.11	0.00	2.77	33.96	92.76	81.11
Natural_Hummock_1C	Natural (Hummock)	1	mossy	C	0.01	2.79	H3	2.76	31.81	4.17	299.00	6.78	3.32	0.00	0.00	0.00	0.00	5.28	0.00	4.33	32.03	82.31	0.00
Natural_Hummock_2A	Natural (Hummock)	2	mossy	A	1.53	3.56	H3	2.61	29.61	4.16	254.80	108.42	3.94	32.39	0.00	0.00	0.00	38.75	0.00	4.66	46.54	27.18	0.00
Natural_Hummock_2B	Natural (Hummock)	2	mossy	B	0.05	NA	H3	3.65	28.74	4.11	296.00	8.90	5.13	0.00	0.00	0.00	0.00	16.10	0.00	4.24	62.65	97.85	96.26
Natural_Hummock_2C	Natural (Hummock)	2	mossy	C	0.01	NA	H2	5.70	37.67	3.99	301.00	73.22	5.74	0.00	0.00	0.00	0.00	87.80	0.00	2.55	56.49	240.82	88.81
Natural_Hollow_3A	Natural (Hollow)	3	mossy	A	0.09	NA	H4	3.10	28.60	4.30	299.00	121.91	8.06	0.00	0.00	0.00	0.00	25.23	5.41	7.46	45.64	20.13	NA
Natural_Hollow_3B	Natural (Hollow)	3	mossy	B	0.55	NA	H3 / H4	3.05	31.91	4.05	290.90	81.79	7.76	0.00	0.00	0.00	0.00	14.31	0.00	4.97	73.61	19.44	0.00
Natural_Hollow_3C	Natural (Hollow)	3	mossy	C	0.03	NA	H3	2.06	37.01	3.98	294.90	25.01	4.54	0.00	0.00	0.00	3.31	39.88	0.00	3.11	73.97	105.91	139.52
Natural_Hollow_4A	Natural (Hollow)	4	mossy	A	0.28	NA	H3 / H4	2.44	26.86	4.06	317.00	93.33	7.04	0.00	0.00	0.00	0.00	69.26	0.00	4.91	44.10	11.78	0.00
Natural_Hollow_4B	Natural (Hollow)	4	mossy	B	0.09	NA	H4	1.00	26.96	4.03	301.00	54.06	3.18	0.00	0.00	0.00	0.00	16.50	0.00	2.52	43.86	79.36	26.04
Natural_Hollow_4C	Natural (Hollow)	4	mossy	C	NA	1.02	H3 / H4	2.26	37.18	3.96	363.00	63.86	3.01	0.00	0.00	0.00	4.37	25.90	0.00	1.69	78.45	166.75	102.26
Natural_Hummock_5A	Natural (Hummock)	5	mossy	A	NA	7.11	H3	2.17	30.20	4.26	197.40	11.90	4.69	0.00	0.00	0.00	0.00	64.67	0.00	3.32	51.60	61.21	NA
Natural_Hummock_5B	Natural (Hummock)	5	mossy	B	0.04	NA	H3	2.35	28.30	4.13	209.60	91.47	6.60	0.00	0.00	0.00	0.00	0.00	0.00	4.71	44.81	50.49	66.06
Natural_Hummock_5C	Natural (Hummock)	5	mossy	C	0.06	5.13	H4	3.06	31.16	4.34	168.50	95.83	7.48	0.00	0.00	0.00	0.00	41.56	0.00	5.51	61.13	15.54	396.37
Natural_Hollow_6A	Natural (Hollow)	6	mossy	A	0.43	5.51	H4	2.00	30.50	4.09	278.80	139.37	6.56	0.00	0.00	0.00	0.00	0.00	4.27	4.87	41.44	14.67	28.72
Natural_Hollow_6B	Natural (Hollow)	6	mossy	B	NA	NA	H4	2.29	32.32	4.00	276.80	132.76	5.90	0.00	0.00	0.00	0.00	23.10	0.00	3.44	63.23	52.31	139.29
Natural_Hollow_6C	Natural (Hollow)	6	mossy	C	NA	2.29	H4	3.16	37.85	4.02	353.00	176.38	3.84	41.54	0.00	0.00	0.00	141.01	0.00	2.07	50.72	134.09	41.46
Restored_2009_M_1A	Restored in 2009	1	mossy	A	0.01	0.59	H3 / H4	2.93	40.20	4.66	321.00	38.35	4.93	12.64	0.00	0.00	0.00	0.00	0.00	2.92	355.79	4.48	1256.44
Restored_2009_M_1B	Restored in 2009	1	mossy	B	0.01	0.96	H5	2.59	40.07	4.66	314.00	36.90	3.91	50.58	0.00	0.00	0.00	0.00	1.59	3.39	472.02	14.92	1255.48
Restored_2009_M_1C	Restored in 2009	1	mossy	C	0.07	1.30	H3 / H4	2.93	32.95	4.69	341.00	83.99	4.08	20.34	0.00	3.43	12.29	13.69	1.65	2.07	265.40	110.14	1026.00
Restored_2009_S_2A	Restored in 2009	2	sedgy	A	4.36	0.68	H4	3.03	27.05	4.78	316.00	306.94	15.20	12.29	32.44	0.00	100.34	12.20	1.26	2.58	61.25	7.41	1076.60
Restored_2009_S_2B	Restored in 2009	2	sedgy	B	2.52	1.25	H4	2.91	28.37	4.77	296.00	134.94	3.82	204.41	0.00	22.22	7.41	18.68	0.00	1.77	370.52	166.81	700.98
Restored_2009_M_3A	Restored in 2009	3	mossy	A	3.71	1.23	H4	5.04	21.35	4.66	263.10	71.73	3.28	99.85	0.00	0.00	2.23	29.28	0.00	2.63	248.11	14.19	221.99
Restored_2009_M_3B	Restored in 2009	3	mossy	B	0.01	1.53	H3 / H4	4.92	30.35	4.49	291.30	62.06	3.57	48.90	0.00	0.00	0.00	24.37	0.00	3.79	305.65	13.31	1275.69
Restored_2009_M_3C	Restored in 2009	3	mossy	C	0.01	0.13	H8	3.20	35.09	4.38	271.30	108.10	2.48	70.89	0.00	1.95	1.70	18.99	1.50	1.92	287.02	26.65	1018.32
Restored_2009_S_4A	Restored in 2009	4	sedgy	A	NA	NA	H4	4.52	23.80	4.65	330.50	79.39	3.38	102.38	0.00	4.66	0.00	7.21	1.67	2.82	269.89	16.78	521.72
Restored_2009_S_4B	Restored in 2009	4	sedgy	B	NA	1.54	H4 / H5	2.50	33.21	4.37	328.00	51.19	2.46	70.18	0.00	0.00	2.32	26.71	0.00	1.93	336.46	12.42	1007.15
Restored_2009_S_4C	Restored in 2009	4	sedgy	C	0.02	0.42	H5	1.98	30.31	4.43	344.00	57.10	1.98	7.24	0.00	0.00	1.63	7.21	0.00	0.00	398.63	18.09	305.43
Restored_2009_S_5A	Restored in 2009	5	sedgy	A	NA	0.54	H6	3.19	14.30	5.06	278.20	77.71	1.57	65.92	0.00	2.06	2.46	22.06	0.00	0.00	132.07	11.81	1045.95
Restored_2009_S_5B	Restored in 2009	5	sedgy	B	0.01	0.04	H4	2.68	14.56	4.99	202.30	27.49	1.93	13.10	0.00	1.59	0.00	7.34	0.00	2.56	73.50	3.06	656.51
Restored_2009_S_5C	Restored in 2009	5	sedgy	C	0.01	NA	H4	1.66	18.03	4.67	281.00	41.35	2.71	6.01	3.67	0.00	8.58	12.47	0.00	1.39	46.25	2.12	1237.76
Restored_2009_M_6A	Restored in 2009	6	mossy	A	0.09	0.29	H5	1.32	15.10	5.44	193.20	48.02	2.03	0.00	0.00	0.00	0.00	6.47	0.00	1.71	127.42	4.12	405.66
Restored_2009_M_6B	Restored in 2009	6	mossy	B	0.09	0.13	H4	1.77	19.40	4.85	222.50	50.23	2.17	0.00	0.00	0.00	0.00	2.63	0.00	1.48	178.31	13.00	540.68
Restored_2009_M_6C	Restored in 2009	6	mossy	C	NA	0.62	H5	1.11	31.84	4.72	262.10	31.88	1.47	0.00	0.00	0.00	0.00	1.69	0.00	0.00	95.81	21.50	335.64
Unrestored_B_2A	Unrestored	2	bare peat	A	0.01	NA	H5	2.60	21.37	4.70	168.10	22.48	57.68	3.22	0.00	0.00	0.00	2.73	2.90	2.91	20.47	4.02	256.04
Unrestored_B_2B	Unrestored	2	bare peat	B	0.08	NA	H4	2.44	21.96	4.47	246.30	26.31	69.49	3.32	0.00	0.00	0.00	3.40	2.52	2.75	29.27	3.29	215.52
Unrestored_B_2C	Unrestored	2	bare peat	C	NA	0.43	H4	2.01	20.40	4.61	256.60	12.16	15.34	0.00	0.00	0.00	0.00	0.00	2.65	3.65	68.36	2.29	182.67
Unrestored_B_3A	Unrestored	3	bare peat	A	NA	NA	H7	1.26	16.39	4.64	180.10	10.25	2.65	0.00	0.00	0.00	0.00	0.00	0.00	1.56	175.48	1.88	832.90
Unrestored_B_3B	Unrestored	3	bare peat	B	0.01	0.41	H8	1.17	16.66	4.56	277.80	8.63	3.22	0.00	0.00	0.00	0.00	0.00	0.00	1.08	193.30	1.29	461.85
Unrestored_B_3C	Unrestored	3	bare peat	C	0.01	0.89	H5	1.25	15.90	4.58	157.30	10.34	2.74	0.00	0.00	0.00	0.00	5.04	1.79	1.98	199.82	1.77	989.35
Unrestored_B_4A	Unrestored	4	bare peat	A	0.03	1.33	H7	0.99	13.48	4.95	298.50	10.90	4.30	0.00	0.00	0.00	0.00	7.30	0.00	1.68	864.89	0.00	740.49
Unrestored_B_4B	Unrestored	4	bare peat	B	0.03	NA	H4	0.63	14.20	4.92	208.70	9.07	8.98	0.00	0.00	0.00	0.00	3.64	0.00	1.60	728.13	0.00	880.09
Unrestored_B_4C	Unrestored	4	bare peat	C	0.01	0.36	H7	0.86	13.65	4.85	276.00	8.30	1.46	0.00	0.00	0.00	0.00	2.69	0.00	5.97	403.84	0.00	422.05

Table A. 2. 3. The absolute abundance of methanogens and methanotrophs in the 63 rarefied peat samples. See Tab. 2.3 for the codes.

Sample	MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8	MG9	MG10	MG11	MG12	MG13	MG14	MG15	MG16	MG17	MG18	MG19	MG20	MG21	MT1	MT2	MT3	MT4	MT5	MT6	MT7	MT8	MT9	MT10	MT11	MT12	MT13	MT14	MT15
NAT_Hummock_M_c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NAT_Hollow_M_b	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	1	0	0	0	0	0	0	0	0	0	0	0
NAT_Hollow_M_c	60	0	3	0	0	0	0	0	24	2	0	0	0	1	0	0	0	0	0	0	0	50	40	0	5	0	0	0	37	0	0	0	0	0	0	0
NAT_Hollow_M_f	15	0	2	0	0	0	0	0	66	9	0	0	0	0	0	0	0	0	0	0	3	13	125	0	24	0	0	0	115	0	0	5	0	0	0	3
NAT_Hollow_M_g	48	1	0	0	0	0	8	0	1053	120	0	0	0	0	0	0	0	0	0	0	15	4	35	0	3	0	0	0	2	0	0	0	0	0	0	0
RES_1991_M_a	0	0	139	23	8	0	67	0	0	0	0	1	28	0	0	0	0	5	0	0	0	0	39	0	1	80	7	0	121	108	16	157	44	0	4	259
RES_1991_M_b	0	0	205	24	5	0	192	0	0	0	0	6	44	17	0	0	0	5	0	0	0	0	64	0	0	43	0	0	84	193	6	77	38	0	0	215
RES_1991_M_c	98	0	192	0	0	0	238	0	0	10	0	5	60	0	0	0	0	15	0	0	0	0	53	0	74	13	0	0	19	21	0	41	0	52	10	27
RES_1991_M_d	59	0	201	4	0	0	203	0	6	47	0	6	70	14	0	0	0	37	0	0	0	4	63	0	3	0	0	5	3	22	0	18	0	0	5	4
RES_1991_M_e	140	11	33	2	0	0	218	0	0	17	0	3	80	12	0	0	0	27	0	0	0	0	20	0	3	2	0	2	4	31	0	9	0	4	5	1
RES_1991_M_i	466	14	26	0	0	0	227	0	0	4	0	3	43	1	0	0	0	42	51	0	0	0	6	0	3	1	0	0	11	59	0	16	0	0	3	14
RES_1991_M_k	36	2	6	0	0	1	171	0	0	25	0	0	27	0	0	2	0	30	44	3	0	0	1	0	0	0	0	0	0	5	0	1	0	0	0	1
RES_1991_S_a	0	0	150	10	3	0	51	0	0	2	0	0	4	4	0	0	0	10	0	0	0	0	40	0	0	5	0	0	16	9	0	37	0	0	6	25
RES_1991_S_b	72	0	739	8	0	0	239	0	0	28	0	3	171	14	0	0	0	19	0	0	0	0	32	0	0	0	0	0	15	975	0	12	0	0	3	26
RES_1991_S_c	92	65	32	1	0	0	68	0	0	50	0	0	36	6	0	0	0	28	0	0	0	0	2	0	1	0	0	0	0	8	0	6	0	0	4	0
RES_1991_S_e	184	48	118	3	0	0	157	0	0	36	0	2	66	6	0	0	0	49	2	0	0	0	38	0	0	0	0	0	22	29	0	8	4	3	5	10
RES_1991_S_j	284	15	94	4	0	0	328	4	0	24	10	0	189	7	0	1	0	37	126	0	0	0	12	0	8	0	0	0	13	39	0	23	0	0	5	9
RES_2009_M_b	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	7	0	0	0	0	0	0	0	0	0	0	9	19	0	0	0	0	0	0
RES_2009_M_c	467	0	0	0	0	0	7	0	0	2	0	0	0	0	0	0	0	5	0	0	0	0	0	0	13	0	0	0	2	573	0	0	0	0	5	0
RES_2009_M_d	0	0	6	3	0	0	17	0	0	0	0	0	0	3	0	0	0	9	0	0	0	0	8	0	9	0	0	0	10	123	0	0	3	0	4	0
RES_2009_M_e	389	0	0	0	0	0	37	0	0	1	0	0	19	0	0	0	0	21	3	0	0	0	0	0	9	0	0	0	0	506	0	0	0	0	47	0
RES_2009_M_f	261	0	0	0	0	0	20	0	0	0	0	0	13	0	0	0	0	11	57	0	0	0	0	0	13	0	0	0	7	318	0	0	0	0	17	0
RES_2009_M_g	273	0	0	0	0	0	86	0	0	2	0	0	8	0	0	0	0	18	350	0	0	0	0	0	41	0	0	0	0	278	0	0	3	0	19	0
RES_2009_M_h	276	0	0	0	0	0	202	0	0	4	0	0	11	0	0	1	0	22	732	0	0	0	0	0	3	0	0	0	0	58	0	0	0	0	4	0
RES_2009_M_i	256	0	0	0	0	0	201	0	0	2	0	0	7	0	2	0	0	29	878	0	0	0	0	0	12	0	0	0	0	54	0	0	0	0	9	0
RES_2009_M_j	113	0	2	0	0	0	157	0	0	0	0	0	9	0	0	3	0	10	1001	0	0	0	0	0	8	0	0	0	6	27	0	0	0	0	0	0
RES_2009_M_k	109	0	0	0	0	0	43	0	0	0	0	0	2	0	0	6	0	7	655	0	0	0	0	0	7	0	0	0	5	154	0	0	0	0	2	0
RES_2009_S_a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RES_2009_S_b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	3	40	0	0	0	0	0	19	56	0	1	5	0	0	0
RES_2009_S_c	0	0	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	16	0	7	0	0	0	18	117	0	0	2	0	0	0
RES_2009_S_d	0	0	9	4	0	0	6	3	0	0	0	0	0	8	0	0	0	2	0	0	0	0	17	0	37	0	0	0	14	35	0	0	4	0	4	0
RES_2009_S_f	7	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	8	0	0	0	8	273	0	0	3	0	0	0
RES_2009_S_g	92	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	3	121	0	0	0	0	0	1	0	0	0	0	2076	0	0	0	0	5	0
RES_2009_S_h	108	0	0	0	0	0	58	0	0	0	0	0	6	0	0	0	0	4	295	0	0	0	0	0	9	0	0	0	0	618	0	0	0	0	3	0
RES_2009_S_i	110	0	0	0	0	0	157	0	0	0	0	0	2	0	0	0	1	7	573	0	0	0	0	0	0	0	0	0	0	138	0	0	0	0	0	0
RES_2009_S_j	150	0	0	0	0	0	118	0	0	8	0	0	7	0	0	18	0	9	1029	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0
RES_2009_S_k	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	8	0	0	12	0	0	0	0	0	0	0	17	0	0	0	0	0	0
RES_2012_M_a	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0
RES_2012_M_b	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	63	0	32	0	0	0	4	0	0	0	0	0	0	0
RES_2012_M_d	0	4	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	7	0	7	0	0	0	2	0	0	0	0	0	0	0
RES_2012_M_e	0	68	0	0	0	0	6	0	0	147	0	0	0	0	0	0	0	1	0	0	0	0	8	0	18	0	0	0	0	0	0	0	0	0	0	0
RES_2012_M_f	0	32	0	0	0	0	1	0	0	42	0	0	0	0	0	0	0	2	0	0	0	0	7	0	3	0	0	0	0	0	0	0	0	0	0	0
RES_2012_M_g	52	191	0	0	0	0	41	0	0	103	0	0	0	0	0	0	0	4	0	0	0	0	0	0	4	0	0	0	0	0	0	34	0	0	0	0
RES_2012_M_h	155	91	0	0	0	0	3	0	0	33	0	0	0	0	0	0	0	13	0	0	0	0	2	0	6	0	0	0	0	0	0	2	0	0	0	0
RES_2012_M_j	446	78	0	0	0	0	142	0	0	17	0	0	31	0	0	0	0	9	0	0	0	0	5	0	9	0	0	0	0	0	0	4	0	0	0	0
RES_2012_M_k	330	6	0	0	0	0	212	0	0	19	0	0	29	0	0	0	0	17	0	0	0	0	5	0	5	0	0	0	0	0	0	0	0	0	0	0
RES_2012_S_a	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	97	0	0	0	0	0	0	0	0	0	0	0	0	0
RES_2012_S_f	3	88	0	0	0	0	0	0	0	151	0	0	0	0	0	0	0	3	0	0	0	0	7	0	82	0	0	0	112	0	0	11	0	0	0	0
RES_2012_S_h	106	40	0	0	0	0	2	0	0	303	0	0	0	0	0	0	0	12	0	0	0	0	12	0	199	0	0	0	52	0	0	7	0	0	0	0
RES_2012_S_i	157	104	0	0	0	0	11	0	0	62	0	0	0	0	0	0	0	17	0	0	0	0	5	0	171	0	0	0	22	0	0	9	0	0	0	0
RES_2012_S_j	194	63	0	0	0	0	50	0	0	54	0	0	8	0	0	0	0	28	0	0	0	0	1	0	622	0	0	0	21	0	0	146	0	0	0	0
RES_2012_S_k	419	20	0	0	0	0	311	0	0	3	0	0	12	0	0	0	0	18	12	0	0	0	2	0	3	0	0	0	44	0	0	4	0	0	0	0
Unrestored_B_a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Unrestored_B_d	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unrestored_B_f	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	11	4	0	0	0	0	0	0	0	0	0	0	0	0
Unrestored_B_g	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0
Unrestored_B_h	12	2	0	0	0	0	0	0	0	47	0	0	0	0	0	0	0	0	0	0	0	0	23	0	320	0	0	0	4	0	0	63	1	0	0	0
Unrestored_B_i	48	31	0	0	0	0	0	0	0	59	0	0	0	0	0	0	0	1	0	0	0	0	6	0	598	0	0	0	47	0	0	239	6	0	0	0
Unrestored_S_c	42	164	0	0	0	0	0	0	0	92	0	0	1	0	0	0	0	5	0	0	0	0	5	0	206	0	0	0	35	0	0	5	20	0	0	0
Unrestored_S_e	72	94	0	0	0	0	1	0	0	57	0	0	6	0	0	0	0	3	0	0	0	0	4	0	552	0	0	0	38	0	0	2	19	0	0	0
Unrestored_S_f	70	47	0	0	0	0	8	0	0	42	0	0	5	0	0	0	0	7	0	0	0	0	2	0	209	0	0	0	14	0	0	10	8	0	0	0
Unrestored_S_h	170	19	0	0	0	0	189	0	0	6	0	0	33	0	0	0	0	12	199	0	0	0	4	0	45	0	0	0	11	0	0	5	0	0	0	0
Active_B_h	145	99	0	0	0	0	125	0	0	231	0	0	0	1	0	0	0	3	0	0	0	0	0	0	13	0	0	0	112	0	0	2	0	0	0	0

Table A. 2. 4. Kruskal-Wallis one-way analysis of variance of physicochemical peat properties between sites (data from 2016).

Variable	H(df)	p-value
DOC	H(6) = 24.107	0.0005
C:N	H(6) = 40.814	3.15E-07
EC	H(6) = 33.657	7.83E-06
pH	H(6) = 51.234	2.66E-09
Von Post	H(6) = 41.689	2.12E-07
LAC	H(6) = 10.168	0.1177
ACE	H(6) = 21.396	0.0016
FOR	H(6) = 14.221	0.0273
SUC	H(6) = 34.772	7.77E-06
PYR	H(6) = 10.529	0.1041
PRO	H(6) = 30.177	3.64E-05
CIT	H(6) = 28.080	9.08E-05
Fe^{3+}	H(6) = 65.588	3.27E-12
NO^{2-}	H(6) = 32.971	1.06E-15
NO^{3-}	H(6) = 14.071	0.0289
SO_4^{2-}	H(6) = 43.883	7.80E-08
PO_4^{3-}	H(6) = 27.139	0.0001

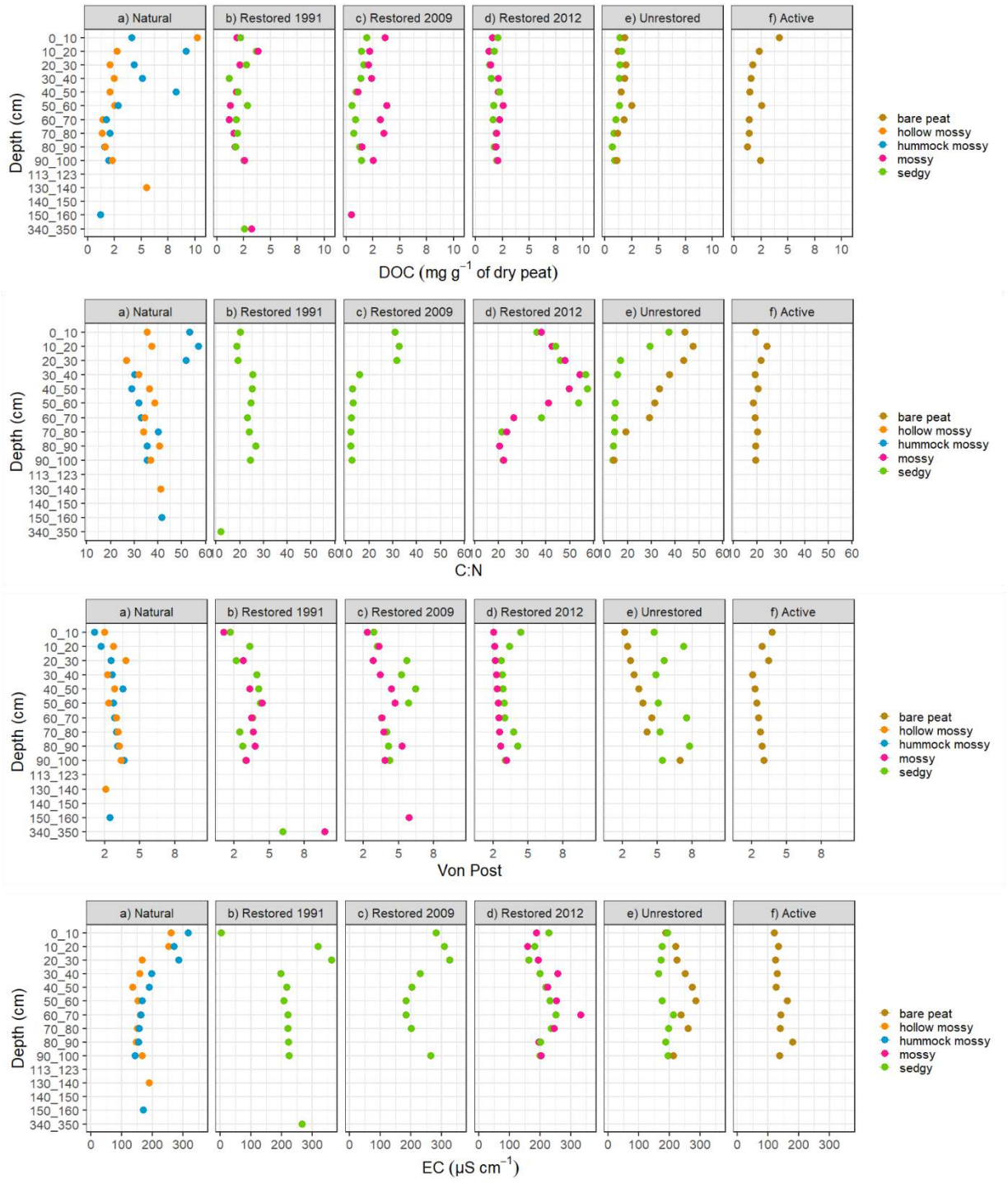


Figure A. 2. 1. Physicochemical properties of peat from the Natural, Unrestored, Active, and three restored sites in Seba Beach horticulture peatland complex. DOC – dissolved organic carbon, C:N – total carbon to nitrogen ratio, Von Post – peat decomposition index in Von Post scale, EC – electrical conductivity.

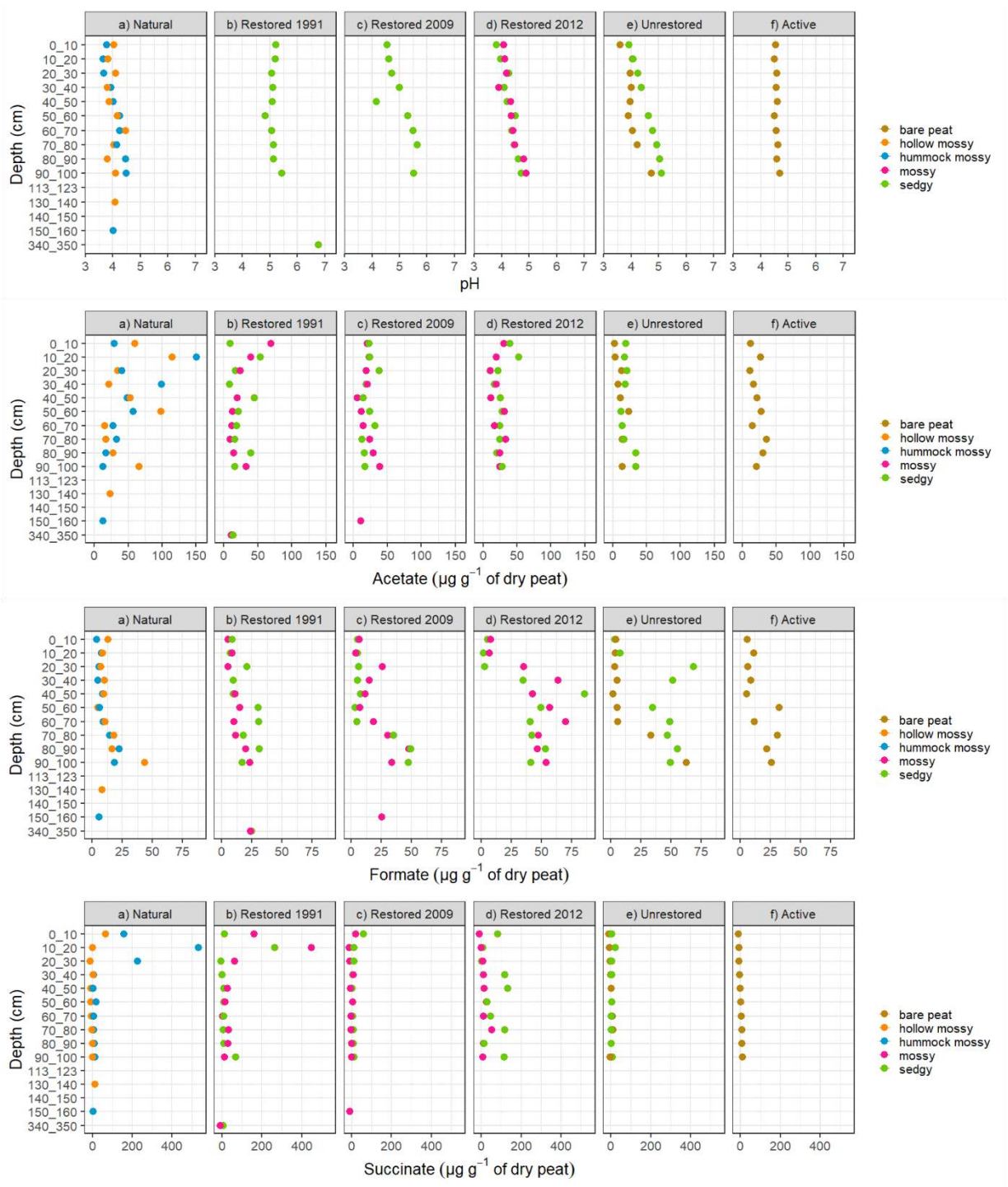


Figure A. 2. 1. [Continuation]

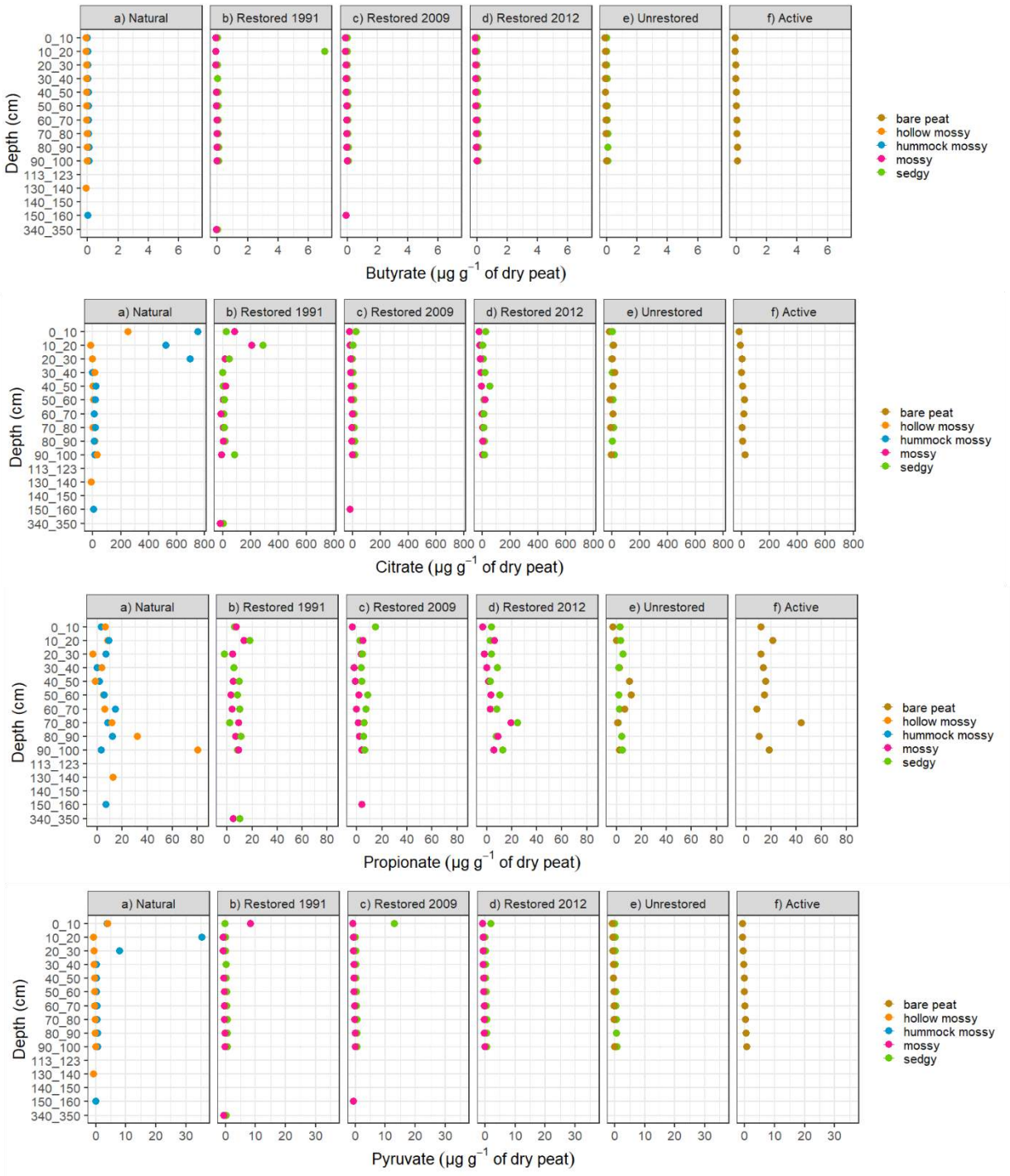


Figure A. 2. 1. [Continuation]

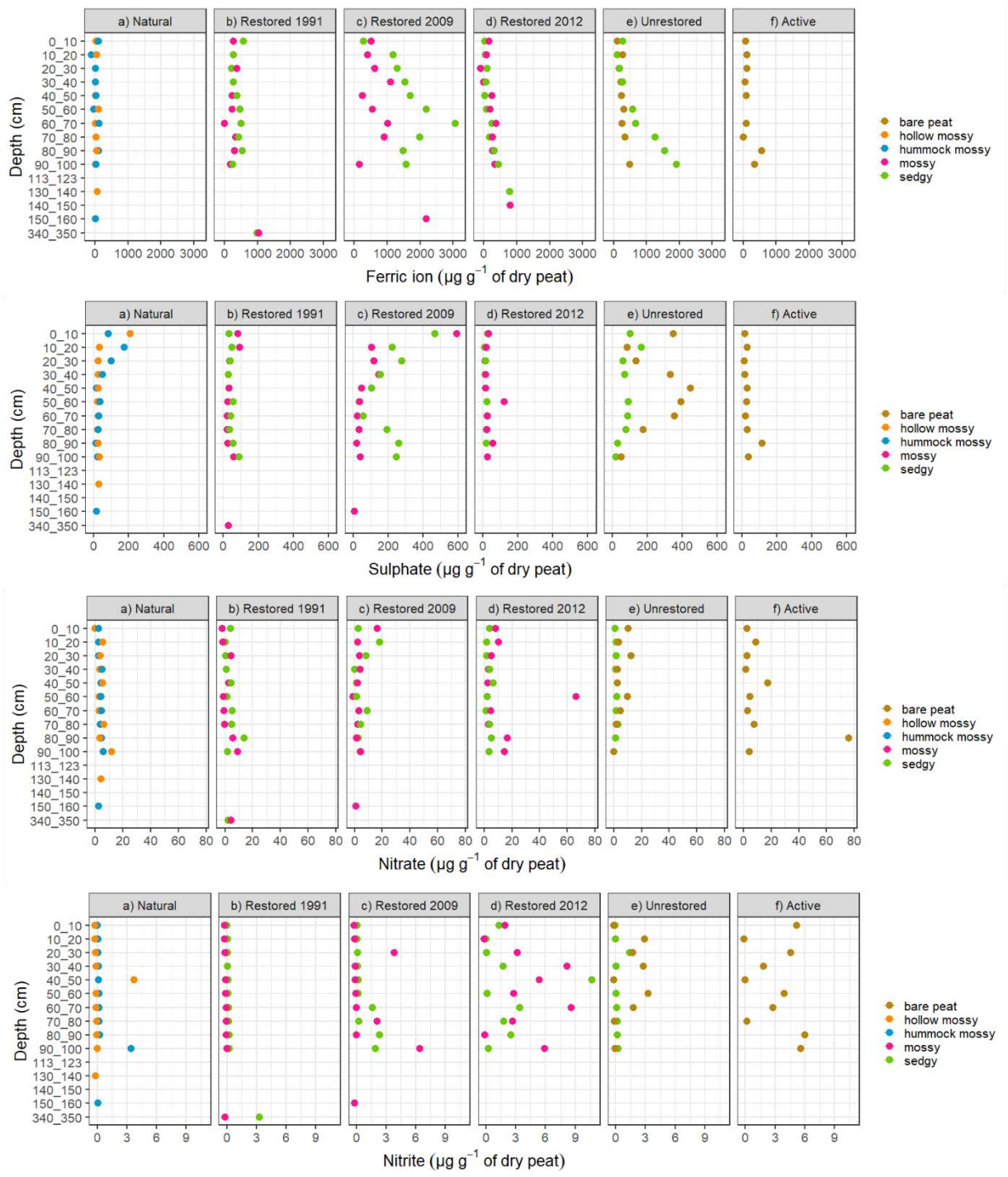


Figure A. 2. 1. [Continuation]

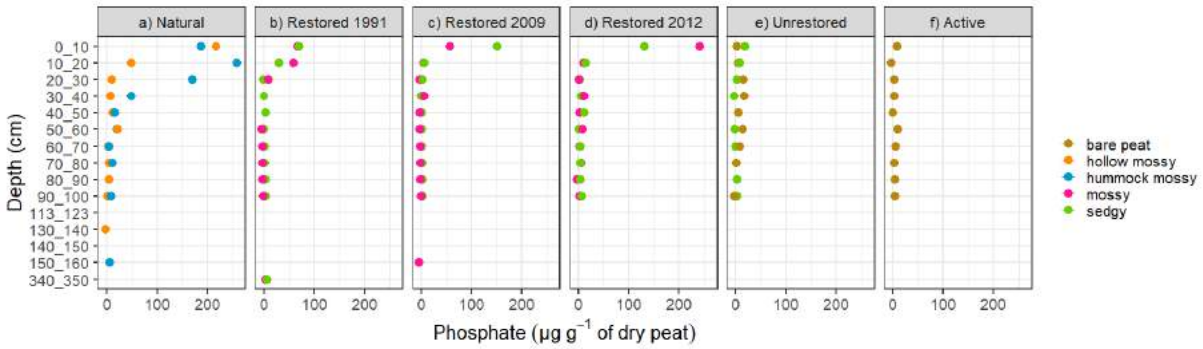


Figure A. 2. 1. [Continuation]

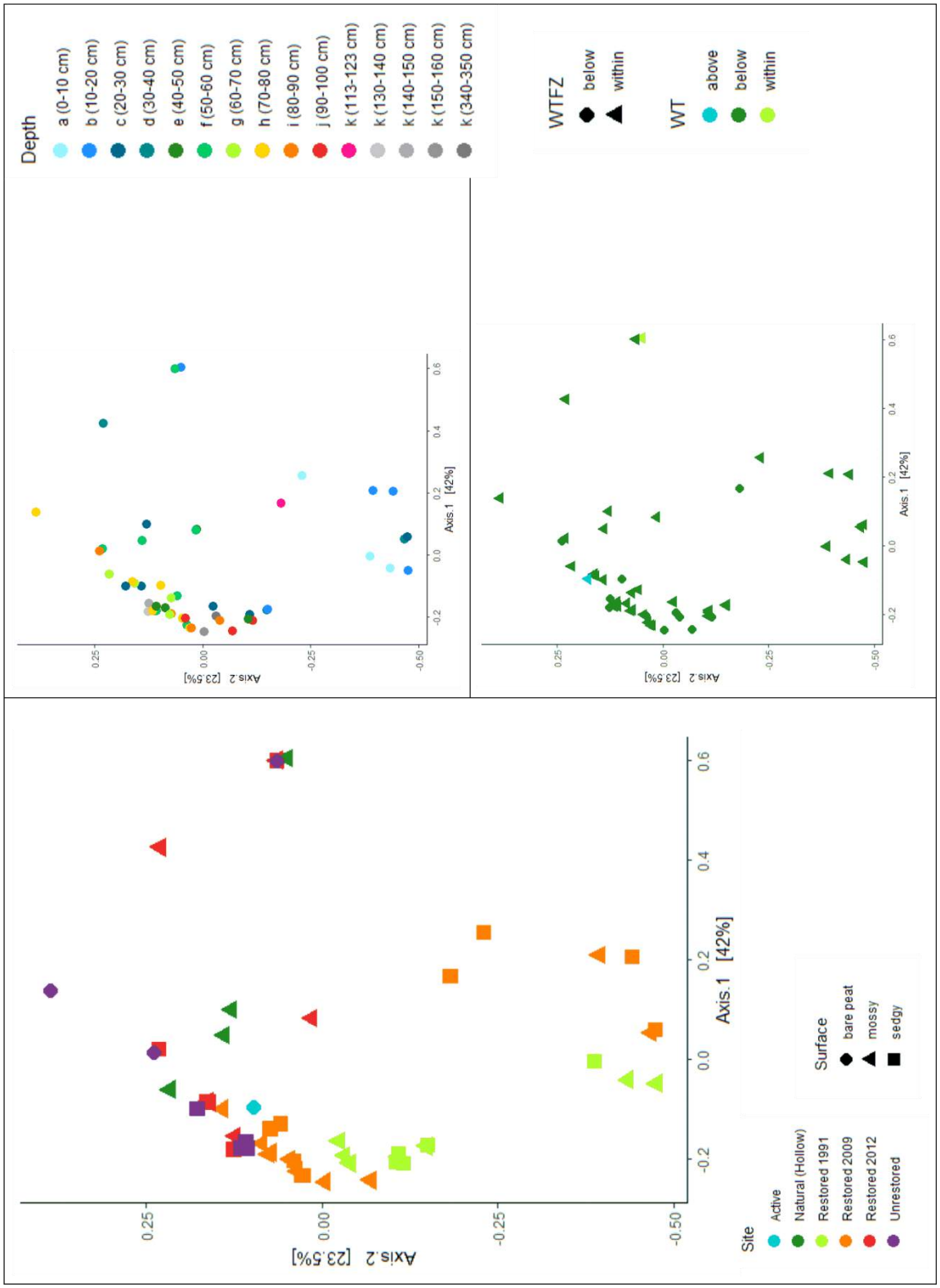


Figure A. 2. 2. Beta diversity visualization. A) Unweighted UniFrac (beta diversity) of methanogens, B) Unweighted UniFrac (beta diversity) of methanotrophs in the Natural, Active, Unrestored, and at three restored peatland sites.

B)

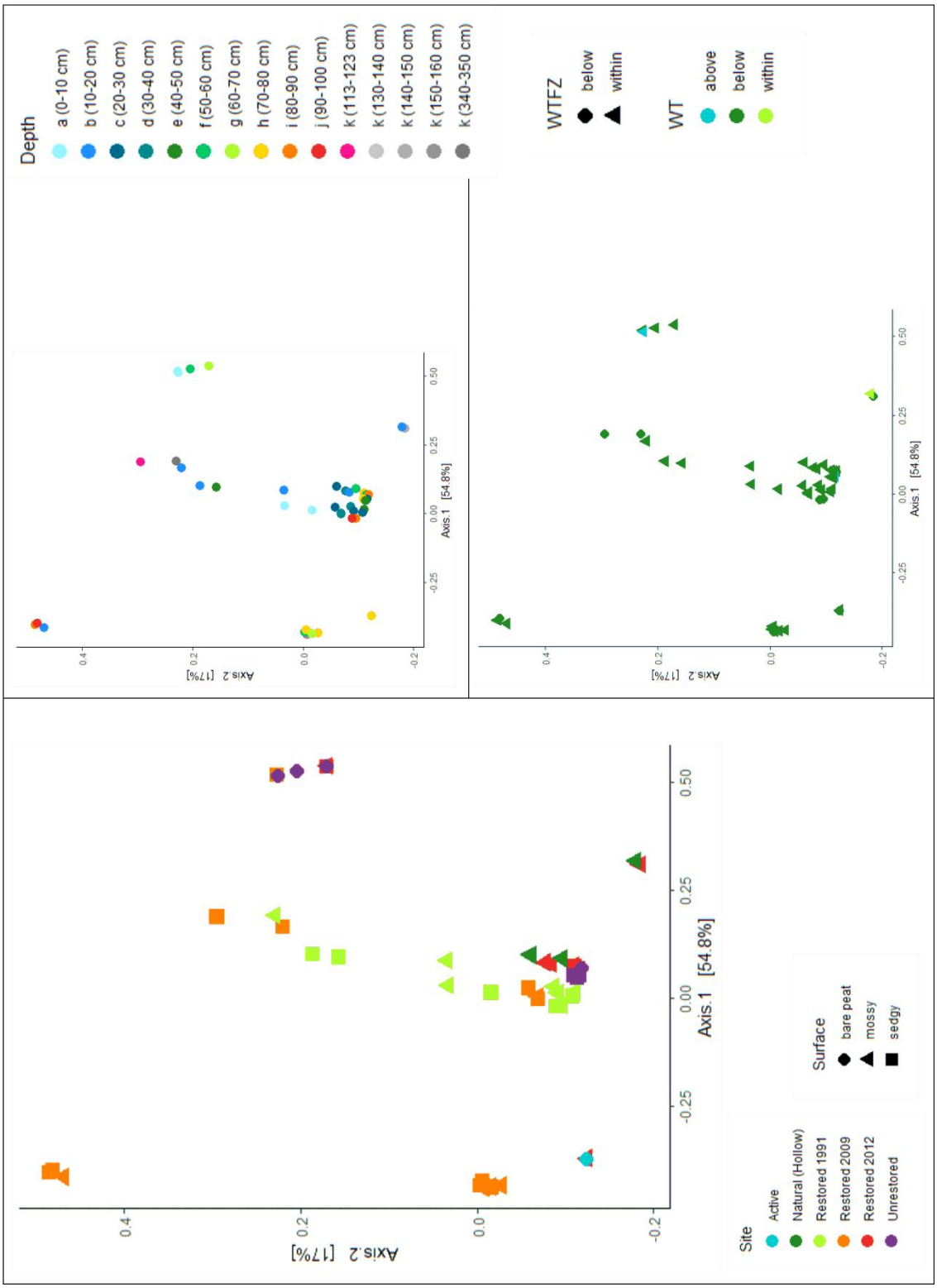


Figure A. 2. 2. [Continuation]

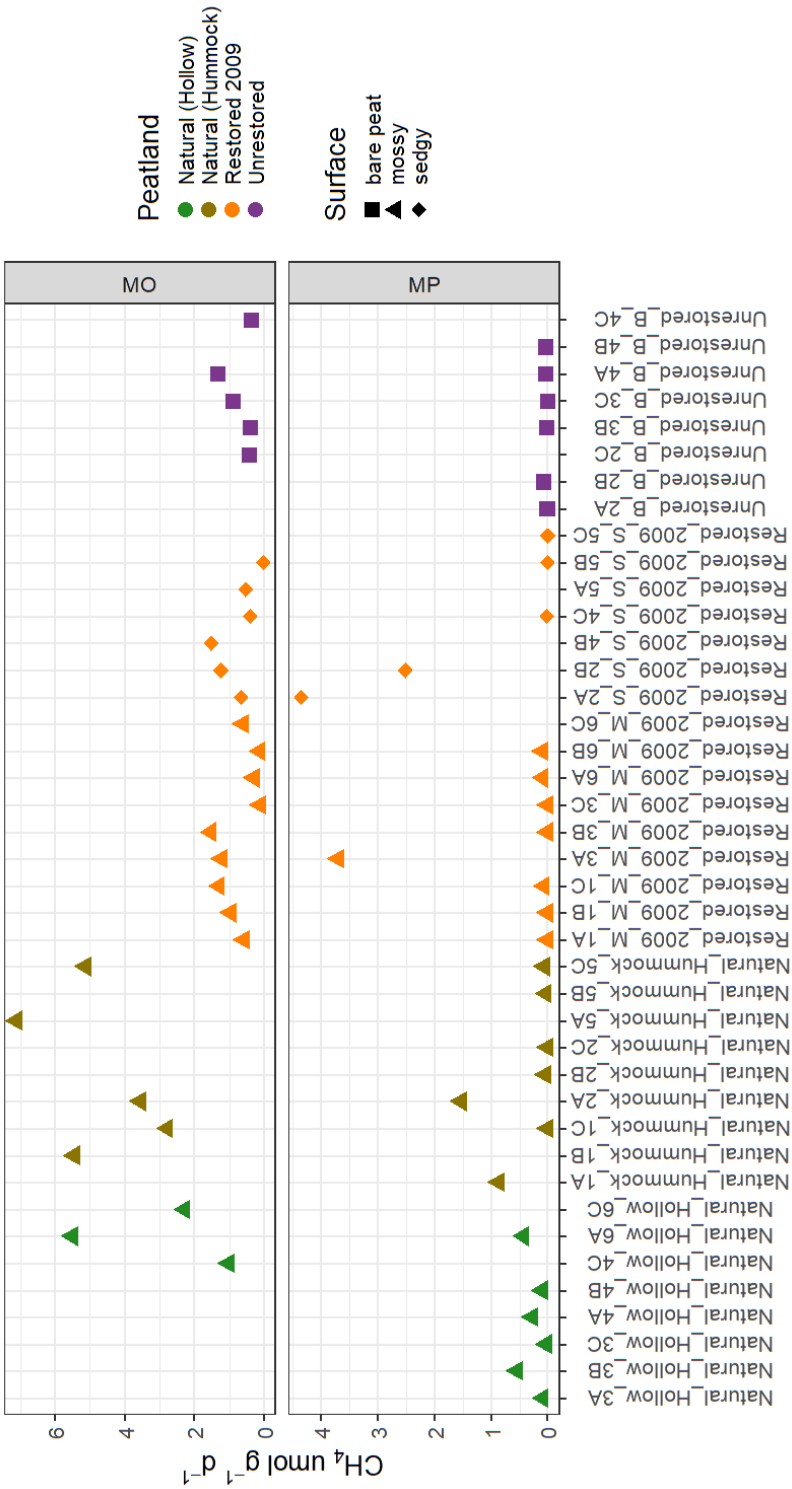


Figure A. 2. 3. Potential CH_4 production rates (MP) and potential CH_4 oxidation rates (MO) in the Natural, restored in 2009 (RES-2009), and Unrestored sites. Letters A – C denote the depth of peat in relation to the water table (WT): A (0 – 10 cm below the WT), B (0 – 10 cm above the WT), C (10 – 20 cm above the WT). M – mossy, S – sedgy, B – bare peat.

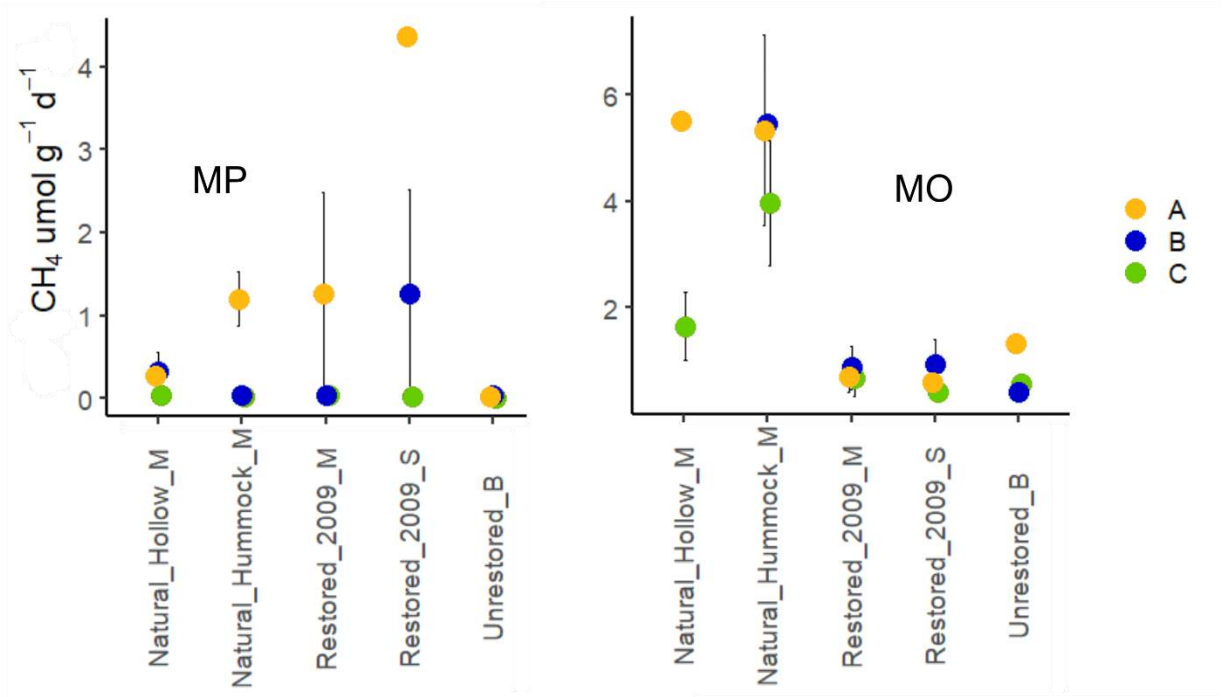


Figure A. 2. 4. Mean potential CH_4 production rates (MP) and mean potential CH_4 oxidation rates (MO) in the Natural, restored in 2009 (RES-2009), and Unrestored sites at depths 0 – 10 cm below the WT (A), 0 – 10 cm above the WT (B), and 20 – 30 cm above the WT (C). M – mossy, S – sedgy, B – bare peat.

Appendix 3

Table A. 3. 1. Steady and ebullitive CH_4 fluxes, CH_4 concentration in pore water, and environmental factors measured at Seba Beach horticulture peatland complex. The table is deposited in: Bieniada, Aneta, 2019, "CH_4 flux, [CH4] in pore water and environmental factors, Seba Beach peatlands", https://doi.org/10.5683/SP2/ZKNAUF, Scholars Portal Dataverse, DRAFT VERSION

Table A. 3. 2. Monthly mean temperature and total precipitation (Tomahawk meteorological station, Government Canada, 2018) in summer 2016 and 2017 and long-term monthly means for summer months (Edmonton Stony Plain station, Government Canada, 2018).

Year	Month	Mean temprature (˚C)	Total precipitation (mm)
2016	May	13.8	9.6
	June	15.0	47.7
	July	16.8	144.3
	August	15.7	173.9
2017	May	12.2	49.6
	June	14.3	137.0
	July	16.4	88.3
	August	15.2	30.5
1981 - 2010	May	10.8	46.6
	June	14.7	80.5
	July	17.0	102.4
	August	16.0	63.4

Table A. 3. 3. Range, mean, and standard deviation of gross ecosystem production (g m^{-2} d^{-1}) during summer 2016 and 2017. RES-1991 – site restored in 1991, RES-2009 – site restored in 2009, RES-2012 – site restored in 2012.

Site	GEP				
	n	min	max	mean	sd
Natural (Hollow)	51	0.0	38.4	14.0	9.3
Natural (Hummock)	55	0.7	42.9	12.3	9.4
RES-1991	106	1.0	146.0	26.3	21.4
RES-2009	113	1.0	84.0	33.1	20.3
RES-2012	97	0.2	125.7	29.2	19.6
Unrestored	101	0.1	35.3	9.0	8.4
Active	9	0.1	1.4	0.6	0.4

Table A. 3. 4. Mean and standard deviation of CH_4 emitted through ebullition at RES-1991 and RES-2012. RES-1991 – site restored in 1991, RES-2012 – site restored in 2012. Collar #2 at RES-2012 was placed at flooded part of the peatland, while # 3 and 4 at moderately wet and # 5 at dry locations.

Collar	Surface cover	n	mean	sd
RES-1991-1	mossy	11	59.5	57.7
RES-1991-2	mossy	12	25.4	24.7
RES-1991-4	sedgy	4	10.8	6.0
RES-1991-5	sedgy	3	65.8	33.8
RES-1991-6	sedgy	4	52.4	34.2
RES-2012-2	sedgy	4	1502.4	2613.4
RES-2012-3	mossy	1	1770.1	NA
RES-2012-4	mossy	1	164.4	NA
RES-2012-5	sedgy	1	8.0	NA

Appendix 4

Table A. 4. 1. Peat porosity at 10-cm increments in the Natural, Unrestored, Active, and three restored sites: RES-1991 – site restored in 1991, RES-2009 – restored in 2009, RES-2012 – restored in 2012. Grey fields indicate depths at which porosity was missing and the values were extrapolated from neighboring depths when in the top peat layer or the bottom layers, otherwise calculated as mean from adjacent depths immediately above and below. Some values are mean of two samples collected at the same depth (indicated with standard error in Fig. 4.1).

Depth (cm)	Natural	RES-1991	RES-2009	RES-2012	Unrestored	Active
0-10	0.96	0.95	0.94	0.79	0.56	0.84
10-20	0.96	0.96	0.92	0.85	0.69	0.90
20-30	0.95	0.96	0.89	0.91	0.81	0.91
30-40	0.96	0.95	0.93	0.93	0.85	0.92
40-50	0.95	0.95	0.90	0.95	0.88	0.93
50-60	0.97	0.98	0.93	0.96	0.91	0.94
60-70	0.95	0.97	0.92	0.94	0.91	0.91
70-80	0.94	0.95	0.92	0.91	0.87	0.93
80-90	0.93	0.96	0.94	0.92	0.87	0.91
90-100	0.96	0.97	0.94	0.93	0.86	0.94
100-130	0.96	0.97	0.93	0.93	0.86	0.94
130-140	0.96	0.97	0.92	0.92	0.86	0.94
140-150	0.93	0.97	0.94	0.93	0.86	0.94
150 - below	0.93	0.97	0.94	0.93	0.86	0.94
340-350	0.93	0.95	x	x	x	x
350 - below	0.93	0.95	x	x	x	x

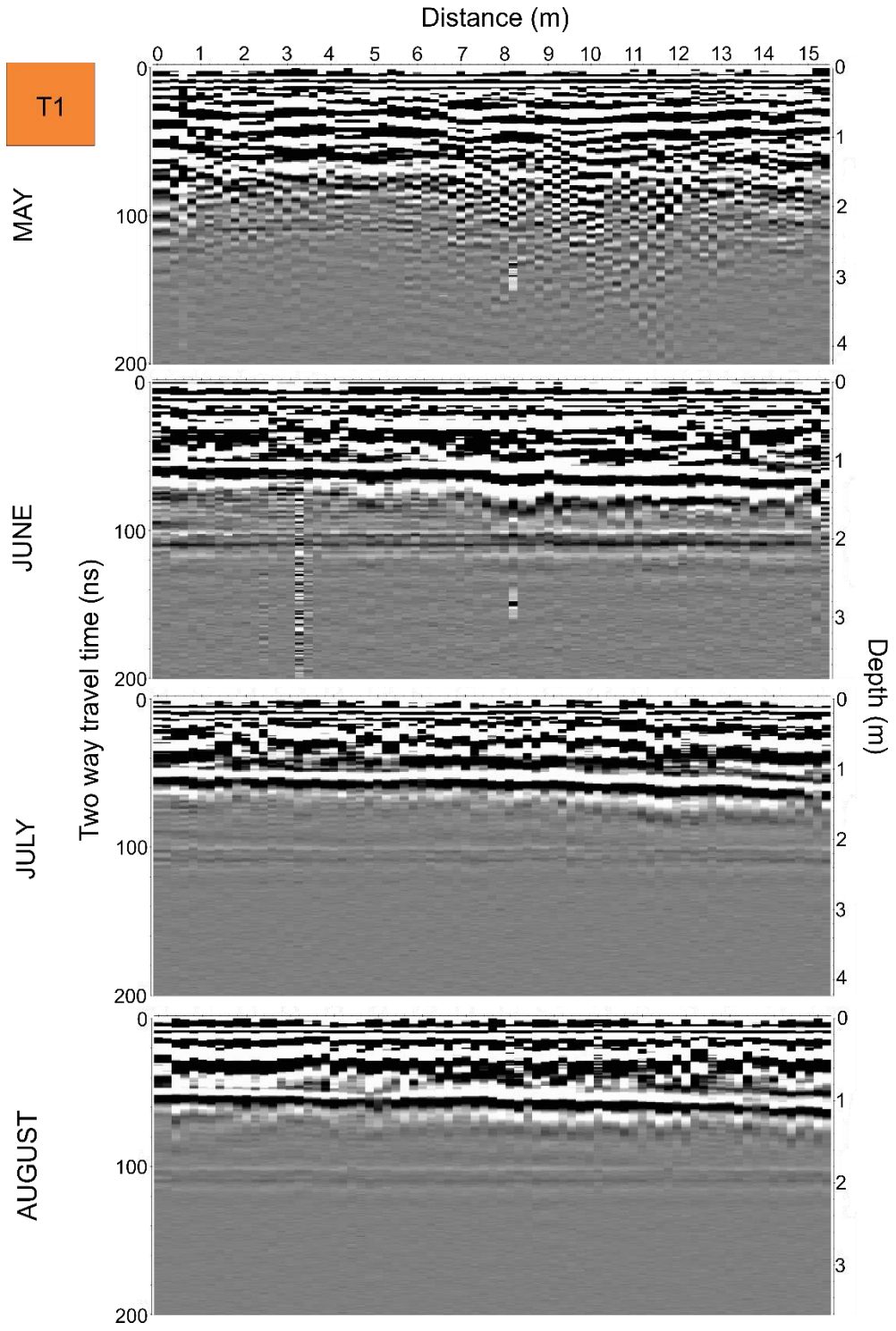


Figure A. 4. 1. Common offset (CO) radargrams of transect T1, T3, and T4 at site RES-2009 in May, June, July, and August showing an example of spatial variability in peat stratification between transects, continuity of layers, and temporal variability depending on changing soil moisture. The depth of peat was calculated based on the average velocity obtained from common midpoint (CMP) surveys, hence individual depth scale on Depth (m) axis. High volumetric gas content (> 20 %) was observed below 2 m depth.

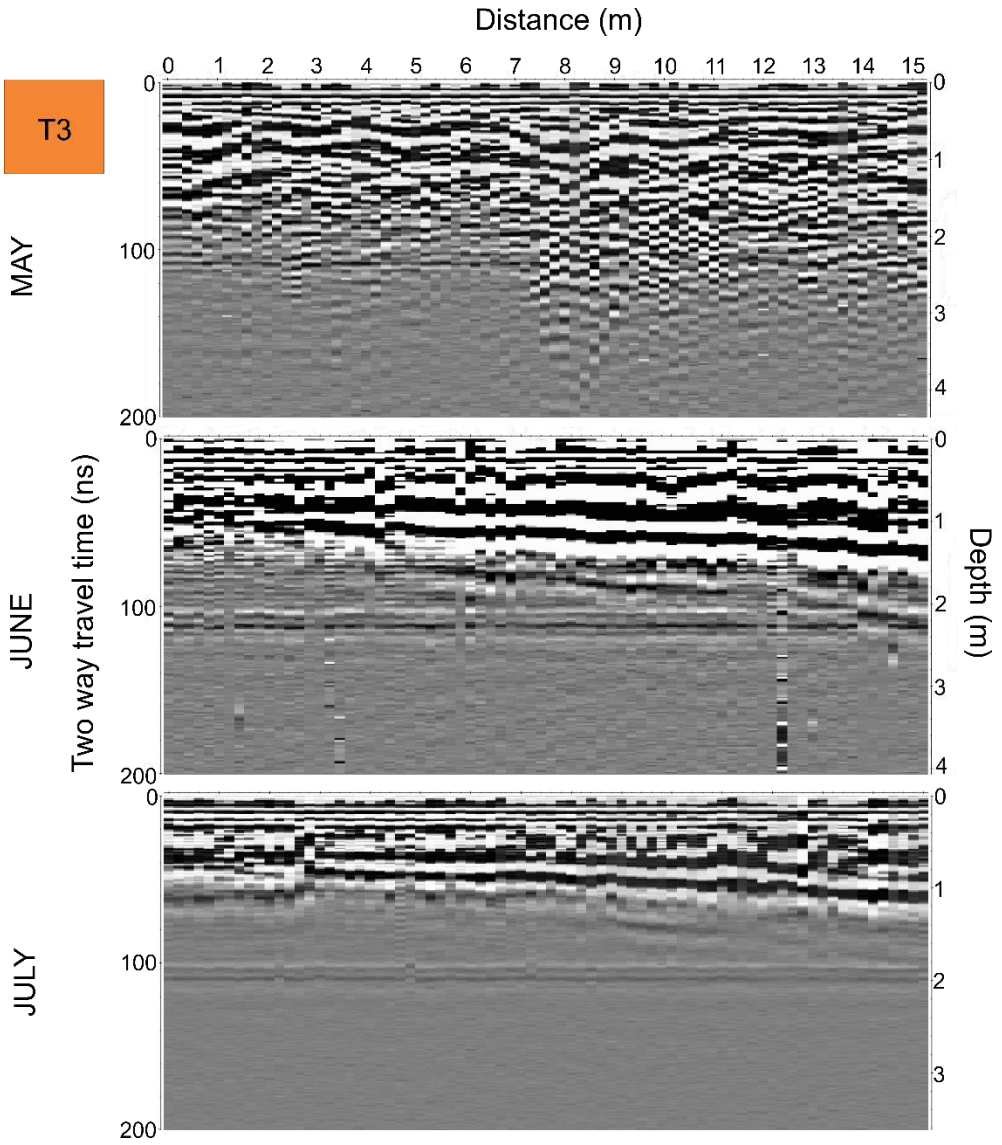


Figure A. 4. 1. [Continuation]. August radargram is not available.

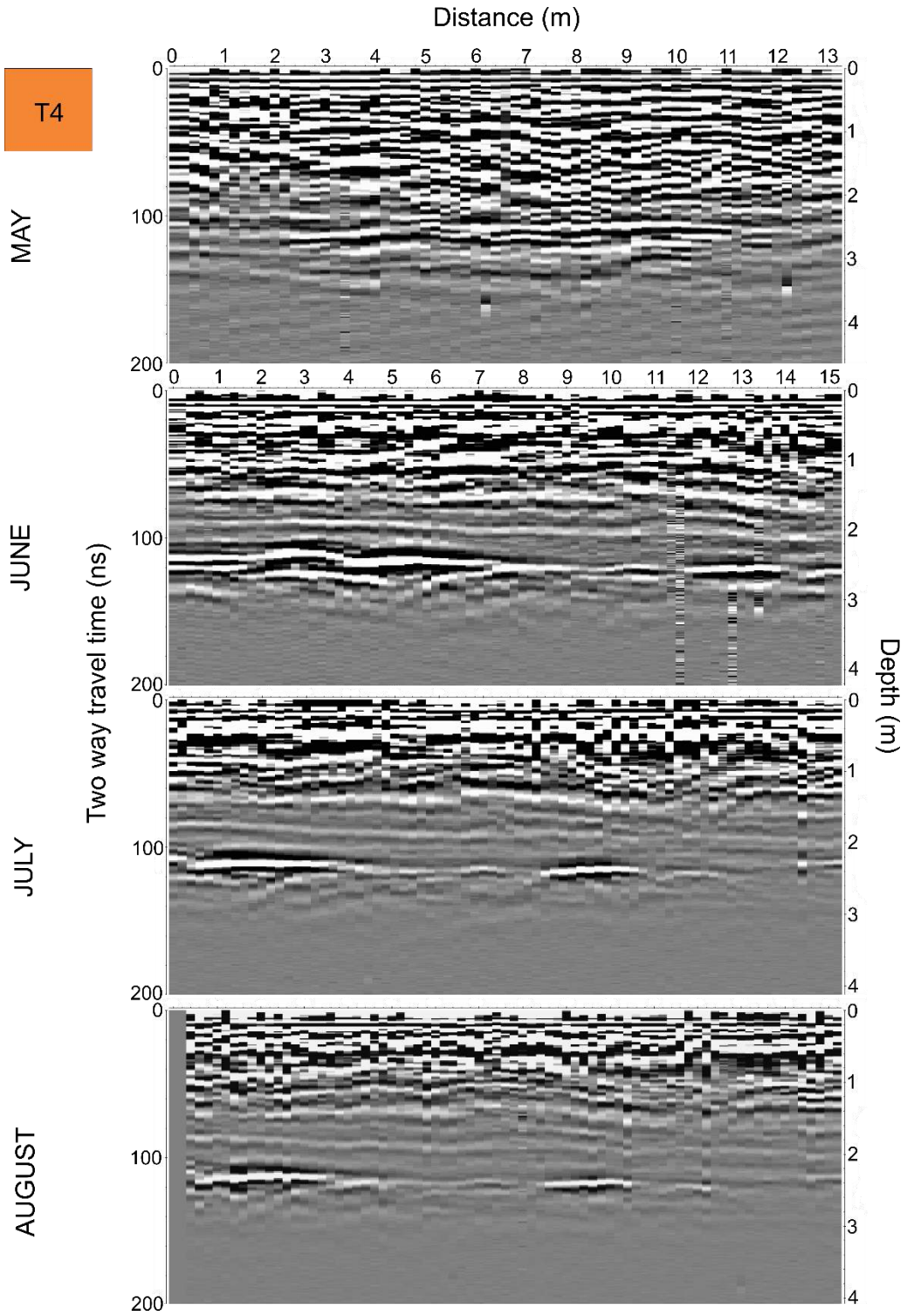


Figure A. 4. 1. [Continuation]