

2013-05-01

# Effect of Drainage on Carbon Biogeochemistry and Microbiological Communities in Western Canadian Boreal Peatlands

Graham, Jaime

---

Graham, J. (2013). Effect of Drainage on Carbon Biogeochemistry and Microbiological Communities in Western Canadian Boreal Peatlands (Master's thesis, University of Calgary, Calgary, Canada). Retrieved from <https://prism.ucalgary.ca>. doi:10.11575/PRISM/25798  
<http://hdl.handle.net/11023/673>

*Downloaded from PRISM Repository, University of Calgary*

UNIVERSITY OF CALGARY

Effect of Drainage on Carbon Biogeochemistry and Microbiological Communities in Western  
Canadian Boreal Peatlands

by

Jaime M. Graham

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BIOLOGICAL SCIENCES

CALGARY, ALBERTA

APRIL, 2013

©Jaime M. Graham 2013

## Abstract

This study compared the bacterial and archaeal communities between a natural peatland and a peatland affected by water table drawdown along a microtopographic position and depth gradient. Peat physicochemical properties, carbon flux and potential CO<sub>2</sub> and CH<sub>4</sub> production as well as CH<sub>4</sub> oxidation were measured to determine which factors affect microbial composition and diversity.

Bacterial and archaeal communities were described by targeting the 16S rRNA gene using pyrosequencing. Physicochemical parameters measured included pH, peat temperature, humification, acetate, formate, sulfate, nitrite and nitrate.

The most predominant methanotroph genus was *Methylocella* and the most predominant methanogen group was *Methanomicrobiales* Rice Cluster II. Diversity indices show humification and position relative to the water table as significant drivers affecting microbial diversity and richness. The control and drained sites differed significantly regarding the physicochemical influences on microbial relative abundance, however, pH affected methanotroph and methanogen relative abundance at both peatland sites.

## **Acknowledgements**

“If the only tool you have is a hammer, you tend to see every problem as a nail” - Abraham Maslow. I am grateful to the many people who enriched this research with their kind encouragement and gave me the tools necessary to be successful. This thesis would not be possible without the tremendous support of mentors, friends and family. In particular, I would like to thank Dr. Maria Strack for always believing in me and allowing me a chance to grow under her encouragement. Her passion for science and dedication to her students is unmatched and has motivated me to succeed. To Dr. Peter Dunfield, thank you for pushing me along even when I was unsure. Your knowledge, time and patient support, has been so incredibly valuable. Thank you to the members of my committee Drs. Lisa Gieg and Alex De Visscher for taking the time to support and encourage my academic efforts. To Dr. Allyson Brady and Christine Sharp, without your willingness and expertise in assisting me with so many aspects of this research, this research would not have been possible. Your active participation and support, was the key to the success of this research. I will be forever grateful! Thanks to Jordanna Branham who has fought the elements with me in the field and in the lab. And finally, thank you to my family and many wonderful friends who have been so incredibly supportive and encouraging throughout this whole process. This research would not have been possible without you all. Thank you!

## Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
Table of Contents.....	iv
List of Tables.....	vii
List of Figures.....	ix
List of Symbols, Abbreviations and Nomenclature.....	xiv
<b>Chapter One: Thesis Introduction .....</b>	<b>1</b>
1.1 Introduction to the Thesis.....	1
1.2 Research Objectives.....	2
1.3 Subsequent Sections of the Thesis.....	3
1.4 Study Sites.....	4
<b>Chapter Two: Literature Review .....</b>	<b>7</b>
2.1 Introduction to Canadian Peatlands and Vegetation.....	7
2.2 Peatland Carbon Balance.....	10
2.3 Water Table Drawdown and Carbon Cycling Response.....	13
2.4 Peatland Microbiology and Function.....	15
2.4.1 Bacterial Communities.....	15
2.4.2 Methanotrophs.....	18
2.4.3 Methanogens.....	21
2.5 Microbial Identification and Characterization.....	26
<b>Chapter Three: Microbial Characteristics of the Wandering River Natural and Drained Bogs .....</b>	<b>29</b>
3.1 Introduction.....	29
3.2 Materials and Methods.....	30

3.2.1 Peat Coring, Water Table Measurements and Flux Sampling.....	30
3.2.2 Incubation Study .....	31
3.2.3 Extraction of Total DNA .....	34
3.2.4 PCR Amplification, Purification and Quantification.....	34
3.2.5 Statistical Analyses .....	35
3.3 Results .....	35
3.3.1 Field Fluxes and Water Table Position.....	35
3.3.2 Potential CO <sub>2</sub> Production .....	37
3.3.3 Potential CH <sub>4</sub> Oxidation (Oxic Incubation Study) .....	41
3.3.4 Potential CH <sub>4</sub> Production (Anoxic Incubation Study).....	42
3.3.5 Microbial Community.....	44
3.3.6 Methanotroph Community.....	49
3.3.7 Putative Anaerobic Methanotrophs.....	52
3.3.8 Methanogen Community .....	54
3.4 Discussion.....	56
3.4.1 CO <sub>2</sub> Flux, WT Position and Potential CO <sub>2</sub> Production.....	56
3.4.2 CH <sub>4</sub> Flux, WT Position, and Potential CH <sub>4</sub> Production/Oxidation.....	58
3.4.3 Bacterial Community.....	59
3.4.4 Methanotroph Community.....	61
3.4.5 Methanogen Community .....	63
<b>Chapter Four: Microbial and Physicochemical Comparisons of the Wandering River Natural and Drained Bogs.....</b>	<b>65</b>
4.1 Introduction .....	65
4.2 Methods .....	66
4.2.1 Study Sites and Sampling .....	66
4.2.2 Humification .....	66
4.2.3 Determination of Anion Concentrations.....	67
4.2.4 Diversity Indices and Statistical Methods.....	67
4.3 Results .....	68

4.3.1 Physicochemical Characteristics.....	68
4.3.2 Anion Concentrations .....	69
4.3.3 Humification .....	73
4.3.4 Effects of Physicochemical Parameters on Microbial Diversity .....	75
4.3.5 Effects of Physicochemical Parameters on the Methanotroph Community .....	81
4.3.6 Effects of Physicochemical Parameters on the Methanogen Community .....	85
4.4 Discussion.....	89
4.4.1 Effect of Temperature and pH on Microbial Community .....	89
4.4.2 Effect of Water Table Position and Humification on Microbial Community .....	90
4.4.3 Effect of Anion Concentrations on Microbial Community .....	92
<b>Chapter Five: Thesis Summary and Conclusions.....</b>	<b>94</b>
References.....	97
Appendix.....	118

## List of Tables

Table 2.1 Taxonomic classification of methanotrophs .....	19
Table 2.2 Summary of a sample of 16S rRNA gene molecular marker studies of methanogens in peatlands. ....	25
Table 3.1 List of 54 samples and categorical attributes used in the incubation experiments and the microbial pyrosequencing. The naming convention is as follows: CH=Control hummock, CW=Control hollow, DH=Drained hummock, DW=Drained hollow. Number values refer to the depth of the sample from surface (i.e., 135150 = 135 to 150 cm). A, B and C are different cores. ....	33
Table 3.2 CO <sub>2</sub> and CH <sub>4</sub> fluxes (mean ± SEM) from the chamber method field flux measurements taken at the Wandering River bogs in July 2010. Positive values are emission from the peatland to the atmosphere while negative values are uptake from the atmosphere to the peatland. ....	36
Table 3.3 Statistical results (general linear model) show significant differences in aerobic and anaerobic potential CO <sub>2</sub> production rates as tested between sites, microforms and WT categories. ....	39
Table 3.4 General linear model results show no significant differences in PMO as tested between sites, microforms and WT categories.....	42
Table 3.5 General linear model results show significant differences in potential CH <sub>4</sub> production (PMP) as tested between sites, microforms and WT categories. ....	42
Table 3.6 Taxonomic descriptions of the ten most abundant families (in which many OTUs were summed to the family level) across all 54 samples (as a percentage of all reads).....	45



Table 4.1 Physicochemical properties (mean  $\pm$  SEM) of the peat samples and measurements taken at the Wandering River bog in July 2010. Soil temperature and pH are the average of section measurements at each microform, i.e., are averaged over depths. .... 69

Table 4.2 General linear model results for concentrations of anions ( $\mu\text{mol g dry peat weight}^{-1}$ ). DT=Drainage Treatment, WT=Water Table (Above/Below)..... 70

Table 4.3 Diversity indices between the control and drained sites along with the respective microforms. The Chao1, Shannon and Simpson indices were all higher at the drained site.75

Table 4.4 General linear model results showing the influencing physicochemical factors of the Chao1, Shannon and Simpson diversity indices. Humification, microforms and position to the water table are significant factors driving microbial richness and diversity..... 76

Table 4.5 General linear model results show physicochemical factors in relation to *Methylocella*, *Methylosinus*/*Methylocystis* and all methanotrophs relative abundance. P values less than 0.05 (bold) were considered significant. Nitrate and pH were significant factors affecting methanotroph relative abundance. DT=Drainage Treatment..... 82

Table 4.6 General linear model results showing the physicochemical factors affecting the Rice Cluster II and acetoclastic methanogen groups. P values less than 0.05 (bold) were considered significant. Nitrate, pH, temperature, humification, position to the WT, formate and sulfate were all significant factors affecting methanogen relative abundance. DT=Drainage Treatment..... 86

## List of Figures

Figure 1.1 Locations of research sites near Wandering River, Alberta. Photo source: Google Earth (public domain), 55°18'38.95"N and 112°28'51.29"W elevation 572 m, 2013..... 5

Figure 1.2 The control site (above) shows stunted *Picea mariana* (Black Spruce) while the drained site (below) shows mature Black Spruce and a higher density of *Ledum groenlandicum* (Labrador tea). Photo: Jaime Graham..... 6

Figure 2.1 Flow diagram of the carbon cycle in peatlands adapted from Faubert (2004)..... 13

Figure 2.2 Flow diagram of anaerobic OM decomposition and methanogenesis modified from Conrad (1999) as cited in Galand (2004) and Juottonen (2008)..... 23

Figure 3.1 Average water table levels from the top of the peat surface (n=3 for each; measured from microform surface). Water table level is shown at 0. .... 36

Figure 3.2 Average aerobic (a) and anaerobic (b) potential CO<sub>2</sub> production with 1 SEM bars by site. The control site had significantly higher aerobic and anaerobic potential CO<sub>2</sub> production compared to the drained site. N=24 for the control site and 30 for the drained site. The asterisks indicate statistically significant differences between sites for aerobic and anaerobic potential CO<sub>2</sub> production (P<0.05) (i.e., aerobic control was significantly higher than aerobic drained and anaerobic control was significantly higher than anaerobic drained). .... 38

Figure 3.3 Average aerobic and anaerobic potential CO<sub>2</sub> production (±1 SEM) by site microform (a and b) and site water table category (c and d). The aerobic potential CO<sub>2</sub> production was higher above the water table than below (GLM Table 3.3). N=12 for control hummocks and control hollows, 15 for drained hummocks and drained hollows. N=10 for control above, 14 for control below, 19 for drained above and 11 for drained below. .... 40

Figure 3.4 Average potential CH<sub>4</sub> oxidation (oxic experiment) ( $\pm 1$  SEM) at the control and drained site microforms (a) and at the microform position as above or below the water table (b). Positive values exhibit potential CH<sub>4</sub> production and negative values indicate potential CH<sub>4</sub> oxidation (consumption). Control hummocks (n=12), control hollows (n=12), drained hummocks (n=15) and drained hollows (n=15). The hummocks above the water table (b) had significantly larger potential CH<sub>4</sub> oxidation than the hollow above or below the water table (Table 3.4). Hummock above (n=16), hummock below (n=11), hollow above (n=13) and hollow above (n=14). ..... 41

Figure 3.5 Potential CH<sub>4</sub> production (anoxic experiment) ( $\pm 1$  SEM) shows a higher potential CH<sub>4</sub> production below the water table as compared to above the water table. Control above (n=10), control below (n=14), drained above (n=19) and drained below (n=11). ..... 43

Figure 3.6 Relative abundance of the most abundant families identified across all samples and organized by control and drained microforms. Samples were pooled as per Table 3.1 and calculated based on the number of reads to the total number of reads within each category. .... 47

Figure 3.7 Relative abundances of the most abundant families identified across all samples and organized into control and drained sites both above and below the water table. Samples were pooled as per Table 3.1 and calculated based on the number of reads to the total number of reads within each category. .... 48

Figure 3.8 Relative abundance of methanotrophs identified across all samples. Overall, there were 930 reads of all methanotrophs of which 61% belong to *Methylocella* followed by 28% *Methylosinus/Methylocystis*. ..... 49

Figure 3.9 16S rRNA gene sequence-based tree showing phylogenetic position of methanotrophs identified in this study (designated with prefix ‘WR’ in bold) assembled as per the Neighbor-Joining method with a Jukes-Cantor correction. Bootstrap support values (% of 1,000 replicates) greater than 50% are indicated at the nodes. A skeleton tree was made from nearly full-length sequences (>1400 nt) and the shorter pyrosequences (450 nt) were added to this tree via parsimony. The sequence alignments were from the ARB-Silva database and calculations were made using ARB (Ludwig *et al.* 2004). The numbers of pyrosequencing reads for the present study are shown in parentheses. The scale bar represents a 0.1 change per nucleotide position..... 50

Figure 3.10 Relative abundance of methanotrophs identified as a percentage of the total community across all samples by control and drained microforms..... 51

Figure 3.11 16S rRNA gene sequence-based tree showing phylogenetic position of the putative anaerobic methanotrophs from the NC10 phylum identified in this study (designated with prefix ‘WR’ in bold) assembled as per the Neighbor-Joining method with a Jukes-Cantor correction. Bootstrap support values (% of 1,000 replicates) greater than 50% are indicated at the nodes. GenBank accession numbers precede the NC10, *Nitrospirae*, *Deferribacteres* and the *Chrysiogenetes* phylum reference sequences. The tree was rooted against a set of sequences from the *Nitrospirae*, *Deferribacteres* and the *Chrysiogenetes* phyla. The scale bar represents 0.1 change per nucleotide position. .... 53

Figure 3.12 Relative abundance of methanogens identified across all samples. Overall, there were 48,467 reads across all methanogens of which 91% belonged to *Methanomicrobiales*, Rice Cluster II methanogens..... 54

Figure 3.13 Relative abundance of methanogens identified across all samples and organized by control and drained microforms.....	55
Figure 4.1 Average anion concentrations between the control (n=24) and drained sites (n=30) show significant differences for acetate, formate and sulfate ( $\pm 1$ SEM). Asterisks represent significant differences between control and drained site as per the general linear model (Table 4.2).....	70
Figure 4.2 Average anion concentrations between the control and drained microforms show significant differences for sulfate ( $\pm 1$ SEM) with letters denoting significant differences. Control hummock and hollow n=12, drained hummock and hollow n=15.....	71
Figure 4.3 Average anion concentrations between the control and drained microforms show significant differences for nitrate ( $\pm 1$ SEM) with letters denoting significant differences. Control above n=10, control below n=14, drained above n=19, drained below n=11. ....	72
Figure 4.4 Regression of von Post index of humification against distance from the water table (shown at zero cm) shows samples below the water table (negative values) are more humified than those above (positive values).....	73
Figure 4.5 Regression plots of von Post index of humification against aerobic potential CO <sub>2</sub> production (a) and potential CH <sub>4</sub> production (PMP) (b) show a negative correlation at both sites. ....	74
Figure 4.6 Regression plots of Shannon and Simpson indices against humification shows a positive correlation at the control site but a negative correlation at the drained site with increasing humification.....	77
Figure 4.7 Regression plots of Chao1, Shannon and Simpson indices against position to water table shows a positive correlation at the drained site but a negative correlation at the control	

site with increasing dryness. Zero is the water table level, negative values are below the water table and positive values are above the water table. .... 78

Figure 4.8 Distance-scaled dendrogram across all samples of the Bray-Curtis similarity index using the QIIME distance matrix and Ward linkage algorithm with Euclidean distances. CH=Control hummock, CW=Control hollow, DH=Drained hummock, DW=Drained hollow. Numbers refer to the depth of the sample from surface (i.e., 135150 = 135 to 150 cm) (Table 3.1). Temperature (T) and pH values are the averages of the samples in each cluster..... 80

Figure 4.9 Regression plots with relative abundances of all methanotrophs and *Methylocella* against nitrate shows a significant and positive correlation at the drained site. .... 83

Figure 4.10 Regression plot with relative abundance of *Methylosinus/Methylocystis* against pH. While pH was not a significant factor affecting relative abundance between sites, pH was negatively correlated with decreasing *Methylosinus/Methylocystis* relative abundance. .... 84

Figure 4.11 Regression plots with relative abundance of Rice Cluster II methanogens against humification (a), nitrate (b), pH (c), temperature (d) and position to the water table (e). Humification and nitrate was positively correlated as a main effect whereas pH was positively correlated as main effect and a site interaction effect. Temperature and position to the water table showed a significant negative correlation at the drained site. .... 87

Figure 4.12 Regression plots with relative abundance of acetoclastic methanogens against temperature (a), formate (b) and sulfate (c). Temperature was significantly and negatively correlated to acetoclastic methanogen relative abundance at both sites. Formate and sulfate concentrations were significantly and positively correlated to acetoclastic methanogen relative abundance at the drained site. .... 88

## List of Symbols, Abbreviations and Nomenclature

<u>Symbol</u>	<u>Definition</u>
ANOVA	analysis of variance
bp	base pair
C	carbon
CO <sub>2</sub>	carbon dioxide
CH	control hummock
CH <sub>4</sub>	methane
CH <sub>3</sub> OH	methanol
CW	control hollow
DH	drained hummock
DIC	dissolved inorganic carbon
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
DW	drained hollow
ER	ecosystem respiration
ET	evapotranspiration
FTIR	Fourier Transform Infrared Spectroscopy
GC	gas chromatographer
GHG	greenhouse gas
H <sub>2</sub>	hydrogen
H <sub>2</sub> O	water
HPLC	high-performance liquid chromatography
ICM	intracytoplasmic membrane
N <sub>2</sub>	nitrogen
N <sub>2</sub> O	nitrous oxide
NEE	net ecosystem exchange
NO	nitric oxide
NPP	net primary production
O <sub>2</sub>	oxygen
OM	organic matter
OTU	operational taxonomic unit
PCR	polymerase chain reaction
Pg	petagram (10 <sup>15</sup> g)
PMO	potential methane oxidation
pMMO	particulate methane monooxygenase
PMP	potential methane production
POC	particulate organic carbon
QIIME	quantitative insights into microbial ecology
RCII	Rice Cluster II methanogen
rRNA	ribosomal ribonucleic acid
SEM	standard error of the mean
sMMO	soluble methane monooxygenase
T-RFLP	terminal restriction fragment length polymorphism
WT	water table

## Chapter One: Thesis Introduction

### 1.1 Introduction to the Thesis

The worldwide coverage of peatlands is only approximately 3% of the earth's surface, but north of 45° approximately 3-4 million km<sup>2</sup> (or 10% of all the land north of 45°N) is covered by peatlands (Basiliko *et al.* 2007; Frohling *et al.* 2009). Northern peatlands are important in the global carbon (C) cycle as a long-term sink of atmospheric carbon dioxide (CO<sub>2</sub>). They contain approximately 200-400 Petagrams (Pg = 10<sup>15</sup>) C representing between 10-20% of total soil C (Frohling *et al.* 2009). While peatlands are considerable long-term CO<sub>2</sub> sinks, in any given year they may act as a C source and typically contribute large amounts of methane (CH<sub>4</sub>) emissions to the atmosphere depending on various environmental factors (Basiliko *et al.* 2007).

Land-use changes in peatlands, including drainage for forestry, agriculture, peat mining or energy exploration and extraction, have increased over the last 50 years. This has important global implications on the functioning of peatlands as long-term CO<sub>2</sub> sinks (Waddington & Price, 2000). Drained peatlands often release CO<sub>2</sub> and a more potent greenhouse gas (GHG) nitrous oxide (N<sub>2</sub>O) due to enhanced aerobic decomposition and denitrification (Turetsky & St. Louis, 2006). The unique biochemistry at any given peatland is largely determined by the long-term feedback mechanisms present between hydrological and ecological dynamics; however, controls on the microbial communities responsible for the net carbon balance and their effects on the biochemistry are poorly understood (Basiliko *et al.* 2007).

Previous research has shown that carbon cycling in peatlands is related to environmental factors such as temperature, local hydrology, vegetation, peat physiochemical properties and the nature of the microbial community (Laine *et al.* 2007; Moore *et al.* 2007; Pelletier *et al.* 2007; Mäkiranta *et al.* 2009; Kotiaho *et al.* 2010; Pihlatie *et al.* 2010). Since peat chemistry and



microbiology affect and are affected by the physical conditions and the vegetation community at a site, these factors should be sensitive to any disturbance. As such, peat chemistry and microbiology may be appropriate indicators for assessing peatland carbon cycling.

As 25% of the world's peatlands are located within Canada, the dominance of this feature on the Canadian landscape is evident (Vitt, 2006). While land-use decisions are changing the coverage of peatlands in Canada, increasing temperatures in recent decades have been stronger at northern latitudes and are affecting water table levels within peatlands (Frolking *et al.* 2009). These changes have important implications on the ability of peatlands to continue to sequester C long-term. Identifying variables that control the nonlinear response of peatlands to environmental forcing is key to improving our understanding of feedbacks on the climate system (Belyea, 2009).

## **1.2 Research Objectives**

The objectives of this research were to collect baseline carbon cycling data in a natural (undisturbed) boreal peatland and then compare these data to a boreal peatland subjected to water table drawdown. This will determine how carbon cycling is affected in a long-term drought scenario. Although some research has investigated drainage effects on peatland carbon cycling, nutrient dynamics and microbiological community, (Waddington & Price, 2000; Juottonen, 2008; Knorr & Blodau, 2009; Macrae *et al.* 2012; Lin *et al.* 2012), the comparisons between these parameters along both a microtopographic and depth gradient in a northern Alberta peatland are yet to be described. It is worth pointing out that this study is not a randomized block design and this may be a limitation in interpreting the results. The drainage of the peatland was not carried out as a part of the experiment; therefore, study sites were limited to what was

available. However, peatland feature is the fixed effect (hummock and hollow microforms) with each feature chosen randomly within a site and is statistically relevant, while the drainage effect is exploratory. While there is appropriate within-site replication, the ideal experimental design would be to collect data from multiple control (undisturbed) peat bogs and drained peat bogs. In practice this was not feasible given the expense and environmental impact of draining multiple sites. Regardless, this research will address the need to investigate the response of carbon cycling in peatlands under disturbance with a local ecosystem approach to determine whether drought affects peat substrate quality and microbial diversity and how these responses vary along a microtopographic and depth gradient. Moreover, this research will provide microbiological, chemical and physical baseline data valuable for monitoring the effects of land management on peatland function.

### **1.3 Subsequent Sections of the Thesis**

The investigation of this knowledge gap will occur in two major sections:

1) Microbial characteristics:

Cores of peat taken from both control and drained sites along a microtopographic position (hummocks and hollows) were divided into sections depending on water table position (two or three sections above and two below the water table). In addition, CO<sub>2</sub> and CH<sub>4</sub> flux measurements were taken at each microform in each bog. An incubation study was completed to characterize the microbial community by determining the potential rates of CO<sub>2</sub> and CH<sub>4</sub> production as well as CH<sub>4</sub> consumption over time using gas chromatography. Bacterial and archaeal communities were described by targeting the 16S rRNA gene using pyrosequencing; and

2) Comparisons between the microbial community and physicochemical characteristics:

The differences in the quality and quantity of substrate along both the microtopographic position and between drainage treatments were determined through the analysis of humification status by the von Post method and the concentrations of major anions such as acetate, formate, nitrite, nitrate and sulfate through the use of High-Performance Liquid Chromatography (HPLC). Additionally, supplementary field physical data such as peat temperature and pH were collected at both the control and drained sites along the microtopographic position and depth gradient.

#### **1.4 Study Sites**

The peatland study area is located in central Alberta near Wandering River, (46°40'N, 71°10'W) on a property managed by Sun Gro Horticulture (Figure 1.1). One site acted as a control in its natural state whereas the other site had been drained in 2003 for preparation for peat harvesting. The nearest meteorological station (approximately 75 km from the study site in Athabasca, AB) reports an annual average temperature of 2.1°C with the warmest and coolest months occurring in July and January at an average 16.2°C and -14.9°C, respectively (Environment Canada, 2010). The annual average precipitation is 503.7 mm with the most rainfall occurring in July (Environment Canada, 2010).

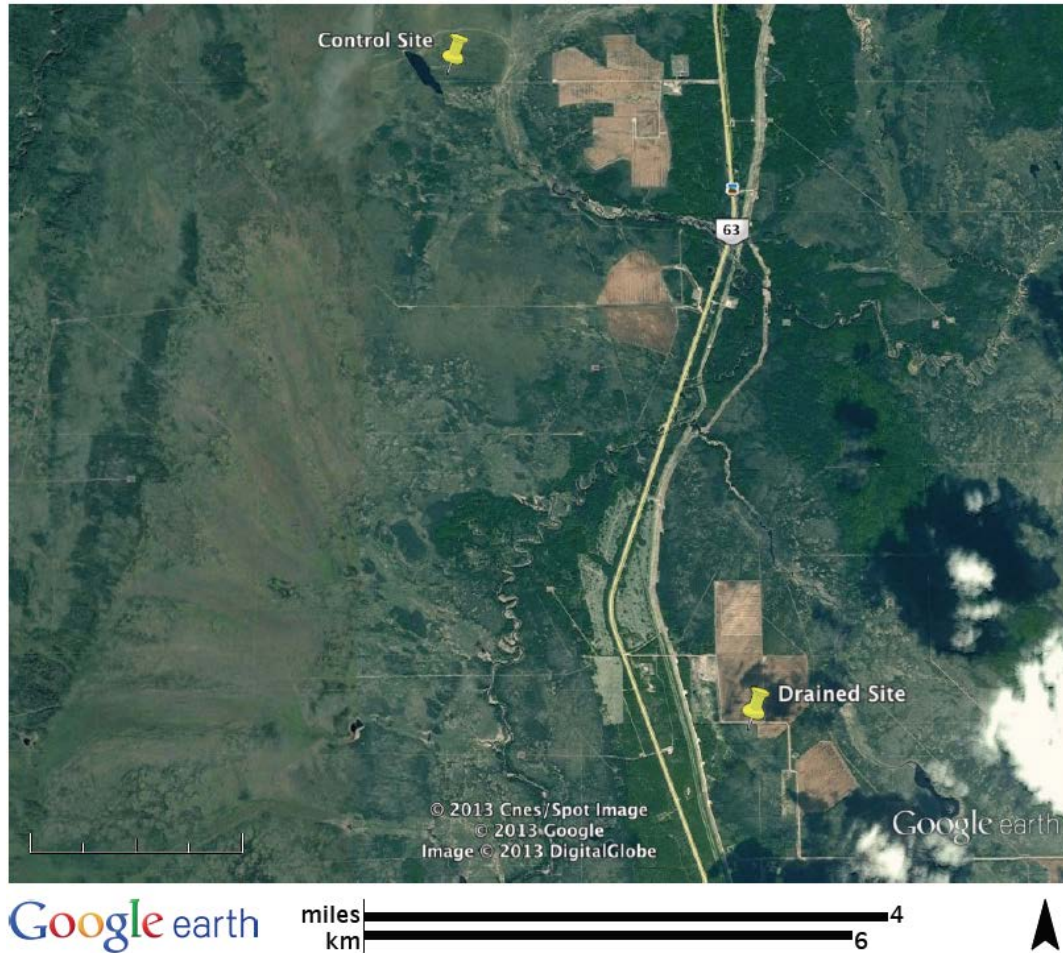


Figure 1.1 Locations of research sites near Wandering River, Alberta. Photo source: Google Earth (public domain), 55°18'38.95"N and 112°28'51.29"W elevation 572 m, 2013.

The control site vegetation is characterized by *Picea mariana*, understory shrubs *Ledum groenlandicum*, *Vaccinium vitis-idaea* and herb *Rubus chamaemorus* and mosses *Sphagnum fuscum* and *Sphagnum angustifolium* (Figure 1.2). The drained site vegetation is characterized by *Picea mariana*, understory shrubs *Ledum groenlandicum* and *Chamaedaphne calyculata* and mosses *Polytrichum strictum*, *Sphagnum capillifolium*, *Sphagnum angustifolium* and *Cladonia stellaris* (lichen) (Figure 1.2). Both sites in this study are classified as ombrotrophic bogs (Chapter 2.1).



Figure 1.2 The control site (above) shows stunted *Picea mariana* (Black Spruce) while the drained site (below) shows mature Black Spruce and a higher density of *Ledum groenlandicum* (Labrador tea). Photo: Jaime Graham.

## **Chapter Two: Literature Review**

### **2.1 Introduction to Canadian Peatlands and Vegetation**

With coverage of approximately 1.136 million km<sup>2</sup> (or 12% of the land area), peatlands across Canada are a dominant physical feature of the landscape (Tarnocai, 2006). Peatlands are any area of waterlogged organic substrate greater than 40 cm thick, resulting from thousands of years in which net primary production (NPP) has exceeded decomposition (Blodau, 2002; Vitt, 2006). The persistence of peatlands depends on a constant, long-term supply of water in which the waterlogged condition of the soil influences the form and the function of peatlands (Vitt, 2006). The waterlogged condition of peatlands leads to a vertical structure where an oxic surface layer known as the acrotelm exists above the water table and an oxygen-depleted layer known as the catotelm exists underneath the acrotelm (Blodau, 2002; Charman, 2002). The thickness of the acrotelm varies depending on microform topography and the position of the water table; however, these differing vertical profiles influence the peat accumulation rates along with the aerobic and anaerobic decomposition rates of organic matter (OM) (Charman, 2002; Vasander & Kettunen, 2006).

Peatlands in Canada are grouped into four basic types: ombrotrophic bogs, minerotrophic fens, intermediate or poor fens and calcareous fens (Blodau, 2002). The distinguishing characteristics are pH, cation concentrations, hydrologic dynamics and dominating vegetation (Blodau, 2002). Fens receive inputs from groundwater and surface runoff (as well as atmospheric precipitation), and therefore tend to be more nutrient-rich and more basic than bogs (Charman, 2002). However, there is a great range in the trophic status of fens making it difficult to precisely differentiate the systems based on geochemical criteria (Charman, 2002). The distinction between a bog and fen was first classified based on vegetation and not by

hydrological or hydrochemical definitions (Wheeler & Proctor, 2000). Distinguishing a bog and fen based on vegetation is problematic as assumptions about the presence of a particular plant species as an indicator of minerotrophy is potentially incorrect (Wheeler & Proctor, 2000). Wheeler and Proctor (2000) noted that multivariate analyses of peatland vegetation corresponded better to a split of bog and poor fen together vs. rich fen. For these reasons, Wheeler and Proctor proposed the differences between a bog and a fen are better defined on the basis of pH and further subdivided by substratum fertility (capacity to support plant growth). Bogs and poor fens are distinguished by low pH (<5) and low calcium concentrations. Rich fens are characterized by high pH (>5.6) and high calcium concentrations (Wheeler & Proctor, 2000; Kulzer *et al.* 2001; Charman, 2002).

In this study, both sites under investigation are classified as ombrotrophic bogs. Since ombrotrophic bogs are influenced primarily by water derived from rain and snow, the water balance and nutrient status depends on the inputs of atmospheric water and evapotranspiration rates from the bog surface (Charman, 2002; Vitt, 2006). The dissolved cations and anions from the water input source along with the evapotranspiration rates exert strong influences on vegetation and function of ombrotrophic bogs (Vitt, 2006). It is important to note that the water chemistry itself cannot be used to predict the type of vegetation at a particular site as two sites with similar water chemistry can support different plant communities. However, the long-term ombrotrophic status of a bog can be visually affirmed by identifying the vegetation that exists at a site (Vitt & Chee, 1990). For instance, normally reliable floristic indicators of ombrotrophy in Canada include *Picea mariana* (Black Spruce), *Rubus chamaemorus* (cloudberry), *Ledum groenlandicum* (Labrador tea) and *Sphagnum fuscum* whereas minerotrophy may be

characterized by vegetation such as *Betula glandulosa* (dwarf birch), *Sphagnum riparium* and numerous regional *Carex* species (grassy sedges) (Vitt, 2006).

*Sphagnum*-dominated peatlands are distinguished from other peat-accumulating wetlands based primarily on acidity (Kulzer *et al.* 2001). *Sphagnum* has several key properties that allow it to influence the physicochemical properties of a peatland. *Sphagnum* takes up mineral nutrients (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  etc.) from water but releases hydrogen ions ( $\text{H}^+$ ) in the process, thus contributing to peatland acidity (Kulzer *et al.* 2001). The increased acidity affects the growth and presence of certain vegetation types such as vascular plants and trees, but also affects the structure of the microbial communities and decomposition rates (Kulzer *et al.* 2001).

While nutrient status affects vegetation between peatland types, vegetation will also differ within peatlands throughout the two broad microtopographic variations (hummocks and hollows). Hummocks and hollows represent elevated and depressed areas, respectively, on the scale of a square meter (Blodau, 2002). The position of the water table relative to the surface of the peat will limit the type of vegetation that can grow. In a poor fen, for example, hummocks are raised above the water table, forming dry microhabitats that are expected to support dense stands of dwarfed shrubs as well as mosses such as *Sphagnum* that continuously cover the microform (St Arnaud, 2007). In the wet, hollow areas of a poor fen, vegetation is expected to be sparse and consist of sedges such as *Carex oligosperma* (St Arnaud, 2007).

Certain types of flora such as sedges are capable of enhancing the emission of  $\text{CH}_4$  produced at depth through root-leaf transport structures as discussed further in Section 2.2 (Kotsyurbenko, 2010). Vascular plants can also enhance  $\text{CH}_4$  production at depth by providing fresh substrate through root exudation and decay, and  $\text{CH}_4$  oxidation by transporting oxygen to the rhizosphere (Bellisario *et al.* 1999; Popp *et al.* 2000).



## 2.2 Peatland Carbon Balance

Peat organic C content varies throughout the peat profile as it is affected by varying inputs from woody, vascular plant and bryophytes (non-vascular plants) and by varying amounts of cellulose and lignin-like compounds from decomposition (Chambers *et al.* 2011). Original litter inputs influence peat bulk density and *Sphagnum*-derived peatlands in western Canada often have lower bulk density than non-*Sphagnum*, sedge and forest peats (Chambers *et al.* 2011). Bulk density values for organic soils range from 0.05 g cm<sup>-3</sup> in very fibric, undecomposed materials to less than 0.5 g cm<sup>-3</sup> in well decomposed materials (Andriessse, 1988). Dark-coloured highly humified peat indicates either fast decomposition or a longer residence time in the acrotelm under dry or warm temperatures (de Jong *et al.* 2010). Conversely, light-coloured unhumified peat indicates a shorter transfer time to the catotelm or a slow rate of decomposition under wet or cool conditions (de Jong *et al.* 2010). Therefore, even though peat is a large reservoir of carbon, the carbon in the peat matrix can be very resistant to decomposition (especially in the anoxic conditions that prevail at depth), as the more labile compounds are preferentially used by microbes and only the more recalcitrant compounds are left behind (Kettunen, 2002).

While peatlands serve many valuable ecological functions, their role as long-term stores of soil carbon is globally important. Peatlands represent an important terrestrial carbon sink with an estimate of 462 Pg C currently stored worldwide (Keller & Bridgham, 2007). However, estimates of peatland coverage, bulk density, mean depth of peat layers and C content vary, so estimates of global peatland carbon pools have a large variation (Vasander & Kettunen, 2006). Canadian peatlands are estimated to contain approximately 147 Pg soil C which represents approximately 56% of the organic C stored in all Canadian soils (Tarnocai, 2006). Despite the

variations in methods assessing peatland carbon pools, peatlands show positive long-term sequestration of large amounts of atmospheric C; however, they simultaneously are the most important global CH<sub>4</sub> source (Vasander & Kettunen, 2006). Because of this, peatlands are globally important to the climate system, considering CH<sub>4</sub> has a global warming potential factor 25 times that of CO<sub>2</sub> over a 100-year time frame (Vasander & Kettunen, 2006; IPCC, 2007).

The storage of carbon is represented by the difference between primary production and decomposition (Clymo 1984). The peatland carbon balance can be represented with the equation:

$$\Delta C = -(NEE + F_{CH_4} + F_{DOC} + F_{DIC} + F_{POC}).$$

Where:  $\Delta C$  is the change in carbon content; NEE is the net flux of carbon as CO<sub>2</sub> from ecosystem to atmosphere;  $F_{CH_4}$  is the net CH<sub>4</sub> flux;  $F_{DOC}$  is the net waterborne exchange of dissolved organic carbon (DOC);  $F_{DIC}$  is the net waterborne exchange of dissolved inorganic carbon (DIC); and  $F_{POC}$  is the net waterborne exchange of particulate organic carbon (POC) (Strack *et al.* 2008). For fluxes, negative values represent a net gain wherein the flux is from the atmosphere to the peatland and positive flux values indicate a net loss wherein the flux is from the peatland to the atmosphere. In addition, Bäckstrand *et al.* (2008) found that non-methane volatile organic carbon emissions (such as plant produced isoprene or terpene) can represent ~5% of the total net carbon exchange at a peatland during a growing season.

Atmospheric CO<sub>2</sub> sequestered into vegetation is the main carbon input into the peatland and while some CO<sub>2</sub> is lost through plant and microbial respiration, the waterlogged condition of the peatland leads to a transformation of some of this carbon to CH<sub>4</sub> in the catotelm (Charman, 2002; Vasander & Kettunen, 2006). The anaerobic decomposition of organic matter resulting in methanogenesis is described in detail in Section 2.4.3. Methane is subject to oxidation via methanotrophy in the acrotelm as this layer is more oxic, producing CO<sub>2</sub> as an end product

(Vasander & Kettunen, 2006). Figure 2.1 shows a flow diagram of the processes and transport mechanisms affecting carbon exchange in a peatland.

Methods for CH<sub>4</sub> transport include diffusion through the peat matrix, ebullition and passage through vascular plants (Vasander & Kettunen, 2006). CH<sub>4</sub> transport by diffusion through the peat matrix is slow and as it passes through the acrotelm, CH<sub>4</sub> is subject to aerobic oxidation (Strack *et al.* 2004). However, transport of CH<sub>4</sub> through peatland vascular plants is an important mechanism of releasing CH<sub>4</sub> to the atmosphere as it bypasses methanotrophic bacteria in the oxic layer (Waddington *et al.* 1996). Additionally, Rosenberry *et al.* (2006) suggested that the sudden release of free-phase gas bubbles into the atmosphere (ebullition) alter hydraulic gradients and movement of water and nutrients within the peatland. Moreover, the presence of CH<sub>4</sub> bubbles affect the local CH<sub>4</sub> diffusive flux as the rate of CH<sub>4</sub> diffusion is related to the CH<sub>4</sub> concentration gradient (influenced by the presence of CH<sub>4</sub> bubbles), (Strack *et al.* 2005).

DOC is produced through the release of exudates from growing plants and the incomplete decomposition of dead plant material (Fenner *et al.* 2007). DOC is leached from peatlands as a result of runoff and can also be leached through the peat profile to the groundwater level (Vasander & Kettunen, 2006). A large portion of exported DOC can be broken down into CO<sub>2</sub> and released into the atmosphere downstream of peatlands (Strack *et al.* 2008).

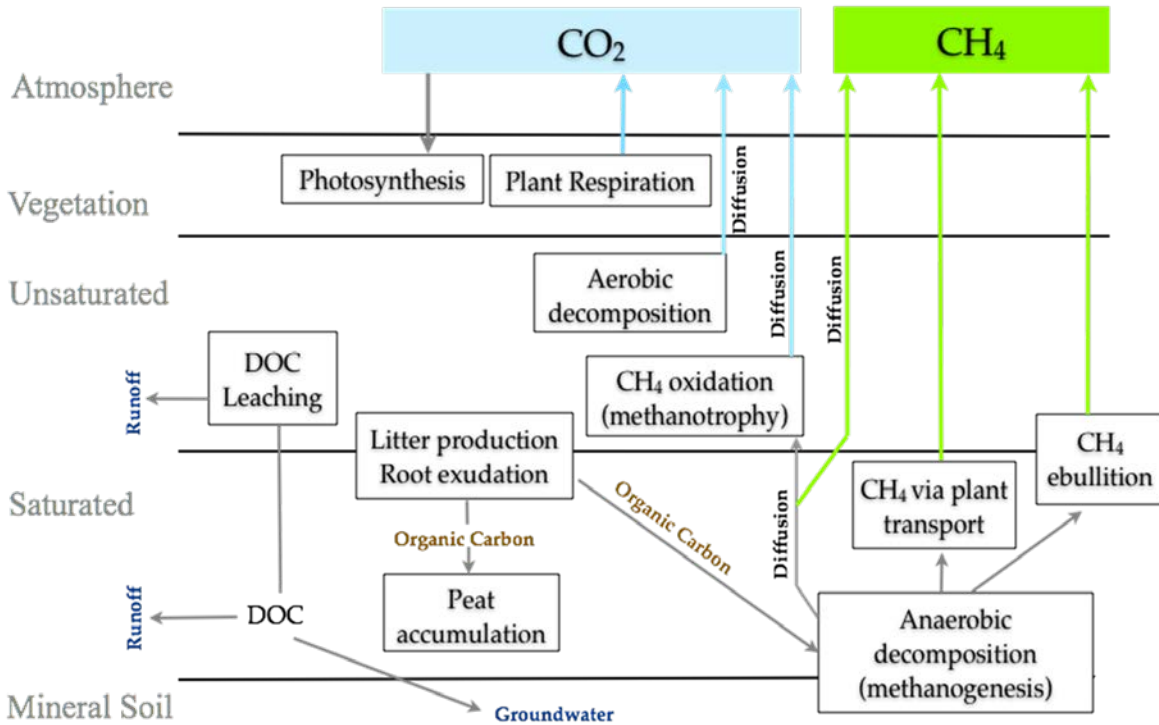


Figure 2.1 Flow diagram of the carbon cycle in peatlands adapted from Faubert (2004).

### 2.3 Water Table Drawdown and Carbon Cycling Response

The average annual air temperature in Canada is expected to increase between 3-5°C by 2020 and between 5-10°C by 2050 (Tarnocai, 2009). As a result of increasing temperatures, ground temperatures will rise, which will increase both evapotranspiration (ET) rates and the drying of non-permafrost peatlands (resulting in water table drawdown) especially in the southern Boreal region (Tarnocai, 2009). While there are several controls on the rates of CO<sub>2</sub> production including temperature and the quality of substrate for decomposition, predicted climate change impacts in the southern Boreal region associated with drought and higher ET rates are expected to result in increased production of CO<sub>2</sub> due to enhanced aerobic decomposition in the acrotelm (Moore & Dalva, 1997; Tarnocai, 2009).

The effects of water table drawdown on CH<sub>4</sub> emissions are more complex as there may be enhanced CH<sub>4</sub> production through root exudates and litter associated with denser vascular plant colonization, but there may also be enhanced transport of CH<sub>4</sub> directly to the atmosphere (Strack *et al.* 2008). However, while CH<sub>4</sub> production may be enhanced, CH<sub>4</sub> oxidation may also be enhanced due to the transport of O<sub>2</sub> to the rooting zone (Sundh *et al.* 1995; Strack *et al.* 2008). Climate change impacts associated with higher air and soil temperatures will affect peatland CH<sub>4</sub> fluxes differently depending on the geographical location of the peatland and local conditions including hydrology, microbial community, substrate quality and substrate availability (Sundh *et al.* 1995; Moore & Dalva, 1997; Strack *et al.* 2008). However, as both CH<sub>4</sub> production and consumption are positively correlated to temperature, but production more so, (Dunfield *et al.* 1993), Strack *et al.* (2008) suggested the increase in atmospheric temperatures (and thus ground temperatures) alone should enhance overall peatland CH<sub>4</sub> emissions.

There are many causes of the slow decay rate of peat, including low concentrations of O<sub>2</sub>, recalcitrant carbon, permafrost, low nutrient supply, low temperature and acidity (Freeman *et al.* 2001; Thomas & Pearce, 2004; Frohling *et al.* 2009). How water table drawdown affects peatland carbon exchange varies depending on the time-scale, vegetation composition and fresh substrate inputs, temperature and soil pH (Peltoniemi *et al.* 2012). However, understanding the interactions that control the nonlinear response of peatlands to environmental forcing is key to improving our understanding of feedbacks on the climate system (Strack *et al.* 2008; Belyea, 2009).

## 2.4 Peatland Microbiology and Function

Microbial diversity in soils is critical for soil functioning and health as microbes are responsible for a wide range of nutrient cycling and geochemical processes (Smalla *et al.* 2007). For instance, the turnover of bacterial populations contributes a net release of nitrogen to soils (DuPont *et al.* 2009) and temporal variability in resource supply can generate pulses of ecosystem activity through major biogeochemical fluxes of energy and nutrients (Lennon & Cottingham, 2008). For these reasons, the study of the interactions between bacteria and their environment can reveal how they would respond to various natural or man-made disturbances (Zhang & Xu, 2008). This is especially relevant in peatlands as increased temperatures from climate change impacts may alter the long-term function of a peatland as a carbon sink by affecting microbial decomposition rates.

### 2.4.1 Bacterial Communities

The acidic environment of *Sphagnum*-dominated bogs coupled with low temperatures, water-saturated conditions and low concentrations of nutrients, provides an extreme habitat for microorganisms (Opelt *et al.* 2007a). Although peatlands are globally important for their role in carbon cycling, bacterial, archaeal and fungal communities are still poorly described and understood (Peltoniemi *et al.* 2009).

The principal aerobic decomposers in peatlands are considered to be fungi, which act upon the surface litter layer, although the role of fungi declines with depth as the moist and anoxic conditions inhibit their growth (Pankratov *et al.* 2011). Consortia of different microorganisms carry out the degradation of plant litter, but their species compositions and roles

are poorly understood (Pankratov *et al.* 2011). The major components of peat carbon are plant polysaccharides such as cellulose and hemicellulose (Tveit *et al.* 2012). These polysaccharides are broken down into simple sugars by hydrolytic extracellular enzymes produced by microorganisms (Tveit *et al.* 2012). The inhibiting effect of phenolic substances, which accumulate in anoxic peat, has been suggested as a major factor in the low rates of organic matter (OM) decomposition in peat soils (Tveit *et al.* 2012). *Actinobacteria* are suggested to be important decomposers as these bacteria can degrade cellulose and some can even metabolize lignin and other complex polymers (Pankratov *et al.* 2006; Peltoniemi *et al.* 2009). Additionally, species of the *Planctomycetes* phylum densely colonize acidic ecosystems including northern peatlands and are thought to function as slow-acting decomposers of plant-derived organic matter (Ivanova & Dedysh, 2012).

*Acidobacteria* have been detected in a wide variety of environments including peatlands, but their broad phylogenetic and functional diversity (and lack of cultures and functional data) make it difficult to determine their functions (Pankratov *et al.* 2008). The *Acidobacteria* phylum is highly diverse comprising 26 distinct phylogenetic subdivisions in which cultured representatives only exist for 5 of the subdivisions (Ward *et al.* 2009). Members of the *Acidobacteria* phylum have been very difficult to isolate and culture as they do not grow on standard media, requiring several days to weeks to form visible colonies on complex, low-nutrient media (Ward *et al.* 2009). These culturing difficulties affect the ability to determine metabolic and physiological traits of *Acidobacteria* (Ward *et al.* 2009). However, Dedysh *et al.* (2006) managed to isolate an acidobacterium that was detected as a predominant member of the community (1 of 24 16S rRNA *Acidobacteria* clones detected). This isolate was taxonomically described as a chemo-organotroph (*Geothrix fermentans*) that utilized various organic acids as

electron donors and humic acids as an alternative electron acceptor. Other recently cultured representatives from the *Acidobacteria* phylum as reviewed by Dedysch (2011), include those of the genera *Granulicella*, *Telmatobacter*, *Bryocella* and *Bryobacter*, all of which are acidophilic chemo-organotrophs that grow at pH values between 3.0 and 6.5-7.5. The ability to degrade various plant-derived polymers varies between different *Acidobacteria* species but *Telmatobacter bradus* is capable of hydrolyzing cellulose (Dedysch, 2011). Dedysch (2011) noted that most peat-inhabiting acidobacteria utilize certain acids produced from the decomposition of *Sphagnum* moss and members of this phylum seem to play an important role in degrading plant-derived polymers in peatlands.

*Proteobacteria* of the genus *Burkholderia* are a typical component of the *Sphagnum* peat bog microbial community due to the high adaptability of *Burkholderia* to northern peat bog conditions (Belova *et al.* 2006; Opelt *et al.* 2007a). *Burkholderia* spp. have been shown to be moderately acidophilic, psychrotolerant, and nitrogen-fixing. Some species can solubilize phosphate (Opelt *et al.* 2007a). *Burkholderia* spp. utilize various organic acids, sugars, alcohols and some aromatic compounds (Pankratov *et al.* 2011). This is of particular importance as various phenolic compounds and derivatives of benzoic acid are major organic compounds produced by mosses and accumulated in *Sphagnum* peat bogs (Belova *et al.* 2006). Additionally, it is thought that *Burkholderia* spp. may survive in methanotrophic communities by metabolizing polysaccharides from methanotrophic capsule material or from the organic matter of dead cells (Belova *et al.* 2006). Belova *et al.* (2006) found that by determination of the partial sequences of 16S rRNA genes in *Sphagnum* bog isolates, members of the genus *Burkholderia* constituted approximately 30% of the bacterial community.



Methanotrophic bacteria as well as the methanogenic archaea in peatland ecosystems can be easily linked to CH<sub>4</sub> cycling as these groups have an obligate metabolism and can be identified on the basis of phylogeny. A detailed discussion on methanotrophs and methanogens is presented in Sections 2.4.2 and 2.4.3.

#### 2.4.2 Methanotrophs

The CH<sub>4</sub> produced anaerobically in peatlands is released to the atmosphere via various pathways as shown in Figure 2.1, unless consumed by aerobic CH<sub>4</sub>-oxidizing bacteria (methanotrophs). Aerobic methanotrophs belong to four different phylogenetic groups including two classes of the *Proteobacteria* phylum: *Alphaproteobacteria* and *Gammaproteobacteria*, which are found in a wide variety of ecosystems (Kip *et al.* 2010). Recently, three newly discovered thermoacidophilic methanotrophs that represent a distinct lineage in the phylum *Verrucomicrobia* are proposed to be included in a new genus termed *Methylacidiphilum* (Dunfield *et al.* 2007; Op den Camp *et al.* 2009). In addition, a newly discovered anaerobic methanotrophic bacterium *Candidatus Methylomirabilis oxyfera* (belonging to the uncultured NC10 phylum) was recently shown to convert two nitric oxide (NO) molecules into N<sub>2</sub> and O<sub>2</sub>. The O<sub>2</sub> was then used to oxidize CH<sub>4</sub> (Ettwig *et al.* 2010). The known taxa in each group of methanotrophs are depicted in Table 2.1 (adapted from Siljanen, 2012).

Table 2.1 Taxonomic classification of methanotrophs.

Phylum	Class	Family	Genus
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Methylocystaceae</i>	<i>Methylosinus</i> <i>Methylocystis</i>
		<i>Beijerinckaceae</i>	<i>Methylocapsa</i> <i>Methyloferula</i> <i>Methylocella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcaceae</i>	<i>Methylobacter</i>
			<i>Methylococcus</i>
			<i>Methylocaldum</i>
			<i>Methylohalobius</i>
			<i>Methylomicrobium</i>
			<i>Methylomonas</i>
			<i>Methylosoma</i>
			<i>Methylosarcina</i>
			<i>Methylosphaera</i>
			<i>Methylothermus</i>
			<i>Methylovulvum</i>
			<i>Crenothrix</i>
			<i>Clonothrix</i>
<i>Verrucomicrobia</i>		<i>Cand. Methylacidiphilum</i>	
NC10		<i>Cand. Methylomirabilis</i>	

The oxidation of CH<sub>4</sub> by aerobic methanotrophs is initiated by methane monooxygenase enzymes that split the O-O bonds of oxygen to reduce one to H<sub>2</sub>O and incorporate the other into CH<sub>4</sub> as CH<sub>3</sub>OH (methanol) (Hanson & Hanson, 1996). There are two types of methane monooxygenases: particulate (pMMO) bound in an intracytoplasmic membrane (ICM) and soluble (sMMO) that is not membrane-bound (Vorob'ev *et al.* 2011). Almost all known methanotrophs possess pMMO with the exception of the genera *Methylocella* and *Methyloferula* which possess only sMMO (Dedysh *et al.* 2000; Vorob'ev *et al.* 2011). The oxidation of CH<sub>4</sub> by aerobic methanotrophs results in a final product of CO<sub>2</sub> (Hanson & Hanson, 1996).

Most aerobic methanotrophic bacteria are capable of growth on only C<sub>1</sub> compounds such as CH<sub>4</sub>, CH<sub>3</sub>OH, methylamine and formate (Dedysh & Dunfield, 2010). However, members of

the genus *Methylocella* are the first fully authenticated facultative methanotrophs shown to utilize CH<sub>4</sub> or some multi-carbon compounds (e.g., acetate and ethanol) as their sole carbon and energy source (Dedysh & Dunfield, 2010). Interestingly, in a study of *Methylocella* by Dedysh *et al.* (2005a), when both acetate and CH<sub>4</sub> substrates were provided in excess, acetate was preferentially used and as a result, CH<sub>4</sub> oxidation was shut down. Therefore, with changing peatland vegetation and thus substrate quality at depth, it is possible that a system dominated with *Methylocella*-like methanotrophs may show an altered CH<sub>4</sub> cycle (e.g., higher CH<sub>4</sub> concentrations) if acetate availability drastically increases (Dedysh & Dunfield, 2010).

Methanotrophs in peat have been investigated using 16S rRNA gene analysis and by functional marker genes such as *mmoX* and *pmoA* (McDonald *et al.* 2008). The *mmoX* and *pmoA* genes encode for subunits of the sMMO and pMMO enzymes, respectively. Since *Methylocella* spp. do not possess sMMO, *mmoX* must be used to detect this methanogen (it will not be detected in *pmoA* based studies) (Kip *et al.* 2010).

One of the most numerically dominant and metabolically active populations in acidic peatlands is *Methylocystis* (Dedysh, 2009). Two representatives of the methanotrophs belonging to the genus *Methylocystis* have been isolated from acidic *Sphagnum* peat bogs (Dedysh, 2009). Interestingly, one strain of *Methylocystis bryophila* is able to grow slowly on acetate in the absence of CH<sub>4</sub> (Dedysh, 2009). Members of the genus *Methylocella* are widely distributed in acidic and neutral peatlands (Dedysh, 2009). Closely related to *Methylocella* spp., members of the genus *Methylocapsa acidiphila* have been detected in acidic peatlands and have been shown to be an obligate methanotroph (Dedysh, 2009). However, *Methylocapsa aurea* is a facultative methanotroph and grows on acetate as well as CH<sub>4</sub> and CH<sub>3</sub>OH (Dunfield *et al.* 2010). Bacteria related to *Methylocapsa* have been linked to the atmospheric consumption of CH<sub>4</sub> (Dedysh,

2009). Members of the *Methylococcaceae* have also been detected in peatlands but do not represent a numerically significant methanotroph population (Dedysh, 2009). Furthermore, it has recently been shown that a nitrite-reducing anaerobic methanotroph from the NC10 phylum has been enriched from a minerotrophic peatland infiltrated by nitrate-rich groundwater (Zhu *et al.* 2012).

### 2.4.3 Methanogens

Methanogens are anaerobic prokaryotes belonging to the domain *Archaea* and are unique in the sense that they obtain energy from selected low molecular weight carbon compounds and hydrogen while producing CH<sub>4</sub> (Juottonen, 2008). Methanogens can be divided into three main nutritional categories: (i) hydrogenotrophs that oxidize H<sub>2</sub> and reduce CO<sub>2</sub> to form CH<sub>4</sub>; (ii) acetoclastic methanogens that utilize the methyl group of acetate to produce CH<sub>4</sub>; and, (iii) methylotrophs that utilize methyl compounds such as CH<sub>3</sub>OH, methylamines or dimethylsulfides to produce CH<sub>4</sub> (Garcia *et al.* 2000; Galand, 2004).

Acetate and H<sub>2</sub>/CO<sub>2</sub> are quantitatively important CH<sub>4</sub> precursors in anoxic peat (Kotsyurbenko, 2010). Most studies suggest that CH<sub>4</sub> formation by acetoclastic methanogens is the most important pathway in nutrient-rich fens whereas CO<sub>2</sub> reduction coupled with H<sub>2</sub> oxidation is the most important methanogenic pathway in *Sphagnum*-dominated bogs (Duddleston *et al.* 2002; Kotsyurbenko, 2010).

There are five known orders of methanogens: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales* and *Methanopyrales* (Garcia *et al.* 2000). Of these orders, members of *Methanosarcinales* are known to contain acetoclastic methanogens although certain species may be exceptions (Garcia *et al.* 2000). The other four orders primarily contain

hydrogenotrophic methanogens, of which some can utilize formate as an alternative to H<sub>2</sub> and some may have additional potential substrates such as methanol (Garcia *et al.* 2000; Galand 2004).

Peat carbon provides a limited substrate for methanogenesis due to the resistant nature of organic matter (OM). Older OM is harder to decompose than newer OM simply because microbial decomposition of OM begins with the fractions that are easier to decompose (Hogg, 1993). However, vascular plants supply fresh substrate through root exudation and litter decay to the anoxic layers of the peatland, enhancing CH<sub>4</sub> production because of the quality and relative ease of decomposition of newer substrate over the older material (Waddington *et al.* 1996).

The first step of anaerobic OM decomposition is hydrolysis (Figure 2.2) in which fermenting bacteria cleave polysaccharide polymers to monomers (Galand, 2004). Following this, fermentation of the monomers results in the release of simple compounds such as fatty acids, H<sub>2</sub>, CO<sub>2</sub> and alcohols (Galand, 2004). Alternatively, monomers can be fermented to acetate by homoacetogenic bacteria. Syntrophic bacteria (e.g., sulfate-reducing bacteria) degrade primary fermentation products into acetate, H<sub>2</sub> and CO<sub>2</sub> (Galand, 2004). Methanogenesis is the final step of anaerobic OM degradation through the three main pathways discussed at the beginning of this section.

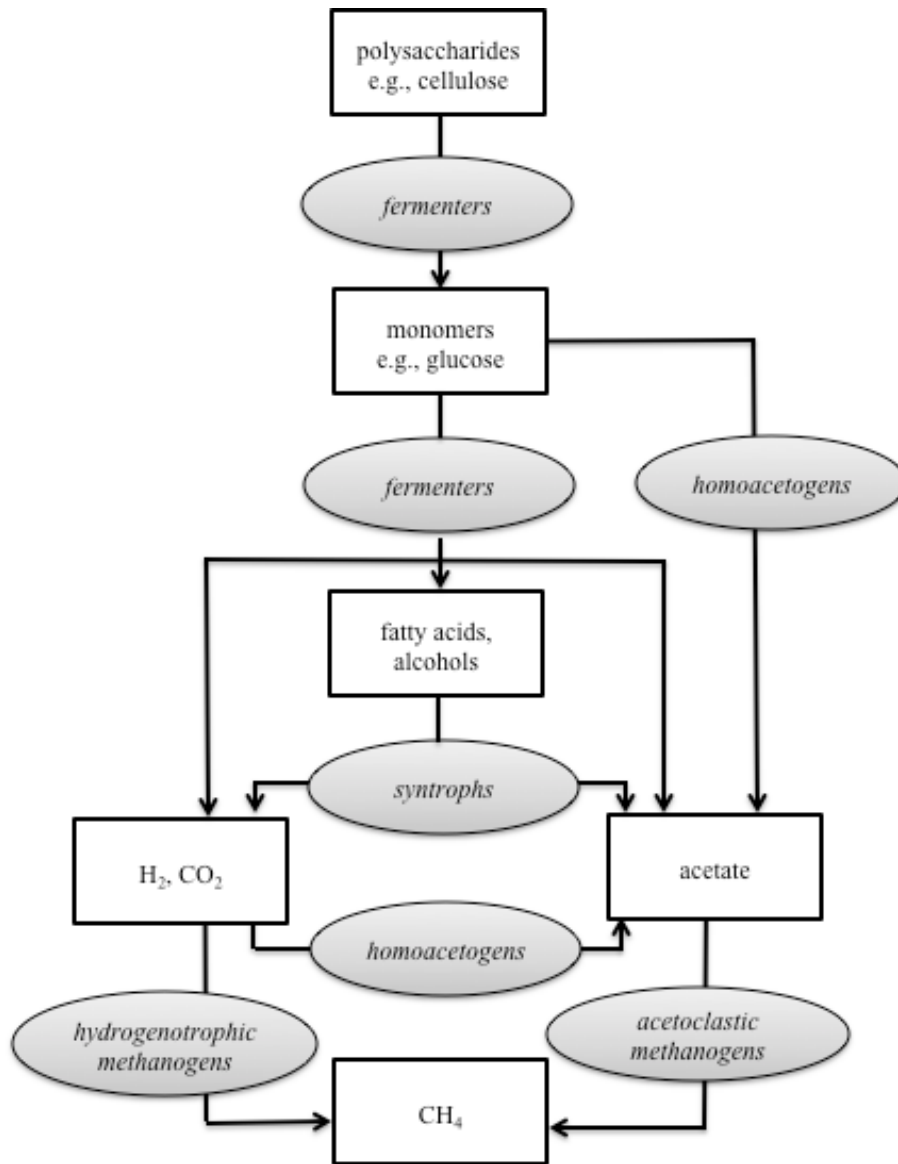


Figure 2.2 Flow diagram of anaerobic OM decomposition and methanogenesis modified from Conrad (1999) as cited in Galand (2004) and Juottonen (2008).

Methanogenesis is a strictly anaerobic process; however, methanogens have adapted to changing moisture conditions in northern peatlands, as they remain attached to peat in pores (Kotiaho *et al.* 2010). In this way, methanogens can survive under more aerated conditions in waterlogged microenvironments in smaller pores above the water table (Kotiaho *et al.* 2010). *In vitro* incubation studies have been used to assess the vertical distribution of methanogenic activity through measuring anaerobic potential CH<sub>4</sub> production (Kotiaho *et al.* 2010). Several studies have resulted in the observation of the highest potential CH<sub>4</sub> production rates within 20 cm below the water table (Sundh *et al.* 1995; Cadillo-Quiroz *et al.* 2006; Juottonen, 2008; Kotiaho *et al.* 2010). Although measurements of CH<sub>4</sub> production can be quantified both in the field and in the laboratory, relatively little is known about the mechanisms of CH<sub>4</sub> production in peatlands and the methanogenic community composition (Kotsyurbenko, 2010).

Methanogens in peat have been investigated with the 16S rRNA gene and the alpha subunit methyl coenzyme M reductase (*mcrA*) functional gene as molecular markers. The *mcrA* enzyme catalyzes the terminal step in methanogenesis (Hallam *et al.* 2003). *Methanomicrobiales*, *Methanobacteriales*, Rice Cluster I (RCI), Rice Cluster II (RCII) and *Methanosarcinales* (members including the *Methanosarcinaceae* and *Methanosaetaceae* families) have frequently been detected in peat (Table 2.2). In addition, an acidophilic methanogen of the *Methanomicrobiales* order (*Methanoregula boonei*) has recently been isolated from an acidic (pH 4.0-4.5) ombrotrophic bog near New York, USA (Bräuer *et al.* 2011). Also, a hydrogenotrophic methanogen of the *Methanomicrobiales* order (*Methanosphaerula palustris*) has been isolated from a minerotrophic fen (Cadillo-Quiroz *et al.* 2009).

Table 2.2 Summary of a sample of 16S rRNA gene molecular marker studies of methanogens in peatlands.

Site and Region	Site pH	Molecular markers / methods	Phylogenetic affiliation	References
Bakchar Bog, West Siberia	4.2 – 4.8	16S rRNA / T-RFLP	Rice Custer II <i>Methanosarcinaceae</i> ; <i>Methanomicrobiaceae</i> ; <i>Methanobacteriaceae</i>	Kotsyurbenko <i>et al.</i> (2004)
Sylvie’s bog north-central AB; continental bog Patuanak, Sask	3.6 – 4.5	16S rRNA / Clone RFLP	Rice Custer II <i>Methanomicrobiales</i> <i>Methanosaetaceae</i> <i>Methanosarcinaceae</i> <i>Methanobacteriaceae</i>	Yavitt <i>et al.</i> (2006)
McLean bog and Chicago bog, New York State	3.5 – 4.5	16S rRNA / T-RFLP	Rice Custer I Rice Custer II <i>Methanomicrobiales</i> <i>Methanosaetaceae</i> <i>Methanosarcinaceae</i>	Cadillo-Quiroz <i>et al.</i> (2006)
15 high-latitude peatlands in Alaska; 3 mid-latitude peatlands in Massachusetts	3.9 – 4.65; 5.61 – 7.01	16S rRNA / DGGE	<i>Methanomicrobiales</i> <i>Methanobacteriaceae</i> <i>Methanosaetaceae</i>	Rooney-Varga <i>et al.</i> (2007)
Six peatlands arrayed 775 km from eastern Ontario, Canada to West Virginia, USA	3.8 – 4.8; 6.8	16S rRNA / Clone RFLP	Rice Custer I Rice Custer II Rice Custer III <i>Methanomicrobiales</i> <i>Methanosaetaceae</i> <i>Methanosarcinaceae</i>	Yavitt <i>et al.</i> (2011)
Rich, intermediate and acidic poor fen, Ontario, Canada	Rich fen 6.31; int. fen 6.15; poor fen 4.77	16S rRNA / T-RFLP	Rice Custer I Rice Custer II <i>Methanosarcinaceae</i> <i>Methanomicrobiales</i> <i>Methanosaetaceae</i>	Godin <i>et al.</i> (2012)



## 2.5 Microbial Identification and Characterization

It is estimated that 1% or fewer of global microorganism species are currently cultured due to the difficulties in reproducing the complex environmental parameters that support some species in the laboratory (Hurst *et al.* 2007). For this reason, microbial community structure and species diversity are best studied with cultivation-independent approaches (Shah *et al.* 2011). 16S rRNA gene sequences have been most commonly used as genetic markers to study bacterial and archaeal phylogeny (Janda & Abbott, 2007). This is primarily due to the fact that the 16S rRNA genes: (i) are present in almost all bacteria and archaea; and (ii) are highly conserved in all bacteria and archaea suggesting that random sequence changes are an accurate measure of evolution (Janda & Abbott, 2007). Molecular analysis of microbial communities begins with DNA extraction from the environmental sample, polymerase chain reaction (PCR) amplification of marker genes (e.g., 16S rRNA), and differentiation of amplicons via a chosen community fingerprinting or cloning technique.

PCR products differentiated by culture-independent pyrosequencing provide a low-cost, high-throughput method for generating sequences. Pyrosequencing generates hundreds of thousands of sequences in a single run and eliminates the need of producing clone libraries (Hamady *et al.* 2008). Adding a unique tag (barcode) to each primer before PCR amplification controls costs and allows for as many as 60 samples to be run in a single pyrosequencing run rather than splitting a single plate across multiple runs (Hamady *et al.* 2008). The vast datasets resulting from pyrosequencing have revealed much greater species diversity in several different environments than previously anticipated (Shah *et al.* 2011). In pyrosequencing, the incorporation of a nucleotide by DNA polymerase during DNA synthesis results in the release of pyrophosphate, which ultimately results (after a number of other reactions) in the release of

visible light (Mardis, 2008). The amount of light produced is captured by a charge-coupled device camera and corresponds to the number of nucleotides incorporated (Mardis, 2008). While the four nucleotides (TCGA) are added sequentially, there is some difficulty in determining the number of incorporated nucleotides following the incorporation of >6 identical nucleotides (Ronaghi, 2001). The intensity of light produced at these long stretches of homopolymeric regions is difficult to correlate to the actual number of nucleotide positions (Mardis, 2008). This typically results in base insertion or deletion errors in homopolymeric regions (Mardis, 2008).

PCR processes and pyrosequencing can introduce errors and lead to inflated diversity estimates (Kunin & Hugenholtz, 2010). Chimeras are PCR artifacts resulting from a prematurely terminated amplicon when it reanneals to a different template DNA (DNA sequences composed of DNA from two or more parents) (Gonzalez *et al.* 2004). Chimeras need to be removed using chimera detection software otherwise the researcher would have the false impression that this is a newly discovered previously unknown species (Kunin & Hugenholtz, 2010). Also, to avoid interpreting sequence errors as naturally occurring populations, pyrosequencing reads are clustered at a sequence identity threshold (typically 97-98%) (Kunin & Hugenholtz, 2010).

There are several software packages available for the comparison and analysis of pyrosequenced datasets. In this study, we utilize QIIME (pronounced “chime”), which stands for Quantitative Insights into Microbial Ecology. QIIME processes pyrosequencing reads by assigning Operational Taxonomic Units (OTUs) based on sequence similarity within the reads. The OTUs are then queried against one of several online large, curated databases of 16S rRNA sequences such as SILVA to identify taxonomy (Haas *et al.* 2011).

While bacterial and archaeal communities can be phylogenetically identified through targeting the highly conserved 16S rRNA gene, the detection of these microbes gives little

information on ecosystem function (Juottonen, 2008). However, the microbes essential to the  $\text{CH}_4$ -oxidizing and  $\text{CH}_4$ -producing functions are relatively well known and therefore the molecular detection of methanotrophs and methanogens can infer function (Juottonen, 2008).

## **Chapter Three: Microbial Characteristics of the Wandering River Natural and Drained Bogs**

### **3.1 Introduction**

While peatlands serve many valuable ecological functions, their role as long-term stores of soil C is globally important. They represent an important terrestrial carbon sink of 462 Pg stored carbon (Keller & Bridgham, 2007). Increasing temperatures in recent decades have been strongest at northern latitudes and are affecting both permafrost and water table levels within northern peatlands (Frolking *et al.* 2009). These changes have important implications on the ability for peatlands to continue to sequester C long-term, because the drawdown of the water table leads to an increased depth of the acrotelm (Worrall & Burt, 2005; Tarnocai 2006). An increased depth of the acrotelm may lead to an increase of CO<sub>2</sub> production as the decay of dead plant material via microbial respiration is enhanced under aerobic conditions (Laiho, 2006). The deeper acrotelm may also lead to an increase of the oxidation of CH<sub>4</sub> as it diffuses from depth to the atmosphere, creating more CO<sub>2</sub> as a byproduct of methanotrophy and reducing the overall CH<sub>4</sub> efflux (Vasander & Kettunen, 2006).

Bacterial and archaeal communities can be phylogenetically identified through targeting the highly conserved 16S rRNA gene. The detection of these microbes forms a starting point from which to understand microbial diversity, but it tells little of ecosystem function (Juottonen, 2008). However, the microbes essential to CH<sub>4</sub> oxidation and CH<sub>4</sub> production are relatively well known and therefore the molecular detection of methanotrophs and methanogens can be linked to function (Juottonen, 2008). Although some research has investigated drought effects on peatland carbon fluxes, the comparison between the microbial community and carbon cycling

along both a microtopographic position and depth gradient in a northern Alberta peatland is yet to be described.

The objective of the research presented in this chapter was to investigate carbon cycling in undisturbed and disturbed peatlands to determine whether long-term water table drawdown affects the microbial community at different microtopographic positions and depth above and below the water table.

## **3.2 Materials and Methods**

### *3.2.1 Peat Coring, Water Table Measurements and Flux Sampling*

At each of the control and drained sites (Chapter 1.4), three hummock and three hollow microforms were chosen to extract peat cores, insert water wells for water table measurements and to measure carbon fluxes (twelve microform sample sites in total; sampling in July 2010). Peat cores were divided into 15-cm intervals that captured 2-3 sections above the water table and 2 sections below. Samples were transported on ice in coolers from the research sites and frozen approximately 6 hours later upon arrival at the lab at  $-80^{\circ}\text{C}$  for samples used for DNA extracts and  $-20^{\circ}\text{C}$  for samples used for other experiments. Twelve aluminum collars (grooved at the top to be filled with water and fitted with a chamber), 60 cm x 60 cm length by width, were inserted approximately 30 cm into the peat at three hummocks and three hollows at both the control and drained sites. Fluxes of  $\text{CO}_2$  and  $\text{CH}_4$  were each measured three times at each sampling plot over a one-week time frame (with the exception of the drained  $\text{CH}_4$  fluxes which were measured twice) using the closed chamber method (e.g., Tuittila *et al.* 2000). A series of gas samples was drawn from the chamber headspace into 20-mL syringes at 7, 15, 25 and 35 minutes after closing the chamber. Carbon dioxide gas mixing ratio was determined in the field using a portable

infrared gas analyzer and CH<sub>4</sub> samples were collected with 20-mL syringes and stored in evacuated vials (Exetainers) (12-mL) sealed with rubber septa (Labco Ltd., UK). These were later analyzed on a gas chromatograph (GC) in the laboratory. Fluxes were determined from the linear change in the gas-mixing ratio within the chamber over time.

A Varian CP-3800 GC was utilized to analyze CH<sub>4</sub> concentration in gas samples stored in the Exetainers from the field chamber measurement as well as the CH<sub>4</sub> and CO<sub>2</sub> from the oxic and anoxic experiments described in Section 3.2.2. Samples (5-mL) were injected via a syringe from the Exetainers into the GC. For quality control, standards and controls were used before and after each set of 12 samples. The standards and controls consisted of a CO<sub>2</sub> and CH<sub>4</sub> mixture gas in which the area calculated under each chromatograph curve (one-point standard curve) in the GC resulted in known values for both gases. The concentration of the standard gas was 52 ppm CH<sub>4</sub> and 500 ppm CO<sub>2</sub> with balance N<sub>2</sub> supplied by Praxair Canada, (Calgary, Alberta).

### *3.2.2 Incubation Study*

Peat core samples were frozen for approximately three months until the commencement of the incubation study. Peat samples were allotted a 48-hour period in which to defrost before being measured into 125-mL glass jars fitted with I-Chem septa lids. The incubation study was divided into two parts, oxic to determine CH<sub>4</sub> consumption and anoxic to determine CH<sub>4</sub> production. CO<sub>2</sub> production was also measured in both the oxic and anoxic experiments. A random replication of six samples for each the oxic and anoxic incubation experiments were taken to ensure results from within a section of the core were within a repeatable range.

After the thawing period, between 11-13 grams (wet weight) of peat from 54 samples (Table 3.1) were placed in 125-mL glass jars fitted with a septum lid, first for the oxic experiment then new samples were prepared the same way for the anoxic experiment. During the oxic study, samples were incubated at approximately 15°C and 20-mL of the standard mixture (resulting in 8.32 ppm CH<sub>4</sub>) was injected into each of the jars to determine CH<sub>4</sub> oxidation as well as CO<sub>2</sub> production. For the anoxic experiment, distilled water was added to each sample until saturation and the samples were left overnight to acclimate in the incubator. The following day, a glove bag was used to flush the samples with N<sub>2</sub> for 15 minutes to simulate anoxic conditions to determine potential CH<sub>4</sub> and CO<sub>2</sub> production. One end of the glove bag was connected to a valve, which was then connected to the N<sub>2</sub> gas. The other end of the glove bag containing the samples was sealed. The N<sub>2</sub> was turned on to fill the bag and when full, the seal at the end of the glove bag was opened to flush the air out and then re-sealed after the procedure was completed. The fill and flush glove bag method was continued for 15 minutes after which the jars were sealed with their lids inside the sealed glove bag.

Samples in both the oxic and anoxic runs were incubated for 5 days and 20-mL of air from the jar headspace was sampled every 12 hours via a syringe during that time period and stored in 12-mL evacuated Exetainers until analyzed. To maintain constant air volume and pressure, the jars were backfilled with 20-mL N<sub>2</sub> via a syringe and the septum seal puncture was resealed with silicone. Prior to the sampling of the anoxic samples, the sample jars were mechanically agitated for ten minutes to mix the gases within peat pore spaces and the jar headspace.

The concentrations of CH<sub>4</sub> and CO<sub>2</sub> were determined through use of a GC (Section 3.2.1) and calculated fluxes were corrected for volume loss during sampling by backfilling of

N<sub>2</sub>. Production rates were determined from the slope of the gas concentrations versus incubation time (linear increases for CO<sub>2</sub> production and PMP and linear decreases for PMO); slopes with r<sup>2</sup> values less than 0.75 were discarded. For further *in vitro* peat incubation methodology, see Waddington *et al.* (2001).

Table 3.1 List of 54 samples and categorical attributes used in the incubation experiments and the microbial pyrosequencing. The naming convention is as follows: CH=Control hummock, CW=Control hollow, DH=Drained hummock, DW=Drained hollow. Number values refer to the depth of the sample from surface (i.e., 135150 = 135 to 150 cm). A, B and C are different cores.

Control Site Sample Name	Microform	Water Table Position	Drained Site Sample Name	Microform	Water Table Position
CHA015	Hummock	Above	DHA015	Hummock	Above
CHA5065	Hummock	Above	DHA7085	Hummock	Above
CHA6580	Hummock	Below	DHA110125	Hummock	Above
CHA8095	Hummock	Below	DHA125140	Hummock	Below
CHB015	Hummock	Above	DHA140155	Hummock	Below
CHB5065	Hummock	Above	DHB015	Hummock	Above
CHB6580	Hummock	Above	DHB7085	Hummock	Above
CHB8095	Hummock	Below	DHB155170	Hummock	Below
CHC015	Hummock	Above	DHB170185	Hummock	Below
CHC110125	Hummock	Below	DHB185200	Hummock	Below
CHC8095	Hummock	Below	DHC015	Hummock	Above
CHC95110	Hummock	Below	DHC7085	Hummock	Above
CWA015	Hollow	Above	DHC85100	Hummock	Above
CWA2035	Hollow	Above	DHC100115	Hummock	Above
CWA3550	Hollow	Below	DHC115130	Hummock	Above
CWA5065	Hollow	Below	DWA015	Hollow	Above
CWB015	Hollow	Above	DWA3550	Hollow	Above
CWB2035	Hollow	Below	DWA7590	Hollow	Above
CWB3550	Hollow	Below	DWA90105	Hollow	Above
CWB5065	Hollow	Below	DWA105120	Hollow	Below
CWC015	Hollow	Above	DWB015	Hollow	Above
CWC5065	Hollow	Below	DWB3550	Hollow	Above
CWC6580	Hollow	Below	DWB105120	Hollow	Above
CWC8095	Hollow	Below	DWB120135	Hollow	Below
			DWB135150	Hollow	Below
			DWC015	Hollow	Above
			DWC3550	Hollow	Above
			DWC105120	Hollow	Below
			DWC120135	Hollow	Below
			DWC135150	Hollow	Below



### 3.2.3 Extraction of Total DNA

In addition to characterizing the potential microbial activity via the incubation study, bacterial and archaeal diversity was analyzed based on recovery of 16S rRNA genes. DNA was extracted from 0.4 g of peat soil for 54 samples (Table 3.1) using the FastDNA<sup>®</sup> SPIN Kit for Soil following the manufacturer's instructions (MP Biomedicals). Additional washes using 5.5 M guanidine thiocyanate were used to remove excess humic acids and to increase DNA recovery (Sharp *et al.* 2012). DNA was suspended in a final volume of 50  $\mu$ L DNase/Pyrogen-free water and stored at -20 °C until used for PCR assays.

### 3.2.4 PCR Amplification, Purification and Quantification

The 16S rRNA gene was targeted using FLX Titanium amplicon primers 454T\_RA\_X and 454T\_F containing the target primers 926f and 1392r at their 3'-ends, along with the adaptors necessary for the Roche Titanium chemistry (Sharp *et al.* 2012). Each reverse primer contained a unique 10-nucleotide identifier barcode sequence that allowed for sequences to be discerned according to the particular sample (Sharp *et al.* 2012). The PCR reaction mixtures contained 0.04  $\mu$ M of the forward primer, 25  $\mu$ L of 2  $\times$  Premix F (Interscience), 1.25 U of *Taq* DNA polymerase (Fermentas), 0.04  $\mu$ M of the reverse primer with its unique barcode sequence for each sample, 1  $\mu$ L of template DNA and nuclease-free water (Qiagen) to make up the total volume of 50  $\mu$ L (Sharp *et al.* 2012).

PCR amplification was performed with a thermal cycler with a temperature profile as follows: initial denaturation at 95° C for 3 min followed by 35 cycles of denaturation at 95° C for 30s, annealing at 55° C for 45s and extension at 72° C for 90s, and a final elongation at 72° C for 10 min.

PCR products were visualized on a 1% agarose gel and then purified using an EZ-10 Spin Column PCR Purification Kit (BioBasic Inc.). For quality control purposes, the purified PCR products were quantified via a Qubit Fluorometer using a Qubit-iT dsDNA HS Assay Kit (Invitrogen) to ensure a sufficient amount of DNA was available for the pyrosequencing (typically 150 ng total DNA). In some cases, the purification step reduced the concentration of DNA necessary for pyrosequencing and additional PCR products had to be combined and added to the sample. PCR products were then sent to Genome Quebec and McGill University Innovation Centre, Montreal, Quebec where they were analyzed by pyrosequencing using a GS FLX Titanium Series Kit XLR70 (Roche Diagnostics Corp.).

### *3.2.5 Statistical Analyses*

Statistical analyses were conducted using Minitab 14.1 (Minitab Inc., PA, USA) and SYSTAT 13 (Cranes Software International, Chicago, IL). The major statistical methods included regression, general linear model and equality of two proportion tests. Resulting P values less than 0.05 were accepted as statistically significant. The analyses of the incubation and microbial community were categorized based on the data presented in Table 3.1.

## **3.3 Results**

### *3.3.1 Field Fluxes and Water Table Position*

The field flux measurements (Table 3.2) measured the CO<sub>2</sub> and CH<sub>4</sub> flux averaged for each microform at the respective control and drained sites. The drained site had the highest CO<sub>2</sub> efflux. Only the control hollow showed CH<sub>4</sub> release while the other microforms consumed atmospheric CH<sub>4</sub> (i.e., negative flux); however, it is important to note the standard error

variability and the number of flux measurements. The CH<sub>4</sub> fluxes were not significantly different from zero with the exception of the drained hollow. The water table position relative to the surface of the microform in each site is depicted in Figure 3.1.

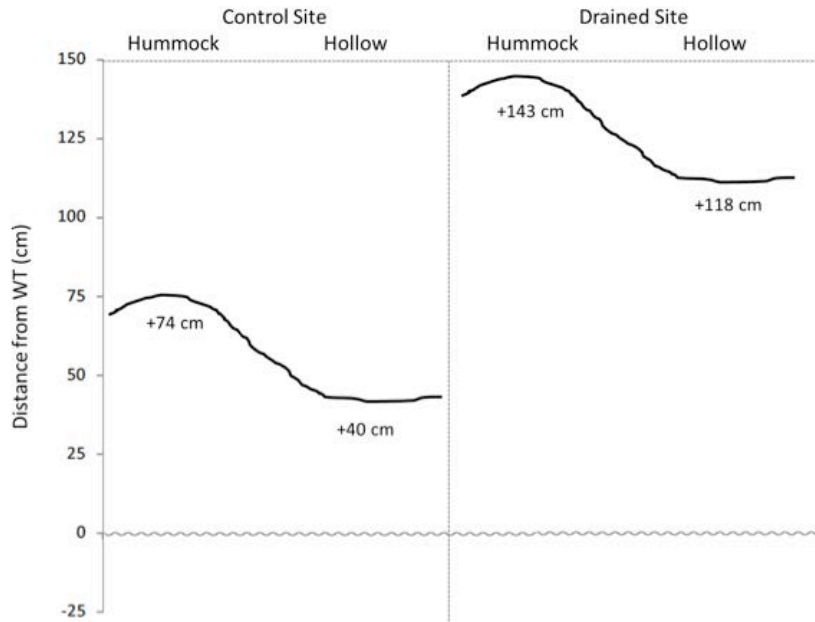


Figure 3.1 Average water table levels from the top of the peat surface (n=3 for each; measured from microform surface). Water table level is shown at 0.

Table 3.2 CO<sub>2</sub> and CH<sub>4</sub> fluxes (mean ± SEM) from the chamber method field flux measurements taken at the Wandering River bogs in July 2010. Positive values are emission from the peatland to the atmosphere while negative values are uptake from the atmosphere to the peatland.

	Control Hummock	Control Hollow	Drained Hummock	Drained Hollow
CO <sub>2</sub> Flux (g CO <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )	25.6 ± 1.6 (n=9)	22.3 ± 2.3 (n=9)	38.6 ± 3.3 (n=9)	30.0 ± 0.9 (n=9)
CH <sub>4</sub> Flux (mg CH <sub>4</sub> m <sup>-2</sup> d <sup>-1</sup> )	- 3.7 ± 4.2 (n=9)	0.5 ± 2.5 (n=9)	-1.6 ± 3.5 (n=6)	-12.0 ± 6.7 (n=6)

CO<sub>2</sub> flux was plotted against the water table levels to determine if a deeper water table (drier conditions) was correlated with higher CO<sub>2</sub> flux. While CO<sub>2</sub> flux increased with drier conditions (deeper WT) in both the control and drained sites, there was no statistically significant correlation (control site:  $F_{1,4}=2.60$ ,  $P=0.182$ ,  $R^2=0.394$ ; drained site:  $F_{1,4}=5.31$ ,  $P=0.083$ ,  $R^2=0.570$ ). Also, CH<sub>4</sub> flux was plotted against the water table levels to determine if a deeper water table (drier conditions) was correlated with lower CH<sub>4</sub> flux. There was no significant correlation between water table and CH<sub>4</sub> fluxes at the control site ( $F_{1,4}=0.17$ ,  $P=0.701$ ,  $R^2=0.410$ ) or the drained site ( $F_{1,3}=8.02$ ,  $P=0.066$ ,  $R^2=0.728$ ).

### *3.3.2 Potential CO<sub>2</sub> Production*

For both the oxic and anoxic experiments, the potential CO<sub>2</sub> production rate was significantly higher at the control site than the drained site (Figure 3.2; Table 3.3). Additionally, potential aerobic CO<sub>2</sub> production was significantly higher above the WT than below the WT at both sites (Figure 3.3; Table 3.3). The average aerobic and anaerobic potential CO<sub>2</sub> production rates at the microforms are also shown although there were no significant differences (Figure 3.3; Table 3.2).

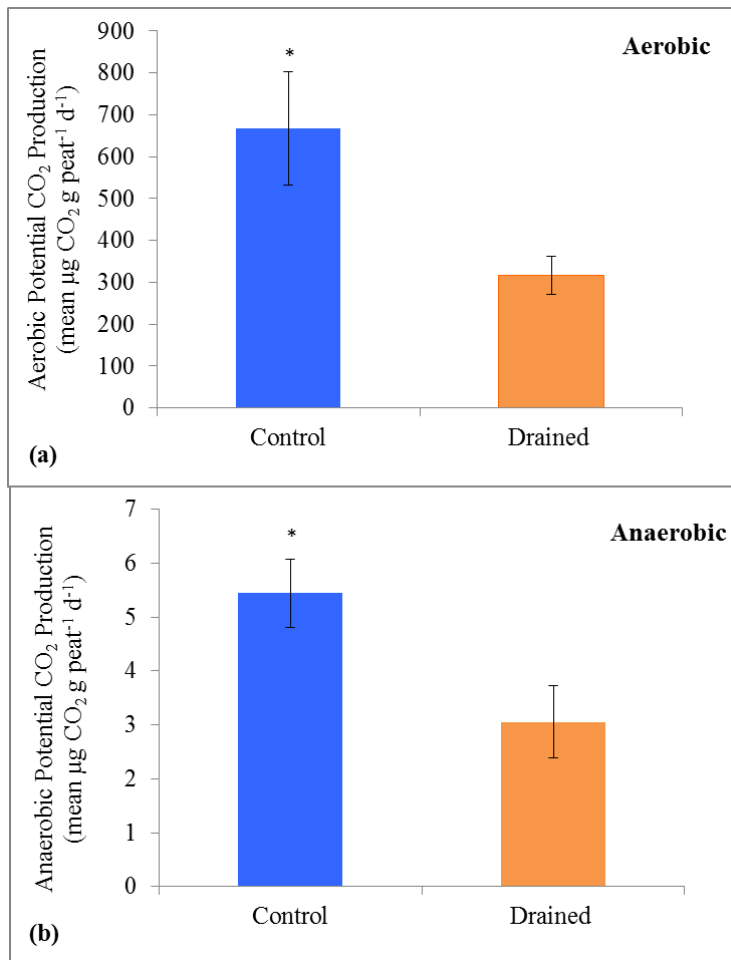


Figure 3.2 Average aerobic (a) and anaerobic (b) potential CO<sub>2</sub> production with 1 SEM bars by site. The control site had significantly higher aerobic and anaerobic potential CO<sub>2</sub> production compared to the drained site. N=24 for the control site and 30 for the drained site. The asterisks indicate statistically significant differences between sites for aerobic and anaerobic potential CO<sub>2</sub> production (P<0.05) (i.e., aerobic control was significantly higher than aerobic drained and anaerobic control was significantly higher than anaerobic drained).

Table 3.3 Statistical results (general linear model) show significant differences in aerobic and anaerobic potential CO<sub>2</sub> production rates as tested between sites, microforms and WT categories.

	Aerobic Potential		Anaerobic Potential	
	CO <sub>2</sub> Production		CO <sub>2</sub> Production	
	<i>F</i> <sub>1,46</sub>	P	<i>F</i> <sub>1,46</sub>	P
Drainage Treatment	9.78	<b>0.003</b>	14.2	<b>0.000</b>
Microform (hummock/hollow)	0.86	0.359	2.41	0.127
WT (above/below)	7.21	<b>0.010</b>	3.09	0.085
Drainage Treatment x Microform	2.09	0.155	0.46	0.503
Drainage Treatment x WT	2.53	0.119	1.40	0.243
Microform x WT	0.19	0.665	0.08	0.780

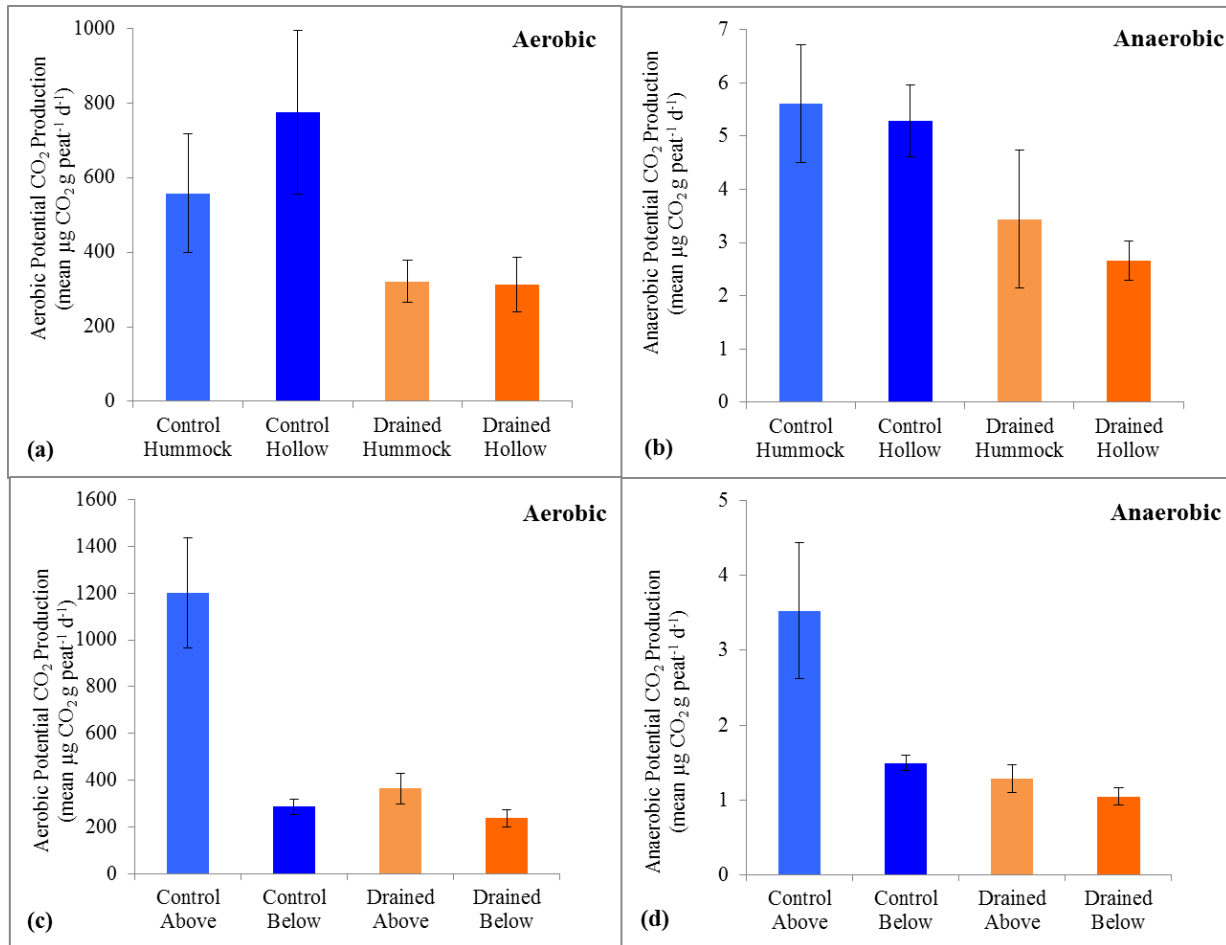


Figure 3.3 Average aerobic and anaerobic potential CO<sub>2</sub> production ( $\pm 1$  SEM) by site microform (a and b) and site water table category (c and d). The aerobic potential CO<sub>2</sub> production was higher above the water table than below (GLM Table 3.3). N=12 for control hummocks and control hollows, 15 for drained hummocks and drained hollows. N=10 for control above, 14 for control below, 19 for drained above and 11 for drained below.

### 3.3.3 Potential CH<sub>4</sub> Oxidation (Oxic Incubation Study)

The results from the oxic incubation study showed net potential CH<sub>4</sub> oxidation (PMO) but only at the hummocks. Unexpectedly, the data showed net CH<sub>4</sub> production (PMP) at the hollow microforms although the standard error was large (Figure 3.4). Statistical tests revealed no significant differences with the exception of the microform and water table interaction (Table 3.4; Figure 3.4). However, there were no significant differences in PMO between the two sites or for control and drained above or below the water table (Table 3.4).

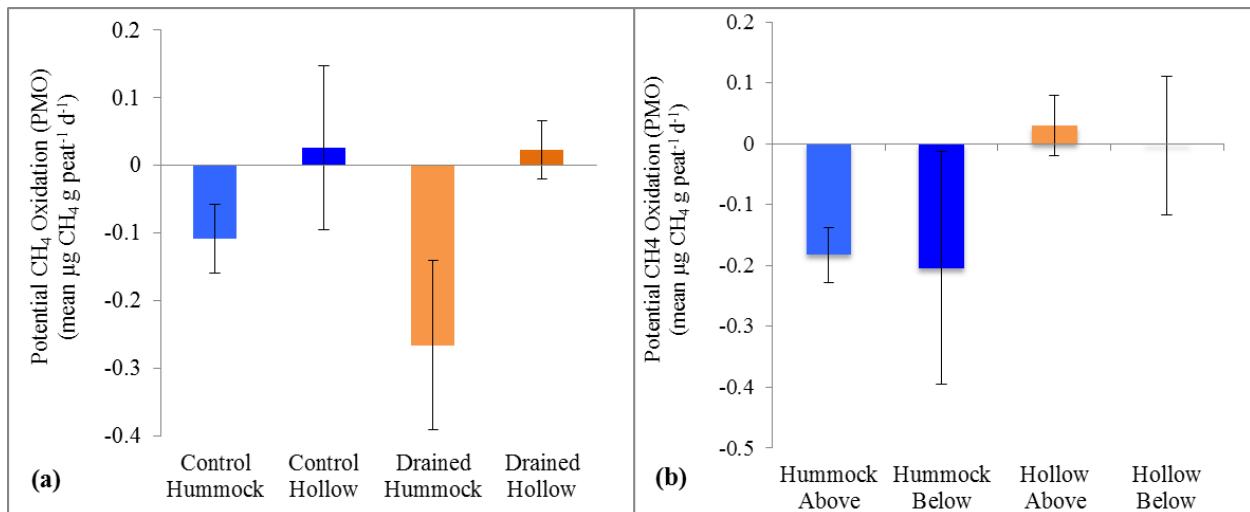


Figure 3.4 Average potential CH<sub>4</sub> oxidation (oxic experiment) ( $\pm 1$  SEM) at the control and drained site microforms (a) and at the microform position as above or below the water table (b). Positive values exhibit potential CH<sub>4</sub> production and negative values indicate potential CH<sub>4</sub> oxidation (consumption). Control hummocks (n=12), control hollows (n=12), drained hummocks (n=15) and drained hollows (n=15). The hummocks above the water table (b) had significantly larger potential CH<sub>4</sub> oxidation than the hollow above or below the water table (Table 3.4). Hummock above (n=16), hummock below (n=11), hollow above (n=13) and hollow above (n=14).



Table 3.4 General linear model results show no significant differences in PMO as tested between sites, microforms and WT categories.

	PMO	
	$F_{1,42}$	P
Drainage Treatment	0.72	0.401
Microform (hummock/hollow)	0.89	0.350
WT (above/below)	0.88	0.351
Drainage Treatment x Microform	0.82	0.776
Drainage Treatment x WT	1.83	0.183
Microform x WT	4.75	<b>0.035</b>

### 3.3.4 Potential CH<sub>4</sub> Production (Anoxic Incubation Study)

There were no significant treatment differences in PMP with the exception of the water table position (Figure 3.5; Table 3.5). The average PMP below the WT was the highest; however there was no interaction between site and WT levels therefore, there were no significant differences between the control and drained sites for PMP.

Table 3.5 General linear model results show significant differences in potential CH<sub>4</sub> production (PMP) as tested between sites, microforms and WT categories.

	PMP	
	$F_{1,42}$	P
Drainage Treatment	0.48	0.493
Microform (hummock/hollow)	0.04	0.853
WT (above/below)	6.00	<b>0.019</b>
Drainage Treatment x Microform	1.64	0.207
Drainage Treatment x WT	0.03	0.860
Microform x WT	0.00	0.938

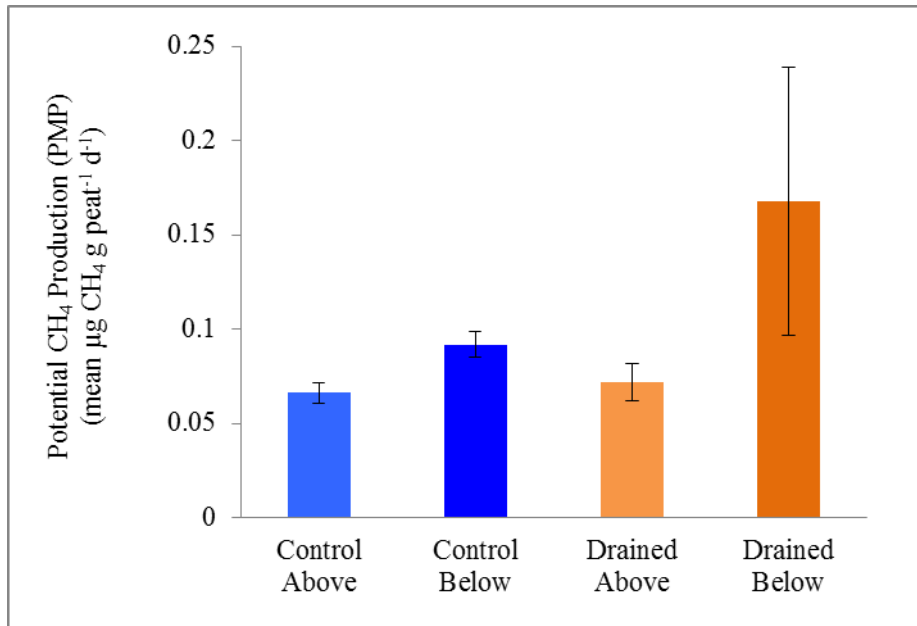


Figure 3.5 Potential CH<sub>4</sub> production (anoxic experiment) ( $\pm 1$  SEM) shows a higher potential CH<sub>4</sub> production below the water table as compared to above the water table. Control above (n=10), control below (n=14), drained above (n=19) and drained below (n=11).

### 3.3.5 Microbial Community

The QIIME software platform (Caporaso *et al.* 2010) was used to analyze the pyrosequenced 16S rRNA genes. QIIME removed low-quality sequences based on a minimum quality score of 25, clustered Operational Taxonomic Units (OTU) based on 97% identity, identified and removed chimeric sequences via ChimeraSlayer, and classified the sequences via the nucleotide BLAST algorithm against the SILVA 108 core database for 16S rRNA gene amplicons (Ramos-Padron *et al.* 2011; Sharp *et al.* 2012).

The results of the pyrosequencing revealed an overall amount of 19,056 OTUs from 437,180 reads across 54 samples. The average number of reads per sample was 8,095 with a maximum of 17,500 and minimum of 2,178. The dominant taxonomic phyla across all samples (>1% of all sequences) were 57.7% *Proteobacteria*, 13% *Acidobacteria*, 11.2% *Euryarchaeota*, 7.9% *Actinobacteria*, 2.9% *Bacteroidetes*, 1.6% *Planctomycetes* and 1.6% *Crenarchaeota*.

Taxonomic orders of the most abundant families (in which many OTUs were summed to the family level) represented as a percentage of all reads are listed in Table 3.6. There was a large relative abundance of the class *Betaproteobacteria* family *Burkholderiaceae*, which constituted nearly 38% of all reads.

Table 3.6 Taxonomic descriptions of the ten most abundant families (in which many OTUs were summed to the family level) across all 54 samples (as a percentage of all reads).

Rank	# of Reads	% of all Reads	Taxonomic Description (Phylum or Class; Order; Family)
1	164,994	37.7%	<i>Betaproteobacteria; Burkholderiales; Burkholderiaceae</i>
2	44,244	10.1%	<i>Methanomicrobia; Methanomicrobiales; Rice Cluster II</i>
3	42,652	9.8%	<i>Acidobacteria; Acidobacteriales; Acidobacteriaceae</i>
4	13,295	3.04%	<i>Alphaproteobacteria; Rhodospirillales; Acetobacteriaceae</i>
5	10,746	2.46%	<i>Actinobacteria; Acidimicrobiia; Acidimicrobiales; TM214</i>
6	10,135	2.32%	<i>Actinobacteria; Actinobacteria; Corynebacteriales</i>
7	9,938	2.27%	<i>Betaproteobacteria; Burkholderiales; Oxalobacteraceae</i>
8	8,536	1.95%	<i>Gammaproteobacteria; Xanthomonadales; Sinobacteraceae</i>
9	7,656	1.75%	<i>Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae</i>
10	6,226	1.42%	<i>Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae</i>

Large differences in the relative abundance from the control site to the drained site include a 12% decrease of *Burkholderiaceae*, a 4% increase of *Acidobacteriaceae* and a 3% increase of *Acetobacteraceae*. Refer to Chapter 4, Section 4.3.4 for a presentation of diversity indices; however, the drained site had higher microbial diversity and evenness (Chao1, Shannon and Simpson indices) as compared to the control site.

The relative abundances of the most abundant families are plotted to show differences in composition between the control and drained microforms (Figure 3.6) and water table categorization (Figure 3.7). Large differences in the relative abundance from the control hummock to the drained hummock included a 14% decrease of *Burkholderiaceae*, a 7% increase of *Acidobacteriaceae* and a 5% decrease of *Corynebacteriales* (Figure 3.6). Also, large

differences in the relative abundance from the control hollow to the drained hollow included an 8% decrease of *Burkholderiaceae*, a 4% increase of *Acetobacteraceae* and a 3.5% decrease of *Corynebacteriales* (Figure 3.6).

Large differences in the relative abundance from the control above the WT to the drained above the WT included a 37% decrease of *Burkholderiaceae*, an 8% increase of *Acidobacteriaceae* and a 3% increase of *Sinobacteraceae* (Figure 3.7). Also, large differences in the relative abundance from the control below the WT to the drained below the WT included a 7% increase of *Burkholderiaceae*, a 7% decrease of *Corynebacteriales* and a 4% increase of *Acetobacteraceae* and (Figure 3.7).

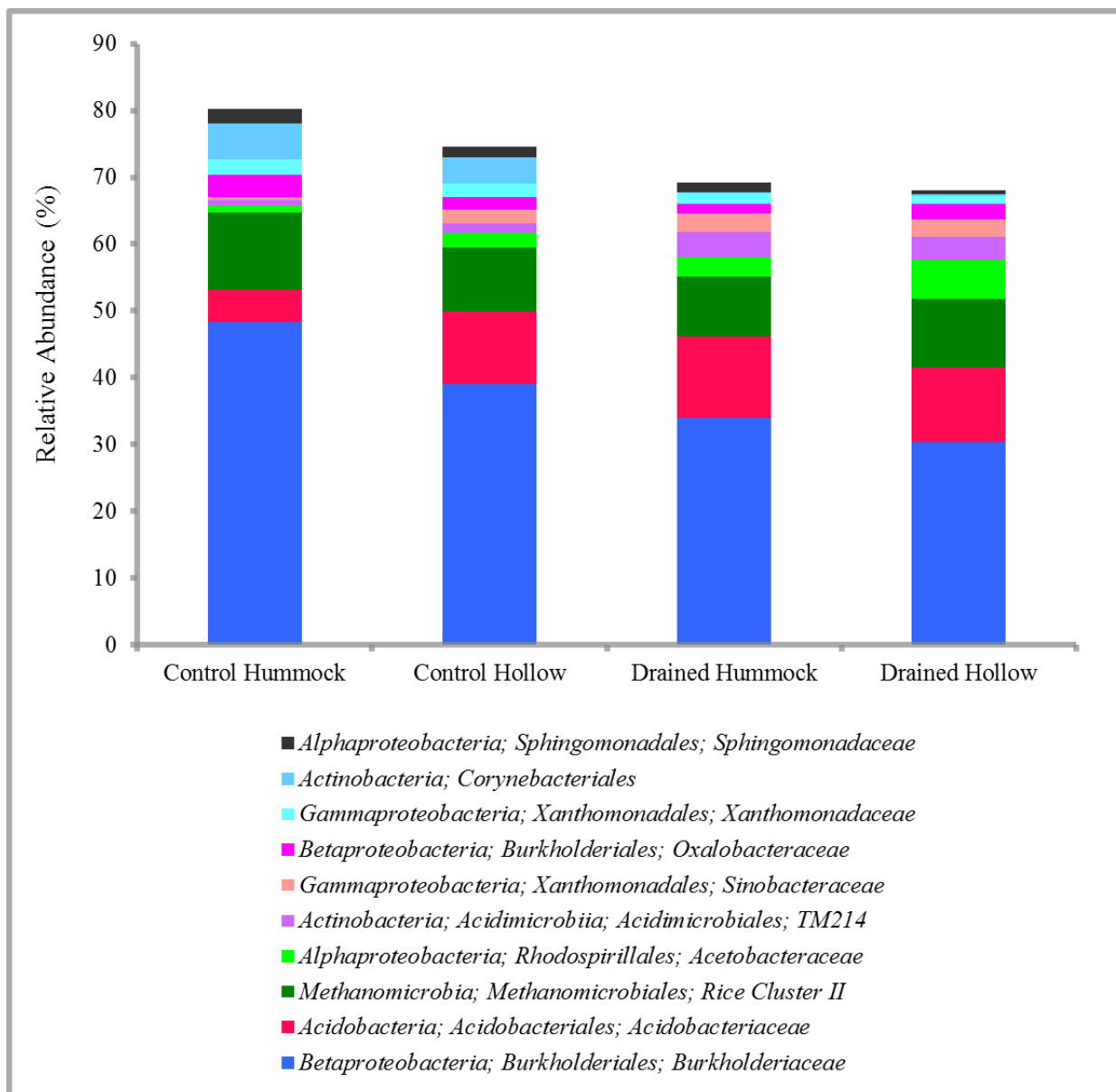


Figure 3.6 Relative abundance of the most abundant families identified across all samples and organized by control and drained microforms. Samples were pooled as per Table 3.1 and calculated based on the number of reads to the total number of reads within each category.

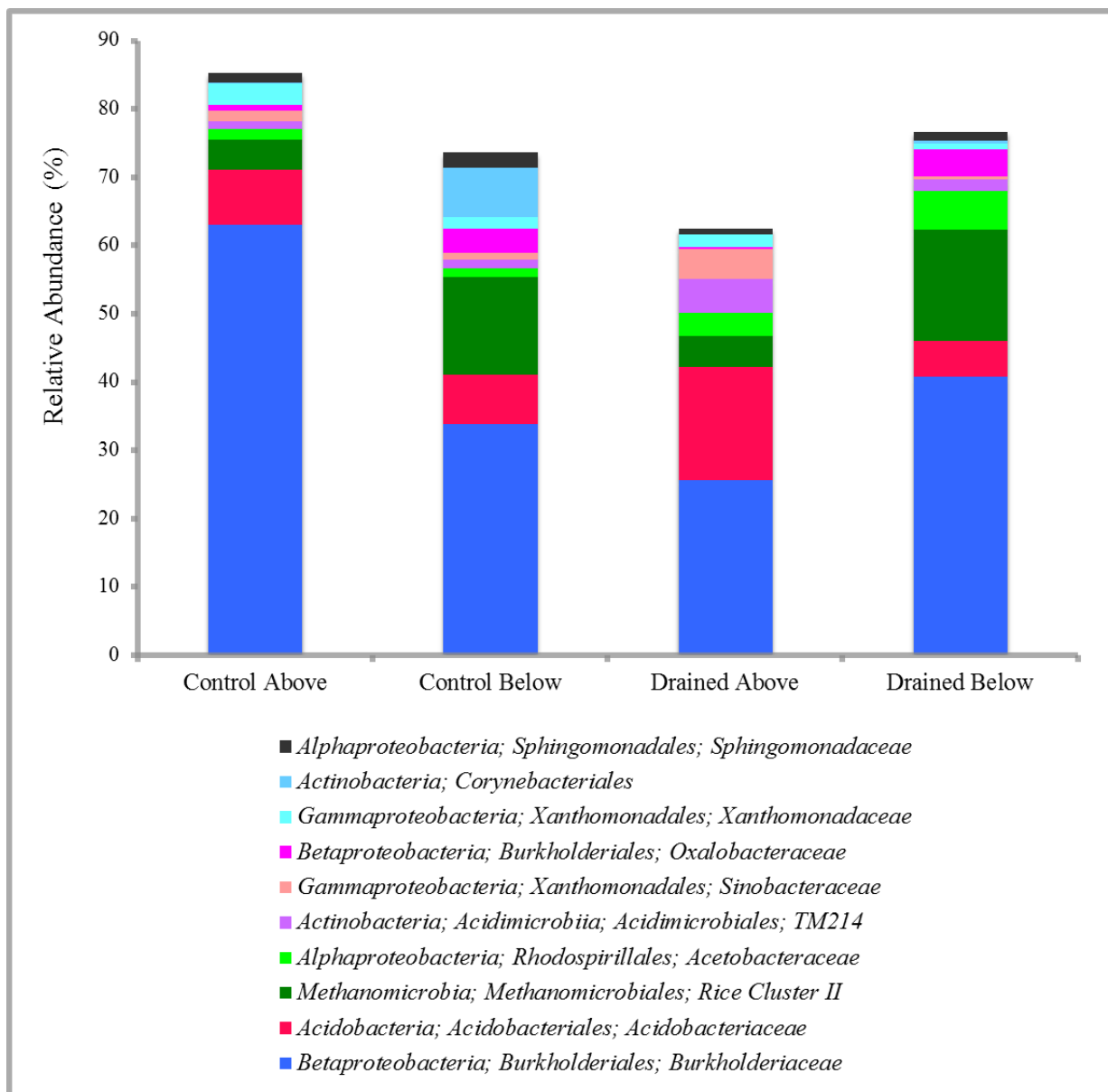


Figure 3.7 Relative abundances of the most abundant families identified across all samples and organized into control and drained sites both above and below the water table. Samples were pooled as per Table 3.1 and calculated based on the number of reads to the total number of reads within each category.

### 3.3.6 Methanotroph Community

The different methanotrophs detected are depicted in Figure 3.8. The Figure represents all of the methanotroph groups identified through pyrosequencing from both the control and drained sites, represented as a percentage of the total methanotroph reads. While a very small number of the *Cand. Methylacidiphilum* methanotrophs were identified a larger group of closely related *Verrucomicrobia* not currently classified as methanotrophs was also noted. Additionally, the phylogenetic position of the methanotrophs identified as per a 16S rRNA sequence-based tree is shown in Figure 3.9.

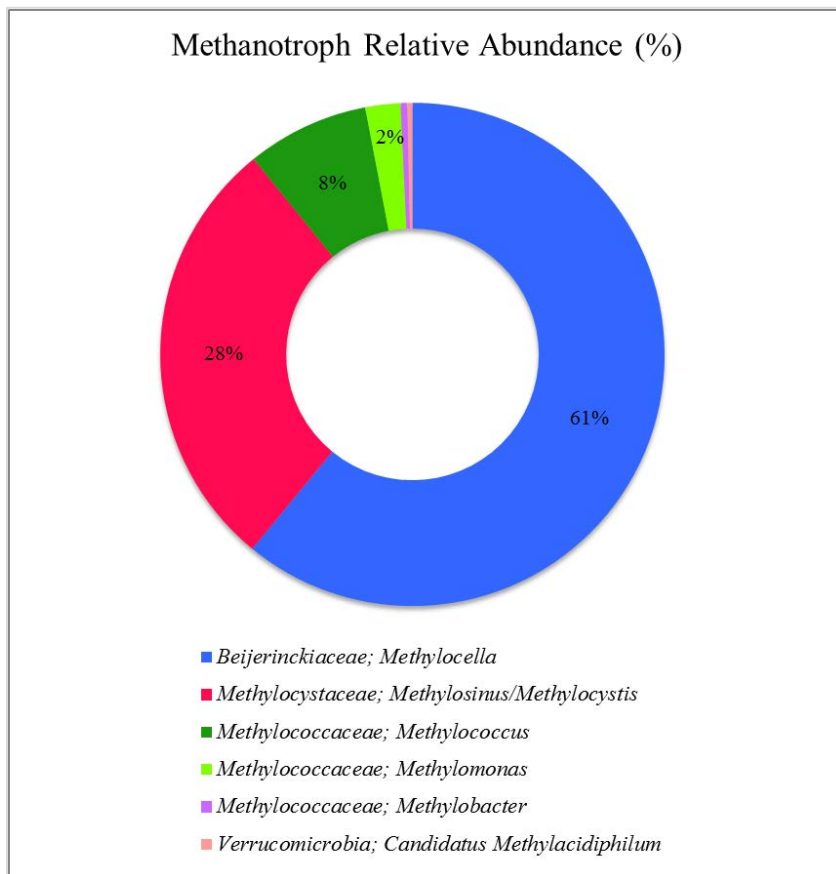


Figure 3.8 Relative abundance of methanotrophs identified across all samples. Overall, there were 930 reads of all methanotrophs of which 61% belong to *Methylocella* followed by 28% *Methylosinus/Methylocystis*.



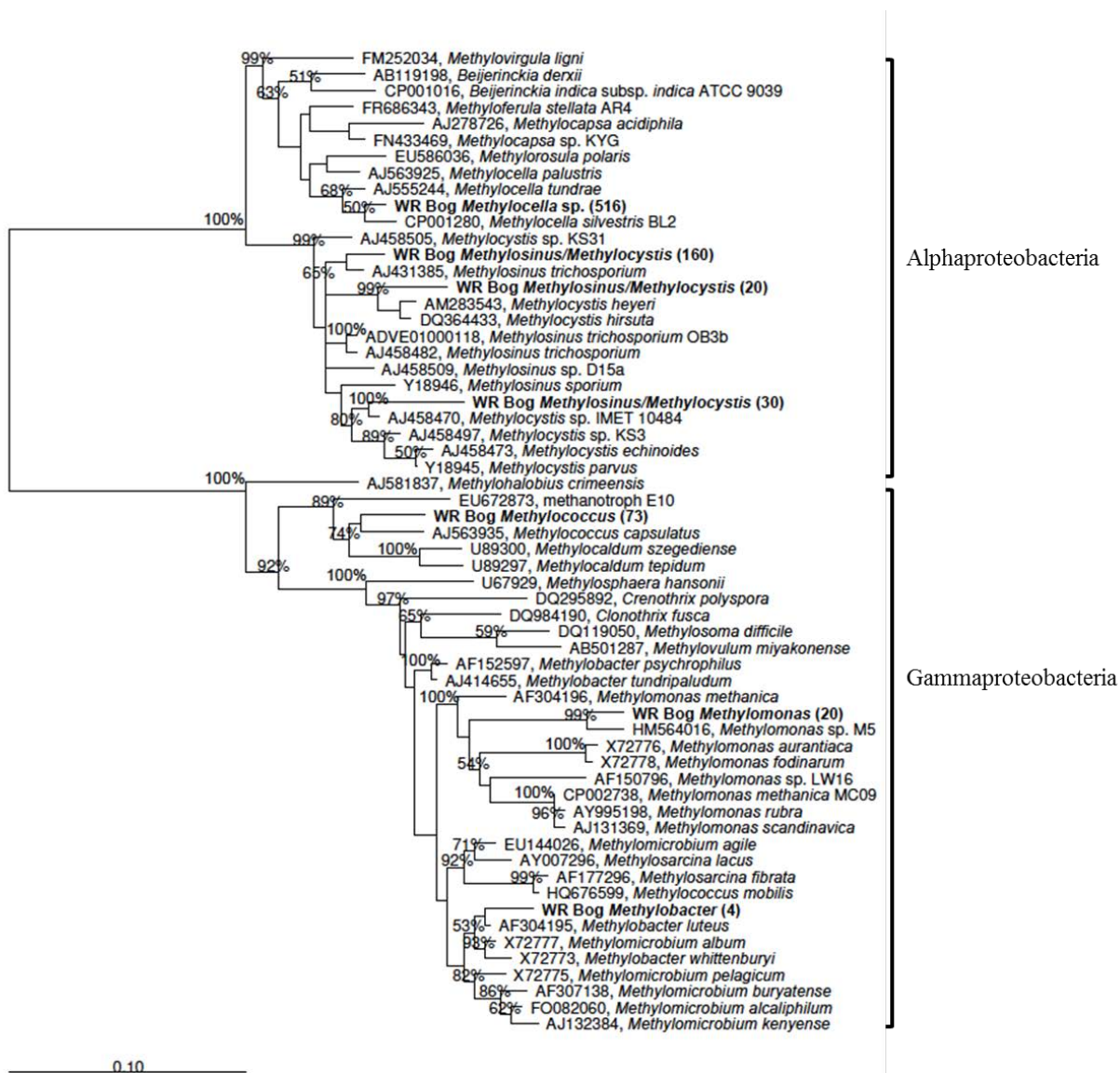


Figure 3.9 16S rRNA gene sequence-based tree showing phylogenetic position of methanotrophs identified in this study (designated with prefix ‘WR’ in bold) assembled as per the Neighbor-Joining method with a Jukes-Cantor correction. Bootstrap support values (% of 1,000 replicates) greater than 50% are indicated at the nodes. A skeleton tree was made from nearly full-length sequences (>1400 nt) and the shorter pyrosequences (450 nt) were added to this tree via parsimony. The sequence alignments were from the ARB-Silva database and calculations were made using ARB (Ludwig *et al.* 2004). The numbers of pyrosequencing reads for the present study are shown in parentheses. The scale bar represents a 0.1 change per nucleotide position.

The relative abundances of *Methylocella* and *Methylosinus/Methylocystis* (as a percentage of the total community) at the control and drained sites were not significantly different (equality of two proportions;  $Z=0.41$ ,  $P=0.682$ ).

The relative abundance of methanotrophs (as a percentage of the total community) based on site microforms, is illustrated in Figure 3.10. Equality of two proportions testing revealed no significant differences ( $P<0.025$  for two-tailed test) in the relative abundance of *Methylocella* and *Methylosinus/Methylocystis* between control and drained hummocks (*Methylocella*:  $Z=0.09$ ,  $P=0.922$ ; *Methylosinus/Methylocystis*:  $Z=-0.82$ ,  $P=0.412$ ) or between control and drained hollows (*Methylocella*:  $Z=0.47$ ,  $P=0.635$ ; *Methylosinus/Methylocystis*:  $Z=1.66$ ,  $P=0.096$ ).

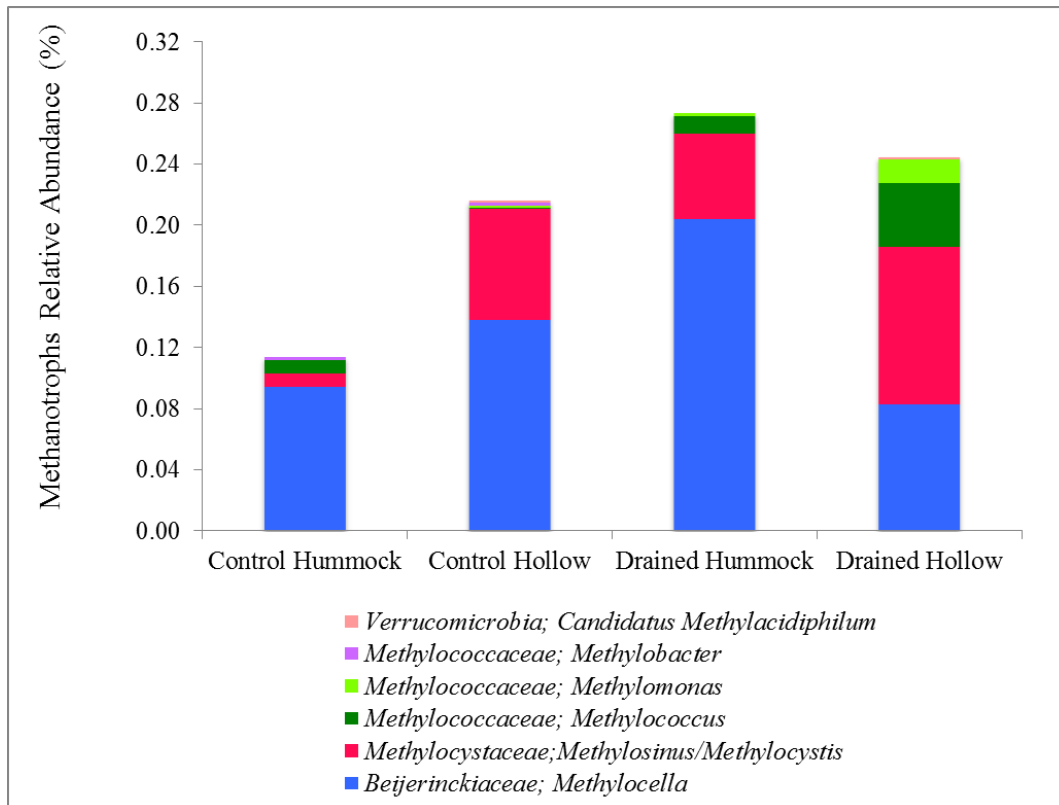


Figure 3.10 Relative abundance of methanotrophs identified as a percentage of the total community across all samples by control and drained microforms.

### 3.3.7 Putative Anaerobic Methanotrophs

There were 66 pyrosequencing reads identified in this study that were affiliated to the NC10 phylum. These sequences were imported into ARB (a centralized database of processed and aligned sequences; Ludwig *et al.* 2004) and compared to the currently known enrichment cultures of nitrite-reducing anaerobic methanotrophic bacteria (*Cand. Methyloirabilis spp.*) from the NC10 phylum (Ettwig *et al.* 2009; Zhu *et al.* 2012). The phylogenetic position of the putative anaerobic methanotrophic bacteria found in this study is shown in the 16S rRNA sequence-based tree (Figure 3.11). The phylogenetic position of the sequences identified in this study as compared to *Cand. Methyloirabilis spp.* was verified with a maximum likelihood tree puzzle method (data not shown) (Strimmer & von Haeseler, 1996; Schmidt *et al.* 2002).

Ettwig *et al.* (2009) screened sediment slurries from floodplain agricultural drainage ditches for NC10 bacteria (inoculum) and subsequently used the sediments to enrich for NC10 bacteria in an anaerobic continuous culture bioreactor (enrichment). Results from Ettwig *et al.* indicated only the enriched group of NC10 bacteria in their study was responsible for anaerobic CH<sub>4</sub> oxidation coupled to nitrite reduction. In the present study, there was better alignment with the inoculum (Ino-F12) NC10 representatives from Ettwig *et al.* (2009) rather than the enrichment culture. Therefore, these pyrosequencing reads can only be referred to as putative anaerobic methanotrophs. Additionally, the sequences did not affiliate closely to NC10 enrichment cultures from a minerotrophic peatland (16S rRNA GenBank accession numbers JX262156- JX262161) Zhu *et al.* (2012).

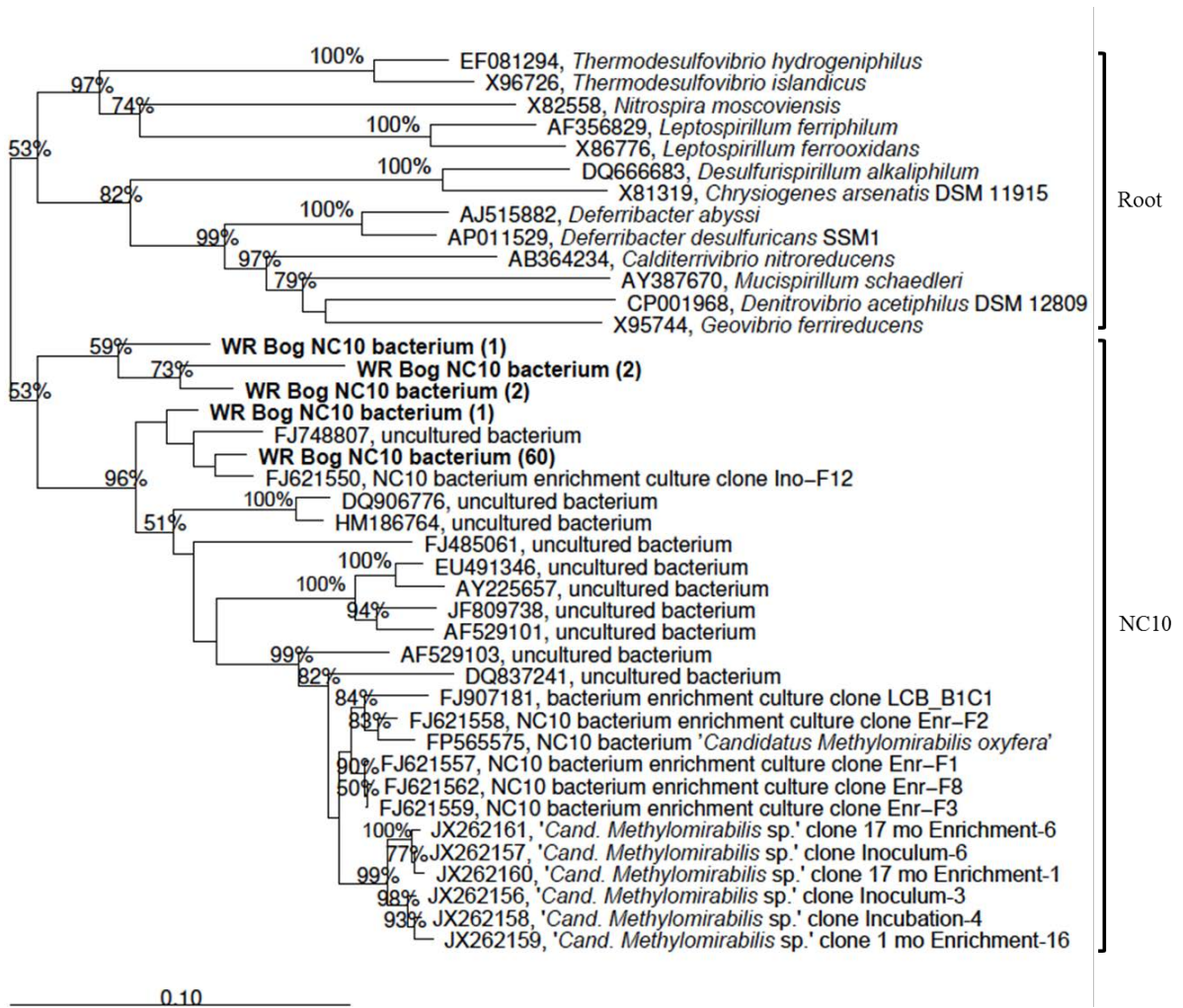


Figure 3.11 16S rRNA gene sequence-based tree showing phylogenetic position of the putative anaerobic methanotrophs from the NC10 phylum identified in this study (designated with prefix ‘WR’ in bold) assembled as per the Neighbor-Joining method with a Jukes-Cantor correction. Bootstrap support values (% of 1,000 replicates) greater than 50% are indicated at the nodes. GenBank accession numbers precede the NC10, *Nitrospirae*, *Deferribacteres* and the *Chrysiogenetes* phylum reference sequences. The tree was rooted against a set of sequences from the *Nitrospirae*, *Deferribacteres* and the *Chrysiogenetes* phyla. The scale bar represents 0.1 change per nucleotide position.

### 3.3.8 Methanogen Community

The different methanogens detected across all samples are shown in Figure 3.12. The figure represents all of the methanogens identified through pyrosequencing from both the control and drained sites and represented as a percentage of the total methanogen reads. The overwhelmingly relatively abundant methanogen is the *Methanomicrobiales* Rice Cluster II (RCII) at 91% of all methanogens detected.

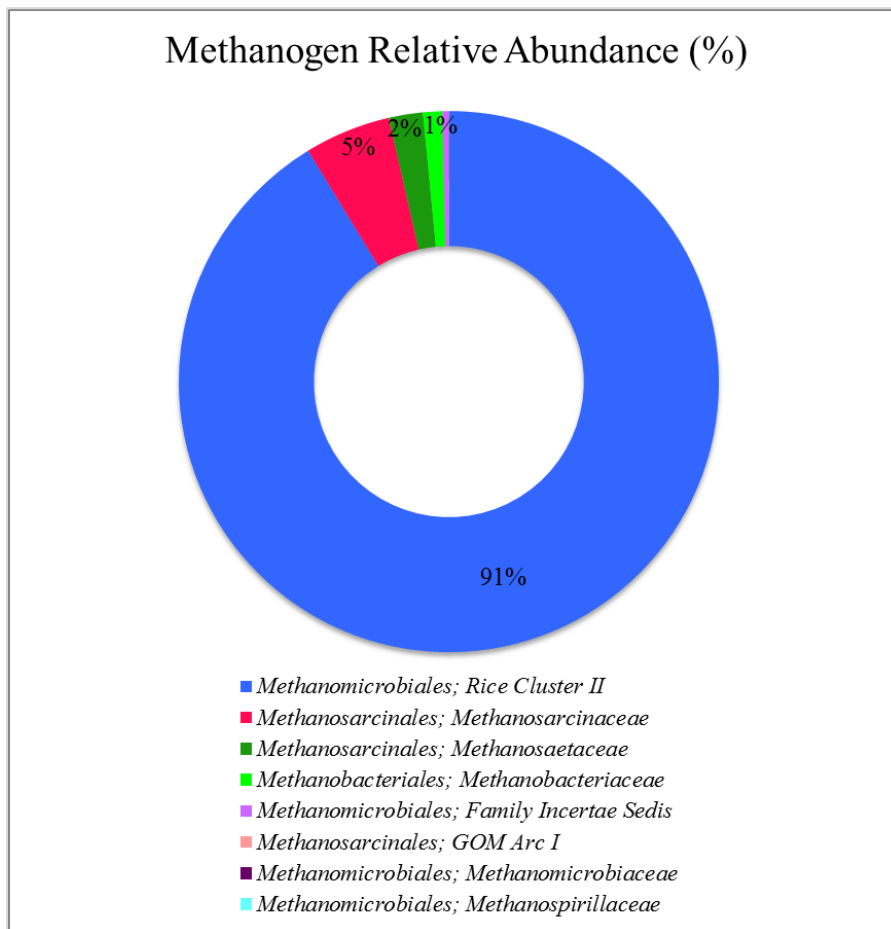


Figure 3.12 Relative abundance of methanogens identified across all samples. Overall, there were 48,467 reads across all methanogens of which 91% belonged to *Methanomicrobiales*, Rice Cluster II methanogens.

The relative abundance of methanogens (as a percentage of the total community) based on site microforms, is illustrated in Figure 3.13. Equality of two proportions testing revealed no significant differences ( $P < 0.025$  for two-tailed test) in the relative abundance of RCII and acetoclastic methanogens between control and drained sites (RCII:  $Z = -1.09$ ,  $P = 0.273$ ; acetoclastic methanogens:  $Z = 4.09$ ,  $P = 0.682$ ), between control and drained hummocks (RCII:  $Z = -0.77$ ,  $P = 0.439$ ; acetoclastic methanogens:  $Z = 0.09$ ,  $P = 0.922$ ) or between control and drained hollows (RCII:  $Z = -0.78$ ,  $P = 0.433$ ; acetoclastic methanogens:  $Z = 0.47$ ,  $P = 0.635$ ).

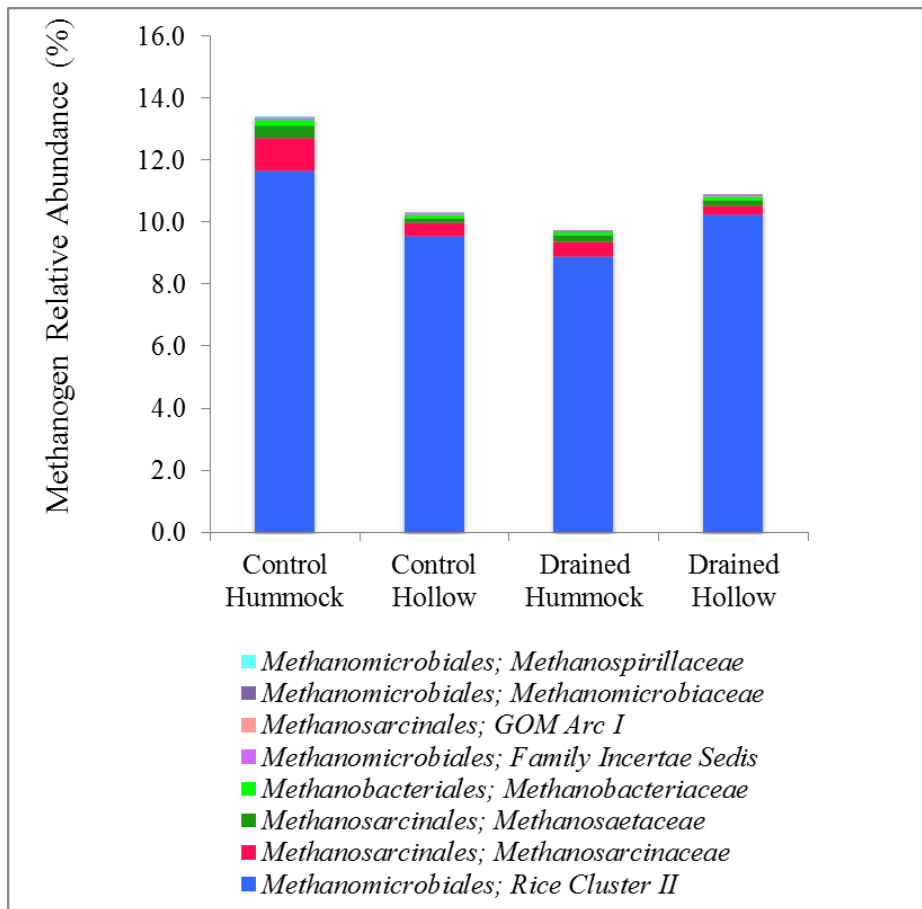


Figure 3.13 Relative abundance of methanogens identified across all samples and organized by control and drained microforms.

### 3.4 Discussion

#### 3.4.1 CO<sub>2</sub> Flux, WT Position and Potential CO<sub>2</sub> Production

There are conflicting results in the literature regarding the relative importance of thermal and hydrological regimes in controlling peatland ecosystem respiration (ER). Lafleur *et al.* (2005) found that growing-season ER in a dry ombrotrophic bog was strongly correlated with peat temperature but only weakly correlated with water table depth. Moreover, in a study by Deppe *et al.* (2010), water table fluctuations had no clear impact on CO<sub>2</sub> flux although this research was based on short-term irrigation regimes and small fluctuations in water table levels on cores in the laboratory. However, in an experimental water table manipulation study in a rich fen, Chivers *et al.* (2009) documented significant changes to ecosystem CO<sub>2</sub> flux wherein the fen changed from a net sink to a net source of atmospheric CO<sub>2</sub> (primarily through reduced plant productivity) in year two after experimental water table drawdown. The conflicting results in previous research may be explained by site-specific plant productivity (as driven by plant community, nutrient status and hydrological regime), which affects the gross ecosystem photosynthesis and therefore the amount of CO<sub>2</sub> taken up and released (Strack *et al.* 2008). While plant productivity is also related to seasonal temperatures, Strack *et al.* (2008) noted that soil respiration is higher under oxic conditions and ER tends to be enhanced with deeper water table positions. Therefore, drier conditions and lowered water tables are expected to increase the zone of oxidation and stimulate aerobic decomposition (Laiho, 2006) although the magnitude of the resulting CO<sub>2</sub> flux will depend on site-specific plant productivity. In this study, average CO<sub>2</sub> flux in both sites was not significantly related to decreasing water table depth (drier conditions) although overall, CO<sub>2</sub> efflux was higher at the drained site. However, this finding must consider the chamber measurement technique and the sampling period.

Chamber measurements performed in this study are of limited value as the installation as well as walking through the bogs from one chamber to another introduces disturbance to the ecosystem and can artificially affect the results. For instance, CH<sub>4</sub> can escape the bog to the atmosphere through diffusion, structural vegetation transport or through ebullition events. The disturbance associated with installing and sampling chambers may cause an ebullition event that would have otherwise not occurred at that time. Additionally, the chamber measurements were only a ‘snapshot’ in time and did not capture complete diurnal ER and seasonal effects.

Contrary to flux measurements, the potential CO<sub>2</sub> production showed that the control site had a significantly higher production potential compared to the drained site. The potential CO<sub>2</sub> production ranged from 0.14 to 2.24 mg CO<sub>2</sub> g peat<sup>-1</sup> d<sup>-1</sup> at the control site and 0.08 to 1.05 mg CO<sub>2</sub> g peat<sup>-1</sup> d<sup>-1</sup> at the drained site. This is in agreement with the ranges published in other studies. In a multi-site study carried out by Glatzel *et al.* (2004), the oxic potential CO<sub>2</sub> production rates ranged from 0.04 to 1.05 mg CO<sub>2</sub> g peat<sup>-1</sup> d<sup>-1</sup>. Additionally, Moore and Dalva (1997) determined oxic potential CO<sub>2</sub> rates to range between 0.08 to 5.0 mg CO<sub>2</sub> g peat<sup>-1</sup> d<sup>-1</sup>.

The lower potential CO<sub>2</sub> production rate at the drained site may be partially explained by the different litters produced by the vegetation communities following long-term drainage and how easily these are decomposed (Laiho, 2006). While the drained site has a larger oxic zone due to drainage, Bridgham and Richardson (1992) noted that peat soils exposed to long periods of aerobic decomposition may be highly resistant to further decomposition due to the recalcitrant nature of the remaining carbon substrate. Therefore, while CO<sub>2</sub> flux was higher at the drained site but potential CO<sub>2</sub> production was higher at the control site, it is possible the substrate is more recalcitrant at the drained site affecting the potential CO<sub>2</sub> results but more of this poor substrate is exposed to oxic conditions leading to the higher net CO<sub>2</sub> efflux. Additionally, tree



root growth and the resulting increase in root respiration at the drained site supports higher CO<sub>2</sub> flux results. This finding is supported by Choi *et al.* (2007) who found that water table drawdown in a minerotrophic fen resulted in an increase in radial growth of trees through enhanced photosynthetic capacity.

#### 3.4.2 CH<sub>4</sub> Flux, WT Position, and Potential CH<sub>4</sub> Production/Oxidation

Methanogenesis is a strictly anaerobic process driven largely by temperature, microbial community present and plant productivity in terms of substrate quantity and quality (Strack *et al.* 2008). However, the flux of trace gases such as CH<sub>4</sub> into the atmosphere is driven by the soil and atmosphere concentration gradient (Conrad, 1996) as well as the transport mechanism. While average CH<sub>4</sub> flux in this study shows large variation and contrasting results between the control and drained sites, the chamber method and temporal resolution is not robust enough to come to any definitive conclusions. However, Sundh *et al.* (1995) also noted a large range in CH<sub>4</sub> flux at both the drier hummocks (-1.9 to 28 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>) and the hollows (42 to 104 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>) in their research sites. Sundh *et al.* observed the consumption of atmospheric CH<sub>4</sub>, as was the case in the present study. While this study does not attempt to determine the carbon balance of the control and drained sites, it is important to note that there are large interseasonal and interannual ranges of CO<sub>2</sub> and CH<sub>4</sub> flux in northern peatlands and resolving these data is difficult without an extensive multi-year study (Roulet *et al.* 2007).

In a multi-site peatland study, Glatzel *et al.* (2004) found no consistent pattern between high rates of PMP relative to peat above or below the water table, and overall PMP had a large range of values from 0.0 to 816 µg CH<sub>4</sub> g peat<sup>-1</sup> d<sup>-1</sup>. Moore and Dalva (1997) also reported a range of PMP over four orders of magnitude from 0.01 to 100 µg CH<sub>4</sub> g peat<sup>-1</sup> d<sup>-1</sup>. In this study,

the PMP was much lower than the results from Glatzel *et al.* (2004) and Moore and Dalva (1997) as the range of PMP extended from 0.02 to 0.88  $\mu\text{g CH}_4 \text{ g peat}^{-1} \text{ d}^{-1}$ . This is consistent with the lower  $\text{CO}_2$  production potential at the drained site, again suggesting a low-quality substrate.

It is typical to see low PMP in bogs as ombrotrophic conditions typically do not provide the optimal pH for methanogen growth (Dunfield *et al.* 1993) and the litter type is often difficult to degrade (Moore & Basiliko, 2006). Actual methanogen abundance was not quantified in this study; therefore, it is difficult to discern whether methanogen abundance is a limiting factor for the PMP rates. However, it is possible that the lack of methanogen diversity is limiting PMP. Yavitt *et al.* (2011) found a significant positive relationship between the diversity of methanogens and potential rates of  $\text{CH}_4$  production (as sampled across six different bogs and fens in NE U.S. and SE Canada). Chapter 4 of this thesis will discuss the physicochemical drivers of the methanogen community in more detail.

In reviewing the results of the PMO experiment, it was determined that the samples were not given an adequate concentration of  $\text{CH}_4$  upon the start of the incubation experiment. This has affected the results to the extent that the samples appear to be producing rather than consuming  $\text{CH}_4$ . Additionally, it is possible that  $\text{O}_2$  penetration within the peat sample was limited particularly in the samples that were wet; however,  $\text{O}_2$  was not measured as a part of the incubation experiments. For these reason, the PMO results are not considered valid and will not be used for any other analyses in this thesis.

### 3.4.3 Bacterial Community

Pyrosequencing was used to compare the bacterial community structure between two *Sphagnum* bogs, two microtopographical positions (3 hummocks and 3 hollows in each bog) and between samples pooled above and below the water table (for a total of 54 samples). The

bacterial communities at the control and drained sites were generally similar in terms of composition but presented some significant differences in terms of relative abundance. The high relative abundance of *Burkholderiaceae* was reduced after drainage and a large increase in both *Acidobacteriaceae* and *Acetobacteraceae* was noted at the drained site.

While not a comparable method to the cultivation-independent pyrosequencing of the present study, Belova *et al.* (2006) found that by determination of the partial sequences of 16S rRNA genes in *Sphagnum* bog isolates (from a direct plating of dilutions of native peat), members of the genus *Burkholderia* constituted approximately 30% of the bacterial community. Since the methods of bacterial identification in the present study vs. Belova *et al.* were much different, the relative abundance of *Burkholderia* is not directly comparable but it is interesting to observe similarly high relative abundances. Approximately 44% of all pyrosequencing reads in the control site and 32% of the reads in the drained site were classed as the *Burkholderiaceae* family, the majority of which consisted of the genus *Burkholderia*. While *Burkholderia* species are known and exploited for their biological and metabolic properties in controlling fungal diseases in plants, little is known about their occurrence and function in natural ecosystems (Vandamme *et al.* 2007). However, some strains of *Burkholderia* form dominant populations on *Sphagnum* mosses and have a high antagonistic potential against fungal pathogens (Vandamme *et al.* 2007).

Most peat-inhabiting *Acidobacteria* can utilize certain acids such as galacturonic and glucuronic acids produced from the decomposition of *Sphagnum* moss, and members of this phylum seem to play an important role in degrading plant-derived polymers in peatlands (Dedysh, 2011). The relative abundance of *Acidobacteria* in the present study increased after drainage from 3.5% at the control site to 6.2% at the drained site. This is considerably lower than

Ausec *et al.* (2009) who observed *Acidobacteria* constituted approximately 25% of 16S rRNA sequences in an acidic (pH 4.5) bog soil and Dedysh *et al.* (2006) found 24 of 84 (~28%) nearly full-length 16S rRNA gene peat clones affiliated with the *Acidobacteria* phylum.

While the relative abundances of bacteria in a peatland may differ from study to study as noted above, bacterial community composition has been shown to be similar. In a study by Morales *et al.* (2006), 24 New England bogs were sampled at surface and anoxic depths to characterize the total bacterial diversity using the 16S rRNA gene and terminal restriction fragment length polymorphism (T-RFLP). As was the case in this study, Morales *et al.* showed all of the ombrotrophic bogs sampled were fairly similar in their bacterial community composition.

#### 3.4.4 Methanotroph Community

The three major methanotrophic bacteria usually found in peat soils are *Methylocella*, *Methylocapsa* and *Methylosinus/Methylocystis* (Dunfield & Dedysh, 2010). This has been shown through the use of methanotroph-specific genes (*pmoA* and *mmoX*) from environmental samples, cultivation studies and also, through species-specific enumeration using oligonucleotide probes in fluorescent in situ hybridization (Dunfield & Dedysh, 2010). This is in agreement with the results of the present study as the majority of methanotrophs identified were *Methylocella* and *Methylosinus/Methylocystis*. However, in contrast to the present study, Kip *et al.* (2011) used pyrosequencing with *pmoA* PCR products from DNA extracted from *Sphagnum* mosses in a Dutch peat bog and found the highest number of reads corresponded to *Methylomonas* spp. (followed by *Methylocystis*). Chen *et al.* (2008) also observed *Methylomonas* spp. at an England bog through *pmoA* and microarray but conversely, not as an abundant group. *Methylomonas* was

detected in the present study but only constituted 20 reads out of the 307 reads total for all methanotrophs. Statistical testing revealed no significant differences in the methanotroph relative abundance between sites or microforms.

While some strains of methanotrophs may not require a large concentration of CH<sub>4</sub> for growth (Knief & Dunfield, 2005), the low concentrations of CH<sub>4</sub> diffusing up through the acrotelm may affect the methanotroph composition. In direct tests, Knief and Dunfield (2005) found most methanotrophs require a range of 100 to greater than 1000 ppmv of CH<sub>4</sub> to grow; however, two *Methylocystis* strains grew at only 10-100 ppmv of CH<sub>4</sub> and one strain oxidized atmospheric CH<sub>4</sub>. Therefore, methanotroph composition at the study sites may be related to the consumption of atmospheric CH<sub>4</sub> or very low levels of produced CH<sub>4</sub> (Knief & Dunfield, 2005). Additionally, members of the genus *Methylocella* have been shown to utilize CH<sub>4</sub> or some multi-carbon compounds (e.g., acetate and ethanol) as their sole carbon and energy source (Dedysh & Dunfield, 2010). As such, a system dominated with *Methylocella*-like methanotrophs may show an altered regulation of CH<sub>4</sub> oxidation potential (Dedysh & Dunfield, 2010).

Anaerobic CH<sub>4</sub> oxidation in freshwater systems and especially in peatlands is largely unexplored. However, Smemo and Yavitt (2007) have demonstrated through *in vitro* assays of peat soil involving methanogenic inhibitors, stable isotope (<sup>13</sup>C-CH<sub>4</sub>) enrichments and natural abundance analysis, that anaerobic CH<sub>4</sub> oxidation consumes a large amount of CH<sub>4</sub> production (mean net anaerobic CH<sub>4</sub> oxidation of 17 ± 2.6 nmol kg<sup>-1</sup>s<sup>-1</sup>). In marine systems, anaerobic CH<sub>4</sub> oxidation is established and coupled primarily to sulfate reduction but in peatland ecosystems, electron acceptors are scarce although this process has been linked to nitrate and nitrite (Zhu *et al.* 2012). While the results of this study show the presence of putative anaerobic methanotrophs,

this group may be related to, and could be affecting, nitrate and nitrite concentrations at the study sites. Nitrate and nitrite concentrations and differences between sites are discussed in Chapter 4.

#### 3.4.5 Methanogen Community

The observed methanogens in this study were dominated by Rice Cluster II (RCII) methanogens but also contained members of *Methanomicrobiaceae*, *Methanosaetaceae* and *Methanosarcinaceae*. Cadillo-Quiroz *et al.* (2006) showed that RCII is phylogenetically placed between *Methanosarcinales* and the obligate CO<sub>2</sub> reducing order *Methanomicrobiales* (via 16S rRNA sequence analysis). While RCII does not have any pure-culture representatives to definitively determine physiological capabilities, Galand *et al.* (2005) observed the related RCI methanogens in sites where methanogenesis was predominately supported by CO<sub>2</sub> reduction. Moreover, an isolate from RCI has been shown to be a CO<sub>2</sub> reducer but not an acetate fermenter (Sakai *et al.* 2007; Sakai *et al.* 2010; Godin *et al.* 2012). For these reasons, the RCII is assumed to be a group of hydrogenotrophic methanogens for the purposes of this research.

In other studies, phylogenetic compositions of peatland euryarchaeota across six different peatlands in eastern Canada and U.S. region revealed the presence of RCII methanogens in four of the six sites (Yavitt *et al.* 2011). In a West Siberian peat bog (pH 4.2-4.8), Kotsyurbenko *et al.* (2004) showed the presence of methanogen members from *Methanomicrobiaceae*, *Methanosarcinaceae* and RCII in which *Methanosarcina* accounted for about half of all the archaeal cells. Furthermore, in two different Finnish fens, researchers noted RCII and *Methanosaetaceae* as major methanogen groups (Putkinen *et al.* 2009). While the distribution of methanogens within and among peatlands is not well known, acetoclastic methanogenesis and therefore acetoclastic methanogens have been documented as major methanogen groups in fens

but not typically at bogs (Juottonen, 2008). However, the lack of sedges, which would provide fresh substrate to the anoxic zone for acetoclastic methanogenesis, may be limiting the relative abundance of acetoclastic methanogens and this could partly explain the overwhelming predominance of hydrogenotrophic methanogens at both sites. As was noted in this study, peat provides a poor habitat for a diverse methanogen community and there is a high relative abundance of RCII methanogens (assumed hydrogenotrophic methanogens), which did not differ significantly between sites or microforms.

## Chapter Four: Microbial and Physicochemical Comparisons of the Wandering River

### Natural and Drained Bogs

#### 4.1 Introduction

The decomposition of organic matter in a peatland is generally controlled by four interacting factors: substrate quality, environmental conditions, decomposers present, and nutrient availability (Laiho, 2006). Although these ecosystems are usually net CO<sub>2</sub> sinks, many studies have shown net CO<sub>2</sub> release from peatlands due to changes in environmental conditions such as drought and melting permafrost (Blodau & Moore, 2003). While the CO<sub>2</sub> release is the greatest immediately following drainage or drought, it slows with time due to decreasing substrate quality (Blodau & Moore, 2003; Turetsky *et al.* 2008). The input of C into the system may also decrease but depending on the site nutrient status, may recover with the response of the plant community (e.g., the transition from sedges to shrubs) (Laiho, 2006). The structure of the plant community may affect the acidic and nutrient-poor environment in a peatland especially if *Sphagnum* moss coverage decreases. This in turn affects the diversity and structure of the microbial communities, which then affects the decomposition rate (Kulzer *et al.* 2001).

When attempting to restore a degraded peatland to a functional and self-sustainable state (e.g., post-harvested sites), monitoring ecologically relevant properties such as biogeochemical cycles, water and peat chemistry along with microbiological analyses is essential to follow the transition of the system over time (Andersen *et al.* 2006). Microbial community composition, diversity and activity are affected by many interacting physicochemical parameters. While there may not be one single clear mechanism driving microbial composition, diversity and activity, the aim of this chapter is to contribute to the existing research an assessment of the physiochemical characteristics of a natural and drained bog over both a microtopographical position and depth



gradient to determine which factors are related to microbial composition, diversity and relative abundance.

## **4.2 Methods**

### *4.2.1 Study Sites and Sampling*

At each of the control and drained sites (Section 3.2.1), three hummock and three hollow microforms were chosen to extract peat cores and to insert water wells for water table measurements (twelve microform sample sites in total; sampling in July 2010). Peat cores were immediately divided in the field into 15-cm intervals and 2-3 sections above and 2 sections below the water table were captured. Peat temperature was measured for all peat core intervals as they were sectioned in the field. Peat samples were saturated with water and slurried prior to measuring the pH with a glass electrode at the field laboratory station (Orion 720A).

### *4.2.2 Humification*

Degree of decomposition was determined on each peat section using a 10-point semi-quantitative index known as the von Post index of humification (Stanek & Silc, 1977). Briefly, the peat samples are observed to describe plant tissues ranging from unaltered (H1) to non-recognizable (H10) (Croft *et al.* 2001). Samples are then pressed in the hand and the appearance of the liquid ranges from clear and colourless (H1) to no free water (H10). The H-value is then used to classify the peat samples as fibric (H1-H4), mesic (H5-H6) and/or humic (H7-H10) (Croft *et al.* 2001).

#### *4.2.3 Determination of Anion Concentrations*

Peat samples were first slurried with 0.5 g peat to 2 mL Milli-Q water before the supernatant was extracted and centrifuged at  $17,970 \times g$  for 5 minutes. The resulting solution was then diluted 1:1 with Milli-Q water for each sample. As a final quality control measure, samples were filtered with a 45- $\mu\text{m}$  sterile Acrodisc® syringe filter with Supor® membrane prior to analyzing with a HPLC equipped with a Dionex IonPac AS18 anion exchange column. Total sample injection volume was 25  $\mu\text{L}$ . Resulting peaks were integrated using Dionex Chromeleon software. Before use, the HPLC was calibrated with linearly increasing standards and checked with laboratory blanks of deionized water.

#### *4.2.4 Diversity Indices and Statistical Methods*

Various indices were used in this study to explore the differences in the microbial community as organized against many factors including drainage treatment, microform type and water table levels. These indices include: (i) the Chao1 index for total species richness as a function of the ratio of singletons and doubletons and will exceed observed species richness as the relative frequency of singletons increases. Singletons are the number of observed species represented by a single individual and doubletons are the number of observed species represented by two individuals; (ii) the Shannon diversity index which increases with greater species richness and a more even distribution; (iii) the Simpson index ( $D$ ) of diversity (represented as  $1 - D$ ), ranges between 0 and 1 and represents the probability that two individuals randomly selected from the sample are different (the index increases as the community diversity increases); and, (iv) the Bray-Curtis similarity index which quantifies all possible differences between two communities for both species identity and relative abundance (a value of one

indicates completely similar communities). The equations used to estimate diversity are listed in the Appendix.

Multivariate statistics were performed using the general linear model in SYSTAT 13 (Cranes Software International, Chicago, IL). Resulting P values less than 0.05 were accepted as statistically significant. Hierarchical cluster analysis was conducted with Ward linkage algorithm and Euclidean distances with SYSTAT 13 (Cranes Software Int., Chicago, IL). The Ward linkage method averages all distances between pairs of objects in different clusters, with adjustments for covariances to decide how far apart the clusters are (SYSTAT 13).

## **4.3 Results**

### *4.3.1 Physicochemical Characteristics*

The physicochemical properties measured for each peat sample are presented in Table 4.1. Average pH was slightly higher at the drained site (pH=4.6) than the control site (pH=4.4) but ranged within all samples from 3.4 to 5.5. Peat soil temperature measurements for all samples ranged from 1.5 to 18.5°C at the control site and from 5.5 to 25.5°C at the drained site.

Table 4.1 Physicochemical properties (mean  $\pm$  SEM) of the peat samples and measurements taken at the Wandering River bog in July 2010. Soil temperature and pH are the average of section measurements at each microform, i.e., are averaged over depths.

Site	Control	Control	Drained	Drained
	Hummock	Hollow	Hummock	Hollow
pH	4.51 $\pm$ 0.19	3.97 $\pm$ 0.17	4.69 $\pm$ 0.11	4.59 $\pm$ 0.15
	(n=12)	(n=12)	(n=15)	(n=15)
Soil Temperature ( $^{\circ}$ C)	6.25 $\pm$ 1.4	8.08 $\pm$ 1.4	13.87 $\pm$ 1.5	12.97 $\pm$ 1.5
	(n=12)	(n=12)	(n=15)	(n=15)

#### 4.3.2 Anion Concentrations

Acetate and sulfate concentrations were significantly higher at the drained site while formate concentrations were significantly higher at the control site (Table 4.2; Figure 4.1). There was also an effect of microform and WT on acetate concentrations but this was not site specific as there were no interaction effects when microform and drainage treatment or WT and drainage treatment was statistically tested (Table 4.2). However, average acetate levels were higher at the hummocks as compared to the hollows (Figure 4.2) and were higher below the WT as compared to above the WT (Figure 4.3). There was a WT effect on nitrate, which was also site-specific as the control below the WT and the drained above the WT had the highest average nitrate concentrations (Table 4.2; Figure 4.3). Sulfate concentrations were significantly different between site microforms, as the drained hummock was significantly higher as compared to the control hummock (Table 4.2; Figure 4.3).

Table 4.2 General linear model results for concentrations of anions ( $\mu\text{mol g dry peat weight}^{-1}$ ).

DT=Drainage Treatment, WT=Water Table (Above/Below).

	Acetate		Formate		Nitrite		Sulfate		Nitrate	
	$F_{1,48}$	P	$F_{1,48}$	P	$F_{1,48}$	P	$F_{1,48}$	P	$F_{1,48}$	P
WT	4.80	<b>0.033</b>	3.39	0.072	0.02	0.880	0.84	0.363	4.24	<b>0.045</b>
DT	12.7	<b>0.001</b>	4.92	<b>0.031</b>	0.08	0.773	19.1	<b>0.000</b>	0.07	0.787
Microform	10.9	<b>0.002</b>	0.85	0.362	3.00	0.089	0.55	0.461	1.93	0.171
Microform x DT	1.79	0.187	0.68	0.412	0.26	0.612	7.06	<b>0.011</b>	0.12	0.727
WT x DT	1.97	0.167	0.46	0.500	2.47	0.123	0.25	0.616	5.33	<b>0.025</b>

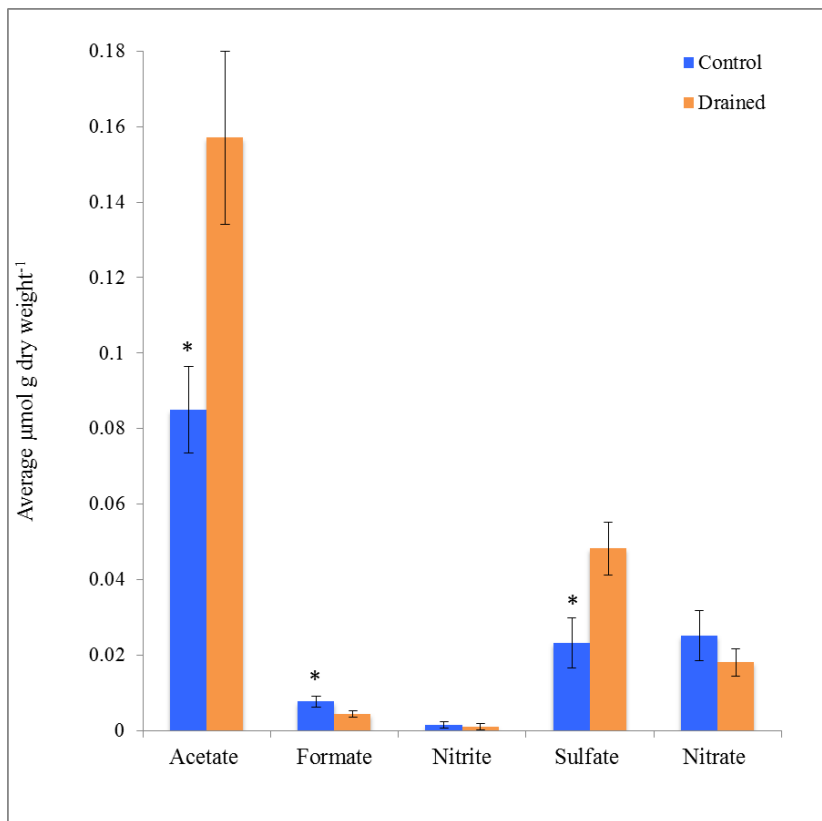


Figure 4.1 Average anion concentrations between the control (n=24) and drained sites (n=30) show significant differences for acetate, formate and sulfate ( $\pm 1$  SEM). Asterisks represent significant differences between control and drained site as per the general linear model (Table 4.2).

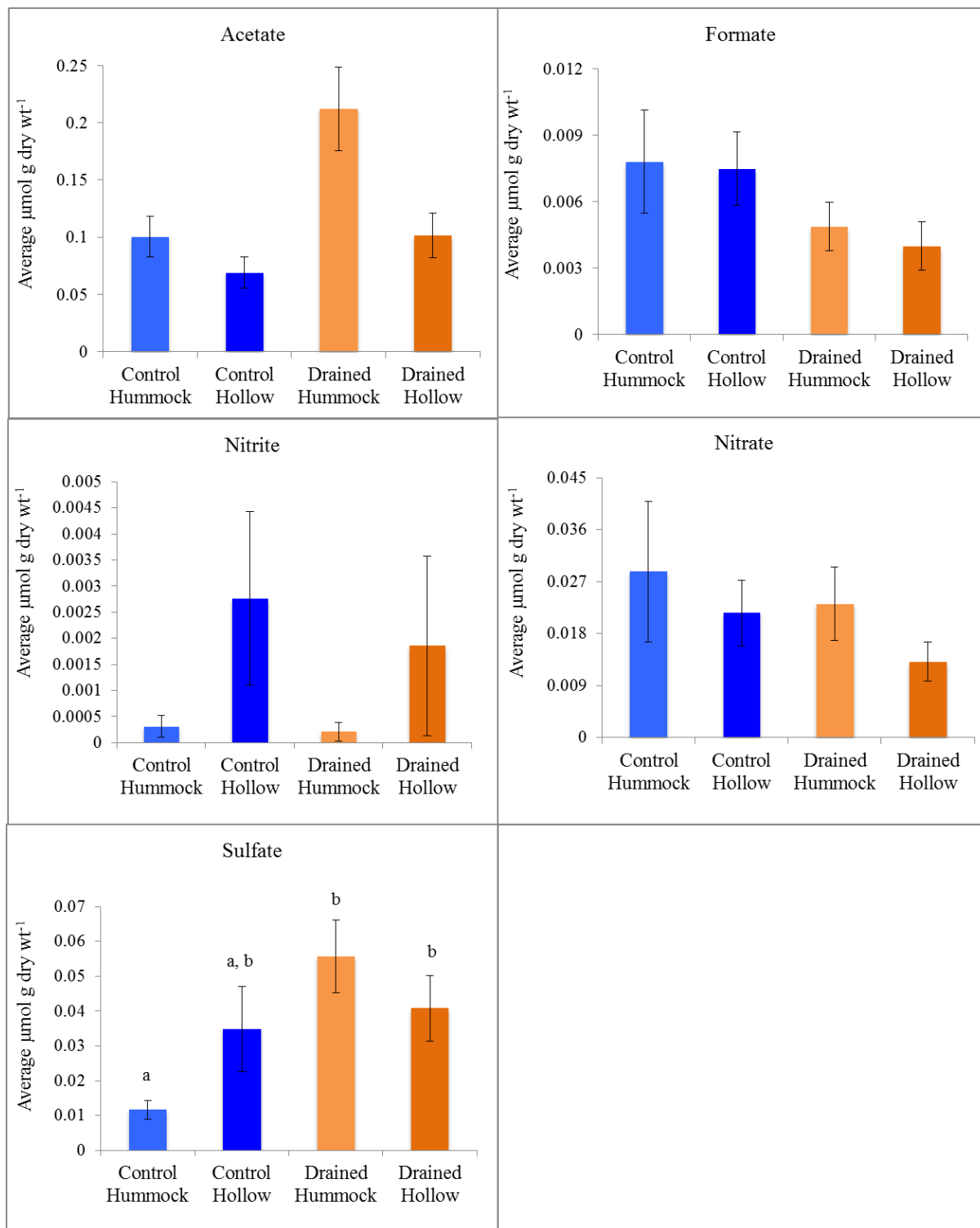


Figure 4.2 Average anion concentrations between the control and drained microforms show significant differences for sulfate ( $\pm 1$  SEM) with letters denoting significant differences. Control hummock and hollow n=12, drained hummock and hollow n=15.

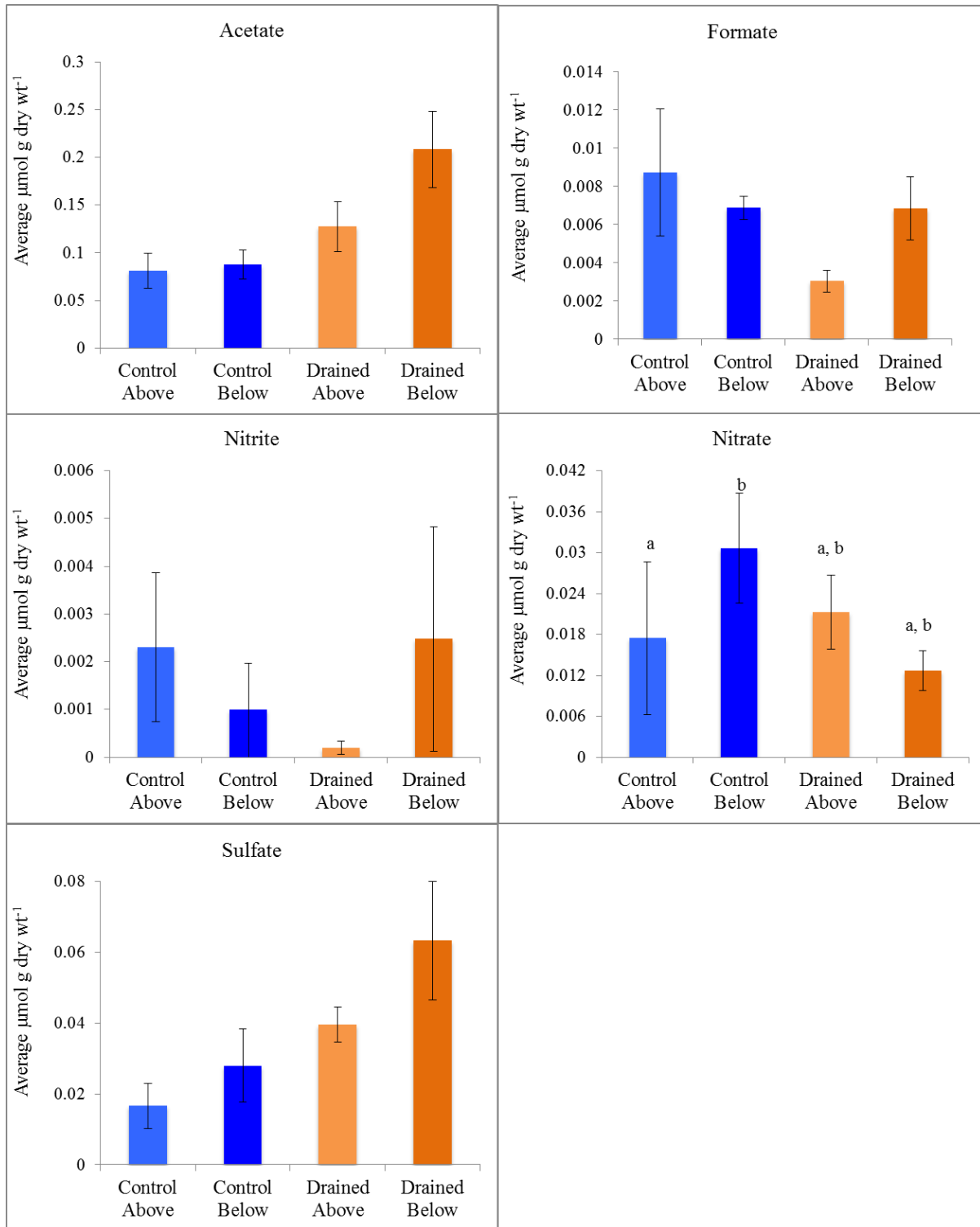


Figure 4.3 Average anion concentrations between the control and drained microforms show significant differences for nitrate ( $\pm 1$  SEM) with letters denoting significant differences. Control above n=10, control below n=14, drained above n=19, drained below n=11.

### 4.3.3 Humification

Of the 24 samples in the control site, 14 were considered to be fibric (von Post index H1 to H4) and 10 were considered mesic (H5 to H6) for a total ratio of 58% fibric and 42% mesic. Interestingly, the drained site was not much different as the 30 samples revealed 17 in the fibric range and another 13 in the mesic range for a total of 57% fibric and 43% mesic. There were no significant differences noted between average levels of humification and the drainage treatment ( $F_{1,52}=0.25$ ,  $P=0.617$ ) or between average levels of humification and the microforms ( $F_{3,50}=0.43$ ,  $P=0.736$ ). However, when plotting humification against the distance to the WT, there was a negative correlation between higher levels of humification (more mesic) with depth below the WT in both the control ( $F_{1,22}=53.67$ ,  $P=0.000$ ,  $R^2=0.71$ ) and drained site ( $F_{1,28}=46.17$ ,  $P=0.000$ ,  $R^2=0.62$ ) (Figure 4.4).

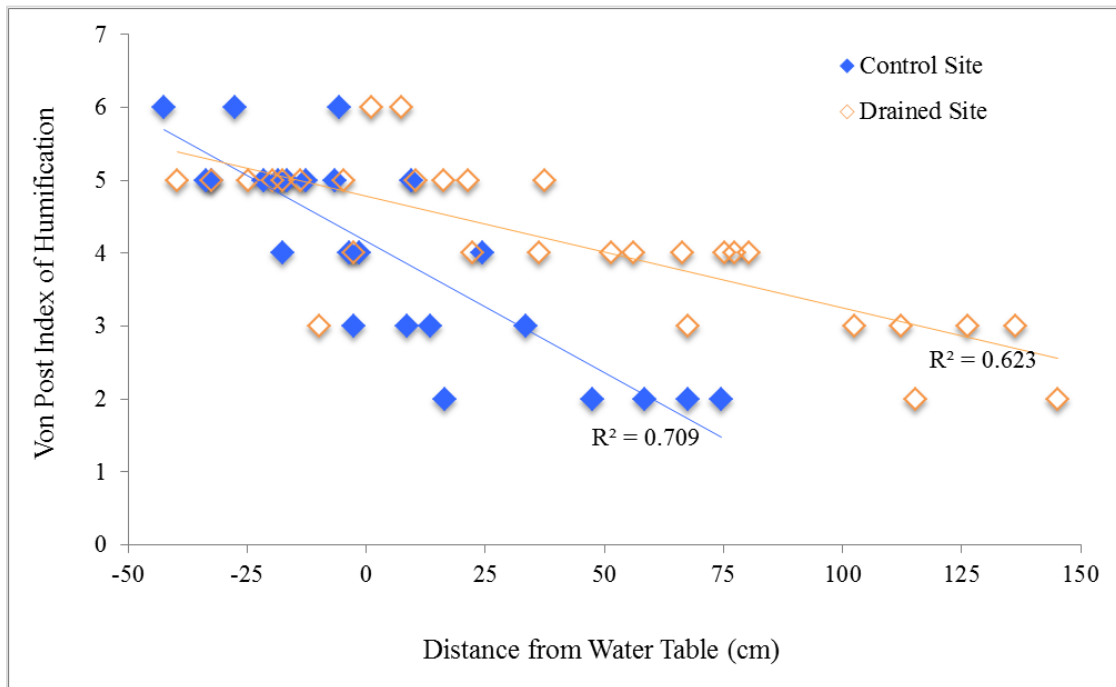


Figure 4.4 Regression of von Post index of humification against distance from the water table (shown at zero cm) shows samples below the water table (negative values) are more humified than those above (positive values).



The von Post index of humification was plotted against the aerobic potential CO<sub>2</sub> production and the PMP (Chapter 3) to determine how humification influenced these factors. Figure 4.5 (a) shows a negative correlation as increasing levels of humification resulted in decreasing aerobic potential CO<sub>2</sub> production (P=0.000, R<sup>2</sup>=0.53 control site; P=0.000, R<sup>2</sup>=0.43 drained site). Figure 4.5 (b) shows PMP decreased and was negatively correlated with increasing humification in the drained site (P=0.089, R<sup>2</sup>=0.06) and at the control site (P=0.025, R<sup>2</sup>=0.47).

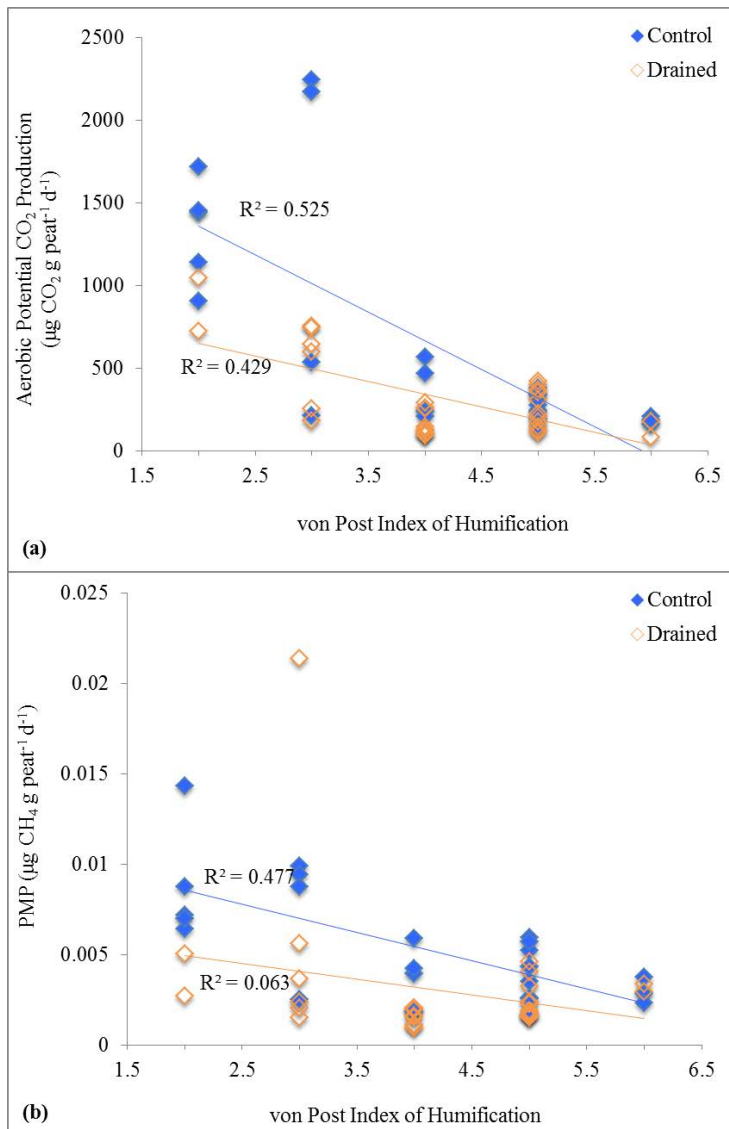


Figure 4.5 Regression plots of von Post index of humification against aerobic potential CO<sub>2</sub> production (a) and potential CH<sub>4</sub> production (PMP) (b) show a negative correlation at both sites.

#### 4.3.4 Effects of Physicochemical Parameters on Microbial Diversity

The Chao1, Shannon and Simpson indices were all calculated using QIIME at a depth of 3142 reads per sample, which eliminated one sample from the data set (n=53). Choosing a sampling depth that is common to most of the samples effectively normalizes the data set and allows for comparison of alpha diversity. In other words, the sampling depth of 3142 maintained the majority of the samples for comparison at a level in which the diversity indices were maximal and no longer influenced by sampling effort. The Chao1, Shannon and Simpson diversity indices were all higher at the drained site over the control site and also in the drained microforms over the control microforms (Table 4.3).

Table 4.3 Diversity indices between the control and drained sites along with the respective microforms. The Chao1, Shannon and Simpson indices were all higher at the drained site.

	Chao1	Shannon	Simpson
Control Site	952 ± 241	5.35 ± 0.66	0.89 ± 0.06
Control Hummock	820 ± 202	5.14 ± 0.55	0.89 ± 0.04
Control Hollow	1100 ± 182	5.62 ± 0.66	0.88 ± 0.08
Drained Site	1185 ± 387	6.60 ± 0.95	0.95 ± 0.04
Drained Hummock	1175 ± 382	6.60 ± 1.01	0.95 ± 0.04
Drained Hollow	1160 ± 422	6.59 ± 0.91	0.96 ± 0.03

To determine which physicochemical characteristics are related to microbial richness and diversity, multivariate statistical testing using a general linear model was utilized against the Chao1, Shannon and Simpson indices and the temperature, pH, humification, position to water table, drainage treatment and microform variables (Table 4.4). Interestingly, temperature and pH

were not found to be significant factors affecting microbial diversity but humification and position to the water table as well as interactions between drainage treatment, microforms, humification and position to the water table were all found to be significant factors affecting diversity. The control and the drained sites showed inverse relationships between the diversity indices and humification and position to the water table. That is, where the control site had a negative correlation with a diversity index and humification or position to the WT, the drained site had a positive correlation (Figures 4.6 and 4.7).

Table 4.4 General linear model results showing the influencing physicochemical factors of the Chao1, Shannon and Simpson diversity indices. Humification, microforms and position to the water table are significant factors driving microbial richness and diversity.

	Chao1		Shannon		Simpson	
	$F_{1,41}$	P	$F_{1,41}$	P	$F_{1,41}$	P
Temperature	0.09	0.758	0.02	0.888	2.21	0.145
pH	0.89	0.351	0.01	0.934	0.24	0.626
Humification	0.49	0.485	12.5	<b>0.001</b>	20.9	<b>0.000</b>
Position to WT	0.51	0.480	7.48	<b>0.009</b>	16.0	<b>0.000</b>
Drainage Treatment	0.21	0.649	2.04	0.160	2.59	0.115
Microform	1.71	0.198	8.13	<b>0.007</b>	7.83	<b>0.008</b>
Microform x Drainage Treatment	0.69	0.413	5.85	<b>0.020</b>	6.73	<b>0.013</b>
pH x Drainage Treatment	0.69	0.412	1.57	0.218	0.63	0.431
Humification x Drainage Treatment	1.33	0.255	0.28	0.603	5.14	<b>0.029</b>
Temperature x Drainage Treatment	3.84	0.057	0.15	0.704	2.05	0.160
Position to WT x Drainage Treatment	7.81	<b>0.008</b>	0.14	0.714	6.43	<b>0.015</b>

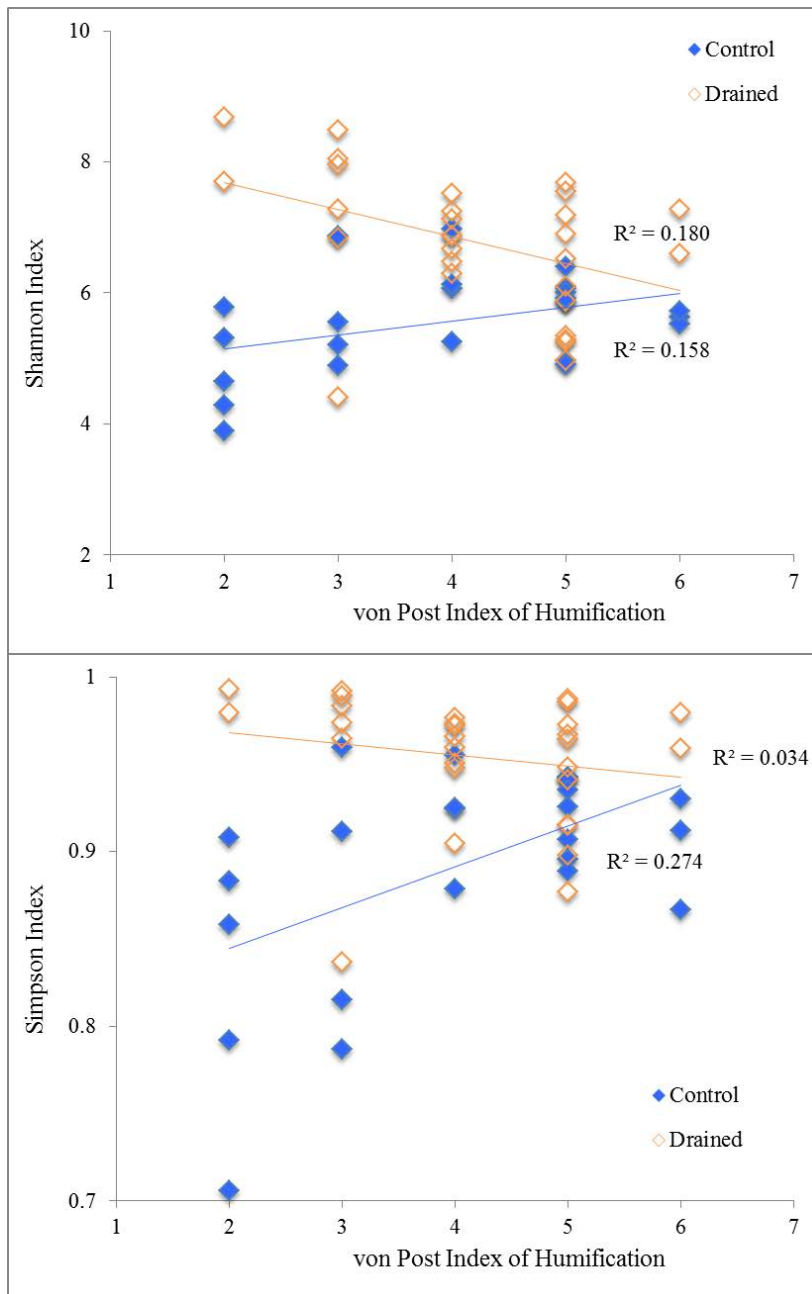


Figure 4.6 Regression plots of Shannon and Simpson indices against humification shows a positive correlation at the control site but a negative correlation at the drained site with increasing humification.

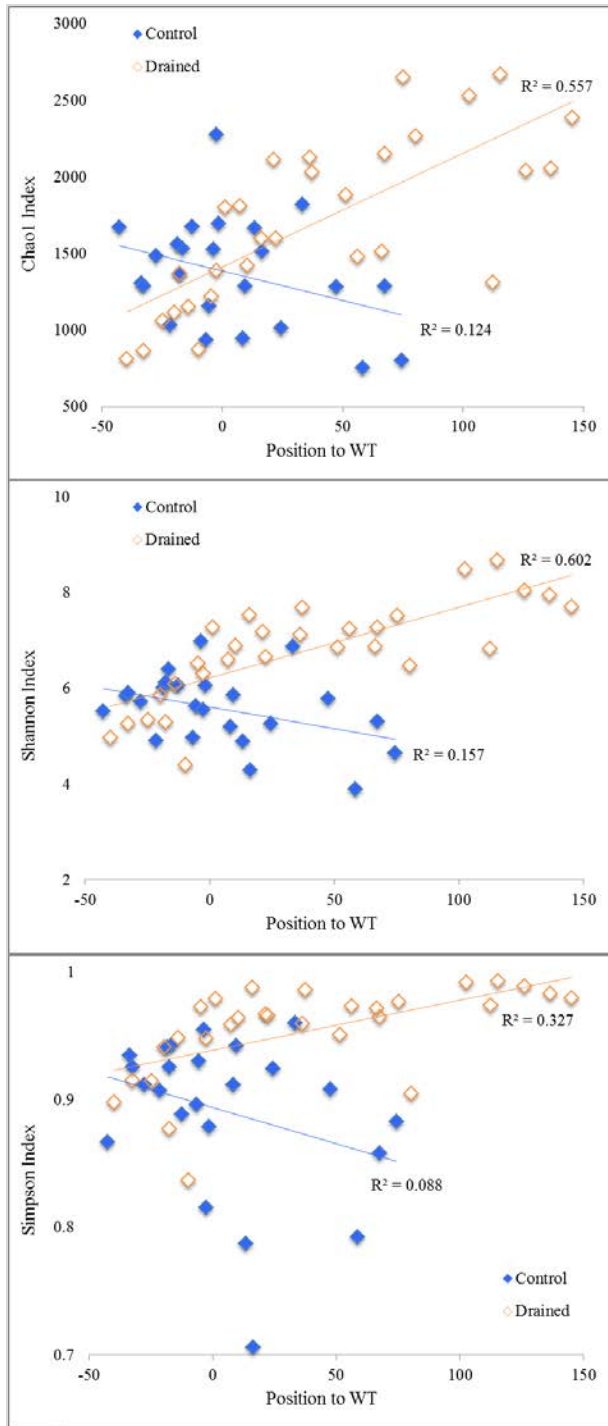


Figure 4.7 Regression plots of Chao1, Shannon and Simpson indices against position to water table shows a positive correlation at the drained site but a negative correlation at the control site with increasing dryness. Zero is the water table level, negative values are below the water table and positive values are above the water table.

The Bray-Curtis similarity index was used to identify those samples in which the microbial species present and their relative abundances were similar. A cluster dendrogram was constructed to show the relative similarities among samples (Figure 4.8). Seven main clusters are denoted which comprise samples with the following attributes:

1. Cluster one is comprised of all control samples that are mostly below the WT. Exceptions are CHA5065 and CHB5065, which are above the WT. Average pH 3.75; average temperature 2.9° C.
2. Cluster two is comprised of all control samples that are mostly above the WT (with the exception of CWB2035) and close to the surface. Average pH 3.99; average temperature 12.4° C.
3. Cluster three contains samples that are mostly from the drained site surface 0-15 cm range and are above the WT with the exception of CWA3550 and CWA5065, which are below the WT. Average pH 4.27; average temperature 18.9° C.
4. Cluster four is all drained site samples of which the majority is above the WT with the exception of DHA125140, which is below the WT. Average pH 4.34; average temperature 13.1° C.
5. Cluster five is deep samples that are mostly from the drained site in which most are above the WT with the exception of DHA140155, DWA105120 and DWB120135, which are below the WT. Average pH 4.69; average temperature 9.6° C.
6. Cluster six is mostly drained site samples all of which are below the WT. Average pH 5.37; average temperature 7.9° C.
7. Cluster seven is mostly control site samples all of which are below the WT. Average pH 5.17; average temperature 5.2° C.

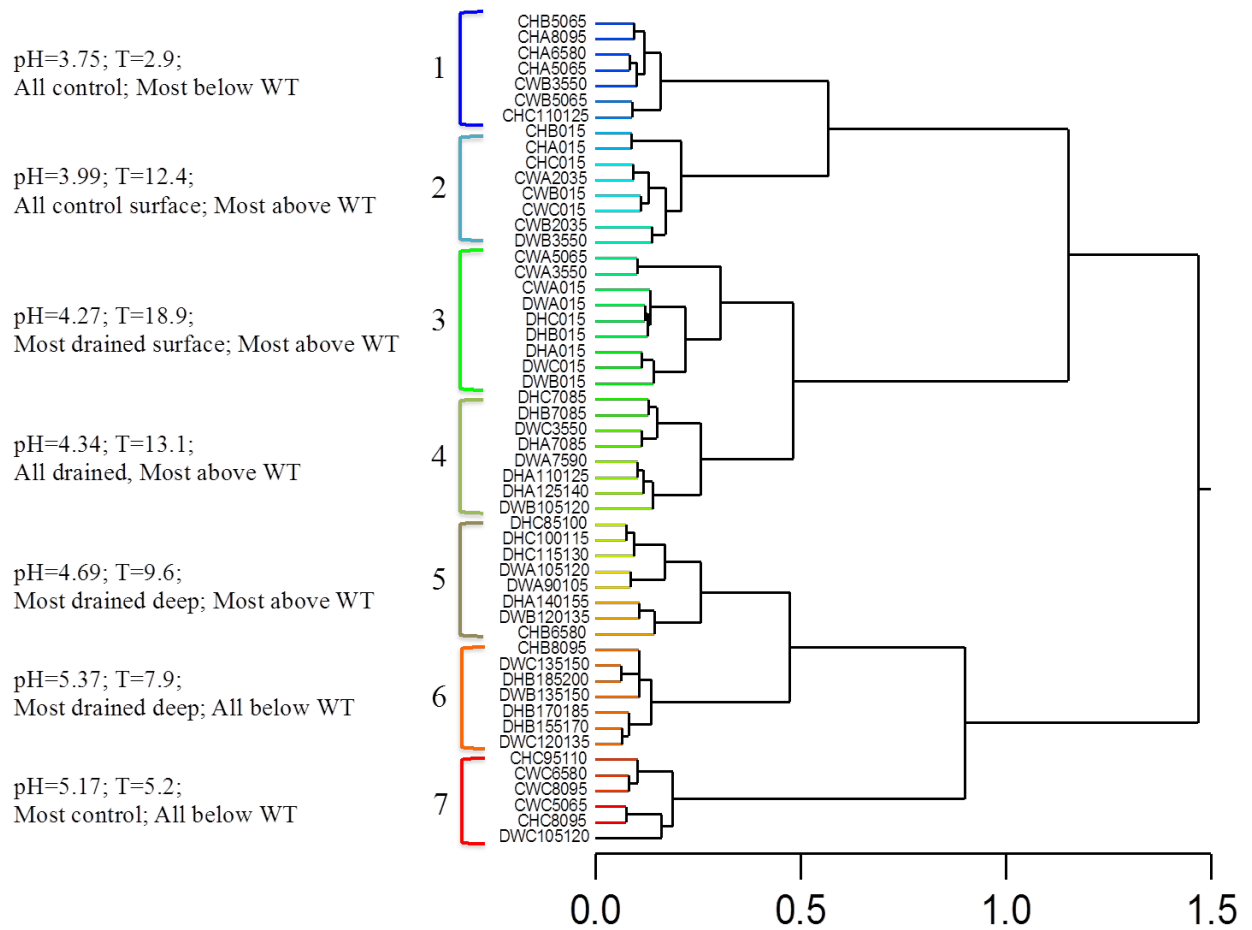


Figure 4.8 Distance-scaled dendrogram across all samples of the Bray-Curtis similarity index using the QIIME distance matrix and Ward linkage algorithm with Euclidean distances. CH=Control hummock, CW=Control hollow, DH=Drained hummock, DW=Drained hollow. Numbers refer to the depth of the sample from surface (i.e., 135150 = 135 to 150 cm) (Table 3.1). Temperature (T) and pH values are the averages of the samples in each cluster.

#### 4.3.5 Effects of Physicochemical Parameters on the Methanotroph Community

General linear model statistical testing was used to determine the physicochemical parameters affecting methanotroph relative abundance within the total microbial community, as well as specifically, the *Methylocella* and *Methylosinus/Methylocystis* groups as the two most abundant methanotroph groups. *Methylocella* and all methanotrophs relative abundance were significantly and positively correlated to nitrate concentrations at the drained site (Table 4.5; Figure 4.9). The drained site showed a stronger positive correlation to nitrate as compared to the control site for the relative abundance of *Methylocella* and all methanotrophs (Figure 4.9). Additionally, *Methylosinus/Methylocystis* relative abundance was significantly and negatively correlated to pH although there was no site-specific difference (Table 4.5; Figure 4.10).



Table 4.5 General linear model results show physicochemical factors in relation to *Methylocella*, *Methylosinus*/*Methylocystis* and all methanotrophs relative abundance. P values less than 0.05 (bold) were considered significant. Nitrate and pH were significant factors affecting methanotroph relative abundance. DT=Drainage Treatment.

	<i>Methylocella</i>		<i>Methylosinus</i> / <i>Methylocystis</i>		All Methanotrophs	
	<i>F</i> <sub>1,36</sub>	P	<i>F</i> <sub>1,36</sub>	P	<i>F</i> <sub>1,36</sub>	P
Position to WT	0.01	0.914	0.40	0.529	0.01	0.929
Humification	1.18	0.286	0.39	0.538	1.48	0.232
Acetate	0.24	0.627	0.32	0.574	0.90	0.350
Formate	0.20	0.655	0.18	0.677	0.02	0.880
Sulfate	0.00	0.995	2.28	0.140	0.07	0.799
Nitrate	10.9	<b>0.002</b>	0.98	0.330	10.7	<b>0.002</b>
pH	0.06	0.806	6.45	<b>0.016</b>	1.43	0.240
Temperature	0.15	0.698	0.00	0.994	0.02	0.892
Drainage Treatment	1.54	0.223	2.17	0.149	0.24	0.631
DT x Position to WT	0.00	0.963	2.17	0.150	0.15	0.701
DT x Humification	0.00	0.989	0.98	0.328	0.14	0.710
DT x Acetate	0.03	0.862	0.12	0.733	0.02	0.881
DT x Formate	0.14	0.710	0.32	0.575	0.00	0.956
DT x Sulfate	0.02	0.879	0.64	0.428	0.02	0.882
DT x Nitrate	8.99	<b>0.005</b>	0.74	0.394	8.29	<b>0.007</b>
DT x pH	1.32	0.258	2.74	0.106	0.12	0.728
DT x Temperature	0.71	0.405	1.97	0.169	0.05	0.828

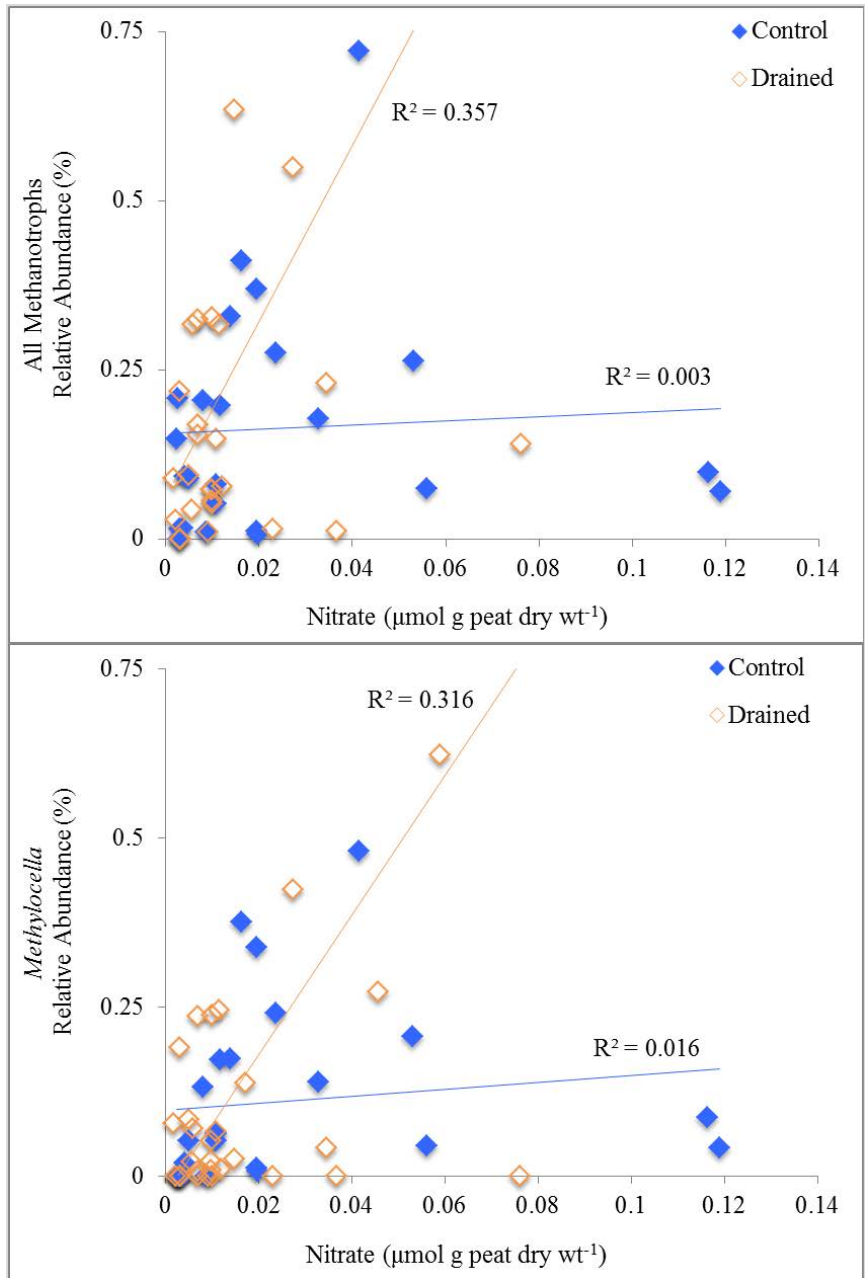


Figure 4.9 Regression plots with relative abundances of all methanotrophs and *Methylocella* against nitrate shows a significant and positive correlation at the drained site.

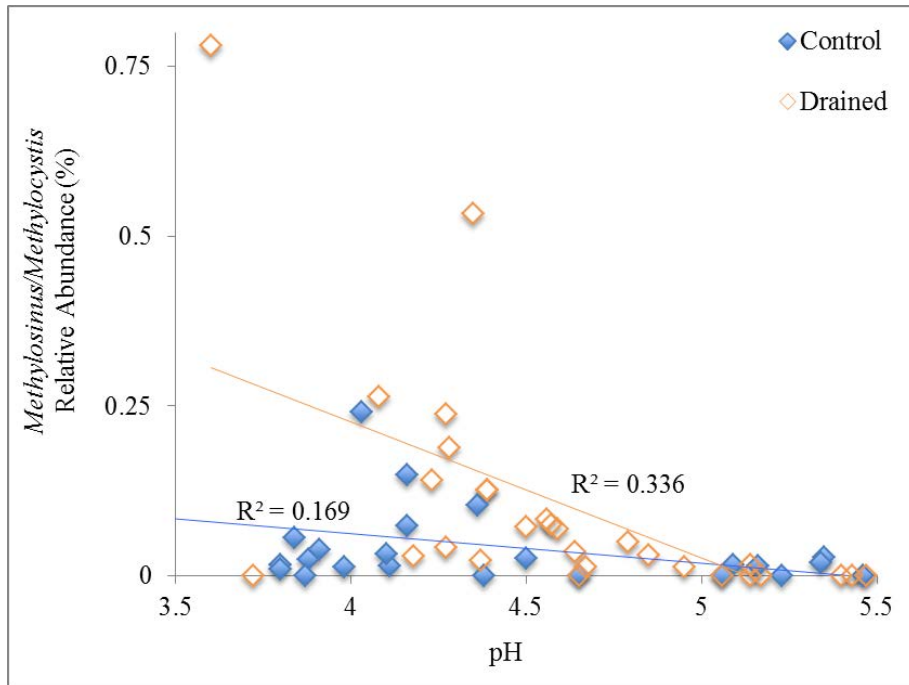


Figure 4.10 Regression plot with relative abundance of *Methylosinus/Methylocystis* against pH. While pH was not a significant factor affecting relative abundance between sites, pH was negatively correlated with decreasing *Methylosinus/Methylocystis* relative abundance.

#### 4.3.6 Effects of Physicochemical Parameters on the Methanogen Community

General linear model statistical testing was used to determine the physicochemical factors affecting methanogen relative abundance compared to the total microbial community for the RCII methanogen group as the most abundant methanogen group and the acetoclastic methanogen group. The acetoclastic methanogens were defined as those reads classified under the *Methanosarcinales* order.

RCII relative abundance was significantly positively correlated to humification and to nitrate concentrations although there was not an interaction effect with drainage treatments (Table 4.6; Figure 4.11). However, there was a main and drainage treatment interaction effect of pH in relation to RCII relative abundance (Table 4.6; Figure 4.11). As pH increased, RCII relative abundance increased at both sites (Figure 4.11). Conversely, temperature and position to the WT were significantly and negatively correlated to RCII relative abundance at the drained site but there was not a main effect (Table 4.6; Figure 4.11).

Acetoclastic methanogen relative abundance was significantly and negatively correlated to temperature although there was not an interaction effect with drainage treatments (Table 4.6; Figure 4.12). However, acetoclastic methanogen relative abundance was significantly and positively correlated to formate and sulfate at the drained site (Figure 4.12).

Table 4.6 General linear model results showing the physicochemical factors affecting the Rice Cluster II and acetoclastic methanogen groups. P values less than 0.05 (bold) were considered significant. Nitrate, pH, temperature, humification, position to the WT, formate and sulfate were all significant factors affecting methanogen relative abundance. DT=Drainage Treatment.

	Acetoclastic		RCII	
	Methanogens		Methanogens	
	$F_{1,36}$	P	$F_{1,36}$	P
Position to WT	0.04	0.853	0.63	0.434
Humification	1.11	0.300	4.56	<b>0.040</b>
Acetate	3.85	0.058	2.24	0.143
Formate	0.07	0.789	0.75	0.393
Sulfate	0.00	0.965	0.44	0.512
Nitrate	0.10	0.760	4.88	<b>0.034</b>
pH	1.18	0.284	21.5	<b>0.000</b>
Temperature	4.43	<b>0.042</b>	0.79	0.377
Drainage Treatment	2.62	0.114	3.72	0.062
DT x Position to WT	0.09	0.768	6.22	<b>0.017</b>
DT x Humification	0.79	0.382	1.01	0.321
DT x Acetate	3.43	0.072	1.70	0.200
DT x Formate	4.51	<b>0.041</b>	0.84	0.365
DT x Sulfate	4.96	<b>0.032</b>	1.10	0.302
DT x Nitrate	0.99	0.324	2.68	0.110
DT x pH	0.67	0.417	12.6	<b>0.001</b>
DT x Temperature	3.76	0.060	6.88	<b>0.013</b>

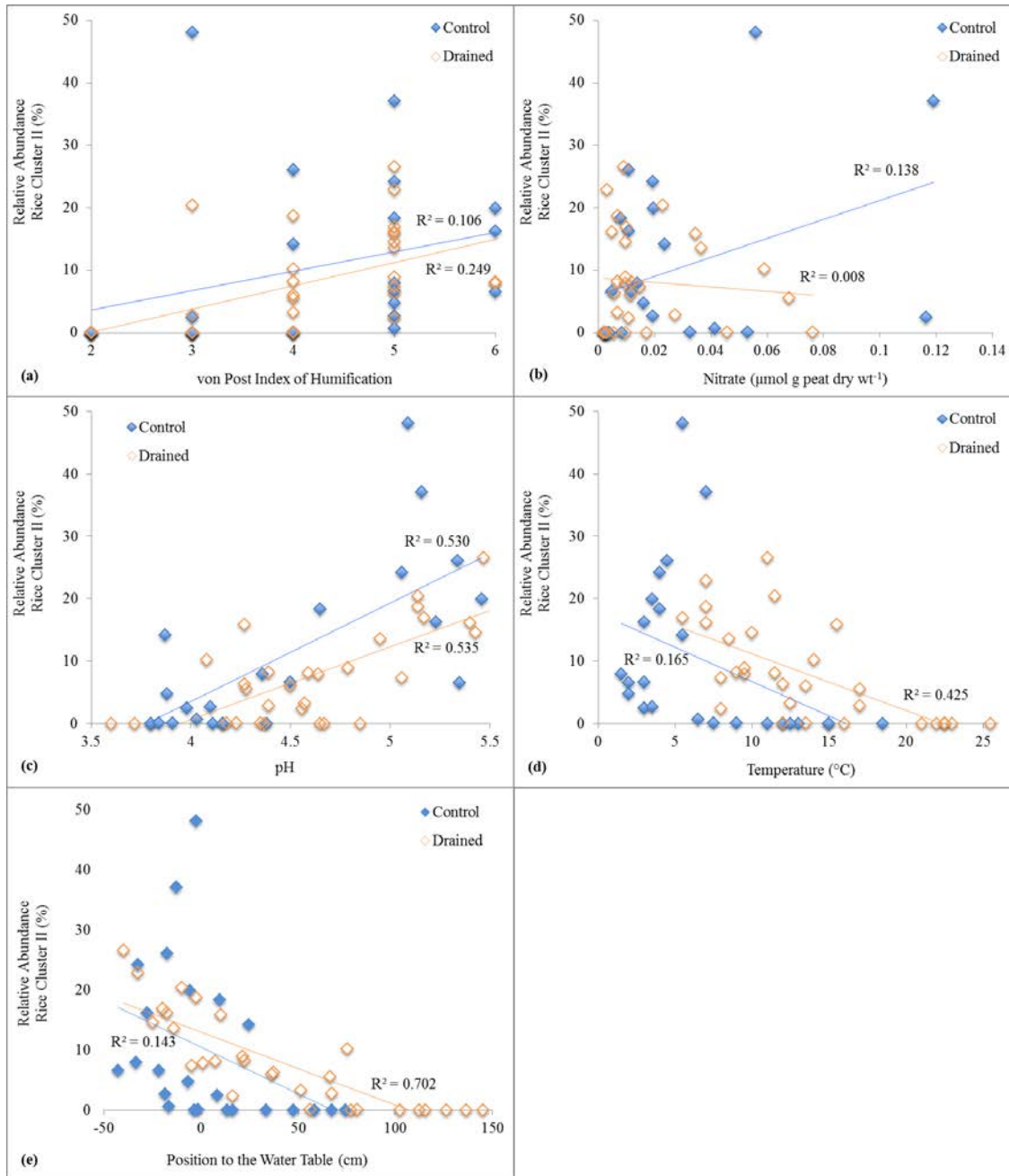


Figure 4.11 Regression plots with relative abundance of Rice Cluster II methanogens against humification (a), nitrate (b), pH (c), temperature (d) and position to the water table (e). Humification and nitrate was positively correlated as a main effect whereas pH was positively correlated as main effect and a site interaction effect. Temperature and position to the water table showed a significant negative correlation at the drained site.

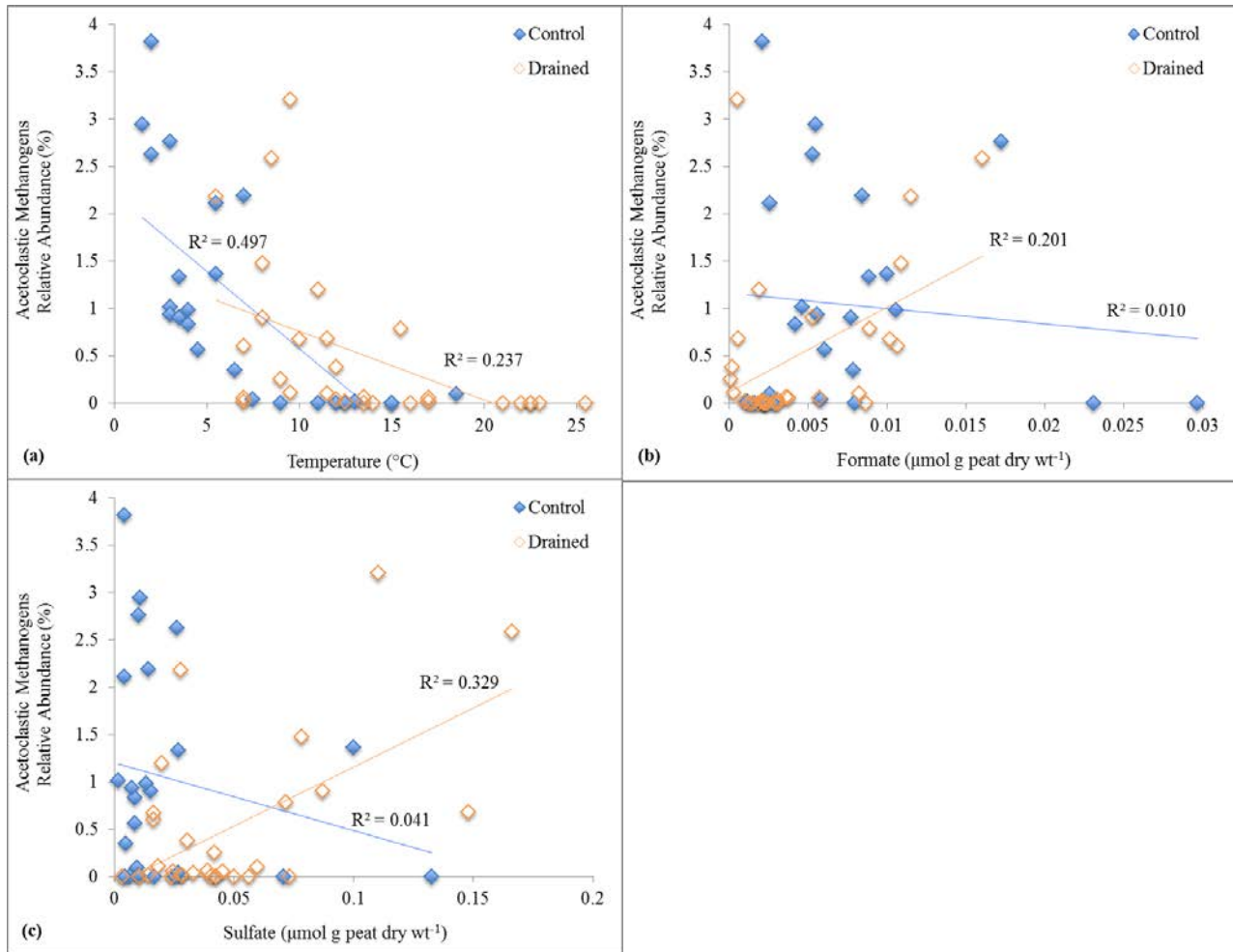


Figure 4.12 Regression plots with relative abundance of acetoclastic methanogens against temperature (a), formate (b) and sulfate (c). Temperature was significantly and negatively correlated to acetoclastic methanogen relative abundance at both sites. Formate and sulfate concentrations were significantly and positively correlated to acetoclastic methanogen relative abundance at the drained site.

## 4.4 Discussion

### 4.4.1 Effect of Temperature and pH on Microbial Community

While the Bray-Curtis index showed a pattern of microbial clustering that appeared to differ with pH and temperature, microbial diversity and richness was not significantly affected by temperature or pH. In a study by Fierer and Jackson (2005), soil pH was observed to be the best predictor of bacterial richness, diversity and composition at a continental scale but vegetation type, carbon availability, nutrient availability and soil moisture were noted to influence microbial communities at a local scale. Additionally, Lauber *et al.* (2009) proposed that soil pH may not directly alter bacterial community structure but may act as an integrated index of soil conditions. For instance, there are a number of soil characteristics related either directly or indirectly to soil pH (e.g., nutrient availability, soil moisture regime, salinity and organic C characteristics) (Lauber *et al.* 2009).

For the methanotrophs, only *Methylosinus/Methylocystis* was significantly and negatively correlated to pH. *Methylosinus/Methylocystis* is mostly neutrophilic although at least one species has been identified as a moderate acidophile (Dunfield & Dedysh, 2010). It is interesting that there is a negative pH effect on the relative abundance of *Methylosinus/Methylocystis* as this group is mostly neutrophilic, suggesting there are other confounding effects influencing relative abundance.

For the methanogens, RCII was significantly and positively correlated to pH at both sites and negatively correlated to temperature at the drained site. While RCII does not have any pure-culture representatives to definitively determine physiological capabilities, an enrichment of the related RCI methanogens was successfully obtained under neutral pH and low temperature conditions (Sakai *et al.* 2007). Additionally, the acetoclastic methanogen relative abundance was



significantly and negatively correlated to temperature at both sites. Kotsyurbenko *et al.* (2007) found the dominance of acetoclastic methanogenesis in peat samples diminished with decreasing temperatures (4°C or 15°C from 25°C) while concurrently the hydrogenotrophic methanogenesis pathway was enhanced. While the present study did not measure and distinguish acetoclastic vs. hydrogenotrophic methanogenesis, the data suggest lowered overall temperatures decrease the relative abundance of acetoclastic methanogens.

In a previous five year experimental warming study of a temperate mountain forest soil, intensive warming of +4°C did not affect microbial biomass or abundance of major microbial groups of the forest topsoil (Schindlbacher *et al.* 2011). The researchers found that shifts in the microbial community composition are not likely to occur until other variables such as substrate become limited (Schindlbacher *et al.* 2011). There was not a significant temperature effect on diversity, evenness and richness on the methanotroph relative abundance; however, temperature and the position to the WT are correlated to one another. As Schindlbacher *et al.* suggest, substrate limitation may be a better explanation for microbial community composition and this is better determined through the analysis presented between position to the WT and humification and the microbial community.

#### *4.4.2 Effect of Water Table Position and Humification on Microbial Community*

Increasing humification was negatively correlated with aerobic potential CO<sub>2</sub> production and PMP. Glatzel *et al.* (2004) also found a significant negative correlation with CO<sub>2</sub> production and CH<sub>4</sub> production and humification suggesting humification is an important control on CO<sub>2</sub> and CH<sub>4</sub> production. While humification was considered to be similar between sites, the method of sampling is semi-quantitative and this data would likely benefit from increased rigor in

technique with the use of Fourier Transform Infrared Spectroscopy (FTIR) to quantify the range of readily metabolized compounds.

Humification was a significant factor correlated to microbial diversity and richness but with inverse relationships at the sites. As humification increased, the microbial diversity and richness increased at the control site but decreased at the drained site. Similarly, position to the WT was significantly correlated with microbial diversity and richness. However, microbial diversity and richness increased with drier conditions at the drained site but decreased at the control site. Peatlands are known to have high densities of both aerobic microorganisms and an abundance of acid-tolerant anaerobic archaea (such as methanogens) adding to overall diversity (Thomas & Pearce, 2004); however, it is interesting to note the inverse effects of drier conditions and diversity measures between the sites although the drained site exhibited a much stronger correlation.

For functional microbial communities, humification had a significant positive correlation with the relative abundance of RCII methanogens at both sites. Additionally, position to the WT had a significant negative correlation with RCII relative abundance at the drained site. However, without distinguishing the principle chemical classes through a more quantitative technique such as FTIR, it is impossible to identify what specific component of the humified material (e.g., fats, aromatics, humic acids) are physiologically affecting the RCII relative abundance at the drained site. Also, it is important to note that the WT is an important control on other factors such as vegetation type, hydraulic conductivity, gas and nutrient exchange that ultimately affect the longevity and types of microbial communities present. For instance, Hornibrook *et al.* (1997), showed acetoclastic methanogenesis is favoured in the upper layers of peat as correlated with labile carbon substrates whereas hydrogenotrophic methanogenesis predominates in the more

recalcitrant deeper peat layers. This suggests both types of methanogens would be significantly affected by the WT position and humification although there was a positive correlation only apparent with the RCII methanogens with humification and position to the WT.

#### *4.4.3 Effect of Anion Concentrations on Microbial Community*

As acetate is a main intermediate during anaerobic decomposition, acetate can accumulate to high levels in a peatland in the absence of acetoclastic methanogenesis (Horn *et al.* 2003). While no significant differences were found in the relative abundances of acetoclastic methanogens between sites, a significantly higher concentration of acetate was noted in the drained site as compared to the control site. Therefore, one possible explanation for the higher concentration of acetate at the drained site may be related to a more active proportion of hydrogenotrophic methanogenesis as compared to acetoclastic methanogenesis. However, there may be other dynamics to take into account including the activity levels of acetate-consuming sulfate-reducing bacteria. Without measuring the fraction of acetoclastic vs. hydrogenotrophic methanogenesis at both sites, it is difficult to say whether higher levels of acetate at the drained site are related to the acetoclastic methanogens or some other confounding effect.

Methane production is usually inhibited by the presence of sulfate, iron or nitrate since these reducers typically outcompete methanogens for common electron donors (Conrad, 1996). However, increasing sulfate concentrations was significantly and positively correlated to the relative abundance of acetoclastic methanogens at the drained site. This is contrary to a study by Minderlein and Blodau (2010), who showed the addition of sulfate inhibited methanogenesis in the peat through bacterial sulfate reduction, which also accelerated anoxic CO<sub>2</sub> production. While the results of the present study also show a higher CO<sub>2</sub> flux at the drained site, it is

unusual that there would be a positive correlation of sulfate concentrations to relative abundance of acetoclastic methanogens and not hydrogenotrophic methanogens. This data suggests as above, there may be a confounding effect of lowered activity levels of sulfate-reducing bacteria.

The drained site relative abundance of acetoclastic methanogens was significantly and positively correlated to the formate concentrations. Formate can be used as an alternative to  $H_2$  in conjunction with  $CO_2$  during hydrogenotrophic methanogenesis and therefore it is surprising that formate concentrations showed a positive correlation with the relative abundance of the drained site acetoclastic methanogens.

Anion concentrations were not a significant factor affecting the relative abundances of methanotrophs with the exception of nitrate concentrations at the drained site. It has been previously established that the introduction of nitrogen in fertilizers or in rainfall deposition has resulted in a loss of  $CH_4$  oxidation activity in oxic soils (Kravchenko, 2002). The effect of nitrate on  $CH_4$  oxidation does not appear to be well documented in peat soils; Kravchenko (2002) found nitrate and other oxidized nitrogen compounds had an inhibiting effect on  $CH_4$  oxidation. However, increasing nitrate concentrations were significantly and positively correlated to the methanotroph relative abundance at the drained site. Bodelier and Laanbroek (2004) provided a review on nitrogen addition as a stimulating factor of  $CH_4$  oxidation and found both the consumption of atmospheric  $CH_4$  and the oxidation of elevated  $CH_4$  concentrations can be enhanced by the addition of nitrate or ammonium. The PMO data would have been useful to determine if  $CH_4$  oxidation is enhanced at the drained site compared to the control site.

## Chapter Five: Thesis Summary and Conclusions

Peatlands have the potential to mediate strong feedbacks on the global climate system but their response to future climate change is uncertain, as they are a complex matrix in which to study carbon cycling. The heterogeneity of these ecosystems produces fluctuations in vegetation, temperature, pH, hydrology, chemistry and redox conditions at many different scales.

Drainage resulted in an increase of vascular plant and tree growth leading to enhanced plant and root respiration at the drained site. While the drained site had a lower potential for CO<sub>2</sub> production due to a lower quality substrate over the control site, the increased growth in trees and shrubs resulted in a higher CO<sub>2</sub> efflux from plant and root respiration. Additionally, PMP was very low in comparison to other studies at both sites and this finding supports the low quality substrate shown in the potential CO<sub>2</sub> production experiment.

The methanotrophs detected were consistent with the major groups found in peat bogs although there was a high relative abundance of just two groups: *Methylocella* and *Methylosinus/Methylocystis*. It is not surprising that *Methylocella* was the major methanotroph detected in both peat bogs as even in an environment in which CH<sub>4</sub> substrate is limited, *Methylocella* is a facultative methanotroph and can utilize other substrates such as acetate and ethanol for growth and survival.

Peat bogs provide a poor habitat for a diverse group of methanogens, but even so, it is interesting that the methanogens detected in these sites were dominated by the RCII group. Since hydrogenotrophic methanogenesis is the primary pathway of methane production in an ombrotrophic peat bog, and no pure cultures of RCII exist to define physiological capabilities, RCII is presumed to be a group of hydrogenotrophic methanogens. The hydrogenotrophic

dominance is consistent with literature, as we would not expect to see a large relative abundance of acetoclastic methanogens in these ombrotrophic peat bogs.

Soil pH has been observed to be the best predictor of diversity and richness on a continental scale but not at a local scale (Fierer and Jackson, 2005). The results of the present study are consistent with this observation, as pH did not affect microbial diversity and richness. However, increasing pH affected individual species: it was negatively correlated with *Methylosinus/Methylocystis* relative abundance but was positively correlated the RCII relative abundance. Additionally, both RCII and the acetoclastic methanogens relative abundance were negatively correlated with increasing temperatures. However this may have more to do with the position to the WT, which is highly correlated to peat soil temperature.

Humification was significantly correlated to microbial diversity and richness but with an inverse relationship between the control and drained sites (negative at the drained site, positive at the control site). Microbial diversity and richness also increased at the drained site with drier conditions but the opposite was found in the control site. Enhanced laboratory methods to improve the quantification of humification is important to resolve this data; however, it is evident that there are confounding effects on microbial diversity and richness at the two sites beyond humification and position to the WT.

Unexpectedly, sulfate and formate showed a positive correlation with the relative abundance of the acetoclastic methanogens but only at the drained site. This is unusual since the addition of sulfate has been shown to inhibit methanogenesis (Minderlein and Blodau, 2010) and formate can be used as an alternative to H<sub>2</sub> in hydrogenotrophic methanogenesis (Garcia *et al.* 2000; Galand, 2004). This finding suggests there are other unmeasured confounding variables

such as the presence and activity levels of sulfate-reducing bacteria, which may be affecting the methanotroph and methanogen communities.

It is apparent that the control and drained sites differed significantly in terms of the physicochemical factors affecting microbial relative abundance. It is likely that the differences between these two sites in terms of the physicochemical factors affecting microbial relative abundance are at least partly due to the temporal resolution of this data as there may be varying interannual and interseasonal effects not shown. Seasonal dynamics have been previously documented to show large variation in peatland biogeochemical fluxes (including CH<sub>4</sub> oxidation, CO<sub>2</sub> and CH<sub>4</sub> production) affecting pH, nutrients, substrate availability and temperature regimes all of which influence microbial community and activity (Regina *et al.* 1999; Strack *et al.* 2004; Kraigher *et al.* 2006; Moore *et al.* 2011). Therefore, as Moore *et al.* (2011) also determined, the relative importance of the driving factors affecting microbial communities and activities at these two peatlands depends on the time scale and spatial measurements of these variables. Additionally, while it is unlikely, it is possible the physical distance between the two sites (9 km) can produce differences in atmospheric inputs, which would affect the results presented in this thesis.

To build on the results found in this study, future research could include the collection of pore water CH<sub>4</sub> to determine the fractions of CH<sub>4</sub> production from acetoclastic and hydrogenotrophic methanogenesis. It would be interesting to see why acetate concentrations were significantly higher at the drained site and if the acetoclastic methanogenesis was inhibited. Additionally, the results presented in this study are based on relative abundances and not actual abundances of bacteria and archaea. It would be useful to correlate methanogenesis and methanotrophy to quantified methanogens and methanotrophs.

## References

- Amaral-Zettler LA, Zettler ER and Amils R (2010). Pyrotag sequencing across three domains and two seasons unveils the Río Tinto's 'rare biosphere.' *Astrobiology Science Conference 2010: Evolution and Life: Surviving Catastrophes and Extremes on Earth and Beyond*. Held April 26-30, 2010 in League City, Texas. LPI Contribution No. 1538, p. 5337.
- Andersen R, Francez A-R and Rochefort L (2006). The physicochemical and microbiological status of a restored bog in Quebec: Identification of relevant criteria to monitor success. *Soil Biology & Biochemistry*, 38: 1375-1387.
- Andriess JP (1988). Nature and management of tropical peat soils. *Food and Agriculture Organization of the United Nations*, FAO Soils Bulletin 59. Rome, 1988.
- Ausec L, Kraigher B and Mandic-Mulec I (2009). Differences in the activity and bacterial community structure of drained grassland and forest peat soils. *Soil Biology & Biochemistry*, 41: 1874-1881.
- Bäckstrand K, Crill PM, Mastepanov M, Christensen TR and Bastviken D (2008). Non-methane volatile organic compound flux from a subarctic mire in Northern Sweden. *Tellus*, 60B: 226-237.
- Basiliko N, Blodau C, Roehm C, Bengtson P and Moore TR (2007). Regulation of decomposition and methane dynamics across natural, commercially mined, and restored northern peatlands. *Ecosystems*, 10: 1148-1165.
- Bellisario LM, Bubier JL, Moore TR and Chanton JP (1999). Controls of CH<sub>4</sub> emissions from a northern peatland. *Global Biogeochemical Cycles*, 13(1): 81-91.



- Belova SE, Pankratov TA and Dedysh SN (2006). Bacteria of the genus *Burkholderia* as a typical component of the microbial community of Sphagnum peat bogs. *Microbiology*, 75(1): 90-96.
- Belyea L (2009). *Nonlinear dynamics of peatlands and potential feedbacks on the climate system*. Baird A, Belyea L, Comas X, Reeve A & Slater L (eds.) American Geophysical Union, Washinton, DC, Geophysical Monograph 184, ISBN: 978-0-87590-449-8 pp. 5-18.
- Blodau C (2002). Carbon cycling in peatlands – a review of processes and controls. *Environmental Reviews*, 10(2): 111-134. National Research Council Canada.
- Blodau C and Moore TR (2003). Micro-scale CO<sub>2</sub> and CH<sub>4</sub> dynamics in a peat soil during a water fluctuation and sulfate pulse. *Soil Biology & Biochemistry*, 35: 535-547.
- Bodelier PLE and Laanbroek HJ (2004). Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiology Ecology*, 47: 265-277.
- Bräuer SL, Cadillo-Quiroz H, Ward RJ, Yavitt JB and Zinder SH (2011). *Methanoregula boonei* gen. nov., sp. nov., an acidiphilic methanogen isolated from an acidic peat bog. *International Journal of Systematic and Evolutionary Microbiology*, 61: 45-52.
- Bridgham SD and Richardson CJ, (1992). Mechanisms controlling soil respiration (CO<sub>2</sub> and CH<sub>4</sub>) in southern peatlands. *Soil Biology & Biochemistry*, 24(11): 1089-1099.
- Cadillo-Quiroz H, Brauer S, Yashiro E, Sun C, Yavitt J and Zinder S (2006). Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York State, USA. *Environmental Microbiology*, 8(8): 1428-1440.

- Cadillo-Quiroz H, Yavitt JB and Zinder SH (2009). *Methanosphaerula palustris* gen. nov., sp. nov., a hydrogenotrophic methanogen isolated from a minerotrophic fen peatland. *International Journal of Systematic and Evolutionary Microbiology*, 59: 928-935.
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsuneko T, Zaneveld J and Knight R (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5): 335-336.
- Chambers FW, Beilman DW and Yu Z (2011). Methods for determining peat humification and for quantifying peat bulk density, organic matter and carbon content for palaeostudies of climate and peatland carbon dynamics. *Mires and Peat*, 7: 1-10.
- Charman D (2002). *Peatlands and environmental change*. West Sussex, England: John Wiley & Sons, Ltd.
- Chen Y, McNamara NP, Dumont MG, Bodrossy L, Stralis-Pavese N and Murrell JC (2008). The impact of burning and *Calluna* removal on below-ground methanotroph diversity and activity in peatland soil. *Applied Soil Ecology*, 40: 291-298.
- Chivers MR, Turetsky MR, Waddington JM, Harden JW and McGuire AD, (2009). Effects of experimental water table and temperature manipulations on ecosystem CO<sub>2</sub> fluxes in an Alaskan rich fen. *Ecosystems*, 12: 1329-1342.
- Choi W-J, Chang SX, Bhatti JS (2007). Drainage affects tree growth and C and N dynamics in a minerotrophic peatland. *Ecology*, 88(2): 443-453.

- Clymo RS (1984). The limits to peat bog growth. *Philosophical Transactions of the Royal Society of London*, 303B: 605–654.
- Croft M, Rochefort L and Beauchamp CJ (2001). Vacuum-extraction of peatlands disturbs bacterial population and microbial biomass carbon. *Applied Soil Ecology*, 18: 1-12.
- Conrad R (1996). Soil microorganisms as controllers of atmospheric trace gases (H<sub>2</sub>, CO, CH<sub>4</sub>, OCS, N<sub>2</sub>O and NO). *Microbiological Reviews*, 60(4): 609-640.
- Conrad R (1999). Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *Federation of European Microbiological Societies Microbiology Ecology*, 28: 193-202.
- Corenblit, D, Steiger, J, Gurnell, A and Tabacchi, E. (2007). Darwinian origin of landforms. *Earth Surface Processes and Landforms*, 32: 2070 – 2073.
- Dedysh SN, Liesack W, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Bares AM, Panikov NS and Tiedje JM (2000). *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. *International Journal of Systematic and Evolutionary Microbiology*, 50: 955-969.
- Dedysh SN, Knief C and Dunfield PF (2005a). *Methylocella* species are facultatively methanotrophic. *Journal of Bacteriology*, 187: 4665-4670.
- Dedysh SN, Pankratov TA, Belova SE, Kulichevskaya IS and Liesack W (2006). Phylogenetic analysis and in situ identification of bacteria community composition in an acidic *Sphagnum* peat bog. *Applied and Environmental Microbiology*, 72(3): 2110-2117.
- Dedysh SN (2009). Exploring methanotroph diversity in acidic northern wetlands: molecular and cultivation-based studies. *Microbiology*, 78(6): 655-669.

- Dedysh SN and Dunfield PF (2010). 27 Facultative methane oxidizers. *Handbook of Hydrocarbon and Lipid Microbiology*, Timmis, K.N. (ed.) Springer-Verlag Berlin Heidelberg, pp. 1968 – 1976.
- Dedysh SN (2011). Cultivating uncultured bacteria from northern wetlands: knowledge gained and remaining gaps. *Frontiers in Microbiology*, Vol. 2 Article 184, pp. 1-15.
- de Jong R, Blaauw M, Chambers FM, Christensen TR, de Vleeschouwer F, Finsinger SF, Johansson M, Kokfelt U, Lamentowicz M, Le Roux G, Mauquoy D, Mitchell EAD, Nichols JE, Samaritani E and van Geel B (2010). Climate and peatlands, Dodson J. (ed.), *Changing Climates, Earth Systems and Society*. Springer Science + Business Media B.V. DOI 10.1007/978-90-481-8716-4, pp. 85-122.
- Deppe M, Mcknight DM and Blodau C, (2010). Effects of short-term drying and irrigation on electron flow in mesocosms of a northern bog and an alpine fen. *Environmental Science & Technology*, 44: 80-86.
- Duddleston KN, Kinney MA, Kiene RP and Hines ME (2002). Anaerobic microbial biochemistry in a northern bog: Acetate as a dominant metabolic end product. *Global Biogeochemical Cycles*, 16(4).
- Dunfield PF, Knowles R, Dumont R and Moore TR, (1993). Methane production and consumption in temperate and subarctic peat soils: Response to temperature and pH. *Soil Biology & Biochemistry*, 25(3): 321-326.
- Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S, Ly B, Saw JH, Zhou Z, Ren Y, Wang J, Mountain BW, Crowe MA, Weatherby TM, Bodelier PLE, Liesack W, Feng L, Wang, L and Alam M (2007). Methane oxidation by an extremely acidophilic bacterium of the phylum *Verrucomicrobia*. *Nature*, 450(6): 879-883

- Dunfield PF, Belova SE, Vorob'ev AV, Cornish SL and Dedysh SN (2010). *Methylocapsa aurea* sp. nov., a facultative methanotroph possessing a particulate methane monooxygenase, and emended description of the genus *Methylocapsa*. *International Journal of Systematic and Evolutionary Microbiology*, 60: 2659-2664
- DuPont, T., Ferris, H. and Van Horn, M. (2009). Effects of cover crop quality on nematode-based soil food webs and nutrient cycling. *Applied Soil Ecology*, 41: 157-167.
- Environment Canada (2010). National Climate Data and Information Archive. Retrieved from: [http://www.climate.weatheroffice.gc.ca/climate\\_normals/results\\_e.html?Province=ALTA&StationName=&SearchType=&LocateBy=Province&Proximity=25&ProximityFrom=City&StationNumber=&IDType=MSC&CityName=&ParkName=&LatitudeDegrees=&LatitudeMinutes=&LongitudeDegrees=&LongitudeMinutes=&NormalsClass=A&SeINormals=&StnId=2467&start=1&end=13&autofwd=0](http://www.climate.weatheroffice.gc.ca/climate_normals/results_e.html?Province=ALTA&StationName=&SearchType=&LocateBy=Province&Proximity=25&ProximityFrom=City&StationNumber=&IDType=MSC&CityName=&ParkName=&LatitudeDegrees=&LatitudeMinutes=&LongitudeDegrees=&LongitudeMinutes=&NormalsClass=A&SeINormals=&StnId=2467&start=1&end=13&autofwd=0)
- Ettwig KF, van Alen T, van de Pas-Schoonen KT, Jetten MSM and Strous M (2009). Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 phylum. *Applied and Environmental Microbiology*, 75(11): 3656-3662.
- Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Manganot S, Kuypers MMM, Schreiber F, Dutilh BE, Zedelius J, de Beer D, Gloerich J, Wessels HJCT, van Alen T, Luesken F, Wu ML, van de Pas-Schoonen KT, Op den Camp HJM, Janssen-Megens EM, Francoijs K-J, Stunnenberg H, Weissenbach J, Jetten MSM and Strous M (2010). Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature*, 464: 543-548.
- Faubert P (2004). *The effect of long-term water level drawdown on the vegetation composition and CO<sub>2</sub> fluxes of a boreal peatland in central Finland*. Master of Science thesis, Université Laval, Quebec, Canada.

- Fenner N, Ostle NJ, McNamara N, Sparks T, Harmens H, Reynolds B & Freeman C (2007). Elevated CO<sub>2</sub> effects on peatland plant community carbon dynamics and doc production. *Ecosystems*, 10: 635-647.
- Fierer N and Jackson RB, (2005). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences*, 103(3): 626-631.
- Fierer N, Bradford MA and Jackson RB, (2007). Toward an ecological classification of soil bacteria. *Ecology*, 88(6): 1354-1364.
- Freeman C, Ostle, N and Kang H (2001). An enzymic 'latch' on a global carbon store. *Nature*, 409, 149, doi:10.1038/35051650.
- Frolking S, Roulet N and Lawrence D (2009). *Issues related to incorporating Northern peatlands into global climate models*. Baird A, Belyea L, Comas X, Reeve A & Slater L (eds.) American Geophysical Union, Washington, DC, Geophysical Monograph 184, ISBN: 978-0-87590-449-8 pp. 19 – 35.
- Galand PE (2004). Methanogenic Archaea in boreal peatlands. Academic dissertation in general microbiology, *Department of Biological and Environmental Sciences, University of Helsinki*.
- Garcia J-L, Patel BKC and Ollivier B (2000). Taxonomic, phylogenetic and ecological diversity of methanogenic Archaea. *Anaerobe*, 6: 205-226.
- Glatzel S, Basiliko N and Moore T, (2004). Carbon dioxide and methane production potentials of peats from natural, harvested and restored sites, eastern Quebec, Canada. *Wetlands*, 24(2): 261-267.

- Godin A, McLaughlin JW, Webster KL, Packalen M and Basiliko N (2012). Methane and methanogen community dynamics across a boreal peatland nutrient gradient. *Soil Biology & Biochemistry*, 48: 96-105.
- Gonzalez JM, Zimmermann J & Saiz-Jimenez C (2004). Evaluating putative chimeric sequences from PCR-amplified products. *Bioinformatics*, 21(3): 333-337.
- Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabb D, Highlander SK, Sodergren E, Methe B, DeSantis TZ, The Human Microbiome Consortium, Petrosino JF, Knight R and Birren BW (2011). Chimeric 16S rRNA sequence formation and detection in Sanger 454-pyrosequenced PCR amplicons. *Genome Research*, 21: 494-504.
- Hallam SJ, Girguis PR, Preston CM, Richardson PM and DeLong EF (2003). Identification of methyl coenzyme M reductase A (*mcrA*) genes associated with methane-oxidizing archaea. *Applied and Environmental Microbiology*, 69(9): 5483-5491.
- Hamady M, Walker JJ, Harris JK, Gold NJ and Knight R (2008). Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nature Methods* 5(3).
- Hanson RS and Hanson TE (1996). Methanotrophic bacteria. *Microbiological Reviews*, 60(2): 439-471.
- Hogg EH (1993). Decay potential of hummock and hollow *Sphagnum* peats at different depths in a Swedish raised bog. *Oikos*, 66: 269-278.
- Hornibrook ERC, Longstaffe FJ and Fyfe WS, (1997). Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence. *Geochimica et Cosmochimica Acta*, 61(4): 745-753.

- Hurst CJ, Crawford RL, Garland JL, Lipson DA, Mills AL and Stetzenbach LD (Eds.) (2007). *Manual of Environmental Microbiology* (3<sup>rd</sup> ed.). American Society for Microbiology Press, Washington, D.C.
- IPCC (2007). Intergovernmental Panel on Climate Change Fourth Assessment Report. *Climate change 2007: the physical science basis*. Colomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M and Miller HL (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.
- Ivanova A and Dedysh SN (2012). Abundance, diversity and depth distribution of Planctomycetes in acidic northern wetlands. *Frontiers in Microbiology*, 3(5): 1-9.
- Janda JM and Abbott SL (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils and pitfalls. *Journal of Clinical Microbiology*, 45(9): 2761-2764.
- Juottonen H (2008). Archaea, bacteria and methane production along environmental gradients in fens and bogs. Dissertation, University of Helsinki, Finland.
- Keller JK & Bridgman SD (2007). Pathways of anaerobic carbon cycling across an ombrotrophic-minerotrophic peatland gradient. *Limnology and Oceanography*, 52(1): 96-107.
- Kettunen A (2002). *Modeling of microscale variations in methane fluxes*. Dissertation, Helsinki University of Technology, Finland.
- Kip N, van Winden JF, Pan Y, Bodrossy L, Reichart G-J, Smolders A, Jetten M, Damsté J and Op den Camp H (2010). Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. *Nature Geoscience*, 3: 617-621.



- Kip N, Dutilh BE, Pan Y, Bodrossy L, Neveling K, Kwint MP, Jetten MSM and Op den Camp HJM (2011). Ultra-deep pyrosequencing of *pmoA* amplicons confirms the prevalence of *Methylomonas* and *Methylocystis* in *Sphagnum* mosses from a Dutch peat bog. *Environmental Microbiology Reports*, 3(6): 667-673.
- Knief C and Dunfield PF, (2005). Response and adaptation of different methanotrophic bacteria to low methane mixing ratios. *Environmental Microbiology*, 7(9): 1307-1317.
- Knorr K-H and Blodau C (2009). Impact of experimental drought and rewetting on redox transformations and methanogenesis in mesocosms of a northern fen soil. *Soil Biology & Biochemistry*, doi:10.1016/j.soilbio.2009.02.030.
- Kotiaho M, Fritze H, Merilä P, Juottonen H, Leppälä M, Laine J, Laiho R, Yrjälä K, Tuittila E-S (2010). Methanogen activity in relation to water table level in two boreal fens. *Biology and Fertility of Soils*, 46: 567-575.
- Kotsyurbenko OR, Chin K-J, Glagolev MV, Stubner S, Simankova MV, Nozhevnikova AN and Conrad R, (2004). Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environmental Microbiology*, 6(11): 1159-1173.
- Kotsyurbenko OR, Friedrich MW, Simankova MV, Nozhevnikova AN, Golyshin PN, Timmis KN and Conrad R, (2007). Shift from acetoclastic to H<sub>2</sub>-dependent methanogenesis in a West Siberian peat bog at low pH values and isolation of an acidophilic *Methanobacterium* strain. *Applied and Environmental Microbiology*, 73(7): 2344-2348.
- Kotsyurbenko OR (2010). 50 Soil, Wetlands, Peat. *Handbook of Hydrocarbon and Lipid Microbiology*, Timmis, K.N. (ed.) Springer-Verlag Berlin Heidelberg, pp. 626 – 634.

- Kraigher B, Stres B, Hacin J, Ausec L, Mahne I, van Elsas JD and Mandic-Mulec I, (2006). Microbial activity and community structure in two drained fen soils in the Ljubljana Marsh. *Soil Biology & Biochemistry*, 38: 2762-2771.
- Kravchenko IK, (2002). Methane oxidation in boreal peat soils treated with various nitrogen compounds. *Plant and Soil*, 242: 157-162.
- Kulzer L, Luchessa S, Cooke S, Errington R, Weinmann F and Vitt D (2001). Characteristics of the low-elevation Sphagnum dominated peatlands of western Washington: A community profile. Retrieved from King County, Washington <http://www.kingcounty.gov/environment/waterandland/stormwater/documents/sphagnum-bogs.aspx>
- Kunin V and Hugenholtz P (2010). PyroTagger: A fast, accurate pipeline for analysis for rRNA amplicon pyrosequence data. *The Open Journal*, Article 1.
- Lafleur PM, Moore TR, Roulet NT and Frohling S, (2005). Ecosystem respiration in a cool temperate bog depends on peat temperature but not water table. *Ecosystems*, 8(6): 619-629.
- Laiho, R. (2006). Decomposition in peatlands: reconciling seemingly contrasting results on the impacts of lowered water levels. *Soil Biology and Biochemistry*, 38: 2011-2024.
- Laine A, Byrne KA, Kiely G and Tuittila E (2007). Patterns in vegetation and CO<sub>2</sub> dynamics along a water level gradient in a lowland blanket bog. *Ecosystems*, DOI: 10.1007/s10021-007-9067-2.
- Lauber CL, Hamady M, Knight R and Fierer N, (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, 75(15): 5111-5120.

- Lennon JT and Cottingham KL (2008). Microbial productivity in variable resource environments. *Ecology*, 89(4): 1001-1014.
- Lin X, Green S, Tfaily MM, Prakash O, Konstantinidis KT, Corbett JE, Chanton JP, Cooper WT and Kostka JE (2012). Microbial community structure and activity linked to contrasting biogeochemical gradients in bog and fen environments of the glacial Lake Agassiz peatland. *Applied and Environmental Microbiology*, 78(19): 7023-7031.
- Ludwig W, Strunk O, Westram R, Richter L, Meier H, Buchner YA, Lai T, Steppi S, Jobb G, Forster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, König A, Liss T, Lubmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A and Schleifer K-H (2004). ARB: A software environment for sequence data. *Nucleic Acids Research*, 32(4): 1363-1371.
- Macrae ML, Devito KJ, Strack M and Waddington JM (2012). Effect of water table drawdown on peatland nutrient dynamics: implications for climate change. *Biogeochemistry*, DOI 10.1007/s10533-012-9730-3.
- Mäkiranta P, Laiho R, Fritze H, Hytönen J, Laine J and Minkkinen K (2009). Indirect regulation of heterotrophic peat soil respiration by water level via microbial community structure and temperature sensitivity. *Soil Biology and Biochemistry*, 41: 695-703.
- Mardis ER (2008). Next-generation DNA sequencing methods. *Genomics and Human Genetics*, 9: 387-402.
- McDonald IR, Bodrossy L, Chen Y and Murrell JC (2008). Molecular ecology techniques for the study of aerobic methanotrophs. *Applied and Environmental Microbiology*, 74(5): 1305-1315.

- Minderlein S and Blodau C (2010). Humic-rich peat extracts inhibit sulfate reduction, methanogenesis, and anaerobic respiration but not acetogenesis in peat soils of a temperate bog. *Soil Biology & Biochemistry*, 42: 2078-2086.
- Moore TR and Dalva M (1997). Methane and carbon dioxide exchange potentials of peat soils in aerobic and anaerobic laboratory incubations. *Soil Biology & Biochemistry* 29(8): 1157-1164.
- Moore T and Basiliko N, (2006). Decomposition. Pages 126-143 in R.K. Wieder & D.H. Vitt (eds.) *Boreal Peatland Ecosystems*, Ecological Studies Vol. 188, Springer-Verlag.
- Moore T, Bubier JL and Bledzki L (2007). Litter decomposition in temperate peatland ecosystems: the effect of substrate and site. *Ecosystems*, DOI: 10.1007/s10021-007-9064-5.
- Moore TR, De Young A, Bubier JL, Humphreys ER, Lafleur PM and Roulet NT, (2011). A multi-year record of methane flux at the Mer Bleue bog, southern Canada. *Ecosystems*, 14: 646-657.
- Morales SE, Mouser PJ, Ward N, Hudman SP, Gotelli NJ, Ross DS and Lewis TA, (2006). Comparison of bacterial communities in New England *Sphagnum* bogs using terminal restriction fragment length polymorphism (T-RFLP). *Microbial Ecology*, 52: 34-44.
- Opelt K, Chobot V, Hadacek F, Schonmann S, Eberl L and Berg G (2007a). Investigations of the structure and function of bacterial communities associated with *Sphagnum* mosses. *Environmental Microbiology*, 9(11): 2795-2809.
- Op den Camp HJM, Islam T, Stott MB, Harhangl HR, Hynes A, Schouten S, Jetten MSM, Birkeland N-K, Pol A and Dunfield PF (2009). Environmental, genomic and taxonomic

- perspectives on methanotrophic *Verrucomicrobia*. *Environmental Microbiology*, 1(5): 293-306.
- Pankratov TA, Dedysh SN and Zavarzin GA (2006). The leading role of *Actinobacteria* in aerobic cellulose degradation in *Sphagnum* peat bogs. *Doklady Biological Sciences*, 410(1): 428-430.
- Pankratov TA, Serkeba YM, Kulichevskaya IS, Liesack W and Dedysh SN (2008). Substrate-induced growth and isolation of *Acidobacteria* from acidic *Sphagnum* peat. *International Society for Microbial Ecology*, 2: 551-560.
- Pankratov TA, Ivanova AO, Dedysh SN and Liesack W (2011). Bacterial populations and environmental factors controlling cellulose degradation in acidic *Sphagnum* peat. *Environmental Microbiology*, 13(7): 1800-1814.
- Pelletier L, Moore TR, Roulet NT, Garneau M and Beaulieu-Audy V (2007). Methane fluxes from three peatlands in the La Grande Rivière watershed, James Bay lowland, Canada. *Journal of Geophysical Research*, 112: 1-12.
- Peltoniemi K, Fritze H and Laiho R (2009). Response of fungal and actinobacterial communities to water-level drawdown in boreal peatland sites. *Soil Biology & Biochemistry*, 41: 1902-1914.
- Peltoniemi K, Strakova P, Fritze H, Iraizoz PA, Pennanen T and Laiho R (2012). How water-level drawdown modifies litter-decomposing fungal and actinobacterial communities in boreal peatlands. *Soil Biology & Biochemistry*, 51: 20-34.
- Pihlatie MK, Kiese R, Brüggemann N, Butterbach-Bahl K, Kieloaho A-J, Laurila T, Lohila A, Mammarella I, Minkkinen K, Penttilä T, Schönborn J and Vesala T (2010). Greenhouse

- gas fluxes in a drained peatland forest during spring frost-thaw event. *Biogeosciences*, 7: 1715-1727.
- Popp TJ, Chanton JP, Whiting GJ and Grant N (2000). Evaluation of methane oxidation in the rhizosphere of a *Carex* dominated fen in north central Alberta, Canada. *Biogeochemistry*, 51: 259-281.
- Putkinen A, Juottonen H, Juutinen S, Tuittila E-S, Fritze H and Yrjala K (2009). Archaeal rRNA diversity and methane production in deep boreal peat. *Federation of European Microbiological Societies Microbiology Ecology*, 70(1): 87-98.
- Ramos-Padron E, Bordenave S, Lin S, Bhaskar IM, Dong X, Sensen CW, Fournier J, Voordouw and Gieg L (2011). Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. *Environmental Science & Technology*, 45: 439-446.
- Regina K, Silvola J and Martikainen PJ, (1999). Short-term effects of changing water table on N<sub>2</sub>O fluxes from peat monoliths from natural and drained boreal peatlands. *Global Change Biology*, 5: 183-189.
- Rooney-Varga JN, Giewat MW, Duddleston KN, Chanton JP and Hines ME (2007). Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands. *Federation of European Microbiological Societies Microbial Ecology*, 60: 240-251.
- Ronaghi M (2001). Pyrosequencing sheds light on DNA sequencing. *Genome Research*, 11: 3-11.
- Rosenberry DO, Glaser PH & Siegel DL (2006). The hydrology of northern peatlands as affected by biogenic gas: Current developments and research needs. *Hydrological Processes*, 20: 3601-3610.

- Roulet NT, Lafleur PM, Richard PJH, Moore TR, Humphreys ER and Bubier J, (2007). Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. *Global Change Biology*, 13: 397-411.
- Sakai S, Imachi H, Sekiguchi Y, Ohashi A, Harada H and Kamagata Y (2007). Isolation of key methanogens for global methane emission from rice paddy fields: a novel isolate affiliated with the clone cluster Rice Cluster I. *Applied and Environmental Microbiology*, 73: 4326-4331.
- Sakai S, Conrad R, Liesack W and Imachi H (2010). *Methanocella arvoryzae* sp nov., a hydrogenotrophic methanogen isolated from rice field soil. *International Journal of Systematic and Evolutionary Biology*, 60: 2918-2923.
- Schindlbacher A, Rodler A, Kuffner M, Kitzler B, Sessitsch A and Zechmeister-Boltenstern S, (2011). Experimental warming effects on the microbial community of a temperate mountain forest soil. *Soil Biology & Biogeochemistry*, 43: 1417-1425.
- Schmidt HA, Strimmer K, Vingron M and von Haeseler A (2002). Tree-puzzle: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics*, 18(3): 502-504.
- Shah N, Tang H, Doak TG and Ye Y (2011). Comparing bacterial communities inferred from 16S rRNA gene sequencing and shotgun metagenomics. *Pacific Symposium on Biocomputing*, 16: 165-176.
- Sharp CE, Stott MB and Dunfield PF (2012). Detection of autotrophic verrucomicrobial methanotrophs in a geothermal environment using stable isotope probing. *Frontiers in Microbiology*, 3(303).

- Siljanen HMP (2012). Activity and diversity of methanotrophs in a littoral wetland of an eutrophic boreal freshwater lake. *Publication of the University of Eastern Finland, Dissertations in Forestry and Natural Sciences* (63). Retrieved from [http://epublications.uef.fi/pub/urn\\_isbn\\_978-952-61-0677-9/urn\\_isbn\\_978-952-61-0677-9.pdf](http://epublications.uef.fi/pub/urn_isbn_978-952-61-0677-9/urn_isbn_978-952-61-0677-9.pdf)
- Stanek W and Silc T (1977). Comparisons of 4 methods for determination of degree of peat humification (decomposition) with emphasis on von Post method. *Canadian Journal of Soil Science*, 57: 109-117.
- St Arnaud C (2007). Dynamique de la vegetation d'un fen pauvre face a une simulation de rechauffement climatique: Reponses potentielles des tourbieres boreales a Sphaignes. M.Sc. Thesis, Université Laval, Quebec, Canada.
- Strack M, Waddington JM and Tuittila E (2004). Effect of water table drawdown on northern peatland methane dynamics: implications for climate change. *Global Biogeochemical Cycles*, 18.
- Strack M, Kellner E and Waddington JM (2005). Dynamics of biogenic gas bubbles in peat and their effects on peatland biogeochemistry. *Global Biogeochemical Cycles*, 19, GB1003, doi:10.1029/2004GB002330.
- Strack M, Waddington JM, Rochefort L and Tuittila E (2006). Response of vegetation and net ecosystem carbon dioxide exchange at different peatland microforms following water table drawdown. *Journal of Geophysical Research*, 111, doi: 10.1029/2005JG000145.
- Strack M, Waddington JM, Turetsky M, Roulet NT, Byrne KA (2008). Northern peatland, greenhouse gas exchange and climate change. *Peatlands and Climate Change*, Strack M. (ed.) International Peat Society, Saarijärven Offset Oy, Saarijärvi, Finland, pp. 40-65.



- Strimmer, K and von Haeseler A (1996). Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. *Molecular Biology and Evolution*, 13: 964-969.
- Smalla K, Oros-Sichler M, Milling A, Heuer H, Baumgarte S, Becker R, Neuber G, Kropf S, Ulrich A and Tebbe C (2007). Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results? *Journal of Microbial Methods*, 69: 470-479.
- Smemo KA and Yavitt JB (2007). Evidence for anaerobic CH<sub>4</sub> oxidation in freshwater peatlands. *Geomicrobiology Journal*, 24: 583-597.
- Sundh, I., Mikkela, C., Nilsson, M. & Svensson, B.H. (1995). Potential aerobic methane oxidation in *Sphagnum* dominated peatland: Controlling factors and relation to methane emission. *Soil Biology & Biochemistry*, 27(6): 829-837.
- Tarnocai C (2006). The effect of climate change on carbon in Canadian peatlands. *Global and Planetary Change*, 53: 222-232.
- Tarnocai C (2009). The impact of climate change on Canadian peatlands. *Canadian Water Resources*, 34(4): 453-466.
- Thomas PA and Pearce ME (2004). Role of cation exchange in preventing the decay of anoxic deep bog peat. *Soil Biology & Biochemistry*, 36: 23-32.
- Tuittila E-S, Komulainen V-M, Vasander H, Nykanen H, Martikainen PJ and Laine J (2000). Methane dynamics of a restored cut-away peatland. *Global Change Biology*, 6: 569-581.
- Turetsky MR and St. Louis V (2006). *Disturbance in boreal peatlands*, pp 359-379. In: RK Wieder and DH Vitt (eds.) *Boreal Peatland Ecology*. Springer-Verlag, Berlin-Heidelberg-New York.

- Turetsky MR, Treat CC, Waldrop MP, Waddington JM, Harden JW and McGuire AD (2008). Short-term response of methane fluxes and methanogen activity to water table and soil warming manipulations in an Alaskan peatland. *Journal of Geophysical Research*, 113, G00A10, doi:10.1029/2007JG000496.
- Tveit A, Schwacke R, Svenning MM and Urich T (2012). Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. *International Society for Microbial Ecology*, 1-13.
- Vandamme P, Opelt K, Knochel N, Berg C, Schonmann S, Brandt ED, Eberl L, Falsen E and Berg G (2007). *Burkholderia bryophila* sp. nov. and *Burkholderia megapolitana* sp. nov., moss-associated species with antifungal and plant-growth-promoting properties. *International Journal of Systematic and Evolutionary Microbiology*, 57: 2228-2235.
- Van Eekeren, N, Bommele, L, Bloem, J, Schouten, T, Rutgers, M, De Goede, R, Reheul, D. and Brussaard, L. (2008). Soil biological quality after 36 years of ley-arable cropping, permanent grassland and permanent arable cropping. *Applied Soil Ecology*, 40: 432-446.
- Vasander H & Kettunen A (2006). Carbon in boreal peatlands. In R.K. Weider & D.H. Vitt (eds.), *Ecological Studies*, 188: 165-181. Springer-Verlag Berlin Heidelberg.
- Vitt DH and Chee W-L (1990). The relationships of vegetation to surface water chemistry and peat chemistry in fens of Alberta, Canada. *Vegetation*, 89(2): 87-106.
- Vitt DH (2006). Functional characteristics and indicators of boreal peatlands, pp. 9-24. In: RK Wieder and DH Vitt (eds.) *Boreal Peatland Ecology*. Springer-Verlag, Berlin-Heidelberg-New York.
- Vorob'ev AV, Baani M, Doronina NV, Brady AL, Liesack W, Dunfield PF and Dedysh SN (2011). *Methyloferula stellata* gen. nov., sp. nov., an acidophilic, obligately

- methanotrophic bacterium that possesses only a soluble methane monooxygenase. *International Journal of Systematic and Evolutionary Microbiology*, 61: 2456-2463.
- Waddington JM, Roulet NT & Swanson RV (1996). Water table control of CH<sub>4</sub> emission enhancement by vascular plants in boreal peatlands. *Journal of Geophysical Research*, 10: 775-785.
- Waddington JM and Price JS (2000). Effect of peatland drainage, harvesting, and restoration on atmospheric water and carbon exchange. *Physical Geography*, 21(5): 433-451.
- Waddington JM, Rotenberg PA & Warren FJ (2001). Peat CO<sub>2</sub> production in a natural and cutover peatland: Implications for restoration. *Biogeochemistry*, 54: 115-130.
- Ward NL, Challacombe JF, Janssen PH, Henrissat B, Coutinho PM, Wu M, Xie G, Haft DH, Sait M, Badger J, Barabote RD, Bradley B, Brettin TS, Brinkac LM, Bruce D, Creasy T, Daugherty SC, Davidsen TM, DeBoy RT, Detter JC, Dodson RJ, Durkin AS, Ganapathy A, Gwinn-Giglio M, Han CS, Khouri H, Kiss H, Kothari SP, Madupu R, Nelson KE, Nelson WC, Paulsen I, Penn K, Ren Q, Rosovitz MJ, Selengut JD, Shrivastava S, Sullivan SA, Tapia R, Thompson LS, Watkins KL, Yang Q, Yu C, Zafar N, Zhou L and Kuske CR (2009). Three genomes from the phylum *Acidobacteria* provide insight into the lifestyles of these microorganisms in soils. *Applied and Environmental Microbiology*, 75(7): 2046-2056.
- Wheeler BD and Proctor MCF (2000). Ecological gradients, subdivisions and terminology of north-west European mires. *Journal of Ecology*, 88: 187-203.
- Worrall, F. & Burt, T. (2005). Predicting the future DOC flux from upland peat catchments. *Journal of Hydrology*, 300: 126-139.

- Yavitt JB, Basiliko N, Turetsky MR and Hay AG (2006). Methanogenesis and methanogen diversity in three peatland types of the discontinuous permafrost zone, boreal western continental Canada. *Geomicrobiology Journal*, 23: 641-651.
- Yavitt JB, Yashiro E, Cadillo-Quiroz H and Zinder SH, (2011). Methanogen diversity and community composition in peatlands of the central to northern Appalachian mountain region, North America. *Biogeochemistry*, 109: 117-131.
- Zhang, L. and Xu, Z. (2008). Assessing bacterial diversity in soil: A brief review. *Journal of Soils and Sediments*, 8: 379-388.
- Zhu B, van Dijk G, Fritz C, Smolders AJP, Pol A, Jetten MSM and Ettwig KF (2012). Anaerobic oxidation of methane in a minerotrophic peatland: Enrichment of nitrite-dependent methane-oxidizing bacteria. *Applied and Environmental Microbiology*, 78(24): 8657-8665.

## Appendix

### Richness and Diversity Indices

**Chao1** estimates total species richness as:

$$S_{\text{Chao1}} = S_{\text{obs}} + \frac{n_1^2}{2n_2}$$

where  $S_{\text{obs}}$  is the number of observed species,  $n_1$  is the number of species captured once and  $n_2$  is the number of species captured twice.

**Shannon** diversity index ( $H$ ):

$$H = -\sum_{i=1}^S (n_i/N) \log_2 (n_i/N)$$

where  $n_i$  is the number of individuals of taxon  $i$ ,  $N$  is the total number of individuals of all species,  $S$  is the total number of species.

**Simpson** index ( $D$ ):

$$D = \sum_{i=1}^S (n_i/N)^2$$

where  $n_i$  is the number of individuals of taxon  $i$ ,  $S$  is the total number of species (OTUs),  $N$  is the total number of individuals of all species.

**Bray-Curtis** similarity index:

$$BC_{ij} = 2C_{ij} / S_i + S_j$$

where  $C_{ij}$  is the sum of the lesser value for only those species common between both sites,  $S_i$  and  $S_j$  are the total number of specimens counted at both sites.