# Greenhouse gas fluxes in a boreal peatland under experimental warming, nitrogen addition, and vegetation composition change

by © Yu Gong

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# Abstract

Although peatlands cover only 3% of land surface over the world, they have stored a large amount of carbon due to the relatively higher rate of net primary production than decomposition. The Intergovernmental Panel on Climate Change (IPCC) shows that net zero carbon dioxide ($CO_2$) emissions should be reached around 2050 to limit warming to 1.5°C above pre-industrial levels. The carbon sink function of peatlands could help to reduce global warming (cooling function). However, it is unclear whether this carbon sink function of peatlands will be altered under future global changes such as climate warming, elevated nitrogen (N) deposition, and vegetation composition change. Moreover, methane ($CH_4$) and nitrous oxide ($N_2O$) are two potent greenhouse gases with 25 and 298 times higher global warming potential than $CO_2$, respectively. Their responses to the three global changes (climate warming, elevated nitrogen deposition, and vegetation composition change) are poorly known in peatland ecosystems, especially the interaction of the three global changes, which leads to an uncertainty in evaluating the cooling function of peatlands in the future.

In this thesis, the three global changes were mimicked in a boreal peatland located in western Newfoundland, Canada. The fluxes of greenhouse gases ($CO_2$, $CH_4$, and $N_2O$) and environmental variables were measured. The results showed that a change in vegetation composition played an essential role in net $CO_2$ uptake. With graminoid removal, net $CO_2$ uptake was significantly decreased, and combined warming and N addition (WN) further decreased $CO_2$ uptake owing to the detrimental effect of N on *Sphagnum* mosses. Shrub removal also decreased net $CO_2$ uptake, but $CO_2$ uptake could recover in the seventh year owing to the growth of graminoids. Warming

and N addition could promote graminoid growth, which might offset the loss of *Sphagnum* moss cover. Consequently, the net $CO_2$ uptake was not altered under the condition of shrub removal.

Graminoid removal significantly decreased $CH_4$ emissions due to the reduction of available carbon for $CH_4$ production and aerenchyma (air channels of some plants) for $CH_4$ transport from soil to the atmosphere. However, this negative effect was not observed under WN conditions, possibly owing to the alteration of temperature sensitivity. Shrub removal significantly decreased $CH_4$ emissions under warming treatment, but this negative effect was also not observed under WN conditions, which could be attributed to the growth of graminoids. The positive impact of graminoid growth on $CH_4$ emission could offset the negative effect of shrub removal.

Nitrogen addition significantly promoted $N_2O$ emissions due to the increase of nitrogen availability for $N_2O$ production. Warming could mitigate the positive effect of N addition under intact vegetation in the middle growing season, which could be attributed to the stimulation of N uptake by plants and less N for $N_2O$ production. With the removal of graminoids or shrubs, WN significantly increased $N_2O$ emissions in the early growing season owing to the alleviation of carbon and nitrogen limitation for $N_2O$ production.

In summary, if the dominant vegetation shifts to shrubs, the net $CO_2$ uptake in peatlands would be decreased under climate warming and elevated N deposition. If the dominant vegetation shifts to graminoids, the net $CO_2$ uptake in peatlands would also be decreased in a short time (~ 3 years) under climate warming and elevated N deposition, but could recover in a long time (~7 years). No matter whether the dominant vegetation shifts to shrubs or graminoids, their impacts on $CH_4$ emissions would be negligible under climate warming and elevated N deposition.  In contrast, no matter whether the dominant vegetation shifts to shrubs or graminoids in the future, the $N_2O$ emissions were significantly promoted under climate warming and elevated N deposition. Overall, taking all three greenhouse gases into account, the cooling function of peatlands would be reduced under future climate warming, elevated N deposition, and vegetation composition change. Besides peatland conservation, other effective measures should be taken in order to slow down the global temperature increase.

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Firstly, I would like to express my sincere appreciation to my supervisor Dr. Jianghua Wu for the consistent encouragement and valuable guidance throughout my research. I appreciate all his contributions of time, ideas, and funding to make my Ph.D. experience productive and stimulating. I would like to thank Dr. Nigel Roulet, Dr. Joe Wroblewski, Dr. Michele Piercey-Normore, Dr. Christine Campbell, and Dr. Carissa Brown for their great support and invaluable advice. I would like to thank Thuong Ba Le, Eissa, Asare Gyimah, Syed Shah Mohioudin Gillani, Jiangqi Wu, and Fan Lu for their field sampling and measurements.

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# CHAPTER 1. Introduction

Peatlands play an important role in global carbon cycling and climate change (Loisel et al., 2017). They have stored approximately 600 Gt (1 Gt =$10^{15}$ g) of carbon (C) (Yu et al., 2010), although peatlands only account for 2.84% of the land surface (Xu et al., 2018). As local and national governments worldwide declare a "climate emergency" (House of Commons Canada, 2019; Welsh Government 2019), peatlands as carbon sinks are of great concern and could be one of the solutions. However, it is unclear whether peatlands will still be the carbon sinks and mitigate climate change in the future.

Previous modeling and manipulated studies have reported that climate warming has the potential to reduce the capacity of peatlands to sequestrate carbon and even switch them to be carbon sources (Dorrepaal et al., 2009; Wu and Roulet, 2014). However, elevated nitrogen (N) and vegetation composition change are not taken into account (Wu and Roulet, 2014), although both of them have been reported to likely change in the future. For instance, N deposition has been predicted to increase by more than ~50% during this century (Reay et al., 2008), and dominant vegetation in peatland ecosystems has the potential to shift from *Sphagnum* mosses to vascular plants (Dieleman et al., 2015).

Peatlands are nutrient-poor ecosystems (Kivimäki et al., 2013; Wang et al., 2014). Elevated N deposition can provide nutrients for vascular plant growth and carbon dioxide ($CO_2$) uptake, thus potentially strengthening the function of peatlands to be carbon sinks (Lund et al., 2009). Nevertheless, it should be noted that elevated N deposition has a detrimental effect on *Sphagnum* mosses, which is an essential plant functional type in peatland ecosystems (Larmola et al., 2013). Due to the complicated effects of elevated N deposition on different plant functional types, their net impacts on the carbon cycling of peatlands are not well understood. Moreover, the change of dominant vegetation shifting from *Sphagnum* mosses to vascular plants could be beneficial for $CO_2$ uptake (Leroy et al., 2019). Nevertheless, the combined effects of climate warming, elevated nitrogen deposition and vegetation composition change on $CO_2$ uptake are unclear.

Methane ($CH_4$) as a greenhouse gas is also an essential component in the carbon cycling of peatlands. Northern peatlands contribute about 10% to $CH_4$ emissions from wetlands which are largest natural sources of $CH_4$ (Mikaloff-Fletcher et al., 2004; Saunois et al., 2016). Vascular plants can provide available carbon for $CH_4$ production via root exudates, and some of them can promote $CH_4$ transport from deep peat to the atmosphere via aerenchyma (Juutinen et al., 2018). Aerenchyma is the tissue in plants, which forms air channels in leaves, stems, and roots. Gases could transport from soil to the atmosphere via aerenchyma. Therefore, $CH_4$ emitted from peatlands would be increased with vegetation composition shifting from *Sphagnum* mosses dominant to vascular plant dominant. Additionally, climate warming or elevated N deposition could increase photosynthesis of vascular plants and root exudates for $CH_4$ production (Granberg et al., 2001). Does that mean climate warming and elevated N deposition would further increase $CH_4$ emission under the condition of vegetation composition change?

Apart from $CO_2$ and $CH_4$, nitrous oxide ($N_2O$) is another potent greenhouse gas and needs to be considered because elevated N deposition can provide available N for nitrification and denitrification, two major biochemical processes for $N_2O$ production (Butterbach-Bahl et al., 2013). Consequently, although elevated N deposition could mitigate climate change by increasing plant growth and $CO_2$ uptake, the increase of $N_2O$ emission may reduce its mitigation owing to its 298 times higher global warming potential than $CO_2$ (IPCC, 2013). Noticeably, climate warming can reduce the positive effect of N deposition on $N_2O$ fluxes through increasing N uptake by vascular plants (Gong et al., 2019). With vascular plant dominant in peatlands, the $N_2O$ fluxes would be further mitigated due to the competition with microbes for available nitrogen. Nevertheless, how these global changes (climate warming, elevated N deposition, and vegetation composition change) interactively affect the $N_2O$ fluxes in peatlands is unknown.

These unclear impacts of the global changes on the carbon cycling and greenhouse gas emissions in peatlands lead to large uncertainty in predicting the function of peatlands. Therefore, the manipulated experiments are urgently needed to advance our understanding of the role of boreal peatlands in the global carbon cycle and global climate system, and formulate peatland management strategies for climate change mitigation.

## 1.1 Objectives of the Research

The interactions of global changes (climate warming, elevated nitrogen deposition, and vegetation composition change) on greenhouse gas fluxes ($CO_2$, $CH_4$, and $N_2O$) are not well known in peatlands. This research aims to fill this knowledge gap. Simulated warming, nitrogen addition, and vegetation composition change have been conducted in a boreal peatland in western Newfoundland, Canada. Three greenhouse gases ($CO_2$, $CH_4$, and $N_2O$) and environmental variables (soil temperature, moisture, water table depth, dissolved organic carbon and total nitrogen) were measured in this research. We investigated the combination of abiotic (warming and N addition) and biotic factors (vegetation composition) on greenhouse gas fluxes, explored the possible underlying mechanisms, and evaluated the primary controls for the greenhouse gases. This research will provide insights for better understanding the greenhouse gas fluxes in peatlands under global change and could help to accurately evaluate the climate mitigation function of peatlands in the future.

## 1.2 Chapter Outline

This thesis is comprised of seven chapters:

Chapter 1 provides an introduction and overview of the thesis.

Chapter 2 is a meta-analysis and review. It synthesizes the current knowledge of three global changes (climate warming, elevated nitrogen deposition, and vegetation composition change) impacts on greenhouse gas fluxes ($CO_2$, $CH_4$, and $N_2O$) in peatlands. The knowledge gaps have also been discussed.

Chapter 3 describes the interactions of climate warming, elevated nitrogen deposition, and vegetation composition change on $CO_2$ fluxes in a peatland. The net $CO_2$ uptake in the peatland under future global change has been evaluated.

Chapter 4 describes the interactions of climate warming, elevated nitrogen deposition, and vegetation composition change on $CH_4$ fluxes in a peatland. The possible underlying mechanisms and the major controls for $CH_4$ fluxes have been investigated.

Chapter 5 describes the interactions of climate warming and nitrogen deposition on $N_2O$ fluxes in a peatland. The possible underlying mechanisms and the major controls have been investigated.

Chapter 6 describes the essential role vegetation composition played in regulating $N_2O$ fluxes in a peatland under climate warming and elevated nitrogen deposition. The biotic control (gross primary production) for $N_2O$ fluxes has been explored.

Chapter 7 is the final chapter of the thesis. It synthesizes the main conclusions, limitations, and further research.

### 1.3 Co-Authorship Statement

I am the principal author of all chapters presented in this thesis. However, my studies could not have been completed without the excellent supervision and guidance of my supervisor Dr. Jianghua Wu, and the help of the other members of Dr. Wu's group. For all the manuscript-format chapters, I did the field samplings and field measurements for the data from 2018 to 2020, and the other years' data came from my supervisor, Dr. Jianghua Wu. The time of greenhouse gas measurements was shown in Table 1.1. For all the manuscripts, I defined all the research questions, analyzed the data, and wrote the first draft under the supervision of Dr. Jianghua Wu. For all the published chapters, I, with the supervision and help of Dr. Jianghua Wu, did all the revision and responded to the comments from the reviewers and editors. Their specific contribution and involvements are recognized here.

For Chapter 2, Dr. Jianghua Wu, Judith Vogt, and Dr. Weiwei Ma provided reviews and comments of the manuscript.

For Chapter 3, Dr. Jianghua Wu contributed to the planning of the research, improvement of the manuscripts, and financial support. Thuong Ba Le, Jiangqi Wu, and Fan Lu assisted in the field sampling and measurements in 2020.

For Chapter 4, Dr. Jianghua Wu contributed to the planning of the research, improvement of the manuscripts, and financial support. Thuong Ba Le assisted in field sampling and field measurements and provided comments on the first draft.

For Chapter 5, Dr. Jianghua Wu contributed to the planning of the research, improvement of the manuscripts, and financial support. Judith Vogt and Thuong Ba Le contributed to the review of the first draft.

For Chapter 6, Dr. Jianghua Wu contributed to the planning of the research, improvement of the manuscripts, and financial support.

Table 1.1 The time of greenhouse gas measurements in chapter three, four, five, and six.

| | Chapter 3 | Chapter 4 | Chapter 5 | Chapter 6 |
|---|---|---|---|---|
| 2015 | | | $N_2O$ | |
| 2016 | $CO_2$ | $CH_4$ | $N_2O$ | $N_2O$ |
| 2017 | | $CH_4$ | | |
| 2018 | | $CH_4$ | | $N_2O$ |
| 2019 | | | | |
| 2020 | $CO_2$ | | | |

## 1.4 Dissemination of Research

All the published papers are attached in the Appendix.

Manuscript # 1 (Chapter 2) has been published in *Environmental Reviews*.

Gong, Y., Wu, J., Vogt, J. and Ma. W., 2020. Greenhouse gas emissions from peatlands under manipulated warming, nitrogen addition, and vegetation composition change: a review and data synthesis. Environmental Reviews, 28(4): 428-437.

Manuscript # 2 (Chapter 3), "Vegetation composition regulates the interaction of warming and nitrogen deposition on net carbon dioxide uptake in a boreal peatland", is to be submitted to *Global Change Biology*.

Manuscript # 3 (Chapter 4) has been published in *Geoderma*.

Gong, Y., Wu, J. and Le T., 2021. Counteractions between biotic and abiotic factors on methane dynamics in a boreal peatland: vegetation composition change vs warming and nitrogen deposition. Geoderma, 395: 115074.

Manuscript # 4 (Chapter 5) has been published in *Science of the Total Environment*.

Gong, Y., Wu, J., Vogt, J. and Le T., 2019. Warming reduces the increase in $N_2O$ emission under nitrogen fertilization in a boreal peatland. Science of the Total Environment, 664:72-78.

Manuscript # 5 (Chapter 6), "Vegetation composition modulates the interaction of climate warming and elevated nitrogen deposition on nitrous oxide flux in a boreal peatland", has been under review in *Global Change Biology*.

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# CHAPTER 2. Greenhouse gas emissions from peatlands under manipulated warming, nitrogen addition and vegetation composition change: a review and data synthesis

## 2.1 Abstract

Peatlands play an essential role in carbon cycling and global warming. However, the feedback of peatlands to global changes is still unclear. Here, we conducted a data synthesis of 236 observations from 52 field experiments to evaluate the effect of three important global changes (warming, nitrogen addition and vegetation composition change) on three major greenhouse gas (GHG) fluxes: $CO_2$, $CH_4$ and $N_2O$. The results showed that (i) GHG responses to warming varied between warming methods, between air temperature increase rates and between warming durations; (ii) GHG responses to N addition varied between peatland types, between N forms, between N concentrations and between experimental durations; (iii) the response rates of GHGs were associated with local environmental parameters (mean annual precipitation, MAP; and water table level, WTL); (iv) the global warming potential (GWP) considerably increased under these global changes, which indicates that cooling function of peatlands will be weakened. Overall, given these global changes occur simultaneously, the interaction of them on GHG fluxes should not be ignored. Our results highlight that a large number of studies in different locations are needed to comprehensively understand and accurately predict GHG emissions from peatlands.

## 2.2 Introduction

Although peatlands cover only 3% of the global terrestrial area, they play an important role in global carbon cycling. Peatlands have been carbon sinks for millennia and stored more than 500

Gt of carbon (Yu, 2012). Carbon dioxide ($CO_2$) in the atmosphere is absorbed by vegetation through photosynthesis, which is an important carbon input in peatland ecosystems (Lees et al., 2018). Decomposition is another essential factor in carbon export. Waterlogged peat and cold temperature are two limiting factors for the decomposition. That is why most peatlands are located in boreal zone (Xu et al., 2018), although peatlands can be also found in tropics with high precipitation rates. Additionally, acid condition and phenolics in *Sphagnum* moss (the builder of boreal peatlands) further slow the decomposition (Rydin and Jeglum, 2013). Besides carbon output in gaseous-phase ($CO_2$ and $CH_4$) via decomposition, there are carbon outputs in liquid-phase and solid-phase from peatlands, including dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and particulate organic carbon (POC) (Lees et al., 2018). Taking all carbon components into account, the carbon accumulation rate is about 10-30 g C $m^{-2}$ $yr^{-1}$ in northern peatlands (Limpens et al., 2008).

However, they are vulnerable to climate change and anthropogenic activities such as global warming, changes in precipitation patterns, fertilization, peat excavation and land-use change (Harenda et al., 2018). Global temperature is projected to increase by up to 2-5 °C within this century (IPCC, 2013), while the total N deposition rate is predicted to increase two- or three-fold in the northern hemisphere (Galloway and Cowling, 2002; Kanakidou et al., 2016; Reay et al., 2008). Moreover, global warming, N deposition and land-use change impact vegetation composition in peatland ecosystems. For instance, global warming and N deposition increase coverage of vascular plants at the expense of bryophytes (Dieleman et al., 2015; Gallego-Sala and Prentice, 2013; Juutinen et al., 2010; Walker et al., 2006). Burning and grazing increase growth of graminoids and decrease growth of shrubs and bryophytes (Ward et al., 2007). Although greenhouse gas (GHG) fluxes in peatlands have been a concern for decades, few models accurately predict them and take all these global changes into account. Thus, field experiments have been conducted to investigate the impacts of warming, N deposition and vegetation composition change on GHG fluxes in peatlands.

Previous studies have reported that warming increases greenhouse gas ($CO_2$, $CH_4$ and $N_2O$) emissions due to increase of substrate availability and microbial activities (Cui et al., 2018; Granberg et al., 2001; Hu et al., 2016; Samson et al., 2018; Turetsky et al., 2008), while some studies have reported that there is negligible or negative effect of warming on them possibly due to alteration of microbial abundance or influence of other environmental factors such as water table and nutrient limitation (Eriksson et al., 2010; Gong et al., 2019; Pearson et al., 2015; Peltoniemi et al., 2016). The controversial results are unlikely to be resolved by these individual studies due to different types of peatlands, methods of warming simulation, and experimental durations.

Besides warming, N addition also has a significant effect on GHG fluxes in peatlands. Gerdol et al. (2008) have reported that ammonium nitrate ($NH_4NO_3$) increases $CO_2$ emissions in an alpine bog due to an increase of N availability for plants and microbes. However, Kivimäki et al. (2013) did not observe the effect of nitrate ($NO_3^-$) or ammonium ($NH_4^+$) on $CO_2$ emission in a temperate bog, which could be owing to phosphorus (P)- or potassium (K)-limitation not N-limitation in their study site. In addition, low N addition (30 kg $ha^{-1}$ $yr^{-1}$) has little or no effect on $CH_4$ emission from bogs and fens due to filter function of mosses (Granberg et al., 2001; Silvola et al., 2003), while high N addition (100 kg $ha^{-1}$ $yr^{-1}$) enhances $CH_4$ emission by increasing coverage of *Eriophorum vaginatum* in a boreal bog (Nykänen et al., 2002). This is because *E. vaginatum* can provide root exudates for $CH_4$ production and has aerenchyma tissue for $CH_4$ transporting directly from soil to the atmosphere. Furthermore, after ~3 years of N addition, no significant effect on $CH_4$ emission from peatlands has been observed (Lund et al., 2009; Silvola et al., 2003), while after 10 years of N addition, there was a significant increase in $CH_4$ emission from a boreal bog due to a shift of vegetation composition from *Sphagnum*-dominated to vascular plant-dominated peatland (Juutinen et al., 2018). These discrepant results suggest that it is necessary to compile all available data to synthesize results from individual studies to reveal the effects of different N forms, N concentrations and experimental durations on the responses of GHGs to N addition.

Vegetation composition is susceptible to global warming, N deposition and anthropogenic activities (drainage, land-use change and peat excavation), and significantly impacts GHG fluxes in peatlands (Leroy et al., 2017; Ward et al., 2013). There are two typical vegetation groups in peatlands: vascular plants (shrubs and graminoids) and bryophytes. The absence of vascular plants reduced ecosystem respiration in a boreal bog (Ward et al., 2013). The presence of graminoids increases GHGs transport from soil to the atmosphere via their aerenchyma tissues (Jørgensen et al., 2012; Nielsen et al., 2017; Ward et al., 2013). However, oxygen is also transported to roots by aerenchyma and decreases the activity of methanogens and denitrification bacteria, thus decreasing $CH_4$ and $N_2O$ production (Jørgensen et al., 2012). Given the complicated effects of vegetation composition on different GHG fluxes in peatlands, it is the utmost importance to collect all available data of them and investigate their net effect on global warming.

In the present study, we compiled the data of GHG emissions from peatlands under simulated three global changes: warming, nitrogen deposition, and vegetation composition change. Our objectives are to determine (i) how GHGs respond to warming in different peatland types, methods of warming simulation, temperature increase rates, and experimental durations; (ii) how GHGs respond to N addition in different peatland types, N forms, N concentrations and experimental durations; (iii) how GHGs respond to vegetation composition change in different peatland types; (iv) How global warming potential is changed under warming, N addition and vegetation composition change; (v) how environmental variables affect the responses of GHGs to the global changes.

## 2.3 Approach

### 2.3.1 Document selection

Peer-reviewed journal articles were searched using Web of Science and Scopus using the keywords: carbon dioxide, methane, nitrous oxide, warming, nitrogen deposition, vegetation composition, fen, bog, mire, peatland. Peatland is defined by peat depth. The minimum peat depth for peatland varies between 30 and 50 cm. For example, the minimum thickness is ~30 cm in England, 50 cm in Scotland (Johnson and Dunham, 1963), and 40 cm in Canada (Rydin and Jeglum, 2013). Therefore, we selected 30 cm to include all peatlands over the world. In this review, peatlands have been classified into bogs and fens. The classification is based on the characteristics of bogs and fens. Bogs are ombrotrophic peatlands that receive water and nutrients only from precipitation (Vitt, 1994). Fens are minerotrophic peatlands and receive water and nutrients not only from precipitation but also from surface water or groundwater (Vitt, 1994). To gain comprehensive coverage, we also checked all references in the papers found in the Web of Science and Scopus search. The database was compiled to compare GHG fluxes among sites. To indicate the direction of the GHG fluxes, we used the atmospheric science sign convention, where a negative sign represents uptake of GHG by the ecosystem. All studies used static chambers to measure GHG fluxes in the field (Luan and Wu, 2014), and sampling was conducted weekly, biweekly, or monthly during the growing season. Laboratory incubation experiments were not considered, as there is a substantial difference in environmental parameters between incubation and field, especially ignoring the impacts of vegetation. Likewise, studies using transplantation or using different locations of peatlands to simulate climate change were excluded because there are many other variables influencing their results, such as different water table depths and microbial compositions. The studies that contained different warming methods, forms and rates of N addition, vegetation species removal or experiment durations were treated as multiple data points. In total, there were 54 data points from 20 published papers about effects of warming on GHG fluxes in peatlands, 92 data points from 22 published papers about effects of N addition on GHG fluxes in peatlands, and 90 data points from 18 published papers about effects of vegetation composition change on GHG fluxes in peatlands. Furthermore, 10 articles focused on the combined effects of them (warming, N deposition or vegetation composition change) on GHG emissions from peatlands (Table S1-S9).

We recorded environmental variables directly from papers or cited papers, including latitude and longitude, mean annual temperature (MAT), mean annual precipitation (MAP), pH, and experimental duration (ED). Negative numbers for water table data indicate a water table position below the peat surface, while positive numbers indicate the water table above the peat surface. Increased air temperature and soil temperature at a depth of 0-5 cm, nitrogen forms, and the ratio between N fertilization and local N deposition were also recorded.

### 2.3.2 Document review and analysis

The data were analyzed using the meta-analysis method described by Hedges et al. (1999), which has been widely used in other synthesis studies (Liu and Greaver, 2009; Lu et al., 2013; Zhou et al., 2014). The effect sizes of warming, N addition and vegetation composition were estimated by

$$L = \ln\left(\frac{X_t}{X_c}\right) = \ln X_t - \ln X_c \tag{1}$$

where Xt and Xc are means of the GHG fluxes in treatment and control groups, respectively. Note that Xt and Xc are in different directions in some studies, where effect sizes were calculated by $L=\ln(|(X_t-X_c)/X_c|)$. The natural logarithm of the response rate (L) were used to calculate the weighted mean of the log response rate (L*), which was widely applied in previous studies (Liu and Greaver, 2009; Lu et al., 2013; Zhou et al., 2014). L* above 0 means the treatment has a positive effect, while L* below 0 means the treatment has a negative effect. The effect of treatments was considered significant if 95% confidence interval (CI) of L* did not overlap 0.

With the purpose of assessing the global warming potential (GWP) of each GHG under different climate change drivers, we also calculated the absolute responsiveness (AR) in $CO_2$-equivalents and fractional importance (FI, %) of each gas species as follows (Carter et al., 2012):

$$AR = (X_t - X_c) \times GWP \tag{2}$$

$$\mathrm{FI} = \frac{|AR_i|}{|AR_{CO2}| + |AR_{CH4}| + |AR_{N2O}|} \times 100\% \tag{3}$$

where GWP is 1, 25 and 298 for $CO_2$, $CH_4$ and $N_2O$, respectively (IPCC, 2013) and $AR_i$ is the numerical value of the absolute responsiveness for each GHG.

Meta-regression was used to detect the relationship between GHG emissions and environmental parameters, including location, mean annual temperature (MAT), mean annual precipitation (MAP), water table level (WTL), pH, ED, ΔAT, and increased soil temperature at 0-5 cm depth (ΔST). Owing to few and unbalance of environmental data, the relationships between GHG and environmental parameters were analyzed separately. All statistical analyses were conducted in R. version 3.5.1 (R Core Team, 2018).

## 2.4 Findings

### 2.4.1 The response rates of GHGs to global warming

As shown in Table 2.1, warming significantly increased ecosystem respiration (ER) and gross primary productivity (GPP), but decreased net ecosystem exchange (NEE) in bogs and fens. In addition, warming significantly increased emissions of $CH_4$ and $N_2O$ in bogs and fens.

Table 2.1 The response rates (L*) of greenhouse gases to experimental warming in peatlands.

| GHG flux | Peatland type | n | L*, Response rate | 95% CI, confidence interval |
|---|---|---|---|---|
| $CO_2$ | | | | |
| ER | Bog | 8 | 0.241 | 0.230, 0.250 |
| | Fen | 12 | 0.200 | 0.190, 0.210 |
| NEE | Bog | 1 | -0.112 | - |
| | Fen | 9 | -0.025 | -0.026, -0.024 |
| GPP | Bog | 1 | 0.063 | - |
| | Fen | 9 | 0.026 | 0.025, 0.027 |
| $CH_4$ | | | | |
| | Bog | 8 | 0.130 | 0.120, 0.160 |
| | Fen | 16 | 0.265 | 0.230, 0.300 |
| $N_2O$ | | | | |
| | Bog | 1 | 0.029 | - |
| | Fen | 6 | 0.452 | 0.446,0.456 |

Note: ER, ecosystem respiration; NEE, net ecosystem exchange; GPP, gross primary productivity.

### 2.4.2 The response rates of GHGs to N addition

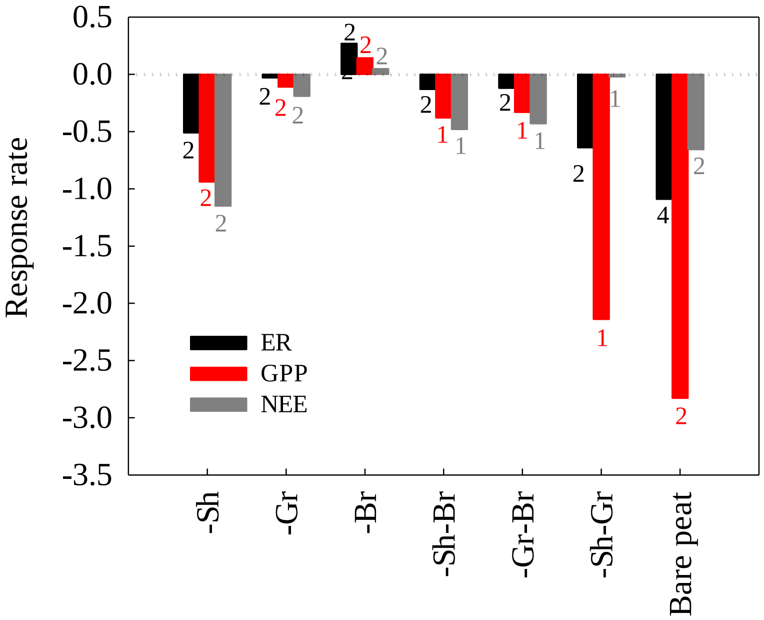
As shown in Table 2.2, N addition slightly increased ER, NEE and GPP both in bogs and fens. Note that the ER, NEE and GPP data in fen were from one study. The effects of N deposition on $CH_4$ emissions varied between peatland types: increased $CH_4$ emissions in bogs and decreased $CH_4$ emissions in fens. Compared with $CO_2$ and $CH_4$, the response rate of $N_2O$ was much higher in fens.

Table 2.2 The response rates (L*) of greenhouse gases to N addition in peatlands.

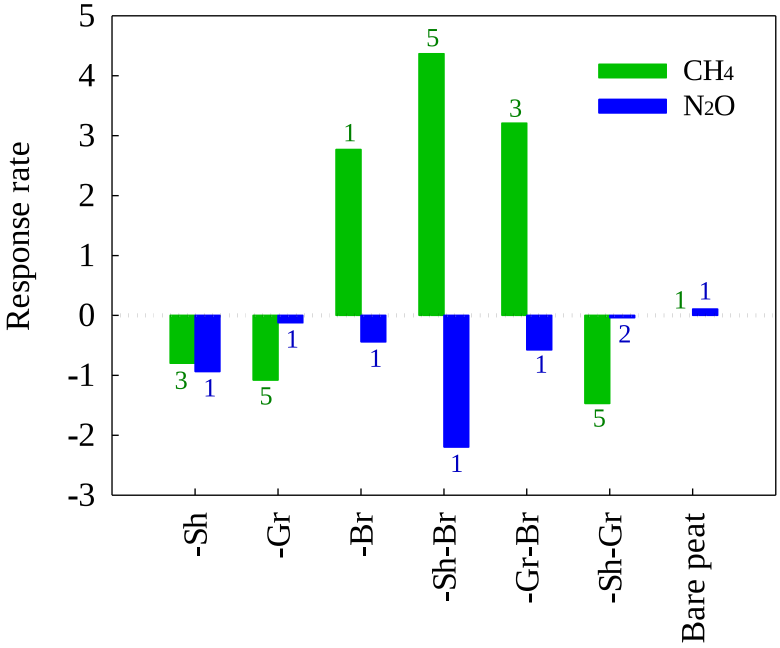
| GHG flux | Peatland type | n | L*, Response rate | 95% CI, confidence interval |
|---|---|---|---|---|
| $CO_2$ | | | | |
| ER | Bog | 39 | 0.028 | 0.027, 0.029 |
| | Fen | 1 | 0.074 | - |
| NEE | Bog | 39 | 0.166 | 0.164, 0.169 |
| | Fen | 1 | 0.097 | - |
| GPP | Bog | 39 | 0.044 | 0.043, 0.046 |
| | Fen | 1 | 0.073 | - |
| $CH_4$ | | | | |
| | Bog | 21 | 0.317 | 0.316, 0.319 |
| | Fen | 2 | -0.073 | -0.075, -0.071 |
| $N_2O$ | | | | |
| | Fen | 2 | 1.462 | 0.804, 2.123 |

### 2.4.3 The response rates of GHGs to vegetation composition change

Vegetation was classified into three typical functional groups: shrubs, graminoids and bryophytes. Absence of vascular plants (shrubs and graminoids) reduced $CO_2$ fluxes (ER, NEE and GPP) in bogs (Figure 2.1a). Removal of vascular plants (shrubs and graminoids) reduced $CH_4$ emissions, while removal of bryophyte increased $CH_4$ emissions in bogs (Figure 2.1b). Likewise, removal of shrubs or graminoids reduced $CH_4$ emission in fens, the response rates of them were -1.19 and -1.93 (data not shown in the figure). Additionally, compared with $CO_2$ and $CH_4$, the impacts of vegetation composition change on $N_2O$ were not obvious in bogs (Figure 2.1b).



(a)



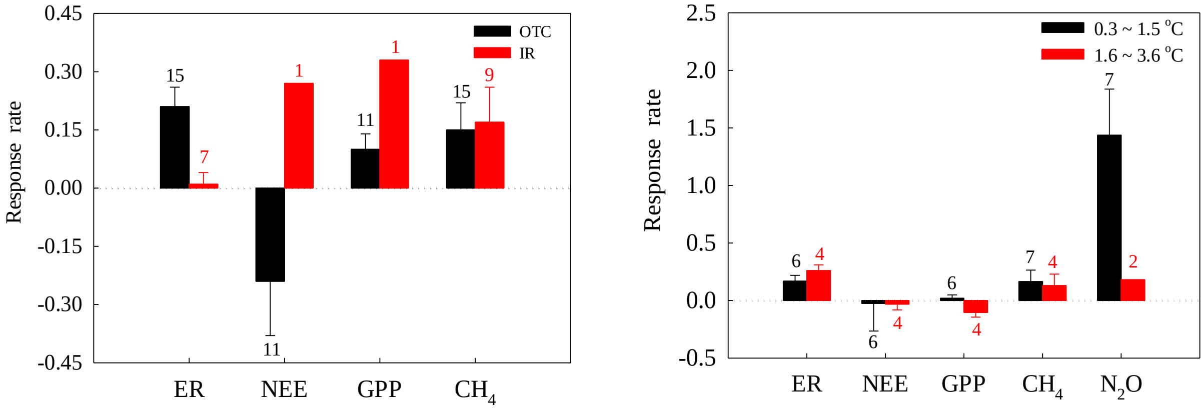
(b)

Figure 2.1 Vegetation composition impacts on the response rates of (a) ER, NEE, GPP, (b) $CH_4$, and $N_2O$ in bogs. The number above the bar represents the number of observations. -Sh represents the removal of shrubs, -Gr represents the removal of graminoids, -Br represents the removal of bryophytes, -Sh-Br represents the removal of shrubs and bryophytes, -Gr-Br represents the removal of graminoids and bryophytes.

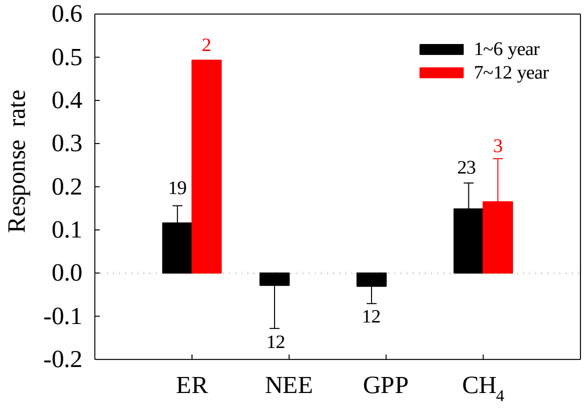
### 2.4.4 Factors influencing the GHG flux responses to experimental warming

Open top chamber (OTC) and infrared lamp (IR) have widely been used to simulate warming climate (Aronson and McNulty, 2009). Both methods had different effects on GHG emissions from peatlands (Figure 2.2a). The response rate of ER under IR treatment was 95% lower than that under OTC treatment, while the response rates of NEE and GPP under IR treatment were about two times higher than that under OTC treatment. Note that the NEE and GPP data under IR treatments were from one study. The $CH_4$ response rates under OTC and IR methods were similar.

ΔAT was increased by OTC and IR from 0.3 $^oC$ to 2.3 $^oC$ for the studies about $CO_2$ emission, from 0.8 $^oC$ to 3.6 $^oC$ for the studies about $CH_4$ emission, and from 0.3 $^oC$ to 2.5 $^oC$ for the studies about $N_2O$ emission. Thus, we divided the temperature increase rates into two categories: 0.3 - 1.5 $^oC$, and 1.6 - 3.6 $^oC$ (Figure 2.2b). The responses of ER slightly increased with ΔAT, while $N_2O$ decreased with ΔAT. There was no significant effect of ΔAT on the response rates of NEE, GPP and $CH_4$. From the perspective of experimental duration, the response rate of ER was increased with warming duration (Figure 2.2c). The impact of warming duration on $CH_4$ response rate was not obvious.



(a) (b)



(c)

Figure 2.2 The impact of different (a) warming methods (OTC, open top chamber; IR, infrared lamp), (b) air temperature increase rates, and (c) experimental duration on ER, NEE, GPP, and $CH_4$ emissions. The number above the bar represents the number of observations. The error bar represents standard error.

As shown in Table 2.3, GHG response rates under experimental warming varied significantly among different locations. There were significant relationships between GHG fluxes and environmental parameters: GPP and ΔAT, GPP and MAP, $CH_4$ and MAP.

Table 2.3 Correlation between GHG response rates and environmental factors under experimental warming.

| | Latitude | Longitude | MAP | MAT | ΔAT | ΔST | WTL | pH |
|---|---|---|---|---|---|---|---|---|
| GPP | 0.067 | -0.069* | 0.553* | -0.324 | 0.564* | 0.161 | 0.370 | -0.196 |
| NEE | -0.046 | 0.027 | 0.392 | 0.041 | 0.476 | 0.138 | 0.449 | -0.900 |
| ER | 0.408 | -0.094 | 0.104 | -0.316 | -0.043 | -0.284 | -0.271 | -0.179 |
| $CH_4$ | -0.425* | -0.695* | 0.609* | 0.141 | -0.285 | 0.018 | -0.139 | -0.295 |
| $N_2O$ | -0.589 | 0.795* | -0.641 | -0.682 | 0.624 | 0.619 | -0.975 | 0.170 |

Note: MAT represents mean annual temperature, MAP represents mean annual precipitation, ΔAT represents increased air temperature and ΔST represents increased soil temperature at 0-5 cm depth. WTL represents water table level. An asterisk (*) represents $P<0.05$.

### 2.4.5 Factors influencing the GHG flux responses to N addition

We calculated the ratio between the concentration of N fertilization and ambient N deposition, and then divided them into three categories: 1-5, 7-12, and 15-20. As shown in Figure 2.3, different rates and forms of N addition had different effects on GHG emissions from peatlands. For low ratios of N addition (1-5), the response rates of ER and GPP were higher under NPK addition than that under $NH_4NO_3$ addition. In addition, there were no considerable effects on $CH_4$ emission from peatlands under low N concentration (1-5). Nevertheless, there was a significant increase in the response rate of $CH_4$ under high ratios of NPK addition (15-20).

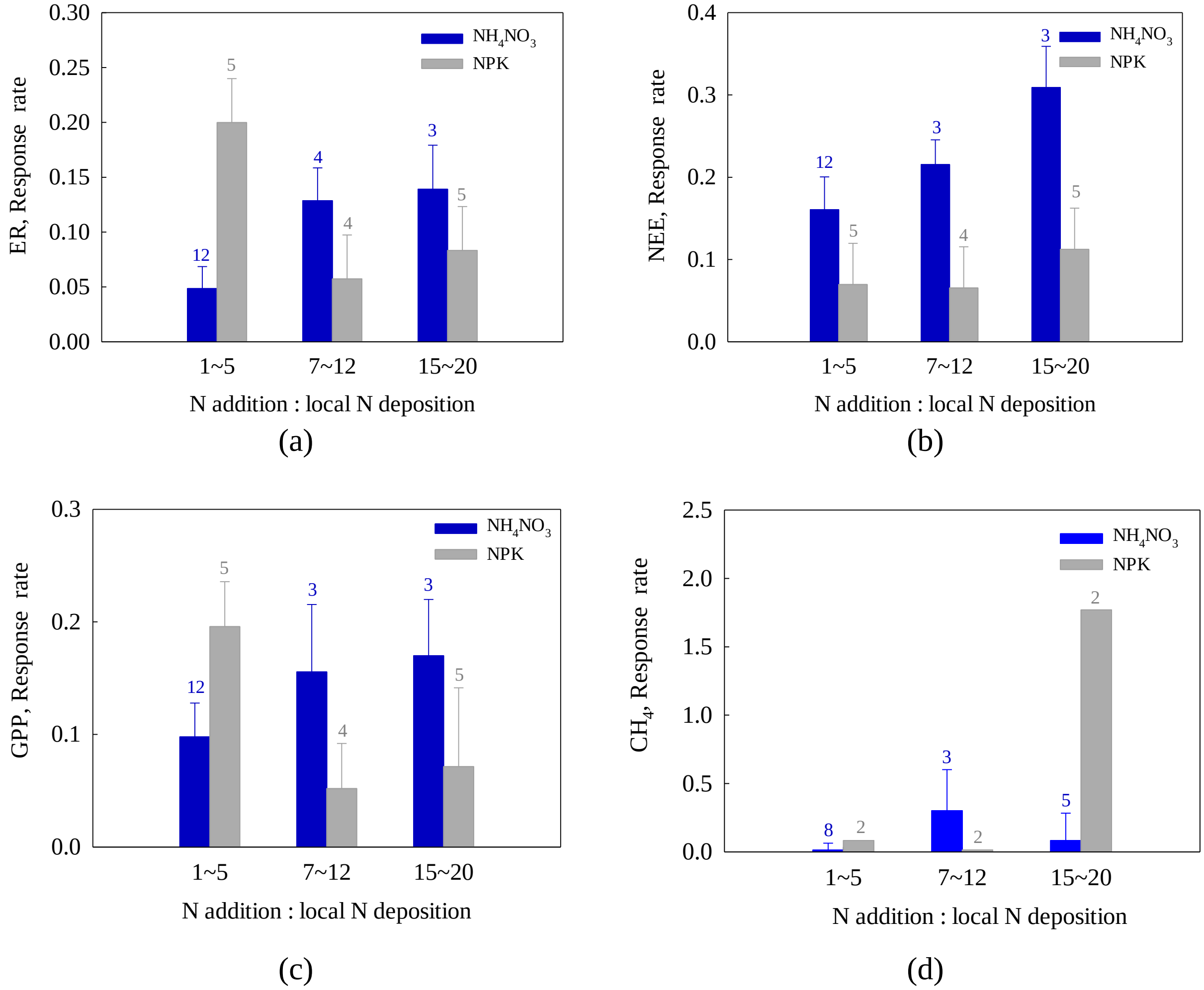
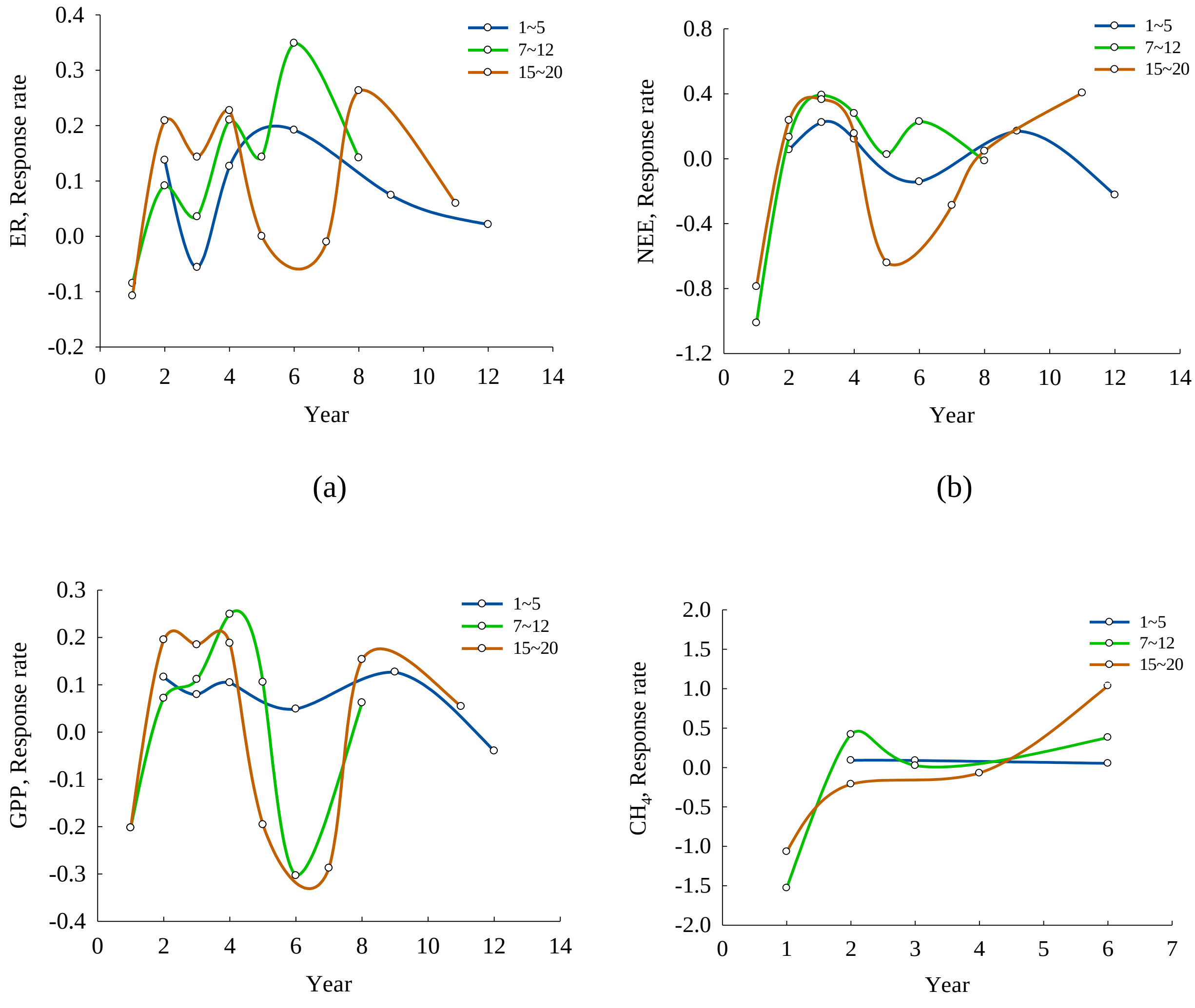


Figure 2.3 Impacts of different forms and ratios of N addition on (a) ER, (b) NEE, (c) GPP, and (d) $CH_4$ emissions. The number above the bar represents the number of observations. The error bar represents standard error.

Experimental durations of N addition also played an essential role in GHG emissions from peatlands. As shown in Figure 2.4, response rates of GHG fluxes under different ratios of N addition varied between experimental durations. The trends of $CO_2$ response rates (ER, NEE and GPP) were similar for different N addition ratios when the experimental durations were shorter than 5 years. The third peak of ER response rate was delayed with the N addition ratio increase, while the second peak of NEE was ahead except under high N addition ratio (15-20). There was a significant fluctuation of the GPP response rate under relative high N addition ratio (7-12 and 15-20), especially in the sixth year. Furthermore, there was no significant difference in the $CH_4$ response rates between three ratios of N addition when the experimental duration was lower than 4 years. After the fifth year of N addition, the response rate of $CH_4$ increased with N addition ratio.



(a) (b)

(c) (d)

Figure 2.4 The relationship between the response rates of GHGs and experimental duration under different ratios of N addition (1-5, 7-12, and 15-20).

As shown in Table 2.4, the response rate of ER significantly varied among different locations. GPP and NEE were not correlated with environmental parameters. However, there was a significant relationship between the $CH_4$ response rate and WTL.

Table 2.4 Correlation between GHG response rates and environmental factors under N fertilization.

| | Latitude | Longitude | MAP | LND | MAT | WTL |
|---|---|---|---|---|---|---|
| GPP | 0.173 | -0.017 | 0.215 | -0.394 | 0.004 | -0.304 |
| NEE | -0.101 | -0.127 | 0.246 | -0.363 | 0.068 | -0.241 |
| ER | 0.439* | 0.339 | -0.254 | 0.357 | 0.045 | -0.463 |
| $CH_4$ | 0.287 | 0.345 | -0.359 | 0.122 | 0.617 | 0.430* |

Note: MAT represents mean annual temperature, MAP represents mean annual precipitation, LND represents local nitrogen deposition, and WTL represents water table level. An asterisk (*) represents $P<0.05$.

### 2.4.6 Global warming potential (GWP)

As shown in Table 2.5, total AR of GHGs was higher under warming than that under N addition. The highest AR of $CO_2$ was found under bare peat. The highest AR of $CH_4$ was found under the absence of graminoids and shrubs. The highest AR of $N_2O$ was found under N addition. When comparing the fractional importance (FI) of each GHG to the total global warming potential, the changes in $CO_2$ emissions dominated the response under warming (99%) and under different vegetation compositions (~99%), while the changes in $N_2O$ emissions dominated the response under N addition (83%). Furthermore, the absence of bryophytes reduced the total GWP.

Table 2.5 Mean absolute responsiveness of each GHG under global changes.

| Global changes | $CO_2$ | $CH_4$ | $N_2O$ | Total |
|---|---|---|---|---|
| Warming | 1748 | -10 | 7 | 1745 |
| N addition | -212 | 3 | 1018 | 809 |
| absence of bryophytes | -308 | 44 | -36 | -299 |
| absence of graminoids | 678 | -183 | -7 | 488 |
| absence of shrubs | 2864 | 113 | -59 | 2918 |
| absence of bryophytes and graminoids | 2890 | -172 | -26 | 2692 |
| absence of bryophytes and shrubs | 2041 | -558 | -41 | 1442 |
| absence of graminoids and shrubs | 4248 | 174 | -2 | 4420 |
| Absence of all vegetation | 4397 | -340 | 3 | 4059 |

## 2.5 Discussion

### 2.5.1 Factors influencing the responses of GHG fluxes to experimental warming

This synthesis showed that peatland types did not impact GHG responses to warming in boreal climate zone. GHGs emissions are regulated by microorganisms and vegetation (Butterbach-Bahl et al., 2013; Minke et al., 2016). Although the microorganisms and vegetation vary among bogs and fens, the tendency of their responses to warming is similar but with different levels owing to their varied temperature sensitivity (Helbig et al., 2019). The narrow temperature increase caused by manipulated warming cannot capture this difference, thereby we only observed the similar trend of GHG responses to warming for boreal bogs and fens. Nevertheless, this conclusion should be extrapolated with caution for NEE and GPP because the data in boreal bog is from one study. To make this conclusion, more research is needed in bogs. In contrast to peatland types, we found that responses of GHGs to warming varied between warming methods, between temperature increase rates, and also between warming durations.

The response rate of ER was higher under OTC treatment than that under IR treatment in this review. This can be attributed to soil drying. Johnson et al. (2013) have reported IR treatment can cause soil drying, which reduces microbial respiration. The other possible explanation is the drying effect of constantly IR heating on vegetation (Johnson et al., 2013), which promotes root growing deeply and reduces the root exudates for microbial respiration in the upper layer. Furthermore, IR treatment can affect the pore water chemistry in boreal bog and fen (White et al., 2008), which has the potential to reduce the microbial activity because they need those chemical substances as electron donor and acceptor (Nielsen et al., 2017). Compared with OTC, IR has a relative high response rate of GPP, possibly because IR is an effective way to increase temperature (Johnson et al., 2013). The efficiency of air temperature increase via OTC depends on solar energy, while IR could increase air temperature constantly. Due to the relative higher response rate of ER than the response rate of GPP under OTC, the OTC has a negative effect on NEE. However, it should be noted that the data about NEE and GPP response rates under IR is from one study, and further research is needed. In addition, we also found that the response rate of ER was much higher during 7-12 years of warming than that during 1-6 years of warming. This is possible because of a considerable change in vegetation composition in the seventh year of warming. The research of Hollister et al. (2005) supports this point. They found that seven years of warming increased cover of graminoids and decreased cover of bryophytes in the dry heath and wet meadow. This change has also been observed in a temperate bog (Kivimäki et al., 2013). This latter study also reported that this pattern of vegetation composition changes increased ER in a temperate bog (Kivimäki et al., 2013). Contrary to ER, we did not find warming durations or warming methods impacted $CH_4$

response rates, but the reasons for the positive response of $CH_4$ to warming in a short-term experiment (1-6 years) and a long-term experiment (7-12 years) are different. The short term of warming (1-6 years) directly increases $CH_4$ emission via increasing microbial activity, while the long term of warming (7-12 years) indirectly increases $CH_4$ emission by changing vegetation composition. With the increase of graminoid cover, more $CH_4$ is emitted through aerenchyma tissue (Nielsen et al., 2017). In addition, we found high temperature increase (1.6-3.6) reduced $N_2O$ response rate. This might be owing to soil drying, which decreases microbial activity and substrates for $N_2O$ production. For instance, soil drying can reduce nitrifier activity or constrain the process of mineralization, which has the potential to decrease the substrate and nutrient availability for nitrification and denitrification, two important biochemical processes for $N_2O$ production (Butterbach-Bahl et al., 2013).

Besides forcing factors (e.g., warming method, warming magnitude, and warming duration), environmental parameters (e.g., latitude, MAT, and MAP) were observed to impact the responses of GHGs to warming in this study. Plant growth needs optimal environmental conditions, such as optimal temperature and optimal soil moisture (Dusenge et al., 2018). Therefore, we found significant relationships between GPP and $\Delta$AT, and between GPP and MAP. Many studies have reported inconsistent responses of GHGs to warming due to spatial and temporal variation, but it remains unclear what is the main factor. Our review and data synthesis partly fill this gap and indicates that MAP is probably the main factor for $CH_4$ fluxes under warming conditions. This can be supported by the previous studies. Shoemaker et al. (2012) demonstrated that rainfall events were related to the degassing of stored $CH_4$ in a boreal fen. Radu and Duval (2018) reported that changing rainfall regimes increased $CH_4$ emissions owing to the water table change in a boreal fen. Brown et al. (2014) also reported that reducing rainfall decreased $CH_4$ emissions due to an increase in water table depth in a boreal bog, which promotes oxygen entering into peat and enhances $CH_4$ oxidation.

### 2.5.2 Factors influencing the responses of GHG fluxes to N addition

Our result showed that N addition increased GPP both in bogs and fens. Considering bogs and fens are nutrient-limited ecosystems, N addition provides much available N for vegetation growth (Rydin and Jeglum, 2013). In addition, we found that the effect of N addition on $CH_4$ was different between bogs and fens. Previous studies have reported that N addition increases vegetation growth, thus providing more root exudates for methanogens and enhancing $CH_4$ transport through aerenchyma (Juutinen et al., 2018). The negative effect of N addition on $CH_4$ in fens in this review is surprising. We rechecked data and found that this negative effect was from one research. They attributed the negative effect to the decrease of deeper root system under N addition, which reduces the substrates for $CH_4$ production at a certain depth (Granberg et al., 2001). This can be supported by previous studies, which has reported that plant root depth can be markedly decreased under nutrient (N and/or P) addition in temperate bogs and fens (Kohzu et al., 2003).

Besides the effects of peatland types, we found that ER and GPP decreased when the concentration of N addition was above 7 times higher than local N deposition. This could be attributed to the acidification, root biomass decrease, and microbial biomass decrease under high N deposition (Chen et al., 2016). In addition, our result showed that ER and GPP increased under NPK addition was more than that under low N addition alone. Bragazza et al. (2004) have reported that coupled with nitrogen deposition, boreal and temperate peatlands (bogs and fens) have the potential to switch from being N-limited to potassium (K) and/or phosphorus (P) co-limited. Accordingly, ER and GPP greatly increased under NPK addition. In addition, phosphorus (P) addition alleviates the negative impact N has on *Sphagnum* by enhancing its capability to assimilate the deposited N in northern fens and bogs (Limpens et al., 2004). Furthermore, temperate and boreal bogs are characterized by N and P co-limitation (Kivimäki et al., 2013; Larmola et al., 2013), thus only N addition cannot considerably increase GPP and ER. Nevertheless, we found that this positive effect of NPK addition was negligible under high N concentration. The possible reason is that the negative effect of high N concentration overrides the positive effect of PK addition.

Theoretically, nitrate directly decreases $CH_4$ production by competing substrates with methanogens, while ammonium increases $CH_4$ emissions by reducing the activity of methanotrophs (Nykänen et al., 2002). Nitrogen addition also indirectly increases $CH_4$ emission by stimulating vascular plant growth, which provides liable substrate for methanogens, and aerenchymatous tissue for $CH_4$ transport (Gray et al., 2013). In this review, N addition slightly increased $CH_4$ emission from peatlands possibly due to the combination of these biogeochemical processes. Moreover, we found that $CH_4$ emission was considerably increased at high rates of NPK addition. This can be attributed to the fact that NPK addition increases vascular plant cover and decreases *Sphagnum* moss abundance, thus altering the quantity and quality of substrate for $CH_4$ production (Juutinen et al., 2018).

From the perspective of experimental duration, we observed similar trends for the response rates of ER, NEE and GPP under different N concentrations. Bubier et al. (2007) have demonstrated that N addition increases vascular plant leaf biomass and reduces moss cover in a boreal bog, thus expecting to increase gross photosynthesis. However, this review showed a reduction in response rate of GPP at the sixth year of N addition, which indicates that the increase in vascular leaf mass is not enough to compensate for the loss of moss photosynthesis. This is in line with previous studies that reported the same results in a boreal bog (Bubier et al., 2007; Juutinen et al., 2010). In addition, because *Sphagnum* moss can filter N deposition and reduces the negative effect of N addition (Chiwa et al., 2016), we observed a weak decrease in the response rate of ER at the third year of N addition. Likewise, the pronounced decrease of the response rate of ER after the sixth year can be attributed to the disappearance of *Sphagnum* moss and soil acidification caused by N addition in a temperate fen (Aerts and De Caluwe, 1999). In contrast to $CO_2$ fluxes, no considerable effect of N addition on $CH_4$ emission was observed during 1-5 years of N addition in this study partly because of *Sphagnum* moss filtration and plant absorption (Song et al., 2007). After 5 years of N addition, we found that the response rate of $CH_4$ was found to increase with the rate of N addition. This result suggests that vascular plants become dominant in peatlands after 5 years of N addition, and high ratios of N addition can stimulate $CH_4$ emission by changing litter quality and root exudates (Juutinen et al., 2018).

From the perspective of environmental parameters (e.g., latitude, MAT, and MAP), ER significantly varied among locations. Therefore, much more studies in various locations are needed to comprehensively understand how N deposition would affect GHG emissions from peatlands. The significant relationship between $CH_4$ and WTL in this review indicates that WTL plays an essential role in $CH_4$ emission under N addition. This is in line with previous studies, which reported that $CH_4$ emission increased with WTL (Minke et al., 2016; Shao et al., 2017; Wang et al., 2017).

### 2.5.3 Factors influencing the responses of GHG fluxes to vegetation composition change

In the present study, there is no difference between peatland types on the response of GHGs to vegetation composition change. We found that absence of vascular plants (shrubs and graminoids) reduced $CH_4$ emissions both in bogs and fens. This reduction can be ascribed to the decrease of root excretes for methanogenesis and reduction of $CH_4$ transport via aerenchyma (Nielsen et al., 2017).

Previous studies have demonstrated that there are interactions between warming, N deposition and vegetation composition change on GHG emissions. Ward et al. (2013) reported that warming effect on NEE was reduced by removing graminoids due to the reduction of photosynthesis in a temperate bog. For $CH_4$, Nielsen et al. (2017) reported that combination of warming and shrub removal tends to increase $CH_4$ emission in a boreal fen. This is attributed to reduced competition between sedges and shrubs for nutrients, which stimulates sedges growth and provides more substrates for $CH_4$ production. For $N_2O$, no significant interactive effect of warming and vegetation removal was observed possibly due to nutrient limitation in boreal and temperate bogs (Gong et al., 2018; Ward et al., 2013). Given global warming, N deposition and vegetation composition change occur simultaneously and affect ecosystem functions interactively, further research should focus on the combined effects of them on GHG emissions from peatlands.

### 2.5.4 Global warming potential (GWP)

Our data synthesis revealed that the absence of bryophytes reduced global warming potential (GWP). Given peatlands might shift from bryophyte-dominated to vascular plant-dominated ecosystems (Juutinen et al., 2018), this result implies that the cooling function of peatland ecosystems will be strengthened. Nevertheless, we also found that warming and N addition considerably increased global warming potential, and this increase overrode the positive effect of bryophyte absence on GWP. Our results indicate that GWP presumably increased in peatlands and cooling function of peatlands will be weakened in the future.

### 2.5.5 Uncertainty

Although the meta-analysis provides a statistical approach to calculate the weighted response rates across the studies, the overall response rates of GHGs may be synthesized with large uncertainties due to the fact that few studies focus on GHG fluxes in peatlands under warming, N deposition and vegetation composition change. In particular, studies conducted over a long-term period (~10 years) and studies focused on NEE and GPP in bogs under climate warming are limited in our database. Therefore, to reduce the uncertainty of predicting how GHG emissions from peatlands respond to global changes (warming, N deposition and vegetation composition change), more studies are urgently needed.

## 2.6 Conclusion

We analyzed the responses of GHG fluxes in peatland ecosystems to manipulated warming, N deposition and vegetation composition change. The results showed that warming methods and warming duration impact the response of GHGs to warming. We also found that different peatland types, N concentrations, N forms and experimental durations remarkably impacted the response of GHGs to N addition. In addition, there were significant relationships between GHGs and local environmental parameters (MAP and WTL). These results suggest that caution is needed when extrapolating the local findings broadly and globally, and more research at different locations is needed to accurately predict GHG emissions from peatlands. Furthermore, the GWP of peatlands was found to increase in future scenarios, which indicates that effective actions are needed to reduce GHG emissions from peatlands.

## 2.7 Acknowledgements

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### 2.9 Supplementary files

Table S2.1 Impacts of warming on $CO_2$ emission from peatlands.

Table S2.2 Impacts of nitrogen deposition on $CO_2$ emission in peatlands.

Table S2.3 Impacts of vegetation composition on $CO_2$ emission in peatlands.

Table S2.4 Impacts of warming on $CH_4$ emission in peatlands.

Table S2.5 Impact of nitrogen deposition on $CH_4$ emission in peatlands.

Table S2.6 Impacts of vegetation composition on $CH_4$ emission in peatlands.

Table S2.7 Impacts of warming on $N_2O$ emission in peatlands.

Table S2.8 Impact of nitrogen deposition on $N_2O$ emission in peatlands.

Table S2.9 Impacts of vegetation composition on $N_2O$ emission in peatlands.

Table S2.1 Impacts of warming on CO$_2$ emission in peatlands.

| **Site Location** | **Years of study** | **Ecosystem classification** | **latitude and longitude** | **Above Sea Level** | **precipitation** | **Mean annual T** | **Increase Air T** | **increase soil T 5cm** | **warming equipment** | **ER** | **ER_W** | **NEE** | **NEE_W** | **GPP** | **GPP_W** | **Reference** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Northern Poland | 2 | Fen | 53° 11′ 15″ N, 18° 18′ 34″ E | 91 | 550 | 8.05 | 0.3 | -0.3 | OTC | 2.66 | 3.04 | ND | ND | ND | ND | (Samson et al., 2018) |
| the Moor House National Nature Reserve, northern England | 4 | Bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | 0.9 | ND | OTC | 5.28 | 3.84 | ND | ND | ND | ND | (Walker et al., 2016) |
| Orivesi, Finland | 3 | Fen | 61°47´N, 24°18´E | ND | 700 | 3.5 | 2.1 | 0.3 | OTC | 44.00 | 35.20 | -30.80 | -31.68 | ND | ND | (Pearson et al., 2015) |
| Virrat, Finland | 3 | Fen | 62°13´N, 23°23´E | ND | 700 | 3.5 | 1.6 | 1.3 | OTC | 61.60 | 52.80 | -27.28 | -25.52 | ND | ND | |
| Kittilä, Finland | 3 | Fen | 67°59´N, 24°12´E | ND | 511 | -1.4 | 0.9 | 0.5 | OTC | 19.36 | 18.48 | -26.40 | -25.96 | ND | ND | |
| the Moor House National Nature Reserve, northern England | 2 | Bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | 0.9 | ND | OTC | 28.16 | 34.32 | -83.60 | -74.80 | ND | ND | (Ward et al., 2013) |
| Michigan, USA | 3 | Fen | 46.85°N 88.37°W | 183 | 833 | 4.5 | ND | 0.5 | OTC | 11.40 | 17.11 | -14.45 | -11.40 | -24.71 | -27.37 | (Johnson et al., 2013) |
| Michigan, USA | 3 | Fen | 46.85°N 88.37°W | 183 | 833 | 4.5 | ND | 0.8 | infrared heaters | 11.40 | 15.21 | -14.45 | -19.01 | -24.71 | -34.21 | |
| Fairbanks, Alaska, USA | 2 | Fen | 64.82°N, 147.87°W | ND | 269 | -2.9 | 0.7 | 0.6 | OTC | 13.15 | 15.17 | ND | ND | -19.31 | -16.38 | (Chivers et al., 2009) |
| Abisko, north Sweden | 4 | Bog | 68°21'N, 18°49'E | ND | 323 | -0.5 | 1 | 1 | OTC | 4.56 | 8.16 | ND | ND | ND | ND | (Dorrepaal et al., 2009) |
| Abisko, north Sweden | 6 | Bog | 68°21'N, 18°49'E | ND | 323 | -0.5 | 1 | 1 | OTC | 12.96 | 14.40 | ND | ND | ND | ND | |
| Abisko, north Sweden | 7 | Bog | 68°21'N, 18°49'E | ND | 323 | -0.5 | 1 | 1 | OTC | 8.16 | 10.80 | ND | ND | ND | ND | |
| Abisko, north Sweden | 8 | Bog | 68°21'N, 18°49'E | ND | 323 | -0.5 | 1 | 1 | OTC | 7.68 | 12.00 | ND | ND | ND | ND | |
| Pituffik (Thule), Greenland | 1 | Fen | 76°33'N, 68°30'W | 150 | 1220 | -11.6 | 2.2 | ND | OTC | 23.95 | 29.65 | -3.04 | -4.56 | -26.61 | -33.07 | (Sullivan et al., 2007) |
| Pituffik (Thule), Greenland | 2 | Fen | 76°33'N, 68°30'W | 150 | 1220 | -11.6 | 2.3 | ND | OTC | 18.63 | 30.41 | -5.70 | -3.42 | -23.57 | -33.07 | |
| Pituffik (Thule), Greenland | 3 | Fen | 76°33'N, 68°30'W | 150 | 1220 | -11.6 | 1.5 | 0.8 | OTC | 12.17 | 19.01 | -7.98 | -8.36 | -22.05 | -29.65 | |
| Duluth,Minnesota, USA | 2 | Fen | 47°N, 92°W | ND | ND | ND | ND | 3 | infrared heaters | 13.20 | 13.38 | ND | ND | ND | ND | (Updegraff et al., 2001) |
| Duluth,Minnesota, USA | 2 | Bog | 47°N, 92°W | ND | ND | ND | ND | 3 | infrared heaters | 12.98 | 13.07 | ND | ND | ND | ND | |
| Duluth,Minnesota, USA | 3 | Fen | 47°N, 92°W | ND | ND | ND | ND | 2.2 | infrared heaters | 17.16 | 17.29 | ND | ND | ND | ND | |
| Duluth,Minnesota, USA | 3 | Bog | 47°N, 92°W | ND | ND | ND | ND | 2.2 | infrared heaters | 17.03 | 17.20 | ND | ND | ND | ND | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Duluth,Minnesota, USA | 4 | Fen | 47°N, 92°W | ND | ND | ND | ND | 1.6 | infrared heaters | 8.80 | 8.93 | ND | ND | ND | ND |
| Duluth,Minnesota, USA | 4 | Bog | 47°N, 92°W | ND | ND | ND | ND | 1.6 | infrared heaters | 9.02 | 9.11 | ND | ND | ND | ND |

Note: the units of precipitation, mean annual Air T and CO$_2$ fluxes were mm year-1, ºC, and g m$^{-2}$ day$^{-1}$, respectively.

## Table S2.2 Impacts of nitrogen deposition on $CO_2$ emission in peatlands.

| Site Location | Years of study | Ecosystem classification | latitude and longitude | precipitation | Mean annual Air | Local N deposition | N concentration | form of N | ER | ER_N | NEE | NEE_N | GPP | GPP_N | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Northern Finland | 67 | fen | 65° N; 24° E | ND | ND | ND | P: 40-350; K: 66-420 | PK | 2.47 | 3.29 | -0.33 | -0.54 | -2.74 | -3.83 | (Ojanen et al., 2019) |
| Southern Finland | 67 | bog | 62°10′ N; 22° 48 E | ND | ND | ND | N:91-201; P: 96-556; K: 60-435 | NPK (Ca-$NH_3$-$NO_2$ and PK) | 3.42 | 3.83 | 1.51 | 1.09 | -1.91 | -2.74 | |
| Västerbotten, Sweden | 20 | Mire | 64° 11′ N, 19° 33′ E | 270 | 523 | 2 | 30 | NH4NO3 | ND | ND | ND | ND | -7.6 | -5.71 | (Peichl et al., 2018) |
| Edinburgh, Scotland | 6 | bog | 3◦16 W, 55◦46 N | 900 | -7.4~25.4 | 8 | 56 | NaNO3 | 2.18 | 2.86 | 1.56 | 2.28 | -1.29 | -0.57 | (Kivimäki et al., 2013) |
| Edinburgh, Scotland | 6 | bog | 3◦16 W, 55◦46 N | 900 | -7.4~25.4 | 8 | 56 | NH4Cl | 2.18 | 2.74 | 1.56 | 2.16 | -1.17 | -0.57 | |
| Edinburgh, Scotland | 6 | bog | 3◦16 W, 55◦46 N | 900 | -7.4~25.4 | 8 | 56 | NO3+PK(P:N,1:14) | 2.18 | 3.36 | 1.56 | 2.112 | -1.8 | -1.24 | |
| Edinburgh, Scotland | 6 | bog | 3◦16 W, 55◦46 N | 900 | -7.4~25.4 | 8 | 56 | NH4+PK(P:N,1:14) | 2.184 | 3.432 | 1.56 | 1.296 | -1.87 | -2.13 | |
| Ottawa, Ontario, Canada | 12 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 16 (5 times) | NH4NO3 | 18.62 | 18.47 | 9.50 | 7.60 | 34.21 | 31.93 | (Larmola et al., 2013) |
| Ottawa, Ontario, Canada | 12 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 5 times | NPK | 18.62 | 19.57 | 9.50 | 7.60 | 34.21 | 33.83 | |
| Ottawa, Ontario, Canada | 11 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 20 times | NPK | 18.63 | 19.76 | 9.50 | 0.68 | 34.21 | 36.11 | |
| Ottawa, Ontario, Canada | 7 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 64 (20 times) | NH4NO3 | 18.25 | 18.05 | 9.12 | 6.84 | 30.41 | 22.80 | |
| Ottawa, Ontario, Canada | 9 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 16 (5 times) | NH4NO3 | 20.45 | 21.66 | -23.56 | -29.65 | -44.02 | -51.32 | (Juutinen et al., 2010) |
| Ottawa, Ontario, Canada | 9 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 50, 63 (5 times PK) | KH2PO4 | 20.45 | 22.01 | -23.56 | -22.81 | -44.02 | -44.82 | |
| Ottawa, Ontario, Canada | 9 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 5 times | NPK | 20.45 | 22.39 | -23.56 | -26.23 | -44.02 | -48.62 | |
| Ottawa, Ontario, Canada | 8 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 10 times | NPK | 20.45 | 23.56 | -23.56 | -23.26 | -44.02 | -46.83 | |
| Ottawa, Ontario, Canada | 8 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 20 times | NPK | 20.45 | 26.61 | -23.56 | -24.71 | -44.02 | -51.32 | |
| Ottawa, Ontario, Canada | 4 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 32 (10 times) | NH4NO3 | 17.86 | 22.04 | -22.42 | -29.65 | -40.30 | -51.70 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ottawa, Ontario, Canada | 4 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 64 (20 times) | NH4NO3 | 17.86 | 22.42 | -22.42 | -26.19 | -40.30 | -48.62 | |
| Fäjemyr, Swedish | 2 | bog | 56◦15'N, 13◦33'E | 700 | 6.2 | 15 | 40 | NH4NO3 | 14.49 | 16.27 | -6.60 | -9.00 | -21.19 | -24.84 | (Lund et al., 2009) |
| Storflaket, Swedish | 2 | bog | 68◦20'N, 18◦58'E | 304 | -0.8 | 2 | 20 | NH4NO3 | 12.288 | 13.46 | -12.86 | -14.68 | -25.87 | -27.79 | |
| Storflaket, Swedish | 2 | bog | 68◦20'N, 18◦58'E | 304 | -0.8 | 2 | 40 | NH4NO3 | 12.288 | 15.14 | -12.86 | -16.29 | -25.87 | -31.44 | |
| Dolomites, Italy | 2 | bog | 46°21′N, 11°44′E | 461 | 14.1 | 8 | 10 | NH4NO3 | 9.6 | 8.88 | -4.32 | -4.56 | -13.92 | -13.44 | (Gerdol et al., 2008) |
| Dolomites, Italy | 2 | bog | 46°21′N, 11°44′E | 461 | 14.1 | 8 | 30 | NH4NO3 | 9.6 | 9.12 | -4.32 | -2.64 | -13.92 | -11.76 | |
| Dolomites, Italy | 3 | bog | 46°21′N, 11°44′E | 531 | 12.1 | 8 | 10 | NH4NO3 | 5.28 | 4.8 | -4.32 | -4.8 | -9.6 | -9.6 | |
| Dolomites, Italy | 3 | bog | 46°21′N, 11°44′E | 531 | 12.1 | 8 | 30 | NH4NO3 | 5.28 | 5.184 | -4.32 | -6 | -9.6 | -11.184 | |
| Dolomites, Italy | 4 | bog | 46°21′N, 11°44′E | 284 | 11.5 | 8 | 10 | NH4NO3 | 6 | 6.192 | -1.44 | -1.296 | -7.44 | -7.488 | |
| Dolomites, Italy | 4 | bog | 46°21′N, 11°44′E | 284 | 11.5 | 8 | 30 | NH4NO3 | 6 | 7.44 | -1.44 | -1.344 | -7.44 | -8.784 | |
| Ottawa, Ontario, Canada | 2 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 16 (5 times) | NH4NO3 | 18.63 | 20.91 | -4.18 | -4.56 | -22.81 | -25.85 | (Basiliko et al., 2006; Bubier et al., 2007) |
| Ottawa, Ontario, Canada | 2 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 50, 63 (5 times PK) | KH2PO4 | 18.63 | 23.76 | -4.18 | -9.88 | -22.81 | -33.83 | |
| Ottawa, Ontario, Canada | 2 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 5 times | NPK | 18.63 | 26.23 | -4.18 | -4.18 | -22.81 | -30.41 | |
| Ottawa, Ontario, Canada | 1 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 10 times | NPK | 18.63 | 17.11 | -4.18 | -1.52 | -22.81 | -18.63 | |
| Ottawa, Ontario, Canada | 1 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 20 times | NPK | 18.63 | 16.73 | -4.18 | -1.90 | -22.81 | -18.63 | |
| Ottawa, Ontario, Canada | 4 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 16 (5 times) | NH4NO3 | 19.77 | 20.15 | -9.50 | -11.78 | -30.03 | -32.31 | |
| Ottawa, Ontario, Canada | 4 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 50, 63 (5 times PK) | KH2PO4 | 19.77 | 22.05 | -9.50 | -11.98 | -30.03 | -34.21 | |
| Ottawa, Ontario, Canada | 4 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 5 times | NPK | 19.77 | 24.71 | -9.50 | -10.26 | -30.03 | -34.59 | |
| Ottawa, Ontario, Canada | 3 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 10 times | NPK | 19.77 | 19.77 | -9.50 | -15.59 | -30.03 | -34.59 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ottawa, Ontario, Canada | 3 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 20 times | NPK | 19.77 | 22.81 | -9.50 | -13.69 | -30.03 | -36.12 | |
| Ottawa, Ontario, Canada | 6 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 16 (5 times) | NH4NO3 | 19.77 | 22.05 | -14.45 | -12.93 | -34.21 | -33.83 | |
| Ottawa, Ontario, Canada | 6 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 50, 63 (5 times PK) | KH2PO4 | 19.77 | 20.15 | -14.45 | -10.26 | -34.21 | -30.41 | |
| Ottawa, Ontario, Canada | 6 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 5 times | NPK | 19.77 | 25.85 | -14.45 | -12.17 | -34.21 | -38.02 | |
| Ottawa, Ontario, Canada | 5 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 10 times | NPK | 19.77 | 22.81 | -14.45 | -14.83 | -34.21 | -38.02 | |
| Ottawa, Ontario, Canada | 5 | bog | 45.40°N, 75.50°W | 944 | 6 | 2 to 12 | 20 times | NPK | 19.77 | 19.77 | -14.45 | -7.60 | -34.21 | -28.13 | |
| Michigan, U.S. | 6 | bog | 46° N, 89° W | ND | ND | 4 | 60 | N: CO(NH2)2 | ND | ND | ND | ND | -0.26[a] | -0.39[a] | (Keller et al., 2005) |
| Michigan, U.S. | 6 | bog | 46° N, 89° W | ND | ND | ND | 20 | P: Ca(H2PO4)2 | ND | ND | ND | ND | -0.26[a] | -0.31[a] | |
| Michigan, U.S. | 6 | bog | 46° N, 89° W | ND | ND | 4 | N:60; P: 20 | (CO(NH2)2 and Ca(H2PO4)2 | ND | ND | ND | ND | -0.26[a] | -0.77[a] | |
| Michigan, U.S. | 6 | fen | 46° N, 89° W | ND | ND | 4 | 60 | N: CO(NH2)2 | ND | ND | ND | ND | -1.79[a] | -1.52[a] | |
| Michigan, U.S. | 6 | fen | 46° N, 89° W | ND | ND | ND | 20 | P: Ca(H2PO4)2 | ND | ND | ND | ND | -1.79[a] | -1.53[a] | |
| Michigan, U.S. | 6 | bog | 46° N, 89° W | ND | ND | 4 | N:60; P: 20 | (CO(NH2)2 and Ca(H2PO4)2 | ND | ND | ND | ND | -1.79[a] | -0.77[a] | |
| Salmisuo, Finland | 3 | fen | 62°47'N, 30°56'E | ND | ND | 2 to 3 | 30 | NH4NO3 | 19.10 | 20.50 | -4.00 | -4.40 | -23.10 | -24.80 | (Saarnio et al., 2003) |

Note: the units of precipitation, mean annual Air T, N concentration and CO$_2$ fluxes were mm year$^{-1}$, ºC, kg N ha$^{-1}$ yr$^{-1}$ and g m$^{-2}$ day$^{-1}$, respectively. “a” indicates the GPP data from ANPP of vascular plants.

Table S2.3 Impacts of vegetation composition on $CO_2$ emission in peatlands.

| Site Location | Years of study | Ecosystem classification | latitude and longitude | Above Sea Level | precipitation | Mean annual Air T | Shrubs | Graminoids | bryophytes | ER | NEE | GPP | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Switzerland | | peatland | 46°33' N, 6°10' E | 1,035 | 1274 | 5.9 | √ | √ | √ | 15.21 | -4.5 | -20.91 | (Gavazov et al., 2018) |
| Switzerland | | peatland | 46°33' N, 6°10' E | 1035 | 1274 | 5.9 | | | √ | 11.02 | 0.31 | -10.26 | |
| Switzerland | | peatland | 46°36' N, 7°58' E | 1885 | 1427 | 1.3 | √ | √ | √ | 11.01 | -11.42 | -22.05 | |
| Switzerland | | peatland | 46°36' N, 7°58' E | 1885 | 1427 | 1.3 | | | √ | 6.84 | -4.36 | -10.64 | |
| the Moor House National Nature Reserve, northern England | 5 | bog | 55°64' N, 2°45' W | 550 | 2016 | 6 | | | √ | 2.06 | ND | ND | (Walker et al., 2016) |
| the Moor House National Nature Reserve, northern England | 5 | bog | 55°64'N, 2°45'W | 550 | 2016 | 6 | | √ | | 4.75 | ND | ND | |
| the Moor House National Nature Reserve, northern England | 5 | bog | 55°64'N, 2°45'W | 550 | 2016 | 6 | √ | | | 2.64 | ND | ND | |
| the Moor House National Nature Reserve, northern England | 5 | bog | 55°64'N, 2°45'W | 550 | 2016 | 6 | | | | 1.37 | ND | ND | |
| the Moor House National Nature Reserve, northern England | 5 | bog | 55°64'N, 2°45'W | 550 | 2016 | 6 | √ | √ | √ | 4.92 | ND | ND | |
| Belarus,Eastern Europe | 3 | fen | 54.10◦ N; 26.29◦ E | ND | 740 | 6.5 | | √ | | 4.82 | -1.05 | -5.86 | (Minke et al., 2016) |
| Belarus,Eastern Europe | 3 | fen | 52.38◦ N;25.21◦ E | ND | 594 | 7.3 | | √ | | 11.17 | -3.59 | -14.77 | |
| Black Law Wind Farm, Scotland | 1 | bog | 55◦ 46'01"N 03◦ 44'20"W | 250 | ND | ND | √ | | | 4.91 | -6.11 | -0.34 | (Armstrong et al., 2015) |
| Black Law Wind Farm, Scotland | 1 | bog | 55◦ 46'01"N 03◦ 44'20"W | 250 | ND | ND | | √ | | 4.38 | -7.19 | -2.17 | |
| Black Law Wind Farm, Scotland | 1 | bog | 55◦ 46'01"N 03◦ 44'20"W | 250 | ND | ND | | | √ | 1.82 | -2.94 | -0.84 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | | √ | √ | 4.56 | -7.2 | ND | (Ward et al., 2013) |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | √ | | √ | 7.44 | -19.2 | ND | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | √ | √ | | 10.08 | -24.6 | ND | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | | | √ | 4.08 | 0.48 | ND | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | | √ | | 6.72 | -14.16 | ND | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | √ | | | 6.96 | -14.88 | ND | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | | | | 2.4 | 0.6 | ND | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | √ | √ | √ | 7.68 | -22.8 | ND | |
| the Moor House National Nature Reserve, northern England | 3 | bog | 54°65′ N, 2°45′ W | 590 | 2012 | 6.1 | | √ | √ | 1.73 | -0.10 | -1.90 | (Ward et al., 2009) |
| the Moor House National Nature Reserve, northern England | 3 | bog | 54°65′ N, 2°45′ W | 590 | 2012 | 6.1 | √ | | √ | 1.08 | -0.072 | -1.20 | |
| the Moor House National Nature Reserve, northern England | 3 | bog | 54°65′ N, 2°45′ W | 590 | 2012 | 6.1 | √ | √ | | 1.01 | -0.08 | -0.94 | |
| the Moor House National Nature Reserve, northern England | 3 | bog | 54°65′ N, 2°45′ W | 590 | 2012 | 6.1 | | | | 0.47 | 0.50 | 0.02 | |
| the Moor House National Nature Reserve, northern England | 3 | bog | 54°65′ N, 2°45′ W | 590 | 2012 | 6.1 | √ | √ | √ | 0.47 | -0.19 | -0.60 | |
| the Moor House National Nature Reserve, northern England | 3 | Bog | 55°64'N, 2°45'W | 550 | 2016 | 5.3 | √ | √ | √ | 3.98 | ND | ND | (Hardie et al., 2009) |
| the Moor House National Nature Reserve, northern England | 3 | Bog | 55°64'N, 2°45'W | 550 | 2016 | 5.3 | | | | 1.42 | ND | ND | |

Note: the units of above sea level, precipitation, mean annual Air T, and $CO_2$ fluxes were m, mm year$^{-1}$, ºC, and g m$^{-2}$ day$^{-1}$, respectively. “√” indicates the present of vegetation.

Table S2.4 Impacts of warming on $CH_4$ emission in peatlands.

| **Site Location** | **Years of study** | **Ecosystem classification** | **latitude and longitude** | **Above Sea Level** | **precipitation** | **Mean annual Air T** | **Increase Air T** | **Increase Soil T 0-5 cm** | **Increase Soil T 15-20 cm** | **warming equipment** | **control mean $CH_4$ flux** | **treatment mean $CH_4$ flux** | **Reference** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Blæsedalen, Disko Island, Greenland | 2 | fen | 69°18'40.9″N, 53°30'40.9″W | 112 | 436 | -3 | 0.89 | 0.18 | ND | OTC | 1.57 | 1.80 | (Nielsen et al., 2017) |
| Orivesi, Finland | 2 | Fen | 61°48'N; 24°19'E | ND | 700 | 3.5 | 1.5 | 0.3 | ND | OTC | 100.00 | 95.00 | (Peltoniemi et al., 2016) |
| Kittila, Finland | 2 | Fen | 67°60'N; 24°12'E | ND | 551 | -1.4 | 1.5 | 0.3 | ND | OTC | 150.00 | 139.50 | |
| Orivesi, Finland | 3 | Fen | 61°47´N, 24°18´E | ND | 700 | 3.5 | 2.1 | 0.3 | ND | OTC | 110.00 | 75.00 | (Pearson et al., 2015) |
| Virrat, Finland | 3 | Fen | 62°13´N, 23°23´E | ND | 700 | 3.5 | 1.6 | 1.3 | ND | OTC | 160.00 | 150.00 | |
| Kittilä, Finland | 3 | Fen | 67°59´N, 24°12´E | ND | 511 | -1.4 | 0.9 | 0.5 | ND | OTC | 230.00 | 200.00 | |
| Alberta, Canada | 1 | bog | 55°21'14.2"N, 112°31'3.7"W | ND | 504 | 2.1 | 1.1 | 0.7 | 0.5 | OTC | 4.50 | 5.50 | (Munir and Strack, 2014) |
| Alberta, Canada | 2 | bog | 55°21'14.2"N, 112°31'3.7"W | ND | 504 | ND | 1.1 | 0.7 | 0.8 | OTC | 4.10 | 4.20 | |
| Alberta, Canada | 3 | bog | 55°21'14.2"N, 112°31'3.7"W | ND | 504 | ND | 1 | 0.4 | 1.4 | OTC | 4.20 | 4.30 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65' N, 2°45' W | 550 | 2048 | 5.8 | 0.8 | ND | ND | OTC | 2.40 | 7.20 | (Ward et al., 2013) |
| Michigan, USA | 3 | fen | 46.85°N 88.37°W | 183 | 833 | 4.5 | ND | 0.5 | ND | OTC | 9.12 | 11.76 | (Johnson et al., 2013) |
| Michigan, USA | 3 | fen | 46.85°N 88.37°W | 183 | 833 | 4.5 | 2.5 | 1.4 | ND | infrared heaters | 9.12 | 10.80 | |
| Västerbotten, Sweden | 12 | fen | 64°11'N, 19°33'E | 270 | 523 | ND | ND | 1.5 | ND | Greenhouse | 98.40 | 79.20 | (Eriksson et al., 2010) |
| Minnesota, USA | 7 | bog | 47°N, 92°W | ND | ND | ND | ND | ND | 1.6 | infrared heaters | 200 | 230 | (White et al., 2008) |
| Minnesota, USA | 7 | fen | 47°N, 92°W | ND | ND | ND | ND | ND | 1.6 | infrared heaters | 70 | 100 | |
| Fairbanks, Alaska, USA | 1 | fen | 64.82°N, 147.87°W | ND | 269 | -2.9 | 1 | 0.7 | 2. | OTC | 75.20 | 118.60 | (Turetsky et al., 2008) |
| Fairbanks, Alaska, USA | 2 | fen | 64.82°N, 147.87°W | ND | 269 | -2.9 | 1 | 0.7 | ND | OTC | 22.30 | 31.50 | |
| Duluth,Minnesota, USA | 2 | Fen | 47°N, 92°W | ND | ND | ND | ND | ND | 1.6 | infrared heaters | 50 | 110 | (Updegraff et al., 2001) |
| Duluth,Minnesota, USA | 2 | bog | 47°N, 92°W | ND | ND | ND | ND | ND | 1.6 | infrared heaters | 130 | 160 | |
| Duluth,Minnesota, USA | 3 | Fen | 47°N, 92°W | ND | ND | ND | ND | ND | 1.6 | infrared heaters | 80 | 130 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Duluth,Minnesota, USA | 3 | bog | 47°N, 92°W | ND | ND | ND | ND | ND | 1.6 | infrared heaters | 160 | 240 | |
| Duluth,Minnesota, USA | 4 | Fen | 47°N, 92°W | ND | ND | ND | ND | ND | 1.6 | infrared heaters | 60 | 80 | |
| Duluth,Minnesota, USA | 4 | bog | 47°N, 92°W | ND | ND | ND | 3.6 | ND | 1.6 | infrared heaters | 160 | 190 | |
| Vasterbotten, Sweden | 3 | fen | 64°11'N, 19°33'E | 270 | 523 | ND | ND | 1.5 | ND | Greenhouse | 35.28 | 32.64 | (Granberg et al., 2001) |

Note: the units of above sea level, precipitation, mean annual Air T, and $CH_4$ flux were m, mm year$^{-1}$, ºC, and mg m$^{-2}$ day$^{-1}$, respectively.

Table S2.5 Impact of nitrogen deposition on CH$_4$ emission in peatlands.

| **Site Location** | **Ecosystem classification** | **Years of study** | **latitude and longitude** | **Above Sea Level** | **precipitation** | **Mean annual Air** | **Local N deposition** | **N concentration** | **form of N** | **control mean CH$_4$ flux** | **treatment mean CH$_4$ flux** | **Reference** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Northern Finland | fen | 67 | 65° N;<br>24° E | ND | ND | ND | ND | P: 40-350; K: 66-420 | PK | 0.68 | -0.54 | (Ojanen et al., 2019) |
| Southern Finland | bog | 67 | 62°10′ N;<br>22° 48 E | ND | ND | ND | ND | N:91-201; P: 96-556; K: 60-435 | NPK (Ca-NH$_3$-NO$_2$ and PK) | 4.6 | 0.01 | |
| Ontario, Canada | bog | 11 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 32 | NH4NO3 | 8.2 | 8.13 | (Juutinen et al., 2018) |
| Ontario, Canada | bog | 11 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 64 | NH4NO3 | 8.2 | 8.91 | |
| Ontario, Canada | bog | 1 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 32 | NH4NO3 | 8.2 | 1.78 | |
| Ontario, Canada | bog | 1 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 64 | NH4NO3 | 8.2 | 2.82 | |
| Ontario, Canada | bog | 6 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 16 | NH4NO3 | 8.60 | 7.08 | |
| Ontario, Canada | bog | 16 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 16 | NH4NO3 | 8.60 | 7.94 | |
| Ontario, Canada | bog | 6 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 16 | NPK(NH4NO3, KH2PO4) | 8.2 | 8.91 | |
| Ontario, Canada | bog | 6 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 32 | NPK(NH4NO3, KH2PO4) | 8.2 | 8.32 | |
| Ontario, Canada | bog | 6 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 64 | NPK(NH4NO3, KH2PO4) | 8.2 | 5.01 | |
| Ontario, Canada | bog | 16 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 16 | NPK(NH4NO3, KH2PO4) | 8.60 | 11.22 | |
| Ontario, Canada | bog | 16 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 32 | NPK(NH4NO3, KH2PO4) | 8.60 | 12.59 | |
| Ontario, Canada | bog | 16 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 64 | NPK(NH4NO3, KH2PO4) | 8.60 | 50.50 | |
| Ontario, Canada | bog | 6 | 45.410017°N, 75.518348°W | ND | 943 | 6 | 5 to 8 | 0 | PK(KH2PO4) | 8.60 | 22.50 | |
| Västerbotten, Sweden | fen | 11 | 64°11'N, 19°33'E | 270 | 523 | 1.2 | 2 | 30 | NH4NO3 | 97.58 | 115.15 | (Eriksson et al., 2010) |
| Fäjemyr, Swedish | bog | 2 | 56◦15'N, 13◦33'E | 140 | 700 | 6.2 | 15 | 40 | NH4NO3 | 10.08 | 11.04 | (Lund et al., 2009) |
| Storflaket, Swedish | bog | 2 | 68◦20'N, 18◦58'E | 380 | 304 | -0.8 | 2 | 20 | NH4NO3 | 5.04 | 7.68 | |
| Storflaket, Swedish | bog | 2 | 68◦20'N, 18◦58'E | 380 | 304 | -0.8 | 2 | 40 | NH4NO3 | 5.04 | 4.08 | |
| Ilomantsi, Finland | mire | 3 | 62°47'N, 30°56'E | 150 | 650 | 16(daily) | 4 | 30 | NH4NO3 | 96.00 | 98.40 | (Silvola et al., 2003) |
| Småland, Sweden | mire | 3 | 57°08'N, 14°30'E | 225 | 800 | 16(daily) | 4 to 39 | 30 | NH4NO3 | 88.80 | 91.20 | |
| Roudsea Wood National Nature | mire | 3 | 54°14'N, 03°01'W | 5 | 1800 | 13(daily) | 4 to 39 | 30 | NH4NO3 | 12.00 | 12.00 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reserve,United Kingdom | | | | | | | | | | | | |
| Drenthe, Netherlands | mire | 3 | 52°49'N, 06°26'E | 13 | 750 | 18(daily) | 39 | 50 | NH4NO3 | 151.20 | 216.00 | |
| Jura, Switzerland | mire | 3 | 47°13'N, 07°03'E | 1000 | 1390 | 15(daily) | 4 to 39 | 30 | NH4NO3 | 98.40 | 96.00 | |
| Orivesi, Finland | mrie | 6 | 61°48'N, 24°19'E | 150 | 709 | 3 | 6 | 30 | NH4NO3 | 14.50 | 18.10 | (Nykänen et al., 2002) |
| Orivesi, Finland | mrie | 6 | 61°48'N, 24°19'E | 150 | 709 | 3 | 6 | 100 | NH4NO3 | 14.50 | 19.60 | |
| Västerbotten,Sweden | fen | 4 | 64°11'N, 19°33'E | 270 | 523 | 1.2 | 2 | 30 | NH4NO3 | 35.28 | 32.88 | (Granberg et al., 2001) |
| East Finland | mire | 2 | 62°47' N, 30°56' E | ND | ND | ND | 4.3 | 30 | NH4NO3 | 4.45 | 5.04 | (Saarnio et al., 2000; Saarnio and Silvola, 1999) |

Note: the units of above sea level, precipitation, mean annual Air T, N concentration, and $CH_4$ flux were m, mm year$^{-1}$, ºC, kg N ha$^{-1}$ yr$^{-1}$, and mg m$^{-2}$ day$^{-1}$, respectively.

Table S2.6 Impacts of vegetation composition on $CH_4$ emission in peatlands.

| Site Location | Years of study | Ecosystem classification | latitude and longitude | Above Sea Level | precipitation | Mean annual Air T | Shrubs | Graminoids | bryophytes | mean $CH_4$ flux | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Blæsedalen, Disko Island, Greenland | 2 | fen | 69°18'40.9″N, 53°30'40.9″W | 112 | 436 | -3 |  | √ | √ | 0.33 | (Nielsen et al., 2017) |
| Blæsedalen, Disko Island, Greenland | 2 | fen | 69°18'40.9″N, 53°30'40.9″W | 112 | 436 | -3 | √ | √ | √ | 1.02 |  |
| Bic Saint-Fabien (BSF) peatland, Rimouski, Quebec, Canada | 2 | fen | 48.322°N, 68.833°W | ND | 959 | −11 to 18(daily) |  | √ |  | 13.8 | (Strack et al., 2017) |
| Bic Saint-Fabien (BSF) peatland, Rimouski, Quebec, Canada | 2 | fen | 48.322°N, 68.833°W | ND | 959 | −11 to 18(daily) |  | √ | √ | 13.8 |  |
| Bic Saint-Fabien (BSF) peatland, Rimouski, Quebec, Canada | 2 | fen | 48.322°N, 68.833°W | ND | 959 | −11 to 18(daily) | √ |  |  | 14.3 |  |
| Bic Saint-Fabien (BSF) peatland, Rimouski, Quebec, Canada | 2 | fen | 48.322°N, 68.833°W | ND | 959 | −11 to 18(daily) | √ |  | √ | 5 |  |
| Bic Saint-Fabien (BSF) peatland, Rimouski, Quebec, Canada | 2 | fen | 48.322°N, 68.833°W | ND | 959 | −11 to 18(daily) |  |  | √ | 1.7 |  |
| Bic Saint-Fabien (BSF) peatland, Rimouski, Quebec, Canada | 2 | fen | 48.322°N, 68.833°W | ND | 959 | −11 to 18(daily) |  |  |  | 0.36 |  |
| Belarus,Eastern Europe | 3 | fen | 54.10◦ N; 26.29◦ E | ND | 740 | 6.5 |  | √ |  | 27.40 | (Minke et al., 2016) |
| Belarus,Eastern Europe | 3 | fen | 52.38◦ N;25.21◦ E | ND | 594 | 7.3 |  | √ |  | 164.38 |  |
| Black Law Wind Farm, Scotland | 1 | bog | 55◦ 46'01"N 03◦ 44'20"W | 250 | ND | ND | √ |  |  | 16 | (Armstrong et al., 2015) |
| Black Law Wind Farm, Scotland | 1 | bog | 55◦ 46'01"N 03◦ 44'20"W | 250 | ND | ND |  | √ |  | 56 |  |
| Black Law Wind Farm, Scotland | 1 | bog | 55◦ 46'01"N 03◦ 44'20"W | 250 | ND | ND |  |  | √ | 0.65 |  |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 |  | √ | √ | 19.2 | (Ward et al., 2013) |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | √ |  | √ | -2.16 |  |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | √ | √ |  | 3.84 |  |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 |  |  | √ | 0.24 |  |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 |  | √ |  | 18.96 |  |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | √ |  |  | 6 |  |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 |  |  |  | 0.24 |  |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’ W | 550 | 2048 | 5.8 | √ | √ | √ | 0.24 |  |
| Great Hing’an Mountains, Northeast China | 1 | Peatland | 52.94◦N, 122.86◦E | ND | 325.9 | -3.9 | √ |  | √ | 5.04 | (Miao et al., 2012) |
| Great Hing’an Mountains, Northeast China | 2 | Peatland | 52.94◦N, 122.86◦E | ND | 325.9 | -3.9 | √ |  | √ | 13.44 |  |

|  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Great Hing’an Mountains, Northeast China | 1 | Peatland | 52.94◦N, 122.86◦E | ND | 493.7 | -3.9 |  | √ |  | 24 |  |
| Great Hing’an Mountains, Northeast China | 1 | Peatland | 52.94◦N, 122.86◦E | ND | 493.7 | -3.9 |  |  |  | 5.28 |  |
| Great Hing’an Mountains, Northeast China | 2 | Peatland | 52.94◦N, 122.86◦E | ND | 493.7 | -3.9 |  | √ |  | 19.2 |  |
| Great Hing’an Mountains, Northeast China | 2 | Peatland | 52.94◦N, 122.86◦E | ND | 493.7 | -3.9 |  |  |  | 4.8 |  |
| Zackenberg, Greenland | 1 | fen | 74°28'N, 20°34'W | ND | ND | -9 to 5.8 |  | √ |  | 273.6 | (Ström et al., 2012) |
| Zoige plateau, China | 1 | fen | 33° 56′ N, 102° 52′ E | 3430 | 650 | 1.7 |  | √ |  | 48.24 | (Chen et al., 2010) |
| St. Charles-de-Bellechasse peatland,Quebec, Canada | 1 | fen | 46°40'N, 71°10'W | ND | 89.8-114.2 | -12.8-16.5 | √ |  | √ | 13.9 | (Strack et al., 2006) |
| St. Charles-de-Bellechasse peatland,Quebec, Canada | 1 | fen | 46°40'N, 71°10'W | ND | 89.8-114.2 | -12.8-16.5 | √ | √ | √ | 41 |  |
| Roudsea Moss, UK | 1 | peatland | 54°N, 3°W | ND | ND | ND | √ | √ | √ | 72 | (Greenup et al., 2000) |
| Roudsea Moss, UK | 1 | peatland | 54°N, 3°W | ND | ND | ND | √ |  | √ | 7 |  |
| Stor-Ämyran, Sweden | 1 | bog | 63°44'N, 20°06'E | 35 | ND | ND | √ | √ | √ | 35.3 | (Waddington et al., 1996) |
| Stor-Ämyran, Sweden | 1 | bog | 63°44'N, 20°06'E | 35 | ND | ND |  |  | √ | 93.4 |  |
| Thompson, Manitoba, Canada | 1 | peatland | 55°55'N,98°25'W | ND | ND | ND |  |  | √ | 75.6 |  |
| Thompson, Manitoba, Canada | 1 | peatland | 55°55'N,98°25'W | ND | ND | ND | √ | √ | √ | 105.6 |  |
| Schefferville, Quebec, Canada | 1 | fen | 54°48'N, 66°49'W | ND | ND | ND |  |  |  | 4.8 | (Whiting and Chanton, 1992) |
| Schefferville, Quebec, Canada | 1 | fen | 54°48'N, 66°49'W | ND | ND | ND | √ | √ | √ | 76.8 |  |

Note: the units of above sea level, precipitation, mean annual Air T, $CH_4$ flux were m, mm year$^{-1}$, ºC and mg m$^{-2}$ day$^{-1}$, respectively. “√” indicates the present of vegetation.

Table S2.7 Impacts of warming on $N_2O$ emission in peatlands.

| **Site Location** | **Years of study** | **Ecosystem classification** | **latitude and longitude** | **Above Sea Level** | **precipitation** | **Mean annual T** | **Increase Air T** | **Increase soil T 5cm** | **warming equipment** | **control mean $N_2O$ flux** | **Treatment mean $N_2O$ flux** | **Reference** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Newfoundland, Canada | 3 | bog | 48º15’46” N, 58º39’21” W | ND | 1340 | 5 | 1.93 | 0.8 | OTC | -0.56 | 0.07 | (Gong et al., 2019) |
| Great Hing'an Mountains, Northeast China | 2 | peatland | 52°94′ N, 122°86′ E | 477 | 452 | −3.9 | 0.6 | 2 | OTC | 0.01 | 0.19 | (Cui et al., 2018) |
| Great Hing'an Mountains, Northeast China | 3 | peatland | 52°94′ N, 122°86′ E | 477 | 452 | −3.9 | 0.6 | 2 | OTC | 0.11 | 0.22 | |
| Great Hing'an Mountains, Northeast China | 4 | peatland | 52°94′ N, 122°86′ E | 477 | 452 | −3.9 | 0.6 | 2 | OTC | 0.02 | 0.16 | |
| Orivesi, Finland | 3 | Fen | 61°47´N, 24°18´E | ND | 700 | 3.5 | 2.1 | 0.3 | OTC | 0.3 | 029 | (Pearson et al., 2015) |
| Virrat, Finland | 3 | Fen | 62°13´N, 23°23´E | ND | 700 | 3.5 | 1.6 | 1.3 | OTC | 0.15 | 0.18 | |
| Kittilä, Finland | 3 | Fen | 67°59´N, 24°12´E | ND | 511 | -1.4 | 0.9 | 0.5 | OTC | 0.3 | 0.22 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | 0.9 | ND | OTC | 216 | -2.4 | (Ward et al., 2013) |

Note: the units of above sea level, precipitation, mean annual Air T, $N_2O$ flux were m, mm year$^{-1}$, ºC and mg m$^{-2}$ day$^{-1}$, respectively.

Table S2.8 Impact of nitrogen deposition on $N_2O$ emission from peatlands.

| **Site Location** | **Ecosystem classification** | **Years of study** | **latitude and longitude** | **Above Sea Level** | **precipitation** | **Mean annual Air** | **Local N deposition** | **N concentration** | **form of N** | **control mean $N_2O$ flux** | **treatment mean $N_2O$ flux** | **Reference** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Northern Finland | fen | 67 | 65° N; 24° E | ND | ND | ND | ND | P: 40-350; K: 66-420 | PK | 0.35 | 0.36 | (Ojanen et al., 2019) |
| Southern Finland | bog | 67 | 65° N; 24° E | ND | ND | ND | ND | N:91-201; P: 96-556; K: 60-435 | NPK (Ca-NH3-NO2 and PK) | 0.34 | 0.36 | |
| Newfoundland, Canada | bog | 3 | 48º15’46” N, 58º39’21” W | ND | 1340 | 5 | 6.4 | 64 | NH4NO3 | -0.56 | 11.79 | (Gong et al., 2019) |
| Scottish Borders | bog | 13 | 3 º 16' W, 55 º 46' N | 282 | 1092 | 8.6 | 8 | 8~64 | NH3 | ~0 | 0.58 | (Leeson et al., 2017) |
| Scottish Borders | bog | 13 | 3 º 16' W, 55 º 46' N | 282 | 1092 | 8.6 | 8 | 8~64 | NH4Cl | ~0 | ~0 | |
| Scottish Borders | bog | 13 | 3 º 16' W, 55 º 46' N | 282 | 1092 | 8.6 | 8 | 8~64 | NaNO3 | ~0 | ~0 | |
| Scottish Borders | bog | 7 | 3 º 16' W, 55 º 46' N | 282 | 1092 | 8.6 | 8 | 56 | NH4Cl | 0.1 | 0.16 | (Sheppard et al., 2013) |
| Scottish Borders | bog | 7 | 3 º 16' W, 55 º 46' N | 282 | 1092 | 8.6 | 8 | 56 | NaNO3 | 0.1 | 0.09 | |
| Scottish Borders | bog | 7 | 3 º 16' W, 55 º 46' N | 282 | 1092 | 8.6 | 8 | 56 | NH3 | 0.1 | 1.09 | |
| Storflaket, Swedish | bog | 2 | 68◦20'N, 18◦58'E | 380 | 304 | -0.8 | 2 | 40 | NH4NO3 | 0.15 | 0.59 | (Lund et al., 2009) |
| Storflaket, Swedish | bog | 2 | 68◦20'N, 18◦58'E | 380 | 304 | -0.8 | 2 | 40 | NP( NH4NO3 and NaH2PO4 | 0.15 | 0.26 | |
| Orivesi, Finland | mrie | 6 | 61°48'N, 24°19'E | 150 | 709 | 3 | 6 | 100 | NH4NO3 | 0.05 | 1.7 | (Nykänen et al., 2002) |
| eastern Finland | fen | 3 | 62°46′N, 30°58′E | ND | 650 | 1.9 | 2.7 | 100 | KNO3 | 1.9 | 2.6 | (Regina et al., 1998) |
| eastern Finland | fen | 3 | 62°46′N, 30°58′E | ND | 650 | 1.9 | 2.7 | 100 | NH4Cl | 1.9 | 3.3 | |
| eastern Finland | fen | 3 | 62°46′N, 30°58′E | ND | 650 | 1.9 | 2.7 | 100 | urea | 1.9 | 3.5 | |

Note: the units of above sea level, precipitation, mean annual Air T, N concentration, and $N_2O$ flux were m, mm year$^{-1}$, ºC, kg N ha$^{-1}$ yr$^{-1}$, and mg m$^{-2}$ day$^{-1}$, respectively.

Table S2.9 Impacts of vegetation composition on $N_2O$ emission from peatlands.

| Site Location | Years of study | Ecosystem classification | latitude and longitude | Above Sea Level | precipitation | Mean annual Air T | Shrubs | Graminoids | bryophytes | mean $N_2O$ flux | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Newfoundland, Canada | 3 | bog | 48º15’46” N, 58º39’21” W | ND | 1340 | 5 | | | √ | 0.36 | (Gong et al., 2018) |
| Newfoundland, Canada | 3 | bog | 48º15’46” N, 58º39’21” W | ND | 1340 | 5 | | √ | √ | 0.34 | |
| Newfoundland, Canada | 3 | bog | 48º15’46” N, 58º39’21” W | ND | 1340 | 5 | √ | | √ | 1.21 | |
| Newfoundland, Canada | 3 | bog | 48º15’46” N, 58º39’21” W | ND | 1340 | 5 | √ | √ | √ | -0.55 | |
| Alberta, Canada | 1 | peatland | 53◦27'17” N, 114◦52'50” W | ND | 550 | 3.5 | | | √ | 0.04 | (Brummell et al., 2017) |
| Alberta, Canada | 1 | peatland | 53◦27'17” N, 114◦52'50” W | ND | 550 | 3.5 | √ | | | -0.04 | |
| Belarus,Eastern Europe | 3 | fen | 54.10◦ N; 26.29◦ E | ND | 740 | 6.5 | | √ | | -0.10 | (Minke et al., 2016) |
| Belarus,Eastern Europe | 3 | fen | 54.10◦ N; 26.29◦E | ND | 740 | 6.5 | | √ | | -0.10 | |
| Belarus,Eastern Europe | 3 | fen | 54.10◦ N; 26.29◦E | ND | 740 | 6.5 | | √ | | -0.31 | |
| Belarus,Eastern Europe | 3 | fen | 52.38◦ N;25.21◦E | ND | 594 | 7.3 | | √ | | 0.62 | |
| Belarus,Eastern Europe | 3 | fen | 52.38◦ N;25.21◦E | ND | 594 | 7.3 | | √ | | -0.10 | |
| Belarus,Eastern Europe | 3 | fen | 52.38◦ N;25.21◦E | ND | 594 | 7.3 | | √ | | 0.62 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | | √ | √ | -336 | (Ward et al., 2013) |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | √ | | √ | 144 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | √ | √ | | -120 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | | | √ | 192 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | | √ | | -168 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | √ | | | -192 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | √ | √ | √ | 216 | |
| the Moor House National Nature Reserve, northern England | 2 | bog | 54°65’ N, 2°45’W | 550 | 2048 | 5.8 | | | | 240 | |

Note: the units of above sea level, precipitation, mean annual Air T, mean $N_2O$ flux were m, mm year$^{-1}$, ºC and mg m$^{-2}$ day$^{-1}$, respectively. “√” indicates the present of vegetation.

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### 2.10 Corrigendum

Corrigendum to “Greenhouse gas emissions from peatlands under manipulated warming, nitrogen addition, and vegetation composition change: a review and data synthesis” [Environ. Rev. 28: 428-437 (2020)]

Recently, when I organized my PhD thesis, I found that the $CH_4$ data under different vegetation compositions in the Table 2.5 are the value of global warming potential. The authors want to change them to the absolute responsiveness (AR). From Table 2.5, the highest AR of $CH_4$ was found under the absence of bryophytes and shrubs. This correction will not change the conclusion of the article that “the absence of bryophytes reduced global warming potential”.

The authors would like to apologize for any inconvenience this may cause.

Dr. Wu, as the correspondence author of this paper, is contacting the Journal’s editorial office to make this correction.

Table 2.5 Mean absolute responsiveness of each GHG under global changes.

| Global changes | $CO_2$ | $CH_4$ | $N_2O$ | Total |
|---|---|---|---|---|
| Warming | 1748 | -10 | 7 | 1745 |
| N addition | -212 | 3 | 1018 | 809 |
| absence of bryophytes | -308 | 33 | -36 | -311 |
| absence of graminoids | 678 | -346 | -7 | 325 |
| absence of shrubs | 2864 | 54 | -59 | 2859 |
| absence of bryophytes and graminoids | 2890 | 53 | -26 | 2917 |
| absence of bryophytes and shrubs | 2041 | 171 | -41 | 2171 |
| absence of graminoids and shrubs | 4248 | -101 | -2 | 4145 |
| Absence of all vegetation | 4397 | 0 | 3 | 4400 |

# CHAPTER 3. Vegetation composition regulates the interaction of warming and nitrogen deposition on net carbon dioxide uptake in a boreal peatland

## 3.1 Abstract

Peatlands are carbon sinks and have the potential to mitigate global warming. However, it is unclear whether peatlands will remain carbon sinks or switch to carbon sources in the future because few studies focus on the interactions of global changes, such as climate warming, elevated nitrogen (N) deposition, and vegetation composition change. In this study, these global changes were mimicked in a boreal peatland for seven years in order to explore the interactions of these global changes on $CO_2$ fluxes. The result showed that warming has a limited effect on net ecosystem production (NEP), while N addition decreased NEP by 65% after seven years of the treatment owing to the detrimental effect on the *Sphagnum* mosses. The negative impact of N addition could be mitigated by warming under intact vegetation. Under the treatment of graminoid removal, combined warming and elevated N addition (WN) decreased NEP by 80 - 106%. Under the treatment of shrub removal, WN decreased NEP by 49% in 2016 but not in 2020. In addition, we also found that soil temperature, moisture, and dissolved organic carbon were the main controls for $CO_2$ fluxes. Overall, this study sheds new light on the interactions of climate warming, elevated N deposition, and vegetation composition change on the $CO_2$ uptake of peatlands; and could help to accurately evaluate the carbon sink function of peatlands under future global change.

## 3.2 Introduction

The Special Report on Global Warming of 1.5 °C (SR1.5) by the Intergovernmental Panel on Climate Change (IPCC) shows that net zero carbon dioxide ($CO_2$) emissions should be reached around 2050 to limit warming to 1.5°C above pre-industrial levels (IPCC, 2018). Peatlands are effective long-term carbon sinks and have stored about one-third of the global soil carbon (Alexandrov et al., 2020). This high capacity of carbon sequestration in peatlands can contribute to the mitigation of global warming. However, we are unaware of whether peatlands will still be the carbon sinks as before or switch to the carbon sources in the future, considering peatlands are vulnerable to global changes such as global warming, elevated N deposition, and vegetation composition change (Gong et al., 2020; Loisel et al., 2021).

Temperature plays an essential role in photosynthesis as well as plant and soil respiration (Jassey and Signarbieux, 2019; Walker et al., 2016). Impacts of warming on the peatland carbon cycle have been experimentally studied in the field (Laine et al., 2019; Voigt et al., 2017; Ward et al., 2013). Generally, warming increases ecosystem respiration (Dorrepaal et al., 2009; Ward et al., 2013), but impacts on gross primary productivity and on net ecosystem $CO_2$ uptake are uncertain, ranging from decreased to increased fluxes (Johnson et al., 2013; Pearson et al., 2015; Ward et al., 2013). It is yet hard to obtain a general conclusion owing to the limited number of studies.

The response of $CO_2$ fluxes to nitrogen deposition in nutrient-limited peatlands exhibits time lags, depending on the response of vegetation (Bubier et al., 2007). Elevated nitrogen availability often increases vegetation growth, but high doses or long-term nitrogen deposition can decrease the moss productivity due to the toxic effect (Limpens et al., 2003). When the growth of vascular plant is higher than the decline of *Sphagnum* moss cover, net ecosystem $CO_2$ uptake would be increased (Bubier et al., 2007). This increase in $CO_2$ uptake can be diminished on a long-term (more than five years) due to the decline of *Sphagnum* moss productivity outweighs the vascular plant growth (Bubier et al., 2007; Juutinen et al., 2010; Larmola et al., 2013). Noticeably, apart from elevated nitrogen deposition, climate warming, and vegetation composition change will also occur in the future. The interactions of them on net $CO_2$ uptake of peatlands are not well known.

Along with climate change and land-use change in the future, peatlands have the potential to shift to shrub-dominated (Bragazza et al., 2015; Kool and Heijmans, 2009) or graminoid-dominated ecosystems (Dieleman et al., 2015). To understand the ecosystem functions under different vegetation compositions, the removal of vegetation is an effective and widely used experimental method (Díaz et al., 2003). Presence of shrubs and/or graminoids promotes net ecosystem $CO_2$ uptake in peatlands due to the greater increase of gross primary productivity than ecosystem respiration (Gavazov et al., 2018; Ward et al., 2013). It should be noted that these studies (Gavazov et al., 2018; Ward et al., 2013) have been conducted less than three-year durations. With the labile vascular plant litter accumulated in the long-term scale, the decomposition and ecosystem respiration can be significantly increased (Dieleman et al., 2016; Walker et al., 2016), suggesting the net ecosystem $CO_2$ uptake would be altered in the future. However, the magnitude of this alteration is unknown.

Furthermore, vegetation composition can regulate the effects of warming and nitrogen deposition on $CO_2$ effluxes. For instance, warming can increase the ecosystem $CO_2$ uptake under shrub presence and graminoid absence but decrease the ecosystem $CO_2$ uptake under graminoid presence (Ward et al., 2013). However, the interaction of nitrogen deposition and vascular plant presence was not observed due to the high capacity of *Sphagnum* mosses to retain N deposition (Leroy et al., 2019). It should be noted that these studies by Leroy et al. (2019) and Ward et al. (2013) have been conducted within three years. It is unclear whether these interactions will be altered on a long-term scale.

To the best of our knowledge, there are no studies focused on the interaction of these global changes (warming, elevated nitrogen deposition, and vegetation composition change) on net $CO_2$ uptake in peatland ecosystems. Here, these changes were mimicked for seven years in a bog in Robinsons, western Newfoundland, Canada. The measurement of $CO_2$ fluxes and ancillary environmental variables were conducted after three (2016) and seven years (2020) of the experiment. The objective of this study was to investigate the interaction of these changes on the net $CO_2$ uptake in the peatland, explore the possible underlying mechanism, and evaluate the carbon sink function of the peatland under these changes. We hypothesized that:

(1) warming would increase net $CO_2$ uptake due to the higher increase of gross primary productivity than ecosystem respiration;

(2) N addition would decrease the net ecosystem $CO_2$ uptake due to the toxic effects of nitrogen on *Sphagnum* mosses, and warming can mitigate this negative effect;

(3) the negative effect of graminoid and/or shrub removal on net ecosystem $CO_2$ uptake would be decreased with experimental duration due to the decrease of labile vascular plant litter and decomposition;

(4) The positive warming effect on the net ecosystem $CO_2$ uptake would be weakened under the condition of graminoid and/or shrub removal due to the decrease of photosynthesis;

(5) The negative effect of N addition on net $CO_2$ uptake would be strengthened under the condition of graminoid and/or shrub removal due to the reduction of their interception and increase of the toxic effect; and

(6) Combined warming and N addition would decrease the net $CO_2$ uptake under the condition of graminoid and/or shrub removal because of the strengthened negative N effect and decreased positive warming effect.

## 3.3 Methodology

### 3.3.1 Study site and experimental design

An ombrotrophic blanket bog located in Robinsons, western Newfoundland, Canada (48°15′46″ N, 58°39′21″ W) has a boreal climate with a mean annual temperature of 5 $^{o}$C and mean annual precipitation of 1340 mm (1981-2010). The climate data were obtained from the nearest weather station in Stephenville, NL, Canada. The mean pH (1:5 soil/water) of this bog was 4.5, and the mean peat depth was 3 m (Luan et al., 2019). The dominant vegetation in the study area consists of graminoids (*Rhynchospora alba* and *Trichophorum cespitosum*), shrubs (*Chamaedaphne calyculata*, *Gaylussacia bigeloviana*, *Vaccinium oxycoccos*, and *Andromeda glaucophylla*), and non-vascular plants (*Sphagnum* spp., liverworts).

Since the spring of 2014, hexagonal open-top chambers (OTC) were installed in the field, which is an effective method to simulate climate warming (Marion et al., 1997). The OTCs were 80 cm along the bottom edge, 62.5 cm along the top edge, and 40 cm in height. The OTCs could increase air temperature by 1.2 - 2.6 $^{o}$C. We have applied ammonium nitrate ($NH_4NO_3$) bimonthly from May to September to simulate elevated nitrogen deposition. Since 2019, in order to simulate N deposition more like the real precipitation regime, we applied ammonium nitrate ($NH_4NO_3$) monthly, instead of bimonthly. Ammonium nitrate was dissolved in 2 L of water from a nearby open pool (10 meters away from the experimental plots) and poured onto the nitrogen addition plots. The same amount of open pool water was poured into the control plots. The rate of nitrogen addition was 6.4 g N $m^{-2}$ $yr^{-1}$ in order to establish non-N-limited conditions for this nutrient-poor bog (Reay et al., 2008). Manual removal of vegetation (graminoids and/or shrubs) was to simulate vegetation composition change. The shoots of shrubs and graminoids were cut back to the litter layer level in early May and early July every year since 2014. During the interval, we also did some clipping, occasionally, when we found the occurrence of graminoids and shrubs in those plots. This removal experiment was effective and useful for understanding the ecosystem effects of vegetation composition change (Díaz et al., 2003). Altogether, there were four abiotic treatments: control (C), warming (W), nitrogen addition (N), and combined warming and nitrogen addition (WN); and four biotic treatments: without removal vegetation (Intact), removal of graminoids (-Gr), removal of shrubs (-Sh), and removal of both (-GS). Each biotic treatment included four abiotic treatments. Therefore, we have sixteen treatments in total. In the field, there were four replicate blocks. Each block included 16 plots (2 m × 2 m). Sixteen treatments were randomly arranged into the plots. The buffer zone between blocks was about 6 meters, and the buffer zone between plots was at least 2 m. This distance between plots was sufficient and similar with previous studies (Eriksson et al., 2010; Ward et al., 2013).

### 3.3.2 Measurements of $CO_2$ fluxes and environmental variables

The difference between $CO_2$ uptake by the vegetation in ecosystems and $CO_2$ emitted by ecosystem respiration (ER) is called net ecosystem production (NEP) (Chapin et al., 2006; Woodwell and Whittaker, 1968). NEP and ER were measured biweekly during the growing seasons (May-October) of 2016 and 2020 from 10:00 to 16:00 local time using paired transparent and opaque chambers (50 cm in height and 26.3 cm in diameter). There were seven sampling rounds in each year. The opaque chamber blocked the light coming into the chamber and thus no photosynthesis occurred during the measurement. Therefore, the $CO_2$ flux measured by the opaque chamber was equivalent to ER. However, light can penetrate the transparent chamber and thus photosynthesis occurred during the measurement. Therefore, the $CO_2$ flux measured by the transparent chamber represented a NEP. During each measurement, we placed the chamber on a PVC (polyvinyl chloride) collar, which was permanently inserted into the peat to a depth of 10 cm in the spring of 2014. The upper part of the collar features a groove to accommodate the water seal needed for the chamber measurements. A fan was fixed on the top of the chamber to mix and cool the air. The $CO_2$ concentration was recorded with a portable greenhouse gas analyzer (Los Gatos Research Inc., USA) at 1 Hz rate during a measurement period of 3 minutes after steady-state conditions were reached. The $CO_2$ fluxes were corrected for chamber volume and air temperature and calculated based on linear regression. There was only about 1-minute break between the measurement from the transparent chamber (for NEP) and from the opaque chamber (for ER). Therefore, it was reasonable and accurate to estimate gross primary production (GPP) from the measurement of NEP and ER at the same plot. Photosynthetically active radiation (PAR) was recorded by the light meter (LI-250A, LI-COR Inc., Nebraska, USA) during the NEP measurements. The sign convention in this study was that positive NEP indicated $CO_2$ uptake and negative NEP indicated $CO_2$ emission, while GPP and ER were given positive signs.

During each gas sampling campaign, we measured soil temperature at 5 cm and 20 cm depth using a soil thermometer (Fisher Scientific Inc., Canada) and soil moisture at 5 cm depth using a soil moisture sensor (ProCheck, Decagon Devices Inc., USA). We also measured water table depth, i.e., the water table level below ground, from dip-wells made of 1 m-long perforated PVC pipes installed at each plot (negative values indicate water levels above the peat surface). In addition, we used a USB temperature logger (Lascar Electronics Ltd., UK) to continually record air temperature at vegetation canopy height every 30 minutes. One temperature logger was installed at a randomly selected warming plot, and the other was installed at a randomly selected control plot. Air temperature and precipitation of the site were measured with a temperature probe (HMP155, Vaisala, Vantaa, Finland) and a tipping bucket rain gauge (TR-525USW, Texas Electronics, Texas, USA), respectively.

Furthermore, concurrent with gas sampling, soil pore water samples at ~10 cm depth in each plot were collected using the MacroRhizons sampler (Rhizosphere Inc., Netherlands). Water samples at 40 cm depth were collected using 60 mL syringes from a perforated PVC tube, with a sealed bottom and a capped top to prevent precipitation from entering the tube, where we only perforated the bottom 5 cm of the tube. The perforated PVC tube was inserted into 40 cm depth before. These water samples were filtered by 0.45 µm syringe filters (Cole Parmer Inc., USA) before analyzing dissolved organic carbon (DOC) and dissolved total nitrogen (TN) with a Shimadzu TOC-LCPH/TN analyzer (Shimadzu Inc., Japan).

### 3.3.3 Data analysis

The effects of treatment on $CO_2$ fluxes (GPP, NEP, and ER) in each year were analyzed by linear mixed effect model. The effects and interactions of treatments (warming, N deposition, and vegetation composition change) were considered as fixed factors. Block and date were considered as random factors. Tukey's post hoc tests were used to determine the differences among vegetation compositions and among treatments. The different abiotic environmental factors (air temperature, soil temperature, soil moisture, WTD, DOC, and TN) between the years were also analyzed by linear mixed effect model. The quantile-quantile plot (QQ plot) was used for normality test. Pearson correlation was used to analyze the relationships between $CO_2$ fluxes and environmental factors. All statistical analyses were performed in the R version 3.5.1 (R Core Team, 2018) with the "lmerTest", "car", "corrplot" and "lsmeans" packages. Structural equation modeling (SEM) was used to examine the direct and indirect effects of global changes on $CO_2$ fluxes, which was performed in AMOS 22.0 (IBM SPSS, Chicago, IL, USA). Given that GPP was calculated from NEP and ER, the correlations between GPP and NEP, and between GPP and ER were called spurious correlation (Brett, 2004). Therefore, bootstrap simulation methodology was used to estimate the significance of these two spurious correlations (Brett, 2004; Lund et al., 2010). In addition, because the significant correlations between NEP, GPP, and ER, we cannot run SEM with all of them included. Therefore, GPP and ER were included in the SEM, while the impacts of environmental variables on NEP were analyzed by linear mixed effect model.

## 3.4 Results

### 3.4.1 Environmental parameters

The open top chamber (OTC) increased air temperature by 1.2 - 2.6 $^{o}$C, which is effective to simulate temperature increase at the end of this century (IPCC, 2014). In addition, the mean air temperature during the growing season at a nearest weather station in Stephenville, NL, Canada was increased by 0.37 $^{o}$C in the last 30 years (1981-2010) (https://climate.weather.gc.ca). Therefore, the temperature increased in this study might simulate the temperature in the future 100 - 200 years.

The mean air temperature (12.9 $^{o}$C) in the growing season of 2020 was slightly higher than it in 2016 (12.4 $^{o}$C) (Figure 3.1). However, the total precipitation in 2020 was 330 mm lower than it in 2016. Due to the dryer and warmer weather in the growing season of 2020, the soil temperature at 5 cm depth was increased by 2.5 $^{o}$C compared with that in 2016, and water table depth was increased by 9.5 cm (Table 3.1). In addition, compared with 2016, the concentrations of dissolved organic carbon (DOC) at 10 and 40 cm depth in 2020 were reduced by 28% and 50%, respectively.

However, the concentrations of total nitrogen (TN) at 10 and 40 cm depth in 2020 were increased by 60% and 12%, respectively.

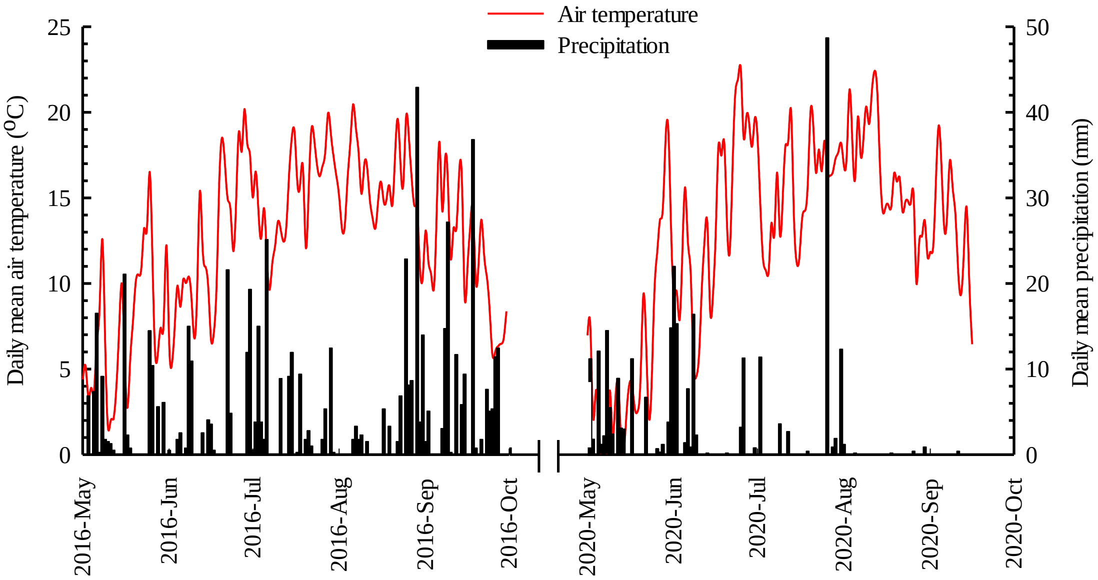

Figure 3.1 The daily mean air temperature and precipitation during the growing seasons (May-October) of 2016 and 2020.

Table 3.1 The different environmental variables (mean ± S.E.) between 2016 and 2020. Soil T represents soil temperature, WTD represents water table depth, DOC represents dissolved organic carbon, and TN represents total nitrogen. The number after the soil T, DOC, and TN represent the depth (cm) of the variables.

| | 2016 | 2020 | P value |
|---|---|---|---|
| Soil T5 (°C) | 16.27±0.14 | 18.74±0.18 | <0.0001 |
| Soil T20 (°C) | 15.3±0.14 | 15.04±0.10 | 0.1190 |
| WTD (cm) | 6.79±0.28 | 16.24±0.49 | <0.0001 |
| Soil moisture (%) | 74.66±0.63 | 48.61±0.9 | <0.0001 |
| DOC10 (mg $L^{-1}$) | 46.56±0.38 | 33.32±0.41 | <0.0001 |

|  |  |  |  |
|---|---|---|---|
| TN10 (mg $L^{-1}$) | 0.87±0.01 | 1.40±0.05 | <0.0001 |
| DOC40 (mg $L^{-1}$) | 50.58±0.44 | 25.31±0.21 | <0.0001 |
| TN40 (mg $L^{-1}$) | 1.55±0.05 | 1.73±0.03 | 0.0019 |

### 3.4.2 Effects of single change on $CO_2$ fluxes

In 2016, warming did not impact the NEP and GPP, but slightly increased ER by 15% (Table 3.2, $F_{1,372} = 4.923$, $P = 0.027$), regardless of N deposition and vegetation composition change (Figure 3.2, Table 3.2). N addition significantly decreased GPP by 16% ($F_{1,372} = 8.241$, $P = 0.004$) and decreased NEP by 26% ($F_{1,372} = 9.343$, $P = 0.002$). Removal of graminoids (-Gr) significantly decreased GPP by 60%, decreased ER by 41%, and decreased NEP by 72% (Tukey's post hoc tests, $P<0.05$). Removal of the shrub (-Sh) significantly decreased GPP by 43%, decreased ER by 34%, and decreased NEP by 49% (Tukey's post hoc tests, $P<0.05$). Removal of graminoids and shrubs (-GS) significantly decreased GPP by 82%, decreased ER by 60%, and decreased NEP by 97% (Tukey's post hoc tests, $P<0.05$).

In 2020, the patterns of change (warming, N addition, and vegetation composition change) impacts on $CO_2$ fluxes were similar to 2016 (Figure 3.3, Table 3.2). Warming did not impact the NEP and GPP, but slightly increased ER by 13% ($F_{1,370} = 6.182$, $P = 0.013$). N addition significantly decreased GPP, ER, and NEP by 26%, 15%, and 36%, respectively. Removal of graminoids (-Gr) and -GS significantly decreased GPP, ER, and NEP. However, there was no significant effect of shrub removal (-Sh) on GPP, ER, and NEP.

Table 3.2 The effects, and interactions, of warming (W), N addition (N), and vegetation composition change (V) on $CO_2$ fluxes (net ecosystem production (NEP), ecosystem respiration (ER), and gross primary production (GPP)).

| | NEP | | | ER | | | GPP | | |
|---|---|---|---|---|---|---|---|---|---|
| | NumDF | DenDF | P value | NumDF | DenDF | P value | NumDF | DenDF | P value |
| 2016 | | | | | | | | | |
| W | 1 | 372 | 0.068 | **1** | **372** | **0.027** | 1 | 372 | 0.670 |
| N | **1** | **372** | **0.002** | 1 | 372 | 0.214 | **1** | **372** | **0.004** |
| V | **3** | **372** | **<0.001** | **3** | **372** | **<0.001** | **3** | **372** | **<0.001** |
| W*N | 1 | 372 | 0.410 | **1** | **372** | **0.038** | 1 | 372 | 0.126 |
| W*V | 3 | 372 | 0.284 | 3 | 372 | 0.355 | 3 | 372 | 0.263 |
| N*V | 3 | 372 | 0.214 | 3 | 372 | 0.628 | 3 | 372 | 0.462 |
| W*N*V | **3** | **372** | **0.018** | 3 | 372 | 0.092 | **3** | **372** | **0.008** |
| 2020 | | | | | | | | | |
| W | 1 | 372 | 0.147 | **1** | **370** | **0.013** | 1 | 374 | 0.688 |
| N | **1** | **371** | **<0.001** | **1** | **370** | **<0.001** | **1** | **375** | **<0.001** |
| V | **3** | **371** | **<0.001** | **3** | **370** | **<0.001** | **3** | **375** | **<0.001** |
| W*N | 1 | 371 | 0.3284 | 1 | 370 | 0.368 | 1 | 374 | 0.532 |
| W*V | 3 | 372 | 0.7161 | 3 | 370 | 0.669 | 3 | 374 | 0.776 |
| N*V | **3** | **372** | **0.017** | 3 | 370 | 0.057 | **3** | **374** | **0.022** |
| W*N*V | **3** | **372** | **0.009** | 3 | 370 | 0.528 | **3** | **374** | **0.015** |

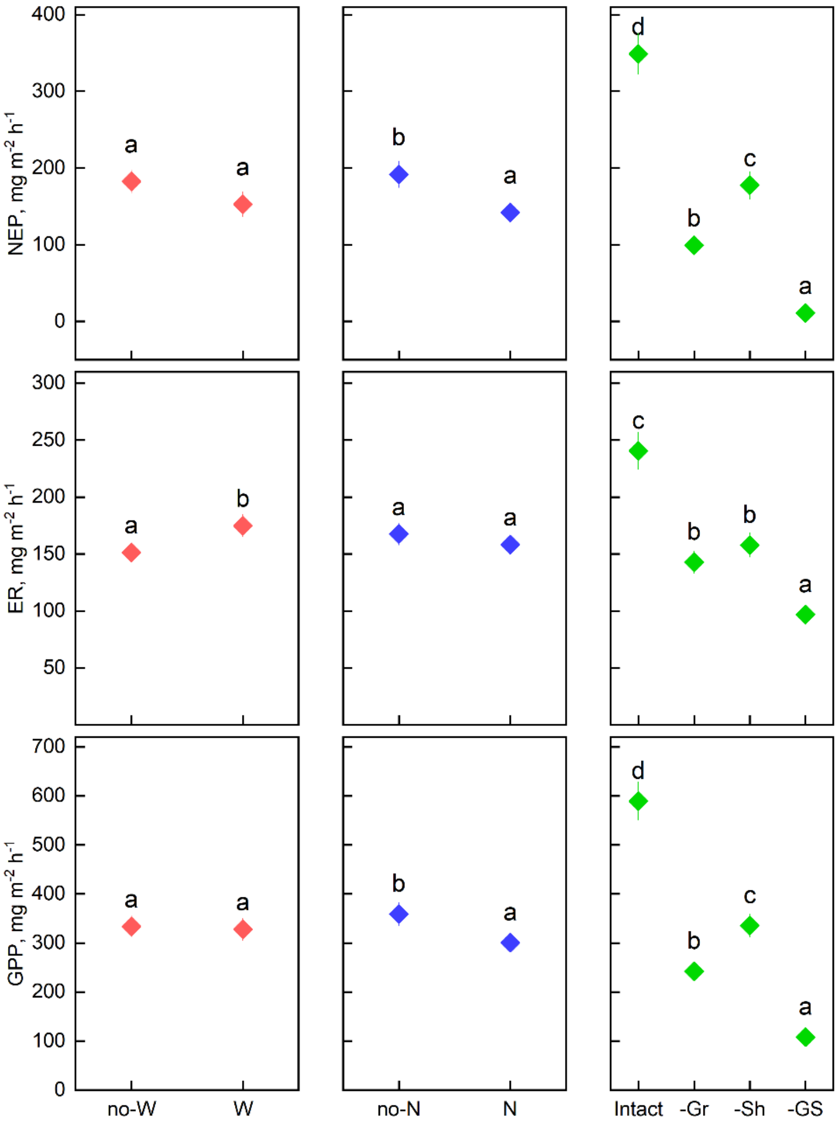

Figure 3.2 The impacts of single change on GPP, ER and NEP in peatlands during the growing season of 2016. The dots represent mean values and error bars represent standard error. The different letters represent the significant difference between the treatments (Tukey's post hoc tests, $P<0.05$). "no-W" represents no warming, "W" represents warming treatment, "no-N" represents no N addition, "N" represents N addition, "Intact" represents without vegetation removal, "-Gr" represents the removal of graminoids, "-Sh" represents the removal of shrubs, and "-GS" represents the removal of graminoids and shrubs.

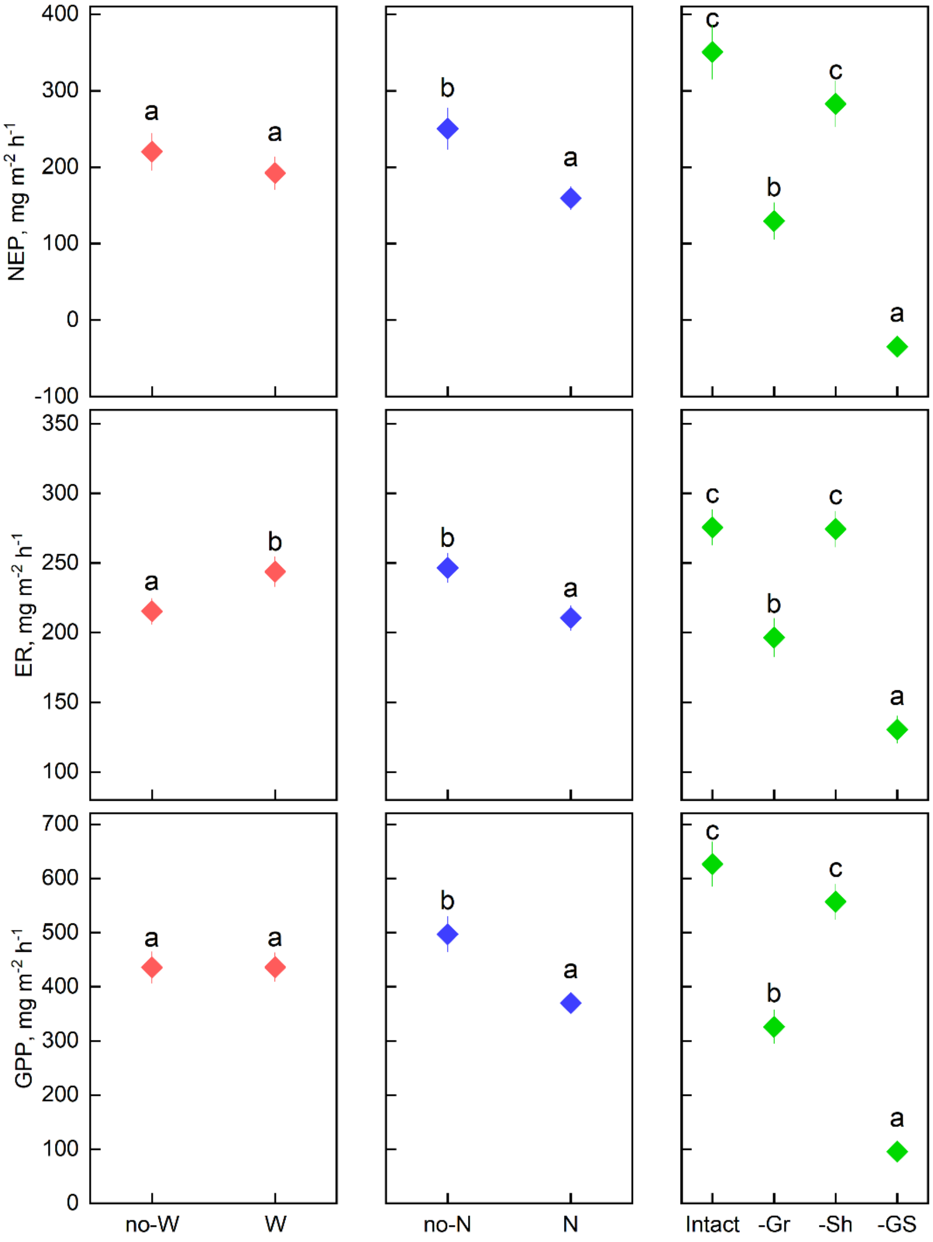

Figure 3.3 The impacts of single change on GPP, ER and NEP in peatlands during the growing season of 2020. The dots represent mean values and error bars represent standard error. The different letters represent the significant difference between the treatments (Tukey's post hoc tests, P<0.05). "no-W" represents no warming, "W" represents warming treatment, "no-N"

represents no N addition, “N” represents N addition, “Intact” represents without vegetation removal, “-Gr” represents the removal of graminoids, “-Sh” represents the removal of shrubs, and “-GS” represents the removal of graminoids and shrubs.

### 3.4.3 Interactions of the changes on $CO_2$ fluxes

The interactions of three changes (W*N*V) on GPP and NEP were observed both in 2016 and 2020 (Table 3.2). In 2016, warming significantly increased GPP by 35% and increased ER by 52% under intact vegetation (Figure 3.4). When graminoids or shrubs were removed, WN significantly decreased GPP by 31% - 45% and decreased NEP by 49 - 80% (Figure 3.4). In addition, there were no interactions of three changes on ER in 2016 except the interaction of warming and N addition (Table 3.2).

In 2020, N addition significantly decreased GPP by 46 - 52% except under the condition of shrub removal (-Sh), and it decreased NEP by 65% and 75% under the conditions of Intact and -Gr, respectively (Figure 3.5). Likewise, WN significantly decreased GPP by 68% and decreased NEP by 106% under the condition of -Gr. In addition, there were no interactions of three changes on ER in 2020 (Table 3.2).

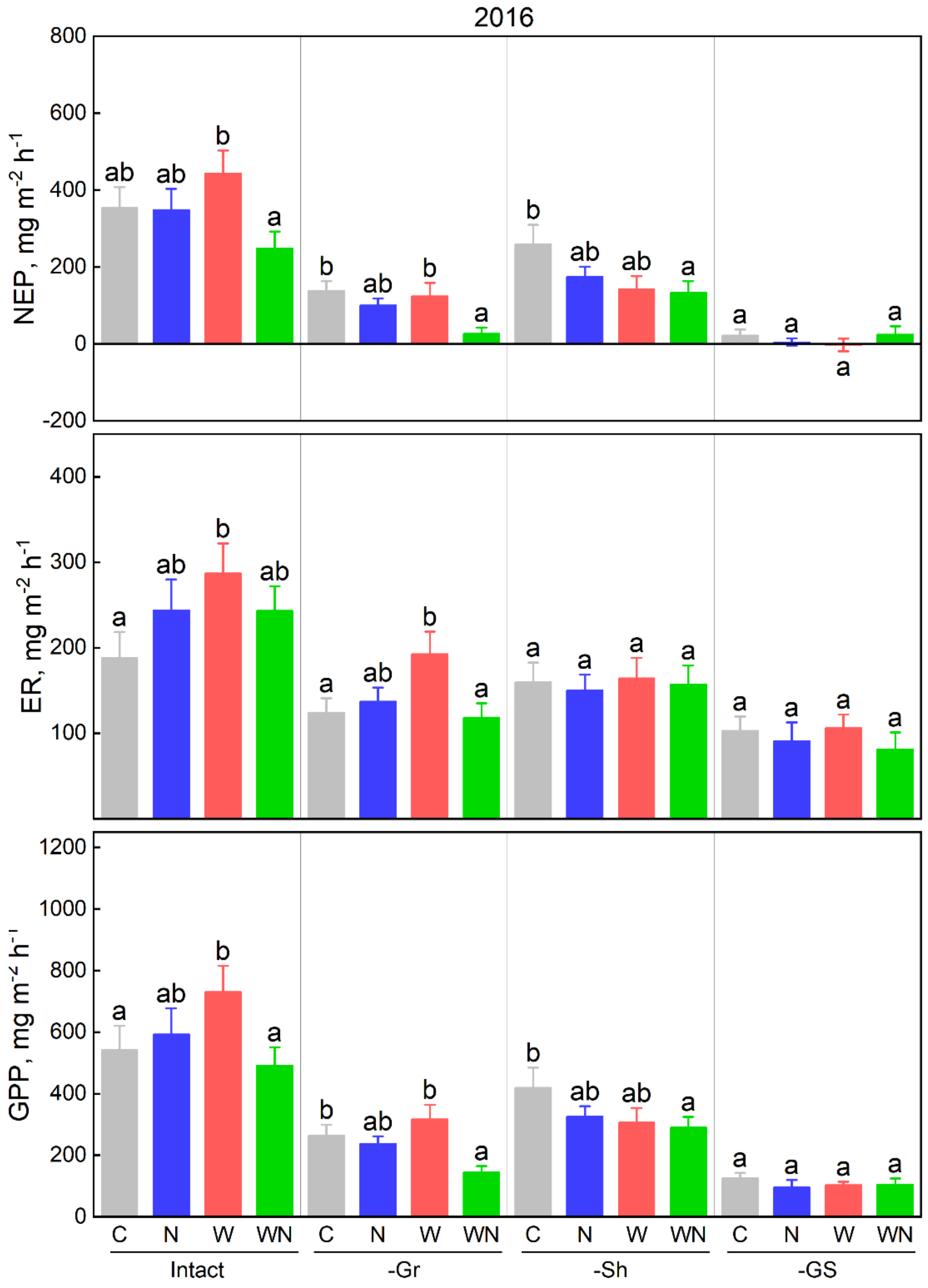

Figure 3.4 Mean $CO_2$ fluxes under different treatments during the growing season of 2016. Error bars represent standard error. The different letters represent the significant difference between the treatments under the same vegetation composition (Tukey's post hoc tests, $P<0.05$). "C" represents control, "W" represents warming treatment, "N" represents N addition, "WN" represents warming and N addition, "Intact" represents without vegetation removal, "-Gr"

represents the removal of graminoids, “-Sh” represents the removal of shrubs, and “-GS” represents the removal of graminoids and shrubs.

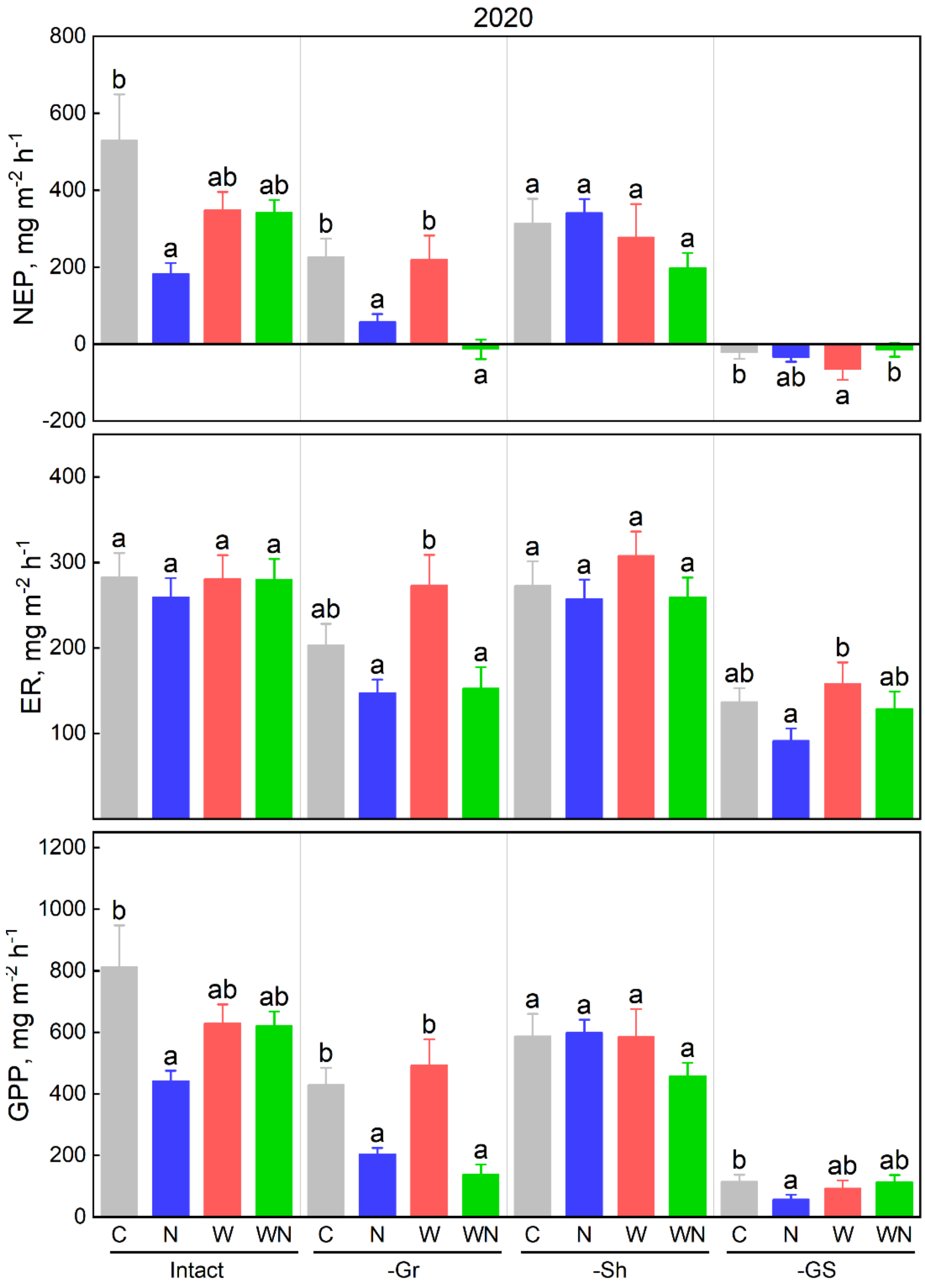

Figure 3.5 Mean $CO_2$ fluxes under different treatments during the growing season of 2020. Error bars represent standard error. The different letters represent the significant difference between the

treatments under the same vegetation composition (Tukey's post hoc tests, $P<0.05$). "C" represents control, "W" represents warming treatment, "N" represents N addition, "WN" represents warming and N addition, "Intact" represents without vegetation removal, "-Gr" represents the removal of graminoids, "-Sh" represents the removal of shrubs, and "-GS" represents the removal of graminoids and shrubs.

### 3.4.4 Relationships between $CO_2$ fluxes and environmental variables

As shown in Figure 3.6, there were significant correlations among $CO_2$ fluxes and environmental variables. After accounting for spurious correlation, the relationships between GPP and NEP, and between GPP and ER were significant ($P < 0.001$). However, these relationships cannot give us the cause-effect relationships among them and the treaments; thereby, the structural equation model (SEM) was applied. We found that warming directly increased ER and had the potential to indirectly increase ER by increasing soil temperature (Figure 3.7). N addition directly decreased GPP and increased ER. It also indirectly impacts ER by increasing soil moisture. Apart from directly decreased the GPP, removal of graminoids or shrubs indirectly impacted ER by increasing soil moisture. The high soil moisture increased the concentration of DOC and TN at 10 and 40 cm depth, which could increase ER. The environmental variables (soil temperature, moisture, and DOC) significantly related to GPP and ER could also impact NEP (Table 3.3).

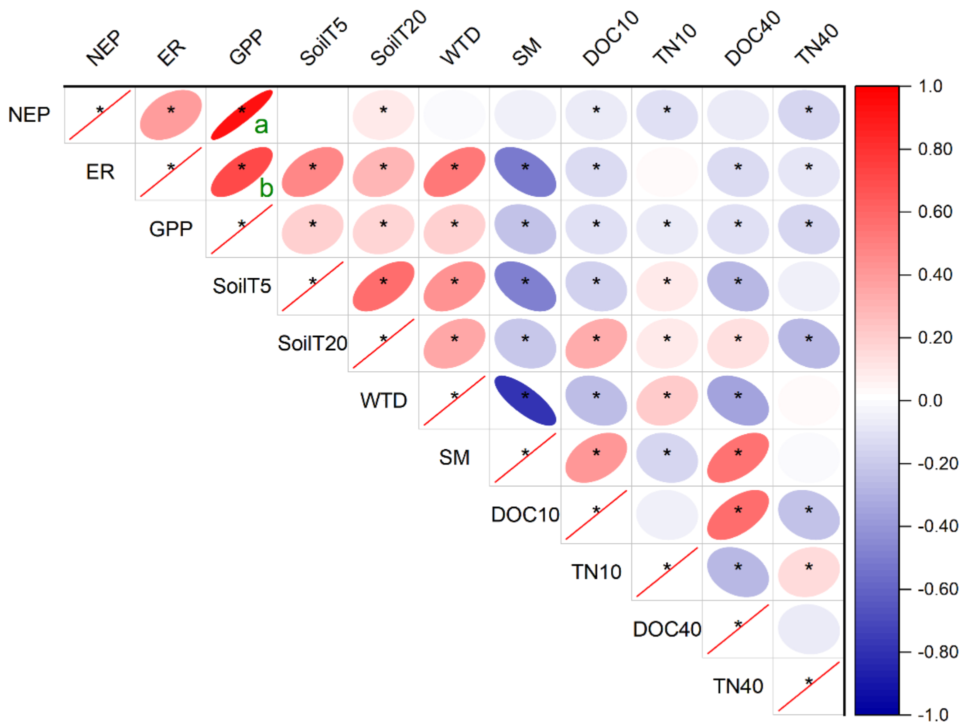

Figure 3.6 The relationships between $CO_2$ fluxes and environment variables. “SM” represents soil moisture. “WTD” represents water table depth. The number after the soil T, TN, and DOC represent the depth (cm) of the variables. “*” represents the significant relationship at the level of 0.05. “a” and “b” represent $P<0.001$ according to the bootstrap simulation method. The red straight line represents the perfect 1:1 relationship.

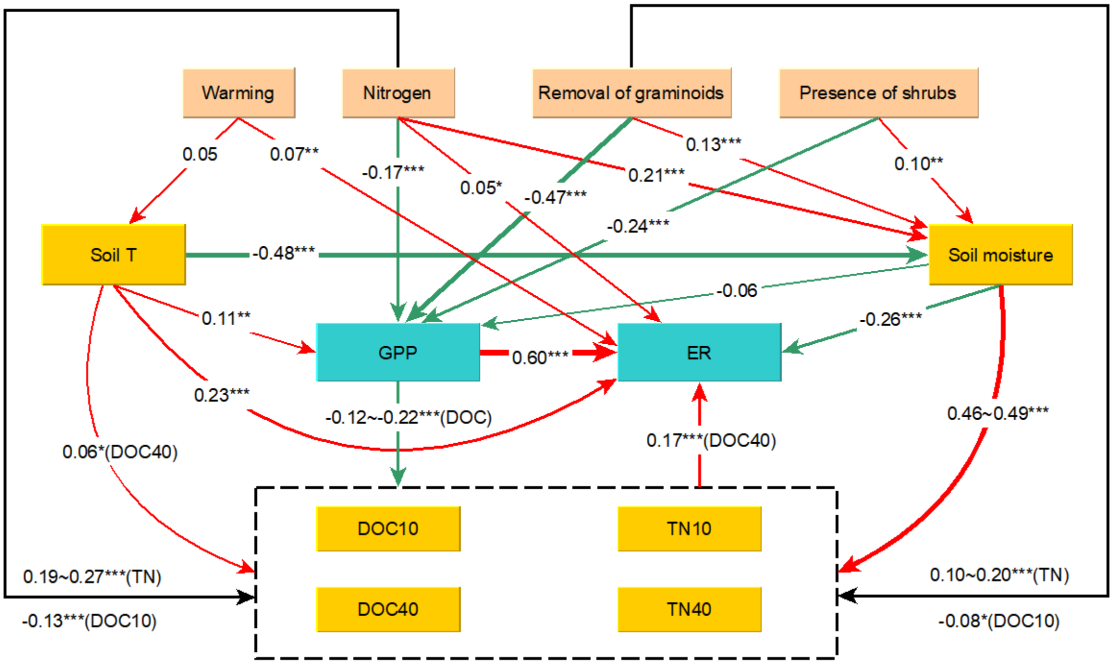

Figure 3.7 Structural equation models for testing the effects of changes in soil temperature and moisture in soils induced by the treatments on $CO_2$ effluxes and nutrients (DOC and TN). The red arrows represent positive effects, and the green arrows represent negative effects. Black lines represent they have both positive and negative effects. The width of the arrows indicates the strength of the relationship. The numbers are standardized path coefficients. "*", "**" and "***" indicate significant effect at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Models satisfactorily fitted to the data, as suggested by the Chi-square value ($\chi^2$) and Goodness of Fit Index (GFI) ($\chi^2$ = 16.864, GFI=0.996, df=13, P=0.206).

Table 3.3 Parameter estimates of the linear mixed-effects model for NEP. The bold font represents significance at $p < 0.05$. The number in the brackets represents the depth below the surface.

| | Estimate | Std. Error | df | t value | P value |
|---|---|---|---|---|---|
| (Intercept) | 45.871 | 83.846 | 612.477 | -0.547 | 0.585 |
| **SoilT5** | **-8.184** | **3.044** | **568.297** | **2.688** | **0.007** |
| **SoilT20** | **26.433** | **5.242** | **570.288** | **-5.042** | **<0.001** |
| **DOC10** | **-4.210** | **1.046** | **575.905** | **4.023** | **<0.001** |
| **DOC40** | **-3.101** | **0.838** | **566.990** | **3.702** | **<0.001** |
| TN10 | -17.805 | 11.445 | 575.195 | 1.556 | 0.120 |
| TN40 | -6.291 | 12.585 | 577.023 | 0.500 | 0.617 |
| **Soil moisture** | **3.119** | **0.552** | **579.614** | **-5.647** | **<0.001** |

## 3.5 Discussion

There is large uncertainty in evaluating the carbon sink function of peatlands under future global change, such as climate warming, elevated N deposition, and vegetation composition change. Vegetation composition plays an essential role in regulating the warming and N addition impacts on net $CO_2$ uptake in peatlands (Figure 3.8). In addition, the interactions of biotic and abiotic changes are not simply additive, which should be considered in the carbon cycling models to accurately project carbon uptake in peatlands.

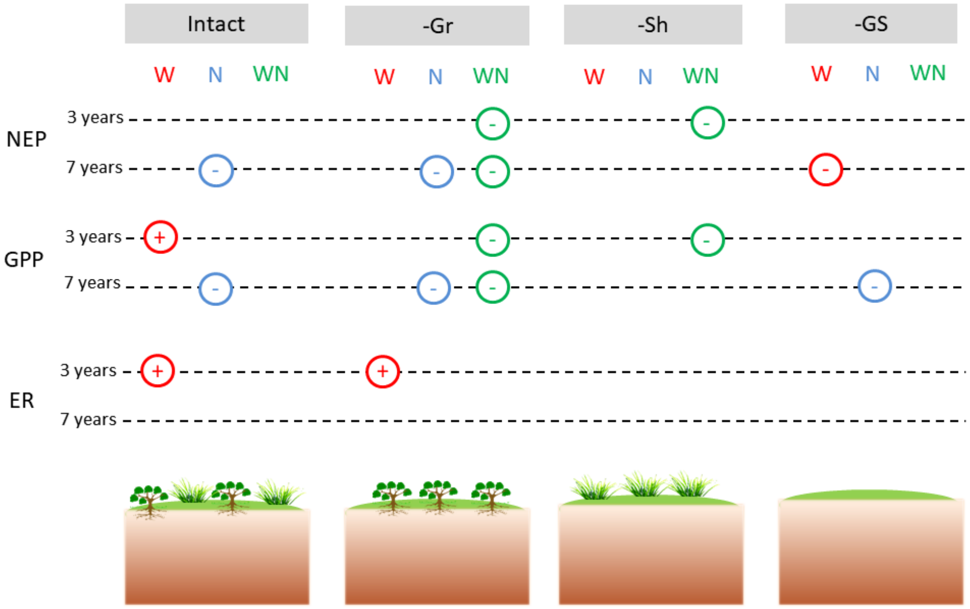

Figure 3.8 The interactions of climate warming, N addition, and vegetation composition change on NEP, ER, and GPP in the bog. "+" in the circle indicates the value is larger than the control under the same vegetation composition, and "-" in the circle indicates the value is smaller than the control under the same vegetation composition. "C" represents control, "W" represents warming treatment, "N" represents N addition, "WN" represents warming and N addition, "Intact" represents without vegetation removal, "-Gr" represents the removal of graminoids, "-Sh" represents the removal of shrubs, and "-GS" represents the removal of graminoids and shrubs.

### 3.5.1 The effect of abiotic changes on $CO_2$ fluxes

In this study, warming did not impact NEP, which is opposite to the first hypothesis that warming increases net $CO_2$ uptake. The reasons are different between the short-term (2016) and long-term scale (2020). We found that warming significantly increased GPP and ER under intact vegetation in 2016, which can be the reason for the unaltered NEP. This phenomenon was not observed in

2020. The non-significant effects of warming on carbon fluxes can be attributed to the dry condition in 2020, which could override the warming effect (Pearson et al., 2015). The dry condition could increase decomposition, thus reducing DOC concentration and increasing the C:N ratio. These results indicate that climate warming has limited impacts on net $CO_2$ uptake in peatlands, regardless of N addition and vegetation composition.

In this study, three years of N addition did not significantly reduce the GPP and NEP under intact vegetation, while seven years of N addition significantly decreased them, which is in line with other studies and can be attributed to the nutrient toxicity and decrease of *Sphagnum* moss cover (Bubier et al., 2007; Juutinen et al., 2010; Larmola et al., 2013). The reduction of GPP and net $CO_2$ uptake were detected after five years of N addition (Bubier et al., 2007; Juutinen et al., 2010). Importantly, we found that combined warming and N addition (WN) did not impact GPP or NEP under intact vegetation, which supports the second hypothesis. The results imply that the carbon sink function of peatlands might not be altered under future climate warming and N deposition. However, vegetation composition change could alter these effects of climate warming and elevated N deposition.

### 3.5.2 The effect of biotic changes on $CO_2$ fluxes

We found that -Gr and -GS significantly decreased GPP, ER, and NEP in both years, which is consistent with the previous studies and can be attributed to the decrease in photosynthesis (Gavazov et al., 2018; Ward et al., 2013). If the peatland shifts to a shrub-dominated ecosystem in the future (Bragazza et al., 2015), the carbon sink function of peatland would be decreased. However, after seven years of shrub removal, it did not impact GPP, ER, and NEP. These results partly support the third hypothesis that the negative effect of graminoid and/or shrub removal would be decreased with experimental duration. Possibly, the reduction of labile litter from shrub removal treatment would decrease decomposition and $CO_2$ emission, which may counteract parts of GPP decrease. This explanation should be taken with caution because we did not observe the change of GPP and ER under -Sh treatment. The other possible reason is that seven years of -Sh could reduce the competition with graminoids for nutrients, which might increase the graminoid growth and offset the negative effect of -Sh on GPP and ER. If peatlands shift to a graminoid-dominated ecosystem in the future (Dieleman et al., 2015), the carbon sink function of peatland would be decreased on a short-term scale (less than 3 years) but recover on a long-term scale (~ 7 years).

### 3.5.3 The interactions of abiotic and biotic changes on $CO_2$ fluxes

Warming slightly decreased NEP under the removal of graminoids and shrubs (-GS) in 2020, which partly supports the fourth hypothesis that the positive warming effect on the net ecosystem $CO_2$ uptake would be weakened under the condition of graminoid and/or shrub removal. Because the positive warming effects on GPP and ER were weakened by the vegetation removal in 2016, the warming impact on NEP was not altered by vegetation composition change. Owing to the dry condition in 2020, the warming effect on GPP, ER, and NEP could be overridden (Pearson et al., 2015). However, our results are inconsistent with the previous study (Ward et al., 2013). They have reported that warming could increase the net $CO_2$ uptake under the presence of shrubs when the graminoids were removed because of the greater increase in photosynthesis relative to respiration, while warming could decrease the net $CO_2$ uptake under the presence of graminoids because of the greater increase in rates of respiration relative to photosynthesis. The inconsistent results could be attributed to the characteristics of different species in bogs (Kool and Heijmans, 2009). In the study of Ward et al. (2013), the dominant shrub species was *Calluna vulgaris*, and the dominant graminoid species was *Eriophorum vaginatum*, which is different from the species at our study site. The dominant shrub species in this study are *Chamaedaphne calyculata*, *Gaylussacia bigeloviana*, *Vaccinium oxycoccos*, and *Andromeda glaucophylla*, while the dominant graminoid species are *Rhynchospora alba* and *Trichophorum cespitosum*. Their different photosynthesis and respiration rates might be attributed to the inconsistent results. Therefore, not only the vegetation composition but also the species characteristics should be taken into account in order to accurately predict net $CO_2$ uptake and evaluate carbon sink function of peatlands under future global change.

The negative effect of N addition on NEP was observed under intact vegetation in 2020. This negative effect was strengthened only under the condition of -Gr, which partly supports the fifth hypothesis, and can be ascribed to the reduction of *Sphagnum* moss cover. The decrease of GPP by N addition under the condition of -Gr could support this statement. It should be noted that N addition did not reduce the GPP under the condition of -Sh. The possible reason is that the removal of shrubs (-Sh) alleviates the competition with graminoids for nutrients, light, and space. This can be supported by a previous study, which has documented that shrubs are stronger competitors than graminoid species in peat bogs under high nutrient conditions (Kool and Heijmans, 2009). Therefore, the photosynthesis of graminoids could be increased after shrubs were removed, which might offset the GPP reduced by the shrub removal and the decrease of *Sphagnum* moss cover under N addition. In addition, although the negative effect of N addition on GPP was observed under -GS, the NEP was not significantly altered. This result could be attributed to the slight reduction of ER. With the reduction of *Sphagnum* moss cover, the respiration of the mosses would be decreased, and the dead *Sphagnum* moss is difficult to be decomposed (Palozzi and Lindo, 2017). Consequently, the decomposition and respiration could be decreased. Indeed, N addition was observed to decrease ER by 33% under -GS, although this effect was not statistically significant.

Combined warming and N addition (WN) significantly decreased NEP under the removal of graminoids (-Gr) and removal of shrubs (-Sh), but this effect was not observed under the removal of graminoids and shrubs (-GS). This result partly supports the sixth hypothesis that WN would decrease the net $CO_2$ uptake under the condition of graminoid and/or shrub removal, and can be attributed to the increase of negative N effect and decrease of the positive warming effect on GPP. If peatlands shift to shrub-dominated ecosystems in the future (Bragazza et al., 2015; Kool and Heijmans, 2009), the carbon sink function might be weakened under future climate change and N deposition. Interestingly, after seven years of -Sh, WN did not significantly decrease NEP, which is opposite to the hypothesis. This result can be attributed to the non-significant change of GPP and ER under WN. The warming effect could be masked by dry conditions, while the negative effect of N addition could be offset by the increase of graminoid photosynthesis. Therefore, if graminoids are the dominant vegetation in peatlands in the future (Dieleman et al., 2015), the carbon sink function of peatlands might be unaltered under climate warming and elevated N deposition.

### 3.5.4 The factors controlling $CO_2$ fluxes

After accounting for spurious correlation, NEP significantly correlated with GPP, suggesting that vegetation composition is more important than other changes (climate warming and elevated N deposition) in regulating the net $CO_2$ uptake. This can be supported by the structural equation modeling (SEM) in Figure 3.7 that the removal of graminoids or shrubs was negatively related to GPP. Elevated N deposition is another essential factor following vegetation composition, because it is also negatively related to GPP but to a less extent than vegetation removal. Compared with vegetation composition and elevated N deposition, climate warming appears to be less important in modulating the net $CO_2$ uptake in peatlands because of its weak correlations with GPP and ER.

Therefore, if vegetation composition and N deposition are not taken into account, there is large uncertainty to project the net $CO_2$ uptake in peatlands. In addition, soil temperature, moisture, and DOC could be the essential controls for NEP and ER under future global change. However, the effect of soil moisture on GPP was not statistically significant, which is inconsistent with previous studies (Ballantyne et al., 2013; Laine et al., 2019). They have reported that dry conditions could increase GPP due to the high oxygen and nutrient availability to plant roots (Ballantyne et al., 2013; Laine et al., 2019). With the removal of vascular plants (graminoids or shrubs) in this study, the dry condition impacts GPP via providing oxygen and nutrient for roots would be weakened. It should be noted that the effect of soil moisture on ER remains strong in this study; thus, the NEP can be reduced considerably under dry conditions.

## 3.6 Conclusion

The net $CO_2$ uptake in peatlands under global change (climate warming, elevated N deposition, and vegetation composition change) is uncertain. This field study showed that climate warming had limited impacts on net $CO_2$ uptake, while elevated N deposition decreased net $CO_2$ uptake on a long-term scale (7 years). Vegetation composition plays a critical role in regulating carbon uptake in peatlands. If shrubs are the dominant vegetation in peatlands, the net $CO_2$ uptake would be significantly decreased. If graminoids to be the dominant vegetation in peatlands, the net $CO_2$ uptake would be decreased on a short-term scale (3 years) and recover on a long-term scale (7 years). Taking future climate warming and elevated N deposition into account, the net $CO_2$ uptake in peatlands under the shrub dominant conditions would decrease, while the net $CO_2$ uptake would not change under the graminoid dominant conditions. Furthermore, these impacts should not be ignored in order to accurately project $CO_2$ fluxes in peatlands and evaluate its carbon sink function.

### 3.7 Acknowledgements

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# CHAPTER 4. Counteractions between biotic and abiotic factors on methane dynamics in a boreal peatland: vegetation composition change vs warming and nitrogen deposition

## 4.1 Abstract

Methane ($CH_4$) fluxes in boreal peatlands are vulnerable to global change, including climate warming, elevated nitrogen (N) deposition, and vegetation composition change. However, few studies focus on the interactions of these factors, particularly combining all of them, which leads to a large degree of uncertainty in predicting $CH_4$ emissions from peatlands in the future. In this study, experimental warming, N addition, and vegetation composition change were conducted for five years at a boreal peatland in western Newfoundland, Canada. We found that graminoid absence substantially decreased $CH_4$ emissions by 72% owing to the reduction of root exudates for $CH_4$ production and aerenchyma for $CH_4$ transport; however, this negative effect disappeared under the combination of warming and N addition, which can be attributed to the elevated temperature sensitivity of $CH_4$. Additionally, we observed that vegetation productivity was the main control when the graminoid was present, but not the major control when the shrub was present only, suggesting the proper parameters should be selected under different vegetation compositions so as to accurately project $CH_4$ emissions in the context of climate change. Compared with the studies focusing on one or two environmental changes, this experiment is valuable for providing empirical evidence on the parameterization of $CH_4$ fluxes in the biogeochemical model of boreal peatlands and in the global coupled climate-carbon model.

## 4.2 Introduction

Methane ($CH_4$) plays an essential role in climate warming due to its high global warming potential (IPCC, 2013). Boreal peatlands have been reported to be important biological sources of atmospheric $CH_4$, with an annual contribution of ~46 Tg $CH_4$-C (Lai, 2009; Mikaloff-Fletcher et al., 2004). Human activities and climate change have resulted in numerous environmental changes, including global warming, elevated nitrogen (N) deposition, and vegetation composition change (Dean et al., 2018; Dieleman et al., 2015). Nevertheless, it remains unclear how the $CH_4$ fluxes in boreal peatlands respond to these changes, and especially the interactions of them.

Although previous studies have focused on the effects of abiotic factors on $CH_4$ fluxes, such as climate warming and nitrogen (N) deposition, the results are inconsistent. Some studies have reported that climate warming increases $CH_4$ emissions from peatlands, owing to the stimulation of root exudates and microbial activity (Granberg et al., 2001; Turetsky et al., 2008; Updegraff et al., 2001; Yang et al., 2014), while some studies did not observe this phenomenon (Johnson et al., 2013; Pearson et al., 2015; Peltoniemi et al., 2016; Verville et al., 1998). Likewise, Gao et al. (2014) demonstrated that N addition decreased $CH_4$ emissions from peatlands due to the positive effect of ammonium and nitrate on $CH_4$ oxidation under the N-limited condition, while some studies have reported that there is an insignificant effect or a positive effect (Juutinen et al., 2018; Keller et al., 2005; Lund et al., 2009; Saarnio & Silvola, 1999; Silvola et al., 2003). The discrepancy of these results could be caused by different peatland types, local environments, forms of added nitrogen and experimental durations (Gong et al., 2020). To generalize the effects of climate warming and N deposition on $CH_4$ emission from peatlands, more research is urgently needed.

Indirectly, warming and N deposition can regulate $CH_4$ emissions through changing vegetation composition. Graminoids, shrubs, and bryophytes are three typical plant functional types in peatlands (Rydin & Jeglum, 2013). Along with warming and N deposition, vegetation composition in peatlands can shift from *Sphagnum*-dominated to graminoid-dominated (Dieleman et al., 2015) or shrub-dominated ecosystems (Bragazza et al., 2015; Bubier et al., 2007; Keller et al., 2005). More seriously, anthropogenic activities (such as drainage, burning, and grazing) pose another threat to vegetation composition. For instance, drainage can decrease the coverage of *Sphagnum* moss on hummocks and facilitate the invasion of sedges on lawns in a poor fen (Strack et al., 2006). Burning and grazing can promote fast-growing graminoids over slower-growing ericaceous shrubs and mosses (Ward et al., 2007). These vegetation changes considerably affect the processes of $CH_4$ production, transportation, and consumption. The presence of graminoids increases $CH_4$ emission by providing root exudates for $CH_4$ production and stimulating $CH_4$ transport through aerenchyma (Armstrong et al., 2015; Green & Baird, 2012; Leroy et al., 2017; Ward et al., 2013). Oxygen can also be transported to the deep peat through aerenchyma, which retards $CH_4$ production and facilitates $CH_4$ consumption (Girkin et al., 2020). Given shrubs can provide root exudates, shrub presence is expected to increase $CH_4$ production. Nevertheless, this positive effect has not been widely observed in peatland ecosystems (Armstrong et al., 2015; Robroek et al., 2015; Ward et al., 2013). Instead, Ward et al. (2013) found a slightly negative effect on $CH_4$ emissions, which may be due to the competition for nutrients with microbes and other vegetation (Nielsen et al., 2017). As mentioned before, both warming and N deposition can increase nutrient content in peat by stimulating microbial activity and decomposition. Consequently, nutrient competition between shrubs and other organisms could be alleviated. This alleviation leads to one critical question, i.e., Will the negative effect of shrub presence on $CH_4$ emissions disappear and a positive effect occur under future climate warming and elevated N deposition?

Given these environmental changes (warming, N deposition increase, and vegetation composition change) occur simultaneously, in the future, investigating their interactions on $CH_4$ emissions is warranted. However, studies focusing on the combined effects of biotic factors and abiotic factors on $CH_4$ emissions in boreal peatlands are very limited. Ward et al. (2013) and Luan et al. (2019) found that the effect of warming on $CH_4$ can be modulated by vegetation composition change, while Nielsen et al. (2017) demonstrated that the combination of warming and shrub removal did not significantly affect $CH_4$ fluxes. It should be noted that the experiments in those studies were conducted only in one or two years, which leaves much uncertainty concerning the long-term effects of these global changes (Dijkstra et al., 2012). Moreover, the combination of these three global changes (warming, N addition, and vegetation composition change) on $CH_4$ fluxes is less known, which further increases the uncertainty in predicting $CH_4$ emission from peatlands.

To fill the knowledge gap, experimental warming, N addition, and vegetation composition change were conducted for five years in a bog in Robinsons, western Newfoundland, Canada. We investigated the combination of abiotic (warming and N addition) and biotic factors (vegetation composition) on $CH_4$ emissions, explored the possible underlying mechanisms, and evaluated the major controls for $CH_4$ emissions. We hypothesized that (1) the absence of graminoids reduces $CH_4$ emissions under the condition of warming and/or N addition due to the reduction of root exudates for $CH_4$ production and aerenchyma for $CH_4$ transport; (2) the absence of shrubs also reduces $CH_4$ emissions under the condition of warming and/or N addition due to the reduction of root exudates; (3) the absence of both of them considerably reduces $CH_4$ emissions under warming and/or N addition. Furthermore, temperature and water table level are two important environmental controls on $CH_4$ flux, but they only explain a small part (~44%) of $CH_4$ variation in bogs (Turetsky et al., 2014) and, currently, variation of $CH_4$ fluxes cannot be adequately explained by environmental conditions alone (Levy et al., 2012). Given the significant relationship between gross primary productivity (GPP) and $CH_4$ fluxes (Lai et al., 2014), we hypothesized that (4) GPP remains the essential factor and is more important than abiotic factors (water table level, soil temperature, and nutrient availability) in regulating $CH_4$ fluxes under global change.

## 4.3 Methodology

### 4.3.1 Study site and experimental design

This study was conducted at an area of oligogenic, ombrotrophic blanket bog, located in Robinsons, western Newfoundland, Canada (48°15′46″ N, 58°39′21″ W). This area is characterized by a boreal climate with annual precipitation of 1340 mm (1981-2010). The daily maximum temperature was 20.6 °C and the daily minimum temperature was -10.7 °C during the last three decades (1981-2010). These climate data were obtained from the nearest weather station in Stephenville, around 30 km away from the site (https://climate.weather.gc.ca). The pH and the depth of peat were 4.5 and 3 m, respectively (Luan et al., 2019). There were three plant functional types: bryophytes, graminoids, and dwarf shrubs. The details of the vegetation information can be found in Luan & Wu (2015). The dominant vegetation in the study area consists of graminoids (*Rhynchospora alba* and *Trichophorum cespitosum*), shrubs (*Chamaedaphne calyculata*, *Gaylussacia bigeloviana*, *Vaccinium oxycoccos* and *Andromeda glaucophylla*), and bryophytes (*Sphagnum* spp., liverwort).

We established a factorial design comprising the manipulation of warming, N deposition, and vegetation composition change in the spring of 2014. There were sixteen treatments in this study: control (C); warming (W); N addition (N); removal of shrubs (-Sh); removal of graminoids (-Gr); removal of graminoids and shrubs (-GS); warming and N addition (WN); warming and removal of shrubs (W-Sh); warming and removal of graminoids (W-Gr); warming and removal of shrubs and graminoids (W-GS); N addition and removal of shrubs (N-Sh); N addition and removal of graminoids (N-Gr); N addition and removal of graminoids and shrubs (N-GS); warming, N addition and removal of shrubs (WN-Sh); warming, N addition and removal of graminoids (WN-Gr); and warming, N addition and removal of graminoids and shrubs (WN-GS). We established four replicate blocks, and each block had sixteen plots (2 m × 2 m). Sixteen treatments were randomly arranged into the plots within each block. The buffer zones between adjoining plots were at least 2 m, and the buffer zones between replicate blocks were about 6 m. Open-top chambers (OTCs) were installed in the field to simulate the warming environment (Marion et al., 1997). N fertilization was applied using ammonium nitrate ($NH_4NO_3$) that was dissolved in 2 L of water from a nearby open pool, about 10 meters away from the experimental plots, and we poured it onto the N fertilization plots bimonthly from May to September each year to simulate atmospheric N deposition. The same amount of open pool water was poured into the control plots. The rate of N addition was 6.4 g N $m^{-2}$ $yr^{-1}$ in order to establish non-N-limited conditions for this nutrient-poor bog (Reay et al., 2008). Vegetation removal was undertaken manually. The shoots of shrubs and graminoids were cut back to the litter layer level in early May and early July every year since 2014. During the interval, we also did some clipping, occasionally, when we found the occurrence of graminoids and shrubs in those plots. This removal experiment was effective and useful for understanding the ecosystem effects of vegetation composition change (Díaz et al., 2003). We did not remove the bryophyte/lichen functional type because of its substrate nature in this ecosystem, and we intended to avoid any soil disturbance. Plots were left to settle for a year before sampling to minimize the effects of decomposition from roots.

### 4.3.2 Measurement of $CH_4$ fluxes and environmental parameters

The measurement methods of $CH_4$ fluxes and environmental parameters have been described in detail in the studies of Luan et al. (2019) and Gong et al. (2019). We only presented them briefly here. Gas samples were taken from opaque chambers (50 cm in height and 26.3 cm in diameter, cover ~0.05 $m^2$) fitted to the groove of the PVC (polyvinyl chloride) collars, which were inserted into the soil to the depth of 10 cm permanently in May 2014. We used four 60-mL syringes to collect gas samples at four intervals during the 30 minutes of chamber closure: immediately upon the closure of the chamber, and 10, 20, and 30 minutes after chamber closure. The measurements were conducted biweekly during the growing season from 2016 to 2018. In total, we have 8 sampling rounds in 2016, 8 sampling rounds in 2017, and 6 sampling rounds in 2018. $CH_4$ fluxes were analyzed by a gas chromatography method. $CH_4$ fluxes were calculated by:

$$F = \frac{dC}{dt} \cdot \rho \cdot \frac{P}{P_0} \cdot \frac{T_0}{T} \cdot \frac{V}{A} \tag{1}$$

where $F$ is $CH_4$ flux (mg $m^{-2}$ $h^{-1}$), $dC/dt$ is the change of concentration over time during the 30 minutes of sampling (ppm $s^{-1}$); $\rho$ represents the gas density (mg $cm^{-3}$). $P$ and $T$ are the atmospheric pressure (Pa) and the air temperature (K) in the chamber over the sampling duration, respectively; $P_0$ and $T_0$ are the atmospheric pressure (Pa) and air temperature (K) under standard conditions, respectively. $V$ is the volume of the chamber ($m^3$), and $A$ is the area covered by the chamber ($m^2$). We used linear regression to find $dC/dt$. Fluxes were accepted if $R^2 \geqslant 0.8$. In order to avoid overestimating the $CH_4$ fluxes, when $R^2 < 0.8$ and the normalized root mean square error (NRMSE) < 0.1, the fluxes were also accepted (Minke et al., 2016; Gong et al., 2019). Therefore, 15% of the total fluxes (1,408) were rejected.

Concurrent with gas sampling, soil pore water samples at ~10 cm depth in each plot were collected using the MacroRhizons sampler (Rhizosphere Inc., Netherlands). Water samples at 40 cm depth were collected using 60 mL syringes from a perforated PVC tube, with a sealed bottom and a capped top to prevent precipitation from entering the tube, where we only perforated the bottom 5 cm of the tube. The perforated PVC tube was inserted into 40 cm depth prior to the collection. These water samples were filtered by 0.45 µm membrane before analyzing dissolved organic carbon (DOC) and dissolved total nitrogen (TN) with a Shimadzu TOC-LCPH/TN analyzer (Shimadzu Inc., Japan).

During each gas sampling campaign, we measured soil temperature at 5 cm and 20 cm depth using a soil thermometer (Fisher Scientific Inc., Canada) and soil moisture at 5 cm depth using a soil moisture sensor (ProCheck, Decagon Devices Inc., USA). After 10 min of chamber closure, a soil thermometer was inserted into the peat at 5 cm depth near the chamber, and we recorded the soil temperature when the number was stable. Subsequently, the thermometer was inserted into the peat of 20 cm depth, and we recorded the soil temperature when the number was stable. Therefore, the recorded temperatures in the middle time of gas sampling (10~20 min after chamber closure) were used to analyze the temperature sensitivity of flux. The soil moisture sensor was calibrated to peat conditions before the measurement. In addition, we measured water table depth, i.e., the water table level below ground, from dip-wells made of 1 m-long perforated PVC pipes installed at each plot (negative values indicate water levels above the peat surface). Furthermore, we used a USB temperature logger (Lascar Electronics Ltd., UK) to continually record air temperature at vegetation canopy height every 30 minutes. One temperature logger was installed at a randomly selected warming plot and the other was installed at a randomly selected control plot.

### 4.3.3 Measurement of gross primary productivity

Given vegetation plays an important role in $CH_4$ fluxes, the relationship between gross primary productivity (GPP) and $CH_4$ was investigated in this study. We measured $CH_4$ flux and $CO_2$ exchange rates of each plot simultaneously by an Ultra-Portable Greenhouse Gas Analyzer (Los Gatos Research, CA, USA), which was connected to a transparent chamber or an opaque chamber. GPP was calculated from the difference between ecosystem respiration (ER), measured by an opaque chamber, and net ecosystem exchange (NEE), measured by a transparent chamber. We measured ER and NEE in the growing season of 2016, biweekly, from 10:00 to 16:00 local time. The opaque chamber blocked the light coming into the chamber and thus no photosynthesis occurred during the measurement. Therefore, the $CO_2$ flux measured by the opaque chamber was equivalent to ER. However, light can penetrate the transparent chamber and thus photosynthesis occurred during the measurement. Therefore, the $CO_2$ flux measured by the transparent chamber represented a NEE, a balance between GPP and ER. Normally only about a 1-minute break occurred between the measurement from the transparent chamber (for NEE) and the opaque chamber (for ER). Therefore, the GPP can be calculated from the measurement of NEE and ER at the same plot. The GPP was only available in 2016 due to the malfunction of the instrument in other years. During each measurement, we placed the chamber on the collar and sealed it with water. A fan was fixed on the top of the chamber to mix and cool the air. The gas concentration was collected at 1 Hz rate during a measurement period of 3 minutes after steady-state conditions were reached. Therefore, during the 3 minutes of chamber closure, we obtained 180 measurements of $CO_2$ and $CH_4$ concentration. The portable greenhouse gas analyzer (Los Gatos Research Inc., USA) has the simultaneous measurement of $CO_2$ and $CH_4$ concentration, so we can analyze the relationship between $CH_4$ fluxes and GPP. For this analysis, we used the $CH_4$ flux calculated from the transparent chamber. The $CO_2$ fluxes and $CH_4$ fluxes were calculated by equation (1) based on a linear regression using all 180 measurements of $CO_2$ and $CH_4$ concentration.

### 4.3.4 Statistical analysis

The linear mixed-effects model was used to test whether $CH_4$ fluxes, GPP, and environmental variables (soil temperature, soil moisture, water table depth, total nitrogen (TN) and dissolved organic carbon (DOC) in water samples) differed between the treatments (warming, N addition, and vegetation composition change). Tukey's post hoc tests were used to determine the differences between treatments. The model was constructed with the whole dataset. The measurement block and date were considered as random factors. In analysis of the variation in $CH_4$ fluxes, the potential fixed predictors, including soil temperature, water table depth, TN, and DOC were added to the model one by one. The AIC value (Akaike information criterion) was used to evaluate whether the addition of a fixed predictor resulted in a better model than before. The measurement block and date were considered as random factors. The residuals were normally distributed around a mean of zero in all the models. All statistical analyses were performed in the R version 3.5.1 (R Core Team, 2018) with the "lmerTest" packages. In addition, redundancy analysis (RDA) was used to determine the relative importance of biotic and abiotic factors for $CH_4$ fluxes. This was performed in the R version 3.5.1 (R Core Team, 2018) with the "vegan" package. Regression analysis between $CH_4$ flux and soil temperature, and between $CH_4$ flux and GPP, were performed in SigmaPlot 12.5 (Systat Software, San Jose, CA).

## 4.4 Results

### 4.4.1 Variation in environmental variables

The open-top chambers effectively increased the average air temperature by 1.2 - 2.6 °C. Regardless of nitrogen addition and vegetation composition change, the average soil temperature was also significantly increased by 0.6 °C at 5 cm depth ($F_{1,\,1335}$ = 36.899, P<0.001) and 0.3 °C at 20 cm depth ($F_{1,\,1335}$ = 28.833, P<0.001) under the warming treatment during the growing seasons of 2016-2018. Along with soil temperature increase, soil moisture was significantly reduced by 6% under the warming treatment ($F_{1,\,1004}$ = 12.846, P<0.001). The mean water table depth (WTD) was ~9 cm during the growing seasons of 2016-2018 (Figure 4.1). The range of WTD was from 2 cm to 28 cm.

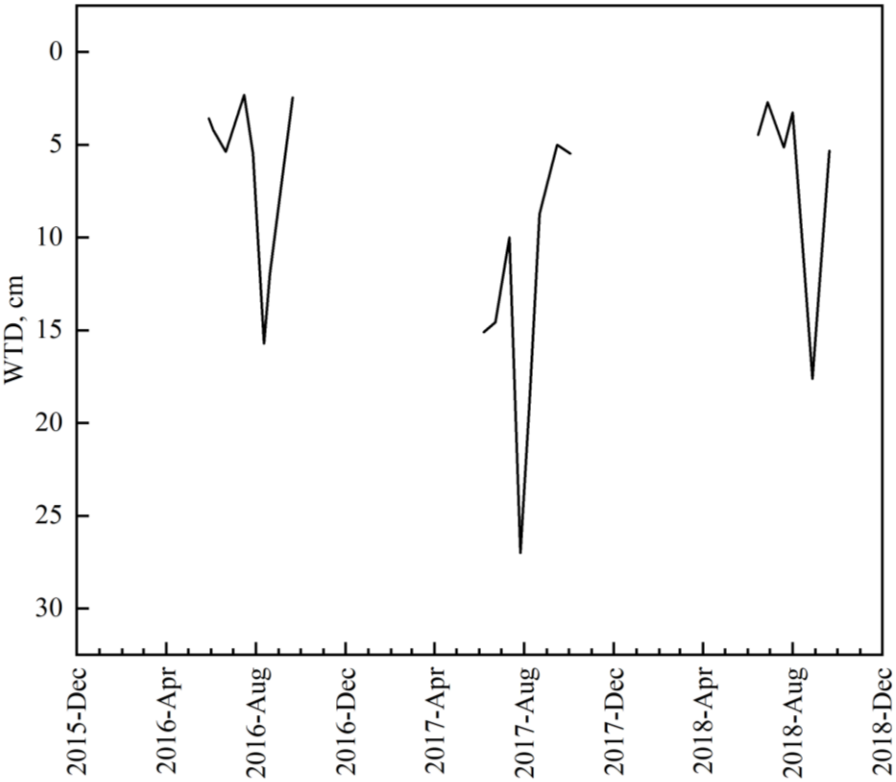


Figure 4.1 The dynamics of water table depth during the growing seasons of 2016-2018.

As shown in Table 4.1, the concentration of DOC at 40 cm depth was increased by 16% under the warming treatment (Turkey's test, P<0.05). The concentration of TN at 40 cm depth was increased by 31% under N addition (Turkey's test, P<0.05). The concentration of TN at 40 cm depth was increased by 51% and DOC at 40 cm depth was increased by 18% under the combination of warming and N addition (WN). The concentration of TN at 10 cm depth and 40 cm depth was increased by 18% - 49% under the combination of N addition and vegetation removal (N-Gr, N-Sh, N-GS). The DOC concentration at 40 cm depth was increased by 10 - 22% (Turkey's test, $P<0.05$) and the TN concentration at 40 cm depth was increased by 36 - 62% (Turkey's test, $P<0.05$) under the combined effect of warming, N addition, and vegetation removal (WN-Gr, WN-Sh and WN-GS).

Table 4.1 The concentration of total nitrogen (TN) and dissolved organic carbon (DOC) at 10 cm and 40 cm depth under different treatments during the growing seasons of 2016-2018 (Mean ± S.E.).

| | **DOC (10 cm)** | **TN (10 cm)** | **DOC (40 cm)** | **TN (40 cm)** |
|---|---|---|---|---|
| C | 37.89±1.00ab | 1.16±0.07abcd | 31.90±1.48a | 1.68±0.12ab |
| C-Gr | 37.49±1.02ab | 1.19±0.06cd | 36.07±1.66abcde | 1.90±0.16bcd |
| C-Sh | 36.89±0.94a | 1.12±0.06abc | 34.41±1.31ab | 1.73±0.11b |
| C-GS | 38.76±1.15ab | 1.35±0.10cd | 35.29±1.42abcde | 1.89±0.15bc |
| W | 37.18±0.92a | 1.00±0.07a | 37.15±1.54bcdef | 1.41±0.11a |
| W-Gr | 37.48±0.95ab | 1.05±0.05abc | 35.01±1.35abc | 1.84±0.11bcde |
| W-Sh | 37.93±1.06ab | 0.96±0.05ab | 34.42±1.53abcd | 1.70±0.12ab |
| W-GS | 36.96±0.95a | 1.22±0.07cd | 34.00±1.26ab | 1.80±0.13b |
| N | 38.09±1.08ab | 1.28±0.10cd | 35.69±1.44abcde | 2.20±0.13cdef |
| N-Gr | 38.31±1.05ab | 1.66±0.15efg | 37.45±1.59cdef | 2.25±0.19def |
| N-Sh | 38.94±1.07ab | 1.37±0.1cde | 36.59±1.36bcdef | 2.22±0.14cdef |
| N-GS | 39.36±1.16b | 1.72±0.13fgh | 36.02±1.52bcdef | 2.50±0.17f |
| WN | 37.79±1.09ab | 1.53±0.13def | 37.53±1.60ef | 2.54±0.20f |
| WN-Gr | 39.22±1.01ab | 2.25±0.20h | 37.1±1.50def | 2.29±0.17ef |
| WN-Sh | 38.22±1.14ab | 1.23±0.08bcd | 35.02±1.26bcdef | 2.30±0.17cdef |
| WN-GS | 38.36±0.95ab | 1.75±0.12gh | 39.10±1.53f | 2.73±0.19f |

Note: C represents control; W represents warming; N represents nitrogen addition; WN represents warming and nitrogen addition; -Sh represents the removal of shrubs, -Gr represents the removal of graminoids, -GS represents the removal of graminoids and shrubs. Different letters represent significant differences ($p < 0.05$) between the treatments according to the Tukey's multiple comparison test.

### 4.4.2 Treatment effects on methane fluxes

Compared with the control, graminoid absence significantly reduced $CH_4$ emissions by 72% (-Gr) and 83% (-GS), respectively (Figure 4.2). Compared with warming alone (W), the combination of warming and vegetation removal considerably decreased the $CH_4$ flux by 56% (W-Gr), by 55% (W-Sh), and by 58% (W-GS). Likewise, compared to nitrogen addition (N) alone, the combination of nitrogen addition and graminoid removal decreased the $CH_4$ flux by 68% (N-Gr) and by 62% (N-GS). However, compared with the combination of warming and N addition (WN), only WN-GS significantly reduced $CH_4$ flux by 50%.

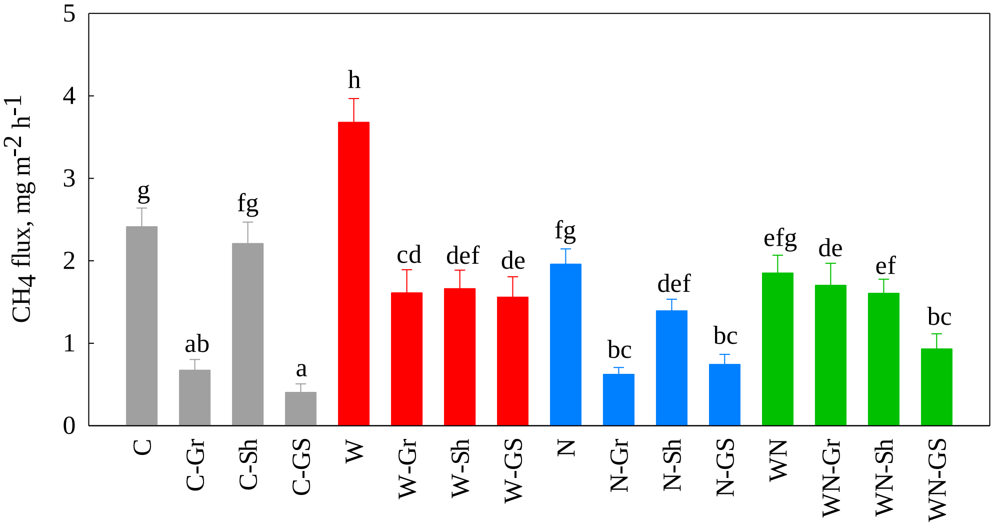


Figure 4.2 Average $CH_4$ fluxes in treatments during the growing seasons of 2016-2018. Error bars represent standard errors (n=75). C represents control; W represents warming; N represents nitrogen addition; WN represents warming and nitrogen addition; -Sh represents the removal of shrubs, -Gr represents the removal of graminoids, -GS represents the removal of graminoids and

shrubs. Different letters represent significant differences ($p < 0.05$) between the treatments according to the Tukey's multiple comparison test.

The combinative effects of biotic (vegetation removal) and abiotic (warming and N addition) factors on $CH_4$ fluxes were consistent among different years (Figure 4.3). Generally, removal of graminoids (-Gr) or all vascular plants (-GS) significantly decreased $CH_4$ fluxes compared with the control. In addition, we also found that the removal of shrubs significantly reduced $CH_4$ fluxes under the warming condition. Furthermore, only removal of all vascular plants (-GS) significantly reduced $CH_4$ fluxes under the combination of warming and N addition, but this effect was not observed in 2018.

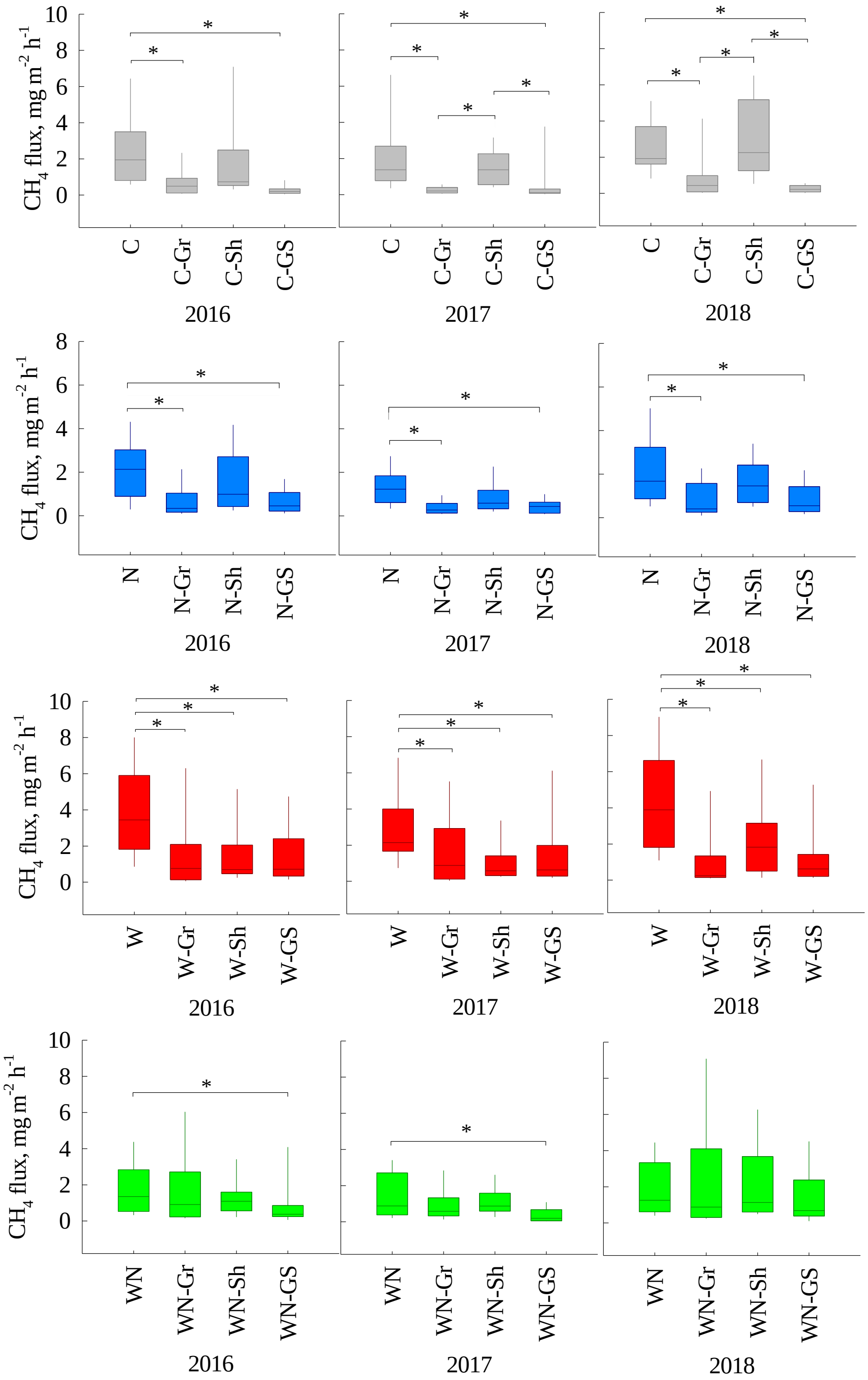


Figure 4.3 Methane fluxes under different treatments during the growing season of 2016, 2017, and 2018. C represents control; W represents warming; N represents nitrogen addition; WN represents warming and nitrogen addition; -Sh represents the removal of shrubs, -Gr represents

the removal of graminoids, -GS represents the removal of graminoids and shrubs. “*” represents significant differences ($p < 0.05$) between the treatments according to the Tukey's multiple comparison test. The range of each box is from the twenty-fifth to seventy-fifth percentile (n=27 for 2016, n=27 for 2017, n=21 for 2018). The solid line in each box indicates the median value.

### 4.4.3 Treatment effects on GPP

GPP varied in different treatments during the growing season of 2016 (Figure 4.4). Compared with the control treatment (C), removal of graminoid (-Gr) and all vascular plants (-GS) considerably decreased the GPP by 50% and 76%, respectively. This pattern was also observed under W, N, and WN conditions. Furthermore, the significantly negative effect of the shrub removal on GPP was only observed under the warming (W) condition.

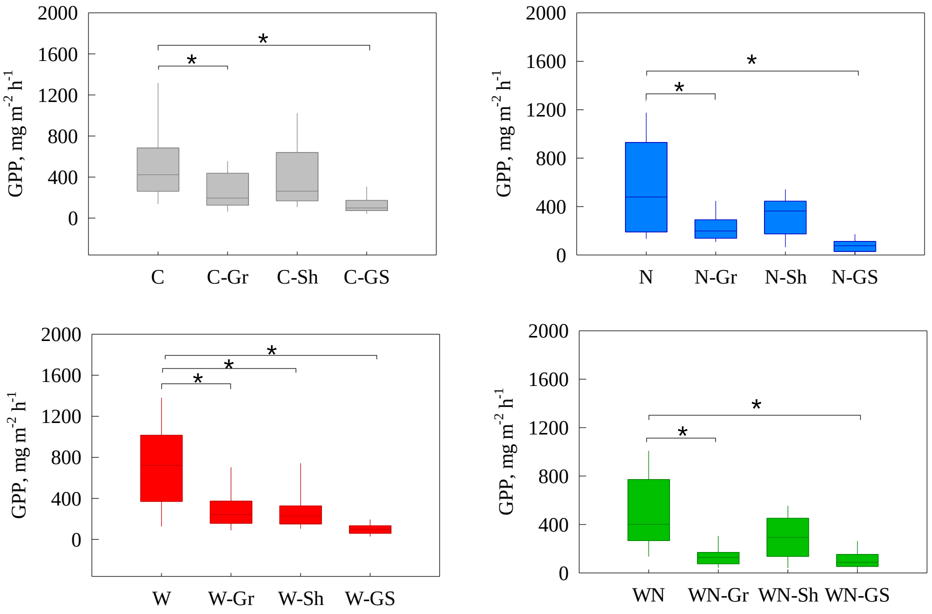


Figure 4.4 Effects of different treatments on GPP during the growing season of 2016. C represents control; W represents warming; N represents nitrogen addition; WN represents

warming and nitrogen addition; -Sh represents the removal of shrubs; -Gr represents the removal of graminoids, -GS represents the removal of shrubs and graminoids. The range of each box is from the twenty-fifth to seventy-fifth percentile (n=27). The solid line in each box indicates the median value. “*” represents significant differences ($p < 0.05$) between the treatments according to the Tukey's multiple comparison test.

### 4.4.4 Environmental controls on methane fluxes

$CH_4$ fluxes showed a significantly positive correlation with soil temperature at 20 cm depth and DOC at 40 cm depth, and negative correlation with WTD, DOC at 10 depth, TN at 10 cm and 40 cm depth (Table 4.2). As shown in Figure 4.5, there was a significantly negative relationship between WTD and $CH_4$ fluxes. Additionally, there was a significant correlation between $CH_4$ fluxes and GPP under the intact vegetation and the absence of shrubs, regardless of warming and N addition (Figure 4.6).

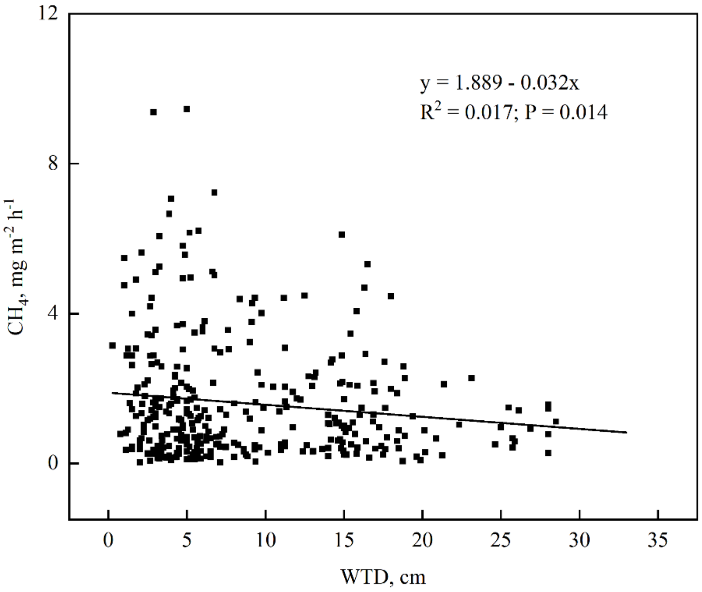


Figure 4.5 The relationship between $CH_4$ fluxes and water table depth (WTD) (n=353).

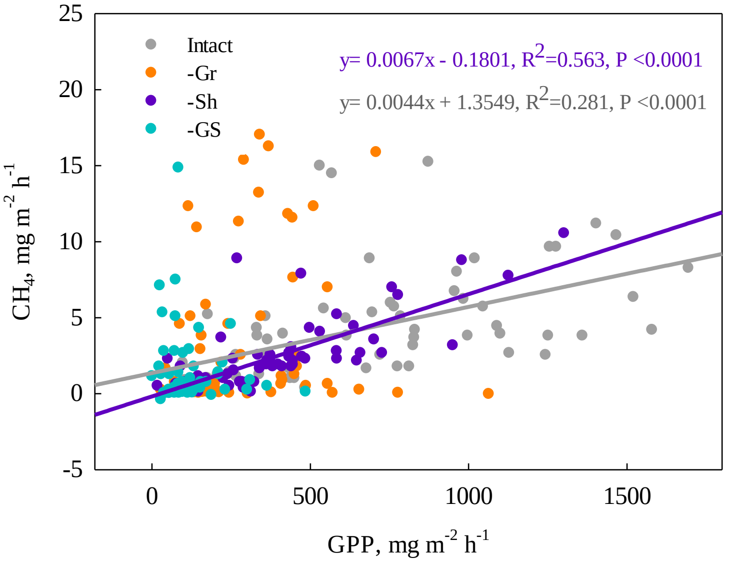


Figure 4.6 The positive relationship between GPP and CH$_4$ fluxes. Intact: without vegetation removal; -Gr: removal of graminoids; -Sh: removal of shrubs; -GS: removal of graminoids and shrubs (n=64).

Table 4.2 Parameter estimates of the linear mixed-effects model for methane flux. The bold font represents significance at $p < 0.05$. The number in the brackets represents the depth below the surface.

| Parameter | Estimate | Std. Error | df | t value | P value |
|---|---|---|---|---|---|
| **(Intercept)** | **-0.8865** | **0.2080** | **39.22** | **-4.2610** | **0.0001** |
| **WTD** | **-0.0101** | **0.0034** | **95.96** | **-2.9840** | **0.0036** |
| **DOC (10 cm)** | **-0.0075** | **0.0032** | **286.70** | **-2.3230** | **0.0209** |
| **TN (10 cm)** | **-0.0592** | **0.0209** | **654.80** | **-2.8390** | **0.0047** |
| **Soil T (20 cm)** | **0.0776** | **0.0146** | **115.00** | **5.3310** | **<0.0001** |
| Soil T (5 cm) | 0.0005 | 0.0088 | 307.10 | 0.0610 | 0.9510 |
| **DOC (40 cm)** | **0.0060** | **0.0026** | **153.60** | **2.3010** | **0.0228** |
| **TN (40 cm)** | **-0.0568** | **0.0172** | **724.70** | **-3.3100** | **0.0010** |

Furthermore, we found an exponential relationship between $CH_4$ fluxes and soil temperature at 20 cm depth (Figure 4.7). However, abiotic and biotic factors can impact this relationship (Figure 4.7; Table 4.3). Removal of vegetation reduced the explanation of soil temperature on $CH_4$ variation, especially the removal treatment of graminoids (-Gr) and all vascular plants (-GS). However, this tendency was not observed under the WN condition.

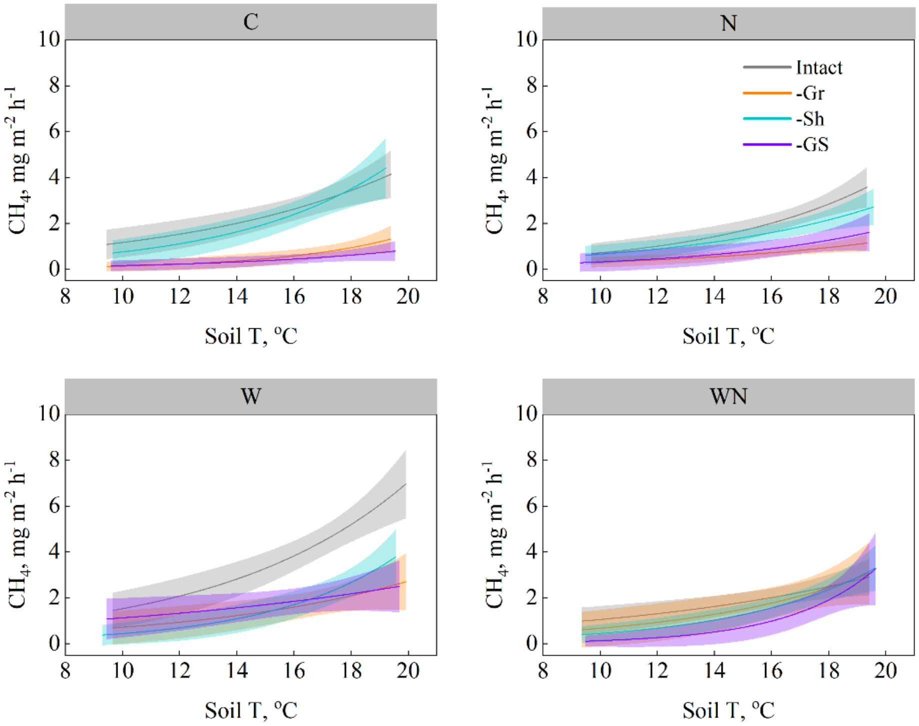
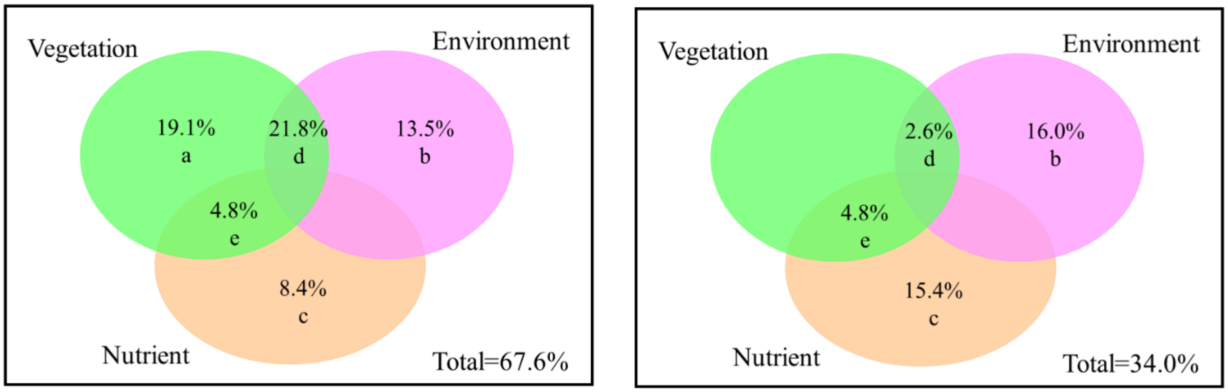


Figure 4.7 The relationships between $CH_4$ flux and soil temperature at 20 cm depth under different treatments (n=24). C: control; N: nitrogen addition; W: warming treatment; WN: warming and N addition; Intact: without vegetation removal; -Gr: removal of graminoids; -Sh: removal of shrubs; -GS: removal of shrubs and graminoids. The shaded areas represent the 95% confidence intervals.

Table 4.3 The regression models between $CH_4$ flux and soil temperature at 20 cm depth under different treatments. C: control; N: nitrogen addition; W: warming treatment; WN: warming and N addition; Intact: without vegetation removal; -Gr: removal of graminoids; -Sh: removal of shrubs; -GS: removal of shrubs and graminoids.

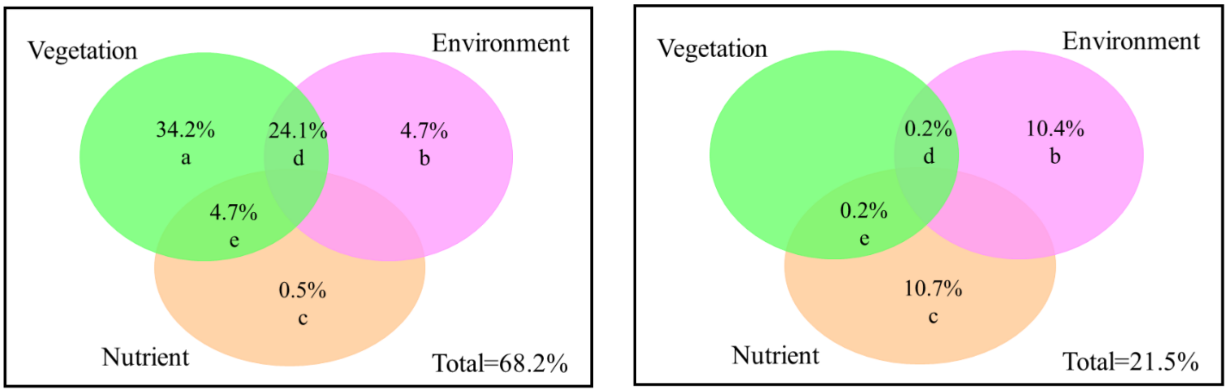
| Abiotic factor | Biotic factor | Formula | $R^2$ | P value |
|---|---|---|---|---|
| C | Intact | y=0.3054*exp(0.1344*x) | 0.67 | <0.01 |
| | -Gr | y=0.013*exp(0.2451*x) | 0.58 | <0.01 |
| | -Sh | y=0.0975*exp(0.1929*x) | 0.68 | <0.01 |
| | -GS | y=0.0522*exp(0.1387*x) | 0.41 | 0.03 |
| N | Intact | y=0.1276*exp(0.1723*x) | 0.70 | <0.01 |
| | -Gr | y=0.0928*exp(0.1298*x) | 0.58 | <0.01 |
| | -Sh | y=0.1203*exp(0.1605*x) | 0.66 | <0.01 |
| | -GS | y=0.0698*exp(0.1644*x) | 0.47 | 0.02 |
| W | Intact | y=0.3321*exp(0.1527*x) | 0.74 | <0.01 |
| | -Gr | y=0.2921*exp(0.1088*x) | 0.39 | 0.04 |
| | -Sh | y=0.0462*exp(0.2246*x) | 0.67 | <0.01 |
| | -GS | y=0.4181*exp(0.0945*x) | 0.37 | 0.05 |
| WN | Intact | y=0.3608*exp(0.1074*x) | 0.53 | <0.01 |
| | -Gr | y=0.0369*exp(0.2377*x) | 0.54 | <0.01 |
| | -Sh | y=0.1824*exp(0.1378*x) | 0.55 | <0.01 |
| | -GS | y=0.0015*exp(0.3826*x) | 0.64 | <0.01 |

Overall, the drivers mentioned above (soil temperature, water table depth, DOC, TN, and GPP) played an essential role in $CH_4$ emissions from the bog. In order to clearly understand the relative importance of these biotic and abiotic factors on $CH_4$ fluxes under different vegetation compositions, we classified them into three categories: environmental conditions (soil temperature, water table depth), nutrient contents (DOC and TN), and vegetation (GPP). As shown in Figure 4.8, vegetation explained 44%~61% of the $CH_4$ variation in the context of graminoid presence (Intact and -Sh). Although environment and nutrients were more important than vegetation under the absence of graminoids (-Gr and -GS), they explained less than 40% of the $CH_4$ variation.



(A) Intact

(B) -Gr



(C) -Sh

(D) -GS

Figure 4.8 Variance in methane fluxes under different vegetation compositions might be explained by vegetation (GPP), nutrient content (DOC and TN), as well as environment (soil temperature and water table), using redundancy analysis. a, b and c are the single effects; d is the interaction effect of environment and nutrient; e is the interaction effect of vegetation and nutrient.

## 4.5 Discussion

Climate warming, elevated N deposition, and vegetation composition change are three important global changes. Although some studies have focused on one or two of them with regard to $CH_4$ emissions from peatlands, the interaction of the three are not well known. This study attempted to fill this knowledge gap and found that the combination of warming and N addition counteracts the negative effect of vegetation absence.

### 4.5.1 Treatment effects on $CH_4$ emission

Consistent with previous studies, the absence of graminoids significantly reduced the $CH_4$ release from peat soils to the atmosphere (Armstrong et al., 2015; Leroy et al., 2017). This result can be attributed to the decrease of root exudates for $CH_4$ production. The decrease of gross primary productivity (GPP) under the treatment of graminoid removal supports this statement (Figure 4.4) because root exudates are closely related to the GPP (Edwards et al., 2018). Moreover, the removal of graminoids can reduce $CH_4$ transport from deep peat to the atmosphere via aerenchyma (Armstrong et al., 2015). In contrast to the removal of graminoids, we did not observe the significant effect of shrub removal on $CH_4$ fluxes, which is in line with previous studies (Nielsen et al., 2017; Riutta et al., 2020). The negligible effect of shrub removal can be ascribed to its negligible effect on GPP and substrate supply for $CH_4$ production (Figure 4.4). Furthermore, given no aerenchyma in shrubs, removal of shrubs does not impact $CH_4$ transport.

The similar patterns of vegetation absence were also observed under N addition: removal of graminoids (N-Gr) or all vascular plants (N-GS) substantially reduced $CH_4$ emissions due to the decrease of root exudates for $CH_4$ production and aerenchyma for $CH_4$ transport, while removal of shrubs (N-Sh) did not significantly impact $CH_4$ fluxes due to the negligible effects on GPP. This result is consistent with the part of the first hypothesis about graminoid absence, but opposite to the part of the second hypothesis about shrub absence. Noticeably, N addition can decrease the coverage of *Sphagnum* mosses due to the toxicity of nitrogen (Bubier et al., 2007; Sheppard et al., 2014), which could reduce GPP and its capacity to filter N (Chiwa et al., 2016). Therefore, much N can enter into the peat and be assimilated by graminoids and shrubs (Limpens et al., 2003). In this case, the GPP of graminoids and shrubs should be increased under N addition. However, we did not find that shrub removal significantly decreased GPP under N addition, which can be attributed to the increase of graminoid photosynthesis that might offset the GPP decrease.

In contrast to N addition, the significantly negative effect of shrub absence on GPP was observed under the warming condition, which is consistent with the part of our second hypothesis. Possibly, shrubs could be the dominant species under warming conditions. This shifting in vegetation composition under climate warming has been widely observed in peatland ecosystems (McPartland et al., 2020; Norby et al., 2019). Therefore, the removal of shrubs significantly reduced the GPP under warming condition in this study. Due to the significantly negative effect of shrub absence on the GPP, the root exudates for $CH_4$ production would be decreased (Edwards et al., 2018). Consequently, we observed a significant reduction of $CH_4$ flux when the shrub was removed under the warming condition. In addition, consistent with our hypothesis, the absence of graminoids (W-Gr) (the first hypothesis) or all vascular plants (W-GS) (the third hypothesis) under warming condition substantially reduced $CH_4$ emissions due to the decrease of root exudates for $CH_4$

production and aerenchyma for $CH_4$ transport. In this study, the open-top chamber (OTC) method increased air temperature by 1.2 - 2.6 $^oC$, and the soil temperature at 5 cm depth by 0.6 $^oC$. The air temperature increased by the OTC is slightly higher than that in previous studies (0.8 - 1.0 $^oC$), but the soil temperature increased by the OTC is within the reported range (0.4 - 1.3 $^oC$) (Munir & Strack, 2014; Turetsky et al., 2008; Ward et al., 2013). Despite the slight difference in air temperature increase, the positive warming impacts on $CH_4$ fluxes are almost consistent. However, it should be noted that the warming effect on $CH_4$ fluxes is related to the water table (Pearson et al., 2015). The water table regulates the boundary of anoxic and oxic layers in peatlands, which plays an essential role in $CH_4$ emission and even masks the positive warming effect (Pearson et al., 2015).

Strikingly, the negative effects of graminid or shrub absence were not obvious under the WN condition, which is opposite to our hypothesis that absence of graminoids (the first hypothesis) or shrubs (the second hypothesis) reduces $CH_4$ fluxes under the condition of warming and N addition. Considering the reduction of GPP when the graminoid was removed, we expected to observe a decrease of $CH_4$ emissions due to reduced root exudates for $CH_4$ production and aerenchyma for $CH_4$ transport. The unexpected result of graminoid removal (-Gr) under the WN condition might be attributed to the alteration of temperature sensitivity of $CH_4$. Methane production has been demonstrated to be exponentially related to soil temperature (Yvon-Durocher et al., 2014). Due to the aerenchyma in graminoids, most of the produced $CH_4$ can be transported from peat to the atmosphere and avoids consumption. Thus, we observed the exponential relationship between $CH_4$ fluxes and soil temperature under intact vegetation, where $CH_4$ fluxes are more determined by the direct production of $CH_4$. After graminoids are removed, however, less $CH_4$ transports from soil to the atmosphere and more $CH_4$ is consumed by methanotrophs, thus the exponential relationship between $CH_4$ flux and soil temperature is weakened. Nevertheless, this effect of graminoids absence was not observed under the WN condition. Therefore, the temperature sensitivity of $CH_4$ might be altered by -Gr under the WN condition, which increases $CH_4$ fluxes and offsets the negative effect of graminoid absence. The other possible reason is that repeated clipping of graminoids might have enhanced the overall level of fresh litter input, which could provide available carbon for $CH_4$ production under the WN condition. If peatlands shift to shrub-dominated ecosystems in the future (Wieder et al., 2019), $CH_4$ emission would be decreased due to the reduction of graminoids. However, the negative effect would be diminished under climate warming and elevated N deposition, based on the result of shrub dominance (graminoid removal) under the WN condition. Consequently, the contribution of $CH_4$ emitted from peatlands to global warming may be underestimated under shrub-dominated conditions.

The underlying mechanism of temperature sensitivity change is likely due to the change of related microbial community composition and abundance (methanogens and methanotrophs) after 5 years of abiotic (warming and N addition) and biotic (vegetation composition change) changes. Previous studies have confirmed that temperature can alter the community and abundance of methanogens and methanotrophs (Cui et al., 2020; Deng et al., 2019). Martí et al. (2019) have reported that warming and N addition increased the abundance of methanogens in a bog. Warming and N addition not only directly impact methanogens and methanotrophs, but also indirectly impact them through altering vegetation. For instance, warming can increase the graminoid coverage (Dieleman et al., 2015) and shrub biomass (McPartland et al., 2020). The effects of N addition on vascular plants and mosses are different: it provides nutrients for vascular plant growth, but it has a detrimental effect on *Sphagnum* mosses due to its toxicity (Bubier et al., 2007; Sheppard et al., 2014). It should be noted that these N effects are associated with the rate of N addition. Under a low N addition rate (< 32 kg N $ha^{-1}$ $yr^{-1}$), the *Sphagnum* mosses have the potential to filter it (Chiwa et al., 2016). Above this rate, *Sphagnum* mosses cannot filter all deposited N (Chiwa et al., 2016). Therefore, some of them enter into the deep peat and become available for vascular plants (Limpens et al., 2003). These vegetation changes can alter the community structures and abundance of methanogens and methanotrophs (Narihiro et al., 2011; Zhang et al., 2019). Although we did not measure the microorganisms in order to avoid soil disturbance, the substantially different relationships between $CH_4$ fluxes and soil temperature were pronounced among different vegetation compositions (Figure 4.7). Therefore, we assume that the absence of graminoids might change the microbial community and abundance, which could result in $CH_4$ fluxes becoming more sensitive to temperature under future scenarios. This alteration of microbial community and abundance suggests that the negative effect of graminoid absence on $CH_4$ fluxes reported before (Leroy et al., 2017; Ward et al., 2013) might be overestimated.

Similarly, the negative effect of shrub removal also has not been observed under the WN condition. This is an unexpected result because the shrub removal significantly reduced the $CH_4$ fluxes under warming alone (Figure 4.3). The possible reason is that the positive effect of graminoids overrides the effect of shrub removal on $CH_4$ fluxes under the WN condition because the temperature and nutrients are adequate for graminoid growth. If *Sphagnum*-dominated peatlands shift to a graminoid-dominated system in the future (Dieleman et al., 2015), $CH_4$ emission would be considerably increased due to the root exudates for $CH_4$ production and aerenchyma for $CH_4$ transport (Armstrong et al., 2015; Green & Baird, 2012; Leroy et al., 2017). Nevertheless, this positive effect can be mitigated under climate warming and elevated N deposition, based on the result of graminoid dominance (shrub removal) under the WN condition. Therefore, the contribution of $CH_4$ emitted from peatlands to global warming may be overestimated under graminoid-dominated conidtions.

In addition, the negative effect of all vascular plant absence (-GS) on $CH_4$ flux was observed in 2016 and 2017 but not in 2018, which is slightly opposite to our third hypothesis that the absence all of them reduces $CH_4$ flux under the WN condition. Comparing the soil temperature among different years, we found that the average soil temperature in 2018 (19.8 $^oC$ at 5 cm and 15.2 $^oC$ at 20 cm depth) was much higher than that in 2016 (15.3 $^oC$ and 14.4 $^oC$) and 2017 (15.5 $^oC$ and 14.5 $^oC$). Therefore, the high soil temperature can increase $CH_4$ production, which may offset part of the negative effect of the -GS treatment, and result in it being statistically insignificant. Notably, the higher plant production in the warm year 2018 can promote $CH_4$ emissions owing to the increase of root exudates for $CH_4$ production.

The great uncertainty in predicting $CH_4$ emission is mainly in modeling complex interactions among hydrological, thermal, biogeochemical processes, and vegetation impacts (Chang et al., 2019). The interactions of warming, N deposition, and vegetation composition change on $CH_4$ fluxes in this study were not simple additive effects. These complicated interactions should be taken into account in modeling $CH_4$ dynamics in boreal peatlands in order to accurately forecast $CH_4$ emissions from peatlands. Furthermore, previous studies have reported that vegetation can modulate the warming effect on $CH_4$ emissions from peatlands based on one or two years of the experiments (Nielsen et al., 2017; Ward et al., 2013). The short-term experiments leave much uncertainty concerning the long-term effects of these environmental changes (Dijkstra et al., 2012). Microorganisms and vegetation could be altered after long-term warming and N addition (Stark et al., 2018; Wiedermann & Nilsson, 2020). For instance, methanogenic activity was substantially increased after 18 years of warming and N addition (Martí et al., 2019). The stoichiometric nutrient demand of soil microorganisms was altered after 19 years of climate warming (Stark et al., 2018). Vascular plant cover was considerably increased after 21 years of warming (Wiedermann & Nilsson, 2020), while *Sphagnum* cover was significantly decreased after >9 years of N addition (Sheppard et al., 2014). These changes in microorganisms and vegetation could impact $CH_4$ production, consumption, and transportation (Armstrong et al., 2015; Leroy et al., 2017; Ward et al., 2013). Consequently, the complicated long-term effect of these global changes on $CH_4$ fluxes cannot be captured in a short-term scale. In this study, the five years' experiment remains in a short-term scale, suggesting that the long-term measurements are urgently needed to further understand the impact of the global changes on $CH_4$ fluxes in peatland ecosystems.

### 4.5.2 The main controls on $CH_4$ emissions under global changes

Apart from the interaction of these global changes (warming, elevated N deposition and vegetation composition change), understanding the essential controls is also critical to accurately project $CH_4$ emissions from peatlands in the future. Consistent with previous studies (Munir & Strack, 2014; Yang et al., 2014), we found that the water table level controls $CH_4$ fluxes (Figure 4.5) because it regulates the boundary between the oxic and anoxic layers; however, this relationship seems messy. The possible reason is that hysteresis occurs in the relationship between water table and $CH_4$ fluxes (Brown et al., 2014). The positive relationship of $CH_4$ flux with DOC at 40 cm depth and soil temperature at 20 depth in this study can be attributed to the elevated carbon availability for $CH_4$ production and the stimulated activity of methanogens, which is in line with previous studies (Nielsen et al., 2017; Zheng et al., 2018). The negative relationship between $CH_4$ fluxes and DOC at 10 cm depth can be owing to the increase of available carbon for methanotrophs near the surface. In addition, we found both TN at 10 cm and 40 cm depth had a negative effect on $CH_4$ flux. This result is consistent with the study of Gao et al. (2014). They stated that there were positive effects of ammonium and nitrate on $CH_4$ oxidation. Moreover, nitrate can reduce the activity of methanogens by increasing the competition between methanogens and microbes that use nitrate as electron acceptors (Chowdhury & Dick, 2013).

Although environmental (soil temperature and WTD) and nutrient variables (DOC and TN) were significantly related to $CH_4$ fluxes, they only explained a small part of the $CH_4$ variation in this study, which coincides with previous study (Turetsky et al., 2014). In order to investigate the other essential factors, GPP has been put forward and verified (Lai et al., 2014). We also observed that GPP explained a large part of the variation in $CH_4$ fluxes when graminoids were present. However, it only explained a small part under the graminoid absence (-Gr and -GS), and the explanation degree of GPP is much lower than that of environmental and nutrient explanators. This is opposite to our fourth hypothesis that GPP is more important than environment variables in regulating $CH_4$ fluxes under global changes. Furthermore, in spite of including all biotic (GPP) and abiotic factors (environmental and nutrient variables), only a small part ($\leq$34%) of $CH_4$ variation was explained under the absence of graminoids (-Gr and -GS). This low degree of explanation indicates other important factors are not included, such as the microbial information. There is growing evidence that peatlands will be shifted from *Sphagnum*-dominated to vascular plant-dominated ecosystems under future global change (Dieleman et al., 2015; Wieder et al., 2019). However, there are two typical vascular plants in peatlands (graminoids and shrubs), and which one will be the dominant plant in the future is uncertain, depending on the climate change scenarios. This uncertainty constrains the accurate estimation of $CH_4$ flux in boreal peatlands under global change. If the graminoids will be the dominant species, the vegetation production should be taken into account in the models about $CH_4$ emissions from peatland ecosystems. If the shrubs will be the dominating species, vegetation production has a limited effect to modify the $CH_4$ dynamics, and other important factors need to be included, such as microbial variables.

## 4.6 Conclusion

In this study, we found that the combination of warming and N addition counteracted the negative effect of graminoid or shrub absence. This pronounced counteraction between biotic and abiotic factors implies that the contribution of $CH_4$ emitted from peatlands to climate change could be overestimated under graminoid-dominated conditions or underestimated under shrub-dominated conditions, if climate warming and elevated N deposition are not taken into account. Furthermore, we found that vegetation productivity played an essential role in regulating $CH_4$ emissions when graminoids were present but exerted no considerable effect with the shrub presence only. Therefore, in order to accurately predict $CH_4$ emissions from peatlands, the proper parameters in the models should be screened according to the different vegetation compositions.

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# CHAPTER 5. Warming reduces the increase in $N_2O$ emission under nitrogen fertilization in a boreal peatland

## 5.1 Abstract

Peatlands are known as $N_2O$ sinks or low $N_2O$ sources due to nitrogen (N) limitation. However, climate warming and N deposition can modulate this limitation, and little is known about the combinative effects of them on $N_2O$ emission from boreal peatlands. In this study, experimental warming and N fertilization treatments were conducted at a boreal peatland in western Newfoundland, Canada. Contrary to previous studies on permafrost peatland and alpine meadows, the effect of warming treatment on $N_2O$ flux was not detectable during the growing seasons of 2015 and 2016. The N fertilization treatment significantly increased the $N_2O$ flux by 1.61 nmol $m^{-2}$ $s^{-1}$ due to increased N availability. Noticeably, warming reduced the effect of N fertilization on $N_2O$ flux with high significance in the middle growing season of 2015. This can be attributed to low N availability caused by stimulated vegetation growth in the warming treatment. In addition, the results showed that total nitrogen was the main control on $N_2O$ emission under N fertilization, while dissolved organic carbon was the main driver under the combined treatment of warming and N fertilization. Due to elevated $N_2O$ emissions under N deposition/fertilization, the contribution of $N_2O$ to global warming and ozone depletion should not be ignored.

## 5.2 Introduction

Nitrous oxide ($N_2O$) is known as an important air pollutant, greenhouse gas and ozone-depleting substance (ODS), which plays a crucial role in human health, global warming and ozone depletion. Even at a non-toxic dose, $N_2O$ can cause cognitive impairment, and disturb the nervous system of humans to some extent (Fluegge, 2016). In addition, $N_2O$ has > 300 times radiative forcing of $CO_2$ over a 100-year time horizon (IPCC, 2013), and is the most important ODS (Ravishankara et al., 2009). Peatlands have the potential to become hot spots of $N_2O$ emission as a result of human activities and climate change (Lund et al., 2009; Marushchak et al., 2011). For instance, global warming and N deposition can alleviate N limitation in peatlands and increase N availability for $N_2O$ production. Global temperature is projected to increase by 0.3-4.8 $^{o}$C at the end of this century (IPCC, 2013), potentially accelerating decomposition and thus increasing N availability (Munir et al., 2017; Sihi et al., 2018). Moreover, atmospheric N deposition is predicted to increase by > 50% by 2030 (Reay et al., 2008). Given these changes, there is an urgent need to assess $N_2O$ emissions from peatlands under warming temperatures and increased N deposition.

In several studies, the effects of warming on $N_2O$ emission from different types of peatlands have been examined but shown different results. A recent study has revealed that warming promoted $N_2O$ emissions in a permafrost peatland by increasing soil temperature and the depth of active layer (Cui et al., 2018). These findings are in accordance with an incubation study undertaken in a mountain peatland by Wang et al. (2017). They found a stronger response of $N_2O$ emissions to warming in moss peat than in mineral sediments due to more labile carbon in the peat (Wang et al., 2017). However, no warming effect on $N_2O$ emissions from fens and bogs has been detected due to N limitation (Pearson et al., 2015; Ward et al., 2013).

In contrast to warming, the impact of N deposition on $N_2O$ emission has been investigated much earlier (Regina et al., 1998). The effect of N deposition depends on the duration, form and concentration of the N deposition treatment. On the one hand, N deposition (ammonium-N, oxidized-N or urea) has been found to increase $N_2O$ emission due to increased N availability in a short-term treatment (1-5 years) (Regina et al., 1998; Lund et al., 2009). These findings are in accordance with further incubation experiment studies (Cui et al., 2016; Lozanovska et al., 2016; Roobroeck et al., 2010). On the other hand, $N_2O$ fluxes were reported to be negligible (Dise, 2001), and peaks only occurred after N treatment in an ombrotrophic boreal peatland (Nykänen et al., 2002). In a long-term treatment (~10 years), a decreased trend of $N_2O$ response to wet N deposition (ammonium-N, oxidized-N) and a significant increase under dry N deposition (ammonia-N) were found (Leeson et al., 2017; Sheppard et al., 2013). Thus, the response of $N_2O$ emission to different N forms depends on their effects on vegetation. While ammonium-N and oxidized-N can be absorbed by vegetation and reduce N availability for related microorganisms, ammonia-N can harm the vegetation and is rather available to denitrifying bacteria (Leeson et al., 2017). $N_2O$ emission from peatlands increases with the N concentration depending on the amount of applied N fertilizer (Lund et al., 2009), which is in accordance with incubation experiments (Cui et al., 2016; Roobroeck et al., 2010). Although global warming and N deposition occur simultaneously, few studies focus on their interactive effects on $N_2O$ emission from boreal peatlands.

In terms of biotic and abiotic controls on $N_2O$ emission from peatland ecosystems, many studies have focused on microorganisms (Lozanovska et al., 2016; Wang et al., 2017), water table depth (Pearson et al., 2015), precipitation, pH, and soil properties, including soil temperature, soil moisture, soil carbon and nitrogen content (Cui et al., 2016; Cui et al., 2018; Lohila et al., 2010; Roobroeck et al., 2010; Ward et al., 2013). Microbial abundance and diversity in peat can be altered by warming, which further affects $N_2O$ emissions (Wang et al., 2017). Microbial biomass content is also reported to be responsible for $N_2O$ dynamics in an incubation experiment (Lozanovska et al., 2016). In relation to abiotic factors, significant relationships were found between $N_2O$ flux and soil temperature (Cui et al., 2018), and between $N_2O$ flux and water table depth (Lohila et al., 2010). A positive correlation with nitrate ($NO_3^-$) (Cui et al., 2016; Regina et al., 1998; Roobroeck et al., 2010), and a negative correlation with C:N ratio in a drained peatland have also been shown (Klemedtsson et al., 2005). In addition, several studies focus on the concentrations of ammonium ($NH_4^+$) and $NO_3^-$ in soil water but reveal only weak correlation with $N_2O$ fluxes (Leeson et al., 2017; Sheppard et al., 2013). Taking climate change into account, it remains unclear if these relationships still stand, and little is known about the main controls on $N_2O$ emission from natural peatlands under climate change.

With the purpose of filling these knowledge gaps, we conducted a warming and N fertilization experiment in a boreal peatland. We hypothesized that: (1) warming stimulates $N_2O$ emission due to increased N availability by facilitated decomposition; (2) N deposition increases $N_2O$ release and peaks occur after N treatment; (3) warming intensifies the effect of N deposition treatment on $N_2O$ release; and (4) soil temperature and water table depth are the main controls for $N_2O$ emission under climate change.

## 5.3 Methodology

### 5.3.1 Study site

This study was conducted at a pristine, ombrotrophic blanket bog located in Robinsons, western Newfoundland, Canada (48°15′44″ N, 58°40′03″ W). The mean annual air temperature was 5 °C and the precipitation was 1340 mm over the last three decades (1981-2010). The pH (1:5 soil/water) of this bog was 4.5 ± 0.01, and the depth of peat was about 3 m. This bog represents the typical type of peatland on the island of Newfoundland, where the vegetation consists of an approximately equal biomass 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 and Wu, 2015).

### 5.3.2 Experimental design

Four different treatments were examined in this study: control (C), N fertilization (N), warming (W), and a combination of warming and N fertilization (WN). Four replicates of each treatment were randomly distributed throughout the sixteen plots. The plots covered an area of 2 m × 2 m, and the distance between the plots was approximately 2-4 m. The open-top chambers (OTCs) were used as a passive warming treatment to simulate a warming environment (Marion et al., 1997). The OTCs were 80 cm along the bottom edge, 62.5 cm along the top edge, 40 cm in height, and covered ~1.66 $m^2$ in area. In May 2014, OTCs were installed permanently. In the N fertilization experiment, ~36.6 g ammonium nitrate ($NH_4NO_3$) in 2 L of water was applied in the N fertilization plots twice a year (June 20th, 2015 and July 20st, 2015; May 15th, 2016 and July 4th, 2016). The amount of the fertilizer was 6.4 g N $m^{-2}$ $a^{-1}$, about ten times higher than the background inorganic wet N deposition in this region (Reay et al., 2008). The same amount of water was applied to the control plots without fertilizer.

### 5.3.3 Gas sampling and analysis

The $N_2O$ flux was measured using the static chamber and gas chromatograph method during the 2015 and 2016 growing season. We divided the entire growing season into three periods according to the growth state of vegetation: the early growing season (EG) from May to June, when vegetation began to grow; the middle growing season (MG) from July to August, when vegetation had a faster growth and reached its maturity; and the late growing season (LG) from September to October, when vegetation started its senescence. Measurements were conducted between 10:00-15:00 local time biweekly from May to October in 2015 and 2016. Gas samples were taken from opaque chambers, which were 50 cm in height and 26.3 cm in diameter, fitted to the groove of the PVC collar, and equipped with a capillary tube to maintain atmospheric pressure inside the chamber. Air samples were taken from the chamber headspace using 60 mL gas syringes at 0 min, 10 min, 20 min and 30 min after closure. Prior to analysis, 8 mL of air sample was injected into pre-evacuated 12 mL Exetainer vials (Labco Inc., UK) for storage. The air samples were analyzed within one week of sampling. 5 mL of sampled gas was injected into a gas chromatograph (Scion 456-GC, Bruker Ltd., Canada) equipped with an electron capture detector, and concentrations of $N_2O$ were analyzed. $N_2O$ fluxes were adjusted for air density, field sampling temperature, headspace volume (Holland et al., 1999), and were calculated by:

$$F = (dC/dt) \times (\rho \times V)/A \tag{1}$$

where $F$ is $N_2O$ flux in nmol $m^{-2}$ $s^{-1}$ (positive values indicate $N_2O$ emission, negative values indicate $N_2O$ absorption), $dC/dt$ is the change of concentration over time in nmol $mol^{-1}$ $s^{-}1$, $\rho$ is the density of air in mol $m^{-3}$, $V$ is the volume of the chamber in $m^3$, and $A$ is the chamber cover area in $m^2$. We adopted similar methods employed in other studies (Silvan et al., 2005; Ward et al., 2013), where linear regression was used to calculate the $N_2O$ flux for the static chamber approach. Normalized root mean square error (NRMSE) was used to screen the samples (Minke et al., 2016). Fluxes were accepted if NRMSE < 0.1, and the number of plots $n \geqslant 3$ (Figure S5.1). 4% of the measurements were therefore rejected. In addition, cumulative seasonal (May-October) release of $N_2O$ was obtained through linear interpolation of biweekly static chamber measurements, as employed in other studies (Cui et al., 2018; Jiang et al., 2010).

### 5.3.4 Water sampling and analysis

Soil pore water samples at ~10 cm depth in each plot were collected using MacroRhizons sampler (Rhizosphere Inc., Netherlands). Water samples at 40 cm were collected using 60 mL syringe from a perforated PVC tube inserted into 40 cm depth before. The water samples were filtered by 0.45 μm membrane prior to analysis. Dissolved organic carbon (DOC) and dissolved total nitrogen (TN) were analyzed with a Shimadzu TOC-LCPH/TN analyzer (Shimadzu Inc., Japan).

### 5.3.5 Environmental parameters

Air temperatures at vegetation canopy height were recorded continually at a 30-minute time step using temperature loggers (Lascar Electronics Ltd., UK). Soil temperature at 5 cm and 20 cm depth, and soil moisture at 5 cm depth were measured by a soil thermometer (Traceable™ Digital Thermometer, Fisher Scientific Inc., Canada) and a soil moisture sensor (ProCheck, Decagon Devices Inc., U.S.). Water table levels were measured from dip-wells made of 1 m-long perforated PVC pipes installed on each plot (Positive values indicate water levels below the peat surface). Soil temperature, soil moisture and water table depth were measured when the gas samples were collected.

### 5.3.6 Statistical analysis

Linear mixed-effects model was used to test whether the $N_2O$ fluxes and environmental variables (soil temperature, soil moisture, water table depth, total nitrogen and dissolved organic carbon in water samples) differed between treatments (warming and N addition). The model was constructed with the whole dataset. $N_2O$ fluxes and each environmental variable were explained by potential fixed predictors of warming and N addition as well as their interaction. The measurement plot was considered as a random factor. Mixed-effects model was also applied to analyze the effect of the environmental parameters on $N_2O$ fluxes. Soil temperature, soil moisture, total nitrogen, water table depth, dissolved organic carbon and time after fertilization were regarded as fixed predictors and the measurement plot was regarded as a random factor. The data were checked for normality and log-transformed where necessary before analysis. All statistical analyses were performed in the SPSS 20.0 statistical software package (SPSS, Inc., Chicago, IL, USA).

## 5.4 Results

### 5.4.1 Environmental parameters

As shown in Figure 5.1, the warming treatment effectively increased the average air temperature by 1.2 °C during the growing season. Soil temperature at 5 cm and 20 cm depths increased by 0.9 °C and 0.5 °C under the warming treatment during the growing seasons of 2015-2016, respectively. Although warming and N treatments did not impact TN concentrations at 40 cm depth independently, their interactive effect significantly increased TN at 40 cm depth by 72.7% compared with the warming treatment. In addition, TN at 10 cm depth showed significant differences between warming and N treatments. The soil moisture, water table depth (WTD) and DOC were not significantly affected in any treatment (Table 5.1).

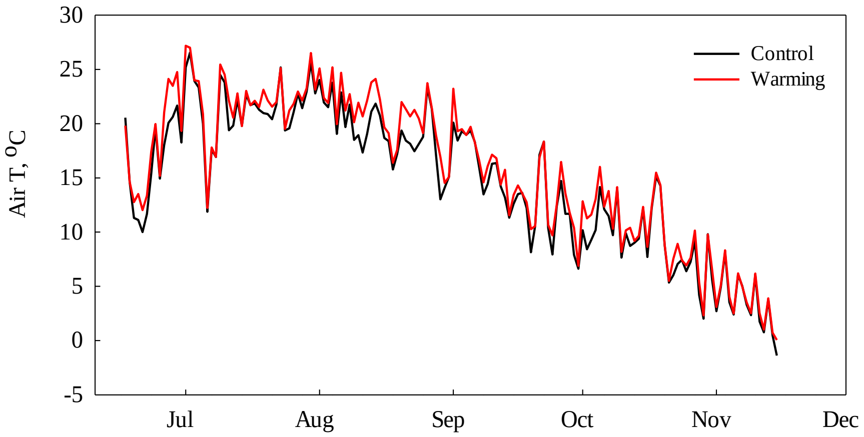


Figure 5.1 Daily mean air temperature in the warming plots (red line) and control plots (black line) in 2014 growing season.

Table 5.1 Environmental parameters under different treatments during the growing seasons of 2015 and 2016 (means ± S.E.).

| Treatment | Soil T (5 cm) | Soil T (20cm) | Soil moisture (5 cm) | WTD | TN (10 cm) | DOC (10 cm) | TN (40 cm) | DOC (40 cm) |
|---|---|---|---|---|---|---|---|---|
| C | $15.9\pm4.2^{a}$ | $14.5\pm3.3^{a}$ | $74.5\pm13.5^{a}$ | $7.6\pm5.2^{a}$ | $0.8\pm0.3^{ab}$ | $40.2\pm9.4^{a}$ | $1.5\pm0.9^{ab}$ | $53.0\pm17.4^{a}$ |
| W | $16.8\pm5.1^{a}$ | $15.0\pm3.4^{a}$ | $68.6\pm9.1^{a}$ | $7.0\pm5.0^{a}$ | $0.6\pm0.1^{a}$ | $39.4\pm7.6^{a}$ | $1.1\pm0.4^{a}$ | $52.8\pm5.4^{a}$ |
| N | $16.0\pm4.6^{a}$ | $14.5\pm3.3^{a}$ | $71.9\pm9.1^{a}$ | $7.2\pm5.0^{a}$ | $0.9\pm0.5^{b}$ | $41.4\pm11.3^{a}$ | $1.7\pm1.0^{ab}$ | $50.0\pm7.8^{a}$ |
| WN | $16.1\pm4.2^{a}$ | $14.9\pm3.2^{a}$ | $70.9\pm9.3^{a}$ | $7.6\pm5.3^{a}$ | $0.8\pm0.3^{ab}$ | $39.4\pm7.6^{a}$ | $1.9\pm1.2^{b}$ | $58.0\pm15.9^{a}$ |

Note: C represents control; W represents warming; N represents nitrogen addition; WN represents warming and nitrogen addition. The units of soil T, soil moisture and water table depth (WTD) were $^{o}$C, % and cm. The unit of TN and DOC was mg/L. The number in the brackets represents the depth below the surface. Different lowercase letters represent significant differences ($p < 0.05$) between the treatments.

### 5.4.2 Treatment effects on $N_2O$ flux

N addition had a significant effect on $N_2O$ flux but warming alone did not significantly affect $N_2O$ flux (Table 5.2). Throughout the entire growing seasons of 2015 and 2016, $N_2O$ emissions varied between the different treatments. There was a trend that $N_2O$ consumption decreased under warming treatment. Compared with the control plot (-0.15 ± 0.12 nmol $m^{-2}$ $s^{-1}$), the plots under N and WN treatments switched from $N_2O$ sinks to $N_2O$ sources and significantly increased by 1.07 nmol $m^{-2}$ $s^{-1}$ under WN treatment and 1.61 nmol $m^{-2}$ $s^{-1}$ under N treatment (Figure 5.2). Moreover, warming reduced the increase of $N_2O$ emission resulted from N fertilization by 0.53 nmol $m^{-2}$ $s^{-1}$.

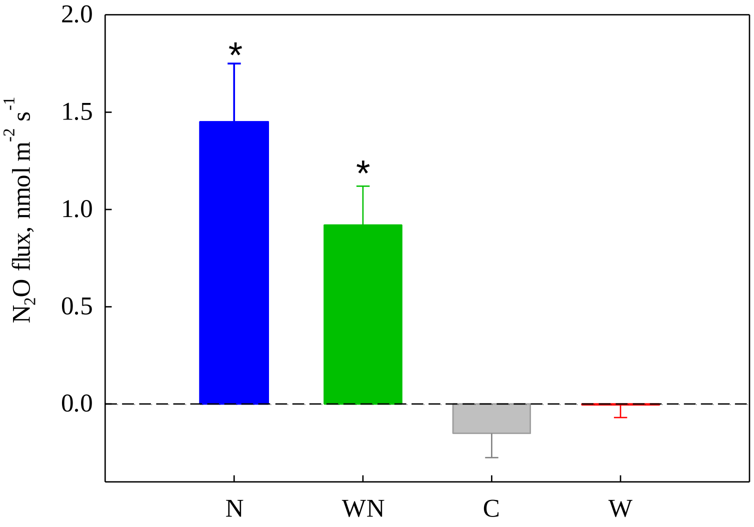


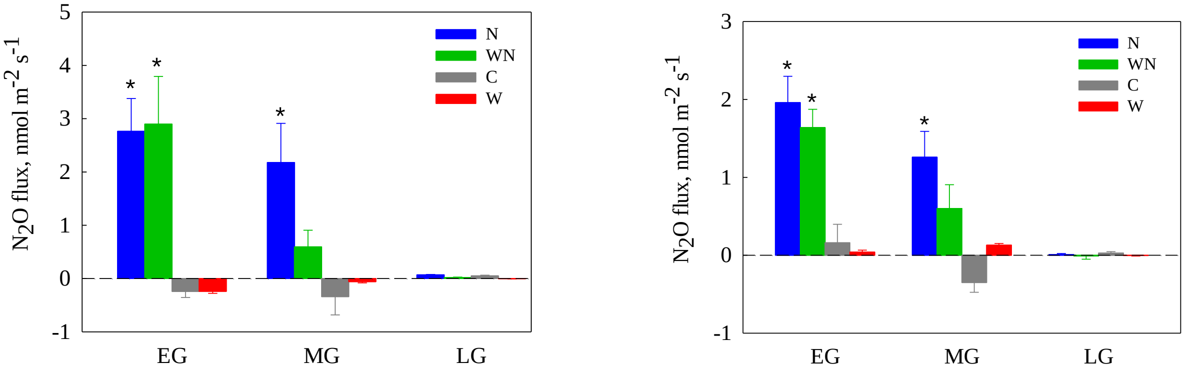
Figure 5.2 Average $N_2O$ flux in the 2015 and 2016 growing seasons under different treatments. C: control; W: warming; N: nitrogen addition; WN: warming and nitrogen addition. The error bar indicates standard error (SE). “*” represents significant differences ($p < 0.05$) with control treatments.

Table 5.2 Parameter estimates of the linear mixed-effects model for $N_2O$ fluxes.

| Parameter | Estimate | Std. Error | df | t | P value |
|---|---|---|---|---|---|
| Intercept | 1.000 | .003 | 236 | 333.638 | .060 |
| Warming | -.001 | .004 | 236 | -.243 | .808 |
| **Nitrogen** | **.010** | **.004** | **236** | **2.450** | **.015** |
| Warming * Nitrogen | .009 | .006 | 236 | 1.539 | .125 |
| Soil T (5 cm) | .157 | .084 | 67 | 1.869 | .061 |
| Soil T (20 cm) | -.205 | .106 | 67 | -1.941 | .083 |
| Soil moisture | .002 | .001 | 67 | 1.482 | .141 |
| WTD | .040 | .015 | 67 | .006 | .664 |
| DOC (10 cm) | -.003 | .011 | 67 | -.257 | .315 |
| TN (10 cm) | 0.110 | .321 | 67 | 3.462 | .068 |
| **DOC (40 cm)** | **.015** | **.011** | **67** | **-1.375** | **.030** |
| **TN (40 cm)** | **.206** | **.104** | **67** | **1.991** | **.003** |
| **Time after fertilization** | **.006** | **.007** | **67** | **.879** | **.040** |

Note: The number in the brackets represents the depth below the surface. The bold font represents significance at $p < 0.05$. * indicated the interaction effect.

Besides N and WN treatments, different periods of the growing season, the date and the time after fertilization also exerted a significant effect on $N_2O$ flux (Table 5.2). Compared with the control treatments in the EG (- 0.24 ± 0.12 nmol $m^{-2}$ $s^{-1}$) and MG periods (- 0.34 ± 0.16 nmol $m^{-2}$ $s^{-1}$) of 2015, the N treatment significantly increased mean $N_2O$ emissions by 3.05 nmol $m^{-2}$ $s^{-1}$ during the EG period and 2.52 nmol $m^{-2}$ $s^{-1}$ during the MG period. Likewise, $N_2O$ emissions under N treatment increased by 1.81 nmol $m^{-2}$ $s^{-1}$ during the EG period and 1.61 nmol $m^{-2}$ $s^{-1}$ during the MG period in 2016. In addition, $N_2O$ emissions under the interactive treatment of warming and N fertilization treatment were 3.14 nmol $m^{-2}$ $s^{-1}$ and 1.88 nmol $m^{-2}$ $s^{-1}$ higher than under the control treatment in the early growing season of 2015 and 2016, respectively (Figure 5.3). Additionally, warming and N fertilization together significantly decreased the $N_2O$ flux by 1.59 nmol $m^{-2}$ $s^{-1}$ compared with the N treatment only during the MG period in 2015 ($F_{1,32} = 4.954$, $P = 0.03$). However, no evidence of a warming effect on $N_2O$ flux was found in this short-term manipulation experiment. During the late growing season, none of the treatments showed a detectable effect on $N_2O$ fluxes.



(a) (b)

Figure 5.3 Average $N_2O$ flux in the 2015 (a) and 2016 (b) early (EG), middle (MG), and late (LG) growing season under different treatments. Error bars show the standard error. “*” represents significant differences ($p < 0.05$) from the control treatment.

The temporal variation of $N_2O$ flux under different treatments during the two growing seasons was shown in Figure 5.4. Two peaks of $N_2O$ flux under N treatment occurred each year, in June (2.76 ± 0.68 nmol $m^{-2}$ $s^{-1}$) and August (4.96 ± 1.46 nmol $m^{-2}$ $s^{-1}$) 2015, and May (2.61 ± 1.05 nmol $m^{-2}$ $s^{-1}$) and July (5.51 ± 1.21 nmol $m^{-2}$ $s^{-1}$) 2016. A similar trend of $N_2O$ flux was found under WN treatment, but the peaks of $N_2O$ were reduced by 3.58 nmol $m^{-2}$ $s^{-1}$ in August 2015, and 2.61 nmol $m^{-2}$ $s^{-1}$ in July 2016, compared with the N treatment. Furthermore, the $N_2O$ flux under the WN treatment was consistently lower than that under the N treatment alone. The trends of $N_2O$ flux for the warming and control treatments were similar.

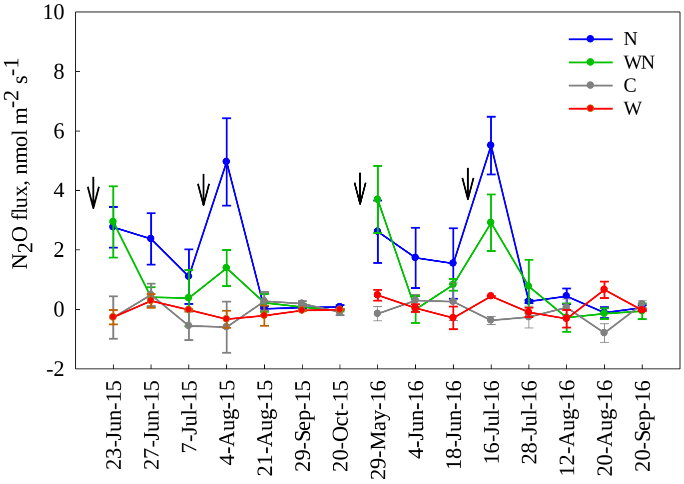


Figure 5.4 Temporal variation of $N_2O$ flux under different treatments. The error bar indicates standard error. “ ↓ ” indicates the time of N addition.

### 5.4.3 Cumulative $N_2O$ flux

As shown in Figure 5.5, the cumulative $N_2O$ fluxes varied with different treatments in 2015 and 2016. The highest cumulative $N_2O$ flux for the whole growing season of 2015 was found for the N treatment, followed by the WN treatment and the control treatment. The lowest cumulative flux of $N_2O$ in 2015 occurred for the warming treatment. For the growing season of 2016, the highest cumulative $N_2O$ flux was found for the N treatment, followed by the WN treatment and the warming treatment. The lowest cumulative flux of $N_2O$ in 2016 occurred under the control treatment. Compared with the control treatment, the effects of N and WN treatments on cumulative $N_2O$ flux were significant in both years. Furthermore, it should be noted that the emission factor (the fraction of nitrogen added that is released as $N_2O$) was about 7.6%, which was higher than 1% (IPCC, 2013) and slightly lower than ~8.5% reported by Leeson et al. (2017).

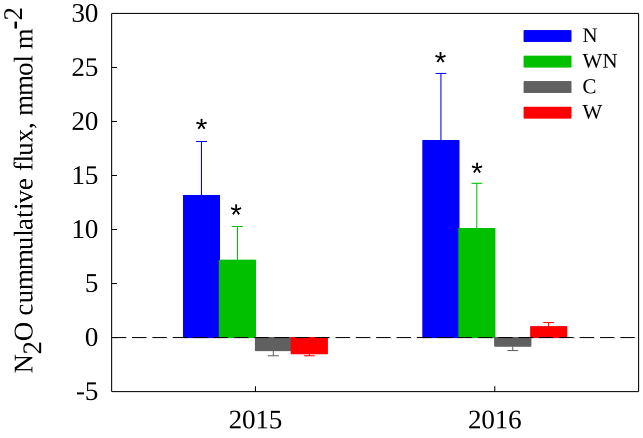


Figure 5.5 Cumulative flux of $N_2O$ under four treatments in 2015 and 2016 growing season. Error bars represent standard error. "*" represents significant differences ($p < 0.05$) from the control treatment.

### 5.4.4 Relationship between $N_2O$ flux and abiotic parameters

After three years of warming and N fertilization treatments, significant correlations between $N_2O$ flux and DOC at 40 cm depth, and between $N_2O$ flux and TN at 40 cm depth were observed, explaining 9.7% and 4.5% of $N_2O$ variation, respectively (Figure 5.6). It should be noticed that all $R^2$ were obtained from the analysis of the log-transformed data. Therefore, the percentage of variance explained in original units was small. Furthermore, the relationships between $N_2O$ flux and other environmental parameters (soil temperature, soil moisture, water table depth, TN and DOC at 10 cm depth) were undetectable in this study.

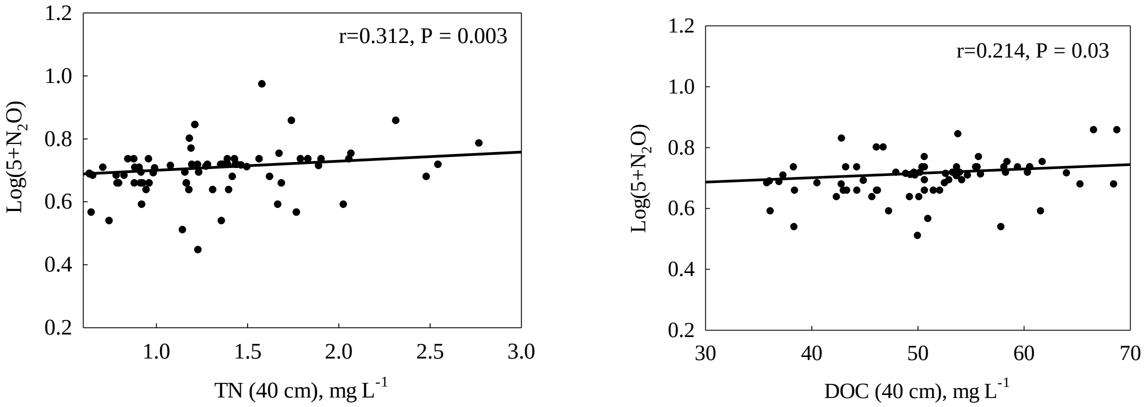


Figure 5.6 Relationship between Log(5+$N_2O$) of all treatments and two environmental variables (dissolved organic carbon (DOC) and total nitrogen (TN) at 40 cm water depths).

## 5.5 Discussion

### 5.5.1 Treatment effects on $N_2O$ flux

After three years of warming treatment in the boreal peatland, no significant effects on $N_2O$ fluxes were found. This failed to confirm our first hypothesis but is consistent with previous studies on fens and bogs (Pearson et al., 2015; Ward et al., 2013). The significant positive effect of warming on $N_2O$ flux has been reported before in a permafrost peatland and is attributed to the increase in soil temperature and active layer depth under the warming treatment (Cui et al., 2018). Although soil temperature was increased due to the passive warming treatment, no effect on $N_2O$ fluxes has been detected in this study. This can be attributed to N limitation (Ward et al., 2013) in this bog. Contrary to the warming treatment, N fertilization significantly increased $N_2O$ emission, which well confirms our second hypothesis. This result is in line with previous studies (Regina et al., 1998; Lund et al., 2009), showing that nitrogen fertilization increases available N for nitrification and denitrification, which are two important pathways for $N_2O$ production (Butterbach-Bahl et al., 2013). The effect of the N treatment is apparent in the temporal variation of $N_2O$ fluxes and different periods of the growing season. Time after N addition had a significant effect on $N_2O$ fluxes (Table 5.2). $N_2O$ fluxes reached their maximum two weeks after N fertilization, and significantly increased in the early and mid-growing season. Due to the absence of N fertilization in the late growing season, the effect of the N treatment was undetectable. A previous study has also reported the same result (Nykänen et al., 2002).

Contrary to the third hypothesis, we found that warming tended to reduce the effect of N fertilization treatment and it was significant in the middle growing season of 2015. This is not in line with previous studies in an alpine meadow system (Chen et al., 2017a; Chen et al., 2017b; Chen et al., 2017c) showing that $N_2O$ flux was increased by the interactive treatment of warming and N addition due to the microbial activity promoted by warming. Nevertheless, the result in this study is in agreement with one study in a high arctic tundra, which reported no significant interactive effects on $N_2O$ flux due to the resistance of microbial communities (Lamb et al., 2011). Due to a lack of strong correlations between environmental factors and $N_2O$ flux among these peatland ecosystems, the different results may be caused by various microbial communities in different ecosystems. In our study, this trend can be attributed to the low N availability under warming treatment because warming promotes vegetation growth and competition with related microorganisms for nitrogen (Brummell et al., 2017; Munir et al., 2017). Munir et al. (2017) also observed a slight decline in N pools and a greater N uptake by plants with warming. The result is also supported by decreased total N in soil water under the warming treatment (Table 5.1). In addition, this effect was significant in the middle growing season, which implies that competition between plants and soil microorganisms for nitrogen becomes fiercer compared with early and late growing season due to faster growth of vegetation.

### 5.5.2 Environmental controls on $N_2O$ flux

Previous studies identified soil temperature and water table depth as the important drivers for $N_2O$ flux changes because soil temperature directly impacts microbial activities (Cui et al., 2018), and water table depth indirectly affects denitrification by controlling anaerobic conditions (Lohila et al., 2010; Pärn et al., 2018). However, no significant relationship between $N_2O$ flux and water table depth was observed. This fails to confirm the fourth hypothesis that soil T and water table depth are the main controls on $N_2O$ flux. A significant correlation between $N_2O$ flux and DOC and TN in soil water at 40 cm depth was found in this study, indicating that denitrification plays a key role in $N_2O$ emission under climate change. Regina et al. (1998) supported this finding by demonstrating that denitrification is a major process of $N_2O$ production in moist peat. Availability of organic substrates and nitrogen are known to limit denitrification (Dodla et al., 2008; Liimatainen et al., 2018). The significant relationships between $N_2O$ flux and DOC and TN at 40 cm depth suggest that N availability and organic substrates are limiting factors for denitrification in this boreal peatland. However, TN and DOC in soil water at 40 cm depth only explained 14.2% of $N_2O$ variation. Therefore, further research is needed to focus on the biotic factors as potential controls of $N_2O$ fluxes.

If comparing between different treatments, TN and DOC were the main controls for $N_2O$. TN was the main control under N treatment. This indicates that N fertilization stimulates $N_2O$ emission by increased N availability. However, the main control shifted from TN to DOC under WN treatment. This can be attributed to two reasons: First, warming stimulates N uptake by vegetation, thus alleviating the effect of N deposition on $N_2O$ flux (Brummell et al., 2017). Second, warming increases root exudates and litter quality, which provides more labile carbon for $N_2O$ production (Bragazza et al., 2013). Therefore, we suggest plant productivity to be a predictor for $N_2O$ fluxes under N deposition. However, to investigate the main factors regulating $N_2O$ fluxes, further research is needed to trace the fate of N in plants and its availability to microbes.

### 5.5.3 Adverse effect on environment

During the growing seasons of 2015 and 2016, the cumulative $N_2O$ flux increased by 8.51 mmol $m^{-2}$ under WN treatment. This suggests that both the global warming potential and ozone depletion potential of $N_2O$ could increase under N deposition/fertilization over a short term in boreal peatlands. Although we did not report all greenhouse gases in this study, the contribution of $N_2O$ to global warming and ozone depletion should not be ignored. Noticeably, our study was conducted in the growing season only. If we also calculated the $N_2O$ flux for the non-growing season, especially during freeze-thaw events, $N_2O$ production would be considerably higher due to the increased N availability (Cui et al., 2016). Furthermore, many models predict that the reduced global cooling function of peatlands under climate change in the future (Foltz et al., 2019; Gallego-Sala et al., 2018; Wu and Roulet, 2014). However, the combinative influence of warming and nitrogen fertilization has not been considered in most models. Consequently, the $N_2O$ fluxes might be overestimated in peatlands as a consequence of climate change.

In this study, the bog is a weak sink of $N_2O$, which is different from the previous study reported that organic soils of the world are important $N_2O$ sources (Pärn et al., 2018). The average $N_2O$ flux under N addition (1.46 nmol $m^{-2}$ $s^{-1}$) and WN treatment (0.92 nmol $m^{-2}$ $s^{-1}$) is much higher than $N_2O$ fluxes emitted from drained organic soils (0.11-1.14 nmol $m^{-2}$ $s^{-1}$) (Pärn et al., 2018). This result suggests that N fertilizer application has more adverse effect on environment than drainage practice, in terms of $N_2O$ fluxes. The emission factor (the fraction of nitrogen added that is released as $N_2O$) amounts to ~7.6%, while the IPCC default emission factor adds up to only 1% (IPCC,

2013) and to ~8.5% as found by Leeson et al. (2017). The high emission factor in this study can be attributed to the application of a highly-dosed N fertilizer twice a year in our manipulative experiment. Under natural conditions, the frequency of wet N deposition depends on precipitation frequencies. Leeson et al. (2017) have added N in 120 events per year to simulate wet N deposition, but no effect on $N_2O$ fluxes has been observed. Consequently, high amounts of N addition in the present study partly explain the high emission factor, as high $N_2O$ fluxes occurred shortly after N addition (Figure 5.4).

Repo et al. (2009) yielded a cumulative $N_2O$ flux of 27.3 ± 6.8 mmol $m^{-2}$ in Arctic bare peat circles during the snow-free season, similar to $N_2O$ emissions from agricultural soils. In this study, we also determined a high cumulative $N_2O$ flux (17.23 ± 6.21 mmol $m^{-2}$) due to the intense N addition. Nevertheless, determining cumulative fluxes via linear interpolation entails large uncertainties due to the large temporal variation of $N_2O$ fluxes in boreal peatlands (Cui et al., 2018). Levy et al. (2017) have provided a robust method for quantifying the uncertainty in estimates of cumulative $N_2O$ fluxes. However, that method requires detailed knowledge of underlying processes and the spatial and temporal distribution of $N_2O$ fluxes first, which is what we are trying to find out in this study.

## 5.6 Conclusion

This study examined the influence of experimental warming and N fertilization on $N_2O$ flux in a peatland ecosystem. The effect of warming treatment on $N_2O$ flux was not detectable, while a significant effect of N fertilization treatment was shown. A trend that warming reduced the effect of N fertilization treatment on $N_2O$ flux was observed, and it was significant in the middle growing season. In addition, the emission factor was very high (7.6%), which can be attributed to low frequency and high concentration of N addition. Among different abiotic factors, TN and DOC in soil water were the main controls for $N_2O$ emission under N and WN treatments, respectively. In this peatland ecosystem, denitrification processes were not only limited by N availability, but also by organic substrates. Furthermore, our result indicates that the contribution of $N_2O$ to global warming and ozone depletion should not be ignored in the future.

## 5.7 Acknowledgments

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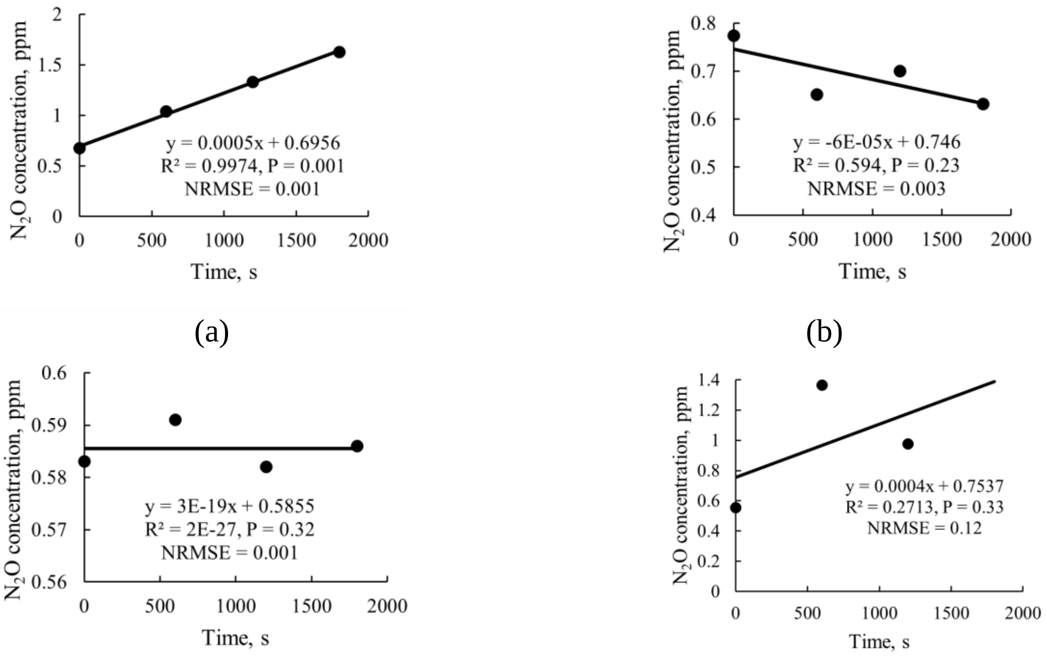
### 5.9 Supplementary files

As shown in Figure S5.1, the normalized root-mean-square error (NRMSE) was used to screen our samples. The linear model fitted well in (a) and the RMSE was lower than 0.1, and n = 4 (number of plot). Therefore, the $N_2O$ flux was accepted. Although the $R^2$ of (b) and (c) were lower than 0.7 and P-value were above 0.1, the NRMSE were less than 0.1 (n=4). Thus, the $N_2O$ fluxes were accepted. However, the NRMSE of (d) was higher than 0.1 (n=3). Thus, the $N_2O$ flux was not accepted.



(a) (b)

(c) (d)

Figure S5.1 $N_2O$ concentration over the sampling time under (a) warming and N addition, (b) warming treatments, (c) control, and (d) N addition.

# CHAPTER 6. Vegetation composition modulates the interaction of climate warming and elevated nitrogen deposition on nitrous oxide flux in a boreal peatland

## 6.1 Abstract

Northern peatlands with large organic nitrogen (N) storage have the potential to be $N_2O$ hotspots under global changes such as climate warming, elevated N deposition, and vegetation composition change. However, the interactions of these three global changes and the primary controls on $N_2O$ fluxes in peatlands are not well known, leading to a large uncertainty in modeling $N_2O$ fluxes in peatland ecosystems. Here, the three global changes were manipulated in a boreal bog in western Newfoundland, Canada for five years. We found that warming mitigated the positive N effect on $N_2O$ fluxes in the mid-growing season under intact vegetation owing to the increase of available N uptake by vegetation and less N for $N_2O$ production. In contrast, under the absence of graminoids or shrubs, warming strengthened the N effect on $N_2O$ fluxes in the early growing season, which can be attributed to the increase of available carbon and nitrogen for $N_2O$ production. It should be noted that these effects were not observed under the condition of low carbon availability. In addition, gross primary production, soil temperature, carbon and nitrogen availability were found as critical controls on $N_2O$ fluxes, which can be applied to reduce the uncertainty in $N_2O$ predicting models. Our findings emphasize the interaction of abiotic (warming and elevated nitrogen deposition) and biotic changes (vegetation composition change) on $N_2O$ fluxes, which should be taken into account in order to project $N_2O$ fluxes in peatland ecosystems accurately.

## 6.2 Introduction

Nitrous oxide ($N_2O$) is a potent greenhouse gas that contributes 6% to global warming (IPCC, 2013) and can cause ozone destruction in the stratosphere (Ravishankara et al., 2009). Northern peatlands have accumulated not only large stocks of organic carbon (C) but also a great amount of organic nitrogen (N), approaching 415 Pg C and 10 Pg N (Hugelius et al., 2020). This large amount of organic N has the potential to be mineralized and stimulate $N_2O$ production under future global changes, including elevated N deposition, climate warming, and vegetation composition change.

Nitrogen deposition has been predicted to increase by 50 - 100% in 2030 owing to the fertilization and fossil-fuel burning (Reay et al., 2008), which can stimulate nitrification and denitrification, two major biochemical processes of $N_2O$ production (Butterbach-Bahl et al., 2013). This positive effect of N deposition on $N_2O$ production has been widely reported in peatland ecosystems, from incubation experiments (Cui et al., 2016; Lozanovska et al., 2016) to field experiments (Gong et al., 2019; Lund et al., 2009).

Global air temperature has also been predicted to increase by ~4.8 $^{o}$C at the end of this century (IPCC, 2013). Temperature is a key factor determining the rates of nitrification and denitrification, and the high temperature can strongly enhance the nitrification and denitrification rates (Dai et al., 2020). The positive warming effect on $N_2O$ fluxes has been observed in a permafrost peatland (Cui et al., 2018) but not in non-permafrost peatlands (Gong et al., 2019; Pearson et al., 2015; Ward et al., 2013). These inconsistent results could be attributed to the relatively high soil temperature increase under warming treatment in the permafrost peatland (Cui et al., 2018).

Moreover, vegetation composition in peatlands is an important factor regulating $N_2O$ fluxes in boreal peatlands (Brummell et al., 2017), and is vulnerable to climate change. For instance, climate warming could shift a *Sphagnum*-dominated peatland to a graminoid-dominated (Dieleman et al., 2015) or shrub-dominated ecosystem (Bragazza et al., 2015). Elevated N deposition can decrease the *Sphagnum* coverage and increase the coverage of vascular plants (Wiedermann et al., 2007). Moreover, land management practices such as burning and grazing further exacerbate vegetation composition changes, causing an increase in graminoid cover and decreased shrub and bryophyte cover (Ward et al., 2007). Simulating these vegetation composition changes in the field is not easy in some areas due to the deep roots and rich species; thus, the research focused on the impacts of vegetation composition change on $N_2O$ fluxes in boreal peatlands is rare. Brummell et al. (2017) stated that the presence of vascular plants in a restored peatland reduced $N_2O$ emissions owing to the mineral N uptake by plant and less N for $N_2O$ production. This result is in line with the study in a pristine bog (Ward et al., 2013), which has reported that shrub presence and bryophyte absence reduced the $N_2O$ flux.

Although the positive effect of N deposition reveals that northern peatlands have the potential to be $N_2O$ hotspots in the future, there is substantial uncertainty due to the few data about the interaction with warming and vegetation composition change. Gong et al. (2019) found warming decreased the positive effect of N addition on $N_2O$ emission in the mid-growing season owing to stimulating much N uptake by plant. Le et al. (2020) reported that graminoid absence reduced the positive effect of N addition due to increased N retention capacity of the moss layer and reduced $N_2O$ transport through plant tissues. Despite these interactions, the combined effect of these three global changes, i.e., warming, increased N deposition, and vegetation composition change, on $N_2O$ flux is still uncertain, which leads to an uncertainty in predicting $N_2O$ emission from peatlands accurately.

In order to improve the models predicting $N_2O$ emissions, not only the interaction of these global changes but also the major controls are needed to be well understood. Although the relationships between $N_2O$ flux and environmental parameters have been reported in peatland ecosystems, there is no definitive conclusion. Marushchak et al. (2011) reported that soil moisture was the major control for $N_2O$ fluxes in permafrost peatlands. Voigt et al. (2017) stated that soil carbon quality and soil moisture were critical regulators for $N_2O$ fluxes in arctic peatlands. Liu et al. (2019) reported that soil bulk density was more important than soil C/N ratio and pH to estimate annual $N_2O$ emissions from degraded peatlands. Cui et al. (2016) found a correlation between nitrate and $N_2O$ fluxes in a permafrost peatland based on an incubation experiment. Gong et al. (2019) demonstrated that dissolved organic carbon and total nitrogen in pore water were related to $N_2O$ fluxes in a boreal peatland. These divergent relationships between $N_2O$ fluxes and environmental parameters cause a large uncertainty in $N_2O$ predicting models. The other issue is that these abiotic variables cannot adequately explain $N_2O$ variations, suggesting other biotic parameters should be taken into account, such as vegetation productivity and microorganisms.

To fill these knowledge gaps, three global changes (warming, N deposition increase, and vegetation composition change) are mimicked for five years in a boreal bog in Robinsons, western Newfoundland, Canada. This study aims to investigate the interactions of abiotic (warming and N addition) and biotic factors (vegetation composition) on $N_2O$ fluxes, explore the possible underlying mechanisms, and evaluate the major controls for $N_2O$ emissions. Gong et al. (2019) reported that warming could reduce the positive effect of N addition on $N_2O$ flux because warming increases nitrogen uptake by plants and less nitrogen for $N_2O$ production. Therefore, we hypothesized that (1) after vascular plants (graminoid and/or shrub) were removed, warming may not mitigate the positive effect of N, and even stimulate $N_2O$ emissions due to the increase of N availability by enhancing soil decomposition and N mineralization. We also hypothesized that (2) vegetation productivity, water table depth, soil temperature, dissolved organic carbon, and nitrogen availability remain the essential controls for $N_2O$ fluxes under global change, and they could explain large part of $N_2O$ flux variation.

## 6.3 Materials and methods

### 6.3.1 Study site and experimental design

This study was conducted at an area of oligogenic, ombrotrophic blanket bog with a pH of 4.5 and a peat depth of 3 m (Luan et al., 2019), located in Robinsons, western Newfoundland, Canada (48°15′46″ N, 58°39′21″ W). This area is characterized by a boreal climate with mean annual precipitation of 1340 mm and mean annual temperature of 5 °C (1981-2010) (climate data from the nearest weather station in Stephenville, https://climate.weather.gc.ca). The dominant vegetation in the study area consists of graminoids (*Rhynchospora alba* and *Trichophorum cespitosum*), shrubs (*Chamaedaphne calyculata*, *Gaylussacia bigeloviana*, *Vaccinium oxycoccos* and *Andromeda glaucophylla*), and non-vascular plants (*Sphagnum* spp., liverworts).

We established a full factorial design comprising the manipulation of warming, N deposition, and vegetation composition change in the spring of 2014. There were sixteen treatments in this study: control (C); warming (W); N addition (N); removal of shrubs (-Sh); removal of graminoids (-Gr); removal of graminoids and shrubs (-GS); warming and N addition (WN); warming and removal of shrubs (W-Sh); warming and removal of graminoids (W-Gr); warming and removal of shrubs and graminoids (W-GS); N addition and removal of shrubs (N-Sh); N addition and removal of graminoids (N-Gr); N addition and removal of graminoids and shrubs (N-GS); warming, N addition and removal of shrubs (WN-Sh); warming, N addition and removal of graminoids (WN-Gr); and warming, N addition, and removal of graminoids and shrubs (WN-GS). We established four replicate blocks, and each block had sixteen plots (2 m × 2 m). Sixteen treatments were randomly arranged into the plots within each block. The buffer zone between adjoining plots was at least 2 m, and the buffer zone between replicate blocks was at least 6 m. Open-top chambers (OTCs) were installed in the field, which was used to simulate the warming environment and can increase air temperature by 1.2-2.6 $^{o}$C. N addition was applied using ammonium nitrate ($NH_4NO_3$) dissolved in 2 L of water from a nearby open pool, about 10 meters away from the experimental plots, and we poured it onto the N addition plots bimonthly (May - September) to simulate elevated nitrogen deposition. The same amount of open pool water was poured to the control plots. The rate of N addition was 6.4 g N $m^{-2}$ $yr^{-1}$ in order to establish non-N-limited conditions for this nutrient-poor bog (Reay et al., 2008). This rate is comparable to the level of N addition treatments used in other peatland studies (Juutinen et al., 2010; Leeson et al., 2017). Vegetation removal was undertaken manually. The shoots of shrubs and graminoids were cut back to the litter layer level in early May and early July every year since 2014. This removal experiment was effective and useful for understanding the ecosystem effects of vegetation composition change (Díaz et al., 2003). Plots were left to settle for a year before sampling to minimize the effects of decomposition from roots.

### 6.3.2 Measurement of $N_2O$ fluxes and environmental parameters

The measurement methods about $N_2O$ fluxes have been described in previous studies (Gong et al., 2019; Luan et al., 2019). Briefly, gas samples were taken from opaque chambers (50 cm in height and 26.3 cm in diameter, cover ~0.05 $m^2$) fitted to the groove of the PVC (polyvinyl chloride) collars, which were inserted into the soil to the depth of 10 cm permanently in May 2014. We used four 60-mL syringes to collect gas samples at four intervals during the 30 minutes of chamber closure: immediately upon the closure of the chamber, and 10, 20, and 30 minutes after chamber closure. The measurements were conducted biweekly during the growing seasons in 2016 and 2018 depending on the weather. In total, we have 512 measurements in 2016, and 384 measurements in 2018. $N_2O$ fluxes were analyzed by gas chromatography method and determined as the slope of the linear regression of headspace $N_2O$ concentrations against time (Gong et al., 2019; Minke et al., 2016).

Concurrent with gas sampling, soil pore water samples at ~10 cm depth in each plot were collected using the MacroRhizons sampler (Rhizosphere Inc., Netherlands). Water samples at 40 cm depth were collected using 60 mL syringes from a perforated PVC tube, with a sealed bottom and a capped top to prevent precipitation from entering the tube, where we only perforated the bottom 5 cm of the tube. The perforated PVC tube was inserted into 40 cm depth before. These water samples were filtered by 0.45 µm syringe filters (Cole Parmer Inc., USA) before analyzing dissolved organic carbon (DOC) and dissolved total nitrogen (TN) with a Shimadzu TOC-LCPH/TN analyzer (Shimadzu Inc., Japan).

During each gas sampling campaign, we also measured soil temperature at 5 cm and 20 cm depth using a soil thermometer (Fisher Scientific Inc., Canada) and soil moisture at 5 cm depth using a soil moisture sensor (ProCheck, Decagon Devices Inc., USA). In addition, we measured water table depth, i.e., the water table level below ground, from dip-wells made of 1 m-long perforated PVC pipes installed at each plot (negative values indicate water levels above the peat surface). In addition, we used a USB temperature logger (Lascar Electronics Ltd., UK) to continually record air temperature at vegetation canopy height every 30 minutes. One temperature logger was installed at a randomly selected warming plot, and the other was installed at a randomly selected control plot.

### 6.3.3 Statistical analysis

The linear mixed-effects model was used to test the effect of three global changes (warming, N addition, and vegetation composition) on $N_2O$ fluxes in 2016 and 2018. The main effects of global changes and their interactions were considered as fixed factors. The measurement block and date were considered as random factors. Tukey's post hoc tests were used to determine the differences between treatments. The linear mixed-effects model was also used to test the significant difference in environmental variables (soil temperature, soil moisture, water table depth, TN, and DOC) between 2016 and 2018. The year was considered as a fixed factor. The measurement block and date were considered as random factors. These analyses were performed in the R version 3.5.1 (R Core Team, 2018) with the "lmerTest", "lsmeans" and "car" packages. The residuals were normally distributed around a mean of zero in the models. Redundancy analysis (RDA) was used to determine the explanation of environmental factors (soil temperature, soil moisture, water table depth, total nitrogen, and dissolved organic carbon) for $N_2O$ fluxes. This analysis was performed in CANOCO 5 (Braak and Smilauer, 2012). In addition, the relationship between gross primary production (GPP) (Text S6.1) and $N_2O$ fluxes was also investigated. Because the measurements of GPP and $N_2O$ fluxes were not conducted at the same time but at the same day, we used the daily mean value of GPP and $N_2O$ fluxes to explore their relationship. This analysis was performed in SigmaPlot 12.5 (Systat, Chicago, IL, USA).

## 6.4 Results

### 6.4.1 The effects of single change on $N_2O$ fluxes

As shown in Figure 6.1, warming did not significantly affect $N_2O$ fluxes, while N addition significantly increased $N_2O$ fluxes in both years. Regardless of warming and N addition, vegetation composition change significantly altered the $N_2O$ fluxes in 2016 (Table 6.1, $F_{(3,442)} = 9.67$, $P < 0.001$), with the lowest emission under the treatment of graminoid and shrub remove (-GS, 0.019 ± 0.015 mg $m^{-2}$ $h^{-1}$). However, the effects of vegetation composition were not observed in 2018 ($F_{(3,357)} = 0.75$, $P = 0.525$).

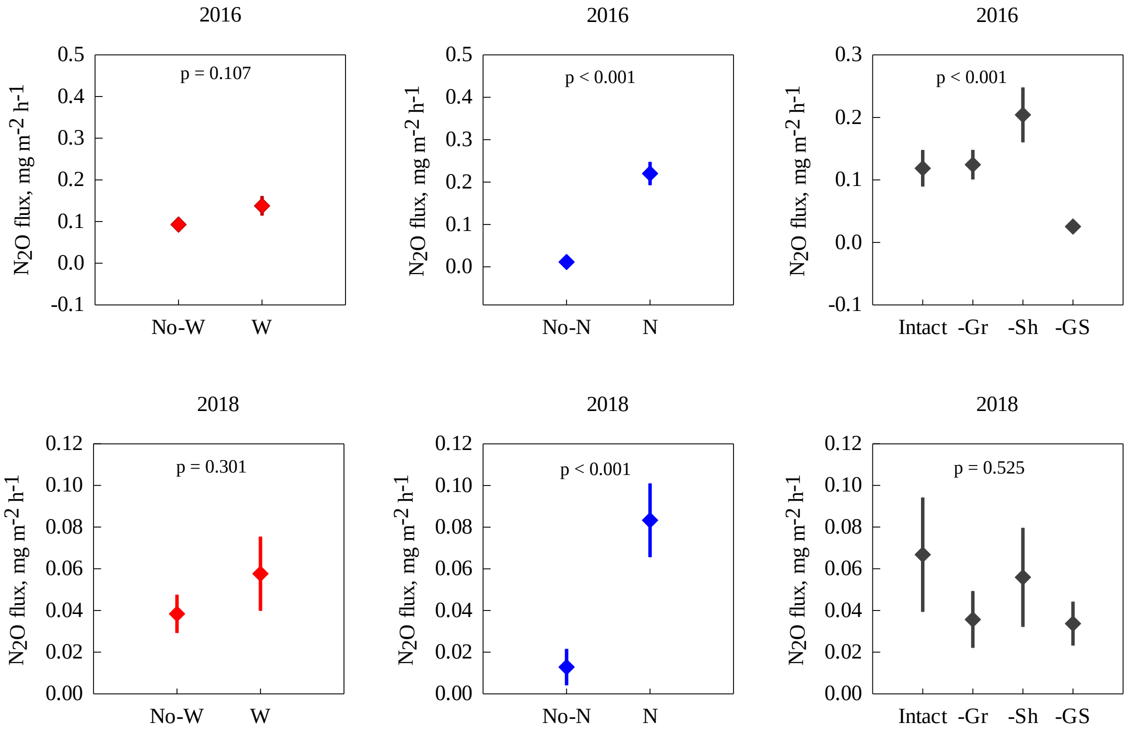


Figure 6.1 The effects of single change (warming, N addition, and vegetation composition change) on $N_2O$ emissions. The dots represent mean values and error bars represent standard error. “No-W” represents no warming treatment, and “No-N” represents no N addition. “Intact”

represents no vegetation removal. "-Gr" represents the removal of graminoids. "-Sh" represents the removal of shrubs. "-GS" represents the removal of graminoids and shrubs.

Table 6.1 Statistical analysis for the effects of, and interactions between, warming, nitrogen addition, and vegetation composition change on $N_2O$ fluxes in the growing seasons of 2016 and 2018.

| | NumDF | DenDF | F value | P value |
|---|---|---|---|---|
| 2016 | | | | |
| Warming | 1 | 442 | 2.61 | 0.107 |
| Nitrogen | 1 | 442 | 57.71 | <0.001 |
| Vegetation | 3 | 442 | 9.67 | <0.001 |
| Warming:Nitrogen | 1 | 442 | 4.64 | 0.032 |
| Warming:Vegetation | 3 | 442 | 2.37 | 0.070 |
| Nitrogen:Vegetation | 3 | 442 | 8.74 | <0.001 |
| Warming:Nitrogen:Vegetation | 3 | 442 | 3.85 | 0.009 |
| 2018 | | | | |
| Warming | 1 | 357 | 1.07 | 0.301 |
| Nitrogen | 1 | 357 | 14.07 | <0.001 |
| Vegetation | 3 | 357 | 0.75 | 0.525 |
| Warming:Nitrogen | 1 | 357 | 4.07 | 0.044 |
| Warming:Vegetation | 3 | 357 | 0.58 | 0.628 |
| Nitrogen:Vegetation | 3 | 357 | 1.37 | 0.250 |
| Warming:Nitrogen:Vegetation | 3 | 357 | 0.30 | 0.828 |

### 6.4.2 The interaction of abiotic and biotic changes on $N_2O$ flux

The significant interaction of abiotic and biotic changes was detected in 2016 (Table 6.1). The positive effect of N addition was not observed under the removal of vegetation (-Gr, -Sh, and -GS) (Figure 6.2). The combination of warming and N addition (WN) significantly increased $N_2O$ fluxes under the condition of -Gr and -Sh. In contrast to 2016, the effects of abiotic factors (warming and N addition) on $N_2O$ flux under different vegetation compositions were not observed in 2018.

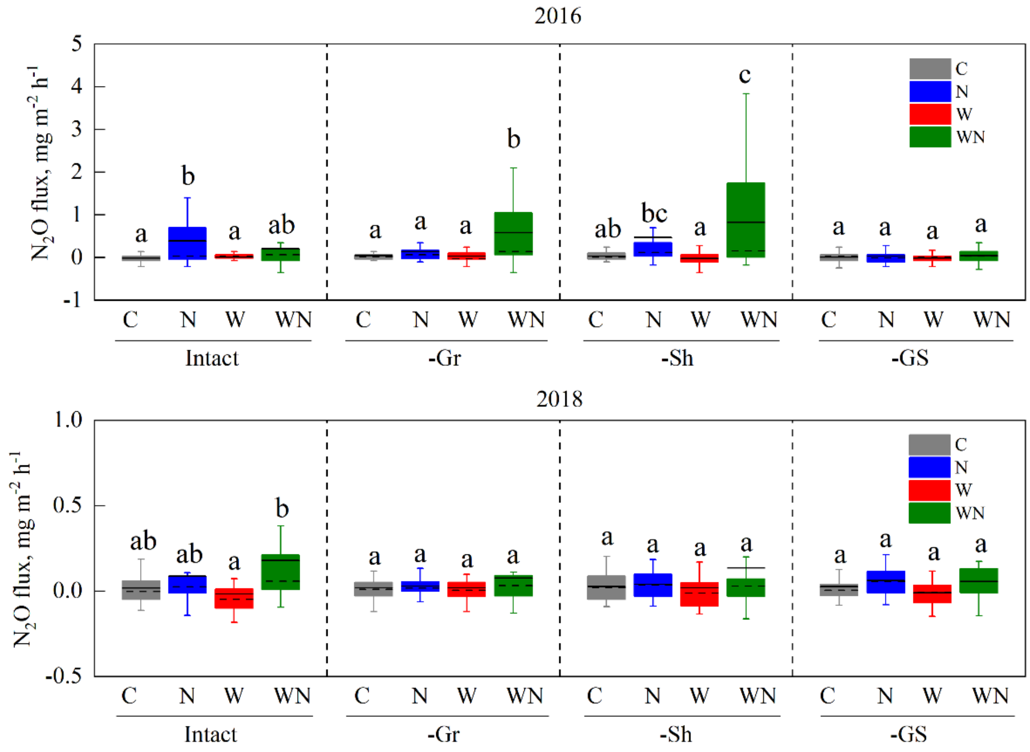


Figure 6.2 The interaction of abiotic factors (warming and N addition) and biotic factors (vegetation composition change) on $N_2O$ fluxes varied between 2016 and 2018. The range of each box is from the twenty-fifth to seventy-fifth percentile. The solid line in each column is the mean value and the dash line is the median value. Different letters above the box represent significant differences ($p < 0.05$) between the treatments according to Tukey's multiple

comparison test.

From the perspective of temporal variation, warming decreased the positive effect of N addition from the end of June to the August under intact vegetation condition in 2016 (Figure 6.3). However, this effect was not evident under the vegetation removal treatment (-Gr, -Sh, and -GS). Instead, the phenomenon that warming stimulated N effect on $N_2O$ fluxes after graminoid removal (-Gr) (Tukey's test, $P<0.05$) or shrub removal (-Sh) (Tukey's test, $P<0.05$) was observed before early June of 2016. Additionally, there was no significant effect of warming and N addition under removal of all vascular plants (-GS). Furthermore, the significant effects of abiotic and biotic changes were not observed in 2018.

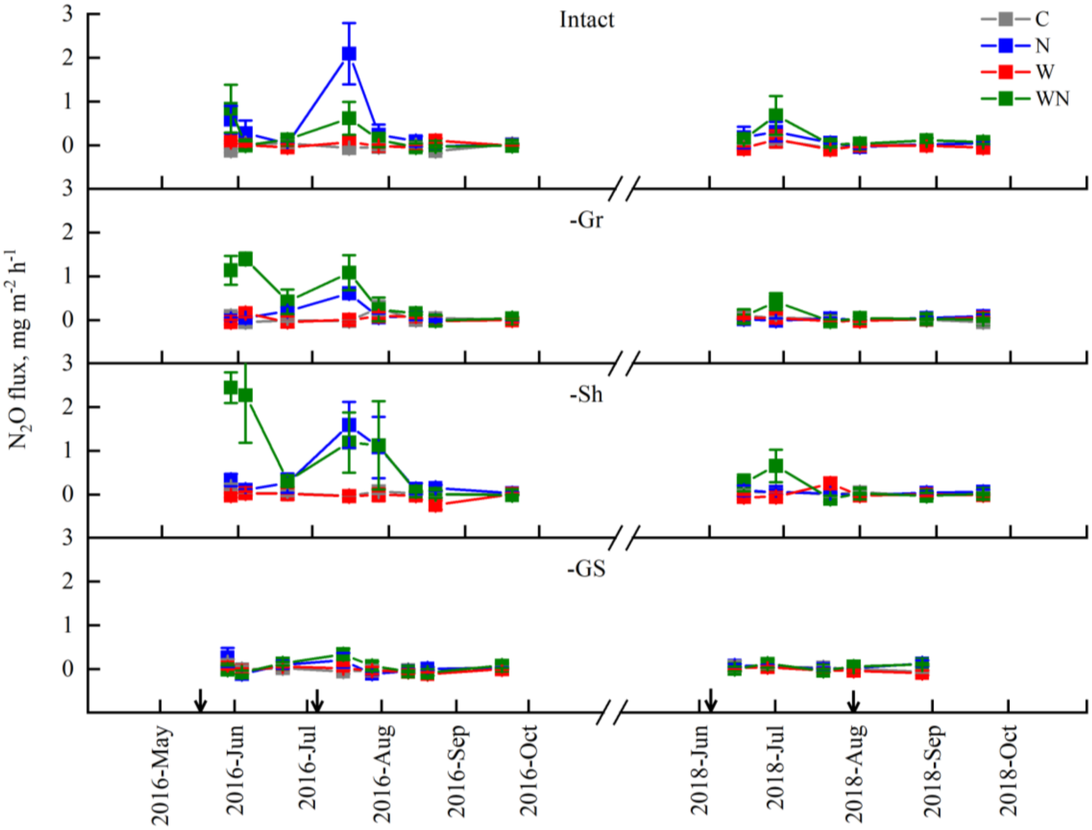


Figure 6.3 The temporary variation of $N_2O$ fluxes under different treatments. Error bar represents standard error. “↓” represents the date of N addition.

### 6.4.3 The controls of $N_2O$ fluxes

As shown in Table 6.2, the variation of $N_2O$ fluxes can be explained by TN at 40 cm depth, DOC at 10 cm depth, and soil temperature at 20 cm depth in the bog. However, it should be noted that these abiotic parameters only explained ~5% of $N_2O$ variation (Table 6.2). In addition, we found that GPP exerted a significant effect on $N_2O$ fluxes under N addition and explained 18.5% of $N_2O$ flux variation (Figure 6.4).

Table 6.2 Explanation of environmental parameters for $N_2O$ fluxes variation based on redundancy analysis (RDA). The number in the brackets represents the depth below the surface.

| | Explains % | Contribution % | P value |
|---|---|---|---|
| Soil T (20 cm) | 2 | 36.4 | 0.010 |
| TN (40 cm) | 1.9 | 34.5 | 0.022 |
| DOC (10 cm) | 1.2 | 21.8 | 0.048 |
| TN (10 cm) | 0.2 | 3.6 | 0.294 |
| DOC (40 cm) | <0.1 | <1.8 | 0.720 |
| Soil T (5 cm) | <0.1 | <1.8 | 0.810 |

Note: because of collinearity, water table depth and soil moisture were not included.

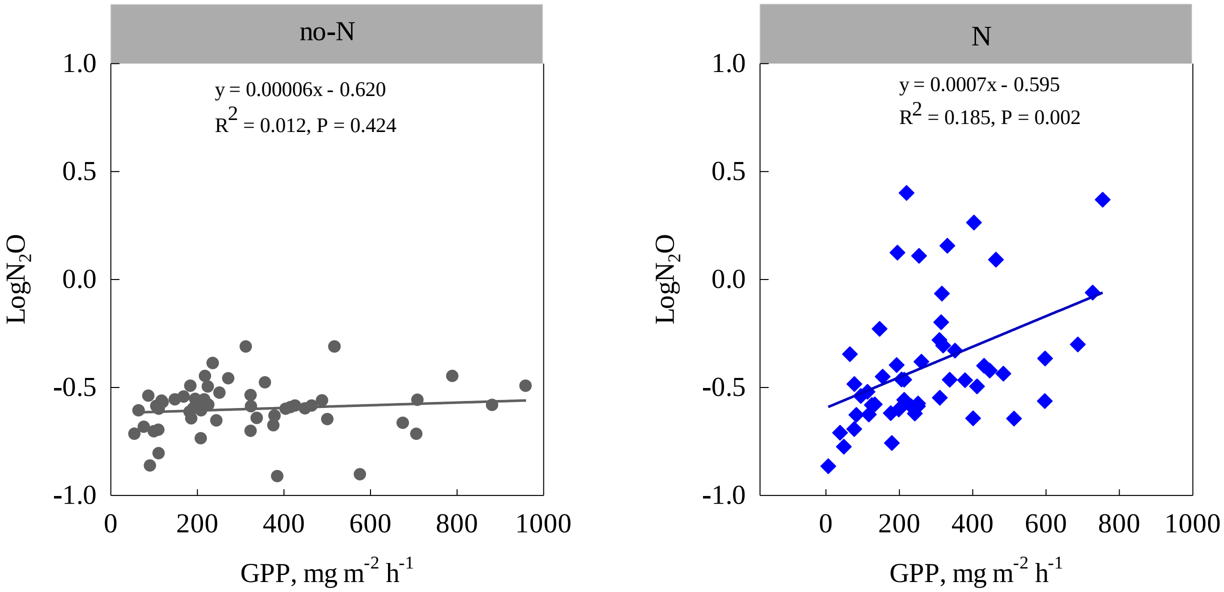


Figure 6.4 The relationship between $N_2O$ flux (log-transformed) and gross primary production (GPP) with nitrogen (N) or without nitrogen (no-N) addition.

## 6.5 Discussion

Peatlands, with a significant amount of N storage, have the potential to become hotspots of $N_2O$ fluxes under global change. Although the effect of elevated N deposition on $N_2O$ emitted from peatlands has been widely observed, the interactions with other global changes (warming and vegetation composition change) are not well known. Moreover, albeit many studies focus on the abiotic controls on $N_2O$ fluxes, their results are inconsistent and the abiotic controls only explain a small part of $N_2O$ flux variation, which further increases the uncertainty in predicting $N_2O$ fluxes in peatlands. This study filled the knowledge gap and revealed that the interaction of warming and N addition on $N_2O$ fluxes was regulated by the vegetation composition change. Furthermore, we also found that GPP was an essential control on $N_2O$ fluxes in peatlands, which can be incorporated in $N_2O$ prediction models.

### 6.5.1 Effects of single change

Warming alone did not affect $N_2O$ flux, probably owing to the low effect on nutrient availability and microbial activity, which is in line with previous studies (Gong et al., 2019; Pearson et al., 2015; Ward et al., 2013). The positive effect of N addition on $N_2O$ fluxes is consistent with previous studies due to the increase of N availability for $N_2O$ production (Leroy et al., 2019; Lund et al., 2009). These results indicate that elevated N deposition can increase $N_2O$ emission and its contribution to global warming and ozone depletion, while climate warming has limited effect.

The effect of vegetation composition change on $N_2O$ fluxes has not been widely investigated. Removal of vascular plant is expected to increase N availability for $N_2O$ production due to reduced competition between plant and microbes for available N (Brummell et al., 2017). However, we did not find the positive effects of graminoid or shrub removal on $N_2O$ fluxes. Instead, the absence of all vascular plants (-GS) significantly decreased $N_2O$ fluxes. This unexpected result can be attributed to the reduction of root exudate, which might decrease the available carbon for $N_2O$ production (Wu et al., 2017). In addition, removal of vascular plants can reduce the $N_2O$ transport via aerenchyma tissues (Jørgensen et al., 2012; Le et al., 2021), which may decrease $N_2O$ emission from the bog. It should be noted that this negative effect of -GS was not observed in 2018. Compared with 2016, DOC concentration in 2018 was considerably lower owing to the different meteorological conditions between the two years (Table S6.1). This low DOC concentration implies the limited carbon availability for $N_2O$ production (Dodla et al., 2008), which could mask the effect of -GS.

### 6.5.2 The interaction of abiotic and biotic changes

Most studies focus on the impacts of abiotic factor (warming, nitrogen deposition, and drought condition) on $N_2O$ fluxes in peatlands (Gong et al., 2019; Lohila et al., 2010; Pearson et al., 2015), while limited studies focus on the interactions of abiotic and biotic factors. These interactions play an essential role in modeling $N_2O$ fluxes. In this study, we did not observe the interaction of warming and vegetation composition on $N_2O$ fluxes, which is in line with a previous study (Ward et al., 2013) and can be ascribed to the low N availability in peatlands.

Vascular plant presence has been reported to reduce the positive effect of N addition on $N_2O$ emissions from peatlands via competition with denitrifies for available nitrogen (Silvan et al., 2005; Leroy et al., 2019). However, we did not observe this phenomenon. In contrast, we found that graminoid absence reduced the positive effect of N addition on $N_2O$ fluxes, which can be attributed to the increase of N retention capacity of *Sphagnum* moss and reduction of $N_2O$ transport through plant tissues (Jørgensen et al., 2012; Le et al., 2021). In addition, this interaction was not observed in 2018, owing to the low DOC concentration and limited carbon availability for $N_2O$ production (Dodla et al., 2008).

Warming reduced the positive effect of N addition under intact vegetation in the middle growing season of 2016, which can be attributed to the stimulated N uptake by vegetation and reduced N availability for $N_2O$ production (Gong et al., 2019). After removal of vascular plants (shrubs and/or graminoids), this mitigation was not evident due to the alleviated competition between plants and microbes for available N. The result supports part of our first hypothesis. Surprisingly, we observed combined treatment of warming and N addition considerably promoted $N_2O$ emission at the early growing season (May - June) when the shrubs or graminoids were removed. Given the low air temperature (~8 $^{o}$C) and soil temperature (~13 $^{o}$C at 5 cm depth and ~9 $^{o}$C at 20 cm depth) during that time, the biochemical processes and vegetation growth could be considerably constrained, which can reduce the available C and N in soil (Munir et al., 2017; Song et al., 2018). With N addition alone, the biochemical processes of $N_2O$ production might be limited by carbon availability. With warming alone, $N_2O$ production in peatlands might be limited by nitrogen availability. Combined warming and N addition can alleviate these two limitations and significantly increase $N_2O$ emission. However, the combined effect was not observed under the removal of all vascular plants, which can be attributed to the reduction of root exudates and available carbon for $N_2O$ production.

Our results emphasize the interaction of abiotic (warming and N addition) and biotic (vegetation composition change) factors on $N_2O$ fluxes in peatland ecosystems. If peatlands shift from *Sphagnum*-dominated to graminoid-dominated (Dieleman et al., 2015) or shrub-dominated systems (Bragazza et al., 2015), the greenhouse gas-$N_2O$ flux would be increased under future climate warming and elevated N deposition, which potentially accelerates global warming and ozone depletion in the stratosphere. In addition, our result supports the point that the temporal variation should not be ignored in $N_2O$ predicting models (Nol et al., 2009). The mitigating effect of warming on the positive N effect was observed in the mid-growing season (July-August), while the enhancement effect of warming on the N effect was observed in the early growing season (May-June). Furthermore, these effects were not observed in 2018 owing to the low DOC for $N_2O$ production (Dodla et al., 2008), suggesting the carbon availability should also need to be considered in the $N_2O$ predicting models.

### 6.5.3 Controls on $N_2O$ fluxes under global changes

Few studies focused on the models of $N_2O$ flux in peatlands (Dinsmore et al., 2009; Nol et al., 2009; Pärn et al., 2018). In order to develop $N_2O$ models in peatlands, the major controls should be well-identified. However, because conclusion about controls for $N_2O$ fluxes in peatland ecosystems is inconsistent, there is large uncertainty in developing models to predict $N_2O$ fluxes under global change. In this study, we found that soil temperature, DOC, and TN were the major abiotic controls for $N_2O$ fluxes, which is slightly opposite to our second hypothesis because the relationship between water table depth and $N_2O$ fluxes was not observed. Water table depth regulates the boundary of the oxic layer and anoxic layer. Considering nitrification is under the oxic condition, and denitrification is under anoxic condition, $N_2O$ fluxes should be related to water table depth. The unexpected result in this study can be owing to the narrow range of water table depth during the measurement (Table S6.1).

Consistent with the previous study (Dinsmore et al., 2009), our results also indicate that soil temperature, carbon and nitrogen availability need to be incorporated into the $N_2O$ predicting models. However, despite including all these controls into the model, they only explain a small part of $N_2O$ flux variation (~5%), indicating other important variables, such as biotic variables, are not included. In this study, we found that GPP was another essential biotic control on $N_2O$ fluxes, which needs to be taken into account and could help to develop the $N_2O$ predicting models. The positive relationship could be owing to the increase of root exudates for $N_2O$ production (Wu et al., 2017). However, it should be noted that this relationship was only observed under N addition. Given N deposition will increase in the future (Reay et al., 2008), GPP will be an essential factor for $N_2O$ fluxes and needs to be taken into account in the $N_2O$ predicting models. However, further studies are needed to specify the governing processes between vegetation production and $N_2O$

emission, and parameterize these processes in peatland ecosystem $N_2O$ modeling. Noticeably, environmental parameters and GPP cannot adequately explain the $N_2O$ variation. The other critical biotic variables may be related to microbes, which is not measured in this study and needs further research.

## 6.6 Conclusion

The unclear interactions among global changes (warming, elevated N deposition, and vegetation composition change) lead to an uncertainty in predicting $N_2O$ fluxes in peatlands. Although the effect of warming on $N_2O$ fluxes was not observed in this study, it reduced the positive N effect under intact vegetation due to the increase of N uptake by plant and the decrease of available N for $N_2O$ production. When graminoids or shrubs were removed, warming did not mitigate the N effect. Instead, it strengthened the N effect on $N_2O$ fluxes. Our results indicate that vegetation composition exerts a significant impact on the $N_2O$ fluxes under climate warming and elevated N deposition. If peatlands shift from *Sphagnum*-dominated to graminoid-dominated or shrub-dominated systems, $N_2O$ flux would be increased under climate warming and elevated N deposition, thus accelerating global warming and ozone depletion in the stratosphere. However, it should be noted that these effects were not observed under the condition of low carbon availability. Coupled with low carbon availability, $N_2O$ emission is constrained even though under high N addition and the change of vegetation composition. These findings imply that not only the interactions of global change but also the temporal variation and carbon availability should be taken into account in order to project $N_2O$ fluxes in peatlands accurately. Furthermore, GPP, soil temperature, carbon and nitrogen availability were found to be the essential controls for $N_2O$ fluxes under global changes. However, they only explain a small part of $N_2O$ fluxes variation. Further research should focus on the microbial parameters in order to improve the $N_2O$ predicting models.

### 6.7 Acknowledgments

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## 6.9 Supplementary files

### Text S6.1 Measurement of gross primary production (GPP)

GPP was calculated from the difference between ecosystem respiration (ER) and net ecosystem production (NEP). We measured ER with an opaque chamber, and NEP with a transparent chamber in the growing season of 2016 biweekly from 10:00 to 16:00 local time. With the opaque chamber, light was blocked and thus no photosynthesis occurred during the measurement. Therefore, the $CO_2$ flux calculated based on the measurements from the opaque chamber can be considered as an ER. With the transparent chamber, light can penetrate the chamber and thus photosynthesis occurred during the measurement. Therefore, the $CO_2$ flux calculated based on the measurements from the transparent chamber can be considered as a NEP, a balance between GPP and ER. There was only about 1-minute break between the measurement from the transparent chamber (for NEP) and from the opaque chamber (for ER). Therefore, it was reasonable and accurate to estimate GPP from the measurement of NEP and ER at the same plot. A Portable Greenhouse Gas Analyzer (Los Gatos Research Inc., USA) was used to measure them. GPP in other years was not measured due to the malfunction of the instrument. During each measurement, we placed the chamber on the collar and sealed it with water. A fan was fixed on the top of the sampling chamber to mix and cool the air. The gas concentration was collected at 1 Hz rate during a measurement period of 3 minutes after steady-state conditions were reached. Therefore, during the 3 minutes of chamber closure, we obtained 180 measurements of $CO_2$ concentration. The $CO_2$ fluxes were calculated based on linear regression.

Table S6.1 Comparing environmental variables (mean ± S.E.) between 2016 and 2018 based on a linear mixed effect model analysis. The number in the brackets represents the depth below the surface.

| | 2016 | 2018 | P value |
|---|---|---|---|
| DOC (10 cm) | 44.88±0.41 | 31.25±0.33 | 0.006 |
| TN (10 cm) | 0.90±0.02 | 1.13±0.05 | 0.161 |
| Soil moisture | 69.60±0.92 | 67.36±0.93 | 0.911 |
| WTD | 6.57±0.26 | 6.45±0.34 | 0.957 |
| Soil T (20 cm) | 14.44±0.14 | 15.21±0.21 | 0.609 |
| Soil T (5 cm) | 15.35±0.14 | 19.76±0.26 | 0.042 |
| DOC (40 cm) | 50.58±0.44 | 24.84±0.40 | <0.001 |
| TN (40 cm) | 1.55±0.05 | 1.61±0.07 | 0.904 |

# CHAPTER 7. Summary and conclusion

Few studies focus on the interactive effects of three global changes (climate warming, elevated nitrogen (N), and vegetation composition change) on greenhouse gases fluxes in peatlands, and that leads to large uncertainty in projecting greenhouse gas fluxes in peatlands and evaluating their function of carbon sink and climate mitigation. In order to reduce this knowledge gap, manipulated warming, nitrogen addition, and vegetation composition change have been conducted at a boreal peatland in western Newfoundland, Canada. The objective of this thesis is to investigate the three potent greenhouse gas fluxes ($CO_2$, $CH_4$, and $N_2O$) in the peatland under simulated global changes, explore the possible underlying mechanisms, and evaluate the major controls for them.

## 7.1 Major findings and significance

### 7.1.1 $CO_2$ fluxes in the peatland under global change

Compared with warming and elevated nitrogen (N) deposition, a change in vegetation composition plays a more critical role in net $CO_2$ uptake of the peatland (Chapter 3). Removal of graminoids and/or shrubs significantly decreased net $CO_2$ uptake, which is in line with previous studies (Gavazov et al., 2018; Ward et al., 2013). Interestingly, after seven years of shrub removal, the net $CO_2$ uptake rate was similar with that under intact vegetation, suggesting that the carbon sink function of peatlands might be not changed if the peatlands shift to graminoid-dominated ecosystems (shrub removal). Although the impacts of warming and N addition on net $CO_2$ uptake were not stronger than vegetation composition change, their effects should not be ignored. The results showed that combined warming and N addition considerably decreased net $CO_2$ uptake of the peatland under the condition of graminoid removal, mainly due to the detrimental effect of N addition on *Sphagnum* mosses. This result indicates that climate warming and elevated N deposition could further decrease the $CO_2$ uptake if the peatlands shift to shrub-dominated ecosystems (graminoid removal). In addition, soil moisture, soil temperature, and dissolved organic carbon were the major controls for net $CO_2$ uptake in the peatland.

### 7.1.2 $CH_4$ fluxes in the peatland under global change

Graminoid removal significantly decreased $CH_4$ emissions from the peatlands due to the reduction of available carbon for $CH_4$ production and aerenchyma for $CH_4$ transport (Nielsen et al., 2017). Nevertheless, this negative effect was not observed under the combination of warming and N addition possibly owing to the change of temperature sensitivity (Chapter 4). This result suggests that shifting the dominant vegetation to shrubs (graminoid removal) might not alter the $CH_4$ fluxes in peatlands in the context of future climate warming and elevated N deposition. In addition, the negative effect of shrub removal on $CH_4$ emission was observed under warming conditions, suggesting that shifting the dominant vegetation to graminoids (shrub removal) would decrease the $CH_4$ fluxes in peatlands under future climate warming. However, this negative effect was not observed under the combination of warming and N addition, which could be attributed to the stimulation of graminoid growth under warming and N addition. The growth of graminoids could increase available carbon for $CH_4$ production and $CH_4$ transport via aerenchyma. This positive effect could offset the negative impact of shrub removal. Therefore, shifting the dominant vegetation to graminoids (shrub removal) might also not change the $CH_4$ fluxes in peatlands in the context of future climate warming and elevated N deposition. Furthermore, water table depth, soil temperature, dissolved organic carbon, and total nitrogen were the major controls for $CH_4$ fluxes in the peatland.

### 7.1.3 $N_2O$ fluxes in the peatland under global change

Nitrogen addition significantly promoted $N_2O$ emissions from the peatland due to the increase of available nitrogen for $N_2O$ production via nitrification and denitrification (Gao et al., 2014). However, warming could reduce its positive impact on $N_2O$ fluxes regardless of vegetation composition change in the middle growing season (Chapter 5), which could be attributed to the stimulation of nitrogen uptake by plants and less nitrogen for $N_2O$ production. In contrast, combined warming and N addition could promote $N_2O$ emissions under the condition of graminoid removal or shrub removal (Chapter 6). It should be noted that the mitigation effect of warming was observed in the middle growing season, while the promotion effect was observed in the early growing season. The possible underlying mechanism is that the biochemical processes of $N_2O$ production are limited by available nitrogen and carbon in the early growing season. With vascular plant removal, processes of $N_2O$ production might be limited by available carbon. In this case, warming could stimulate plant growth and provide available carbon for $N_2O$ production, while N addition could provide available N for $N_2O$ production. These results indicate that $N_2O$ emitted from peatlands would be considerably increased under global changes. Furthermore, the gross primary production, soil temperature, dissolved organic carbon, and total nitrogen were the major controls for $N_2O$ fluxes.

### 7.1.4 Cooling function of the peatland under global changes

Peatlands can mitigate global warming by regulating greenhouse gas fluxes (Leifeld et al., 2019). This cooling function could be altered under future global change. If the peatlands shift to shrub-dominated ecosystems in the future, the net $CO_2$ uptake would be decreased. Moreover, climate warming and elevated N deposition would further decrease net $CO_2$ uptake from the result, indicating that net $CO_2$ uptake in boreal peatlands will be reduced under future global change.

Although peatlands shifting to the shrub-dominated ecosystems could decrease $CH_4$ emitted from peatlands, the negative effect was not observed under warming and N addition condition. What's worse, warming and elevated N deposition considerably increased $N_2O$ fluxes under shrub-dominated conditions. Given that $N_2O$ has ~300 times higher global warming potential than $CO_2$ (IPCC, 2013), the cooling function of peatlands will be substantially reduced in the future.

If the peatlands shift to graminoid-dominated ecosystems in the future, the net $CO_2$ uptake would be decreased. However, the capacity of $CO_2$ uptake can be recovered on a long-term scale (~ 7 years); and climate warming and elevated N deposition have limited effects on it. This result implies that the net $CO_2$ uptake in boreal peatlands might not be impacted under global change. In addition, graminoid-dominated conditions do not affect $CH_4$ fluxes under climate warming and elevated N deposition. Nevertheless, climate warming and elevated N deposition could significantly increase $N_2O$ emitted from peatlands under the graminoid-dominated conditions. Taking three greenhouse gases into account, the cooling function of peatlands will be weakened under future global change.

## 7.2 Limitations and further research

This research sheds new light on the impacts of three crucial global changes (climate warming, elevated N deposition, and vegetation composition change) on greenhouse gas fluxes in peatlands. However, the effects of global change during the non-growing season are not investigated due to the bad weather conditions, which leads to an uncertainty in accurately evaluating the carbon sink and cooling function of peatlands. Moreover, although we put forward some possible underlying mechanisms, these impacts are not fully elucidated. The information of microorganisms could help to fill this knowledge gap. Because of avoiding soil disturbance, microorganisms were not measured in this study. The production and consumption of greenhouse gases are closely linked to microorganisms. For example, $CH_4$ production is related to methanogens, while $CH_4$ oxidation is related to methanotrophs. $N_2O$ production and consumption is related to microorganisms in N cycling such as nitrifiers and denitrifiers. In this thesis, the environmental variables and GPP cannot fully explain the variation of greenhouse gas fluxes. The presumably reason is that the activity, abundance and community structure of the related microorganisms have been altered. Therefore, further research should focus on the global change impacts on microorganisms in order to elucidate the underlying mechanisms of greenhouse gas fluxes in peatlands.

## 7.3 Conclusion

This thesis has investigated three potent greenhouse gas fluxes ($CO_2$, $CH_4$, and $N_2O$) in a boreal peatland under three simulated global changes (climate warming, elevated N deposition, and vegetation composition change). The interactions of these changes are not simple additive, and should be taken into account in the greenhouse gas predicting models to accurately evaluate the climate mitigation function of peatlands. In addition, to improve the models predicting greenhouse gas dynamics in peatlands, not only the interaction of these global changes but also the major controls are needed to be well understood. Therefore, the main biotic and abiotic controls found in this research could help to reduce the uncertainty in the models.

## 7.4 References

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