Response of Methane Emission and Dissolved Organic Carbons to Warming and Nitrogen Fertilization in Boreal Peatlands

By

Albert Adu Sey

A thesis submitted to Memorial University

in partial fulfillment of the requirements of the degree of

Master of Science

Boreal Ecosystems and Agricultural Sciences

School of Science and Environment

Grenfell Campus

Memorial University

May, 2019

St. John's, Newfoundland and Labrador, Canada

Response of Methane Emission and Dissolved Organic Carbons to Warming and Nitrogen Fertilization in Boreal Peatlands

By

Albert Adu Sey

A Thesis submitted to the School of Graduate Studies

in partial fulfillment of the requirements for the degree of

Master of Science

Boreal Ecosystems and Agricultural Sciences

Approved:

Dean of the Graduate School

Supervisor

Date

Committee member:

Dr. Robert Scott

Abstract

Although most sources of greenhouse gases (GHG) have been extensively studied, relatively little is understood about the interactive effect of warming and nitrogen addition on methane (CH_4) emissions from boreal peatland. Since methane is a considerably more potent GHG compared to carbon dioxide (CO_2), studies investigating what physical and biological factors control methane emissions in boreal peatlands are becoming increasingly important as an increase in temperature increases the rate of CH_4 emission as well as enhancing the decomposition of dissolved organic matter (DOM). In this experiment, I investigated the response of CH_4 emissions and dissolved organic carbon (DOC) concentrations and optical characteristics to warming and nitrogen fertilization in boreal peatlands as a result of global climate change at a peat bog complex in western Newfoundland, Canada. I found that climate warming increased CH_4 emission and DOC concentration at 40 cm peat depth. In contrast, nitrogen (N) addition did not increase CH_4 emission in a boreal peatland. However, the interactive effect of warming and nitrogen addition decreased the rate of CH_4 emission due to the reducing effect of N-addition which counteracted the positive effect of warming in increasing the rate of CH_4 emission from boreal peatlands. This unexpected trend suggests unforeseen factors are involved in the process of methanogenesis that were beyond the scope of this experiment. However, the interactive effect of warming and N addition synergistically increased the DOC and total nitrogen (TN) concentrations at the 40 cm depth due to root exudates favoring DOC mineralization in boreal peatlands. The results further indicate that specific ultraviolet absorbance ($SUVA_{254}$) increased peat aromaticity. Furthermore, fluorescence

index (FI) indicated the peat was of microbially (1.53-1.65) processed dissolved organic matter (DOM) over vascular plant derived DOM in peatland. Humification index (HIX) values for the treatment effects ranged between 0.15-0.17, indicating the peat carbon was not highly degraded and composed of less condensed molecules, indicating a decrease in recalcitrant humified carbon production.

***Keywords**:* Peatland, Methane, Nitrogen, Warming, CO_2, DOC, TN, DOM, FI, $SUVA_{254}$, HIX

Acknowledgments

The research would not have been made possible without the support of the following funding agencies: Natural Sciences and Engineering Research Council of Canada (NSERC)-Discovery Grant, Canada Foundation for Innovation-John R. Evans Leaders Fund, Research & Development Corporation (RDC, NL)-Leverage R&D, RDC-Ignite R&D, RDC-RCRI (Regional Collaborative Research Initiative), Humber River Basin Research Initiative of NL, Vice-President Research Fund, and the Graduate Student Stipend funding from the Institute for Biodiversity, Ecosystem Science, and Sustainability (IBES, NL). Received partial financial support from the Graduate Student Baseline Fellowship from the School of Graduate Studies, Memorial University and GF2, Gov NL, Department of Fisheries and Land Resources.

My sincere thanks and appreciation to my supervisor Dr. Jianghua Wu, for his supervision, encouragements, support and criticism which helped bring out the best in me. I could not have come this far without him imparting so much knowledge in me and also giving me the opportunity to achieving one of my greatest goals in life.

Special thanks to Syed Gillani and Maryam Hajheidari who assisted me with field sampling and Dr. Tao Yuan, Dr. Robert Scott and Dr. Charles Manful for assisting in data analysis and interpretation as well as Gabriel K. Tei and Dr. Thomas Halford for their reviews.

To Dr. Christabel O. Sey, I couldn't have hoped for a better life partner on this journey. There are no words to express how grateful I am to have you by my side every

step of the way on this successful adventure. In so many tangible ways this accomplishment is as much yours as it is mine.

Albert Adu Sey

Table of Contents

Response of Methane Emission and Dissolved Organic Carbons to Warming and Nitrogen Fertilization in Boreal Peatlands 2
Abstract 3
Acknowledgments 5
Table of Contents 7
List of Tables 10
List of Figures 11
List of Abbreviations and Symbols 14
Chapter 1 17
1.0 Introduction and overview 17
1.1 Introduction 17
1.2 Research questions and objectives 19
1.3 Thesis outline 20
1.4 Contribution statement 21
Chapter 2 22
2.0 Literature review 22
2.1 Peatland characteristics 22
2.2 Peatlands role in global C cycling 23
2.3 Influence of N deposition in CH_4 emission 24
2.4 Effect of temperature on CH_4 emission 25
2.5 Effect of water table depth on CH_4 emission 26
2.6 DOC exchange and transport 27
2.7 Specific ultraviolet absorbance ($SUVA_{254}$) 27
2.8 Fluorescence index (FI) 28
2.9 Humification index (HIX) 29
2.10 Potential drivers of change in DOC 29
2.10.1 Temperature change 29
2.10.2 Water table depth 30

Chapter 3 31
3.0 Response of methane emission to warming and nitrogen fertilization in a boreal peatland 31
3.1 Introduction 31
3.2 Materials and methods 34
3.2.1 Site description 34
3.2.2 Experimental design 35
3.2.3 Gas sampling and analysis 37
3.2.4 Methane analysis and emission rate calculations 39
3.2.5 Data analysis 40
3.3 Results 40
3.3.1 Environmental variables influencing CH_4 emission 40
3.3.2 Effect of N fertilizer application on CH_4 flux 44
3.3.3 Effect of warming on CH_4 flux 47
3.3.4 Interactive effect of warming and nitrogen addition on CH_4 flux. 50
3.4 Discussion 53
3.4.1 Impact of environmental variables 53
3.4.2 Response of CH_4 flux to N addition 54
3.4.3 Response of CH_4 flux to warming 56
3.4.4 Interactive effect of warming and N addition on CH_4 flux 57
3.5 Conclusion 58
Chapter 4 60
4.0 Response of Dissolved Organic Carbon (DOC) to Experimental Warming and Nitrogen Fertilization in Boreal Peatland 60
4.1 Introduction 60
4.2 Materials and methods 64
4.2.1 Site description and experimental design 64
4.2.2 Water sampling 65
4.2.3 DOC and UV-vis analysis 65
4.2.4 Fluorescence and humification index analysis 67

4.2.5 Data analysis 68
4.3 Results 68
4.3.1 Treatment effects on DOC and TN concentrations 68
4.3.2 Spectroscopy index of DOC 71
4.3.2.3 Humification 76
4.4 Discussion 78
4.4.1 The effects of warming treatment on DOC 78
4.4.2 The effect of N addition on DOC 80
4.4.3 The interactive effects of warming and N addition on DOC 81
4.5 Conclusion 83
Chapter 5 86
5.0 Summary and Conclusion 86
References 88
Appendices 104

List of Tables

Table 3. 1 Three years mean values of environmental variables over the growing season (2015-2017)..... 41

Table 3. 2 Pearson correlation (r) coefficients between $Log_{10}(CH_4)$ flux and environmental variables in a northern natural peatland. 42

Table 4. 1 Mean DOC, TN concentration and spectroscopy characteristics of, $SUVA_{254}$, FI and HIX for 3 years of warming and nitrogen addition. 70

Table A. 1 Environmental variables and CH4 fluxes under different treatments and years for the entire growing season..... 104

Table A. 2 Pearson correlation (r) coefficients between dissolved organic carbon (DOC) and the spectroscopy indexes. 103

List of Figures

Figure 3. 1 Plot 2 x 2 m, buffer zone 4 m, red filled means warming treatment (W), blue filled means nitrogen addition (N)(6.4g N/m^2/yr), green filled means warming and N addition (W+N) (6.4g N/m^2/yr) and control (C) treatment. 36

Figure 3. 2 Daily mean air temperature in the open top chamber (red line) and the ambient (black line) for 2017 growing season. 42

Figure 3. 3 Non-linear relationship between CH_4 emission and peat soil temperatures (^oC) at different soil depth when all treatments were pooled together. Temperature at 5 cm (T5 cm) (A) and temperature at 20 cm depth (T20 cm) (B). 43

Figure 3. 4 Overall CH_4 flux for three years under different treatment effects. Control (C), nitrogen addition treatment (N), warming treatment effect (W) and interactive effect of warming and nitrogen addition (W+N). The solid bar represents the means and error bar indicates standard error (means±SE), sample size (n=80). Non-identical letters mean corresponding treatments are significantly different ($p \leq 0.001$). 44

Figure 3. 5 Non-linear regressions of CH_4 flux and peat soil temperature at 5 cm (A) and 20 cm (B) depth in the N- treatment effect. 46

Figure 3. 6 Monthly fluctuations of CH_4 flux under control (C), nitrogen addition (N), warming (W) and interactive effect of warming and nitrogen addition (W+N) treatment effects for the 3years. 48

Figure 3. 7 Non-linear regressions of CH_4 flux and peat soil temperature at 5 cm (A) and 20 cm (B) depth in the W- treatment effect. 49

Figure 3. 8 Response of CH_4 flux (mg m^{-2} h^{-1}) to various treatment effects for the growing seasons. Control (C), nitrogen addition (N), warming (W) and interactive effect of warming and nitrogen addition (W+N) treatment effect. Error bars represent standard errors (SE). Letters on error bar indicates significant differences (p <0.05) between treatment effect within the respective year. 51

Figure 3. 9 Non-linear regressions of CH_4 flux and peat soil temperature at 5 cm and 20 cm depth in the W+N treatment effect. 52

Figure 4. 1 Monthly mean value of peatland dissolved organic carbon (DOC) (A) and total nitrogen (TN) (B) monthly concentrations for the three-year period. Treatment effects are represented as: control (C), nitrogen addition treatment (N), warming treatment (W) and interaction effect of warming and nitrogen addition treatment (W+N). 71

Figure 4. 2 Relationship between specific ultraviolet absorbance ($SUVA_{254}$) and DOC (mg L^{-1}) at 10 cm(A) and 40 cm (B) peat depth. Control (C) (gray solid), nitrogen addition (N) (blue solid), warming (W) (red triangle) and interactive effect of warming and nitrogen addition (W+N) (green triangle). 73

Figure 4. 3 Relationships between dissolved organic carbon (DOC) concentrations and fluorescence index (FI). Legend represents; control (gray solid), nitrogen addition (blue solid), warming treatment (red triangle) and interactive effect of warming and nitrogen addition (green triangle) treatment at 10 cm (A) and 40 cm (B) peat soil depth. 75

Figure 4. 4 Relationship between humification index (HIX) and DOC (mg L^{-1}) at 10 cm (A) and 40 cm (B) peat depth. Control (C) (gray solid), nitrogen addition (N) (blue solid),

warming (W) (red triangle) and interactive effect of warming and nitrogen addition (W+N) (green triangle). 77

Figure A. 1 Relationship between specific ultra-violet absorbance ($SUVA_{254}$) and DOC (mg L^{-1}) at 10 cm (A) and 40 cm (B) peat depth when all treatment effects were pooled 106

Figure A. 2 The average annual treatment effect of $SUVA_{254}$ and HIX at 10 cm peat depth. Legend labels represents control (C), nitrogen addition (N), warming (W) and interactive effect of warming and nitrogen addition (W+N) treatment effect. Bars represent standard error bars (SE)..... 107

Figure A. 3 The average annual treatment effect of $SUVA_{254}$ and HIX at 40 cm peat depth. Legend labels represents control (C), nitrogen addition (N), warming (W) and interactive effect of warming and nitrogen addition (W+N) treatment effect. Bars represent standard error bars (SE)..... 108

List of Abbreviations and Symbols

ABS - Acrylonitrile butadiene styrene

ANOVA - Analysis of variance

C - Carbon

CH_4 - Methane

CO_2 - Carbon dioxide

DIC - Dissolved inorganic carbon

DOC - Dissolved organic carbon

DOM - Dissolved organic matter

DON - Dissolved organic nitrogen

FI - Fluorescence index

GLM - General linear model

HIX - Humification index

IPCC - Intergovernmental panel on climate change

KHP - Potassium hydrogen phthalate

Mha - Million hectare

N - Nitrogen

N_2O - Nitrous oxide

NH_4NO_3 - Ammonium nitrate

OTC - Open top chamber

Pg - Petagram

PVC - Polyvinyl chloride

RCBD - Randomized complete block design

SD - Standard deviation

SE - Standard error

SMC - Soil moisture content

SUVA - Specific ultraviolet absorbance

T - Measured soil temperature (^oC)

Tg - Teragram

TN - Total nitrogen

TOC - Total organic carbon

UVA - Ultraviolet absorbance

WTD - Water table depth

Chapter 1

1.0 Introduction and overview

1.1 Introduction

Peatlands are carbon (C) rich ecosystems with slower decomposition rates of peat organic matter under water logged conditions (Turunen, 2002; Yu *et al*., 2010) and plays an important role in C cycling. Peatlands also store more carbon than any other terrestrial ecosystem and covers about 3% (400 million ha) of Earth's land area (Turunen *et al*., 2002; Wu, 2012), of which 350 million (ha) are found in the northern hemisphere of the north America, Russia and Europe (Strack, 2008). Nearly 60% of these peatlands can be found in Canada (Tarnocai, 2009). They further account for 5-10% of the global methane (CH_4) in the atmosphere (Blodau, 2002; Mikaloff-Fletcher *et al*., 2004). Ombrotrophic peatlands receives all of its water and nutrient from precipitation, rather than from streams or springs and are often dominated by *Sphagnum* mosses. Fens on the other hand gain nutrient and water through groundwater and surface precipitation which results in a nutrient rich and a more alkaline ecosystem with a pH greater than 6 (Keddy, 2011). In addition, the peat pore water dissolved organic carbons (DOC) accounts for about 24% net ecosystem C uptake and plays an important role in the ecological, chemical functions as well as serving as microbial substrates. Spectroscopy techniques have led to the use of optical characteristic such as fluorescence and absorbance to assess dissolved organic matter (DOM) composition to infer the source. Little information is available to assess the impact of warming and N deposition to determine whether they provide any unique changes that can be linked to original DOM source in peatlands. Peatlands which serve as

C sinks are currently being threatened by increasing global atmospheric temperature causing positive C feedbacks. Also, studies show that atmospheric nitrogen deposition can change the release of CH_4 not only through increased vegetation growth but also by changing the species cover in the peatland (Nykänen *et al*., 2002). In addition, it is indicative that climate change could reduce the ability of northern peatlands to sequester atmospheric carbon (Wu & Roulet, 2014) which may be dependent on how the interactions with climate change affect CH_4 emissions. Anthropogenic activities have increased the global atmospheric concentration of CH_4 by 145% based on the International Panel on Climate Change (IPCC) data (Greatorex, 2000). However, there is still a great deal of uncertainty on the interactive effect of warming and nitrogen addition on CH_4 emission and DOC concentration in boreal peatland ecosystems. This may in turn affect plant productivity and species composition involved in the production, oxidation and transport of CH_4 (Joabsson *et al*., 1999; Lai *et al*., 2014) in boreal peatland. There have been some contradictory statements Saarnio *et al*. (2000a,b) being reported that N addition (fertilizer application) to peatlands does not have any effect on CH_4 emission despite Nykänen *et al*. (2002) reporting an increase in CH_4 emission. The contradicting responses may come as a result from changes in the soil microbial C use efficiency following N addition, thus the proportion of microbially assimilated C that may be incorporated into the microbial biomass, and the different temperature and moisture conditions. The N addition introduced in peatlands is to mimic the atmospheric nitrogen deposition associated with climate change. In view of this, carefully accounting for CH_4

fluxes and DOC concentration and their environmental controls is essential for an accurate assessment of the climate impacts of boreal peatlands.

1.2 Research questions and objectives

1.2.1 Research Questions

I seek to address the following research questions:

(i) Which environmental variable, such as water table, soil temperature and soil moisture, alters the CH_4 emission and DOC concentration from boreal peatlands?

(ii) How does atmospheric nitrogen deposition affect the rate of CH_4 emission from boreal peatlands?

(iii) How can climate warming affect CH_4 emissions from boreal peatland?

(iv) How does the interactive effect of warming and N addition affect the rate of CH_4 emissions from boreal peatlands?

(v) What effect does warming have on the DOC concentration in a peatland ecosystem?

(vi) How does N fertilization influence the DOC concentration in a boreal peat land?

(vii) How does the interactive effect of warming and N addition influence the soil pore water DOC and TN concentration and composition in an ombrotrophic peatland?

1.2.2 Hypothesis:

I hypothesized that: i) Addition of nitrogen fertilizer increases CH_4 emissions by enhancing vascular plant cover, which can stimulate plant root exudation and plant-mediated transport of CH_4; ii) Climate warming will significantly reduce the capacity of

ombrotrophic peatlands to sequester atmospheric carbon; iii) The effect of each of the single factors may change in combination with each other; thus the positive effect of warming treatments being counteracted by increased addition of nitrogen fertilizer; iv) warming, N addition and the interactive effect of warming and N addition would increase the DOC concentration by increasing the decomposition of soil organic matter and alter the optical characteristics of microbial and plant derived DOC.

1.3 Thesis outline

This thesis consists of five chapters.

Chapter #1 is an introductory chapter to the research.

Chapter #2 is the literature review where the characteristics of peatlands, C cycling, effect of temperature and water table on CH_4 emission, atmospheric nitrogen deposition, transport and exchange of DOC, influence of water table and DOC, the spectral characteristics of peat pore water was explained.

Chapter #3 and #4 are the main contribution of the thesis, of which each chapter would be considered for submission for publication in peer-review journals. Chapter #3 presents the results of the response of methane emission to warming and nitrogen addition in a boreal peatland. Chapter #4 presents the response of dissolved organic carbon to experimental warming and nitrogen fertilization in a boreal peatland.

Chapter #5 is made up of the summary and conclusion of the important findings from my research which will contribute to the global scientific body.

1.4 Contribution statement

The research objectives were conceived and defined under the supervision of Dr. Jianghua Wu who also provided the funding for the research to be carried out. I personally wrote the first draft of my thesis and the two manuscripts that make up the core of my thesis. Dr. Jianghua Wu comprehensively reviewed and revised my thesis. My committee member, Dr. Robert Scott reviewed both drafts of my research paper and steered me in the right direction of how to analyze my raw data and the possible output of my data presentation. Maryam Hajheidari helped develop the protocol used for analyzing the humification and fluorescence indices.

Chapter 2

2.0 Literature review

2.1 Peatland characteristics

Peatlands are carbon (C) rich ecosystems with slower rates of decomposition of peat organic matter under water logged conditions (Turunen, 2002) and play an important role in C cycling. The recalcitrant *Sphagnum* moss decomposition under anoxic condition greatly contributes to the peatland formation and C sequestration (Luan & Wu, 2014). Peatlands typically cover nearly 3% (400 Mha) of the entire world and hold about 470-620 Pg of C, making them the single largest terrestrial C store (Turunen *et al*., 2002; Lavoie *et al*., 2005; Porcal *et al*., 2009; Page *et al*., 2011). However, a total of 350 Mha of these peatlands are located in the Northern hemisphere, including the North America, Russia and Europe (Strack, 2008). With the North America contributing a staggering 186.5 Mha (Wang *et al*., 2017) and Canada alone contributing nearly 60% (124 Mha) of the total peatlands in the northern hemisphere (Tarnocai, 2009, Wang *et al*., 2018). These peatlands are often characterized base on their nitrogen (N) depositions and water levels. Ombrotrophic peat bogs normally receive nutrients and water by means of precipitation resulting in a nutrient poor and acidic conditions with pH <5 (Keddy, 2011). Fens on the other hand gain nutrient and water through groundwater and surface precipitation which results in a nutrient rich and a more alkaline ecosystem with a pH greater than 6 (Keddy, 2011). Peatlands vegetation is mostly made up of sedges, shrubs and *Sphagnum* moss. However, *Sphagnum* moss serves as the largest ground cover for any or all peatlands ecosystem Lund & Wu, 2014).

2.2 Peatlands role in global C cycling

2.2.1 Peatlands C pool

Natural peatlands have long been regarded as C sinks due to the imbalance between primary production and decomposition (Gorham, 1991). The C pools in peatlands ranges from 234 to 547 Gt C (Turunen, *et al*., 2002; Yu *et al*., 2010) with maximum accumulation in early Holocene in response to high insolation and stronger summer-winter seasonality. Yu *et al*. (2010) found that the C accumulation is controlled by increased plant production during warm summers and reduced peat C respiration during the cold winter in Alaska peatlands. Although global peatland C sink intensity has varied greatly over time, peatlands have accumulated >600 Gt C over the Holocene, serving as a long-term persistent C sink of >5 Gt C per century on average (Yu *et al*., 2010).

2.2.2 CH_4 exchange in peatland ecosystem

CH_4 is produced in the anaerobic zones of submerged soils by methanogens, oxidized to form CO_2 by methanotrophs in the aerobic zones, and is emitted to the atmosphere when the balance between the production and consumption is positive (Abdalla *et al*., 2016). CH_4 directly produced in peat can be oxidized as an energy source, or used for biosynthesis by methanotrophs. In a recent review, Turetsky *et al*. (2014) concluded that the CH_4 flux from fens is more sensitive to the vegetation type present and less sensitive to soil temperature than fluxes from bog or swamp ecosystems. However, the major controlling factors of CH_4 emission in northern pristine peatlands such as the peat soil temperature and the water table, but the vegetation composition (vascular plants)

may to some extent override the transport (Abdalla *et al*., 2016) of CH_4 emission to the atmosphere. Thus avoiding the oxidation stage of CH_4 emission in an ombrotrophic peatland due to the presences of sedges and shrubs which serves as conduits for the CH_4 emission. Other studies also identify soil moisture content, atmospheric N deposition, peat pH and the availability of quality substrates to influence the rate or control the CH_4 emission (Granberg *et al*., 1997; Bodelier & Laanbroek, 2004). However, there is some uncertainty with pH on CH_4 emission because the measured pH in mesocosms may differ from field pH, which could be due to the presence of other environmental factors since most works have been done in laboratory mesocosms. CH_4 emission from spring thaw for instance, may contribute up to 11% of the annual emission (Tokida *et al*., 2007) while other frozen sources emit CH_4 to the atmosphere in the form of ebullition. In view of this, higher CH_4 emissions could lead to a positive feedback on climate change and thereby further disturbance of peatland C stocks (Friedlingstein *et al*., 2006; Abdalla *et al.*, 2016).

2.3 Influence of N deposition in CH_4 emission

Nitrogen deposition in peatland ecosystems can alter soil biogeochemistry (Aber *et al*., 1998) and increase plant N concentration and growth (Fu & Shen, 2016), as well as N losses through soil gaseous emissions and nitrate leaching to waters. Nitrogen plays a regulatory role in CH_4 consumption (Bodelier & Laanbroek, 2004). Since pre-industrial times, the global nitrogen cycle has been greatly influenced by anthropogenic activities (Fowler *et al*., 2015). With elevated N deposition having been observed to influence the emission of some greenhouse gases over the last couple of decades and these N depositions favor most nitrophilic plant species, such as graminoids and vascular plant

species in peatland ecosystems. Some studies have indicated N addition focus on inhibiting CH_4 emission by direct denitrification and indirectly by microbes (Bodelier, 2011; Crill *et al*., 1994). The tight coupling between methane and nitrogen cycling and the associated implications for atmospheric methane concentrations has called to mind numerous studies such as Nykänen *et al*. (2002) and Bodelier (2011) to assessing N deposition effects on methane emission, consumption and underlying microbial processes.

2.4 Effect of temperature on CH_4 emission

Temperature has long been said to be correlated with CH_4 flux in peatlands. However, increasing temperature may not only increase CH_4 production but also CH_4 oxidation to some extent (van Winden *et al*., 2012). Dinsmore *et al.* (2009) reported that high water table increased CH_4 emission with increasing temperature in peat mesocosms and under-low water table and low temperature. At high water table conditions, methanogens increase CH_4 emissions as microbial activities increase with respect to increasing peat temperature in the anoxic zone. Abdalla *et al*. (2016) suggested that the response of CH_4 emission in peatlands to temperature appeared to be unpredictable, despite other studies (Christensen *et al*., 2003; Updegraff *et al*., 2001) reporting a clear dependence of CH_4 emission intensities on the peat soil temperature. Turetsky *et al*. (2008) reported an increase in CH_4 emission in a warming treatment in an Alaskan wetland whiles Updegraff *et al*. (2001) also reported similar findings in bogs and fens in northern Minnesota, USA.

2.5 Effect of water table depth on CH_4 emission

Water table is one of the three most important controlling factors of CH_4 emission in pristine peatland ecosystems as it determines the oxic and anoxic boundary and redox level within the peat (Dinsmore *et al*., 2009). Water table of peatlands controls the CH_4 emission by mediating the volume of peat substrate which is exposed to oxygen, influencing the microbial activities and decomposition (Teh *et al*., 2005). It has often been reported that at depth >20 cm below peat surface, a near zero CH_4 emission occurs or a net uptake from the atmosphere (Couwenberg *et al*., 2010). Water table can cause certain plant species to transport O_2 from the atmosphere to the rhizosphere and vice versa, completely by-passing the aerobic peat horizon (Minkkinen & Laine, 2006) and accounts for >80% of the CH_4 emissions especially from rice fields (Yu *et al*., 1997; Butterbach-Bahl *et al*., 1997). Lowering the water table in peatlands increases C mineralization and decrease the CH_4 emission (Dinsmore *et al*., 2009) and may often lead to a net increase in N_2O emissions (Aerts & Ludwig, 1997). Saturated water table in sedges/hummock mesocosms has been found to increase CH_4 emissions (Dinsmore *et al*., 2009), indicating a seasonal shift in the balance of positive and negative effect of vascular plants on CH_4 emission. Abdalla *et al.* (2016) also reported a significant decrease in CH_4 emission in a drained peatland irrespective of the vegetation cover or land use, while rewetting increased CH_4 flux by an average of 1.3±6.5 g C m^{-2} $year^{-1}$. Water table can act as an "on-off" switch in regulating the rate of CH_4 and CO_2 emission in peatlands as reported by Luan & Wu (2015), thus making peat substrate swell and shrink depending on water inputs (Carlson *et al*., 2015). This water fluctuation may

encourage other important environmental factors to influence CH_4 emission if water tables drop below a certain threshold (Dinsmore *et al*., 2009; Luan & Wu, 2015). Therefore, the relationship between water table and CH_4 flux can be complicated under field conditions.

2.6 DOC exchange and transport

Peatlands are substantial source of dissolved organic carbon (DOC) to surface water (Clark *et al*., 2005) and contributes up to about 35% to the overall peatland carbon budget (Worrall *et al*., 2003). Dissolved organic matter (DOM) consists of a continuum of organic substances ranging from defined small molecules to highly polymeric humic substances. The DOM continues to contribute greatly to carbon, nitrogen, phosphorus and nutrient budget (Michalzik & Matzner, 1999) and the transport of metals (Guggenberger *et al*., 1994). Recent report indicate that vascular plants influence the quality of peatland DOC by enriching it with low molecular weight (LMW) compounds, which increased heterotrophic microbial activity in the peat (Robroek *et al*., 2016). In addition, an increase of vascular plant cover in response to climate change can potentially alter the organic matter (OM) in peatlands, leading to increased C losses.

2.7 Specific ultraviolet absorbance ($SUVA_{254}$)

Specific UV absorbance (SUVA 254), defined as the UV absorbance at 254 nm normalized for DOC concentration (L mg C^{-1} m^{-1}), is used for estimating the aromaticity of each DOC fraction aromaticity index (Weishaar *et al*., 2003). Report indicates that $SUVA_{254}$ from DOM increases with temperature due to increased microbial activity

leading to the partial decomposition in a lysimetric experiment (Karavanova & Milanovskiy, 2016). It has been reported that, aromaticity of DOM depends on the season and source as they often tend to differ, thus DOM eluted from plant residues such as leaves and grasses has high aromaticity which could be due to the presence of tannin compared to DOM from chernozen (black-colored soil) (Karavanova & Milanovskiy, 2016).

2.8 Fluorescence index (FI)

Fluorescence spectra are used to assess the origin and transformation degree of DOM through calculation of several fluorescence indices. McKnight *et al*. (2001**)** found the fluorescence index (FI) to have a significant relationship with DOC concentrations. However, the FI values may vary within different flow path with little change in the DOC concentration. It has been reported that increasing FI values for increasing residence time of water within a terrestrial ecosystem, decreases the DOC concentration with increasing hydrologic residence time (Johnson *et al*., 2011). These differences have enabled researchers to characterize the DOC sources with the use of fluorescence spectroscopy in a diverse range of settings. Johnson *et al*. (2011) reported that FI value in a base flow was inversely related to the DOC concentration. In addition, FI value between 1.5-1.8 may indicate a microbial derived source of DOM and 1.2-1.4 for plant-derived source of DOM (McKnight *et al*., 2001). An intermediate values suggest a contribution of a combination of the two sources (Johnson *et al*., 2011). A lower DOC: DON (dissolved organic nitrogen) ratio is indicative of newer and more labile carbon inputs in the form

of recently synthesized plant exudates, whereas older terrestrially derived DOM is usually nitrogen poor (Fenner *et al*., 2007).

2.9 Humification index (HIX)

Humification index (HIX) is a sensitive and simple parameter, which is often used to characterize DOM and calculated from the fluorescence emission spectra obtained at excitation wavelength of 254 nm. It was first introduced by Zsolnay *et al*. (1999) and later modified by Ohno (2002) to estimate the degree of maturation of soil DOM and has since been used to by several scientists. The HIX increases with increasing degree of DOM aromaticity in the soil.

2.10 Potential drivers of change in DOC

2.10.1 Temperature change

Laboratory studies have consistently shown a positive influence of temperature on soil DOC production (Moore & Dalva, 2001), and positive within-year correspondence between DOC and temperature has been observed in field studies of a range of soil waters (Michalzik & Matzner, 1999). However, the influence of temperature on temporal variations at an individual site may appear very different. The production of DOC is a microbially mediated, oxidative decomposition of the organic matter in the peat. All microbially mediated reactions will increase in rate with increasing temperature, and so the rate of production of labile carbon will increase as temperature increases.

2.10.2 Water table depth

One of the main controls of DOC is the water table depth as it determines the depth of the oxic/anoxic boundary and redox level within the soil. Increasing precipitation generate additional runoff from wetland areas and thus increased DOC flux. In a controlled laboratory experiments, Clark *et al*. (2009) observed that water table draw-down can cause an increase in net DOC production. The general consensus from these studies is that lowering the water table increases C mineralization and decreases CH_4 emissions (Aerts & Ludwig, 1997).

Chapter 3

3.0 Response of methane emission to warming and nitrogen fertilization in a boreal peatland

3.1 Introduction

Methane (CH_4) is one of the most important greenhouse gases (GHG) as it has a major role in warming of the Earth's climate (IPCC, 2013). CH_4 is a potent GHG that is 86 and 25 times more effective than CO_2 in absorbing long wave radiation in the atmosphere with a 20-year and 100-year time horizon (Forster *et al*., 2007; IPCC, 2013). Global CH_4 emission is about 500-600 Tg CH_4 per year (Wang *et al.*, 2004; Conrad, 2009; Bruhwiler *et al*., 2014) with approximately 40% of the emissions coming directly from natural sources such as wetlands (Abdalla *et al*., 2016). Over the last two centuries, methane concentrations in the atmosphere have more than doubled, largely due to human related activities (EPA, 2017). Cold climates in boreal peatlands slow down decomposition rates of organic matter (Mikaloff-Fletcher *et al*., 2004, Luan & Wu, 2015). These decomposition rates are regulated by saturated, anaerobic soils and the cool climate conditions associated with relatively northern latitudes where peatlands are predominantly found (Luan & Wu, 2015). Thus, boreal peatlands emit methane that is produced under anaerobic conditions (Frolking *et al*., 2006) and contribute approximately 5-10% of global CH_4 emitted to the atmosphere (Mikaloff-Fletcher *et al*., 2004). For most CH_4 sources, the rate of production is usually much larger than the rate of CH_4 emission since CH_4 is consumed by microorganisms before entering the atmosphere (Hanson & Hanson, 1996; Conrad, 2009).

CH_4 can be transported from the anoxic peat via three main pathways: diffusion, ebullition, and plant mediated transport (Lai, 2009). CH_4 is transported to the atmosphere by diffusion due to the concentration gradient from the peat to the atmosphere. This diffusion process, despite being slower compared to the other modes of CH_4 transport, is an important component of the biogeochemical cycle that controls the rate of microbial CH_4 consumption (Lai, 2009). About 64% of the total CH_4 flux in northern peatlands is transported via ebullition when there is a sudden drop in atmospheric pressure (Tokida *et al*., 2007), a rise in temperature (Fechner-Levy & Hemond, 1996), and a reduction in hydrostatic pressure (Strack *et al*., 2005). Aerenchymatous tissues of some vascular plants serve as conduits for transporting CH_4 from roots in the anaerobic zone to the atmosphere, therefore bypassing the aerobic and methane oxidizing peat layers (Whalen, 2005). Mechanism controlling CH_4 emission in boreal peatland may include, but are not limited to, the quality of substrate for methanogenesis (Lai, 2009), water table depth (Dise *et al*., 1993; Bellisario *et al*., 1999), peat temperature (Granberg *et al*., 2001; Lai, 2009), soil pH (Dunfield *et al*., 1993), and peat vegetation (Bubier *et al*., 1995). Studies show that atmospheric nitrogen deposition can change the release of CH_4 not only through increased vegetation growth but also by changing the species cover on the peatland (Nykänen *et al*., 2002). Although it is well-known that N fertilizer is beneficial to both methanogens and methanotrophs (Bodelier, 2011), there is still a great deal of uncertainty on the interactive effects of warming and N fertilization on CH_4 flux in boreal wetlands because CH_4 flux largely depends on environmental and physical factors (Granberg *et al*., 2001; Olefeldt *et al*., 2013; Luan & Wu, 2015). For example, CH_4 flux

within a wetland shows large spatial heterogeneity, in which hollows are noted for being CH_4 sources, hummocks CH_4 sinks, and lawns the transition zones between the two (Luan & Wu, 2015; Lozanovska *et al*., 2016). Global temperature is predicted to increase by 0.3~4.8 ^oC at the end of this century (IPCC, 2013), causing an increase in the rate of decomposition. However, the current average increase across land and ocean surface areas has reached a record high of 0.94 °C in 2016, above the 20th century average of 13.9 °C, surpassing the previous record warmth of 2015 by 0.04 °C in the last 41 years (NOAA, 2018) with a 4% increment in temperature. Furthermore, atmospheric N deposition rates are predicted to continuously increase and may double from current values by 2050 (Bobbink *et al*., 2010; Phoenix *et al*., 2011), greatly increasing the number of regions receiving damaging levels of N inputs. Due to these anticipated changes, it has become necessary to study the interactive effect of warming and nitrogen deposition on CH_4 emissions from boreal peatlands.

Chen *et al*. (2017) found warming suppressed the rate of CH_4 emission due to a decrease in the soil moisture content in an alpine meadow. Earlier research also indicated that warming (van Winden *et al*., 2012) can have significant effects on ecosystem CH_4 emissions in peatlands via increase of the methanogens both in the number and abundance at surface soil. Turetsky *et al*. (2014) found that CH_4 flux from bogs is less sensitive to soil temperature while flux from fens is more sensitive to the vegetation type present. Ward *et al*. (2013) also found, CH_4 emission in a boreal peatland was more strongly controlled by the vegetation composition than by warming. However, warming

may accelerate changes in soil microbial processes, vegetation dynamics and chemistry of pore water, all of which affect CH_4 cycling (Weltzin *et al*., 2003).

Despite the fact that global warming and N deposition may occur simultaneously, very few studies have actually focused on their interactive effects on CH_4 emission from boreal peatlands. In order to prevent over estimation of the rate of CH_4 emission from boreal peatlands due to climate change, I conducted a warming and N deposition experiment in a peatland complex. Therefore, the present study was intended to contribute to understanding the effects of warming and N deposition on CH_4 flux in peatland ecosystems to help to assess the effects of N and warming globally to make an informed decision on future climate change. I hypothesized: i) that addition of nitrogen fertilizer increases CH_4 emissions by enhancing vascular plant cover, which can stimulate plant root exudation and plant-mediated transport of CH_4; ii) climate warming will significantly reduce the capacity of ombrotrophic peatlands to sequester atmospheric carbon; iii) that the effect of each of the single factors may change in combination with each other; thus the positive effect of warming treatments being counteracted by increased addition of nitrogen fertilizer.

3.2 Materials and methods

3.2.1 Site description

The study was carried out at the Robinson peat bog (48°15’ 44” N, 58°40’ 03” W), ~100 km southwest of Corner Brook, Newfoundland and Labrador (NL). The experimental site has a temperate climate with an annual precipitation of 1340 mm and yearly average temperature of 5 °C, with January and July temperature of between −9 and

−2 °C and 13 and 20 °C respectively (1981–2010 averages; data from the nearest weather station in Stephenville, ~50 km away from our site). The manipulative experiment was established at the natural peatland. The natural peatlands are a lot wetter than the drained peatlands, which include some wet depressions and peatland pools (permanently inundated with about 40–60 cm of standing water from about 10–200 m^2 in area). Vegetation consists of an approximately equal distribution of graminoids (*Trichophorum cespitosum, Carex chordorrhiza*) and dwarf shrubs (*Gaylussacia baccata, Rhododendron groenlandicum, Andromeda glaucophylla, Ledum palustre ssp*.), with bryophytes (*Sphagnum spp., Hylocomium splendens, Aulacomnium turgidum*) (Luan & Wu, 2015). *Sphagnum* moss serves as the major ground cover for the peat bog and its sponge-like characteristics gives its unique ability to absorb water and trap gases within its structure.

3.2.2 Experimental design

The experiment was conducted using randomized complete block design (RCBD) to analyze the effects of four treatments with four replicates. In detail, it consisted of four (4) main plots of 16 m×16 m dimensions separated from each other by a 4 m buffer space. Of these, each individual main plot consisted of four 2 m×2 m subplots with 2 m buffer space between the subplots. Within each main plot, subplots were assigned to one of four treatments: warming (W), nitrogen fertilizer application (N), interactive effect of warming and nitrogen fertilizer application (W+N) and a control treatment (C). Each treatment was replicated four times to cover the scope of the research. Boardwalks are installed next to each plot to reduce disturbance during sampling without necessarily compressing the surrounding peat, which could enhance the escape of the trapped gases.

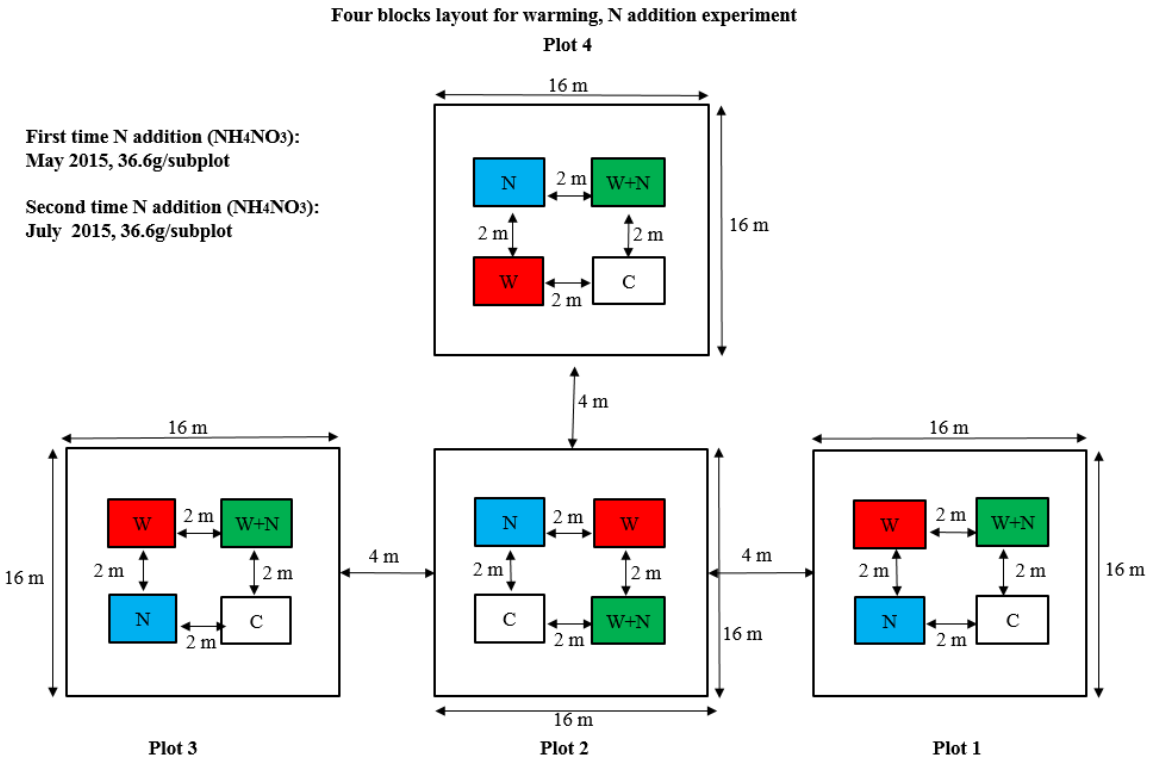

Figure 3. 1 Plot 2 x 2 m, buffer zone 4 m, red filled means warming treatment (W), blue filled means nitrogen addition (N)(6.4g N/m^2/yr), green filled means warming and N addition (W+N) (6.4g N/m^2/yr) and control (C) treatment.

The manipulative warming was achieved using an open top chamber (OTC) (Marion *et al*., 1997; Teh *et al*., 2011; Ward *et al*., 2013) constructed with 0.125″ thickness of transparent plexiglass acrylic sheet paper. The OTC was expected to achieve an average warming of about ~1-2 °C during the growing season. The method offers a strong means to examine the effects of warming without the need for a power supply and has been used repeatedly in peatland ecosystems (Walker *et al*., 2006; Dorrepaal *et al*., 2009) because it is inexpensive and easy to construct. At each subplot, a piezometer made of perforated 1.5 m ABS (Acrylonitrile Butadiene Styrene) pipe with a sealed bottom was inserted into

the peat to measure the water table depth. The peatland water table was measured from the perforated pipes with a ruler. Peat temperatures at different depth (5 cm and 20 cm) were measured with temperature probes (Digi-sense calibrated long stem, Quebec, Canada), moisture at 5 cm depth below peat surface was measured regularly with GS3 probe connected to a ProCheck reader (Decagon Devices Inc, Washington, USA) during gas sampling. Furthermore, the Lascar USB temperature/humidity data logger (Lascar Electronics Ltd., UK) at a warming subplot and a non-warming subplot continuously measured air temperature at a half-hourly average basis.

N fertilizer was added in the form of ammonium nitrate (NH_4NO_3) dissolved in a 5.678 L of water drawn from a nearby pool. A total amount of 36.6 g N per subplot (corresponding with 6.4 g N m^{-2} $year^{-1}$) which is ten times higher than the N deposition in the region (Reay *et al*., 2008) was added to each N treatment subplot and W+N treatment subplots one week prior to the first gas sampling using a 7.571 L capacity watering can. Fertilizer application was done twice in each growing season (May and July) to mimic the atmospheric N deposition in the region and to conform to other research works being carried out (Luan & Wu, 2014; Gong *et al*., 2019). The unfertilized plots were watered with the same amounts of water drawn from the pool. N inputs were intended to stimulate the microbial community and result in broad differences in fluxes of CH_4 thus providing a better dataset on which to compare the fluxes from the treatments.

3.2.3 Gas sampling and analysis

Gas sampling ensued from June to September in 2015 and 2016 and June to October in 2017. Gas samples were taken between the midday times of 10:00 and 16:00

every two weeks. Gas sampling at each subplot was collected using static opaque chambers made of polyvinyl chloride (PVC) of 50 cm height and 26.3 cm diameter. A collar of 26 cm inner diameter with a groove on the top was permanently inserted into the peat at depth of 10-15 cm at least two weeks prior to my first sampling (i.e. during the field maintenance stage). During each sampling, the chamber was fitted on a water-filled groove on the collar, enabling an airtight seal between the headspace air and surrounding atmosphere. The openings on the covers remained open to avoid pressurizing the chamber air, which has the potential to cause a large measurement error (Hutchinson & Livingston, 2001). The concentration of the emitted gas in the chamber increases with time and gas exchange rate can be estimated based on its concentration changes in the chamber headspace during the sampling (Kutzbach *et al*., 2007). In addition, as samples are drawn from the chamber, it is important to replace the sampled air by an equal volume of air so as to avoid any artifacts of negative pressure within the chamber as low pressure will draw more gas from the soil to compensate. In doing so, the chamber air was circulated during flux measurements by syringe pumping (mixing). Four gas samples were taken within intervals of 0, 10, 20 and 30 min after the immediate deployment of the opaque chambers on the grooves of the collars, after which CH_4 concentrations were measured according to Yu *et al*. (2013) and Luan & Wu (2014). Samples were temporarily stored in a 60 mL polypropylene syringe fitted with a polycarbonate/nylon stopcock and subsequently analyzed using a gas chromatograph (Scion 456-GC, Bruker Ltd., Canada) equipped with a flame ionization detector (FID) to analyze the CH_4

concentration within 24 h. An amount of 8 mL gas is transferred into a pre-vacuumed 12 mL glass vial, which is sealed with a butyl rubber septum.

3.2.4 Methane analysis and emission rate calculations

CH_4 flux was calculated from the linear regression slope of the concentration change in the chambers over time. Three criteria were used to reject erroneous or insignificant methane flux measurements. First, to avoid introduction of artifacts due to disturbance during the chamber deployment, all measurement series with a starting value above twice the ambient methane concentration were rejected. Second, CH_4 fluxes were calculated by performing a linear regression (Holland *et al*., 1999) and using the slope of the linear regression to calculate the flux:

$$F = S \bullet V \bullet A^{-1} \qquad (1)$$

where F is the flux (mg m^{-2} h^{-1}), S is the slope of the regression (dc/dt)(s), V is the chamber volume (L), and A is the chamber area (m^2). Thirdly, data points were evaluated based on goodness of fit through visual inspection and excluding points showing signs of plateau. I used a minimum of three time points for flux calculation, established a consistent protocol and rejected any time series that failed to meet that protocol's standards for linearity to minimize introduction of long-term errors due to possible diurnal variation in CH_4 emission. A non-linear regression analysis was used to explore the trend of CH_4 emission with the environmental variables.

3.2.5 Data analysis

I used Minitab17 (Minitab 17 statistical software) and Sigma plot 12.5 for data exploration, conditioning and analysis. CH_4 flux values were log_{10} transformed to normalize the data before any statistical analysis was performed to minimize heterogeneity of variance effect. The data used were the treatment means of each sampling day for the three years. General linear model (GLM) was used for the repeated measures ANOVA to determine the effect of warming and nitrogen deposition on CH_4 emission and environmental variables were treated as covariates. A post hoc Tukey's test was used to determine the differences between means of the treatment. A non-linear regression was used to observe the relationship between CH_4 fluxes and environmental variables.

3.3 Results

3.3.1 Environmental variables influencing CH_4 emission

The average air temperature was 16.8 ^oC and 14.3 ^oC for the warming plot and ambient (control) for the 2017 growing season (**Figure 3. 2**), which suggested that my OTC increased the air temperature by 2.5 ^oC. However, peat soil temperature decreased by 0.4 ^oC at the 5 cm and increased by 0.2 ^oC at the 20 cm depth in the same growing season (2017) (**Table A. 1**). The peat soil temperature increased by 0.4 ^oC at both 5 cm and 20 cm depth with respective to control during the growing season when all data was pooled together (**Table 3. 1**). There was no significant difference in peat soil temperature at 20 cm depth ($F_{3,68} = 0.09$, $p = 0.97$) but significant difference between the years was observed ($F_{2,68} = 4.34$, $p = 0.017$). In addition, there was no significant difference in the

water table depth ($F_{3,68}$ = 0.04, p = 0.99) but there was significant difference between the years ($F_{2,68}$ = 7.38, p = 0.001). Also no significant difference was observed at the peat soil temperature at 5 cm ($F_{3,68}$ = 0.17, p = 0.92). Soil moisture content at the boreal peat land was not significant ($F_{3,52}$ = 042, p = 0.74) between the treatment and the years ($F_{2,52}$ = 0.69, p = 0.5). There was a correlation between the following environmental variables and $Log_{10}(CH_4)$ flux: the peat soil temperature at different depths ($T_{5\ cm}$ and $T_{20\ cm}$) were positive and significantly correlated at (p = 0.001) for $T_{5\ cm}$ and (p < 0.001) at $T_{20\ cm}$ **(Table 3. 2)** when all treatments were pooled together in a Pearson's correlation**.** Water table depth on the other hand, was not significantly correlated with methane flux when all treatment effects were pooled (p = 0.339). The environmental variables explained 64% of the variation in the CH_4 emission when treatment and year was used as factors when all the data was pooled in a GLM.

Table 3. 1 Three years mean values of environmental variables over the growing season (2015-2017).

Treatment	Soil Moisture Content (%)	Soil Temp. $_{5\ cm}$ (^oC)	Soil Temp. $_{20\ cm}$ (^oC)	Water Table Depth (cm)
C	66.7±14.9^a	16.1±3.5^a	14.7±2.9^a	9.0±7.0^a
N	66.7±14.1^a	16.1±3.6^a	14.7±2.9^a	9.0±6.7^a
W	61.7±13.2^a	16.5±3.9^a	15.1±2.9^a	8.3± 6.1^a
W+N	65.3±13.5^a	15.9±3.7^a	14.8±2.9^a	9.2±7.5^a

Data values are means±SD for the different treatment effect. Control (C), Nitrogen addition (N), Warming (W) and Warming and Nitrogen addition (W+N). Similar lowercase letters represent no significant differences (p > 0.05) between the treatments.

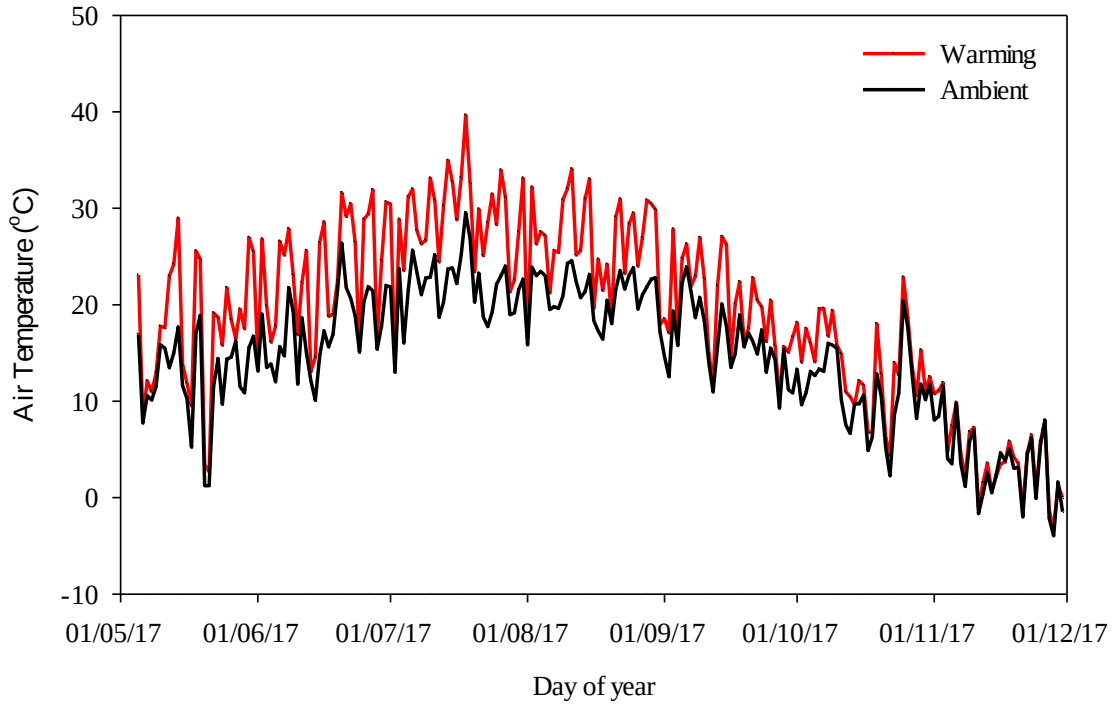

Figure 3. 2 Daily mean air temperature in the open top chamber (red line) and the ambient (black line) for 2017 growing season.

Table 3. 2 Pearson correlation (r) coefficients between $Log_{10}(CH_4)$ flux and environmental variables in a northern natural peatland.

	$Log_{10}(CH_4)$ Flux (mg m^{-2} h^{-1})	Soil Moisture Content (%)	Soil $T_{5\ cm}$ (^oC)	Soil $T_{20\ cm}$ (^oC)	Water Table Depth(cm
Soil Moisture Content (%)	-0.0.39				
Soil $T_{5\ cm}$ (^oC)	0.361***	-0.456***			
Soil $T_{20\ cm}$ (^oC)	0.509***	-0.362**	0.883***		
WTD (cm)	-0.109	-0.736***	0.454***	0.339**	

Coefficient values without (*) are not significant. *p= 0.05, ** $p \leq 0.01$, *** $p \leq 0.001$. Soil moisture content (SMC), $Log_{10}(CH_4)$ flux (mg m^{-2} h^{-1}), Soil temperature ($T_{5\ cm}$ and $T_{20\ cm}$), Water table depth (WTD).

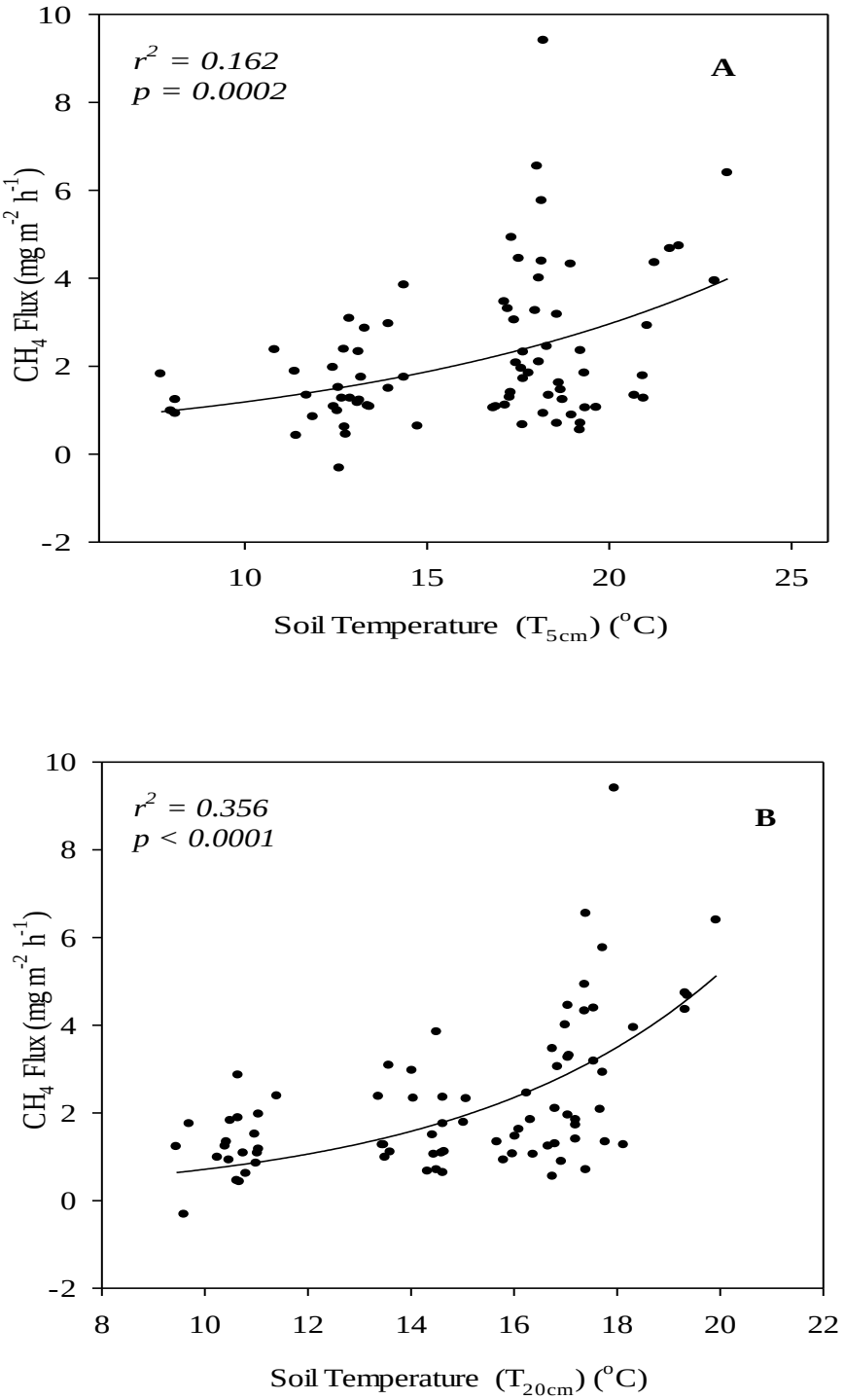

Figure 3. 3 Non-linear relationship between CH_4 emission and peat soil temperatures (^oC) at different soil depth when all treatments were pooled together. Temperature at 5 cm (T5 cm) (A) and temperature at 20 cm depth (T20 cm) (B).

A non-linear regression analysis showed CH_4 flux increased with increasing $T_{5\ cm}$ (r^2 = 0.162, p = 0.0002) when all treatment was pooled together while an increasing $T_{20\ cm}$ led to an increase in the CH_4 flux over the duration of the experiment (r^2 =0.356, $p < 0.0001$) (**Figure 3. 3**). A non-linear regression between the cumulative CH_4 flux and water table depth (WTD) for the three (3) years showed no correlation ($F_{1,78}$ = -2.5, p = 1.0) and soil moisture content was not correlated to cumulative CH_4 flux (mg m^{-2} h^{-1}) ($F_{1,62}$ = -1.15, p = 1.0).

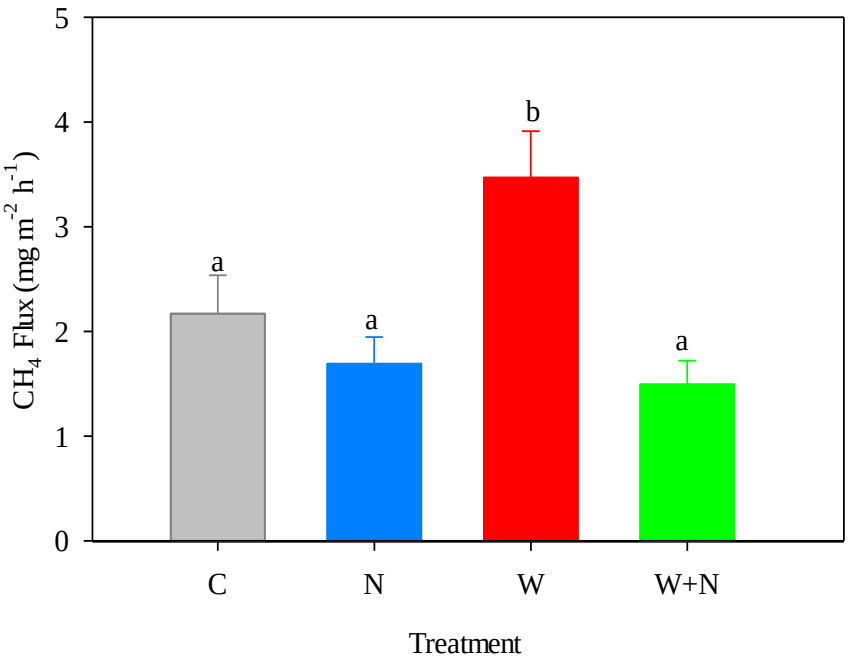

Figure 3. 4 Overall CH_4 flux for three years under different treatment effects. Control (C), nitrogen addition treatment (N), warming treatment effect (W) and interactive effect of warming and nitrogen addition (W+N). The solid bar represents the means and error bar indicates standard error (means±SE), sample size (n=80). Non-identical letters mean corresponding treatments are significantly different ($p \leq 0.001$).

3.3.2 Effect of N fertilizer application on CH_4 flux

The cumulative mean CH_4 flux in the N-treatment effect was 1.7 mg m^{-2} h^{-1} for the duration of the experiment. The average CH_4 emission in the N-treatment effect for

the growing season was 2.0±1.6 mg m^{-2} h^{-1}, 1.9±1.1 mg m^{-2} h^{-1} and 1.3±0.8 mg m^{-2} h^{-1} for the year 2015, 2016 and 2017 respectively (**Table A. 1**). CH_4 emission decreased by 23% in nitrogen addition treatment relative to the control treatment. CH_4 emission in the N addition treatment effect decreased by 32% from 2016–2017 and decreased by 5% from 2015-2016, compared to the control treatment effect which also increased by 14% from 2015-2016 and decreased by 17% from 2016-2017. Further, a non-linear regression indicated a relationship between CH_4 flux and soil temperature at 20 cm (r^2 = 0.361, p < 0.0001) and 5 cm (r^2 = 0.176, p = 0.007) in the N-treatment **(Figure 3. 5)** but no significant relationship was observed in the remaining environmental variables. Also, peat soil temperature at 5 cm and 20 cm depth explained 18% and 36% of the variation of CH_4 flux in the N addition treatment effect. Emissions from the N-treatment followed the same pattern as the rest of the treatment effect with peaks of CH_4 flux occurring in the summer periods in 2015 (**Figure 3. 6**) and decreased towards the end of the summer period of the subsequent years. My data indicated that N addition alone did not have a significant effect on CH_4 flux. Furthermore, data indicates a significant difference between W and N-addition treatment (t = 4.11, p = 0.001) and no significant difference was observed with C and N treatment (t = -1.11, p = 0.68) (**Figure 3. 4**).

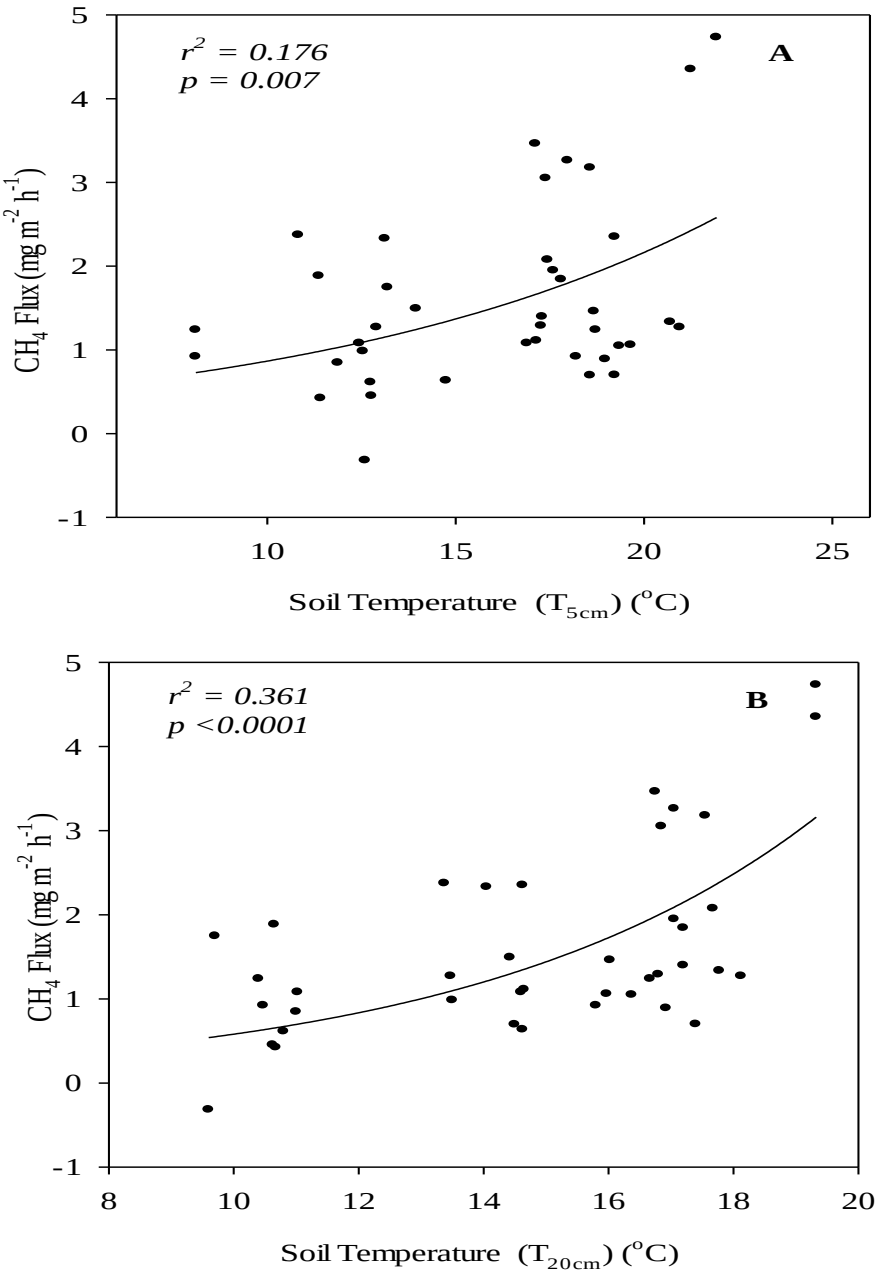

Figure 3. 5 Non-linear regressions of CH_4 flux and peat soil temperature at 5 cm (A) and 20 cm (B) depth in the N-treatment effect.

3.3.3 Effect of warming on CH_4 flux

The cumulative mean CH_4 flux for the warming treatment was 3.5±2.0 mg m^{-2} h^{-1} and the highest instantaneous CH_4 flux recorded was 4.1±2.7 mg m^{-2} h^{-1} which occurred in 2016, followed by a 3.4±1.9 mg m^{-2} h^{-1} in 2015 and the least recorded was 2.9±1.2 mg m^{-2} h^{-1} in 2017 (**Table A. 1**). A post hoc Tukey's test indicated that, there was a significant difference in the cumulative CH_4 flux between W and W+N ($t = -4.15$, $p = 0.001$), W and N ($t = 4.11$, $p = 0.001$) and W and control ($t = 3.0$, $p = 0.019$) respectively during the three-year manipulative experiment. Also, CH_4 emission increased by 59% in the W-treatment effect with respect to the control for the duration of the experiment. CH_4 flux decreased by 29% between 2016-2017 in the warming treatment. However, there was a 21% increase in the CH_4 flux in the W-treatment between 2015 and 2016 growing season. But no significant difference ($F_{3,16} = 0.89$, $p = 0.469$) in CH_4 flux was observed between the W-treatment effect and the control, N and W+N treatment effect in 2015, but there was a significant difference between W and W+N treatment effect ($t = -3.02$, $p = 0.029$) in 2016 and also a significant difference between W and N addition ($t = 3.09$, $p = 0.022$) and W and W+N ($t = -2.89$, $p = 0.035$) treatment effects in 2017 respectively. A non-linear regression analysis in the W-treatment effect showed a positive non-linear relationship between CH_4 flux and soil temperature at 20 cm depth ($r^2 = 0.475$, $p = 0.001$) and relationship for soil temperature at 5 cm was not significant ($r^2 = 0.156$, $p = 0.085$) (**Figure 3. 7**). Furthermore, no significant correlation ($p > 0.05$) was observed in the warming treatment effect between the CH_4 flux and the environmental variables.

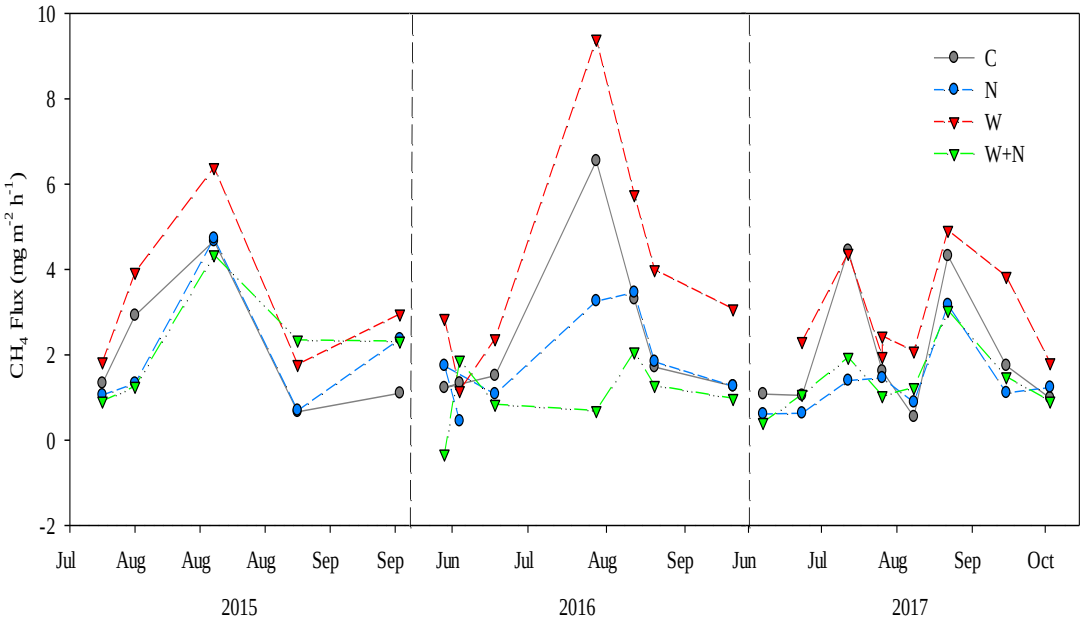

Figure 3. 6 Monthly fluctuations of CH_4 flux under control (C), nitrogen addition (N), warming (W) and interactive effect of warming and nitrogen addition (W+N) treatment effects for the 3years.

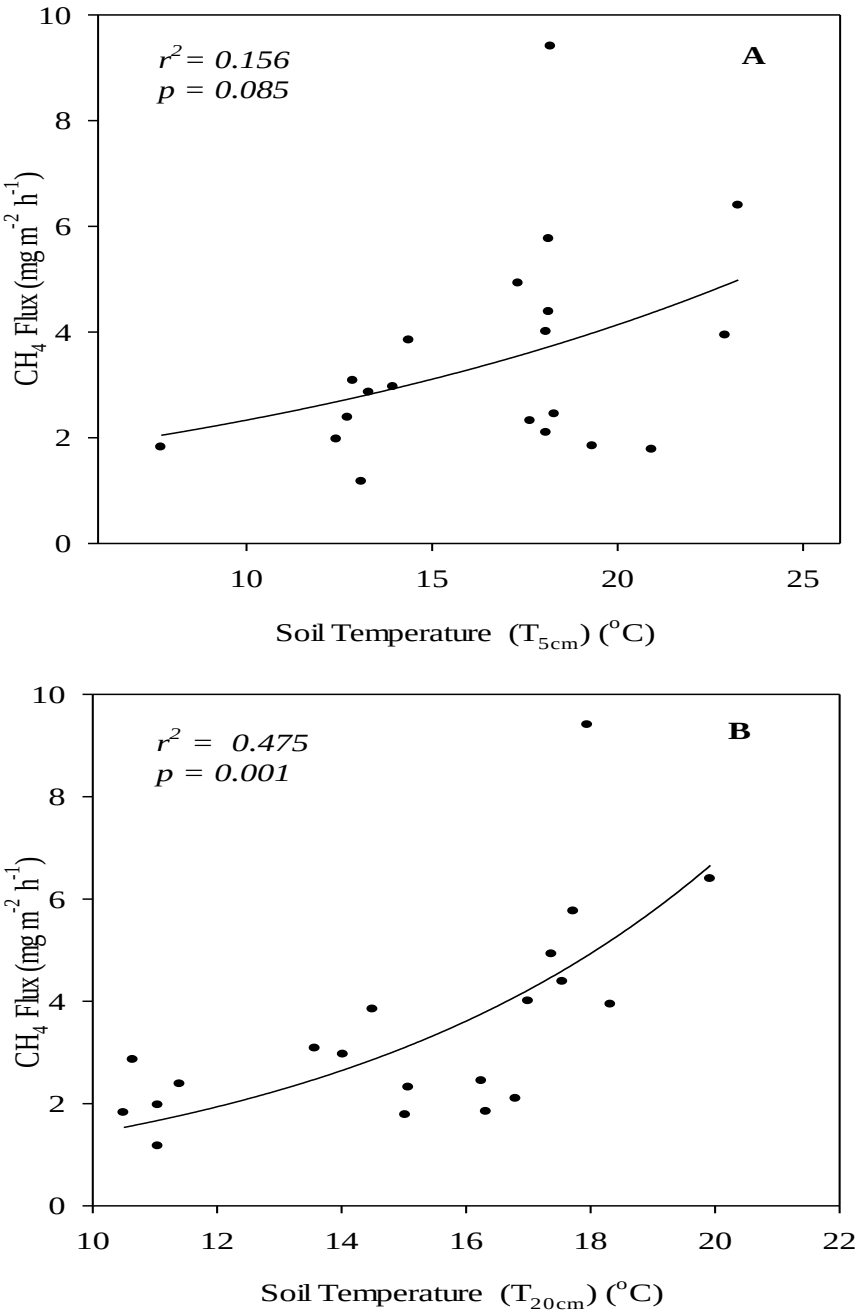

Figure 3. 7 Non-linear regressions of CH_4 flux and peat soil temperature at 5 cm (A) and 20 cm (B) depth in the W-treatment effect.

3.3.4 Interactive effect of warming and nitrogen addition on CH_4 flux.

The mean CH_4 flux for the W+N treatment effect was 1.5±1.0 mg m^{-2} h^{-1} for the duration of the experiment. The highest CH_4 flux in the W+N treatment was recorded in 2015. However, the interactive effect of warming and nitrogen addition in the manipulative experiment showed a decrease in CH_4 emission by 32% with respect to the control for the entire duration of the experiment. In addition, CH_4 emission in the W+N treatment effect decreased by 51% from the year 2015–2016 and increased by 31% from 2016–2017 growing season (**Figure 3. 8**). There was no significant difference between the W+N treatment effect and the control (t = -1.19, p = 0.637), W+N and N addition (t = -0.09, p = 1.0) but there was a significant difference between the W+N and W-treatment (t = -4.15, p = 0.001). There was also a significant but non-linear relation between CH_4 flux and soil temperature at 20 cm depth (r^2 = 0.331, p = 0.008) and there was no significant relationship between CH_4 flux and soil temperature at 5 cm depth (r^2 = 0.186, p = 0.057) **(Figure 3. 9)**. There was no significant correlation between CH_4 flux and the environmental variables (soil moisture content and water table) at the W+N treatment plot.

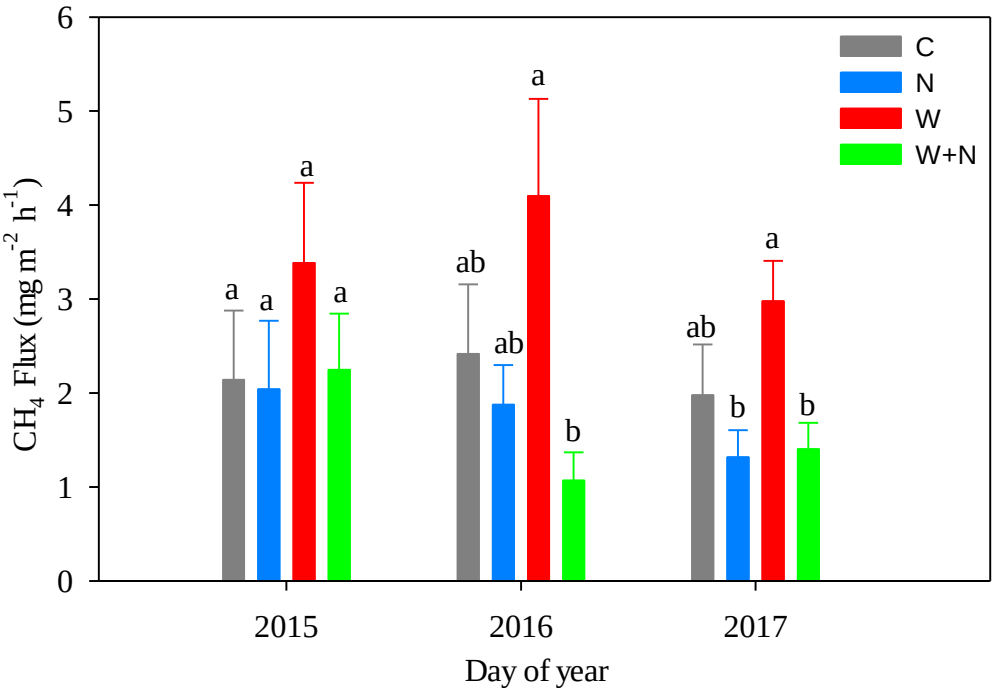

Figure 3. 8 Response of CH_4 flux (mg m^{-2} h^{-1}) to various treatment effects for the growing seasons. Control (C), nitrogen addition (N), warming (W) and interactive effect of warming and nitrogen addition (W+N) treatment effect. Error bars represent standard errors (SE). Letters on error bar indicates significant differences (p <0.05) between treatment effect within the respective year.

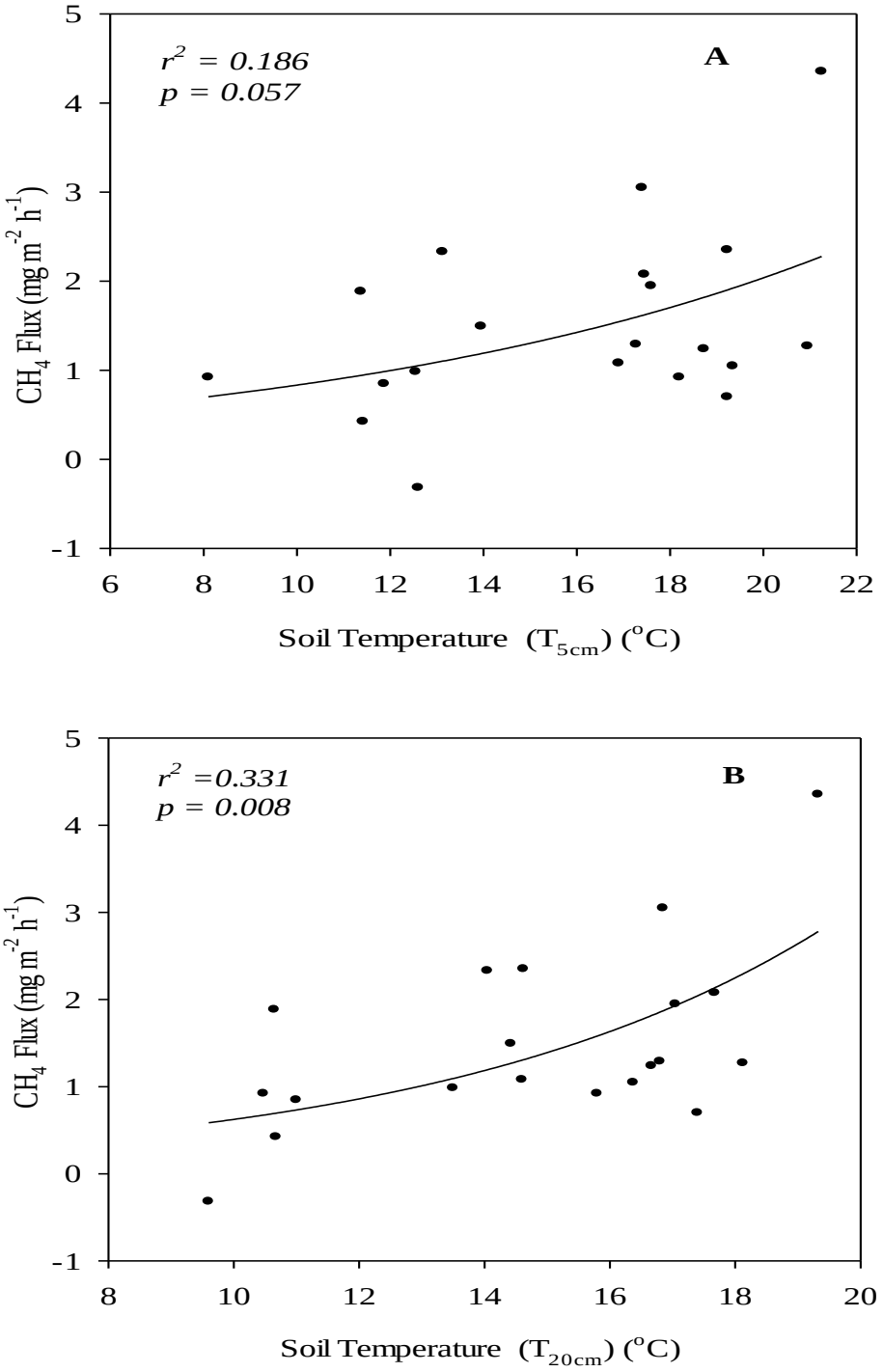

Figure 3. 9 Non-linear regressions of CH_4 flux and peat soil temperature at 5 cm and 20 cm depth in the W+N treatment effect.

3.4 Discussion

3.4.1 Impact of environmental variables

A number of environmental variables have been known to control CH_4 emissions in northern peatlands such as temperature, water table and moisture content. My study used a unique experimental setup that simultaneously examined the effect of warming and nitrogen addition in natural boreal peatland over the growing season and the environmental conditions that altered CH_4 flux. The OTC in my manipulative experiment generated an increased air temperature of 2.5 ^oC similar to what was reported in Gong *et al*., (2019). The OTC allowed excess heat to dissipate via the top opening by free convection aided by the forced convective flow of wind preventing the OTC from over heating as both moisture and gas concentrations could be potentially affected by the degree of enclosure. After three years of the warming and nitrogen addition experiment, peat soil temperatures were the only major contributing factor that influenced the rate of CH_4 fluxes in my experiment which is in accordance with previous studies conducted (Granberg *et al*., 2001; Luan & Wu, 2015), where temperature contributed to about 18% of the total variation in CH_4 flux during the wet season (Luan &Wu, 2015). CH_4 has often been known to be produced under anoxic conditions in boreal peatlands. However, our results indicated neither water table nor soil moisture content had a significant effect on CH_4 flux ($F_{1,53} = 1.33$, $p = 0.254$ and $F_{1,53} = 0.03$, $p = 0.857$ respectively). This indicates that the water table may function as an "on-off switch'' depending on the variability of CH_4 flux (Luan & Wu, 2015) in peatland ecosystems. Similar results were observed by Dinsmore *et al*. (2009) in a mesocosm experiment with *Juncus*/hummock,

sedge/hummock and hollows. My research allowed me to identify the seasonal CH_4 flux response to warming and N addition in a manipulation experiment over the growing season (**Figure 3. 8**). For the entire growing seasons, fluctuations of CH_4 was often increased days after nitrogen fertilization but was not significant and periods where the temperature conditions increased thereby enhancing the activities of some microorganisms.

3.4.2 Response of CH_4 flux to N addition

In general, N addition tended to decrease CH_4 flux over the duration of the manipulative experiment (**Figure 3. 8**) compared to the control. Similar findings have also been reported by Bodelier (2011) who found nitrogen addition to decrease CH_4 emission. The N addition effect mainly focused on the direct inhibition by denitrification or the indirect inhibition of microbes or plants by increasing their biomass. A possible explanation for the lower CH_4 emission following N addition may be due to simulation of CH_4 consumption rather than CH_4 production in the ombrotrophic peatland. This is similar to a previous study by Crill *et al*. (1994) in a drained peatland where they observed N addition inhibiting CH_4 emission. This may have reduced carbon sequestration due to the shift towards more easily decomposable litter (Berendse *et al*., 2001; Malmer & Wallén, 2005) mainly during the summer periods **(Figure 3. 6).** Aerts *et al*. (2001) also argued that N addition may initially ease the nutrient limitation to plant growth, while in the long term other environmental factors such as temperature and dissolved organic carbons (DOC) may become increasingly important.

In view of this, continuous N deposition to natural peatlands over prolong period reduces or has no effect on CH_4 emission (Watson & Nedwell, 1998; Granberg *et al*., 2001) despite rapid increase in sedge coverage after fertilizer application. This may have occurred when the *Sphagnum* filters were no longer able to trap the high rate of N deposition and the excess N leached through the *Sphagnum* filters, which was later made available for vascular plant uptake (Lamers *et al*., 2000; Rydin & Jeglum, 2006). There are several contradictory statements made about how N addition in peatland can affect the rate of CH_4 emission (Nykänen *et al*., 2002). For example, Nykänen *et al*., (2002) reported N addition increased the rate of CH_4 emission in an ombrotrophic peatland due to the rapid coverage of *Eriophorum* (sedges), despite Frenzel & Rudolph (1998) reporting that the *Eriophorum* lacked the ability to oxide CH_4. Also Dise & Verry (2001) reported no significant difference in the N-addition treatment effect and the control treatment in relation to CH_4 emission but observed an enhanced plant productivity.

Furthermore, studies by Saarnio & Silvola (1999) and Dise & Verry (2001) showed no effect of nitrogen addition increasing CH_4 emission in peatland. In view of this, nitrogen addition may be seen to have both positive and negative effects on the CH_4 flux similar to what was observed in an alpine meadow (Chen *et al*., 2017). Other research suggests that increase in N leaching is unlikely to occur in the short term (Wright *et al*., 2001) but may likely lead to the emission of the third most important greenhouse gas N_2O which has a global warming potential of 298 over an atmospheric lifespan of 114 years (Nykänen *et al*., 2002; IPCC, 2013). However, this was beyond the scope of my research at this point since I did not focus on N_2O emission.

3.4.3 Response of CH_4 flux to warming

The positive effect of CH_4 emission due to increased temperature in the warming treatment was closely related to the presence of some vascular plants such as sedges and shrubs. And this effect is mainly due to increased CH_4 production in the anoxic region of the peatland as my research showed air temperature stimulate CH_4 production directly, or indirectly via effects on the vegetation present. Emission of CH_4 were high throughout the growing season and the highest CH_4 peak was observed in the latter part of the summer of 2016 (**Figure 3. 6**). It has long been anticipated that carbon cycling processes in peatlands are susceptible to changes in climate and I found that there was a significant difference between CH_4 emission in the W-treatment and control for the entire duration of the manipulative experiment. Emissions in the W-treatment followed similar pattern as the W+N treatment effect in the first year. Throughout the growing season, the warming plot was observed to be a continuous CH_4 source (**Figure 3. 6**) in my manipulative experiment. This was interpreted as an increase in CH_4 production in the anoxic regions of the peat (Granberg *et al*., 2001) directly and indirectly via the effects on the plants (sedges and shrubs) which modulate the transport of CH_4. Thus increased temperature may enhance the photosynthetic carbon fixation rates and also increasing the root exudation by the sedges.

Recent studies showed that climate warming affected CH_4 flux more than did water table depth (Lafleur *et al*., 2005) and this was confirmed by our experiment as CH_4 flux was seen to positively correlate with peat soil temperature at $T_{20\ cm}$ compared to the water table (**Figure 3. 7**) and my result is similar to what was observed by Rinne *et al*.

(2018). However, another study found peat temperature at 30 cm depth to be the best predictor of CH_4 emission in peatlands (Nykänen, *et al*., 1998). Peat warming caused an increasing proportion of easily decomposable peat components to decay leading to peatlands becoming sources of CH_4 (Malmer *et al*., 2005) as observed in our manipulative experiment. In addition, Moore (1995) showed that warming contributes to about 2.3 x 10^{12} g of CH_4 m^{-2} yr^{-1}emission from boreal peatlands in Canada. In view of this, final observations in our experiment support our hypothesis that warming will increase the rate of CH_4 emission from boreal peatland making them C source.

3.4.4 Interactive effect of warming and N addition on CH_4 flux

Warming and nitrogen addition has often been reported in the literature to influence or alter the rate of CH_4 emission. However, its interactive effects (W+N) are seldom examined. Our manipulative experiment showed a decrease (32%) in the rate of CH_4 emission in the W+N treatment effect with respect to the control. In addition, CH_4 emission decreased by 57% between the W-treatment and the interactive effect of W+N treatment and the percentage change in the rate of CH_4 emission was not due entirely to the environmental variables in the peatland ecosystem. The decrease in CH_4 emission in the W+N treatment was due to the additive effect experienced in the W+N treatment effect by the addition of ammonium based nitrogen fertilizer. In part, this counteracting effect of N addition on the positive effect of warming may be related to the changes in the temperature regime and vegetation composition. This may also indicate a biogeochemical effect, in which the competition from nitrogen reducers on CH_4 production counteracted the positive effect of warming induced changes in vegetation.

From all indication, the N addition may have led to higher oxidation potentials of CH_4 in our manipulative experiment and hence one of the reasons for the reduced CH_4 emission in the treatment. Looking at the nature and trend of our W+N treatment, we can anticipate an increase in CH_4 emission occurring subject to a decreasing warming effect in future.

Therefore, if care is not taken when interpreting results of the interactive effect of W+N, we may be over estimating the rate of CH_4 emission in peatlands since our results has shown N addition to have a negative effect on peatland ecosystem. However, the negative effect of N addition on CH_4 emission may likely lead to the emission of the third most important greenhouse gas N_2O which has a global warming potential of 298 over an atmospheric lifespan of 114 years (Nykänen *et al*., 2002; IPCC, 2013), however this was not considered in our present study.

3.5 Conclusion

The primary goal of this study was to examine the role of increasing warming and N addition on the CH_4 fluxes of an ombrotrophic peatland under climate warming. Our findings showed that CH_4 emission in the ombrotrophic peatland was highly correlated with peat soil temperature at 20 cm depth. Also, our finding showed that increasing N addition tend to decrease the rate of CH_4 emission in peatland over a prolong period of time and warming continuously increased the rate of CH_4 emission. The present study further demonstrates the interactive effect of increasing warming and N addition can make a substantial contribution to the GHG budget in peatland ecosystems. These

findings will prevent over estimating CH_4 emission due to the counteractive effect of the presence of nitrogen.

In addition, in order to draw general conclusion about experimental treatment effects, it is essential to evaluate findings over several field seasons, since response patterns can be strongly confounded by interannual variations in the weather. It is evident that short-term manipulative experiment mainly demonstrated transient response patterns and may not be convictive enough hence the need for a long-term manipulative experiment. This should not be ignored because of the larger soil organic carbon pool and higher temperature sensitivity of the peatland ecosystem. The present study deepens our understanding of the interactive effect of warming and N addition impacts on GHG balance in the peatland ecosystem, and help us assess the global C effects in order to avoid the over estimation of the global CH_4 emission.

Chapter 4

4.0 Response of Dissolved Organic Carbon (DOC) to Experimental Warming and Nitrogen Fertilization in Boreal Peatland

4.1 Introduction

Dissolved organic carbon (DOC) is a ubiquitous component of the freshwater carbon cycle, produced by the decomposition of dead plant material and the release of exudates from growing plants (Hope *et al*., 1997; Fenner *et al*., 2004; Freeman *et al*., 2004, Dieleman *et al*., 2016; Wang *et al*., 2017). DOC concentrations and characteristics vary over time based on climate, hydrology and catchment due to seasonal changes in production, consumption and transport (Fellman *et al*., 2009; Broder *et al*., 2017). DOC concentration in peatland pore water accounts for about 24% of the net ecosystem carbon (C) uptake (Dinsmore *et al*., 2010) and plays several ecological and chemical functions as well as serving as microbial substrate (Porcal *et al*., 2009; Strack *et al*., 2015; Pinsonneault *et al*., 2016). The transfer of fresh DOM from the peat surface to deep layers can induce methanogensis or microbial decomposition in temperate peatlands, thereby producing carbon dioxide (CO_2) and methane (CH_4) deep in the peat. In addition, DOC producing peatlands occupy approximately 3% of the Earths total land surface and hold about 470-620 Pg of C, making them the single largest terrestrial carbon store (Lavoie *et al*., 2005; Porcal *et al*., 2009; Page *et al*., 2011). However, peatlands are at risk due to climate change (Clark *et al*., 2010; Gallego-Sala & Prentice, 2013) as a result of global warming, N deposition, and changes in hydrology, affecting peatlands

substantial loss of carbon due to increased CO_2 released from decomposition and lower carbon accumulation rates (McDowell *et al*., 1998; Yano *et al*., 2000; Jorgenson *et al*., 2013; Selvam *et al*., 2017) and the increased CH_4 emissions to the atmosphere (Roulet *et al*., 1992). There are numerous pathways for releasing carbon from peatlands which are related to changes in the depth of the water table. For instance, increased decomposition following increased aerobic condition as the water table depths increase (Dieleman *et al*., 2016) and nitrogen supply changes may cause the shifts in carbon storage (Weltzin *et al*., 2003). Among these pathways of carbon release is the flux of DOC and therefore, the control of DOC release is important in managing carbon balance in peatlands.

The principal controls on production and consumption of DOC in peatland are complex, and include vegetation composition, hydrology, and soil chemistry. For instance, vegetation composition in peatland ecosystems may influence the local hydrology and soil temperature, and plants stimulate microbial activity through the release of root exudates (Bubier *et al*., 1995; Peacock *et al*., 2014). In addition, there is collective evidence of increasing carbon turnover, which indicates that peatlands are on the verge of switching from being a carbon sink to a carbon source due to climate change (Boothroyd *et al*., 2015).

Several studies have indicated that enhanced temperature and microbial activities result in a rise in DOC as a consequence of higher peat decomposition (Freeman *et al*., 2001; Clark *et al*., 2005; Dinsmore *et al*., 2013; Ward *et al*. 2013; Dieleman *et al*., 2016; Yuan *et al*, 2018). The sensitivity of DOC production to temperature is affected by the water table within the soil (Clark *et al*., 2009). As climate change scenarios suggest that

increased temperature and a reduction in summer rainfall across the northern hemisphere could simulate decomposition of organic matter leading to an increase in the production and release of DOC. However, increasing evapotranspiration in peatland due to climate change may negate and perhaps lower DOC export despite increasing temperatures (Pastor *et al*., 2003, Kane *et al*., 2014) and potentially drive these ecosystems towards acting as net carbon sources, rather than sinks.

Very few studies have been conducted *in situ,* most of the experiments carried out so far were done in laboratory mesocosms (Dieleman *et al*., 2016) and with very few studies covering forest ecosystems (Dillon & Molot, 2005). Also, how far these laboratory findings can actually be replicated under field conditions is uncertain. Compared to laboratory studies, findings from field monitoring often tend to show contradictory results. Increasing atmospheric nitrogen deposition has also been linked to increase DOC concentrations in peatland (McDowell *et al*., 1998; Yano *et al*., 2000). The amount of atmospheric N deposition has continued to increase significantly (Yuan *et al*., 2018) due to increasing industrialization over the past couple of decades. This has resulted in the mineralization of nitrogen content in peatland vegetation and has affected DOC cycling. Concerns regarding the impact of N deposition has led to several studies over the last two decades with focus on those simulating increased N deposition via application of N to experimental plots (Worrall *et al*., 2006). Increases in anthropogenic N deposition can increase productivity, C assimilation and also release C if decomposition rates are accelerated through changes in N availability or vegetation composition. According to a report by IPCC (2013), N deposition has become one of the

major contributors to global climate warming for more than half a century. Scheffer *et al*. (2001) documented that mineralization of N is faster in *Sphagnum* spp. dominated peatland compared to vascular plant and bryophyte dominated peatlands. However, Aerts *et al*. (1999) suggest there are no clear differences in N mineralization in bogs and fens of North America and Europe, indicating that the various dominant plant community has a stronger influence on nutrient cycling dynamics in peatlands. Recent studies have acknowledged that N deposition increased DOC production (McDowell *et al*., 1998; Yano *et al*., 2000) while other studies have shown no significant increase in DOC production and export (Porcal *et al*., 2009). These contradictory findings indicate that other mechanisms may play a major role in the changes in DOC concentration and export.

Seasonal variations in both quantity and quality of DOC have been observed and such changes will have ecological repercussions (Peacock *et al*., 2014). The quality of DOC as a microbial substrate may influence the balance between C sequestration and its mobilization in peatlands (Holden, 2005; Limpens *et al*., 2008). In view of this, warming and nitrogen deposition may have the potential to alter the dissolved organic carbon under the influence of other drivers (Froberg *et al*., 2013, Luan & Wu, 2014). Therefore, a comprehensive understanding of how warming, nitrogen deposition and its interactive effects influence DOC characteristics is lacking in peatland ecosystems.

In this study, I report three years of data on DOC concentration from the natural peat bog complex with the aim of determining the response to warming, N deposition, and interactive effect of warming and N addition on the quantity, quality and spectral

characteristics of DOC. I hypothesized that warming, N addition and the interactive effect of warming and N addition would increase the DOC concentration by increasing the decomposition of peatland and alter the optical characteristics of microbial and plant derived DOC. To the best of my knowledge, my research is the first to investigate the interactive effects of warming and N addition in an *in situ* manipulative experiment in northern peatlands. Although, some research has been conducted in laboratory mesocosms (see Dieleman *et al*., 2016) and in a forest ecosystem (Dillon & Molot, 2005), this study will provide a better understanding for elucidating the effects of global changes on DOC in peatland ecosystems.

4.2 Materials and methods

4.2.1 Site description and experimental design

The study was carried out at the same site as mentioned earlier in Chapter #3 of this research paper along with the experimental design. Also, a pipe with 1.8 cm internal diameter was inserted at a 40 cm depth into the peatland and MacroRhizon (Rhizosphere, The Netherlands) coupled with a female luer was subsequently inserted at a 10 cm depth to collect soil pore water samples at 10 cm depth for DOC and total nitrogen (TN) measurement. Measurement of all environmental variables has been reported in Chapter #3 of this research paper. In addition, N inputs were intended to stimulate the microbial community and result in broad differences in DOC and TN fluxes thus providing a better dataset on which to compare treatment effects. In the first year (2015) of the manipulative experiment, no water samples were collected at 40 cm depth for the DOC and TN analysis.

4.2.2 Water sampling

The water samples were collected using a 60 mL air tight syringe with a female luer attachment and wells were flushed out at 40 cm depth and allowed to refill. Water samples were also collected from the MacroRhizon (Rhizosphere, The Netherlands) at 10 cm. Once collected, the samples were transported immediately to the laboratory and stored in refrigerator at 4 °C until DOC and TN measurement was carried out. Water samples were pre-filtered with a 0.45 µm syringe filter (VWR International, Edmonton, Canada) to remove particulates and the filtrates transferred into a 24 mL glass vial for DOC and TN analysis. Each water sample was divided into three (3) aliquots to be used for the different analysis. All samples, regardless of sampling time, were subjected to cold storage which has been shown to ensure reasonable preservation of DOC between time of sampling and analysis (Cook *et al*., 2016). However, DOC concentrations and quality (absorbance and fluorescence properties) may change in stored water samples over time even after acidification or freezing (Spencer *et al.*, 2007; Fellman *et al.*, 2008; Cook *et al.*, 2016).

4.2.3 DOC and UV-vis analysis

DOC concentrations were calculated using a calibration curve ranging from 0 to 100 mg L^{-1} based on potassium hydrogen phthalate (KHP) (Sigma–Aldrich, Ontario, Canada) and potassium nitrate (Sigma–Aldrich, Ontario, Canada) standards. The DOC analysis was carried out using a Shimadzu TOC-LCPH/TN analyzer (Mandel Scientific Company Ltd, Guelph, Canada). Additionally, all water samples were acidified and sparged to remove dissolved inorganic carbon (DIC), except for samples to be analyzed

using Agilent 8453 UV-Visible spectrophotometer (Agilent Technology, San Diego, USA). Sparging is crucial as the presence of DIC in water samples cause interferences leading to large errors with the determination of total organic carbon (TOC) and DOC using Shimadzu TOC-LCPH/TN analyzer (Mandel Scientific Company Ltd, Guelph, Canada). The organic carbon is oxidized to CO_2, which when released from the sample is detected and reported as mg L^{-1} or ppm TOC or DOC.

The ultra-violet absorbance (UVA) procedure also required that the sample be passed through a 0.45 μm syringe filter and transferred to a quartz cuvette with 1 cm path length. UV measurements were performed using distilled water as a blank at 25 °C at a wave length 254 nm and absorbance reported as cm^{-1}. The DOC and UVA determinations are then used in the calculation of the specific ultra-violet absorbance (SUVA) (Tipping *et al*., 2009; Peacock *et al*., 2014). The $SUVA_{254}$, an indicator of aromatic C content (Weishaar *et al*., 2003) is calculated by dividing the UV absorbance at 254 nm (cm^{-1}) by the DOC concentration of the sample (mg L^{-1}) and then multiplying by 100. SUVA is reported in units of L mg^{-1} m^{-1} as shown in equation 1.

$$[SUVA_{254} = Abs_{254}\ (cm^{-1}) / DOC\ (mg\ L^{-1}) \times 100] \qquad (1)$$

Where $SUVA_{254}$ is the specific ultraviolet absorbance at 254 wavelengths (L mg^{-1} m^{-1}), Abs_{254} is the absorbance at 254 wavelengths (cm^{-1}), DOC is the dissolved organic carbon (mg L^{-1}). Increasing $SUVA_{254}$ values indicate a higher aromaticity (recalcitrant carbon compound) and lower values indicate more labile compounds (Hansson *et al*., 2010). This was used to determine the quality of DOC in peatlands.

4.2.4 Fluorescence and humification index analysis

Water samples were further analysed using the Cytation3 imaging reader (BioTek, Vermont, USA). Samples were placed in a 96-well microplate and analysed alongside blanks and without blanks. However, only the data analysed without the blanks was used for our research in order to conform to the previous year data analysis (thus Raman scattering was not included). Fluorescence spectroscopy used to distinguish between different classes of organic matter (Senesi, 1992; Alberts & Takacs, 2004). Emission at 470-520 nm for excitation at 370 nm (Cory & McKnight, 2005) with 10 nm increment was used to determine the fluorescence index (FI):

$$FI = I_{470} \, x \, (I_{520})^{-1} \qquad (2)$$

Where fluorescence index (FI), intensity at different wavelength (I) for excitation at 370 nm. The FI was used to differentiate between plant derived (FI=1.2-1.4) and microbial derived DOM (FI=1.5-2.0) (McKnight *et al*., 2001; Cory & McKnight, 2005) as the ratio represents a decrease in emission with increasing wavelengths of microbial derived DOM. Emissions at 435-480 nm with a 5 nm wavelength increment were used to determine excitations at 254 nm for the humification index (HIX) respectively. HIX was calculated according to Ohno (2002) as sum of fluorescence intensities from 435 to 480 nm divided by the sum of fluorescence intensities from 300 to 345 nm and from 435 to 480 nm. HIX values in this formula range from 0 to 1 with increasing degree of humification:

$$\mathbf{HIX} = \sum_{\mathbf{n=435}}^{\mathbf{480}} \mathbf{In} \times \left[\sum_{\mathbf{300}}^{\mathbf{345}} \mathbf{In} + \sum_{\mathbf{435}}^{\mathbf{480}} \mathbf{In} \right]^{-1} \qquad (3)$$

Where, $(\Sigma I_x \rightarrow y)$ is the sum of the fluorescence intensity at emission wavelengths $x \rightarrow y$ nm at an excitation of 254 nm and I is the fluorescence intensity at each wavelength (Ohno, 2002).

4.2.5 Data analysis

Normality was tested using the Anderson-Darling test. Repeated measure ANOVA(GLM) was applied to compare the means of the DOC flux between the treatments across the growing season. Further, Tukey's significant ($p \leq 0.05$) difference test was employed to identify differences among means. Pearson's correlation was also carried out to determine correlations between DOC and spectral characteristics. Minitab 17 (statistical software) and sigma plot 12.5 software were used for all data analysis and preparing graphs/charts. Error estimates presented in this paper are standard error unless otherwise stated based on the data being analyzed.

4.3 Results

4.3.1 Treatment effects on DOC and TN concentrations

The average air temperature was 16.8 ^{o}C and 14.3 ^{o}C for the warming and ambient (control) plot for the 2017 growing season as mentioned earlier in Chapter #3. The OTC warming increased the air temperature by 2.5 ^{o}C in the manipulative experiment. There was no significant difference between the treatment effect of C, N, W and W+N treatment effect on DOC concentrations at 10 cm depth ($F_{3,48} = 0.15$, $p = 0.927$) (**Table 4. 1**). Also, there was no significant difference between the treatment

effects at $DOC_{40\ cm}$ ($F_{3,23}$ = 2.19, p = 0.117). Pair t-test between $DOC_{10\ cm}$ and $DOC_{40\ cm}$ indicated a significant difference (t = 2.23, p = 0.031) between the two peat soil depths. DOC in the W-treatment decreased by 1% and increased by 13% relative to the control at10 cm and 40 cm peat depth respectively. The N addition treatment effect also increased by 0.8% and 13% in the 10 cm and 40 cm peat depth respectively. DOC at the W+N treatment effect increased by 1.3% and 20% in the 10 cm and 40 cm peat depth respectively. In general, the DOC concentrations in our experimental system increased with increasing peat soil depth. Monthly fluctuation of the $DOC_{10\ cm}$ concentration in all treatments followed a similar pattern with mostly (high DOC) peaks being observed in August (summer) of 2016 and 2017, and a decline in DOC occurring in part of the Fall season (**Figure 4. 1A**). There was a difference in $TN_{40\ cm}$ ($F_{3,39}$ = 3.43, p = 0.026) between the W+N and W-treatment effect but no significant difference at the $TN_{10\ cm}$ ($F_{3,76}$ = 2.25, p = 0.089) and a paired t-test also showed a significant difference between $TN_{10\ cm}$ and $TN_{40\ cm}$ (t = -7.76, p < 0.001) peat soil depth. Conversely, TN concentrations in the treatment effect increased with increasing peat depth except the control at 40 cm. The W-treatment decreased the total nitrogen concentration by 8.8% and 25.2% in the 10 cm and 40 cm peat depth, and the N addition treatment also increased TN by 11.8% and 10% in the 10 cm and 40 cm depth respectively. There was also an increase of 33.3% and 26.2% in the TN concentration at the W+N treatment at 10 cm and 40 cm peat depth. The $DOC_{40\ cm}$ and $TN_{40\ cm}$ concentration data included only 2 years (2016 and 2017) since no data was recorded for the first year of sampling at 40 cm peat depth.

Table 4. 1 Mean DOC, TN concentration and spectroscopy characteristics of, $SUVA_{254}$, FI and HIX for 3 years of warming and nitrogen addition.

Peat soil depth (cm)	Treatment	DOC (mg/L)	TN (mg/L)	$SUVA_{254}$ (L mg^{-1} m^{-1})	FI	HIX
10	C	38.60±7.71[a]	1.02±0.34[a]	2.79±0.64[a]	1.53±0.06[a]	0.17±0.07[a]
	N	38.89±9.01[a]	1.14±0.48[a]	2.66±0.58[a]	1.55±0.11[a]	0.15±0.07[a]
	W	38.21±6.57[a]	0.93±0.45[a]	2.76±0.75[a]	1.56±0.06[a]	0.16±0.07[a]
	W+N	39.11±6.82[a]	1.36±0.85[a]	2.86±0.56[a]	1.56±0.09[a]	0.17±0.07[a]
40	C	34.91±9.17[a]	2.02±0.63[ab]	3.02±0.65[a]	1.59±0.12[a]	0.15±0.05[a]
	N	39.50±5.05[a]	2.22±0.98[ab]	2.79±0.41[a]	1.59±0.14[a]	0.16±0.06[a]
	W	39.44±8.29[a]	1.51±0.47[a]	2.79±0.33[a]	1.61±0.08[a]	0.16±0.05[a]
	W+N	41.76±7.63[a]	2.55±0.90[b]	2.65±0.41[a]	1.65±0.29[a]	0.17±0.05[a]

Treatment symbols represent: control (C), nitrogen addition effect (N), warming effect (W), and interactive effect of warming and nitrogen addition (W+N). Spectroscopy characteristics are fluorescence index (FI), humification index (HIX), specific ultraviolet absorbance ($SUVA_{254}$), total nitrogen (TN) and dissolved organic carbons (DOC). Data values are (mean ± SD) of the respective treatment effects and superscripts represents significant differences ($p < 0.05$) between treatments.

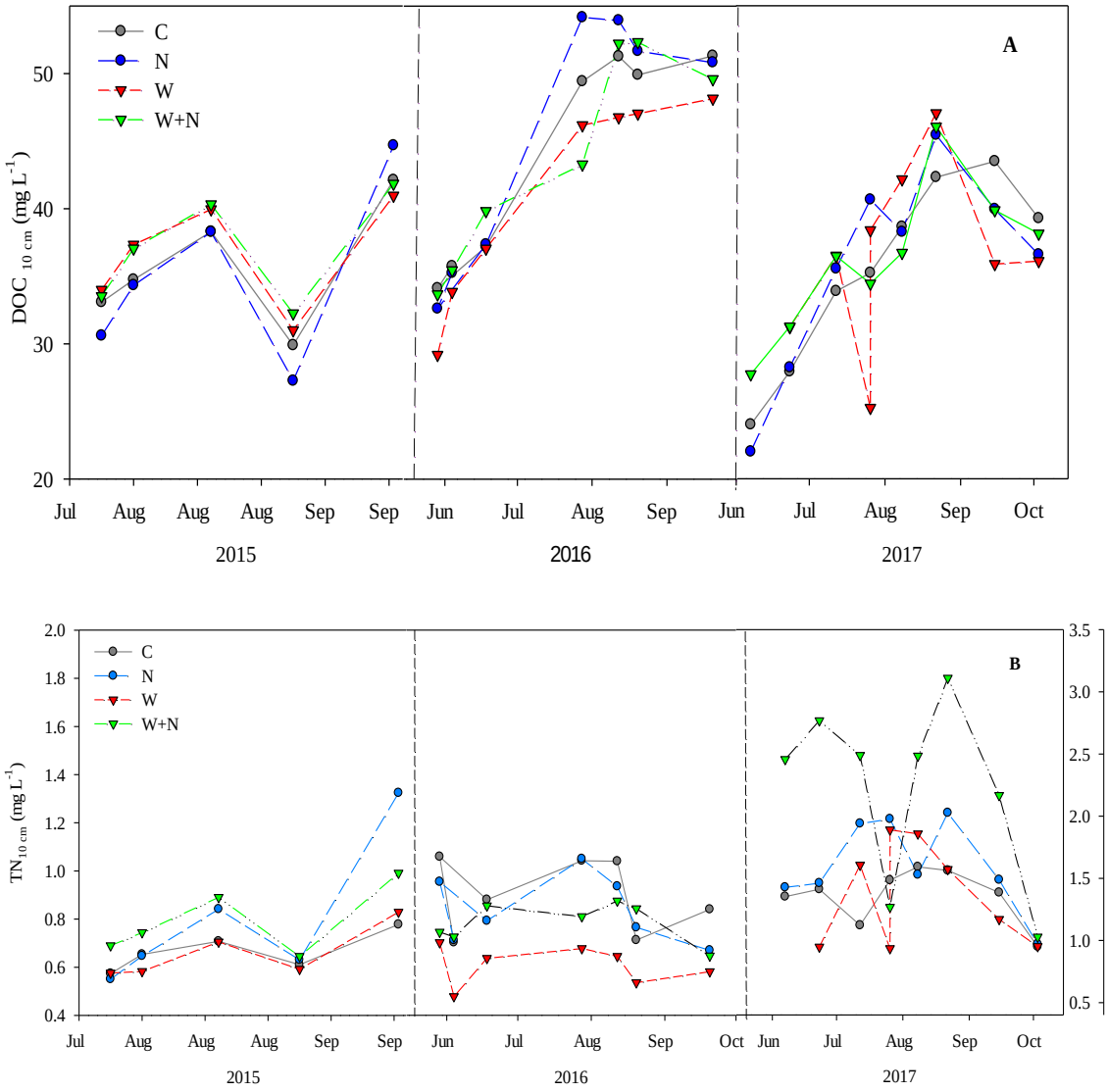

Figure 4. 1 Monthly mean value of peatland dissolved organic carbon (DOC) (A) and total nitrogen (TN) (B) monthly concentrations for the three-year period. Treatment effects are represented as: control (C), nitrogen addition treatment (N), warming treatment (W) and interaction effect of warming and nitrogen addition treatment (W+N).

4.3.2 Spectroscopy index of DOC

4.3.2.1 Specific ultraviolet absorbance (SUVA)

The mean $SUVA_{254}$ in the treatments at 40 cm were higher compared to $SUVA_{254}$ at 10 cm (**Table 4. 1**) with the exception of W+N (2.65) which decreased at 40 cm. The

highest mean $SUVA_{254}$ values at the different peat depth were found in W+N treatment effect at 10 cm and control treatment effect at 40 cm peat depth (**Table 4. 1**). $SUVA_{254}$ in the W-treatment decreased by 1.1% and 7.6%, and in the N addition treatment by 4.7% and 7.6% in the 10 cm and 40 cm peat soil depth respectively relative to the control. Also, the interaction treatment effect increased $SUVA_{254}$ by 2.5% and decreased by 12.3% at 10 cm and 40 cm peat depth respectively. There was no significant difference ($t = -0.36$, $p = 0.721$) found between $SUVA_{254}$ for the two peat depths. A significant decrease in $SUVA_{254}$ ($r^2 = 0.082$, $p = 0.01$) in DOM aromaticity for 10 cm depth, and significant decrease $SUVA_{254}$ ($r^2 = 0.308$, $p = 0.0001$) for the 40 cm was shown (**Figure A. 1)** when all treatments were pooled with respect to DOC concentration at both peat depths.

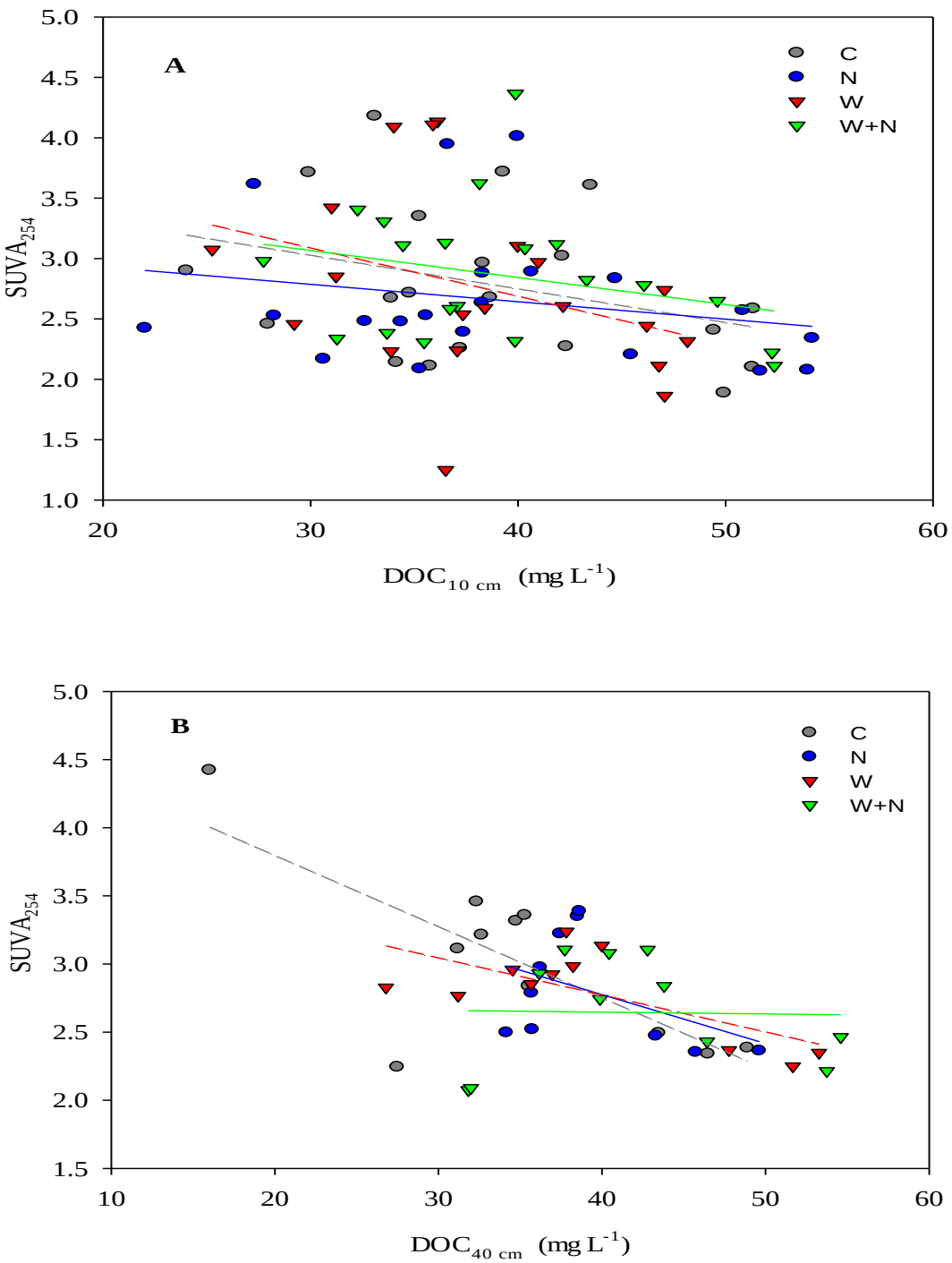

Figure 4. 2 Relationship between specific ultraviolet absorbance ($SUVA_{254}$) and DOC (mg L^{-1}) at 10 cm(A) and 40 cm (B) peat depth. Control (C) (gray solid), nitrogen addition (N) (blue solid), warming (W) (red triangle) and interactive effect of warming and nitrogen addition (W+N) (green triangle).

4.3.2.2 Fluorescence index (FI)

The fluorescence index increased with increasing peat soil depth and ranged between 1.53 to 1.65. The fluorescence index increased by 2%, 1.3%, and 2% in the W-treatment, N addition treatment and interactive treatment effect of W+N respectively at 10 cm soil depth (**Table 4. 1**). Also, the W-treatment effect increased FI by 1.3% and W+N treatment effect by 3.8%. However, there was no change in the FI values in the N-treatment at the 40 cm soil depth with respect to the control treatment effect. There was significant difference ($t = -3.14$, $p = 0.003$) between the fluorescence index at different depth based on paired t-test. Furthermore, the fluorescence index decreased with increasing DOC concentration at both 10 cm and 40 cm peat depth in all treatment effects. In general, the FI values of all treatment effect varied between 1.53 and 1.65 indicating a domination of microbial or microbially processed DOM over vascular-plant-derived DOM in the peatland.

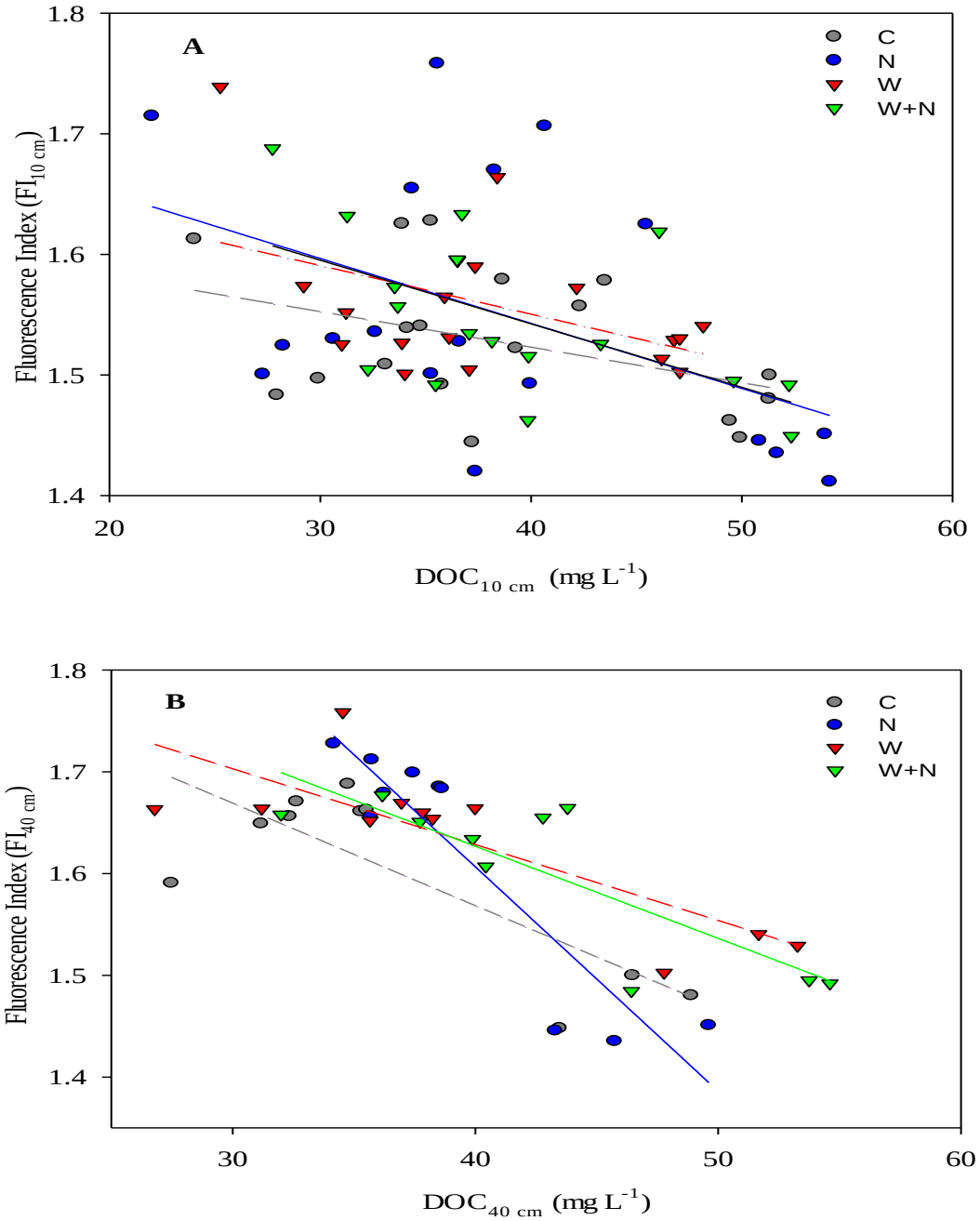

Figure 4. 3 Relationships between dissolved organic carbon (DOC) concentrations and fluorescence index (FI). Legend represents; control (gray solid), nitrogen addition (blue solid), warming treatment (red triangle) and interactive effect of warming and nitrogen addition (green triangle) treatment at 10 cm (A) and 40 cm (B) peat soil depth.

4.3.2.3 Humification index (HIX)

HIX values ranged between 0.15 and 0.17, which is within the range of reported values for DOM with a principal humic nature (**Table 4. 1**) (Ohno, 2002). The HIX decreased by 5.9% and increased by 6.7% in the W-treatment at the 10 cm and 40 cm peat depth respectively. Similarly, HIX decreased by 11.8% and increased by 6.7% at the 10 cm and 40 cm depth in the N addition treatment. There was no change in HIX at the 10 cm depth of the W+N treatment effect with respect to the control treatment but increased by 13.3% at the 40 cm peat depth. There was no significant difference ($t = 1.70$, $p = 0.095$) between HIX at different peat depth. In addition, HIX in all the treatment effects were <1 (**Table 4. 1**) and the mean HIX values in the control and W+N treatment effect was the same at the 10 cm depth and mean HIX values in the N and W-treatment effect were also the same at the 40 cm peat depth. Pearson's correlation between DOC and HIX are weakly correlated ($r = 0.262$, $p = 0.026$) in the 10 cm peat depth but no significant correlation was observed at the 40 cm peat depth ($r = 0.175$, $p = 0.262$) **(Table A2).** A linear regression subsequently showed HIX increase with increasing DOC concentration at the N addition treatment ($r^2 = 0.102$, $p = 0.197$) **(Figure 4. 4A**), W+N treatment effect ($r^2 = 0.0725$, $p = 0.279$) at the 10 cm peat depth **(Figure 4. 4A**) and was not reciprocated at the 40 cm peat depth. However, the linear regression for the W-treatment indicated a decreasing trend of HIX with increasing DOC concentration at the 40 cm peat depth ($r^2 = 0.246$, $p = 0.121$) **(Figure 4. 4B)** and HIX at 10 cm showed an increase but not significant relationship with increasing $DOC_{10\ cm}$ concentration (**Figure 4. 4A**).

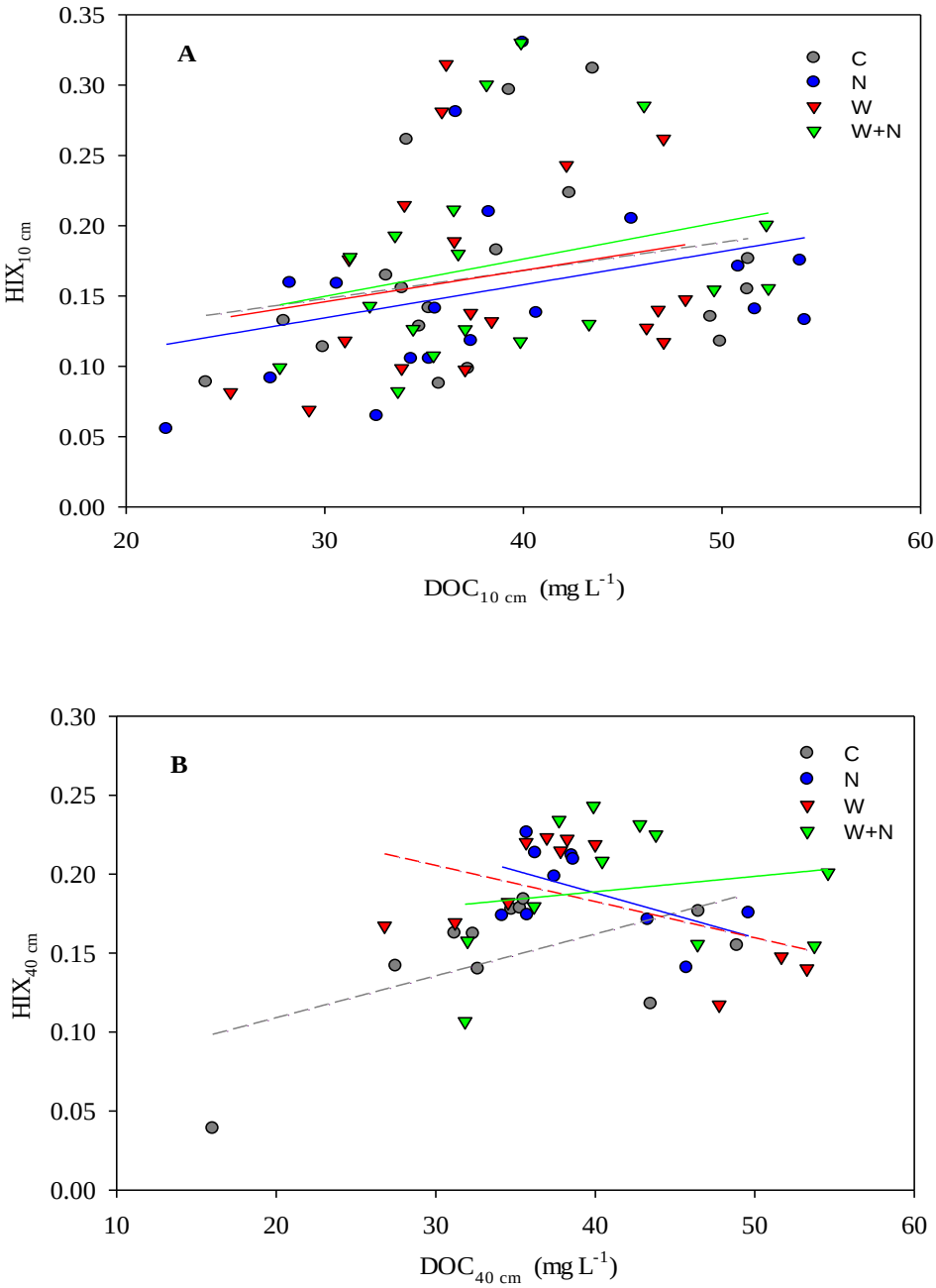

Figure 4. 4 Relationship between humification index (HIX) and DOC (mg L^{-1}) at 10 cm (A) and 40 cm (B) peat depth. Control (C) (gray solid), nitrogen addition (N) (blue solid), warming (W) (red triangle) and interactive effect of warming and nitrogen addition (W+N) (green triangle).

4.4 Discussion

4.4.1 The effects of warming treatment on DOC

Plant litter quality, microbial decomposition of peat, and its mineralization interactively defined the change of DOC concentrations in my experimental set-up. Warming was found to increase the DOC concentration but not significant at 40 cm depth in my study which is in accordance with several studies (Fenner *et al*., 2007; Worrall & Burt, 2004; Ward *et al*., 2013; Delarue *et al*., 2014; Dieleman *et al*., 2016; Yuan *et al*., 2018) despite a decrease of 1% in DOC at 10 cm peat depth. Delarue *et al*. (2014) also found DOC concentration to decrease at the subsurface due to DOC consumption in the process of *in situ* mineralization rather than the production of DOC associated with peat decomposition at 10 cm. This finding is in agreement with a study by Hu *et al.* (2009) which concluded that warming can increase nitrogen mineralization in soils by providing the vegetation cover with the needed nutrients and by improving the microbial activities. While Dieleman *et al*. (2016) also found high temperatures and lower water table elevations in northern peatlands to increase DOC production. One study reported that increasing microbial activities may often produce two different patterns in DOC concentration (Delarue *et al*., 2014) which could be one of the reasons for the decrease in the DOC concentration at the 10 cm peat depth. This further explains why Pastor *et al*. (2003) also observed warming reduced the DOC flux in soil cores in a laboratory experiment under temperatures between 1.6-4 ^{o}C. Worrall & Burt (2004) and Dillon & Molot (2005) suggested that increased rates of precipitation due to climate change will equally lead to increased DOC concentration and DOC export from the soil surface

irrespective of the warming condition (temperature) in the soil. Moreover, these factors (temperature and hydrology) may drive both positive and negative feedbacks to DOC export (O'Donnell *et al*., 2012) in the soil. Warming decreased the total nitrogen concentration at both 10 cm and 40 cm peat soil depth by 8.8% and 25.2% relative to the control. However, the decrease in TN concentration at W-treatment could be due to the rapid growth and utilization of the mineral nitrogen available for plant development in the warming treatment with respect to their individual controls at 10 cm and 40 cm peat depth. Peatlands are reported to be N-limited (Luan & Wu, 2014), and it is possible the little nitrogen in the peat was immediately used up by the plants and or microorganisms or even leached further in to deeper peat layer.

$SUVA_{254}$ values, indicating the amount of aromatic carbon compounds in the peatland where high values indicate more recalcitrant carbon compound and low values indicate highly labile compounds (Hansson *et al*., 2010), decreased by 7.6% at 40 cm depth compared to the control. A negative linear relationship between $SUVA_{254}$ and DOC at 10 cm peat depth and 40 cm at peat depth (**Figure 4. 2**) indicated that increasing DOC concentration (thus increase in decomposition) reduce the aromaticity by the decomposition of labile compounds (Moore & Clarkson., 2007; Hansson *et al*., 2010).

The FI of the W-treatment indicated that the peatland DOC at that region was of a microbial derived source throughout the peat layers and was linearly correlated with DOC (**Figure 4. 3**). The humification index increased by 6.7% at 40 cm depth despite a 5.9% decrease at 10 cm depth which was due to warming and demonstrate the extent of the DOM humification. A decrease in HIX value at the 10 cm depth indicates that humic

content did not provide enough suitable substrate for microbial demand. In general, the HIX in our study ranged between 0.15-0.17 which indicate the carbon has less condensed molecules and not entirely degraded as an increasing HIX increased the humification (Dieleman *et al*., 2016).

4.4.2 The effect of N addition on DOC

Increase in DOC concentration at the 40 cm depth despite not being significant could be due to an initial increase in peatland vegetation growth such as sedges and shrubs and the decomposition of dead plant materials on peat surface (Fenner, *et al*., 2007). This supports our hypothesis that increased N addition contributes to high DOC biodegradability. Increased DOC concentration enhance labile carbon processing while inhibiting recalcitrant carbon processing (Liu *et al*., 2018). Microbial communities in peatlands are likely to respond more rapidly than plants to the changes of nutrient enrichment because of their higher turnover rates (Lund *et al*., 2009). N addition unequivocally increased the TN concentrations by 11.8% and 10% in the 10 cm and 40 cm depth with respect to the control. I anticipated that water table may have played a role but found no differences between WTD at the control and N addition treatment (see Chapter #3). This increase may be due to mineralization of N which has been found to be much faster in *Sphagnum* spp. dominated peatland compared to vascular plants and bryophyte dominated peatlands (Scheffer *et al*., 2001).

$SUVA_{254}$ values decreased by 4.7% and 7.6% in the 10 cm and 40 cm peat depth. Tfaily *et al*. (2013) also reported similar findings but at a different $SUVA_{254}$ range of ~3 and ~4 L mg C^{-1} m^{-1} in peatland. The linear relationship indicated that $SUVA_{254}$

decreased with increasing peat DOC concentrations (**Figure 4. 2**), which means more of the labile compounds are being decomposed increasing the DOC concentration while reducing the aromaticity.

Despite the percentage decrease (11%) in the HIX at 10 cm peat depth with respect to control treatment effect, a linear relationship between HIX and $DOC_{10\ cm}$ exhibited correlation but no significant at 40 cm depth (**Figure 4. 4**). This may indicate that decomposition was too low to be recognized at the 40 cm depth since peatland has consistently been described as having highly humified and recalcitrant characteristics (Dieleman *et al*., 2016). Also, HIX values were less than 1 at both peat depths which indicates the lower decomposition rates of DOM **(Table 4. 1)**.

4.4.3 The interactive effects of warming and N addition on DOC

DOC concentration for the interaction effects W+N increased by 1.3% in the 10 cm peat soil depth compared to 20% in the DOC concentration at the 40 cm peat depth in the *in situ* manipulative experiment (**Table 4. 1**). At the 40 cm depth, increased DOC concentration could be due to root exudates favoring DOC mineralization as reported by Delarue *et al*. (2014). Also, increased DOC in the 40 cm depth may be due to increased rates of leaching from the peat surface (Hongve *et al*., 2004; Porcal *et al*., 2009). DOC and TN concentrations at the W+N treatment effects were the highest achieved in the *in situ* manipulative experiment at both peat soil depths indicating a synergistic effect. However, the increment in the DOC concentration was not significant ($p > 0.05$). Some studies have suggested the rising DOC concentrations are a result of hydrological changes including increased precipitation, climate warming (Freeman *et al*., 2001) and N

deposition (Findlay, 2005). Nitrogen addition in the interaction effect W+N, increased DOC concentration by 0.56% and warming by 2.36% at 10 cm in the manipulative experiment. This indicates that, nitrogen applied in the interactive treatment effect was mostly used up immediately by the vegetation (sedges and shrubs) since peatlands are noted to be N-limiting hence the low impact on DOC concentration at the 10 cm peat depth compared to warming in the interactive effect. The 2.36% increment in the DOC concentration attributed to warming at the 10 cm peat depth was due to decomposition of labile plant litter by micro-organisms and water table. In addition, DOC concentration in the 40 cm was increased by 5.7% due to nitrogen addition and 5.9% due to warming in the interactive effect W+N, but this increment was not significant at the 40 cm peat depth and could be attributed to the water table level. The interactive effect of W+N treatment increased the TN concentration at the 10 cm and 40 cm peat depth respectively. Interestingly, a decreasing trend of TN concentration with increased DOC concentration in our manipulative experiment despite our anticipation that an increased TN will cause increased DOC concentrations at different peat depth**.** The highest mean $SUVA_{254}$ value 2.86 L mg C^{-1} m^{-1} was found at 10 cm peat depth, which may be associated with high iron concentrations in the peat as suggested by Moore & Clarkson (2007). However, our research did not cover the scope of determination of iron concentrations in the peatland at our experimental site. W+N had the lowest mean $SUVA_{254}$ value 2.65 L mg C^{-1} m^{-1} which could be due to the high DOC concentration at the 40 cm depth and the possibility of a nonlinear absorbance at high DOC concentration (Moore & Clarkson, 2007). Furthermore, the fluorescence index increased with increasing peat depth suggested a

more microbially derived source (1.5–2.0) of DOM over plant derived DOM (1.2–1.4) (Mcknight *et al*., 2001; Cory & Mcknight, 2005). However, FI decreased with increasing DOC concentration at the 10 cm (r^2 = 0.329, p = 0.016) and at the 40 cm (r^2 = 0.690, p = 0.0029) (**Figure 4. 3**) because the DOM lacks lignin and is less aromatic and more aliphatic (low $SUVA_{254}$ and high FI). The HIX of the peat DOM is often characterized by the increasing degree of humification and our results indicated that HIX increased with increasing peat depth as was the case at 40 cm peat depth **(Table 4. 1)** while there was no change in the HIX at the 10 cm depth with respect to the control treatment effect. However, the result indicate the peat was not entirely degraded at both depths (Dieleman *et al*., 2016). The maximum water holding capacity of the peat may give a fairly accurate picture of the degree of humification. Higher water tables, caused by wetter and/or cooler climatic conditions, resulted in less decomposed peats. The low oxygen concentration in the peat due to flooding inhibits the activity of phenol oxidase causing accumulation of phenol compounds which inhibit the activity of hydrolase enzymes responsible for the decomposition. However, lower water tables, occurring in warmer and/or drier climates cause the peat to break down more as it sits in the acrotelm for longer period.

4.5 Conclusion

Several potential mechanisms are responsible for the changes in DOC concentrations, including climate warming, N deposition, atmospheric CO_2 concentration and hydrological changes in peatland ecosystems.

Increasing global air temperatures due to climate change have a strong impact on boreal peatland ecosystems. In view of this, warming increased DOC concentration at 40 cm depth but decreased DOC and TN concentration in the peat surface (10 cm) possibly by reducing enzyme activities and microbial biomass and was not influenced by the water table depth. Enhanced decomposition rates may slow down or reverse peat accumulation compromising the stability of the peatland by exporting DOC rich pore water to other ecosystems altering the environmental conditions. These will eventually lead peatlands to become a carbon source instead of being a carbon sink. Warming also decreased $SUVA_{254}$ in both soil layers and HIX at 10 cm but increased the HIX at 40 cm peat soil depth. Conversely nitrogen addition increased the DOC and TN concentration in the peat layer but decreased the aromaticity at both peat depths and the degree of humification at 10 cm and increased HIX at 40 cm depth. Likewise, the interactive effect of W+N addition led to increased DOC and TN concentrations, decreased aromaticity, increased HIX in the 40 cm depth but a high $SUVA_{254}$ value was indicative of an increased aromatic fraction of DOC at the 10 cm peat depth was observed in the treatment effect. The FI values of all treatment effect varied between 1.53 and 1.65 indicating a domination of microbial or microbially processed DOM over vascular plant derived DOM in peatland (Cory & McKnight, 2005), and was significantly correlated to DOC concentration and increased with increased peat depth. In general, the HIX values for the treatment effects also ranged between 0.15-0.17, indicating the peat carbon was not highly degraded and composed of less condensed molecules, which in other words indicate a decrease in recalcitrant humified carbon production (HIX <1). HIX did not

consistently increase with peat depth as findings were in agreement with several studies (Zaccone *et al*., 2018; Magnan *et al*., 2018) despite the different environmental conditions associated with peat accumulation. In view of this, long-term simulation experiments are now needed to elucidate the exact nature of the response of peatlands to climate warming in regional carbon cycling under current and future climate change.

Chapter 5

5.0 Summary and Conclusion

The purpose of this research was to examine the role of increasing warming, N addition and their interactive effect of warming and nitrogen addition on CH_4 emission, and DOC concentration and the optical characteristics of DOM in boreal peatland ecosystems under a manipulative field experiment.

In chapter #3, I found that, environmental variables such as peat soil moisture content, water table depth not to have a significant effect on CH_4 emission despite previous research indicating water table to be one of the major controlling factor of CH_4 emission. However, I also found peat soil temperature at 20 cm to significantly influence the CH_4 emission in my experimental site and the OTC chamber increased the atmospheric air temperature by 2.5 ^oC leading to an increase in the rate of CH_4 emission in the warming treatment. Furthermore, I found N addition to have a reduced effect on CH_4 emission relative to the control but was not statistically significant. This was in contrast with Nykänen *et al*. (2002) who reported an increase in CH_4 emission due an increase in *Eriophorum* coverage. Research conducted in an alpine meadow showed N addition to have both positive and negative effect on CH_4 emission (Chen *et al*., 2017), therefore long-term observations must be conducted to confirm the result as N deposition may be in a transient state for the duration of the experiment. In addition, to previous observations, I found the interactive effect of warming and nitrogen deposition to have an addictive effect rather than a synergistic effect in my manipulative experiment. Implying that if care is not taken we will continuously be over estimating CH_4 emission in boreal

peatlands leading to incorrect estimation of the global GHG budget due to climate change.

In chapter #4, warming increased DOC concentration at 40 cm depth but decreased DOC and TN concentration in the peat surface (10 cm) possibly by reducing enzyme activities and microbial biomass following three years of warming and N addition experiment. The effect of warming on DOC has been observed in field, and laboratory studies where temperature differences were above 5 ºC (Dieleman *et al.*, 2016). It can be said that climate change may have the potential to produce more microbial-like, labile carbon compounds in the surface of the peat thereby altering the DOC quality. Enhanced decomposition rates may slow down or reverse peat accumulation made up of the stability of peatland by exporting DOC-rich pore water to other ecosystems altering the environmental conditions. These will eventually lead peatlands to become carbon source instead of being a carbon sink. N addition in the short-term was beneficial for maintenance, by supplying the available nutrients to peat vegetation. The interactive effect of W+N addition led to increased DOC and TN concentrations, decreased aromaticity, increased HIX in the 40 cm depth but a high $SUVA_{254}$ value was indicative of an increased aromatic fraction of DOC at the 10 cm peat depth was observed in the treatments. Given the rate of increased N loading due to anthropogenic activities coupled with warming, long-term simulation experiments are now needed to elucidate the response of peatlands to climate warming in regional carbon cycling under current and future climate change.

References

Abdalla, M., Hasting, A., Truu, J., Espenberg, M., Mander, Ü., Smith, P., 2016. Emissions of methane from northern peatlands: A review of management impacts and implications for future management options. Ecology and Evolution, 6:7080-7102.

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. BioScience 48, 921–934.

Aerts, R., Ludwig, F., 1997. Water-table changes and nutritional status affect trace gas emissions from laboratory columns of peatland soils. Soil Biol Biochem 29:1691–1698.

Aerts, R., Verhoeven, J.T.A., Whigham, D.F., 1999. Plant-mediated controls on nutrient cycling in temperate fens and bogs. Ecology, 80 (7), pp. 2170-2181.

Aerts, R., Wallén, B., Malmer, N., De Caluwe, H., 2001. Nutritional constraints on sphagnum growth and potential decay in northern peatlands. Journal of Ecology 89, 292–299.

Alberts, J.J., Takacs, M., 2004. Total luminescence spectra of IHSS standard and reference fulvic acids, humic acids and natural and the role of carbon substrate quality. Journal of Geophysical Research: Biogeosciences, 123, 591–609.

Bellisario, L.M., Bubier, J.L., Moore, T.R., 1999. Controls on CH_4 Emissions from a Northern peatland. Global Biogeochemical Cycles, *13*, 81–91.

Berendse, F., *et al*., 2001. Raised atmospheric CO_2 levels and increased N deposition cause shifts in plant species composition and production in sphagnum bogs. Global Change Biology, Vol. 7, No. 5, Pp. 591–98.

Blodau, C., 2002. Carbon cycling in peatlands-A review of processes and controls. Environ. Rev. Vol.10, pp 111-135.

Bobbink, R., Hicks, K., Galloway, J., *et al*., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications, 20, 30–59.

Bodelier, P.L., 2011. Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils. Environmental Sustainability. 3:379–388.

Bodelier, P.L.E., Laanbroek, H.J., 2004. Nitrogen as a regulatory factor of methane oxidation in soils and sediments. FEMS Microbiology Ecology, 47, 265–277.

Boothroyd, I.M., Worrall, F., Allott, T.E.H., 2015. Variations in dissolved organic carbon concentrations across peatland hillslopes. Journal of Hydrology 530:372 – 383.

Broder, T., Knorr, K.H., Biester, H., 2017. Changes in dissolved organic matter quality in a peatland and forest headwater stream as a function of seasonality and hydrologic conditions. Hydrol. Earth Syst. Sci., 21, 2035–2051.

Bruhwiler, L., Dlugokencky, E., Masarie, K., Ishizawa, M., Andrews, A., Miller, J., ⋯ Worthy, D., 2014. Carbontracker-CH_4: An assimilation system for estimating emissions of atmospheric methane. Atmospheric Chemistry and Physics, 14, 8269–8293.

Bubier, J.L., 1995. The relationship of vegetation to methane emission and hydrochemical gradients in northern peatlands. Journal of Ecology, Vol. 83, No. 3, Pp. 403–20.

Bubier, J.L., Moore, T.R., Juggins, S., 1995. Predicting methane emission from bryophyte distribution in northern Canadian peatlands. Ecology,76(3), pp. 677-693.

Butterbach-Bahl, K., Papen, H., Renneberg, H., 1997. Impact of gas transport through rice cultivars on methane emission from rice paddy fields. Plant cell Environ 20:1175-1183.

Carlson, K.M., Goodman, L.K., Tobin, C.C.M., 2015. Modeling relationships between water table depth and peat soil carbon loss in Southeast Asian plantation. Environ. Res. Lett. 10 074006.

Chen, X., Wang, G., Zhang, T., Mao, T., Wei, D., Song, C., *et al*., 2017. Effects of warming and nitrogen fertilization on GHG flux in an alpine swamp meadow of a permafrost region. Sci. Total Environ. 601-602, 1389-1399.

Christensen, T.R., Ekberg, A., Ström, L., Mastepanov, M., Panikov, N., Öquist, M., Oskarsson, H., 2003. Factors controlling large scale variations in methane emissions from wetlands. Geophysical Research Letter, 30, 1414.

Clark, J. *et al*., 2010. Assessing the vulnerability of blanket peat to climate change using an ensemble of statistical bioclimatic envelope models. Clim. Res. 45 (Uplands Special Issue), 131-150.

Clark, J.M., Ashley, D., Wagner, M., Chapman, P.J., Lane, S.N., Evans, C.D., Heathwaite, A.L., 2009. Increased temperature sensitivity of net DOC production from ombrotrophic peat due to water table draw-down. Glob. Change Biol. 15 (4),794–807.

Clark, J.M., Chapman, P.J., Adamson, J.K., Lane, S.N., 2005. Influence of drought-induced acidification on the mobility of dissolved organic carbon in peat soils. Global Change Biol 11:791–809.

Conrad, R., 2009. The global methane cycle: recent advances in understanding the microbial processes involved. Environmental Microbiology Reports. 1(5) 285 – 292.

Cook, S., Peacock, M., Evans, C.D., Page, S.E., Whelan, M., Gauci, V., Khoon, K.L., 2016. Cold storage as a method for the long-term preservation of tropical dissolved organic carbon (DOC). Mires Peat 18, 1- 8.

Cory, R., McKnight, D., 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. Environ. Sci. Technol., 39(21), 8142–8149.

Couwenberg, J., Dommain, R., Joosten, H., 2010. Greenhouse gas fluxes from tropical peatlands in South–East Asia Glob. Change Biol. 16 1715–32.

Crill, P.M., Martikainen, P.J., Nykänen, H., Silvola, J., 1994. Temperature and N fertilization effects on methane oxidation in a dried peatland soil. Soil Biology and Biochemistry 26:1331–1339.

Delarue, F., Gogo, S., Buttler, A., Bragazza, L., Vincent, E., Jassey, J., Bernard, G., Laggoun-Défarge, F., 2014. Indirect effects of experimental warming on dissolved organic carbon content in subsurface peat. J Soils Sediments 14:1800–1805.

Dieleman, C.M., Lindo, Z., McLaughlin, J.W., Craig, A.E., Branfireun, B.A., 2016. Climate change effects on peatland decomposition and porewater dissolved organic carbon biogeochemistry. Biogeochemistry.128: 385– 396.

Dillon, P.J., Molot, L.A., 2005. Long-term trends in catchment export and lake retention of dissolved organic carbon, dissolved organic nitrogen, total iron and total phosphorus: The Dorset, Ontario study, 1978–1998. J Geophys Res-Biogeosciences 110, No. G01002,

Dinsmore, K.J., Billett, M.F., Dyson, K.E., 2013. Temperature and precipitation drive temporal variability in aquatic carbon and GHG concentrations and fluxes in a peatland catchment. Glob. Change Biol. 19 (7), 2133–2148.

Dinsmore, K.J., Billett, M.F., Skiba, U.M., Rees, R.M., Helfter, C., 2010. Role of the aquatic pathway in the carbon and greenhouse gas budgets of a peatland catchment, Global Change Biol., 16, 2750–2762.

Dinsmore, K.J., Skiba, U.M., Billet, M.F., Rees, R.M., 2009. Effect of water table on greenhouse gas emission from peatland mesocosms. Plant Soil. 318: 229- 242.

Dise, N.B., *et al*., 1993. Environmental factors controlling methane emissions from peatlands in northern Minnesota. Journal of Geophysical Research, Vol. 98, No. D6, P. 10583.

Dise, N.B., Verry, E.S., 2001. Suppression of peatland methane emission by cumulative sulfate deposition in simulated acid rain. Biogeochemistry, Vol. 2, pp. 143-160.

Dorrepaal, E., Toet, S., Van Logtestijn, R.S.P., Swart, E., Van De Weg, M.J., Callaghan, T.V. *et al*., 2009. Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. Nature, 460, 616–619.

Dunfield, P., Knowels, R., Dumont, R., Moore, T.R., 1993. Methane production and consumption in temperate and subarctic peat soils: Response to temperature and pH. Soil Biology and Biochemistry. Vol. 25. Issue 3, 321-326.

EPA., 2017. Global methane initiative. U.S. Environmental protection Agency, Washington, DC, USA.

Fechner-Levy, E.J., Hemond, H.F., 1996. Trapped methane volume and potential effects on methane ebullition in a northern peatland. Limnol. Oceanogr. 41 (7): 1 375–1 383.

Fellman, J.B., D'Amore, D.V., Hood, E., 2008. An evaluation of freezing as a preservation technique for analyzing dissolved organic C, N, and P in surface waters. Sci Total Environ 392(2):305–12.

Fellman, J.B., Hood, E., D'Amore, D.V., Edwards, R.T., White, D., 2009. Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds, Biogeochemistry, 95, 277–293.

Fenner, N., Freeman, C., Lock, M.A., Harmens, H., Reynolds, B., Sparks, T., 2007. Interactions between elevated CO_2 and warming could amplify doc exports from peatland catchments. Environmental Science & Technology.; 41(9):3146±3152.

Fenner, N., Ostle, N., Freeman, C., Sleep, D., Reynolds, B., 2004. Peatland carbon efflux partitioning reveals that *Sphagnum* photosynthate contributes to the doc pool. Plant Soil 259(1– 2):345–54.

Findlay, S.E.G., 2005. Increased carbon transport in the Hudson River: unexpected consequence of nitrogen deposition? Front Ecol Environ 3:133–137.

Forster, P., *et al*., 2007. Changes in atmospheric constituents and in radiative forcing, in *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, Edited by S. Solomon *et al*., Pp. 129–234, Cambridge Univ. Press, Cambridge, U. K.

Fowler, D., Steadman, C.E., Stevenson, D. *et al*., 2015. Effects of global change during the 21st century on the nitrogen cycle. At. Chem. Physics 15, 13849-13893.

Freeman, C., Evans, C.D., Montheith, D.T., Reynolds, B., Fenner, N., 2001. Export of organic Carbon from peat soils. *Nature,* 412,785.

Freeman, C., Fenner, N., Ostle, N.J., Kang, H., Dowrick, D.J., Reynolds, B., Lock, M.A., Sleep, D., Hughes, S., Hudson, J., 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. Nature 430:195–198.

Frenzel, P., Rudolph, J., 1998. Methane emission from a wetland plant: the role of CH_4 oxidation in Eriophorum. Plant Soil 202, 27–32.

Friedlingstein, P., Cox, P., Betts, R., Bopp, L., von Bloh, W., Brovkin, V., ⋯Zeng, N. 2006. Climate–carbon cycle feedback analysis: Results from the C4MIP model intercomparison. Journal of Climate, 19, 3337–3353.

Froberg, M., Grip, H., Tipping, E., Svensson, M., Stroèmgren, M., Dan, B.K., 2013. Long-term effects of experimental fertilization and soil warming on dissolved organic matter leaching from a spruce forest in northern Sweden. Geoderma. 200-201(S 200±201):172±179.

Frolking, S., Roulet, N., Fuglestvedt, J., 2006. How northern peatlands influence the earth's radiative budget: Sustained methane emission versus sustained carbon sequestration. J. Geophys. Res., 111, P. G01008.

Fu, G., Shen, Z.X., 2016. Response of alpine plants to nitrogen addition on the Tibetan Plateau: A meta-analysis. J. Plant Growth Regul. 35, 974-979.

Gallego-Sala, A.V., Prentice, C.I., 2013. Blanket peat biome endangered by climate change. Nat. Clim. Change, 3, 152–155.

Gong, Y., Wu, J., Vogt, J., Le, T.B., 2019. Warming reduces the increase in N2O emission under nitrogen fertilization in a boreal peatland. Science of the Total Environment, 664, 72-78.

Gorham, E., 1991. Northern peatlands: role in the carbon cycle and probable responses to climatic warming. Ecological applications, 1 (2): 182-195.

Granberg, G., Mikkelä, C., Sundh, I., Svensson, B. H., & Nilsson, M. 1997. Source of spatial variation in methane emission from mires in northern Sweden: A mechanistic approach in statistical modeling. Global Biogeochemical Cycles, 11, 135–150.

Granberg, G., Sundh, I., Svensson, B. H., Nilsson, M., 2001. Effects of temperature, and nitrogen and sulfur deposition, on methane emission from a boreal mire. *Ecology*, *82*, 1982–1998.

Greatorex, James Michael., 2000. A Review of Methods for Measuring Methane, Nitrous Oxide and Odour Emissions from Animal Production Activities, p. 27.

Guggenberger, G., Glaser, B., Zech, W., 1994. Heavy metal binding by hydrophobic and hydrophilic dissolved organic carbon fractions in a spodosol A and B horizon. Water Air and Soil Pollution 72: 111–127.

Hanson, R.S., Hanson, T. E., 1996. Methanotrophic bacteria. Microbiol. Rev. 60 (2): 439–471.

Hansson, K., Kleja, D.B., Kalbitz, K., Larsson, H., 2010. Amounts of carbon mineralised and leached as DOC during decomposition of Norway spruce needles and fine roots. Soil Biology & Biochemistry 42: 178 – 185.

Holden, J., 2005. Peatland hydrology and carbon release: Why small-scale process matters. Philos Trans R Soc A 363(1837):2891–913.

Holland, E.A., Robertson, G.P., Greenberg, J., Groffman, P.M., Boone, R.D., Gosz, J.R., 1999. Soil CO_2, N_2O, and CH_4 exchange, Standard soil methods for long-term ecological research. Oxford University Press, Oxford: 185-201.

Hongve, D., Riise, G., Kristiansen, J.F., 2004. Increased colour and organic acid concentrations in Norwegian forest lakes and drinking water—a result of increased precipitation? Aquat Sci 66:231–238.

Hope, D., Billett, M.F., Milne, R., Brown, T.A.W., 1997. Exports of organic carbon in British rivers. Hydrological Processes, 11,325–344.

Hu, L.J., Yang, H.J., Wang, W.W., Guo, J.X., 2009. Soil nutrient responses to one year of simulated global warming and nitrogen deposition on the Songnen meadow steppes, Northeast China. International Conference on Bioinformatics and Biomedical Engineering. 24:1±6.005. V. 127. PP. 177–187.

Hutchinson, G.L., Livingston, G.P., 2001. Vents and seals in non-steady-state chambers for measuring gas exchange between soil and the atmosphere. Eur. J. Soil Sci. 52:675–682.

IPCC., 2013. Summary for policymakers. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, P. M. Midgley (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I To The Fifth Assessment Report Of The Intergovernmental Panel On Climate Change* (Pp. 1535). Cambridge, UK And New York, NY, USA: Cambridge University Press.

Joabsson, A., Christensen, T.R., Wallén, B., 1999. Vascular Plants controls on methane emissions from Northern peat forming wetlands. Trends in Ecology & Evolution 14(10): 385-388.

Johnson, M.S., Couto, E.G., Abdo, M., Lehmann, J., 2011. Fluorescence index as an indicator of dissolved organic carbon quality in hydrologic flowpaths of forested tropical watersheds. Biogeochemistry 105: 149-157.

Jorgenson, M.T. *et al*., 2013. Reorganization of vegetation, hydrology and soil carbon after permafrost degradation across heterogeneous boreal landscapes. Environmental Research Letters 8.

Kane, E.S., Mazzoleni, L.R., Kratz, C.J., Hribljan, J.A., Johnson, C.P., Pypker, T.G., Chimner, R., 2014. Peat porewater dissolved organic carbon concentration and lability increase with warming: a field temperature manipulation experiment in a poor-fen. Biogeochemistry 119 (1-3).

Karavanova, E., Milanovskiy, E., 2016. Aromaticity and humification of dissolved organic matter (Lysimetric experiment). Retrieved from https://www.semanticscholar.org/paper/.

Keddy, P.A., 2011. Wetland Ecology-priciples and conservation [M]. Cambridge University Press.

Kutzbach, L., Schneider, J., Sachs, T., Giebels, M., Nyakanen, H., Shurpali, N.J., Martikainen, P.J., Alm, J., Wilmking, M., 2007. CO_2 flux determination by closed-chamber methods can be seriously biased by inappropriate application of linear regression. Biogeosci., 4, 1005-1025.

Lafleur, P.M., Moore, T.R., Roulet, N.T., Frolking, S., 2005. Ecosystem respiration in a cool temperate bog depends on peat temperature but not water table. Ecosystem., Vol. 8, No. 6, Pp. 619-629.

Lai, D.Y.F., 2009. Methane dynamics in northern peatlands: A Review. *Pedosphere*, *19*(4), 409–421.

Lai, D.Y.F., Roulet, N.T., Moore, T.R., 2014. The spatial and temporal relationships between CO_2 andCH_4 exchange in a temperate ombrotrophic bog. Atmospheric Environment, vol. 89, pp. 249–59.

Lamers, L.P.M., Bobbink, R., Roelofs, J.G.M., 2000. Natural nitrogen filter fails in polluted raised bogs. Global Change Biology, Vol. 6, No. 5, Pp. 583–86.

Lavoie, M., Paré, D., Bergeron, Y., 2005. Impact of global change and forest management on carbon sequestration in northern forested peatlands. Environ Rev 13:199–240.

Limpens, J., Berendse, F., Blodau, C., Canadell J.G., Freeman, C., Holden, J., Roulet, N., Rydin, H., Schaepman-Strub, G., 2008. Peatlands and the carbon cycle: From

local processes to global implications—A Synthesis. Biogeosciences 5(5):1475–91.

Liu, F., Chen, L., Zhang, B., Wang, Q., Qin, S., 2018. Ultraviolet radiation rather than inorganic nitrogen increases dissolved organic carbon biodegradability in a typical thermo-erosion gully on the Tibetan Plateau. Science of the Total Environment 627, 1276–1284.

Lozanovska, I., Kuzyakov, Y., Krohn, J., Parvin, S., Dorodnikov, M., 2016. Effects of nitrate and sulfate on greenhouse gas emission potentials from microform-derived peats of a boreal peatland: a 13C tracer study. Soil Biol. Biochem. 100, 182–191.

Luan, J., Wu, J., 2014. Gross photosynthesis explains the 'artificial bias' of methane fluxes by static chamber (opaque versus transparent) at the hummocks in a boreal peatland. Environ. Res. Lett. 9,105005.

Luan, J., Wu, J., 2015. Long-term agricultural drainage stimulates CH_4 emissions from ditches through increased substrate availability in a boreal peatland. Agriculture, Ecosystems & Environment, Vol. 214, Pp. 68–77.

Lund, M., Christensen, T.R., Mastepanov, M., Lindroth, A., Stro¨m, L., 2009. Effects of N and P fertilization on the greenhouse gas exchange in two northern peatlands with contrasting N deposition rates. Biogeosciences 6:2135–2144.

Magnan, G., *et al*., 2018. Impact of the little ice age cooling and 20th century climate change on peatland vegetation dynamics in central and northern Alberta using a multi-proxy approach and high-resolution peat chronologies. Quat. Sci. Rev. 185, 230–243.

Malmer, N., Johansson, T., Olsrud, M., Christensen, T.R., 2005. Vegetation, climatic changes and net carbon sequestration in a North-Scandinavian subarctic mire over 30 years, Global Change Biology 11 (1) 1895–1909.

Malmer, Nils., Wallén, Bo., 2005. Nitrogen and phosphorus in mire plants: Variation during 50 years in relation to supply rate and vegetation type. Oikos, Vol. 109, No. 3, Pp. 539–54.

Marion, G.M., Henry, G.H.R., Freckman, D.W., Johnstone, J., Jones, G., Jones, M.H. *et al*., 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. Glob. Change Biol., 3, 20–32.

McDowell, W.H., Currie, W.S., Aber, J.D., Yano, Y., 1998. Effects of chronic nitrogen amendments on production of dissolved organic carbon and nitrogen in forest soils. Water Air Soil Poll 105:17–182.

McKnight, D.M., Boyer, E.W., Westerhoff, P.K., Doran, PT, Kulbe, T., Andersen, D.T., 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol Oceanogr 46 (1):38–48.

Michalzik, B., Matzner, N., 1999. Fluxes and dynamics of dissolved organic nitrogen and carbon in a spruce (Picea abies Karst.) forest ecosystem. Eur. J. Soil Sci. (in press).

Mikaloff-Fletcher, S.E., Tans, P.P., Bruhwiler, L.M., Miller, J.B., Heimann, M., 2004. CH_4 sources estimated from atmospheric observations of CH_4 and it's C-13/C-12 Isotopic Ratios: 1. Inverse modeling of source processes. Vol. 18, No.4.

Minkkineen, K., Laine, K., 2006. Vegetation heterogeneity and ditches create spatial variability in methane fluxes from peatlands drained for forestry. Plant Soil 286: 289-304.

Moore, B., 1995. Global carbon cycle. Encyclopedia of Environmental Biology. San Diego: Academic Press, pp 215–223.

Moore, T.R., Clarkson, B.R., 2007. Dissolved organic carbon in New Zealand peatlands. New Zealand Journal of Marine and Freshwater Research. Vol. 41: 137-141.

Moore, T.R., Dalva, M., 2001. Some controls on the release of dissolved organic carbon by plant tissues and soils. Soil Science, 166, 38–47.

NOAA National Centers for Environmental Information, State of the Climate: Global Climate Report for Annual 2017, published online January 2018, retrieved on December 8, 2018 from https://www.ncdc.noaa.gov/sotc/global/201713.

Nykänen, H., Alm, J., Silvola, J., Tolonen, K., Martikainen, P.J., 1998. Methane fluxes on boreal peatlands of different fertility and the effect of long-term experimental lowering of the water table on flux rates. Global Biogeochemical Cycle. Vol. 12, No. 1, Pp 53-69.

Nykänen, H., Vasander, H., Huttunen, J.T., Martikainen, P.J., 2002. Effect of experimental nitrogen load on methane and nitrous oxide fluxes on ombrotrophic boreal peatland. Plant and Soil 242: 147–155.

O'Donnell, J.A., Aiken, G.R., Walvoord, M.A., Butler, K.D., 2012. Dissolved organic matter composition of winter flow in the Yukon River basin: Implications of permafrost thaw and increased groundwater discharge. Global Biogeochemical Cycles, Vol. 26, GB0E06.

Ohno T., 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. Environ Sci Technol 36(4): 742–746.

Olefeldt, D., Turetsky, M.R., Crill, P.M., Mcguire, A.D., 2013. Environmental and physical controls on northern terrestrial methane emissions across permafrost zones. Global Change Biology 19, 589–603.

Page, S.E., Rieley, J.O., Banks, C.J., 2011. Global and regional importance of the tropical peatland carbon pool Glob. Change Biol. 17 798–818.

Pastor, J., Solin, J., Bridgham, S.D., Updegraff, K., Harth, C., Weishampel, P., Dewey, B., 2003. Global warming and the export of dissolved organic carbon from boreal peatlands. Oikos 100:380–386.

Peacock, M., Evans, C.D., Fenner, N., Freeman, C., Gough, R., Jones, T.G., Lebron, I., 2014. UV-visible absorbance spectroscopy as a proxy for peatland dissolved organic carbon (DOC) quantity and quality: considerations on wavelength and absorbance degradation. Environ. Sci. Process. Impacts 16, 1445e1461.

Phoenix, G.K., Emmett, B.A., Britton, A.J., Caporn, S.J.M., Dise, N., *et al*. 2011. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. Global Change Biology 18:1197–1215.

Pinsonneault, A. J., Moore, T. R., Roulet, N.T., 2016. Effects of long-term fertilization on peat stoichiometry and associated microbial enzyme activity in an ombrotrophic bog. Biogeochemistry 129:149–164.

Porcal, P., Koprivnjak, J.F., Molot, L.A., Dillon, P.J., 2009. Humic substances: The biogeochemical of dissolved organic carbon and its interactions with climate. Environ Scie Pollut Res. 16: 714-726.

Reay, D.S., Dentener, F., Smith, P., Grace, J., Feely, R.A., 2008. Global nitrogen deposition and carbon sinks. Nat. Geosci. 1, 430-437.

Rinne, J., Tuittila, E.S., Peltola, O., Li, X., Raivonen, M., Alekseychik, P., *et al*., 2018. Temporal variation of ecosystem scale methane emission from a boreal fen in relation to temperature, water table position, and carbon dioxide fluxes. Global Biogeochemical Cycles, 32.

Robroek, B.J.M., Albrecht, R.J.H., Hamard, S., Pulgarin, A., Bragazza, L., Buttler, A., Jassey, V.EJ., 2016. Peatland vascular plant functional types affect dissolved organic matter chemistry. Plant Soil 407: 135-143.

Roulet, N., Moore, T., Bubier, J., Lafleur, P., 1992. Northern fens: methane flux and climate change. Tellus, 44B, 100-105.

Rydin, H., Jeglum, J., 2006. The Biology of Peatlands, Oxford Univ. Press, New York, USA.

Saarnio, S., Saarinen, T., Vasander, H., Silvola, J., 2000a. A moderate increase in the annual CH_4 efflux by raised CO2 or NH_4NO_3 supply in a boreal oligotrophic mire. Global Change Biol. 6, 137–144.

Saarnio, S., Silvola J., 1999. Effects of increased CO2 and N on CH_4 efflux from a boreal mire: a growth chamber experiment. Oecologia 119, 349–356.

Saarnio, S., Silvola, J., Foot, J.P., Sundh, I., Greenup, A., Heijmans, A., Joabsson, A., Mitchell, A., Van Breemen, N., 2000b. Effects of elevated CO_2 and N deposition on CH_4 emissions from European bogs. *In* Abstracts, Invited Papers Symposium 73, Millennium Wetland event, Québec, August 6 – 12.

Scheffer, R.A., Logtestijn, R.S.P., Verhoeven, J.T.A., 2001. Decomposition of Carex and *Sphagnum* litter in two mesotrophic fens differing in dominant plant species. - Oikos 92: 44-54.

Selvam, B.P., Lapierre, J.F., Guillemette, F., Voigt, C., Lamprecht, R.E., Biasi, C., Christensen, T.R., Martikainen, P.J., Berggren, M., 2017. Degradation potentials of dissolved organic carbon (DOC) from thawed permafrost peat. Scientific Reports. 7: 45811.

Senesi, N., 1992. Application of electron spin resonance and fluorescence spectroscopies to the study of soil humic substances. In: Humic substances in soil, sediment, and water (Ed. J. Kubat), Pp. 13–52. John Wiley and Sons, New York.

Spencer, R.G.M., Bolton, L., Baker, A., 2007. Freeze/thaw and pH effects of freshwater dissolved organic matter fluorescence and absorbance properties from a number of UK locations. Water Res., 41, 2941–2950.

Strack, M. 2008. Peatlands and Climate Change. International Peat Society, Finland.

Strack, M., Kellner, E., Waddington, J.M., 2005. Dynamics of biogenic gas bubbles in peat and their effects on peatland biogeochemistry. Glob. Biogeochem. Cy. 19: GB1003.

Strack, M., Zuback, Y., McCarter, C., Price, J., 2015. Changes in dissolved organic carbon quality in soils and discharge 10 years after peatland restoration. Journal of Hydrology 525:345-354.

Tarnocai, C. 2009. The Impact of Climate Change on Canadian Peatlands. Canadian Water Resources Journal / Revue canadienne des ressources hydriques, 34, 453-466.

Teh, Y., Silver, W., Sonnentag, O., Detto, M., Kelly, M., Baldocchi, D., 2011. Large greenhouse gas emissions from a temperate peatland pasture. Ecosystems 14, 311–325.

Teh, Y.A., Silver, W.L., Conrad, M.E. 2005. Oxygen effects on methane production and oxidation in humid tropical forest soils Glob. Change Biol. 11 1283–97.

Tfaily, M.M., Hamdan, R., Corbett, J.E., Chanton, J.P., Glaser, P.H., Cooper, W.T., 2013. Investigating dissolved organic matter decomposition in Northern peatlands using complimentary analytical techniques. Geochimica et Cosmochimica Acta 112:116-129.

Tipping, E., Corbishley, H.T., Koprivnjak, J.-F., Lapworth, D.J., Miller, M.P., Vincent, C.D., Hamilton-Taylor, J., 2009. Quantification of natural DOM from UV absorption at two wavelengths, Environ. Chem., 2009, 6, 472–476.

Tokida, T., Miyazaki, T., Mizoguchi, M., Nagata, O., Takakai, F., Kagemoto, A., Hatano, R., 2007. Falling atmospheric pressure as a trigger for methane ebullition from peatland. Glob. Biogeochem. Cy. 21: GB2003.

Turetsky, M., Treat, C.C., Waldrop, M.P. *et al*., 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: Biogeosciences, 113 (G3): 10.1029/2007JG000496

Turetsky, M.R., Kotowska, A., Bubier, J., *et al*., 2014. A synthesis of methane emissions from 71 northern, temperate, and subtropical wetlands. Global change biology, 20 (7): 2183-2197.

Turunen, J., Tomppo, E., Tolonen, K. & Reinikainen, A., 2002. Estimating carbon accumulation rates of undrained mires in Finland-application to boreal and subarctic regions. The Holocene, 12 (1): 69-80.

Updegraff, K., Bridgham, S. D., Pastor, J., Weishampel, P., & Harth, C. 2001. Response of CO2 and CH4 emissions from peatlands to warming and water table manipulation. Ecological Applications, 11(2), 311–326.

Van Winden, J.F., Reichart, G.J., Mcnamara, N.P., Benthien, A., Damsté, J.S.S., 2012. Temperature-induced increase in methane release from peat bogs: A mesocosm experiment. Plos One, 7(6): E39614.

Walker, M.D., Wahren, C.H., Hollister, R.D., Henry, G.H.R., Ahlquist, L.E., Alatalo, J.M., *et al*., 2006. Plant community responses to experimental warming across the tundra biome. Proc. Natl Acad. Sci. USA, 103, 1342–1346.

Wang, J.S., Logan, J.A., McElroy, M.B., Duncan, B.N., Megretskaia, I.A., Yantosca, R.M., 2004. A 3-D model analysis of the slowdown and inter annual variability in the methane growth rate from 1988 to 1997 [Review]. Global Biogeochem Cycles 18: B3011.

Wang, M., Wu, J., Luan, J., Lafleur, P., Chen, H., Zhu, X., 2017. Near-zero methane emission from an abandoned boreal peatland pasture based on Eddy covariance measurements. Plos One 12(12): e0189692.

Wang, M., Wu, J., Luan, J., Lafleur, P., Chen, H., Zhu, X., 2018. Can abandoned peatland pasture sequester more carbon dioxide from the atmosphere than an adjacent pristine bog in Newfoundland, Canada? Agricultural and Forest Meteorology. Vol. 248, pp 91-108.

Ward, S.E., Ostle, N.J., Oakley, S., Quirk, H., Henrys, P.A., Bardgett, R.D., 2013. Warming effects on greenhouse gas fluxes in peatlands are modulated by vegetation composition. Ecol. Lett. 16, 1285–1293.

Watson, A., Nedwell, D.B., 1998. Methane production and emission from peat: the influence of anions (sulphate and nitrate) from acid rain. Atmospheric Environment 32: 3239–3245.

Weishaar, J.L, Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ Sci Technol 37(20):4702–8.

Weltzin, J.F., Bridgham, S.D., Pastor, J., Chen, J., Harth, C., 2003. Potential effect of warming amd drying on peatland plant community composition. Global Change Biology. 9, 141-151.

Weltzin, J.F., Loik, M.E., Schwinning, S., Williams, D.G *et al*., 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. BioScience. Vol. 53 No. 10, 941-953.

Whalen, S.C., 2005. Biogeochemistry of methane exchange between natural wetlands and the atmosphere. Environ. Eng. Sci. 22(1): 73–94.

Worrall, F., Burt, T.P., 2004. Time series analysis of long term river dissolved organic carbon records. Hydrological Processes 18, 893–911.

Worrall, F., Burt, T.P., Adamson, J., 2006. Do nitrogen inputs stimulate dissolved organic carbon production in upland peat bogs? Global Biogeochemical Cycles, Vol. 20, GB3013.

Worrall, F., Reed, M., Warburton, J., Burt, T.P., 2003. Carbon budget for British upland peat catchment. Sci Tot Environ; 312:133 –146.

Wright, R.F., Alewell, C., Cullen, J. M., Evans, C.D., Marchetto, A., Moldan, F., *et al*., 2001. Trends in Nitrogen Deposition and Leaching in Acid-Sensitive Streams in Europe. Hydrology and Earth System Sciences, 5, 299–310.

Wu, J & Roulet, N.T., 2014. Climate change reduces the capacity of northern peatlands to absorb the atmospheric carbon dioxide: The different responses of bogs and fens: peatlands switch to C sources by 2100. Global Biogeochemical Cycles, vol. 28, no.10, pp.1005–24.

Wu, J., 2012. Response of peatland development and carbon cycling to climate change: a dynamic system modeling approach. Environ. Earth Sci. 65, 141–151.

Yano, Y., McDowell, W.H., Aber, J.D., 2000. Biodegradable dissolved organic carbon in forest soil solution and effects of chronic nitrogen deposition. Soil Biol Biochem 32(11–12):1743–51.

Yu, J., Anderson, R.F., Jin, Z.D., Rae, J., Opdyke, B.N., Eggins, S., 2013. Responses of the deep ocean carbonate system to carbon reorganization during the last glacial-interglacial cycle. Quat. Sci. Rev. 76, 39e52.

Yu, K.W., Wang, Z.P., Chen, G.X., 1997. Nitrous oxide and methane transport through rice plants. Biol Fertil Soils 24:341-343.

Yu, Z., Loisel, J., Brosseau, D.P., Beilman, D.W. and Hunt, S.J., 2010. Global peatland dynamics since the Last Glacial Maximum. Geophysical Research Letters, 37 (13):10.1029/2010GL043584

Yuan, X, Si, Y., Lin, W., Yang, J., Wang, Z., Zhang, Q., *et al*., 2018. Effects of short-term warming and nitrogen addition on the quantity and quality of dissolved organic matter in a subtropical *Cunninghamia Lanceolata* plantation. Plos One 13 (1): E0191403.

Zaccone, C., Plaza, C., Ciavatta, C., Miano, T.M., Shotyk, W., 2018. Advances in the determination of humification degree in peat since Achard (1786): Applications in geochemical and paleoenvironmental studies. Earth-Science Reviews 185: 163-178.

Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B., Saccomandi, F., 1999. Differentiating with fluorescence spectroscopy the source of dissolved organic matter in soils subjected to drying. Chemosphere, Vol. 38, No.1, pp.45-50.

Appendices

Table A. 1 Environmental variables and CH4 fluxes under different treatments and years for the entire growing season

Year	Treatment	Soil Moisture Content (%)	Soil Temp. $_{5\ cm}$ (^oC)	Soil Temp. $_{20\ cm}$ (^oC)	Water Table Depth (cm)	CH_4 Flux (mg m^{-2} h^{-1})
2015	C	67.6±9.4	18.4±3.3	16.1±2.4	6.2±8.5	2.1±1.7
	N	67.4±9.4	18.3±4.4	16.2±2.4	6.6±7.0	2.0±1.6
	W	60.6±13.4	20.1±3.8	16.7±2.4	7.0± 6.1	3.4±1.9
	W+N	63.0±14.4	18.6±3.3	16.4±2.3	6.8±8.0	2.2±1.3
2016	C	71.0±16.3	14.7±2.8	13.7±3.5	6.9±5.0	2.4±2.0
	N	68.8±10.5	14.9±2.6	13.7±3.3	6.4±5.0	1.9±1.1
	W	64.9±6.9	15.2±2.8	14.2±3.3	6.1±4.6	4.1±2.7
	W+N	70.3±4.3	14.6±3.2	13.8±3.5	6.7±5.0	1.07±0.8
2017	C	64.0±17.4	15.9±3.8	14.7±2.8	12.7±6.6	2.0±1.5
	N	65.3±18.3	15.8±3.8	14.8±2.8	12.8±6.8	1.3±0.8
	W	60.7±16.5	15.5±3.8	14.9±2.8	11.0±6.8	2.9±1.2
	W+N	63.9±16.5	15.4±4.0	14.6±2.7	12.8±8.4	1.4±0.8

Data values are means±SD for the different treatment effect; Control (C), Nitrogen addition (N), Warming (W), Warming and Nitrogen addition (W+N).

Table A. 2 Pearson correlation (r) coefficients between dissolved organic carbon (DOC) and the spectroscopy indexes.

	DOC (mg/L) $_{10\ cm}$	DOC (mg/L) $_{40\ cm}$	TN (mg/L) $_{10\ cm}$	TN (mg/L) $_{40\ cm}$	SUVA $_{10\ cm}$	SUVA $_{40\ cm}$	$FI_{10\ cm}$	$FI_{40\ cm}$	$HIX_{10\ cm}$	$FI_{40\ cm}$
DOC $(mg/L)_{40\ cm}$	0.843***									
TN $(mg/L)_{10\ cm}$	-0.064	-0.364*								
TN $(mg/L)_{40\ cm}$	-0.392**	-0.434**	0.713***							
SUVA $_{10\ cm}$	-0.286*	-0.367*	0.040	0.338*						
SUVA $_{40\ cm}$	-0.322*	-0.556***	0.250	0.439**	0.337*					
$FI_{10\ cm}$	-0.450***	-0.411**	0.521***	0.472***	0.099	0.366*				
$FI_{40\ cm}$	-0.506***	-0.531***	0.563***	0.552***	0.309*	0.166	0.606***			
$HIX_{10\ cm}$	0.262*	-0.040	0.358**	0.319*	0.502***	0.252	-0.051	0.092		
$HIX_{40\ cm}$	0.235	0.175	0.402***	0.474***	0.429***	0.117	0.207	0.062	0.779***	

Coefficient values without (*) are not significant. *p= 0.05, ** $p \leq 0.01$, *** $p \leq 0.001$. Correlation of dissolved organic carbon (DOC), total nitrogen (TN), specific ultraviolet absorbance (SUVA),fluorescence index (FI) and the humification index (HIX) at different peat depth.

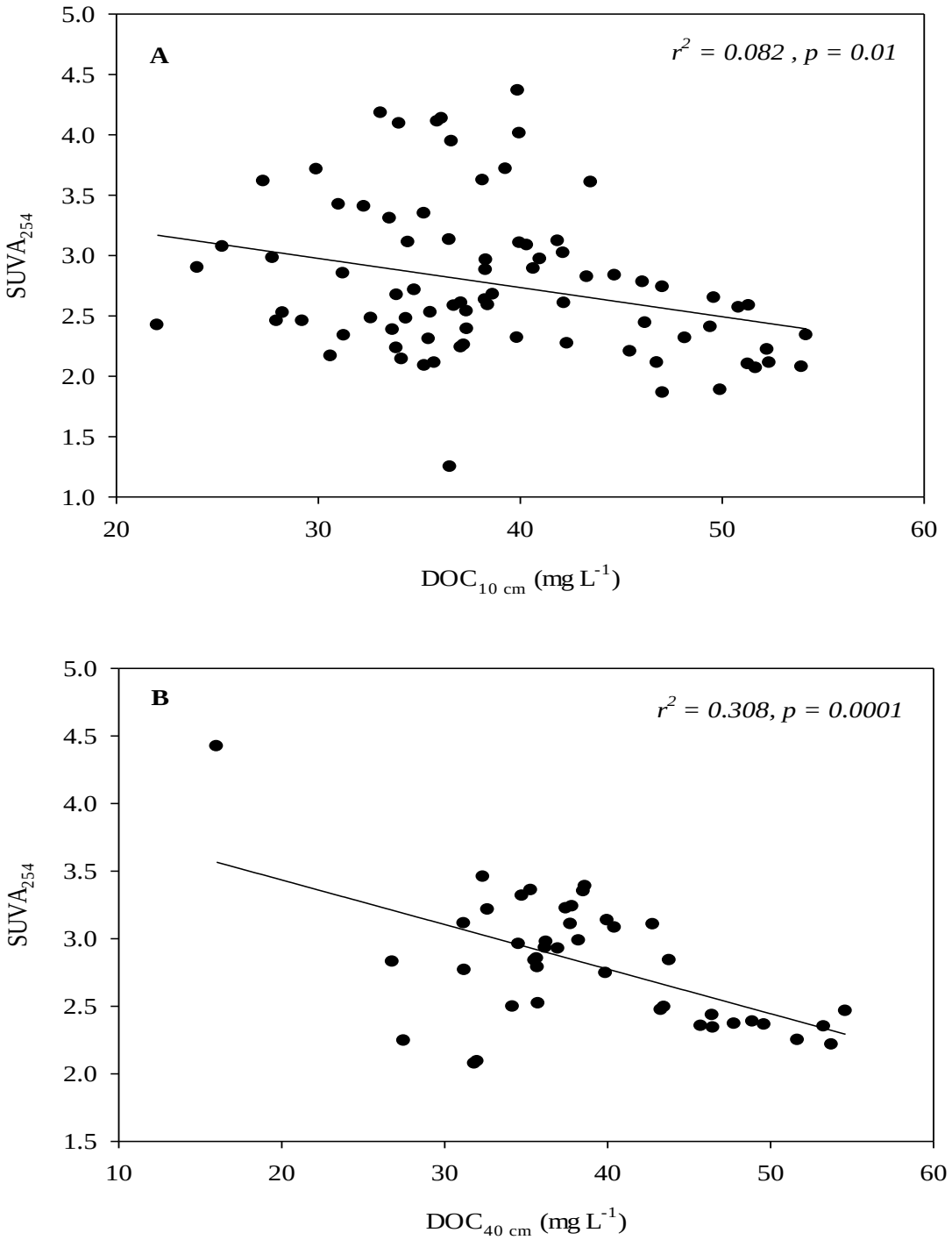

Figure A. 1 Relationship between specific ultra-violet absorbance ($SUVA_{254}$) and DOC (mg L^{-1}) at 10 cm (A) and 40 cm (B) peat depth when all treatment effects were pooled

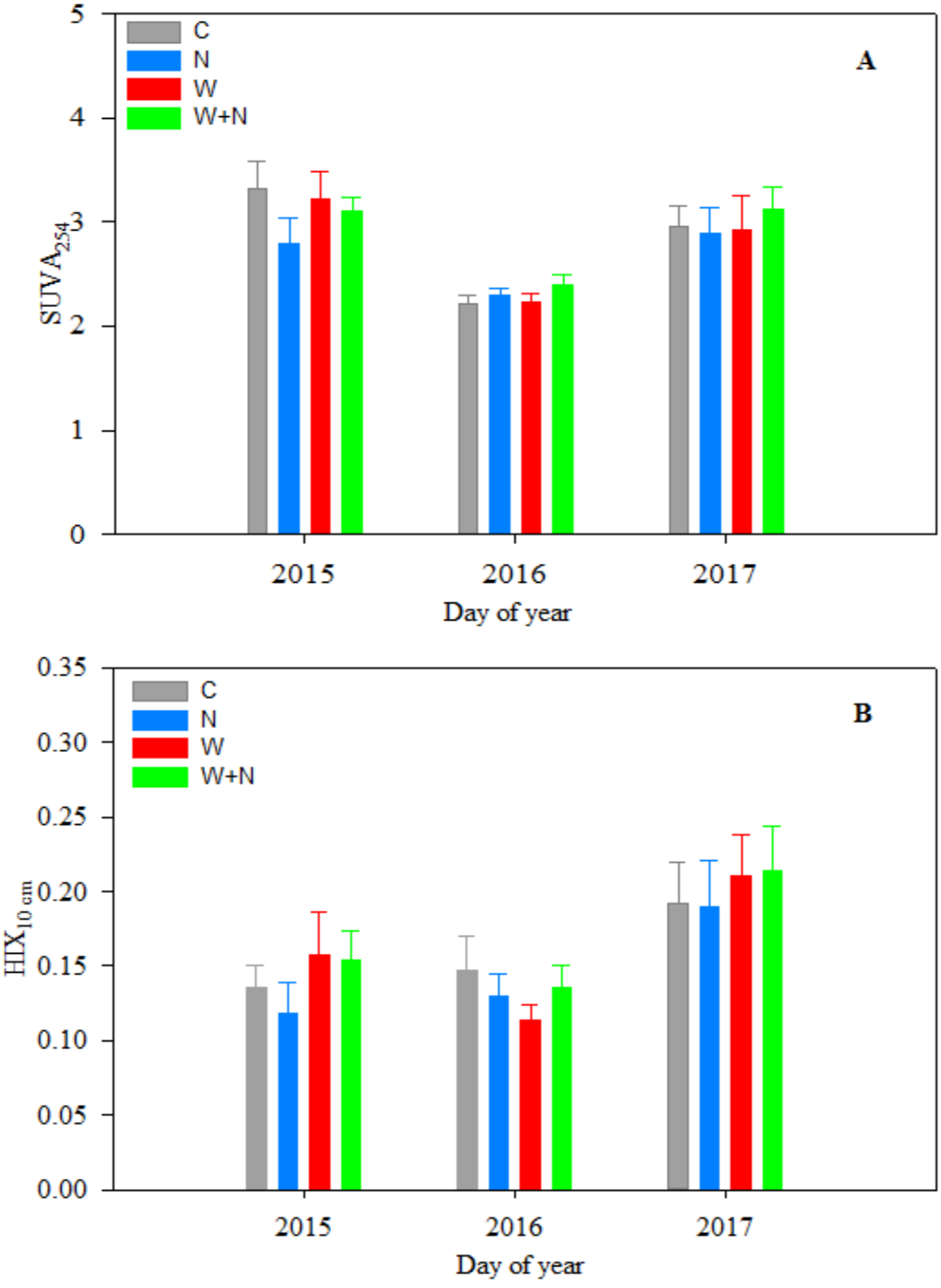

Figure A. 2 The average annual treatment effect of $SUVA_{254}$ and HIX at 10 cm peat depth. Legend labels represents control (C), nitrogen addition (N), warming (W) and interactive effect of warming and nitrogen addition (W+N) treatment effect. Bars represent standard error bars (SE).

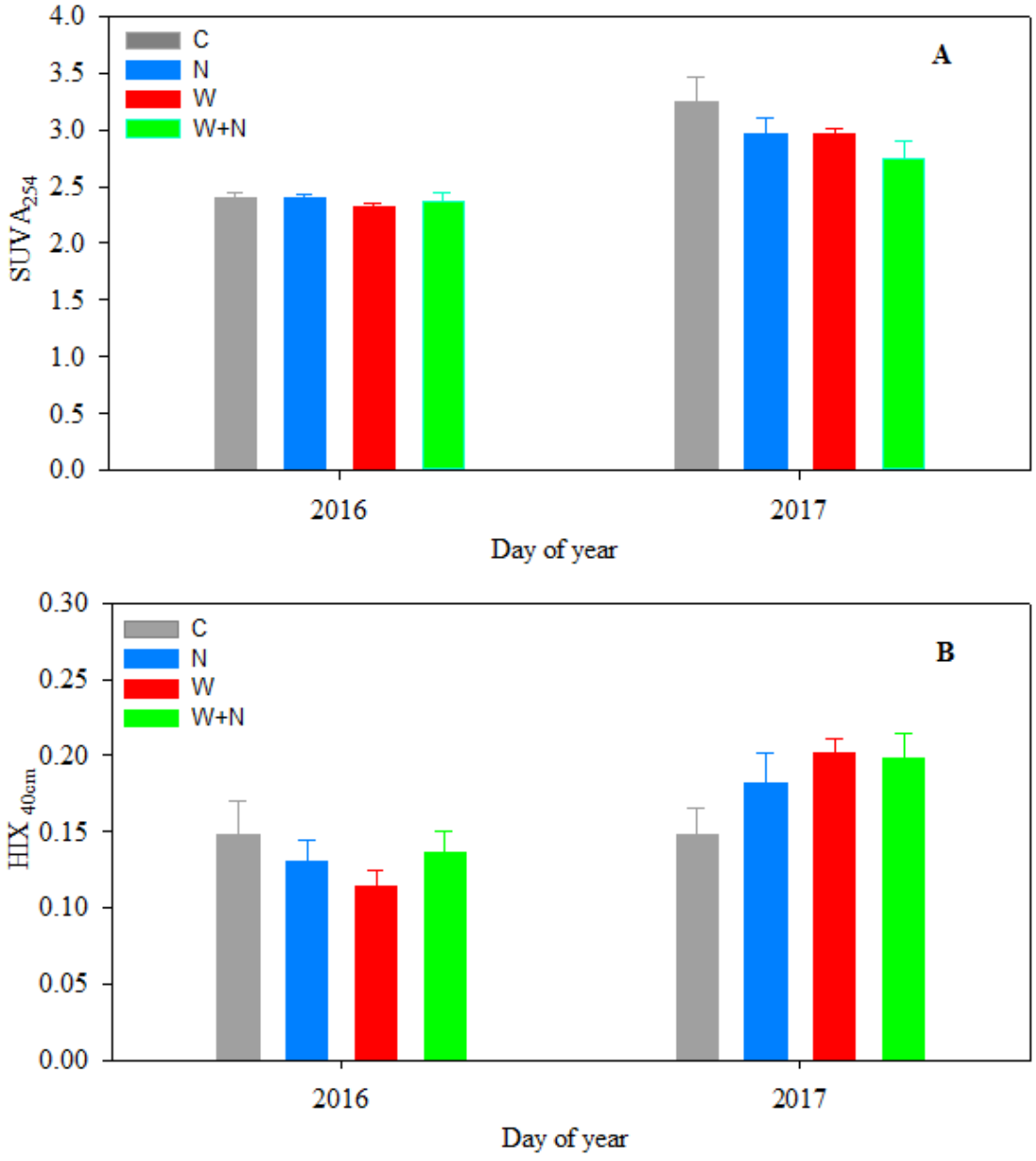

Figure A. 3 The average annual treatment effect of $SUVA_{254}$ and HIX at 40 cm peat depth. Legend labels represents control (C), nitrogen addition (N), warming (W) and interactive effect of warming and nitrogen addition (W+N) treatment effect. Bars represent standard error bars (SE)