FUNCTIONS AND DYNAMICS OF BOREAL BOG VEGETATION UNDER GLOBAL CHANGES

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Abstract

Vegetation with a dominance of *Sphagnum* and low cover of vascular plants have been recognized as a core component that controls several biogeochemical processes and vital services of boreal bogs, especially in greenhouse gas emissions. The boreal bog plants are long-term adapted to cold, low available nutrient conditions. Therefore, global warming and increases in nitrogen (N) availability may change the boreal bog vegetation and alter the primary services of these boreal ecosystems. This study utilized long-term field experiments to examine the function of vegetation composition in regulating N_2O emissions and dynamics of boreal bog vegetation to the projected global changes. Results show that graminoids accelerate N_2O emissions from boreal bogs under N-enriched conditions. This study also indicates that the long-term warmer (W) and N-enriched conditions enhance vascular plant growth, especially graminoids. By contrast, these environmental changes result in rapid losses of *Sphagnum* in boreal bogs. Vascular plants mitigate the decline of *Sphagnum*, although the facilitation by vascular plants can not eliminate the decreases in *Sphagnum* mosses due to the warming and high N availability. These findings suggest that vegetation might switch to the dominance of vascular plants under warmer, N-enriched conditions, stimulating N_2O emission in boreal bogs.

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Chapter 1 Introduction and overview

1.1. Overview and objectives

Boreal bogs are crucial ecosystems generating several vital services, including carbon sequestration, greenhouse gas regulation, water supply, biodiversity protection, natural risk mitigation, food and fuel sources, and recreation opportunities (De Groot et al. 2002, Kimmel and Mander 2010, Page and Baird 2016). Previous studies indicated that vegetation modulated several biogeochemical processes and vital services within boreal bogs: carbon sequestration (Belyea and Malmer 2004, Potvin et al. 2015, Kolari et al. 2021), decomposition rate (Dorrepaal et al. 2005, Ward et al. 2015b, Wiedermann et al. 2017, Bell et al. 2018, Gavazov et al. 2018), microbial activities (Bragazza et al. 2015, Robroek et al. 2015, Robroek et al. 2016, Dieleman et al. 2017) and nitrogen cycle (Aerts et al. 1999). Notably, vegetation also determines greenhouse gas emissions from boreal bogs (Laine et al. 2012, Ward et al. 2013b, Robroek et al. 2015, Dieleman et al. 2017, Riutta et al. 2020, Whitaker et al. 2021) that are increasingly important under global climate changes. Specifically, intact boreal bogs with a dominance of *Sphagnum* and a low cover of vascular plants (Malmer et al. 2003, Turetsky et al. 2012b) have been characterized as significant sinks of CO_2 (Gorham 1991, Blodau 2002, Turunen et al. 2004, Yu et al. 2009), important sources of CH_4 (Mikaloff Fletcher et al. 2004, Lai 2009, Turetsky et al. 2014), and negligible sources of N_2O (Bobbink et al. 1998b, Limpens et al. 2006, Francez et al. 2011, Frolking et al. 2011).

Increasing evidence suggests that boreal bogs are subject to global changes, including warmer climate, high CO_2 concentration (Stocker et al. 2014), and high level of nitrogen availability due to high nitrogen deposition (Reay et al. 2008, Ackerman et al. 2019) and rapid decomposition rate under the warmer conditions (Ward et al. 2015). For example, the environmental changes may strongly affect vegetation that long-term adaptation to cold and poor nutrient availability conditions of boreal bogs (Bobbink et al. 1998b). The warmer climate benefits vascular plants and limits the growth of *Sphagnum* (Gunnarsson et al. 2004, Bragazza 2008, Jassey et al. 2013, Walker et al. 2015, He et al. 2016, Norby et al. 2019, Whitaker et al. 2021). The increase of nitrogen availability also enhances the abundance of vascular plants, while the nitrogen enrichment may lead to rapid losses of *Sphagnum* in boreal bogs (Gunnarsson and Rydin 2000, Van der Heijden et al. 2000, Berendse et al. 2001, Gunnarsson et al. 2004, Limpens et al. 2004, Bubier et al. 2007, Gerdol et al. 2007, Limpens et al. 2009, Bu et al. 2011a, Limpens et al. 2011, Fritz et al. 2012, Granath et al. 2012, Larmola et al. 2013, Wieder et al. 2019, Wieder et al. 2020). Recent research also indicates that the boreal bog vegetation growth is also affected by elevated CO_2 concentration levels (Tjoelker et al. 1998, Saarnio et al. 2003, McPartland et al. 2019, Ward et al. 2019). However, the combined effects of these environmental changes on boreal bog vegetation are still understudied. More importantly, the environmental changes may alter the interaction between plants that regulate the composition of communities and ecosystems (Brooker 2006). Despite this, whether changes in plant-plant interactions will modify the effects of environmental changes on boreal bog vegetation is unknown (Figure 1-1).

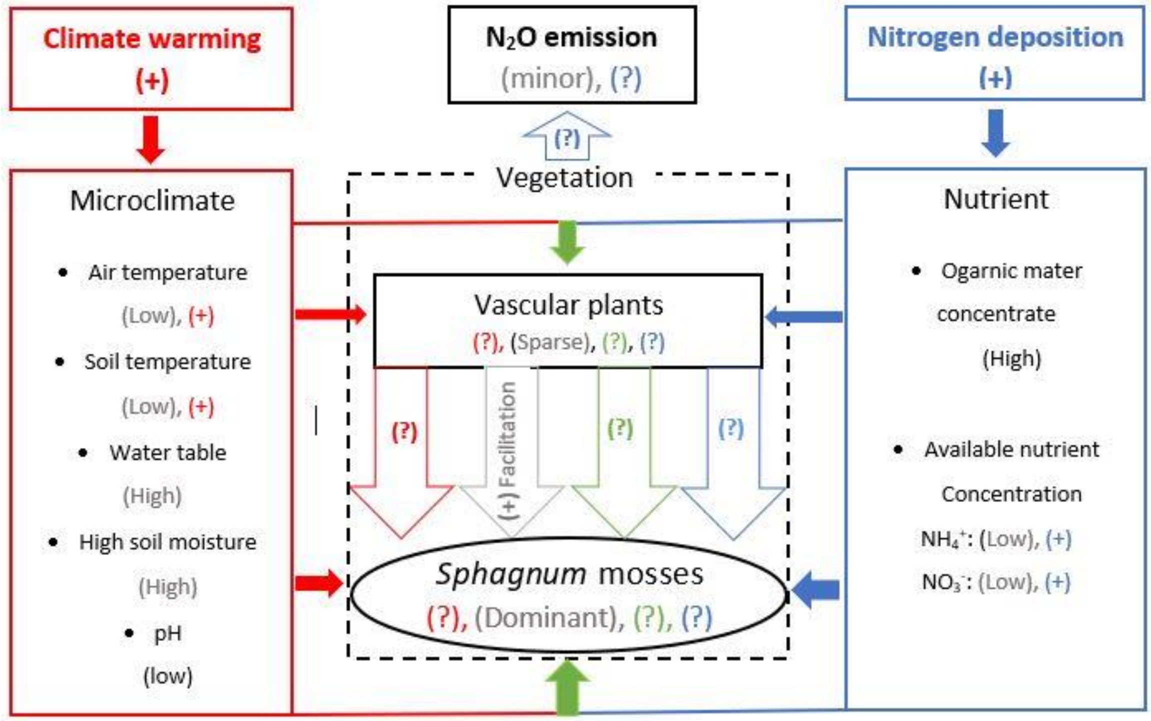

Figure 1-1. Knowledge gaps in potential effects of warming (red), enriched nitrogen availability (blue), and their combination (green) on vegetation and N_2O emissions on boreal bogs. The grey color indicates the boreal bog characteristics without disturbances.

The changes in vegetation regulate the effects of environmental changes on CO_2 (Ward et al. 2013a, Oke and Hager 2020, Whitaker et al. 2020) fluxes and CH_4 emissions (Ward et al. 2013a, Gong et al. 2021) in boreal bogs. These ecosystems will emit more N_2O under enriched nitrogen availability (Nykänen et al. 2002, Gao et al. 2015, Gong et al. 2019). However, the role that vegetation plays in regulating N_2O emission due to the enriched nitrogen conditions is unknown (Figure 1-1).

This thesis will (1) examine combined effects of both vegetation composition and nitrogen addition on N_2O, which contributes to comprehensively understanding the role that boreal bogs play in greenhouse gas regulation under nitrogen-enriched conditions; (2) examine responses of vascular plants to warmer and nitrogen-enriched conditions as well as changes in interaction between the plants under these environmental changes; (3) investigate responses of *Sphagnum* to warmer and nitrogen-enriched conditions as well as roles of vascular plants in regulating these responses of boreal bog *Sphagnum* to these environmental changes; (4) determine responses of foliar photosynthesis and respiration of dominant boreal bog plants to scenarios of future CO_2 emission and temperature increases under nitrogen-enriched conditions.

1.2. Thesis outline

In addition to this introductory chapter, this thesis includes the following chapters:

Chapter 2: Graminoid removal reduces the increase of N2O fluxes due to nitrogen fertilization in a boreal peatland. This chapter was published in Ecosystems, 24(2), 261-271, https://doi:10.1007/s10021-020-00516-5.

Chapter 3: Effects of warming and nitrogen addition on vascular plants on a boreal bog.

Chapter 4: Vascular plants regulate responses of boreal bog *Sphagnum* to climate warming and nitrogen addition. This chapter was published in Science of The Total Environment, 2021, 152077, ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2021.152077.

Chapter 5: Long-term warming and nitrogen addition regulate responses of dark respiration and net photosynthesis in boreal bog plants to short-term increases in CO_2 and temperature.

Chapter 6: Conclusions

Co-authorship statement

In this thesis, four chapters from 2 to 5 were completed with the cooperation of co-authors, wherein I significantly contributed during the preparation of these manuscripts. In detail, in manuscript # 1 (chapter 2)- "*Graminoid removal reduced the increase of* N_2O *fluxes due to nitrogen fertilization in a boreal peatland*" by Thuong Ba Le, Jianghua Wu (JW), Yu Gong (YG), Judith Vogt (JV), I am the first author who analyzed data, wrote, and improved the manuscript. JW conceived, designed, and provided funding for this study. JW, YG, JV, and I carried out the study. JW, YG, and JV edited and commented on the first draft of the manuscript. All authors were involved in the revision.

In manuscript # 2 (chapter 3)- " *Effects of warming and nitrogen addition on vascular plants in a boreal bog*", manuscript # 3 (chapter 4)-"*Vascular plants regulate responses of boreal bog Sphagnum to climate warming and nitrogen addition*" and manuscript # 4 (chapter 5)-" *Long-term warming and nitrogen addition regulate responses of dark respiration and net photosynthesis in boreal bog plants to short-term increases in* CO_2 *and temperature*" prepared by Thuong Ba Le, Jianghua Wu (JW) and Yu Gong (YG), I designed experiment methods, collected and analyzed data, wrote, and improved these manuscripts. JW designed the experiment and edited the manuscripts. YG contributed to writing and improving the manuscripts.

Chapter 2 Graminoid removal reduces the increase of N_2O fluxes due to nitrogen fertilization in a boreal peatland

2.1. Abstract

The increase of nitrogen (N) deposition may affect nitrous oxide (N_2O) emission in boreal peatlands by increasing N availability and/or altering vegetation composition. However, the effects of changes in vegetation composition (due to the increase in N deposition) on N_2O emission are still unknown. Therefore, we used a factorial design, comprising the removal of plant functional groups and N fertilization, to investigate their independent effects and combined effects on N_2O fluxes at an ombrotrophic blanket bog in Western Newfoundland, Canada. The results reveal that N_2O emission significantly increased with N fertilization. Notably, the effect of N addition on N_2O fluxes was modulated by the removal of graminoids. In particular, the removal of graminoids reduced 69% of the N_2O emission increases due to N fertilization in the third year of the experiment. This result suggests that the enhancement of graminoid abundance due to increases of N deposition/fertilization may dramatically increase N_2O emissions in boreal peatlands leading to stronger impacts on the global climate and stratospheric ozone depletion in the future if no countermeasures to reduce reactive N emission are undertaken.

2.2. Introduction

Nitrous oxide (N_2O) is one of the major greenhouse gases, and it has a 100-year global warming potential that is 298 times that of carbon dioxide (IPCC 2013). N_2O is also the single most crucial depleting substance of the stratospheric ozone (Ravishankara 2009). In the last decade, the atmospheric N_2O concentration increased by 0.9 ppb yr^{-1} and reached 328.9 ppb in 2016, exceeding the pre-industrial level by around 22%; this was mainly caused by excessive use of nitrogen fertilizers in agriculture, resulting in higher soil N_2O emission (WMO 2017).

Boreal peatlands, storing ca. 30% of global soil carbon in an area equivalent to only 3% of the Earth surface (Gorham 1991), have long been recognized as weak sinks or negligible sources of N_2O (Bobbink et al. 1998b, Limpens et al. 2006, Francez et al. 2011, Frolking et al. 2011). Small N_2O fluxes in these ecosystems can be explained by reduced microbial activity, owing to the low availability of N and the impacts of *Sphagnum* mosses (Regina et al. 1996, Drewer et al. 2010). Being a dominating species in boreal ombrotrophic peatlands, *Sphagnum* mosses uptake most of the N deposited from the atmosphere and strongly acidify the environment, thereby hampering microbial decomposition (Limpens et al. 2003b, Sheppard et al. 2013).

However, the significant increase of atmospheric N deposition since the Industrial Revolution (Matson et al. 2002, Galloway et al. 2008, IPCC 2013) has resulted in higher N loads, particularly to boreal ecosystems (Ackerman et al. 2019). This may cause changes in the available N-status of boreal peatlands and thus influence N_2O emissions by i) stimulating microbial activity with increased N availability (Francez et al. 2011) and ii) changing plant productivity and vegetation composition (Matson et al. 2002, Juutinen et al. 2010, Wu and Blodau 2015).

Francez et al. (2011), in a laboratory experiment, indicated that the increase of inorganic N deposition from the atmosphere triggered the activity of the denitrification in the bacterial community, resulting in an enhancement of N_2O emissions in peatlands. However, previous *in situ* studies in boreal peatlands have shown contradictory results on the response of N_2O emission to synthetic N fertilization. Both N_2O emission increase (Nykänen et al. 2002, Gao et al. 2015, Gong et al. 2019) and no effect (Lund et al. 2009, Leeson et al. 2017) have been reported.

Changes in plant productivity and vegetation composition due to N addition have been observed in several previous studies. High doses of N fertilization might increase vascular plant growth in boreal peatlands (Tomassen et al. 2004, Limpens et al. 2009, Wiedermann et al. 2009) and reduce the cover and productivity of *Sphagnum* mosses owing to the toxic effect of NH_4^+ on moss cells (Limpens et al. 2003b, Limpens et al. 2011), or as a result of the intense competition of vascular plants (Heijmans et al. 2001, Tomassen et al. 2004, Bubier et al. 2007). The changes in vegetation may lead to increases in the amount of N available in boreal peatlands by reducing the N retention capacity of moss layers (Aldous 2002, Limpens et al. 2003b) and by increasing decomposition rate (Sheppard et al. 2013). Hence, the increase of N deposition can have a similar effect on vegetation changes and thus may lead to elevated N_2O emissions. However, the effects of changes in vegetation composition on N_2O fluxes under enriched-N availability are still unknown.

In response to this gap in knowledge, we established a factorial experiment comprising both plant functional group removal and N fertilization in 2014 at an ombrotrophic blanket bog in western Newfoundland, Canada. We hypothesize that: (1) N fertilization will increase N_2O emission; (2) the removal of vascular plants will decrease N_2O

emission as a result of less available N due to the increase in cover and productivity of *Sphagnum* mosses; (3) the removal of vascular plants will reduce the N-fertilization-induced increase of N_2O emissions due to enhanced N retention capacity of mosses, increased soil N_2O uptake, and reduced tissular transport and release by vascular plants.

2.3. Methods

2.3.1. Study Site

This study was conducted at an ombrotrophic blanket bog located in western Newfoundland, Canada (48°15′44″ N latitude, 58°40′ 03″ W longitude). The annual air temperature and precipitation (1981-2010) were 5 °C and 1340 mm. In the 2015 and 2016 growing seasons (from May 1 to October 31), the mean air temperature was 12.6 and 12.5 °C, and the total precipitation was 481.3 and 608.8 mm, respectively (Figure 2-1).

The depth of peat is approximately 3 m, while the pH is 4.5. This bog is a typical peatland on the island of Newfoundland, presenting a matrix of three main plant functional groups, including bryophytes (*Aulacomnium turgidum, Hylocomium splendens,* and *Sphagnum spp.),* graminoids (*Rhynchospora alba, Trichophorum cespitosum*), and dwarf ericaceous shrubs (*Andromeda polifolia var. glaucophylla, Chamaedaphne calyculata, Vaccinium myrtilloides, Vaccinium oxycoccos, Gaylussacia baccata*, *Gaylussacia bigeloviana, Rhododendron tomentosum,* and *Rhododendron groenlandicum*).

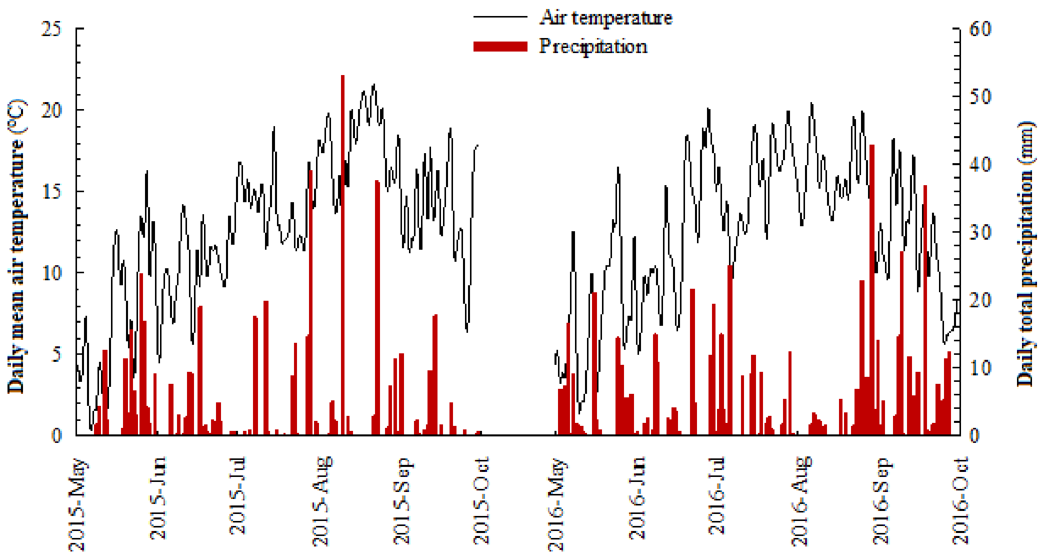

Figure 2-1. Daily mean air temperature and total precipitation in the study site during the 2015 and 2016 growing seasons.

2.3.2. Experimental design and treatments

In this study, a plant removal approach was applied to measure the effects of plant functional groups (Díaz et al. 2003, Ward et al. 2009, Ward et al. 2013b). There were 4 plant removal levels: control with all vegetation present (C); graminoid removal (G); shrub removal (S); and both graminoid and shrub removal (GS). To avoid the significant impact of vegetation removal on soil, we did not remove the ground cover of *Sphagnum* mosses in this experiment. Removal of graminoids and shrubs was implemented using the method modified from Ward et al. (2013b). In this study, the shoots of these plants were first trimmed in May 2014. To minimize the effects of root decomposition, we only started to sample in the 2015 growing season. In 2015 and 2016, we first cut the new shoots of the removed plant group in May, about two weeks before sampling, and then cut their new shoots, whenever there were any, during the sampling campaigns.

In the N-enriched treatments, we applied 6.4 g N $m^{-2}yr^{-1}$, approximately 10 times the ambient annual N deposition (0.5 -0.6 g N m^{-2} yr^{-1}) (Reay et al. 2008) to simulate N-saturated conditions for this N-limited ecosystem. This is comparable to the N addition level treatments used in other studies in boreal peatlands (Nykänen et al. 2002, Leeson et al. 2017). More specifically, 36.6 g of ammonium nitrate (NH_4NO_3) was diluted in 2 L of pool water from the same site, and then the solution was sprinkled in the plots. In the plots without N addition, the same amount of water (without the N fertilizer) was applied. The water for fertilization dilution and watering was taken from nearby peatland pools. The fertilizer regime was implemented twice a year in each growing season. Therefore, our experiment had 8 treatments, including C, N+C, G, N+G, S, N+S, GS and N+GS. These treatment plots were randomly distributed within one block. Four replicates of each treatment were located in 4 blocks with a 4 m buffer zone between each block. Each plot covered an area of 2 m × 2 m, separated by a 2m buffer zone between the plots.

2.3.3. Gas sampling and analysis

A static chamber method was used to collect gas samples. In each plot, we installed one permanent collar fitting with opaque chambers by a groove (Luan et al. 2019). To balance atmospheric pressure inside and outside the chambers, we installed a capillary tube in the chamber lids. We used 60 mL syringes to collect gas samples at four intervals: 0, 10, 20, and 30 minutes after chamber closure. The gas samples were collected between 10:00-16:00 local time, biweekly, from May to October in the 2015 and 2016 growing seasons. Within one week after sampling, the samples were analyzed by gas chromatography (GC) using a Scion 456-GC (Bruker Ltd., Canada) equipped with an electron capture detector. N_2O flux in each plot was calculated by using a linear regression between concentration and time.

2.3.4. Soil environmental variable measurement

Soil temperature (ST) at a depth of 5 cm and 20 cm was measured with a soil thermometer (Traceable™ Digital Thermometer, Fisher Scientific Inc., Canada). A soil moisture sensor (ProCheck, Decagon Devices Inc., U.S.) was used to measure soil moisture (SM) at 5 cm depth. In each plot, one perforated PVC pipe was installed to measure water table depth. These soil environmental variables were measured soon after gas samples were collected.

2.3.5. Pore water sampling and analysis

One MacroRhizons sampler (Rhizosphere Inc., Netherlands) was used to collect soil pore water at ~10 cm depth in each plot, while soil pore water samples at 40 cm in each plot were collected with 60 mL syringes from a perforated PVC tube with a sealed bottom permanently inserted into 40 cm depth. The PVC tube was perforated at the bottom 5 cm, with the top opening sealed using a cap to prevent any precipitation from entering the PVC tube. The water samples were filtered through 0.45 μm membranes before analyzing dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) with a Shimadzu TOC-LCPH/TN analyzer (Shimadzu Inc., Japan).

2.3.6. Data analytical methods

Data were processed and analyzed with R ver. 3.5.1 (https://www.r-project.org/) with the following packages: car (Fox and Weisberg 2011), ggplot2 (Wickham 2016), multcomp (Hothorn et al. 2008), nlme (Pinheiro et al. 2018), PerformanceAnalytics (Peterson et al. 2018), Johnson (Fernandez 2014), and tidyr (Wickham and Henry

2018). Data were checked for normality using a residual plot method, and Johnson Su transformation was used where necessary before analysis. The effects of experimental N fertilization, plant removal, and their interactions were analyzed by running repeated-measures ANOVA as a mixed effect model, with experimental plots as a random effect. The repeated-measures ANOVA was also used to examine the effects of treatments on the soil environmental variables. The relationships between N_2O fluxes and the soil environmental variables within and between studied plots were determined by Pearson's correlation method.

2.4. Results

2.4.1. Soil environmental variables

Mean soil temperature (at 5 cm and 20 cm depth) and water table depth was not significantly different between treatments (Table 2-1). In contrast, significant differences in soil moisture at 5 cm between treatments were detected ($p<0.01$). More specifically, SM varied from 58.42±2.51 to 70.72±1.90 %, with the lowest value measured in GS treatment, which was 19% lower than that of the control plots (C).

Table 2-1. Mean soil temperature at 5 cm depth (ST_5) and at 20 cm depth (ST_{20}), mean soil moisture at 5 cm depth (SM_5), and mean water table depth (WTD) under different treatments in the 2015 and 2016 growing seasons. C: control (no removal); G: graminoid removal; S: shrub removal; GS: graminoid + shrub removal; + N: N fertilization. Data are presented as mean ± standard error (SE). Different lowercase letters indicate the significant differences ($p < 0.05$) between the treatments.

Treatments	ST_5 (°C)	ST_{20} (°C)	SM_5 (%)	WTD (cm)
C	16.56±0.49a	14.90±0.40a	72.25±2.41a	7.79±0.74a
N+C	16.62±0.56a	14.96±0.39a	70.72±1.90a	7.49±0.66a
G	16.24±0.47a	14.64±0.39a	64.49±2.53ab	8.00±0.77a
N+G	16.32±0.48a	14.79±0.39a	68.57±2.03a	7.24±0.64a
S	16.97±0.55a	15.04±0.40a	67.67±2.62ab	8.28±0.75a
N+S	16.23±0.48a	14.89±0.40a	69.95±2.20a	8.01±0.64a
GS	15.98±0.49a	14.57±0.40a	58.42±2.51b	9.44±0.68a
N+GS	16.03±0.49a	14.55±0.40a	68.16±2.45ab	7.07±0.66a

As shown in Table 2-2, concentrations of DOC (10 and 40 cm depths) and DTN (40 cm depth) in soil pore water did not differ significantly between the studied treatments ($p>0.05$). By contrast, the DTN of soil pore water at 10 cm depth of N+GS treatment was significantly different from the others ($p<0.05$). Notably, DTN ranged from 0.71±0.04 to 1.14±0.11 mg L^{-1} with the greatest value at N+GS treatment, which was 36 % higher than at the control plots. We did the statistical analysis using sampling time as an independent variable and found that the sampling time had no significant effects on the differences in environmental variables among the treatments.

Table 2-2. Mean dissolved organic carbon (DOC), mean dissolved total nitrogen (DTN) of soil pore water at 10 cm depth (DOC_{10}, DTN_{10}) and 40 cm depth (DOC_{40}, DTN_{40}) under different treatments during the growing season in 2015 and 2016. C: control (no removal); G: graminoid removal; S: shrub removal; GS: graminoid + shrub removal; + N: N fertilization. Data are presented as mean ± standard error (SE). Different lowercase letters indicate the significant differences ($p < 0.05$) between the treatments.

Treatments	DOC_{10} (mg/L)	DOC_{40} (mg/L)	DTN_{10} (mg/L)	DTN_{40} (mg/L)
C	41.70±1.24a	58.33±6.03a	0.84±0.04a	1.62±0.24a
N+C	42.27±1.46a	50.95±1.95a	0.79±0.05a	1.65±0.24a
G	42.15±1.29a	53.81±2.60a	0.82±0.04a	1.32±0.15a
N+G	41.56±1.47a	54.19±1.10a	0.82±0.04a	1.26±0.07a
S	40.09±1.15a	51.62±4.46a	0.71±0.04a	1.54±0.24a
N+S	44.65±1.14a	52.93±1.96a	0.82±0.05a	1.42±0.11a
GS	44.95±1.30a	54.98±3.53a	0.76±0.04a	1.66±0.28a
N+GS	44.45±1.43a	56.26±3.81a	1.14±0.11b	1.64±0.21a

2.4.2. Variation of N_2O fluxes and drivers

We found that N_2O fluxes varied by the timing of fertilization (Figure 2-2). The N_2O fluxes at the non-fertilization plots were near zero in all treatments; N_2O peaks were observed in the N-applied plots following N fertilization events.

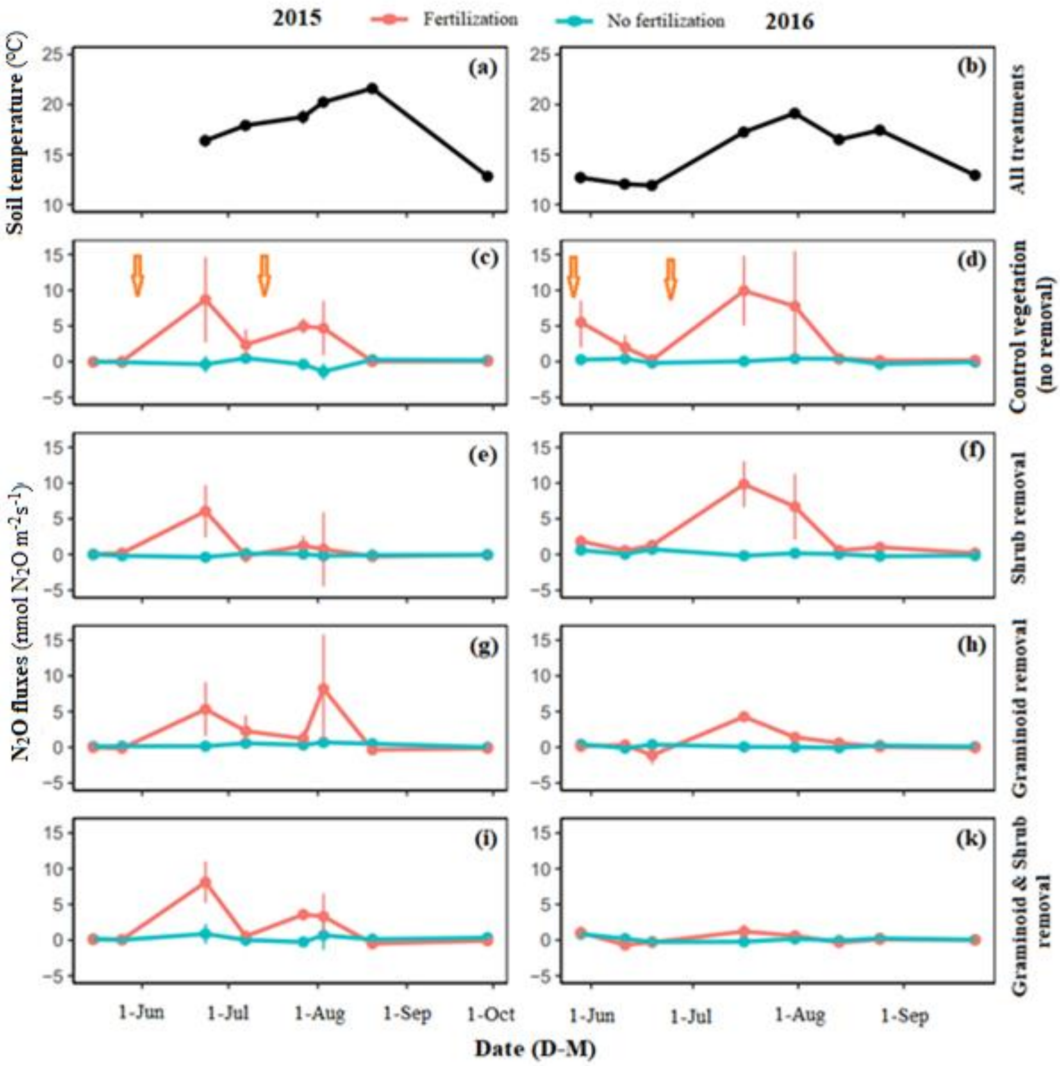

Figure 2-2. The soil temperature at 5 cm and the variation of N_2O fluxes during the 2015 and 2016 growing seasons. The error bars represent standard error of the mean (n = 4), and arrows indicate N fertilization times in all treatments.

Figures 2-2a and 2-2b showed the variation of ST_5 over time that remained below 15 ^oC from May to early June, increased rapidly in late June and July, peaked around 20 ^oC in August, and dropped quickly to approximately 12 ^{o} C in September. The temporal dynamics of ST_5 explained 6.25 % of N_2O variation in the N-treated plots (r=0.25, $p<0.05$, Figure 2-3). In contrast, no correlation was detected between N_2O fluxes and

ST_{20} , SM, WTD, DOC_{10}, DOC_{40}, DTN_{10} and DTN_{40}, both with N addition and without N fertilization treatments.

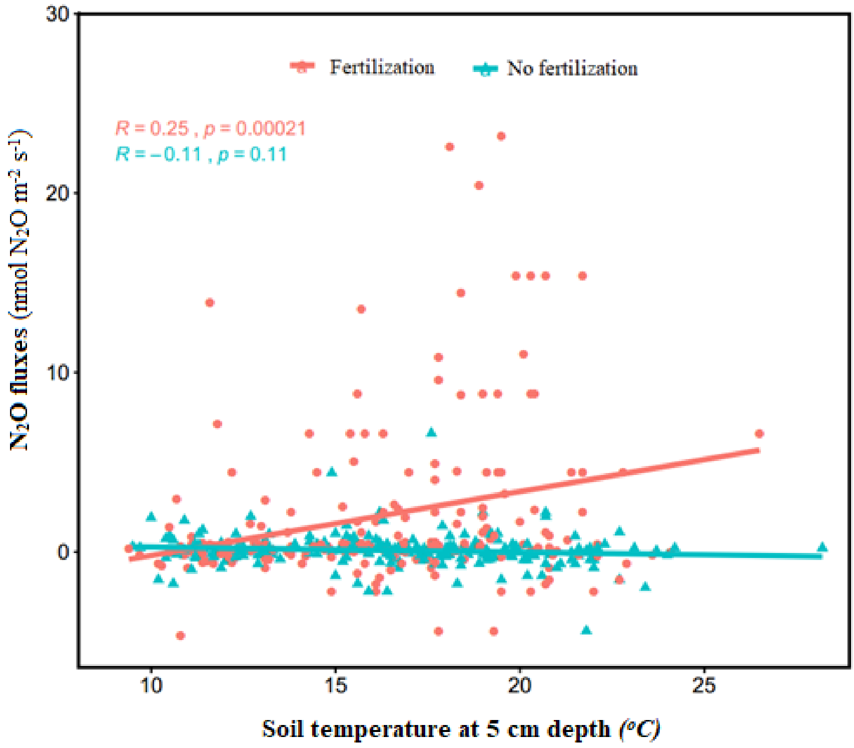

Figure 2-3. Relationship between N_2O fluxes and soil temperature at 5 cm depth with and without N fertilization. Data from all treatments (n= 432) were used for this correlation.

2.4.3. Effects of N fertilization and plant removals on N_2O fluxes

Our results showed that the addition of N significantly increased N_2O emission ($F_{1,512}$=23.98, p<0.0001, Figure 2-4b, Table 2-3). However, the significant increase of N_2O emission due to N addition was only detected in the third year (2016) of the experiment (p<0.05) (Figure 2-4b). In 2016, the N fertilization increased the N_2O emission in the N+C (control) and N+S (shrub removal) treatments by 3.02 and 2.72 nmol $m^{-2}s^{-1}$, respectively (Figure 2-4b). In contrast, the N addition did not significantly impact N_2O emissions in other treatments with plant removal (N+G and N+GS; Figure 2-4b). In 2015, we did not find significant effects of the fertilization on N_2O fluxes for any of the vegetation treatments (Figure 2-4a).

Table 2-3. Statistical analysis (F-test) for effects of, and interactions between, N fertilization and the removal of graminoids and the removal of shrubs on N_2O fluxes. The bold numbers present statistical significance ($p<0.05$).

Variables	N_2O fluxes		
	Df	F	P
	n = 512		
N treatments (fertilization/ no fertilization)	**1**	**23.98**	**<0.0001**
Graminoids (removal/no removal)	1	1.09	0.29
Shrub (removal/no removal)	1	1.97	0.16
N fertilization x Graminoids	**1**	**8.61**	**0.0035**
N fertilization x Shrubs	1	0.05	0.82
Graminoids x Shrubs	1	1.31	0.57
N fertilization x Graminoids x Shrubs	1	0.13	0.72

We found an interactive effect of N addition and the graminoid removal on N_2O fluxes ($F_{1,512}$=8.61, p< 0.01) (Table 2-3). The interaction effect on N_2O fluxes was observed in 2016 when the removal of graminoids reduced 69% of the mean increase in N_2O fluxes due to N fertilization (Figure 2-5b); the interactive effects were not detected in 2015 (Figure 2-5a).

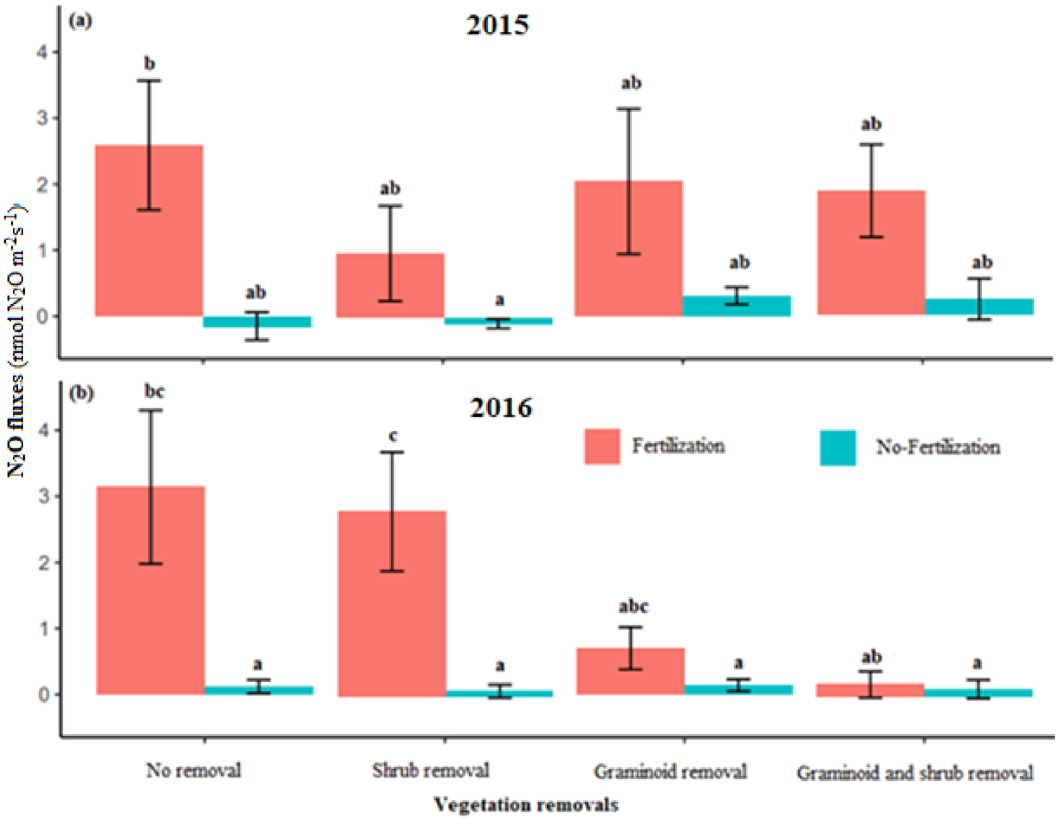

Figure 2-4. Mean N_2O fluxes ± SE from all treatments in 2015 (a), in 2016 (b). Different lowercase letters indicate the significant differences ($p < 0.05$) between the treatments

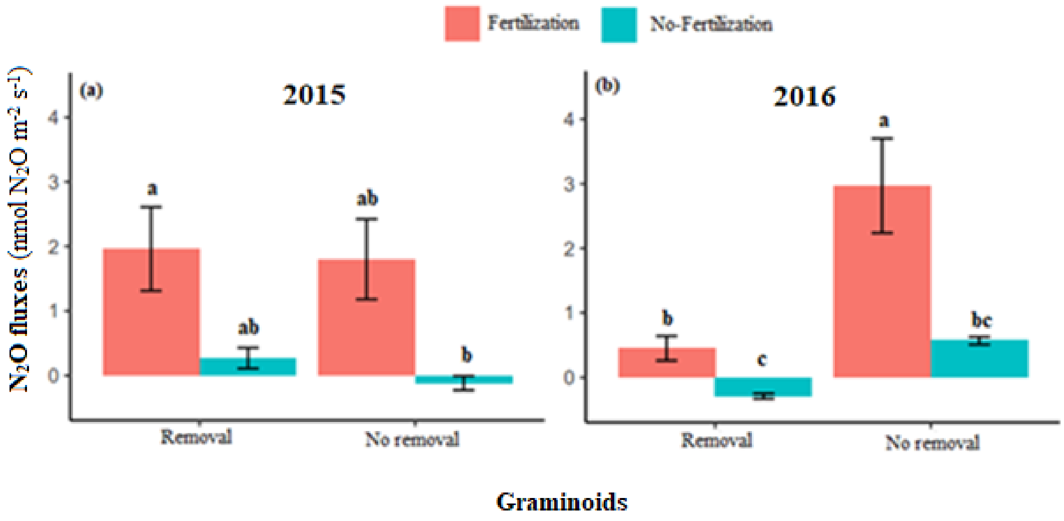

Figure 2-5. Mean N_2O fluxes (± SE) from studied plots with (no removal) and without graminoids (removal) in 2015 (a) and in 2016 (b). Different lowercase letters indicate the significant differences ($p < 0.05$) between the treatments

2.5. Discussion

2.5.1. Effect of N fertilization on N_2O fluxes

In this study, we have found that N fertilization substantially increased N_2O emission (Table 2-3, Figure 2-4). The increase of N_2O due to N fertilization has been well documented for many ecosystems, especially in tropical forests and agricultural sites (Hall and Matson 1999, Liu and Greaver 2009, Kim et al. 2013, Shcherbak et al. 2014, Cheng et al. 2016, Cowan et al. 2019). In peatlands, however, previous studies have shown extremely wide ranges of N_2O emission in response to N fertilization, namely, varying from a substantial increase to no response. The differences in application rate, frequency, and timing of N fertilization may account for these different results. For example, Zhang et al. (2007) found a significant increase of N_2O fluxes under N fertilization in a 2-year study at a Chinese peatland, when 24 g NH_4NO_3-N $m^{-2}yr^{-1}$ was applied within 7-9 events. Nykänen et al. (2002) found a slight short-term increase of N_2O fluxes upon adding 10 g NH_4NO_3-N $m^{-2}yr^{-1}$ within 6 applications over a 6-year study at a boreal *Sphagnum fuscum* pine bog in Finland with an atmospheric N deposition rate of 6 kg N ha^{-1} yr^{-1}. In contrast, Leeson et al. (2017) did not find any significant response of N_2O fluxes to an experimental KNO_3-N or NH_4Cl-N fertilization (at rates of 0.8 g – 6.4g-N $m^{-2}yr^{-1}$ split in 120 applications over a 13-year study) in a Scottish peat bog receiving ca. 8 kg N ha^{-1} yr^{-1} through N deposition from the atmosphere.

Net N_2O fluxes between soil and the atmosphere are governed by key processes (and affected by their rates) of N_2O production, consumption within soils and transportation through vascular plant tissues, release from soils and plants to the atmosphere (Butterbach-Bahl et al. 2013). The N_2O production pathways such as nitrification and denitrification highly depend on the availability of inorganic N in soil and pore water (Regina et al. 1998, Silvan et al. 2002, Butterbach-Bahl et al. 2013, Heil et al. 2016), while the transportation of N_2O relies on diffusion and plants as conduits (Chang et al. 1998). In peatlands, moss layers have high efficiency in capturing N deposition (Heijmans et al. 2002b). Williams et al. (1999) pointed out that mosses can take up 11% to 100% of the added N in two weeks after its application. However, the N retention capacity of the moss layer seems to be inversely dependent on the ambient N deposition rate (Bragazza et al. 2005). Therefore, the effects of N fertilization on dissolved inorganic N concentrations in pore water and N_2O production are likely to be significant at high rates of N application (fertilization and/ or deposition), *i.e.*, those exceeding the N retention threshold of the moss layer. In this study, under the condition favored for denitrification, such as high WTD and high SM (Table 2-1), the high dose of N fertilization (6.4 g N $m^{-2}yr^{-1}$) may increase N availability leading to an increase in N_2O production. Moreover, the addition of N may enhance the activity of the microbial community (Gilbert et al. 1998), thereby increasing N_2O production. Gilbert et al. (1998) indicated that N addition at different rates to the soil increased microbial biomasses, while significant changes in microbial composition were only observed at higher rates (≥ 5 g $m^{-2}yr^{-1}$). This may be another explanation for elevated N_2O emissions under high N application rates (6.4 g N $m^{-2}yr^{-1}$) in this study.

Moreover, N fertilization may increase the abundance of vascular plants and decrease the cover of *Sphagnum* mosses (Fritz et al. 2014, Wu and Blodau 2015). The change of vegetation composition may lead to an increase in N_2O production because of a decrease in N retention and an increase of litter decomposition rate. A higher abundance of vascular plants may improve the N_2O transport capacity from soil to the atmosphere via their tissues (Yu et al. 1997, Chang et al. 1998, Xu et al. 2002). Therefore, the N addition could increase both soil N_2O production and soil-plant N_2O transportation, which may account for the significant increase of N_2O fluxes in this study.

2.5.2. Effect of vegetation removal on N_2O fluxes

We have clearly demonstrated that graminoid or shrub removals did not affect N_2O fluxes without N fertilization (Table 2-3). Our findings have coincided well with Ward et al. (2013b), who also did not find any significant effect of graminoids or shrubs removal on N_2O emission in a poor N available ombrotrophic bog. Without N fertilization, most of the atmospheric N deposition is absorbed by the layers of mosses (Aldous 2002, Limpens et al. 2003b). Therefore, the presence or absence of graminoids and shrubs may have negligible effects on available N, explaining why there was no significant response of N_2O emission to plant removals in our study.

2.5.3. Combined effect of N fertilization and vegetation removal on N_2O fluxes

Our experimental results provided evidence of the importance of vegetation composition in modulating effects of N fertilization on N_2O emissions. We have shown that the graminoid removal reduced 69% of the N_2O emission that increased with N fertilization in the third year of our experiment (Figure 2-5b). One can suggest that a time lag (at least 3 years) might be required for adaptation/ changes of microbial composition in response to plant composition change (from moss to vascular plants) and adaptation to new N-saturation status of formerly N-limited peatland to detect distinctive effects on N_2O emissions. Similarly to us, Heijmans and others (2001) only detected significant effects of N addition on *Sphagnum* mosses and vascular plants in the third year of their experiment. Nykänen et al. (2002) also only found increased N_2O emission until the sixth year of their experiment, with 10 g NH_4NO_3-N $m^{-2}yr^{-1}$ being applied within 6 events over a 6-year study at a boreal bog in Finland. Our findings from our experiments where we studied the combined effects of vegetation composition and N fertilization on N_2O emission are in line with the results of Leeson et al. (2017). They found that N_2O fluxes from plots dominated by sedges were higher than fluxes from plots covered by *Sphagnum* mosses after adding 0.8 g – 6.4g-N $m^{-2}yr^{-1}$ within 120 doses over a 13-year study in a Scottish peat bog. In this study, the removal of graminoids might improve the N retention capacity of the moss layer via eliminating the shading effect, *i.e.* competition for light (Limpens et al. 2003b). Thereby dissolved inorganic N (DIN) concentrations in soil pore waters of N+GS and N+G plots could be decreased compared to that of N+C and N+S (those data were not measured in this study). We have suggested that the decrease of DIN availability in graminoid-free plots (N+GS and N+G) may explain the lower increase of N_2O emission upon N fertilization in comparison with the plots where graminoids were present (N+C and N+S). Also, the removal of graminoids may reduce the transportation of soil N_2O through plant tissues and inhibit the direct release of N_2O to the atmosphere (Yu et al. 1997, Chang et al. 1998, Xu et al. 2002), *i.e.* mitigate fertilization-induced N_2O emission from boreal peatlands (Figure 2-5).

Contrary to the graminoid removal from N fertilized plots (N+G and N+GS), we have not observed a similar effect following the removal of shrubs alone (N+S), most probably owing to relatively low shrub abundance and their biomass at the studied peatland ecosystem.

We have found no significant pair-correlations between N_2O fluxes and soil environmental variables for all studied plots, except those with soil temperatures at 5 cm depth (ST_5) for N-treated sites (N+C, N+G, N+GS, N+S; Figure 2-3). However, ST_5 was estimated to explain only 6.25% of N_2O emission variation. We assumed that DIN concentrations (nitrate, nitrite, and ammonium) of soil pore waters, changes in microbial composition and their functional gene abundance/ activities, pH fluctuations, pathways of chemical N transformations in soil pore waters, and N-retention rates by plant functional groups, which were unfortunately not measured/ accounted for in this study, might allow us to understand better mechanisms impacting N_2O emission from boreal peatland under N fertilization and/ or removal of functional plant groups (Ward et al. 2013b, Leeson et al. 2017).

2.6. Conclusions

N fertilization was shown to increase N_2O emission in boreal peatlands. We have found that the effect of N addition was modulated by graminoid presence. Notably, the presence of graminoids substantially increased N_2O emission under N-enriched conditions. One can conclude that an increased abundance of graminoids, which are less vulnerable than *Sphagnum* mosses to elevated N deposition tending to increase in the nearest future, may enhance N_2O emission in boreal peatland ecosystems affecting climate change and stratospheric ozone depletion. Further comprehensive studies on reactive N and greenhouse gas fluxes from boreal peatlands and the impact of N deposition on boreal peatlands are urgently needed to mitigate N_2O emissions from these natural ecosystems. Moreover, although the mechanisms explaining our findings should be investigated in further research, our results highlight the importance of vegetation composition in modulating the effects of N deposition on N_2O emissions in boreal peatlands.

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Chapter 3 Effects of warming and nitrogen addition on vascular plants in a boreal bog

3.1. Abstract

Vascular plants are sparse in boreal bogs because of limitations from *Sphagnum*. The warmer and/or nitrogen-enriched conditions may significantly affect boreal bog vascular plants and interactions between the plants. The changes in vascular plant interactions may modify the responses of the plants to the environmental changes. Here, we combined vegetation removal treatments with warming and nitrogen addition experiments to investigate responses effects of these environmental changes on the vascular plants and the plan interactions. We revealed that the warming and nitrogen addition significantly enhanced the growth of boreal bog vascular plants, especially graminoids. We also found that competition from graminoids reduces shrub growth under nitrogen-enriched conditions, whereas graminoids facilitate shrub growth under warmer conditions. These findings suggest a rapidly increasing trend of boreal bog graminoids under future warmer, nitrogen-enriched conditions. This changing trend of boreal bog vegetation may significantly alter the vital services of these ecosystems.

3.2. Introduction

Boreal bogs are crucial natural ecosystems providing many vital services such as carbon sequestration, water supply, biodiversity protection, natural risk mitigation, food, and fuel provision, and recreation opportunities (De Groot et al. 2002, Kimmel and Mander 2010, Page and Baird 2016). Notably, boreal bogs have been characterized as significant sinks of CO_2 because decomposition rates are lower than the carbon amount absorbed by vegetation in these ecosystems (Gorham 1991, Blodau 2002, Turunen et al. 2004, Yu et al. 2009). The vital services from boreal bogs are provided and controlled by boreal bog vegetation wherein *Sphagnum* are dominant plants (Malmer et al. 2003, Turetsky et al. 2012b). Previous studies have indicated that the vegetation is a crucial factor regulating several biogeochemical processes and services in boreal bogs, including carbon sequestration (Belyea and Malmer 2004, Potvin et al. 2015, Kolari et al. 2021), greenhouse gas emission (Laine et al. 2012, Ward et al. 2013b, Robroek et al. 2015, Dieleman et al. 2017, Riutta et al. 2020, Gong et al. 2021, Le et al. 2021, Whitaker et al. 2021), decomposition rate (Dorrepaal et al. 2005, Ward et al. 2015b, Wiedermann et al. 2017, Bell et al. 2018, Gavazov et al. 2018), microbial activities (Bragazza et al. 2015, Robroek et al. 2015, Robroek et al. 2016, Dieleman et al. 2017) and nitrogen cycle (Aerts et al. 1999). Therefore, any changes in the vegetation community may alter the boreal bogs and services of these ecosystems (Turetsky et al. 2012b, Armstrong et al. 2015, Dyderski et al. 2016, Oke and Hager 2020, Riutta et al. 2020, Whitaker et al. 2020).

There is increasing evidence that boreal bog vegetation might be significantly affected by warmer or nitrogen-enriched conditions resulting from global warming (Weltzin et al. 2003, Loisel et al. 2014, Stocker et al. 2014, Dieleman et al. 2015, Dieleman et al. 2016, Malhotra et al. 2020) and/or increasing levels of nitrogen deposition (Bobbink et al. 1998a, Bubier et al. 2007, Galloway et al. 2008, Kivimäki et al. 2013, Ackerman et al. 2019). The faster warming rate in boreal bogs (Stocker et al. 2014) may benefit vascular plants (McPartland et al. 2020, Whitaker et al. 2021) and depress *Sphagnum* (Heijmans et al. 2008, Heijmans et al. 2013, Oke and Hager 2020). Similarly, increases in nitrogen concentration might interrupt nitrogen competition from *Sphagnum* (Lamers et al. 2000), leading to even more abundance of boreal bog vascular plants (Berendse et al. 2001, Limpens et al. 2003a, Bubier et al. 2007, Larmola et al. 2013, Oke and Hager 2020). Therefore, the concomitant warmer and nitrogen-enrichment conditions may speed up the positive feedback of the existing boreal bog vascular plant community.

The warmer, nitrogen-enriched conditions might alter interactions between vascular plants in boreal bogs (Juutinen et al. 2010). Changes in these interactions could modulate the responses of the plants to environmental changes (Brooker 2006, McPartland et al. 2019, McPartland et al. 2020, Kolari et al. 2021). However, the responses of dominant vascular plants and their interactions to the combined effects of these abiotic and biotic variables are unknown. To fill this knowledge gap, we established a full factorial experiment comprising warming, N addition, and removing graminoids and shrubs in a coastal blanket bog in Newfoundland, Canada. We hypothesize that (1) Nitrogen addition and warming treatments will interactively stimulate the growth of dominant bog graminoids and shrubs; (2) These environmental changes will enhance competition between graminoids and shrubs, which could reduce the positive responses of these plants to warming and nitrogen addition treatments.

3.3. Materials and methods

3.3.1. Study site

The study site is a coastal blanket bog in western Newfoundland, Canada (48°15′44″ N, 58°40′03″ W). In this study area, the mean annual air temperature and level of precipitation for the last 30 years were approximately 5 °C and 1340 mm, respectively (Gong et al. 2019). Locally, the average air temperature was 10.6 in 2019 and 12.9 °C in 2020 growing seasons. During the data collection times, mean precipitation was 514.4 in the 2019 growing season and 286.2 mm in the 2020 growing season. In the study ecosystem, peat depth is 3 m, while pH of underground water is 4.5 (Gong et al. 2019). The study site is a typical ombrotrophic boreal bog in Newfoundland, where *Sphagnum fuscum* (Schimp.) H. Klinggr and *Sphagnum rubellum* generate a carpet covering almost all ground surfaces. The sparse vascular plant community consists of dwarf shrubs (*Andromeda glaucophylla, Chamaedaphne calyculata, Gaylussacia bigeloviana, Vaccinium oxycoccos, Gaylussacia baccata, Rhododendron tomentosum, and Rhododendron groenlandicum*), and graminoids (*Rhynchospora alba* (L.) Vahl, *Trichophorum cespitosum* (L.) Hartman).

3.3.2. Experimental design

In the blanket bog, we established 48 square 4 m^2 plots in 4 blocks separated by 6 m buffer zones. In 12 plots of each block, we randomly arranged 12 treatments that were generated from 3 levels of vascular plant removal (no removal, shrub removal, and graminoid removal), 2 levels of nitrogen addition (with and without addition), and 2 levels of warming (with and without warming treatment). To increase temperature, we used open-top chambers (OTCs) comprised of six glass sheets (80 cm along the bottom edge, 62.5 cm along the top edge, 40 cm in height) to cover an area of 1.66 m^2. Two USB temperature loggers (Lascar Electronics Ltd., UK) were utilized to record air temperature at vegetation canopy height every 30 minutes in the warming and control plots. The OTCs increased the mean daytime air temperature during the 2019 growing season and 2020 growing season by 2.10 and 2.12 ^{o}C, respectively. To improve nitrogen availability, we annually applied 6.4 g N m^{-2}. yr^{-1} via two doses from 2014 to 2018 and by four doses between 2019 and 2020. Fertilizer was added in the soluble form (NH_4NO_3) dissolved in 2 L of same site pool water. The exact amount of water without the nitrogen addition also was added in no nitrogen treatment plots.

To determine the effects of graminoids and shrubs on the growth of each other, we used the removal method with 3 treatments of vascular plant removal, including No (No removal with the presences of both graminoid and shrub), G (Graminoid removal), and S (Shrub removal). The shoots of graminoids and/or shrubs were manually pruned to the moss layer in early May 2014 and then re-cut biweekly if the vascular plants grew again.

3.3.3. Vegetation selection and measurement

Five dominant plant species representing functional plant types were selected. Specifically, two evergreen shrubs (*A. glaucophylla and C. calyculata*), one deciduous shrub (*G. bigeloviana*), and two graminoids (*R. alba* and *T. cespitosum*) were selected. Non-destructive methods were used to assess the growth of these selected plants by measuring tissue extension on the major growth axis of the selected plant species (Walker et al. 2015). In each plot, three medium individuals of each shrub were selected and labeled to measure new shoot increment, number of new shoots, and new leaf. The selected individuals were at least 50 cm apart to avoid growing from the same genets. With *T. cespitosum,* three tussocks were selected to measure leaf length increment. For *R. alba,* we selected the three highest plant density areas to measure leaf length increment because of its scattered growth. These measurements were conducted late the 2019 and 2020 growing seasons to ensure the plants had reached maximum size. In addition, we measured the cover of vascular plants and *Sphagnum* mosses by point interception methods (Floyd and Anderson 1987) in the 2020 mid-growing season.

3.3.4. Environmental measurement

We biweekly measure soil environmental variables. Specifically, soil temperature at 5 and 20 cm depth was measured with a soil thermometer (Fisher Scientific Inc., Canada). We also measured soil moisture at 5 cm depth using a soil moisture sensor (ProCheck, Decagon Devices Inc., USA). Water table depth was also surveyed by using a tube permanently settled in each plot. Also, soil water samples were biweekly collected at 10 cm and 40 cm depth using MacroRhizon samplers (Rhizosphere Inc., Netherlands) and perforated PVC tubes installed at all plots. The water samples were filtered through 0.45-µm membranes before analyzing $NH_4^+$and NO_3^- concentration via flow injection analysis methods (Lachat Instruments, Inc. USA).

3.3.5. Data analysis

Data was processed and analyzed in R software ver. 3.6.3 (R Core Team 2020). Data was checked for normality, and Johnson transformation was used where necessary by *jtrans* package (Wang 2016) before analysis. Effects of warming, nitrogen addition, and shrub removal on graminoid growth, and effects of warming, nitrogen addition, and graminoid removal on shrub growth were analyzed by running ANOVAs as mixed effect models, with experimental blocks as a random effect in *nlme* package (Pinheiro et al. 2018). The ANOVA tests with the *nlme* package were also utilized to examine the effects of warming, nitrogen addition, and vascular plant removal on environmental variables. Tukey's post hoc tests were also conducted by *multcomp* package (Hothorn et al. 2008) to determine the differences in vascular plant removal treatments when a significant effect on these environmental factors was detected. Figures were created by *ggplot2* (Wickham 2016) and *ggpubr* (Kassambara 2020) packages.

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3.4. Results

3.4.1. Effects of shrubs, nitrogen addition, and warming on graminoids

Table 3-1. Effects of shrubs, nitrogen addition (N), and warming (W) on leaf length increment of two dominant boreal bog graminoids: *Rhynchospora alba* and *Trichophorum cespitosum*.

Sources of variation	Df	*a, Rhynchospora alba*		*b, Trichophorum cespitosum*	
		F	P	F	p
Shrubs	1	3.4	0.0714	0.2	0.6514
N	1	13.2	0.0006	0.3	0.5963
W	1	39.4	<.0001	3.0	0.0869
Shrub x N	1	0.1	0.8195	2.6	0.1138
Shrub x W	1	3.6	0.0640	0.5	0.4768
N x W	1	10.6	0.0020	0.1	0.7858
Shrub x N x W	1	0.0	0.9505	0.1	0.7766

Results show that nitrogen addition and warming interactively affected the length increment of *R. alba* (Table 3-1b), while shrubs removal had no effects on *R. alba* *(*Figure 3-1a, table 3-1a). Specifically, nitrogen addition increased the length increment of *R.* alba by 12% (F=13.2, p=0.0006), and warming treatments enhanced the *R. alba* growth by 22% (F=39.4, p<0.0001). Furthermore, combined nitrogen addition and warming treatments stimulated *R. alba* leaf length increment by 38% (F=10.6, p=0.002). By contrast, we did not find significant responses of *T. cespitosum* to nitrogen addition, warming, and shrub removal (Figure 3-1b, table 3-1b).

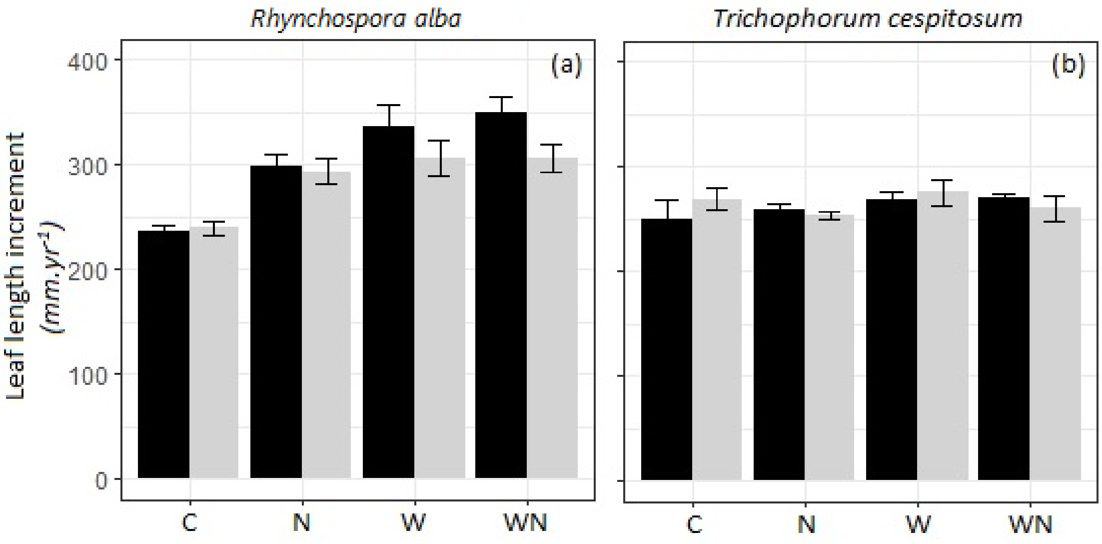

Figure 3-1. Leaf Length increment (mean ± SE (mm. yr^{-1})) of (a) *R. alba* and (b) *T. cespitosum*. Black and grey bars represent the growth of these graminoids with and without the presence of shrubs.

3.4.2. Effects of graminoids, nitrogen addition, and warming on the growth of shrubs

Results indicate that the absence or presence of graminoids did not affect the shoot length increment of all dominant shrubs, while the graminoids altered *A. glaucophylla* new shoot, new leaf numbers, and new shoot number in *C. calyculata* (Figure 3-2, table 3-2). Graminoid removal increased mean number of new shoots (F=16.8, p=0.0001) and new leaves of *A. glaucophylla* (F=10.3, p=0.0042) by 34% and 47%, respectively (Figure 3-2d, g). Similarly, graminoid removal also resulted in an 18% increase in the mean new shoot number of *C. calyculata (*Figure 3-2e).

Table 3-2. Effects of graminoids, nitrogen addition (N), and warming (W) on boreal bog shrubs

Sources of variation	df	*a, Andromeda glaucophylla* F	p	*b, Chamaedaphne calyculata* F	P	*c, Gaylussacia bigeloviana* F	P
Shoot length increment							
Graminoid	1	1.0	0.3257	2.2	0.1463	0.1	0.7803
N	1	18.9	0.0001	0.0	0.9810	2.4	0.1291
W	1	4.0	0.0505	8.2	0.0060	7.7	0.0076
Graminoid x N	1	1.3	0.2635	0.1	0.7156	0.2	0.6395
Graminoid x W	1	0.6	0.4239	1.1	0.3048	0.3	0.5801
N x W	1	1.1	0.3052	0.8	0.3647	1.5	0.2283
Graminoid x N x W	1	2.1	0.1492	1.9	0.1781	0.2	0.6927
Number of new shoots							
Graminoid	1	16.8	0.0001	7.0	0.0109	0.1	0.7095
N	1	8.1	0.0063	6.7	0.0125	0.4	0.5139
W	1	3.3	0.0760	5.2	0.0273	8.2	0.0060
Graminoid x N	1	6.2	0.0163	1.9	0.1769	0.1	0.7295
Graminoid x W	1	4.1	0.0473	0.1	0.7907	0.0	0.8594
N x W	1	0.1	0.7674	3.7	0.0593	3.0	0.0896
Graminoid x N x W	1	11.6	0.0013	0.5	0.4911	0.7	0.4018
Number of new leaves							
Graminoid	1	10.3	0.0042	0.0	0.8294	0.1	0.7872
N	1	20.1	0.0002	0.4	0.559	0.1	0.7114
W	1	2.6	0.1217	0.0	0.8719	7.9	0.0106
Graminoid x N	1	8.0	0.0102	0.7	0.4123	0.6	0.4593
Graminoid x W	1	4.8	0.0390	0.0	0.8764	0.5	0.4936
N x W	1	2.7	0.1184	0.2	0.7017	0.1	0.8004
Graminoid x N x W	1	4.9	0.0374	0.3	0.5718	0.1	0.7152

Nitrogen addition significantly affected *A. glaucophylla* growth, while the nitrogen treatment had no effects on *G. bigeloviana* (Figure 3-2, table 3-2). In detail, nitrogen addition significantly increased *A. glaucophylla* new shoot increment by 47% (F=18.9, p=0.0001, figure 3-2a), number of new shoots by 19% (F=8.1, p=0.0063, figure 3-2d) and number of new leaves by 51% (F=20.1, p=0.0002, figure 3-2g). Nitrogen addition also increased the number of new shoots of *C. calyculata* by 18% (F=6.3, p=0.0125, figure 3-2e).

By contrast, warming did not affect *A. glaucophylla*, but the warmer conditions stimulated *G. nigeloviana* growth (Figure 3-2, table 3-2). Warming increased mean new shoot increment by 22% (F=7.7, p=0.0076, figure 3-2c), number of new shoots by 22% (F=8.2, p=0.0060, figure 3-2i) and number of new leaves by 37% (F=7.9, p=0.0106, figure 3-2f) on *G. nigeloviana*. The warmer conditions also led to significant increases in mean shoot length increment (F=8.2, p=0.0060) by 37% (Figure 3-2b) and mean number of new shoots in *C. calyculata* (F=5.2, p=0.0273) by 18% (Figure 3-2e).

More importantly, we found that nitrogen addition and warming altered the effects of graminoid removal on new shoots and new leaves of *A. glaucophylla* (Table 3-2). Specifically, nitrogen addition enhanced mean increases of *A. glaucophylla* new shoot number and new leaf number result from graminoid removal by 3.5 and 4.9 times, respectively. In contrast, the positive effects of graminoid removal on new shoot number and new leaf number of *A. glaucophylla* were reduced by warming, wherein the mean increase of new shoot and new leaf number after removing graminoids were 59% and 86% lower in the warmer plots than in plots without the warming treatment. In plots treated with both warming and nitrogen addition, the increases in *A. glaucophylla* new shoot number and new leaf number after removing graminoids were 17% and 22% higher than the increases in plots with control conditions (Figure 3-2d, g).

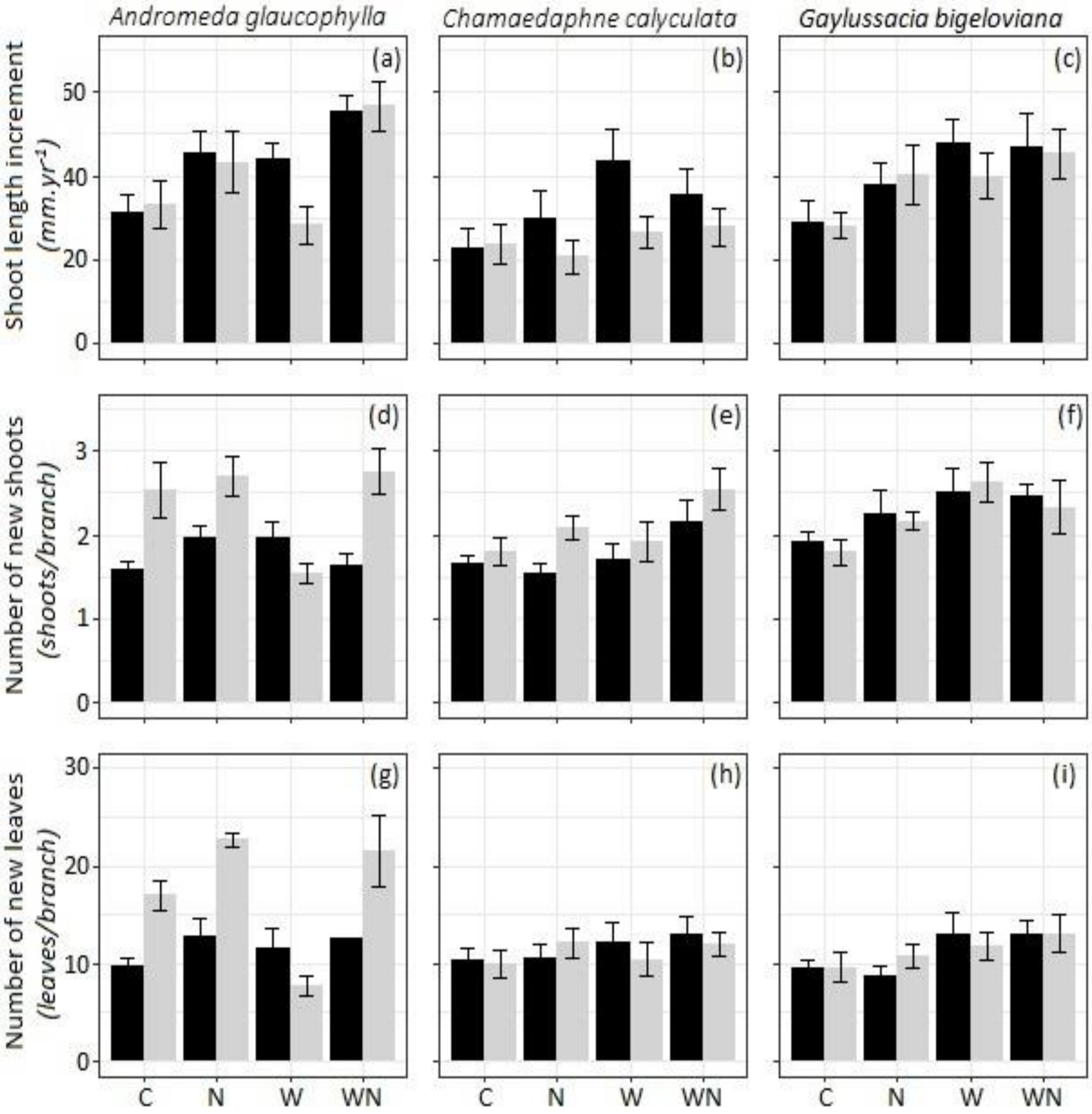

Figure 3-2. Shoot length increments (mean ± SE (mm. yr^{-1})) of (a) *A. glaucophylla*, (b) *C. calyculata*, (c) *G. bigeloviana*; new shoot number (mean ± SE (shoots. branch $^{-1}$)) of (d) *A. glaucophylla*, (e) *C. calyculata*, (f) *G. bigeloviana* and new leaf number (mean ± SE (leaves. branch $^{-1}$)) of (g) *A. glaucophylla*, (h) *C. calyculata*, (i) *G. bigeloviana*. Black and grey bars represent growth of these shrubs with and without presence of graminoids.

3.4.3. Effects of treatments on environmental variables

Graminoid and shrub removal significantly affected vascular plant cover, soil moisture, and concentrations of NH_4^+ at 40 cm depth, while the removal of vascular plants did not influence other soil environments (Table S 3-1). Graminoid removal reduced the mean cover of vascular plants by 56%, while removal of shrubs reduced vascular plant cover by 26% (Table 3-3). Mean soil moisture in the plots with graminoid removal was 13% higher than in the plots without removal of vascular plants. NH_4^+ concentration in the graminoid removal plots was 36% higher than in the shrub removal plots (Table 3-3).

Nitrogen addition altered vascular plant cover, *Sphagnum* cover, soil moisture, concentrations of NH_4^+ and NO_3^-, while the nitrogen treatment did not affect soil temperature and water table depth (Table S3-1). Nitrogen addition increased the mean cover of vascular plants by 17%, whereas nitrogen fertilization reduced the mean cover of *Sphagnum* by 74%. The nitrogen addition enhanced mean soil moisture by 22%. Also, nitrogen addition substantially improved mean NH_4^+ concentration at 10 cm depth by 126%, at 40 cm depth by 37%, and mean NO_3^- concentration at 10 cm depth by 27% (Table 3-3).

The warming treatment significantly influenced vascular plant cover, *Sphagnum* cover, and soil temperature at 10 cm depth, while the temperature treatment did not affect other soil environmental variables (Table S 3-1). Specifically, the warming treatment reduced *Sphagnum* cover by 14%, whereas vascular plant cover was increased by 19% in warmer conditions. In addition, warming treatments increased soil temperature at 10 cm depth by 0.9 ^{o}C during the growing seasons (Table 3-3).

Table 3-3. Cover of *Sphagnum* mosses (COS), cover of vascular plants (COV), mean soil temperature (ST) at 5 and 20 cm depth, soil moisture (SM), water table depth (WTD), the concentration of ammonium (NH_4^+) and nitrate (NO_3^-) in soil solution at 10 and 40 cm depth.

Treatment	COS (%)	COV (%)	ST_5 (^{o}C)	ST_{20} (^{o}C)	SM (%)	WTD (cm)	NH_4^+ (mg. l^{-1})		NO_3^- (mg. l^{-1})	
							10 cm	40 cm	10 cm	40 cm
Control condition										
No	93.8±2.7	77.0±6.4	16.9±0.4	14.9±0.4	43.6±2.6	14.9±1.4	0.37±0.06	0.68±0.08	0.082±0.038	0.042±0.017
G	82.5±4.6	32.3±1.3	17.5±0.4	14.7±0.4	46.4±2.5	14.7±1.5	0.49±0.07	1.02±0.05	0.053±0.016	0.039±0.018
S	87.5±4.0	52.0±4.1	17.8±0.5	15.2±0.4	48.5±2.8	15.9±1.5	0.44±0.09	0.69±0.10	0.031±0.009	0.034±0.009
Nitrogen addition										
No	33.0±2.8	91.0±3.4	17.3±0.4	15.0±0.4	49.9±2.8	15.2±1.4	0.90±0.15	1.01±0.08	0.212±0.147	0.030±0.007
G	23.5±3.3	30.8±5.9	17.7±0.4	14.7±0.4	57.6±2.8	12.9±1.3	0.96±0.10	1.17±0.08	0.238±0.100	0.031±0.008
S	34.3±4.9	70.7±8.3	17.3±0.5	15.0±0.4	55.8±2.8	13.6±1.4	0.65±0.10	0.99±0.06	0.088±0.020	0.020±0.004
Warming										
No	90.8±2.8	90.8±1.9	18.2±0.5	14.9±0.3	38.4±2.5	14.1±1.3	0.28±0.05	0.47±0.08	0.095±0.045	0.034±0.009
G	81.8±2.0	42.5±8.0	18.5±0.4	14.9±0.3	48.0±2.7	15.7±1.4	0.36±0.07	0.86±0.04	0.049±0.017	0.028±0.007
S	84.0±4.5	62.0±7.4	18.3±0.5	15.0±0.4	40.8±2.3	16.0±1.5	0.41±0.05	0.58±0.06	0.062±0.024	0.035±0.011
Nitrogen addition and warming										
No	8.5±2.2	97.0±2.5	17.9±0.5	14.7±0.4	54.0±2.8	15.1±1.6	0.90±0.11	1.00±0.07	0.203±0.072	0.026±0.007
G	16.0±3.7	49.0±3.0	18.6±0.5	14.9±0.3	57.2±3.2	13.9±1.5	1.31±0.29	1.30±0.11	0.192±0.082	0.032±0.007
S	22.3±2.6	77.8±2.6	18.2±0.5	14.9±0.3	50.2±2.9	15.2±1.6	0.91±0.16	0.91±0.06	0.102±0.027	0.032±0.011

No: No removal with presences of both graminoids and shrubs; G: Graminoid removal; S: Shrub removal. Data values are mean ± standard error.

Table S 3-1. Summary of models of warming treatment (W), nitrogen addition (N) and vascular plant removal (V), and their combined effects on percentage cover of *Sphagnum* (COS), percentage cover of vascular plants (COV), soil temperature (ST), soil moisture (SM), water table depth (WTD), the concentration of ammonium (NH_4^+) and nitrate (NO_3^-) in soil solution at 10 and 40 cm depth at a boreal bog.

Source of variation	Df	COS (%)		COV (%)		ST_5 (^{o}C)		ST_{20} (^{o}C)		SM (%)		WTD (cm)		NH_4^+ (mg. l^{-1})				NO_3^- (mg. l^{-1})			
														10cm		40cm		10cm		40cm	
		F	p	F	p	F	p	F	p	F	p	F	P	F	p	F	p	F	p	F	P
V	2	2.4	0.11	69.4	<.01	2.7	0.07	2.9	0.06	6.9	<.01	0.8	0.45	1.9	0.16	4.0	0.03	0.4	0.65	0.3	0.75
N	1	162.2	<.01	12.0	<.01	0.1	0.74	0.8	0.36	58.0	<.01	2.5	0.12	44.5	<.01	11.4	<.01	8.7	0.01	1.3	0.27
W	1	9.2	<.01	9.1	<.01	26.9	<.01	0.7	0.39	2.9	0.09	0.7	0.41	0.0	0.94	0.1	0.75	0.4	0.56	0.1	0.74
V x N	2	5.2	0.01	2.0	0.15	0.6	0.55	0.3	0.71	0.4	0.70	1.9	0.15	1.7	0.20	0.1	0.92	0.6	0.55	0.5	0.61
V x W	2	2.5	0.09	0.6	0.57	0.4	0.68	2.6	0.08	3.0	0.05	0.6	0.56	0.9	0.41	0.1	0.91	0.7	0.52	0.6	0.58
N x W	1	0.3	0.57	0.0	0.95	0.2	0.68	0.5	0.50	1.5	0.22	0.5	0.50	1.4	0.24	0.3	0.59	0.0	0.93	0.6	0.46
V x N x W	2	0.0	0.99	0.1	0.90	0.9	0.40	1.0	0.39	1.6	0.21	0.2	0.86	0.1	0.94	0.2	0.80	0.7	0.52	0.0	1.00

3.5. Discussion

Boreal bog vegetation is characterized by the dominance of *Sphagnum* that can limit vascular plants by generating low available nutrient conditions (van Breemen 1995, Turetsky et al. 2012b). However, previous experiments indicated that existing boreal bog vascular plants might grow better and become more abundant under warmer conditions (Buttler et al. 2015, Dieleman et al. 2015, Richardson et al. 2018, Lyons et al. 2020, McPartland et al. 2020) or nitrogen-enriched conditions (Berendse et al. 2001, Heijmans et al. 2001, Limpens et al. 2003a, 2004, Bubier et al. 2007, Wieder et al. 2019). Therefore, the concomitant warmer and nitrogen-enriched conditions due to climate warming and/or high nitrogen (Bell et al. 2018, Wang et al. 2018) may significantly increase the growth of vascular plants in boreal bogs. In addition, the higher abundance of vascular plants due to warmer and nitrogen-enriched conditions may enhance their interaction, which may modulate responses of boreal bog vascular plants to these environmental changes. However, the combined effects of warmer, nitrogen-enrichment and plant-plant interactions on existing boreal bog vascular plants are unknown. This study examined the interactive effects between warming, nitrogen-enrichment, and shrub removal on two boreal bog graminoids. Furthermore, this study investigated the combined effects of warming, nitrogen-enrichment, and graminoid removal on three dominant boreal bog shrubs.

3.5.1. Effects of shrub removal, nitrogen addition, and warming on dominant graminoids.

This study revealed that the combined effects of warming and nitrogen addition stimulated the growth of *R. alba* (Table 3-1a). Specifically, leaf length increment of *R. alba* due to concomitant warming and nitrogen addition treatments was 16 % and 26% higher than increases due to individual nitrogen addition treatments or warming treatments, respectively (Figure 3-1a). By contrast, these environmental changes did not affect *T. cespitosum* (Figure 3-1b, table 3-1b). These findings partly support the first hypothesis that the combination of warming and nitrogen addition would increase graminoid growth. In this study, nitrogen addition and warming increased the soil temperature and NO_3^- at 10 cm depth, while these treatments did not affect these factors at 40 cm depth (Table 3-3, S3-1). This might be the primary explanation for these findings, wherein significant responses were only detected in the shallow root graminoid – *R.alba* (Ohlson and Malmer 1990, Limpens et al. 2003a), whereas no responses were observed at a high-density tussock, deep root graminoid – *T. cespitosum*. The resistance of high-density tussock, deep root graminoids under environmental changes, has been documented in other studies (Thormann and Bayley 1997, Tomassen et al. 2004, Buttler et al. 2015, Walker et al. 2015). However, the increases in soil temperature and nitrogen available concentration in the deeper layers are expected under longer-term treatments (Buttler et al. 2015). Therefore, the positive responses of graminoids, even in deep root plants, might be detected in the future warmer and nitrogen-enriched conditions. The additive effects of warming and nitrogen addition on the graminoid growth suggest that projections modeling individual effects of warmer or increasing nitrogen concentration levels on the existing boreal bog graminoid community could be underestimated.

We also found that the presence or absence of shrubs did not affect the growth of boreal bog graminoids even under warmer, nitrogen-enriched conditions (Figure 3-1, table 3-1). In this study area, both *R. alba* and *T. cespitosum* are the upper canopy layer, explaining no competition for light from shrubs and no responses of these graminoids to the presence or absence of shrubs. Furthermore, most graminoid roots are in deeper layers (40 to 50 cm depth), while shrub roots are present in shallow layers (0-10 cm depth) in boreal bogs (Murphy et al. 2009). The differences in root distribution between shrubs and graminoids may explain the weak effects of shrubs on graminoids. These findings suggest that shrub community dynamics might not affect graminoids under nitrogen-enriched and warmer conditions.

3.5.2. Effects of graminoid removal, nitrogen addition, and warming on shrubs

Warming and nitrogen addition treatments affected boreal bog shrubs; however, the individual effects of these treatments on the shrubs are species-specific (Table 3-2). Specifically, the evergreen shrubs (*A. glaucophylla* and *C. calyculata*) are likely more sensitive to nitrogen addition than a deciduous shrub (*G. bigeloviana),* whereas the deciduous shrub is more responsive to warming treatment than the evergreen boreal bog shrubs (Figure 3-2). The positive effects of nitrogen addition on *A. glaucophylla* and *C. calyculata* are consistent with many previous findings (Thormann and Bayley 1997, Wiedermann et al. 2007, Wieder et al. 2019). Effects of these environmental changes on *G. bigeloviana* are unknown, although better growth and more abundance under warmer conditions have been found in many other deciduous boreal shrubs (Givnish 2002, Moore and Bubier 2020, Standen and Baltzer 2021). The differences in traits and strategies to adapt to the environments of these plant species may partly explain the specific responses of these shrubs to environmental changes (Weltzin et al. 2003, Lin et al. 2020, Warren et al. 2021). Deciduous plants only grow in short seasons with favorable conditions in both temperature and nutrient availability, whereas these plants cease growing during the unfavorable season to minimize the costs of leaf and root respiration (Givnish 1986, 2002). By contrast, evergreen plants with long-lived leaves can grow over a more extended period even under low-temperature conditions during early spring and late fall (Waring and Franklin 1979, Givnish 2002). Therefore, warming treatments that may expand growing seasons by increasing temperature (Richardson et al. 2018) and improving nutrient availability (Vitt et al. 1995) could benefit deciduous plants more than evergreen plants. In contrast, nitrogen addition might benefit evergreen plants more than deciduous plants when it only enhances nutrients without temperature changes (Table 3-3).

This research also found that graminoid removal increased new leaf and/or new shoot number of *A. glaucophylla* and *C. calyculata*, while the graminoid presence had no effects on *G. bigeloviana* (Table 3-2). Elimination of intense light competition from a high density of graminoids (Table 3-3) might be a primary reason for the positive effects of graminoid removal on the lower shrubs in this study. By contrast, the consistency of *G. bigeloviana* to graminoid removal might be interpreted by low competition of graminoids to this plant. Surrounding *G. bigeloviana* bushes is a thick litter layer of dead leaves. The litter layer reduced the density of graminoids nearby *G. bigeloviana* bushes leading to lower local competition between graminoids and this shrub.

More interestingly, this study revealed that nitrogen addition treatments enhanced the positive effects of graminoid removal on *A. glaucophylla* (Figure 3-2 d, g, table 3-2a). This result indicates that *A. glaucophylla* growth was negatively affected by graminoids under nitrogen-enriched conditions. A significant increase in cover of these vascular plants in nitrogen addition treatments (Table 3-3, S3-1) is likely a primary explanation for the light competition between graminoids and *A. glaucophylla* with an obvious vulnerability of *A. glaucophylla* due to its smaller stature (Lee and Caporn 1998, Van den Berg et al. 2005). By contrast, our results showed that warming reduced the positive effects of graminoid removal on *A. glaucophylla* (Figure 3-2g, d, table 3-2a), suggesting facilitation from graminoids to the boreal bog shrub under warmer conditions. Generally, boreal bog vascular plants positively respond to warmer conditions (Buttler et al. 2015, Dieleman et al. 2015, Richardson et al. 2018, Lyons et al. 2020, McPartland et al. 2020) by increasing nutrient availability generated by faster decomposition rates (Ward et al. 2015a, Asemaninejad et al. 2017, Bell et al. 2018) and an extended growing season (Richardson et al. 2018). In addition, the warmer conditions may also benefit the growth of vascular plants by improving photosynthetic efficiency when the increasing temperature is lower than optimum temperature, while higher temperatures decrease plant growth by reducing photosynthesis (Kirschbaum 2004). Reich et al. (2018) indicated that warmer conditions only increase photosynthesis of boreal bog plants when soil moisture is higher than 50%; otherwise, warmer conditions limit plant photosynthesis. In this study, warming treatments not only increased temperature but also decreased soil moisture to less than 50% (Table 3-3); therefore, warming treatments may depress the photosynthetic activity of *A. glaucophylla*. Thus, graminoid presence could reduce the intensity of temperature increases due to warming treatments; thereby, graminoids mitigated stressors of warming and facilitated *A. glaucophylla* growth under warmer plots.

Although the mechanisms explaining these findings require further research, these findings indicate the substantial advantage of graminoids under warmer and nitrogen-enriched conditions, suggesting a high potential dominance of the plants in boreal bogs. The trend in vegetation dynamics of boreal bogs can significantly affect vital ecosystem services. Previous studies indicate that graminoids stimulate greenhouse gas emissions under environmental changes, including N_2O (Le et al. 2020) and CH_4 emissions (Ward et al. 2013b, Gong et al. 2021, Standen and Baltzer 2021).

3.6. Conclusions

This study provides evidence from a field manipulation experiment that the combination of warming and nitrogen addition enhances the growth of graminoids. Our results suggest that evergreen shrubs might be benefited from nitrogen-enrichment conditions more than deciduous shrubs; meanwhile, deciduous shrubs are benefited from warming more than evergreen plants. Furthermore, we found that competition from graminoids reduced shrub growth under nitrogen-enriched conditions, whereas graminoids facilitated shrub growth under warmer conditions. These findings suggest a rapid increase of vascular plants, especially graminoids, under future warmer, nitrogen-enriched conditions in boreal bogs.

This change in boreal bog vegetation could enhance greenhouse gas emissions in the ecosystems.

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Chapter 4 Vascular plants regulate responses of boreal peatland *Sphagnum* to climate warming and nitrogen addition

4.1. Abstract

Boreal peatland *Sphagnum* may be affected by climate warming and elevated nitrogen availability directly and indirectly via altering interaction with vascular plants. Here, we used a field experiment of nitrogen addition, warming, and vascular plant removal to investigate the effects of these factors on *Sphagnum* in a Canadian blanket boreal bog. We revealed that significant effects of warming and nitrogen addition on *Sphagnum* were regulated by vascular plant interaction. The intense competition of vascular plants accelerated an adverse effect of warming on *Sphagnum*, while facilitation of vascular plants reduced detrimental losses of the *Sphagnum* due to high dose of nitrogen addition and both warming and the nitrogen addition. These findings indicate the crucial role of vascular plants in regulating the effects of environmental changes on existing *Sphagnum* in boreal peatlands.

4.2. Introduction

Ombrotrophic boreal peatlands provide several vital ecosystem services, including water supplying and carbon sequestration (Gorham 1991, Limpens et al. 2008, Kuiper et al. 2014b, Loisel et al. 2014, Waddington et al. 2015, Wiedermann et al. 2017). In boreal peatlands, *Sphagnum* mosses are keystone plants that regulate the critical biogeochemical processes and essential services of these ecosystems (Breeuwer et al. 2008, Turetsky et al. 2012b, Kuiper et al. 2014a, Buttler et al. 2015). The mosses create acidic, low available nutrient, anoxic environments (Limpens and Berendse 2003, Malmer et al. 2003, Rydin et al. 2006, Soudzilovskaia et al. 2010), that lead to the low decomposition rate and high carbon sequestration ability of boreal peatlands. The *Sphagnum* mosses also generate up to 90% of the peat in boreal peatlands (Kuhry 1997, Hájek et al. 2011). Therefore, changes to *Sphagnum* mosses may significantly influence the biogeochemical processes and vital ecosystem functional services of the boreal peatlands.

In intact ombrotrophic boreal peatlands, vegetation is a matrix of *Sphagnum* mosses and vascular plants (Heijmans et al. 2002a, Le et al. 2020). *Sphagnum* limits the overgrowth of vascular plants by creating a low nutrient availability condition (Aldous 2002, Malmer et al. 2003). Simultaneously, the low cover rate of vascular plants facilitates the *Sphagnum* mosses by forming a firm matrix to support the vertical growth and spongy biomass of the mosses (Malmer et al. 1994, Fenton and Bergeron 2006, Pouliot et al. 2011) and by creating stable microclimates (Heijmans et al. 2002a). The domination of *Sphagnum* mosses with the facilitation from vascular plants ensures the stability of boreal peatlands and their carbon sequestering function (Malmer et al. 2003, Pouliot et al. 2011, Waddington et al. 2015, Oke and Hager 2020).

Recent evidence suggests that boreal peatlands are subject to rapid climate warming (Stocker et al. 2014). Boreal peatland vegetation, especially *Sphagnum* mosses that have long-term adapted to low temperature, may significantly be affected by increasing temperature (Gunnarsson et al. 2004, Bragazza 2008, Jassey et al. 2013, Walker et al. 2015, He et al. 2016, Norby et al. 2019). An elevated temperature may directly affect *Sphagnum* mosses (Robroek et al. 2007, Bragazza 2008, Breeuwer et al. 2008, Norby et al. 2019). The warmer conditions may lead to more abundant vascular plants in boreal peatlands (Heijmans et al. 2008, Jassey et al. 2013, Carrell et al. 2019, McPartland et al. 2020) by increasing available nutrients if decomposition rate is increased with elevated temperature (Gerdol et al. 2007, Dieleman et al. 2016).

Boreal peatlands are also sensitive to increased atmospheric nitrogen deposition because of long-term adaptation to low available nutrients (Bobbink et al. 1998a, Galloway et al. 2008, Whitaker et al. 2020). An increase of available nitrogen due to the high nitrogen deposition may substantially influence the ecosystems, especially *Sphagnum* mosses (Gunnarsson and Rydin 2000, Van der Heijden et al. 2000, Berendse et al. 2001, Gunnarsson et al. 2004, Limpens et al. 2004, Bubier et al. 2007, Gerdol et al. 2007, Limpens et al. 2009, Bu et al. 2011a, Limpens et al. 2011, Fritz et al. 2012, Granath et al. 2012, Wieder et al. 2019, Wieder et al. 2020). The increase in available nitrogen may directly affect *Sphagnum* mosses (Van der Heijden et al. 2000, Limpens and Berendse 2003) but may also indirectly impact *Sphagnum* mosses by increasing the coverage and growth of vascular plants (Juutinen et al. 2010, Wu and Blodau 2015).

Although both climate warming and elevated nitrogen deposition might alter the boreal peatland *Sphagnum* mosses, the combined effects of these environmental changes on the mosses are little studied. In addition, the changes in environments significantly influence vascular plants that have been recognized as a pivotal factor facilitating growth of the boreal peatland mosses (Malmer et al. 1994, Malmer et al. 2003, Pouliot et al. 2011, Turetsky et al. 2012a). However, the combined effects of the abiotic and biotic changes on the boreal peatland *Sphagnum* are unknown. In this study, we designed a full factorial experiment including warming, nitrogen addition and vascular plant removal to examine the effects of these factors on *Sphagnum* mosses. Using this full-factorial approach, the experiment could be used to determine how the interaction between *Sphagnum* and vascular plants changed responses of *Sphagnum* to warmer and N-enriched conditions. We hypothesize that the warmer and elevated nitrogen conditions will change the interaction between vascular plants and *Sphagnum* from facilitation to competition because of increases in vascular plant coverage, accelerating the decreases of *Sphagnum* due to these environmental changes.

4.3. Material and methods

4.3.1. Study site

The study site is located at a boreal ombrotrophic blanket peatland in western Newfoundland, Canada (48°15′44″ N, 58°40′03″ W), with a constant peat depth of 3 m. Mean annual air temperature and precipitation over the last 30 years of this study area were approximately 5 °C and 1340 mm, respectively (Gong et al. 2019). The average air temperature was 10.6 °C during the 2019 growing season and 12.9 °C during the 2020 growing seasons. During the data collection times, mean precipitation was 514.4 mm in the 2019 growing season and 286.2 mm in the 2020 growing season. In the study ecosystem pH of porewater water is 4.5 (Gong et al. 2019). The study site is a typical ombrotrophic boreal peatland in Newfoundland, where a *Sphagnum* blanket is dominated by *Sphagnum fuscum* and *Sphagnum rubellum*. The sparse vascular plants consist of dwarf shrubs (*Andromeda glaucophylla, Chamaedaphne calyculata, Gaylussacia bigeloviana, Vaccinium oxycoccos, Gaylussacia baccata, Rhododendron tomentosum, and*

Rhododendron groenlandicum), and graminoids (*Rhynchospora alba* (L.) Vahl, *Trichophorum cespitosum* (L.) Hartman).

4.3.2. Experimental design

The experimental design was described by Luan et al. (2019). In this experiment, 16 treatments were generated from 4 levels of vascular plant removal, 2 levels of nitrogen addition (with and without addition), and 2 levels of warming (with and without warming treatment). We created 64 plots in 4 blocks, representing 4 replicates, with 16 plots in each block. 6m buffer zones separated the blocks; meanwhile, the plots in these blocks were disparted by 2 m buffer zones. Each plot covers an area of 4 m^2 (2 m × 2 m). The 16 treatments were randomly arranged at 16 plots in each block (Figure 4-S1).

To increase air temperature, we used open-top chambers (OTCs) constructed from six plexiglass sheets (80 cm along the bottom edge, 62.5 cm along the top edge, 40 cm in height) to cover an area of 1.66 m^2. The warming treatments increased the mean daytime air temperature by 2.10 ± 1.52 ^oC during the 2019 growing season and by 2.02± 1.11 ^oC during the 2020 growing season. The effects of warming on air temperature were assessed using two USB temperature loggers (Lascar Electronics Ltd., UK) that recorded air temperature at vegetation canopy height every 30 min in the warming and control plots.

To establish nitrogen non-limited conditions, we applied 3.2 g $N.m^{-2}$ in early June and the exact amounts of nitrogen in early August from 2014 to 2018. During 2019-2020, we monthly applied 1.6 g $N.m^{-2}$ from June to September in N addition plots. These treatments added 6.4 g N. m^{-2}. yr^{-1} that is equivalent to 10 times of inorganic nitrogen background deposition in this study area (Reay et al. 2008, Le et al. 2020). This dose of nitrogen addition is comparable to the level of nitrogen addition in other studies on boreal peatlands (Nykänen et al. 2002, Lund et al. 2009, Leeson et al. 2017, Juutinen et al. 2018, Levy et al. 2019). Fertilizer was given in the soluble form (NH_4NO_3) dissolved in 2 L of same site pool water. The same amount of pool water also watered no nitrogen treatments without the nitrogen addition.

To determine the roles of vascular plants in the growth and cover of *Sphagnum*, we used the “removal” method with 4 treatments of vascular plant removal: (1) No (No vascular plants removed), (2) G (removed graminoids), (3) S (removed shrubs), and (4) GS (removed both graminoids and shrubs). The shoots of graminoids and/or shrubs were cut back to the moss layer in early May 2014 and then re-cut biweekly if new vascular plant shoots regrew.

4.3.3. Sphagnum moss measurement

The height increment of the *Sphagnum* layer was determined by the cranked wire method (Clymo 1970). In detail, three cranked wires were inserted into the moss carpet at all 64 experimental plots in May; and the linear growth of *Sphagnum* plants was measured in October 2019 and in 2020. The *Sphagnum* cover in all plots was also measured by a point interception method (Jonasson 1983) in the 2020 mid-growing season. After measuring both height increment and cover of *Sphagnum*, three cores of the *Sphagnum* in each plot were also collected in the 2020 late growing season. These *Sphagnum* cores were separated into capitulum (0 -1 cm) and stem (1-2 cm) before drying at 60°C to calculate the bulk density of these sections. The bulk densities and the height increment were used to calculate the annual biomass production of the *Sphagnum* in the below equation (Limpens et al. 2004):

$$\text{Biomass production } (g.m^{-2}.\ yr^{-1}) = H_s * B_s + H_c * ((B_c - B_c \text{ control})/7) \qquad (1.1)$$

Where H_s is the stem height increment (m. yr^{-1}); H_c is the length of Sphagnum capitulum (0.01 m); B_s and B_c are bulk densities of stem and capitulum section (g.m^{-3}); B_c control is capitulum bulk density (g.m^{-3}) in the control environment without vascular plant removal; 7 is the number of years during the experiment from 2014 to 2020; The negative values of the biomass production indicate the decreases, while the positive values present the increases in *Sphagnum* biomass.

4.3.4. Vascular plant interaction with Sphagnum mosses

We used the biomass production to compute vascular plant interaction with *Sphagnum* via extended relative interaction index (RIIe) modified from Armas et al. (2004):

$$RIIe=(Bw-Bo)/ABS(Bw+Bo) \qquad (1.2)$$

Bw and Bo present *Sphagnum* biomass production with vascular plants without vascular plants. Equation 1.2 enables us to calculate the relative interaction index event when Bw and/or Bo get the negative value that may occur when environmental changes stress plant communities. RIIe values range from $-\infty$ (Competition) through 0 (No interaction) to $+\infty$ (Facilitation). The more negative RIIe values indicate the more intense competition, while higher positive values present the more intensity of facilitation.

4.3.5. Environmental measurement

The cover of vascular plants was measured by the point intercept method (Floyd and Anderson 1987) at the peak of vegetation biomass in mid-August 2020. Soil temperature and soil moisture at 5 cm depth were measured biweekly by utilizing a soil thermometer (Fisher Scientific Inc., Canada) and a soil moisture sensor (ProCheck, Decagon Devices Inc., USA). Also, 10 cm porewater samples were collected biweekly using MacroRhizon samplers (Rhizosphere Inc., Netherlands); meanwhile, perforated PVC tubes were used to collect the samples at 40 cm depth. The water samples were filtered through 0.45-µm membranes before analyzing NH_4^+ and NO_3^- concentration levels via flow injection analysis (Lachat Instruments, Inc. USA).

4.3.6. Statistical analysis

Data was processed and analyzed utilizing R software ver. 3.6.3 (R Core Team 2020). Before analyzing, a mean value of three measured points obtained at each plot is used as a single value. The plot data was checked for normality and was transformed by *jtrans* package (Wang 2016) where it is necessary. Effects of warming, nitrogen addition, and vascular plant removal on the cover, bulk density, biomass production of *Sphagnum* moss, vascular plant cover, RIIe, and environmental variables were analyzed by running ANOVAs as mixed effect models, with experimental blocks as a random effect in *nlme* package (Pinheiro et al. 2018). Because *Sphagnum* growth data was measured in two years, the year was added as another random variable during examining the effects of treatments on *Sphagnum* growth. Adjusted R^2 of the models was computed by the *rsq* package (Zhang 2020). Tukey’s post hoc tests were conducted via *multcomp* package (Hothorn et al. 2008) to determine the effects of vascular plant removal treatments on analyzed variables when a significant effect of the vascular plant removal on the variables was detected. Figures were created by *ggplot2* (Wickham 2016) and *ggpubr* (Kassambara 2020) packages.

4.4. Results

4.4.1. Effects of warming, nitrogen addition, and vascular plant removal on Sphagnum mosses

Without disturbances of warming, N addition, and vascular plant removal, the *Sphagnum* mosses layer covered 93.75±2.69 % (mean± standard error) of boreal peatland surface (Figure 4-1a), with 23.7±2.8 kg.m^{-3} of bulk density (Figure 4-1d). The *Sphagnum* moss layer annually increased by 5.67±0.20 mm in height (Figure 4-1b), contributing to an increase of the *Sphagnum* biomass by 60.1±2.0 g.m^{-2}. yr^{-1} (Figure 4-1e) in the control conditions. The cover and growth of *Sphagnum* were significantly affected by vascular plant removal (Table 4-1). In control conditions, the removal of both graminoids and shrubs reduced *Sphagnum* cover by 32% (Figure 4-1a), height increment by 38% (Figure 4-1b), and biomass production by 77% (Figure 4-1d).

The warming treatment significantly affected *Sphagnum* growth; however, the treatment did not impact the moss cover (Table 4-1). The elevated temperature increased the mean height increment of *Sphagnum* mosses by 17% (F=7.1, p=0.0087, Figure 4-1b). In contrast, the 7-year treatment of temperature reduced mean bulk density of the moss layer by 39% (F=11.9, p=0.0012, Figure 4-1c). As a result, the warming treatment reduced biomass production of the *Sphagnum* by 92% (F=20.2, p<0.0001, Figure 4-1d).

The adverse effect of the warming on biomass production of *Sphagnum* mosses was mitigated by the vascular plant removal (F=3.5, p=0.0239). In no vascular plant removal plots (No), *Sphagnum* biomass increased by 60.1±2.0 g.m^{-2}. yr^{-1} in control conditions and decreased by 16.4±0.8 g.m^{-2}. yr^{-1} in warming conditions. The loss of *Sphagnum* biomass due to warming treatment was significantly decreased when vascular plants were removed, wherein *Sphagnum* biomass only decreased by 4.0 ±3.9 g.m^{-2}. yr^{-1} in shrub removal plots (S), increased by 6.4 ±2.0 g.m^{-2}. yr^{-1} in all vascular plant removal plots (GS) and by 28.1±12.0 g.m^{-2}. yr^{-1} in graminoid removal plots (G) (Figure 4-1d).

Table 4-1. Summary of models of warming (W), nitrogen addition (N), vascular plant removal (V), and their combined effects on the cover, height change, bulk density, and biomass change of *Sphagnum* mosses at a boreal peatland.

Source of variation	Df	a, Cover (%) (n=64)		b, Height increment (mm. yr^{-1}) (n=128)		c, Bulk density (Kg.m^{-3}) (n=64)		d, Biomass increment (g.m^{-2}. yr^{-1}) (n=64)	
		F	p	F	p	F	P	F	P
W	1	3.9	0.0556	7.1	0.0087	11.9	0.0012	20.2	<.0001
N	1	173.6	<.0001	809.7	<.0001	262.1	<.0001	472.7	<.0001
V	3	15.4	<.0001	36.9	<.0001	5.7	0.0021	6.9	0.0006
W x N	1	0.2	0.6543	2.9	0.0894	6.2	0.0162	21.0	<.0001
W x V	3	1.9	0.1452	2.4	0.0737	2.4	0.0819	3.5	0.0239
N x V	3	3.8	0.0159	0.9	0.4500	6.3	0.0011	6.6	0.0009
WxNxV	3	0.0	0.9985	7.6	0.0001	2.4	0.0806	3.0	0.0423
Full model R^2			0.56	0.88		0.66		0.82	

Nitrogen addition significantly decreased the *Sphagnum* growth and cover (Table 4-1). The addition of nitrogen caused a "negative" height increment of the *Sphagnum* layer (F=809.7, p<0.0001, Figure 4-1b). The fertilization with nitrogen also reduced mean cover by 71% (F=173.6, p<0.0001, Figure 4-1a) and mean bulk density of the *Sphagnum* layer by 93%

(F=262.1, p<0.0001, Figure 4-1c). These decreases in height and bulk density due to N addition resulted in 50 g.m^{-2}. yr^{-1} loss in biomass of the moss layer (F= 524.5, p<0.0001, Figure 4-1d).

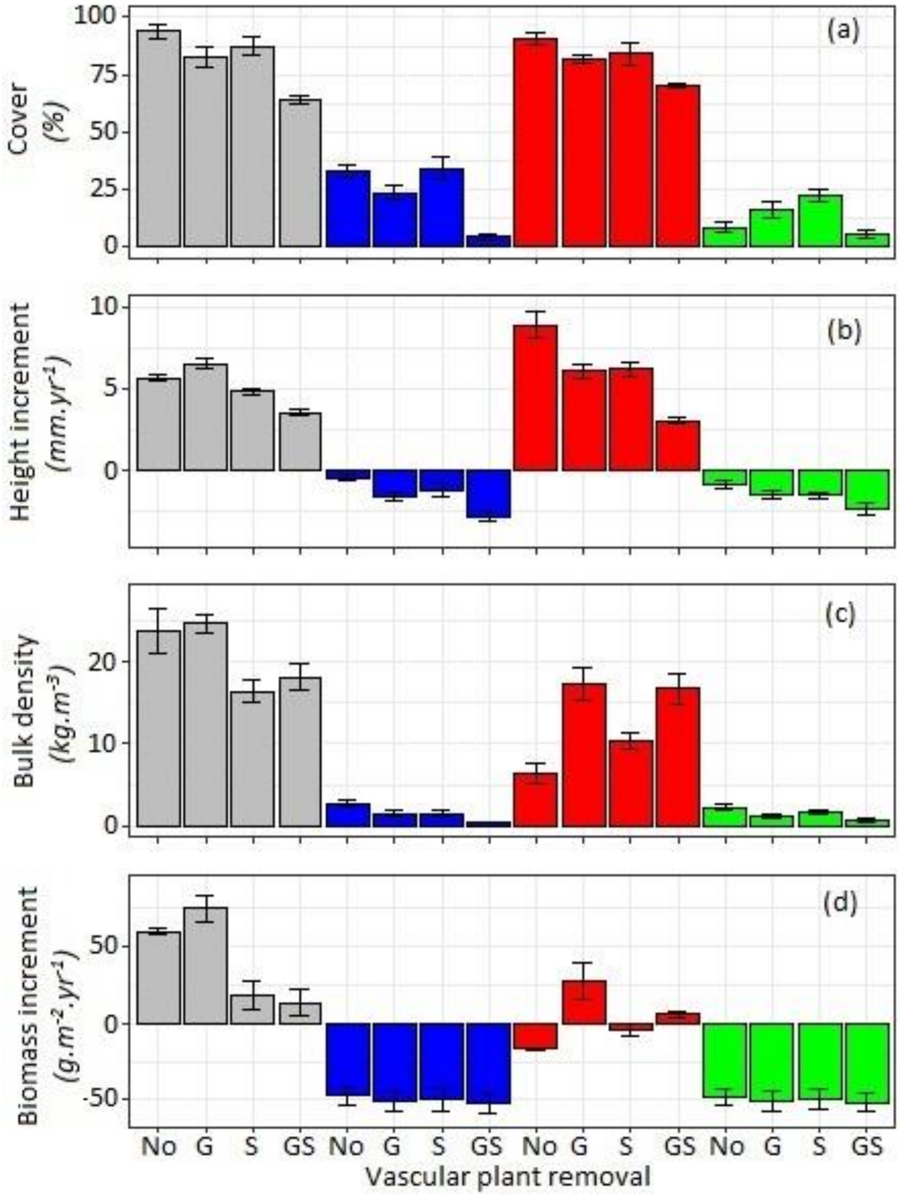

Figure 4-1. Cover (a), height increment (b), bulk density (c), and biomass increment (d) of *Sphagnum* mosses when no vascular plant (No), graminoids (G), shrubs (S), and both

graminoids and shrubs (GS) were removed under conditions of control (Grey bars), N addition (Blue bars), warming (Red bars) and both N addition and warming (Green bars) conditions.

We also found that vascular plant removal stimulated the adverse effects of N addition on the *Sphagnum* cover (F=3.8, p=0.0159), bulk density (F=6.3, p=0.0011), and biomass production (F=6.6, p=0.0009) of *Sphagnum* mosses (Table 4-1). Without vascular plant removal, nitrogen addition reduced *Sphagnum* coverage from 94% to 33% (Figure 4-1a) and bulk density from 23.7 ±2.8 kg.m^{-3} to 2.7 ±0.1 kg.m^{-3} (Figure 4-1c). The addition of nitrogen also resulted in a 47.3 ±6.3 g.m^{-2}. yr^{-1} biomass loss of *Sphagnum* (Figure 4-1d) when all vascular plants presented. Under the nitrogen addition conditions, removing both graminoids and shrubs reduced *Sphagnum* cover to only 4% (Figure 4-1a), bulk density to 0.4 ±0.1 kg.m^{-3} (Figure 4-1c), and enhanced biomass production loss of the moss to nitrogen addition by 10% (Figure 4-1d).

This study found significant combined effects of warming and nitrogen addition on *Sphagnum* bulk density (F=6.2, p=0.0162) and biomass production (F=21.0, p<0.001) (Table 4-1). Nitrogen addition increased the reduction of *Sphagnum* bulk density due to warming treatment, wherein bulk density of the *Sphagnum* layer was reduced by 39% in warming treatments and by 93% in treatments of both warming and nitrogen addition (Figure 4-1c). The addition of nitrogen also enhanced the loss of *Sphagnum* biomass production from 92% in warming treatments to 1.9 times in the combined warming and nitrogen treatment (Figure 4-1d).

More importantly, we found that vascular plant removal modified the combined effects of warming and nitrogen addition on annual height increment (F=7.6, p=0.0001) and annual biomass production (F=3.0, p=0.0423) of the *Sphagnum* (Table 4-1). The absence of both graminoids and shrubs increased a negative height increment by approximately twice as much in plots where both warming and nitrogen addition were applied (Figure 4-1b). Similarly, removing all vascular plants expanded annual biomass loss of the *Sphagnum* layer by 7% in warming and N addition plots.

4.4.2. Effects of nitrogen addition and warming on interaction of vascular plants with Sphagnum mosses.

Under control conditions, the presence of graminoids and shrubs facilitated the growth of *Sphagnum* mosses with intensely positive extended relative interaction indices (RIIe) (Figure 4-2). However, the high facilitation of vascular plants for *Sphagnum* mosses was altered by warming and nitrogen fertilization separately and in combination (Table 4-2, Figure 4-2).

Table 4-2. Statistical analysis of warming (W) and nitrogen addition (N) effects on extended relative interaction index (RIIe) of vascular plants with *Sphagnum* mosses.

Source of variation	Df	Graminoids		Shrubs		Graminoids and Shrubs	
		F	P	F	P	F	P
W	1	14.2	0.0044	2.2	0.1752	115.4	<.0001
N	1	1.5	0.2451	23.6	0.0009	0.4	0.5333
W x N	1	18.2	0.0021	0.0	0.8396	46.2	0.0001

Nitrogen addition reduced the facilitation of shrubs for *Sphagnum* mosses (F=23.6, p=0.0009, Figure 4-2b). Warming switched the strong facilitation of graminoids for

Sphagnum mosses to intense competition (F=12.4, p=0.0044, Figure 4-2a). The significant competition of both graminoids and shrubs to *Sphagnum* was also detected in the warmer conditions (F=115.4, p<0.0001, Figure 4-2c). Interestingly, the intense competition from graminoids and graminoids and shrubs together to *Sphagnum* in warming conditions were reversed to low-intense facilitation when both warming and nitrogen treatments were combined (Table 4-2, Figure 4-2a, 4-2c).

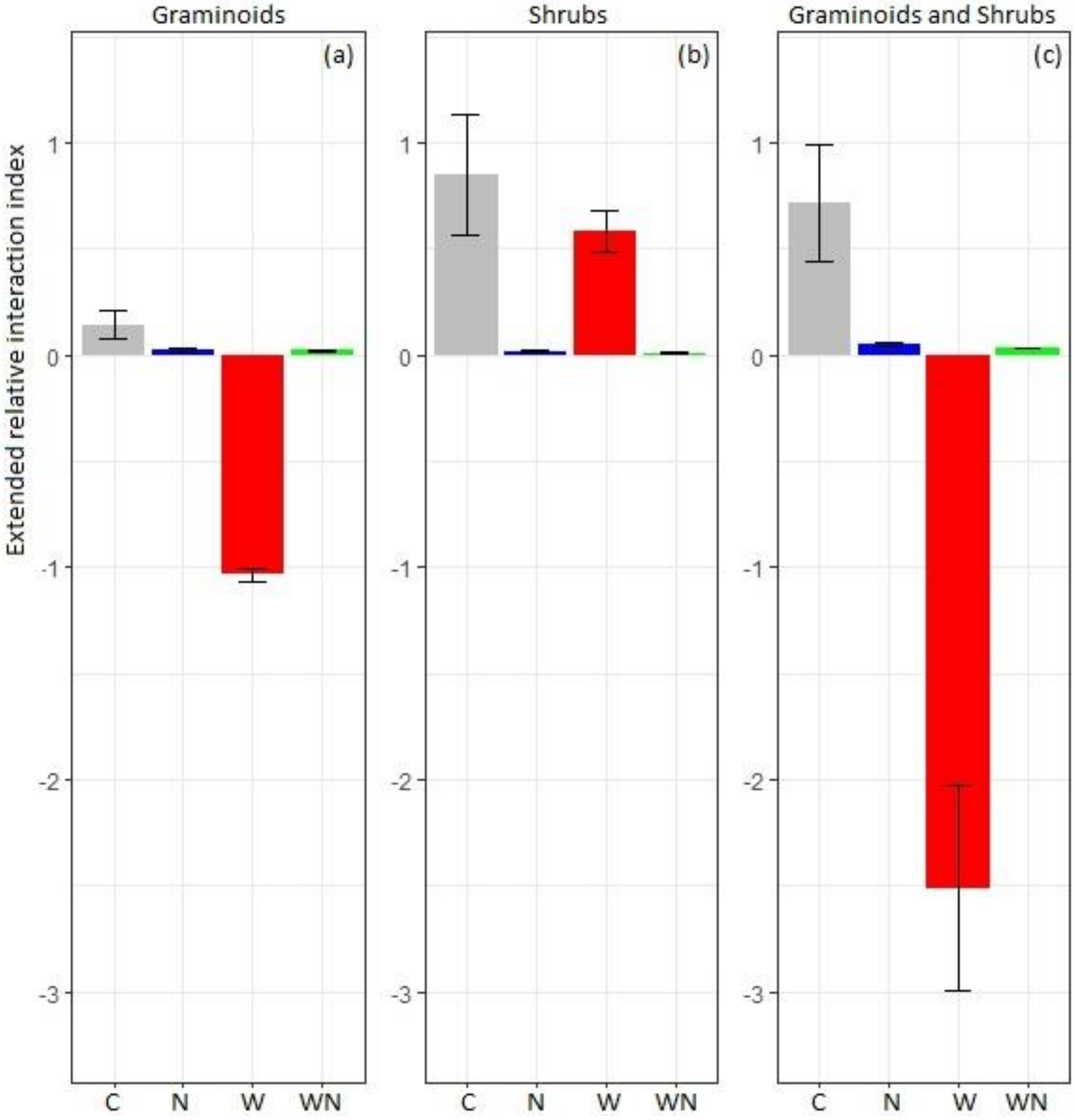

Figure 4-2. Extended relative interaction index (RIIe) of (a) graminoids, (b) shrubs, and (c) both graminoids and shrubs with *Sphagnum* mosses in control (C), nitrogen addition (N), warming (W), and both N addition and warming (WN) conditions.

4.4.3. Effects of the treatments on soil environment and cover of vascular plants

The warming treatment significantly increased vascular plant cover and soil temperature, while this treatment did not affect soil moisture, $NH_4^{+,}$ and NO_3^- concentration levels in soil water samples (Table 4-S1). The warming treatment increased the mean cover of vascular plants by 19%. Also, the mean annual soil temperature was increased by 0.9 ^oC in warming plots (Table 4-3).

Nitrogen addition altered vascular plant cover, soil moisture, NH_4^+, and NO_3^- concentration, while the nitrogen treatment did not affect soil temperature (Table 4-S1). Specifically, the nitrogen addition increased the mean cover of vascular plants by 17% and annual soil moisture by 20% (Table 4-3). The addition of nitrogen also enhanced NH_4^+ concentration in 10 and 40 cm depth by 149% and 32%, respectively (Table 4-3). Annual NO_3^- concentration at 10 cm depth increased by 113% in N treatments, while nitrogen treatment did not change annual NO_3^- concentration at 40 cm depth (Table 4-3).

Table 4-3. Cover of vascular plants (COV), mean soil temperature (ST), soil moisture (SM), concentration of ammonium (NH_4^+) and nitrate (NO_3^-) in soil solution at 10 and 40 cm depth.

Treatment	COV (%) (n=64)	ST (^{o}C) (n=896)	SM (%) (n=896)	NH_4^+ (mg. l^{-1})		NO_3^- (mg. l^{-1})	
				10 cm (n=270)	40 cm (n=275)	10 cm (n=312)	40 cm (n=289)
Control condition							
No	77.0±6.4	16.90±0.42	43.55±2.62	0.37±0.06	0.68±0.08	0.082±0.038	0.042±0.017
G	32.3±1.3	17.50±0.44	46.40±2.54	0.49±0.07	1.02±0.05	0.053±0.016	0.039±0.018
S	52.0±4.1	17.82±0.46	48.50±2.76	0.44±0.09	0.69±0.10	0.031±0.009	0.034±0.009
GS	0	17.34±0.44	49.88±2.27	0.46±0.07	1.07±0.06	0.050±0.016	0.024±0.004
Nitrogen addition							
No	91.0±3.4	17.28±0.40	49.92±2.81	0.90±0.15	1.01±0.08	0.212±0.147	0.030±0.007
G	30.8±5.9	17.67±0.40	57.55±2.80	0.96±0.10	1.17±0.08	0.238±0.100	0.031±0.008
S	70.7±8.3	17.31±0.45	55.77±2.83	0.65±0.10	0.99±0.06	0.088±0.020	0.020±0.004
GS	0	17.54±0.45	59.19±3.03	1.37±0.16	1.37±0.07	0.524±0.241	0.036±0.011
Warming							
No	90.8±1.9	18.24±0.47	38.37±2.48	0.28±0.05	0.47±0.08	0.095±0.045	0.034±0.009
G	42.5±8.0	18.49±0.44	47.99±2.66	0.36±0.07	0.86±0.04	0.049±0.017	0.028±0.007
S	62.0±7.4	18.28±0.49	40.77±2.25	0.41±0.05	0.58±0.06	0.062±0.024	0.035±0.011
GS	0	18.50±0.44	50.53±2.96	0.51±0.09	1.04±0.08	0.031±0.008	0.034±0.008
Warming and nitrogen addition							
No	97.0±3.5	17.92±0.49	53.99±2.84	0.90±0.11	1.00±0.07	0.203±0.072	0.026±0.007
G	49.0±3.0	18.55±0.46	57.22±3.19	1.31±0.29	1.30±0.11	0.192±0.082	0.032±0.007
S	77.8±2.6	18.17±0.51	50.21±2.85	0.91±0.16	0.91±0.06	0.102±0.027	0.032±0.011
GS	0	18.19±0.49	56.71±2.74	1.64±0.21	1.23±0.07	0.270±0.117	0.031±0.008

No: Graminoid and Shrub presences without removal; G: removing graminoids; S: removing shrubs; GS: removing both graminoids and shrubs. Data values are mean ± standard error.

Table S 4-1. Summary of models of warming treatment (W), nitrogen addition (N) and vascular plant removal (V), and their combined effects on cover of vascular plants (COV), soil temperature a(ST), soil moisture (SM), concentration of ammonium (NH_4^+) and nitrate (NO_3^-) in soil solution at 10 and 40 cm depth at a boreal peatland.

Source of variation	df	COV (%) (n=64)		ST (°C) (n=128)		SM (%) (n=128)		NH_4^+ at 10cm (mg. l^{-1}) (n=64)		NH_4^+ at 40cm (mg. l^{-1}) (n=64)		NO_3^- at 10 cm (mg. l^{-1}) (n=64)		NO_3^- at 40 cm (mg. l^{-1}) (n=64)	
		F	p	F	p	F	P	F	p	F	P	F	p	F	p
W	1	9.1	0.0041	42.0	<.0001	2.8	0.0957	1.1	0.3007	0.4	0.5245	0.0	0.9427	0.0	0.8484
N	1	12.0	0.0012	0.2	0.6814	69.9	<.0001	41.3	<.0001	14.7	0.0004	6.5	0.0142	0.6	0.4417
V	3	163.6	<.0001	2.1	0.1068	9.4	<.0001	4.4	0.0083	7.2	0.0005	0.8	0.5169	0.1	0.9804
W x N	1	0.0	0.9453	0.7	0.3917	0.5	0.4798	2.1	0.1527	0.1	0.7162	1.1	0.3031	0.1	0.7501
W x V	3	1.5	0.2222	0.3	0.8218	2.1	0.1024	0.2	0.8622	0.3	0.8439	0.8	0.4996	0.7	0.5737
N x V	3	3.1	0.0364	0.5	0.7107	0.5	0.7092	2.5	0.0724	0.1	0.9753	4.2	0.0100	0.7	0.5651
W x N x V	3	0.1	0.9619	0.8	0.4787	1.6	0.2006	0.1	0.9441	0.3	0.7911	0.3	0.8606	0.5	0.6539

Nitrogen addition and warming treatments did not interactively affect the soil environment and vascular plant cover (Table 4-S1). However, this study found significant interaction effects of nitrogen addition and vascular plant removal on vascular plant cover and NO_3^- concentration at 10 cm depth (Table 4-S1). In detail, nitrogen fertilization increased vascular plant cover by 17 % with graminoid presence, 4% with shrub presence, and 9% when both the plant groups were presented. The addition of nitrogen also increased mean NO_3^- concentration at 10 cm depth soil water samples by 59% without vascular plant removal, while the mean NO_3^- concentration was enhanced by 10 times in N addition plots when all vascular plants were removed (Table 4-3).

4.5. Discussion

We provide evidence that the responses of *Sphagnum* mosses to both warming and nitrogen addition were modified by vascular plant removal. This finding revealed that the changes in vascular plant interaction with *Sphagnum* in the warmer and/or nitrogen-enriched conditions altered the environmental change effects on the existing boreal peatland *Sphagnum*. Based on these findings, we created a conceptual model of S*phagnum* moss growth in an ombrotrophic boreal peatland (Figure 4-3). Our model indicates the effects of warming and increased nitrogen deposition on boreal peatland *Sphagnum* mosses directly and indirectly by altering vascular plant interaction. We found that vascular plants played a pivotal role in the growth of *Sphagnum* mosses (Figure 4-2, 4-3) and regulated responses of *Sphagnum* mosses to environmental stresses, such as warming and increased nutrients.

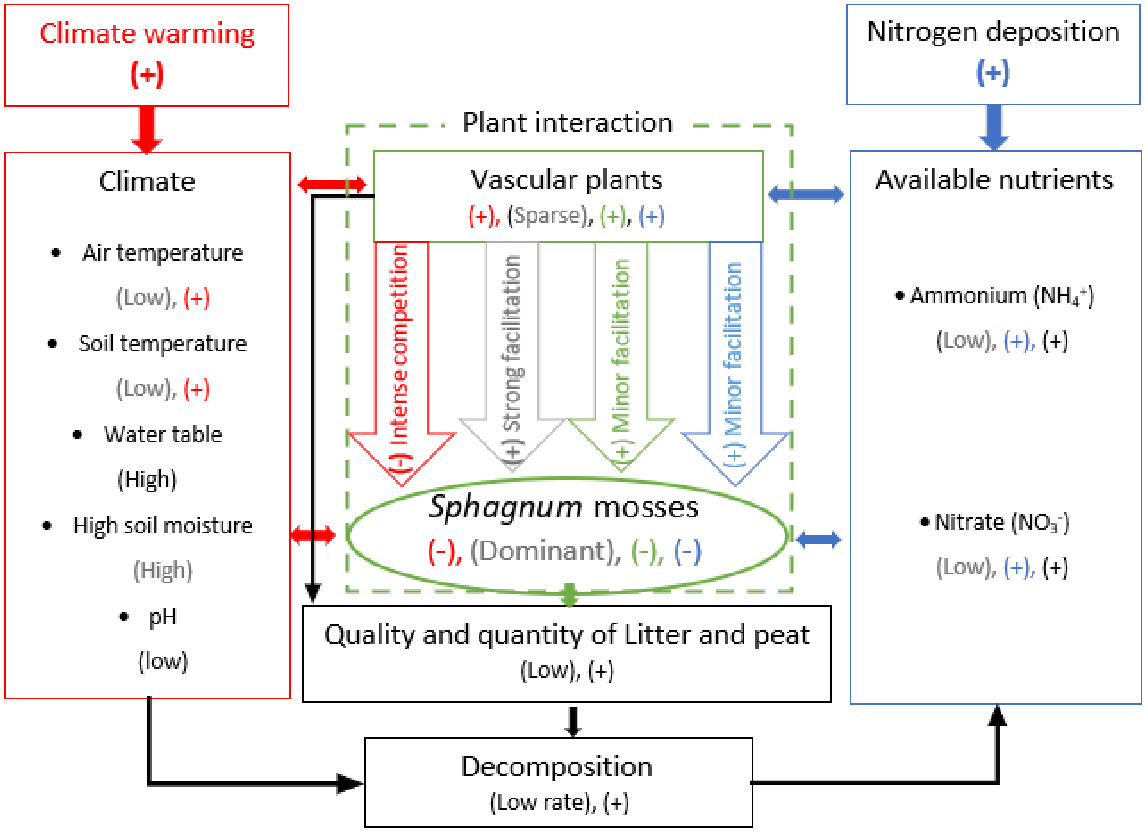

Figure 4-3. Schematic showing the potential effects of climate warming and nitrogen deposition increase in an ombrotrophic boreal peatland. The grey, red, blue, and green indicate the control, warming, nitrogen addition, and warming and nitrogen addition conditions found in this study. The black presents condition and potential effects of warming and nitrogen addition finding in the literature.

Substantial decreases in cover (Figure 4-1a), annual height increment (Figure 4-1b), and biomass production (Figure 4-1e) of *Sphagnum* mosses due to the removal of vascular plants, especially the removal of shrubs, suggest significant facilitation of vascular plants for *Sphagnum* mosses (Figure 4-2). The facilitation from vascular plants to the growth of *Sphagnum* mosses in undisturbed boreal peatlands was observed in previous studies

(Pouliot et al. 2011, Walker et al. 2015). In the boreal ecosystems, vascular plants can form a firm matrix to support the vertical growth and spongy biomass of the mosses (Malmer et al. 1994, Fenton and Bergeron 2006, Pouliot et al. 2011). The presence of vascular plants may also create high air moisture and stable microclimates that facilitate the growth of *Sphagnum* mosses (Heijmans et al. 2002a). The results indicated that the strong facilitation of vascular plants might contribute to the growth and dominance of *Sphagnum* mosses in intact boreal peatlands.

Our results show that boreal peatland *Sphagnum* mosses were significantly affected under warmer conditions (Table 4-1, Figure 4-1, Figure 4-3). Previous studies showed a wide range of *Sphagnum* responses to climate warming, from significant increases to substantial decreases. The increases or minor responses of the *Sphagnum* were observed in experiments where effects of vascular plants were ignored due to removal or no changes (Robroek et al. 2007, Breeuwer et al. 2008, Buttler et al. 2015). By contrast, other results in literature show negative responses of *Sphagnum* mosses to temperature increases (Gunnarsson et al. 2004, Bragazza 2008, Jassey et al. 2013, Walker et al. 2015, He et al. 2016, Norby et al. 2019). Our study found that the height increment of *Sphagnum* mosses was increased by warming treatment (Figure 4-1b). However, the temperature increases significantly reduced bulk density (Figure 4-1c), leading to a substantial reduction in biomass production of the *Sphagnum* in the warmer conditions (Figure 4-1d). In this study, significant increases in air temperature, soil temperature, and especially in vascular plant covers (Table 4-3, S1) might be critical interpretations for the decreases in bulk density and biomass of the *Sphagnum* in warming treatments (Bragazza 2008, Jassey et al. 2013, He et al. 2016). The higher height increment of *Sphagnum* in the warming plots could be explained by adaptation of the mosses to intense light competition conditions due to the increase of vascular plant cover under the warmer conditions (Gruntman et al. 2017).

We also found that vascular plants modified the significant effects of warming treatment on the *Sphagnum* by altering the vascular plant interaction with the boreal peatland mosses (Table 4-1). Our results showed that *Sphagnum* biomass decreased in the warmed plots where graminoids or graminoids and shrubs were present (Figure 4-1d). The high cover of vascular plants with the presence of graminoids and both graminoids and shrubs under the elevated temperature treatment (Table 4-3), which further exceed 60% - the threshold of vascular plant cover needed to reduce *Sphagnum* growth due to light competition (Malmer et al. 2003), might be a primary explanation for the reduction of *Sphagnum* biomass. Our extended relative interaction index confirmed that the strong facilitations from graminoids or graminoids and shrubs in control conditions were converted to intense competition in warmer conditions (Figure 4-2a, 4-2c). By contrast, the decreases of vascular plant cover to less than 50% after removing graminoids or both graminoids and shrubs could eliminate the light competition of the vascular plants (Table 4-3), which interpreted the lower decreases of *Sphagnum* biomass in these plots under warmer conditions (Figure 4-1d). These findings suggest that the conversion of vascular plant interaction from facilitation to competition might be a primary mechanism interpreting the negative effects of warming on the boreal peatland *Sphagnum*, consistent with Jassey et al. (2013) and He et al. (2016). However, the elimination of light competition from graminoids and both graminoids and shrubs could not erase the adverse effects of climate warming when the annual biomass production of the *Sphagnum* was significantly lower in warmer conditions where graminoids or both graminoids and shrubs were removed (Figure 4-1d). This finding suggests that temperature increase may also limit the growth of *Sphagnum* mosses by other mechanisms other than the interaction with vascular plants (Brooker 2006), such as extensive desiccation (Norby et al. 2019) or high mortality (Bragazza 2008).

The high dose of nitrogen load significantly damaged *Sphagnum* mosses (Table 4-1, Figure 4-1). We detected substantial decreases in cover (Figure 4-1a), annual height increment (Figure 4-1b), bulk density (Figure 4-1d), and biomass production of *Sphagnum* (Figure 4-1e) in nitrogen addition plots. The adverse effects of high dose nitrogen addition or nitrogen pollution on *Sphagnum* mosses have been observed in many previous experiments (Gunnarsson and Rydin 2000, Van der Heijden et al. 2000, Berendse et al. 2001, Gunnarsson et al. 2004, Limpens et al. 2004, Bubier et al. 2007, Gerdol et al. 2007, Limpens et al. 2009, Bu et al. 2011a, Limpens et al. 2011, Fritz et al. 2012, Wieder et al. 2019, Wieder et al. 2020). The significant increases in NH_4^+ and NO_3^- concentration of peat water samples in N addition plots (Table 4-2, 4-3) suggest that the dose of nitrogen addition in this study exceeded the nitrogen absorb capacity of *Sphagnum* moss layers. The significant accumulation of toxic NH_4^+ in capitulum cells due to a high dose of NH_4NO_3 fertilization (Limpens and Berendse 2003) can be a primary factor that sustainably reduced growth and cover of *Sphagnum* mosses in our study. Van der Heijden et al. (2000) indicated that the high nitrogen content in *Sphagnum* capitular might lead to photosynthesis reduction, water content decrease, and necrosis of the mosses.

Our results also revealed that the detrimental effects of nitrogen addition on *Sphagnum* mosses were mitigated by presences of vascular plants (Table 4-1, Figure 4-1). Several previous studies indicated that the increase of intense light competition from vascular plants is a crucial mechanism depressing the *Sphagnum* in peatlands (Berendse et al. 2001, Tomassen et al. 2004, Van der Wal et al. 2005, Wiedermann et al. 2009, Wieder et al. 2019, Wieder et al. 2020). In this study, nitrogen addition treatments also increased vascular plant cover to higher than 70% when graminoids or both graminoids and shrubs were presented (Table 4-3, 4-4). The high cover of vascular plants may limit *Sphagnum* by light competition (Malmer et al. 2003, Limpens et al. 2011). However, our results showed a higher cover (Figure 4-1a), lower height reduction (Figure 4-1b), more significant bulk density (Figure 4-1c), and lower significant biomass losses of *Sphagnum* mosses (Figure 4-1d) when all vascular plants were presented. Our extended relative interaction index analysis indicated that vascular plants facilitated *Sphagnum* mosses (Figure 4-2) in nitrogen addition treatments. These findings indicated that vascular plants benefitted *Sphagnum* mosses, although the vascular plant cover was significantly increased after adding nitrogen availability. Vascular plants could absorb an amount of available nitrogen, leading to a lower available nitrogen concentration of porewater samples in plots without vascular plant removal than in vascular plant removal plots after adding nitrogen (Table 4-3). This vascular plant performance could be a primary mechanism that reduces the harmful effects of high-dose nitrogen addition on *Sphagnum* mosses. These results revealed that the increase of vascular plants postponed the adverse effects of high-dose nitrogen load by reducing available nitrogen in boreal peatlands.

More importantly, we discovered that vascular plant removal regulated the combined effect of warming and nitrogen addition on the growth of *Sphagnum* mosses (Table 4-1). The combined effects of enriched nitrogen and warmer conditions on the *Sphagnum* in boreal peatlands are understudied. Gunnarsson et al. (2004) showed a significant decrease in *Sphagnum* production within plots where both N and W were applied, which was also detected by Breeuwer et al. (2009). However, these studies did not consider the effects of vascular plants that have been recognized as a key meditate effect of N and W on *Sphagnum* mosses (Berendse et al. 2001, Heijmans et al. 2002a, Van der Wal et al. 2005, Heijmans et al. 2008, Jassey et al. 2013, Dieleman et al. 2015, He et al. 2016, Küttim et al. 2020). Our results revealed that removing all vascular plants significantly enhanced the negatively combined effects of both warming and nitrogen addition on height and biomass production of the *Sphagnum* (Table 4-1, Figure 4-1b, 4-1d). In the warmer and enriched available nitrogen conditions, vascular plants may limit *Sphagnum* mosses due to light competition (Table 4-3). However, the vascular plants also reduced available nitrogen concentration (Table 4-3), which mitigated the adverse effects of high-dose nitrogen load on the *Sphagnum* (Figure 4-1). Taken together, vascular plants facilitated the growth of the *Sphagnum* mosses in warmer and increased available nitrogen conditions (Figure 4-1, 4-2). This finding is consistent with previous studies suggesting that plants usually tend to be facilitative under environmental stresses, including water, nutrients, light, salinity, cold, and wind exposure (Bruno et al. 2003, Lortie and Callaway 2006, Holmgren and Scheffer 2010, He et al. 2013). However, the facilitation from vascular plants is insufficient to eliminate the detrimental effects of both warming and nitrogen addition treatments on the *Sphagnum*.

The significant interaction effects of warming, nitrogen addition, and vascular plant removal suggest that projections modeling effects of warmer and/or nitrogen deposition increases on boreal peatland *Sphagnum* without considering interaction with vascular plants remain uncertain. Specifically, the effects of warming on *Sphagnum* may be underestimated when the projections do not involve intense competition from vascular plants. By contrast, the prediction of detrimental effects of high dose nitrogen load or effects of both warmer and available nitrogen increase on the boreal peatland mosses can be overestimated without factoring in the facilitation of vascular plants. Therefore, any model projecting responses of existing *Sphagnum* to climate warming and/or nitrogen deposition increase in boreal peatlands should consider the interaction from vascular plants.

4.6. Conclusion

Our findings revealed evidence that warming and nitrogen addition altered interactions of the vascular plants with *Sphagnum*, which regulated the effects of these factors on *Sphagnum*. Adverse effects of warmer conditions on the *Sphagnum* were strengthened with intense competition from vascular plants. Meanwhile, the facilitation by vascular plants reduced the harmful effects of high dose nitrogen load on the *Sphagnum* mosses. The vascular plant facilitation also mitigated a rapid decline of the boreal peatland *Sphagnum* under warmer and high doses of nitrogen addition conditions. While further experiments are required to examine responses of *Sphagnum* to multiple levels of warming, nitrogen addition, and their combinations to examine the dynamics of *Sphagnum* mosses to many scenarios of environmental changes, our results highlight the crucial role of vascular plants in regulating the effects of environmental changes in *Sphagnum* mosses in boreal peatlands.

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Chapter 5 Long-term warming and nitrogen addition regulate responses of dark respiration and net photosynthesis in boreal bog plants to short-term increases in CO_2 and temperature

5.1. Abstract

Boreal bog plants adapted to low temperatures and low available nutrients might be strongly affected by global changes, including elevated CO_2, warming (W), and increasing nitrogen (N) availability. Here, we examined responses of dark respiration (R_d) and net photosynthesis (A_{net}) in four dominant bog plants to five levels of short-term elevations of both CO_2 and temperature (eCT); and the effects of long-term (7 years) W and N addition on these responses. Results indicated that eCT increased R_d, meanwhile the eCT decreased A_{net} in the boreal bog plants. Long-term N addition simulated the R_d increases and the A_{net} decreases in one graminoid, while W mitigated the R_d increases in two shrubs and the A_{net} decreases in one shrub. These findings highlight the importance of long-term W and N addition in regulating responses of boreal bog plants to short-term increases in both CO_2 and temperature, suggesting the necessity to investigate long-term effects when projecting responses of boreal bog vegetation to global changes.

5.2. Introduction

Photosynthesis and respiration are key physiological processes that regulate the carbon balance of individual plants, ecosystems, and the global carbon cycle (Tjoelker et al. 1999, Reich et al. 2016). Both are strongly affected by global warming resulting from increasing levels of atmospheric CO_2 and other greenhouse gas concentrations (Tjoelker et al. 1999, Kirschbaum 2004, Mathur et al. 2014, Tkemaladze and Makhashvili 2016). Therefore, the responses of physiological processes to global warming should be viewed in direct relation to elevated CO_2 (eCO_2) conditions (Hamilton III et al. 2008, Albert et al. 2011, Bu et al. 2011b, Li et al. 2013b, Dusenge et al. 2019). The photosynthesis and the respiration of plants are also greatly influenced by the soil environment, particularly its composite nutrients (Ainsworth and Rogers 2007, Bubier et al. 2011, Liang et al. 2020). These may be strongly influenced by high nitrogen deposition (Galloway et al. 2008, Leeson et al. 2017, Le et al. 2021). Hence, the effects of the concomitant increase in atmospheric CO_2 and air temperature on plant photosynthesis and respiration should be examined in evolving soil environments (Kirschbaum 2004, Ainsworth and Rogers 2007, Wang et al. 2012, Dusenge et al. 2019).

Boreal bogs are crucial ecosystems in the global carbon cycle (Bu et al. 2011b, Frolking et al. 2011), storing approximately 500 Gt carbon in belowground layers (Loisel et al. 2014). Plant photosynthesis and respiration levels are low in "harsh" environments, especially in ombrotrophic bogs containing permanent water saturation, low temperature, low pH, and deficient nutrients (Gorham 1991, Joosten and Clarke 2002, Page and Baird 2016). Long-adapted to such environments, plants in these unique ecosystems are susceptible to predicted environmental changes, including global warming (Walker et al. 2015) and increasing levels of available nitrogen concentration (Bobbink et al. 1998a). Bubier et al. (2011) indicated the physiological stress of boreal bog plants under conditions of high N accumulation. Reich et al. (2016) showed strong acclimation of respiration to warming in five boreal plants. Ward et al. (2019) found species-specific responses of photosynthesis and dark respiration in boreal bog plants to the combination of warming and elevated CO_2

concentration. However, the responses of photosynthesis and respiration of dominant boreal bog plants to accompanying increases in atmospheric CO_2 concentration and temperature under the enriched available soil nitrogen have not been studied.

In response to this knowledge gap, we designed a full factorial experiment including warming and nitrogen addition in 2014 at a typical boreal bog in Western Newfoundland, Canada. By utilizing portable gas exchange analyzers (Li-6400 XT), the experiment enabled us to investigate responses of A_{net} and R_d in four dominant species (i.e., one *Sphagnum* moss, two shrubs, and one graminoid species) to the short-term concomitant eCO_2 and air temperature increases after the plants were exposed to the long-term (7 years) warming and enriched nitrogen conditions. We hypothesize that: 1) Short-term eCT will increase the R_d in these boreal bog plants, and the positive effects of the short-term eCT on R_d will be accelerated by long-term N addition, meanwhile, be reduced by warming; 2) the short-term eCT will increase A_{net} in vascular plants (two shrubs and one graminoid), the positive effects of eCT on A_{net} will be stimulated by long-term warming and N addition; 3) the short-term eCT will decrease A_{net} in *sphagnum*, the responses of A_{net} in the moss will be reduced by the long-term exposure to warming and be stimulated by long-term N addition.

5.3. Materials and methods

5.3.1. Study site

The study site is situated at an ombrotrophic blanket bog located in Robinsons, western Newfoundland, Canada (48°15′44″ N, 58°40′03″ W). The last 30-year annual air temperature and precipitation were approximately 5 °C and 1340 mm, respectively (Gong et al. 2019). In the 2020 growing season (from May 1 to September 30), the mean air temperature was 12.9 ^{o}C, and the total precipitation was 286.2 mm, respectively. The bog has a 3 m depth peat with a pH of 4.5 in underground water (Gong et al. 2019). The study site is a typical boreal bog in Newfoundland, where a bryophyte layer of *Sphagnum* mosses dominates vegetation. The vascular plant community consists of dwarf shrubs (*Andromeda glaucophylla, Chamaedaphne calyculata, Gaylussacia bigeloviana, Vaccinium oxycoccos, Gaylussacia baccata, Rhododendron tomentosum, and Rhododendron groenlandicum*), and graminoids (*Rhynchospora alba, Trichophorum cespitosum*).

5.3.2. Experimental design

The experiment was conducted in 2014 and was described by Gong et al. (2019). Specifically, four different treatments: Control (C), nitrogen fertilization (N), warming (W), and a combination of nitrogen fertilization and warming (NW), were randomly arranged in 3 blocks with 4 plots in each block. Each plot covers an area of 4 m^2 (2 m × 2 m) and is separated by 2m buffer zones.

To simulate warming, we used open-top chambers (OTCs) composed of six glass sheets (80 cm along the bottom edge, 62.5 cm along the top edge, 40 cm in height) to cover an area of 1.66 m^2. The warming treatments increased the average daily air temperature during growing seasons by 2.10 and 2.12 ^{o}C in 2019 and 2020, respectively.

To enrich the available nitrogen in N and NW treatments, we applied two doses of 3.2 g N m^{-2} in early June and July, each year from 2014 to 2018. In 2019 and 2020, four doses of 1.6 g N m^{-2} were applied monthly from June to September. The applications added 6.4 g N m^{-2} y^{-1} to the N and NW treatments. Fertilizer was given in the soluble form (NH_4NO_3)

dissolved in 2 L of same site pool water. The C and W treatments were also watered by 2 L of water taken from the same pool at the same site without the nitrogen addition.

5.3.3. Foliar photosynthesis and dark respiration measurements

We selected four dominant plant species, including *Sphagnum fuscum* (*S. fuscum*), *Trichophorum cespitosum (T. cespitosum*), *Andromeda glaucophylla* (*A. glaucophylla*), and *Gaylussacia bigeloviana* (G. *bigeloviana*). In each plot, two average and healthy individuals of *A. glaucophylla* and *G. bigeloviana*, one tussock of *T. cespitosum,* and 1 point of *S. fuscum* were labeled for 2-time gas exchange measurements when the leaves of the species were mature. In each plot of this experiment, one leaf of *G. bigeloviana*, 3-5 leaves of *A. glaucophylla*, and six leaves of *T. cespitosum* from the selected individuals /tussock were used to measure A_{net} and R_d, without cutting. In addition, six capitula (1cm from the top) of *S. fuscum* were cut from the labeled point, and these samples were used for A_{net} and R_d measurements in the moss.

To measure the foliar gas exchange in vascular species (*A. glaucophylla*, *G. bigeloviana*, and *T. cespitosum*), we used a Li-6400 XT (Li-COR Biosciences, Lincoln, NE, USA) with a red-blue light-emitting diode (LED) light source (6400-02B) and the standard 2 cm × 3 cm chamber. A bryophyte chamber (6400-24) with a light source (6400-18A RGB) was used to measure A_{net} and R_d of *S. fuscum*. To examine the responses of A_{net} and R_d to the eCT in the plants, we set up five-chamber conditions: C400T0, C421T1.5, C538T2.3, C670T3.3, and C936T5.2. C400T0 indicates a present condition of CO_2 (400 ppm) and temperature at the beginning of measurement in control plots (T_{oc}), C421T1.5 (CO_2:421 ppm, T: T_{oc}+ 1.5°C), C538T2.3 (CO_2: 538 ppm, T: T_{oc} +2.3), C670T3.3 (CO_2: 670 ppm, T:

T_{oc} +3.3), C936T5.2 (CO_2: 936 ppm, T: T_{oc} +5.2) indicate the climate change scenarios: RCP2.6, RCP4.5, RCP6.0, and RCP8.5, respectively in the year 2100 at this study are (Meinshausen et al. 2011, Zhang et al. 2019). Other conditions, such as photosynthetically active radiation (PAR) and relative humidity (RH), were constantly controlled during the leaf gas exchange measurements. Light saturation conditions (1500 µmol photons.m^{-2}. s^{-1} in PAR) and 55-65% RH were stably controlled during A_{net} and R_d measurements in vascular plants. A lower PAR (500 µmol photons.m^{-2}. s^{-1}) and a higher RH (65-75%) were established during the A_{net} and R_d measurements in *S. fuscum*.

A_{net} was measured in the order of C400T0, C421T1.5, C538T2.3, C670T3.3, C936T5.2, and R_d measurement occurred at least 5 min after switching the light source off. Data was logged when reference CO_2, chamber air temperature, and gas exchange levels were stabilized (CV<1% over 20 s). After the measurements, the vegetation samples were collected and dried at 60 °C until the constant weight was used in computing A_{net} and R_d. These gas exchange measurements were conducted in two periods of the growing season, including the mid-growing season (from late July to early August) and the late-growing season (from late August to early September), when the plant leaves were mature.

5.3.4. Environmental measurements

Air temperature (T_{air}) at the canopy level in each plot was measured at the beginning of A_{net} measurements. During the A_{net} and R_d measurement, a ProCheck sensor (Decagon Devices Inc., USA) was used to measure soil moisture (M_{soil}) and soil temperature (T_{soil}) at 5 cm depth. Also, one MacroRhizon sampler (Rhizosphere Inc., Netherlands) and a perforated PVC tube (sealed in the bottom permanently and capped at the top) were installed in each plot to collect soil pore water at 10 cm and 40 cm depths, respectively. In each A_{net} and R_d measurement sequence, the soil water samples were collected twice (the first collection two weeks before and the second one during the sequence). The water samples were filtered through 0.45-µm membranes before analyzing NH_4^+ and NO_3^- concentration utilizing flow injection analysis methods (Lachat Instruments, Inc. USA).

5.3.5. Statistical analysis

Data were processed and analyzed by R software ver. 3.6.3 (R Core Team 2020). The effects of eCT, N, W, and their interactions on A_{net} and R_d of 4 dominant boreal bog plants during the two periods of the growing season were analyzed utilizing ANOVA as mixed effect models, with experimental blocks as a random effect in *nlme* package (Pinheiro et al. 2018). The data were tested for normality, and Johnson transformation was used where necessary before analysis. Adjusted R^2 of the models was computed by the *rsq* package (Zhang 2020). To test the effects of eCT levels on A_{net} and R_d, a posthoc analysis was conducted by Tukey test in *multcomp* package (Hothorn et al. 2008). Mixed effect models were also used to examine the effects of nitrogen addition and W on the total cover of vegetation and environmental variables with experimental blocks as a random effect in the two periods of the growing season. All figures were created using *ggplot2* (Wickham 2016) and *ggpubr* (Kassambara 2020) packages.

5.4. Results

5.4.1. The response of dark respiration

The eCT significantly affected the R_d in four dominant boreal bog plant species (*A. glaucophylla*, *G. bigeloviana*, *S. fuscum*, and *T. cespitosum)* during both mid and late-growing seasons ($p<0.0001$) (Table 5-1). The rise of eCT from C400T0 to C421T1.5 increased R_d in *A. glaucophylla* by 27% during the late-growing season (Figure 5-1h), while this change of eCT did not affect R_d in *A. glaucophylla* during the mid-growing seasons (Figure 5-1g) and R_d in other plants during both mid-and late-growing seasons (Figure 5-2g, 5-2h, 5-3g, 5-3h, 5-4g, 5-4h). Rd in the four boreal bog plants dramatically increased when the eCT increased to C538T2.3, C670T3.3, C936T5.3 with an average of approximately 1.5 times, 3.2 times, 7.6 times, respectively.

Moreover, R_d increases due to the eCT were regulated by N and W (Table 5-1). A significant interactive effect ($F_{1,38}=4.1$, $p=0.0071$) between N and eCT on R_d was detected in *T. cespitosum* during the mid-growing season when N accelerated the increases R_d due to C670T3.3 and C936T5.2 by 30%, 25%, respectively (Figure 5-4g). In contrast, W reduced the positive effects of eCT on R_d in *A. glaucophylla* at mid-growing season ($F_{4,38}=7.9$, $p=0.0076$) and at *G. bigeloviana* in the late growing season ($F_{4,38}=10.6$, $p=0.0045$). In detail, W dampened the increase of R_d in *A. glaucophylla* in C670T3.3 by 23% and C936T5.2 by 30% during the mid-growing season (Figure 5-1g). W also modified the effects of eCT on R_d in *G. bigeloviana* during the late growing season in the C670T3.3 and C936T5.2 conditions with 29%, 30% decreases, respectively (Figure 5-2h).

Table 5-1. Summary of models of elevated CO_2 and temperature (eCT), warming treatment (W) and nitrogen fertilizer addition (N), and their combined effects on dark foliar respiration (R_d) in four dominant plant species during the mid and late-growing season at a boreal bog.

		R_d (µmol. kg $^{-1}$. s $^{-1}$)			
Source of variation	df	Mid growing season		Late growing season	
		F	P	F	P
A. glaucophylla (n=60)					
eCT	4	91.3	<.0001	297.3	<.0001
N	1	0.2	0.6508	9.4	0.0039
W	1	2.1	0.1520	10.7	0.0023
eCT x N	4	1.2	0.3380	0.7	0.6025
eCT x W	4	2.7	0.0450	0.2	0.9565
N x W	1	7.9	0.0076	0.3	0.5716
eCT x N x W	4	0.7	0.6088	0.1	0.9773
Full model adjusted R^2		0.864		0.953	
G. bigeloviana (n=60)					
eCT	4	77.7	<.0001	133.5	<.0001
N	1	2.2	0.1453	0.1	0.8215
W	1	2.2	0.1506	22.1	<.0001
eCT x N	4	1.4	0.2566	0.3	0.8771
eCT x W	4	0.8	0.5088	4.5	0.0045
N x W	1	10.6	0.0024	1.5	0.2352
eCT x N x W	4	1.9	0.1275	0.7	0.5943
Full model adjusted R^2		0.847		0.907	
S. fuscum (n=60)					
eCT	4	44.9	<.0001	73.5	<.0001
N	1	2.8	0.1044	7.4	0.0099
W	1	1.2	0.2721	2.0	0.1699
eCT x N	4	0.1	0.9948	0.2	0.9194
eCT x W	4	0.1	0.9929	0.4	0.8217
N x W	1	95.8	<.0001	2.5	0.1258
eCT x N x W	4	0.1	0.9827	0.3	0.878
Full model adjusted R^2		0.816		0.831	
T. cespitosum (n=60)					
eCT	4	229.0	<.0001	96.4	<.0001
N	1	11.2	0.0018	10.0	0.0031
W	1	1.7	0.2052	0.1	0.7916
eCT x N	4	4.1	0.0071	0.2	0.9551
eCT x W	4	0.5	0.7030	0.4	0.8265
N x W	1	9.6	0.0037	7.1	0.0115
eCT x N x W	4	0.1	0.9723	1.7	0.1766
Full model adjusted R^2		0.941		0.870	

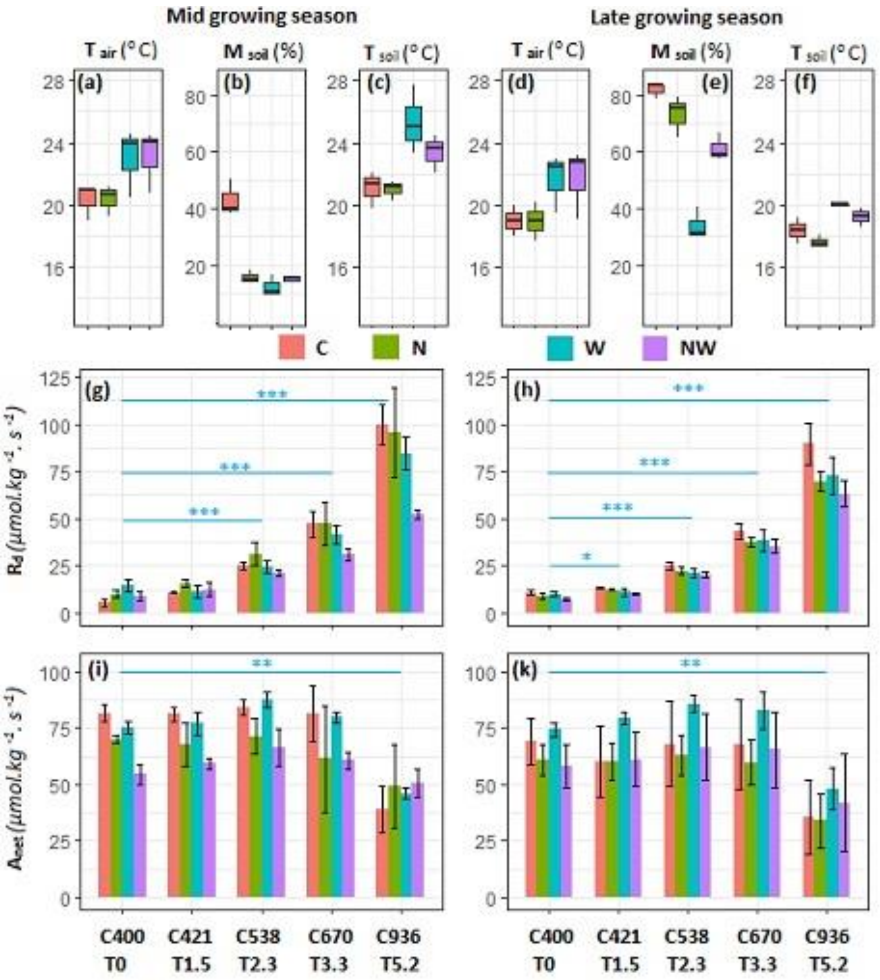

Figure 5-1. The dark respiration (R_d) (g,h) and net photosynthesis (A_{net}) (i,k) of *A. glaucophylla* in the elevated CO_2 and air temperature conditions (eCT) under simulated future scenarios for the year 2100 in the Control (C-red bars), N addition (N-*green bars*), warming (W-blue bars) and both N addition and warming (NW-purple bars) plots. Error bars represent the standard error of the mean (n = 3). Stars indicate the p-value of the Tukey test for significant differences of R_d and A_{net} between levels of eCT (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$). Box plots show environmental variables, including air temperature (T_{air}), soil moisture (M_{soil}), and soil temperature (T_{soil}) during the mid (a,b,c) and late (d,e,f) growing season.

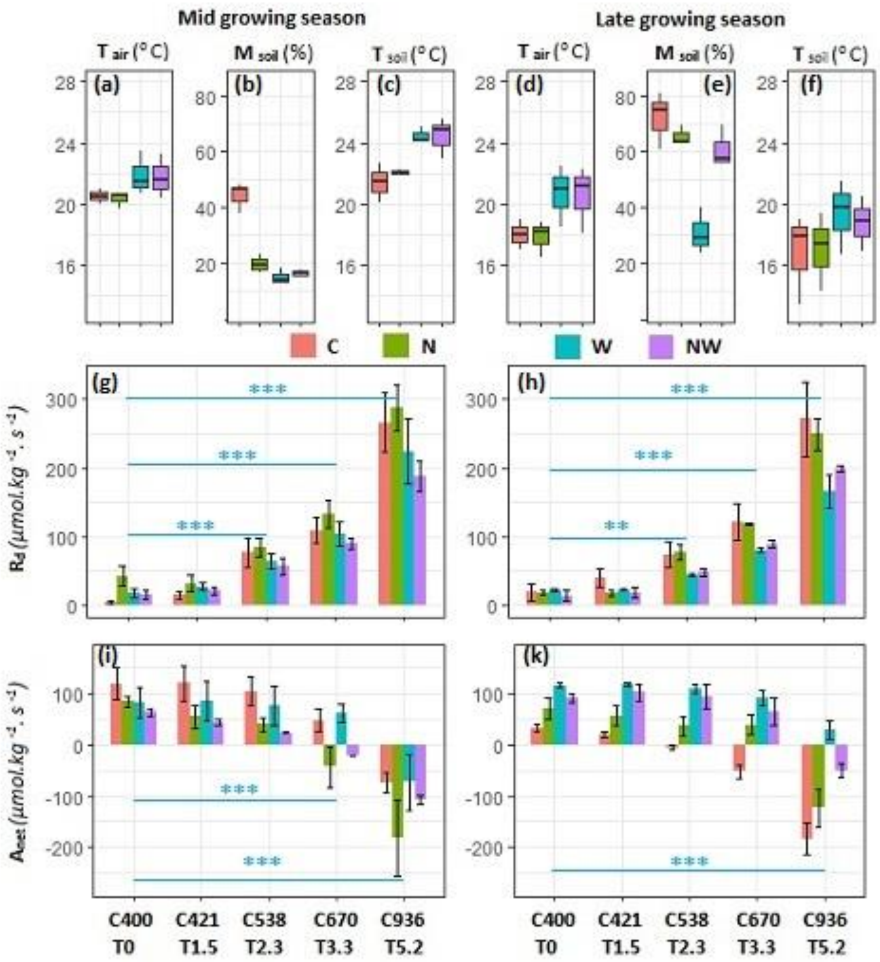

Figure 5-2. The dark respiration (R_d) (g, h) and net photosynthesis (A_{net}) (i, k) of *G. bigeloviana* in the elevated CO_2 and air temperature conditions (eCT) under simulated future scenarios for the year 2100 in the Control (C-red bars), N addition (N-*green bars*), warming (W-blue bars) and both N addition and warming (NW-purple bars) plots. Error bars represent the standard error of the mean (n = 3). Stars indicate the p-value of the Tukey test for significant differences of R_d and A_{net} between levels of eCT (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$). Box plots show environmental variables, including air temperature (T_{air}), soil moisture (M_{soil}), and soil temperature (T_{soil}) during the mid (a, b, c) and late (d, e, f) growing season.

5.4.2. The response of Net photosynthesis

Results show that A_{net} of 4 dominant boreal bog plants was significantly influenced by eCT during mid-and late-growing seasons ($p<0.0001$) (Table 5-2). A_{net} of the plants had no change when eCT increased from C400T0 to C421T1.5 (Figure 5-1, 5-2, 5-3, 5-4). The increase of eCT to C538T2.3 only decreased the A_{net} of *S. fuscum* by 2.3-times during the late growing season (Figure 5-3k). C670T3.3 decreased A_{net} in all plants (Figure 5-2i, 5-3i, 5-3k, 5-4i, 5-4k), except *A. glaucophylla* during both periods of the growing season (Figure 5-1i, 5-1k) and *G. bigeloviana* during the late-growing season (Figure 5-2k). The lowest values of A_{net} were observed at C936T5.2 in all plants (Figure 5-1, 5-2, 5-3, 5-4). Of the four plants, S. *fuscum* showed the most significant A_{net} decreases due to eCT, with 3-times and 9-times lower at C936T5.2 than C400T0 during the mid and late-growing seasons, respectively (Figure 5-3i, 5-3k). By contrast, A. *glaucophylla* had the smallest decreases of A_{net} with 34% and 39% decreases at the highest level of eCT during the mid and late-growing seasons, respectively (Figure 5-1i, 5-1k).

Table 5-2. Summary of models of elevated CO_2 and temperature (eCT), warming treatment (W) and nitrogen fertilizer addition (N), and their combined effects on foliar net photosynthesis (A_{net}) in four dominant plant species during the mid and late-growing seasons at a boreal bog.

		A_{net} (µmol. kg^{-1}. s^{-1})			
Source of variation	df	Mid growing season		Late growing season	
		F	P	F	p
A. glaucophylla (n=60)					
eCT	4	8.4	0.0001	4.6	0.0042
N	1	12.2	0.0013	3.6	0.0649
W	1	1.2	0.2783	2.6	0.1153
eCT x N	4	1.9	0.1327	0.1	0.9824
eCT x W	4	0.4	0.7928	0.1	0.9739
N x W	1	0.6	0.4276	1.0	0.3174
eCT x N x W	4	0.0	0.9978	0.0	0.9952
Full model adjusted R^2		0.429		0.185	
G. bigeloviana (n=60)					
eCT	4	31.7	<.0001	53.4	<.0001
N	1	34.4	<.0001	1.6	0.2097
W	1	1.7	0.1955	114.7	<.0001
eCT x N	4	0.8	0.5616	0.7	0.5983
eCT x W	4	1.2	0.3093	3.5	0.0166
N x W	1	0.8	0.3658	27.8	<.0001
eCT x N x W	4	0.1	0.9853	1.2	0.3333
Full model adjusted R^2		0.751		0.859	
S. fuscum (n=60)					
eCT	4	68.0	<.0001	56.0	<.0001
N	1	6.8	0.0129	16.1	0.0003
W	1	0.4	0.5261	0.7	0.4037
eCT x N	4	0.1	0.9816	1.1	0.3879
eCT x W	4	0.2	0.9322	0.5	0.7114
N x W	1	116.2	<.0001	1.1	0.3114
eCT x N x W	4	1.2	0.3294	1.1	0.3873
Full model adjusted R^2		0.867		0.799	
T. cespitosum (n=60)					
eCT	4	99.1	<.0001	50.3	<.0001
N	1	30.0	<.0001	2.8	0.1026
W	1	8.9	0.0049	9.7	0.0035
eCT x N	4	5.3	0.0017	2.3	0.0755
eCT x W	4	2.3	0.0736	0.7	0.5730
N x W	1	0.0	0.962	0.0	0.8582
eCT x N x W	4	1.1	0.388	4.5	0.0045
Full model adjusted R^2		0.889		0.792	

Moreover, we found that long-term N and W regulated the responses of A_{net} to eCT (Table 5-2). N accelerated the decreases in A_{net} of *T. cespitosum* by 34% and 95% during the mid-growing season when eCT increased to C670T3.3 and C936T5.2, respectively (Figure 5-4i). By contrast, W reduced the decrease of A_{net} in *G. bigeloviana* due to C936T5.2 by 93% during the late-growing season (Figure 5-2k).

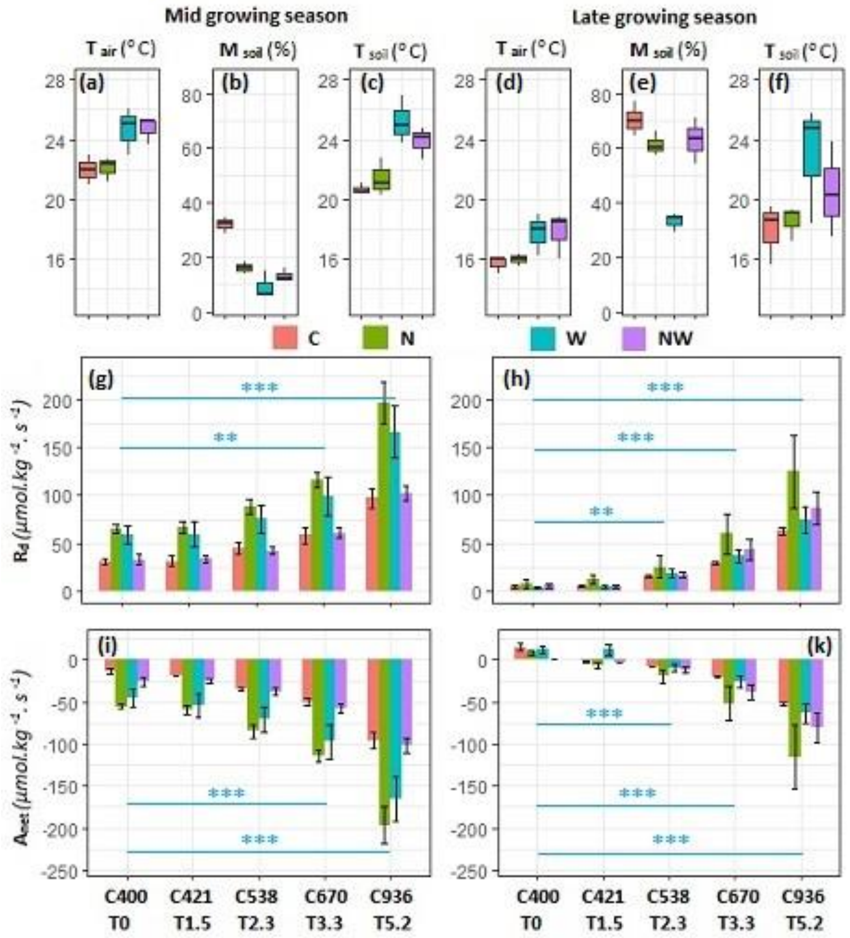

Figure 5-3. The dark respiration (R_d) (g, h) and net photosynthesis (A_{net}) (i,k) of *S. fuscum* in the elevated CO_2 and air temperature conditions (eCT) under simulated future scenarios for the year 2100 in the Control (C-red bars), N addition (N-*green bars*), warming (W-blue bars) and both N addition and warming (NW-purple bars) plots. Error bars represent the

standard error of the mean (n = 3). Stars indicate the p-value of the Tukey test for significant differences of R_d and A_{net} between levels of eCT (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$). Box plots show environmental variables, including air temperature (T_{air}), soil moisture (M_{soil}), and soil temperature (T_{soil}) during the mid (a, b, c) and late (d, e, f) growing season.

Finally, a combined effect of N and W on the responses of A_{net} to eCT was found *in T. cespitosum* during the late-growing season ($F_{4,38}$=4.5, $p<0.01$). W reduced the negative effect of nitrogen addition on A_{net} in *T. cespitosum* due to C936T5.2 during the late-growing season (Figure 5-4k).

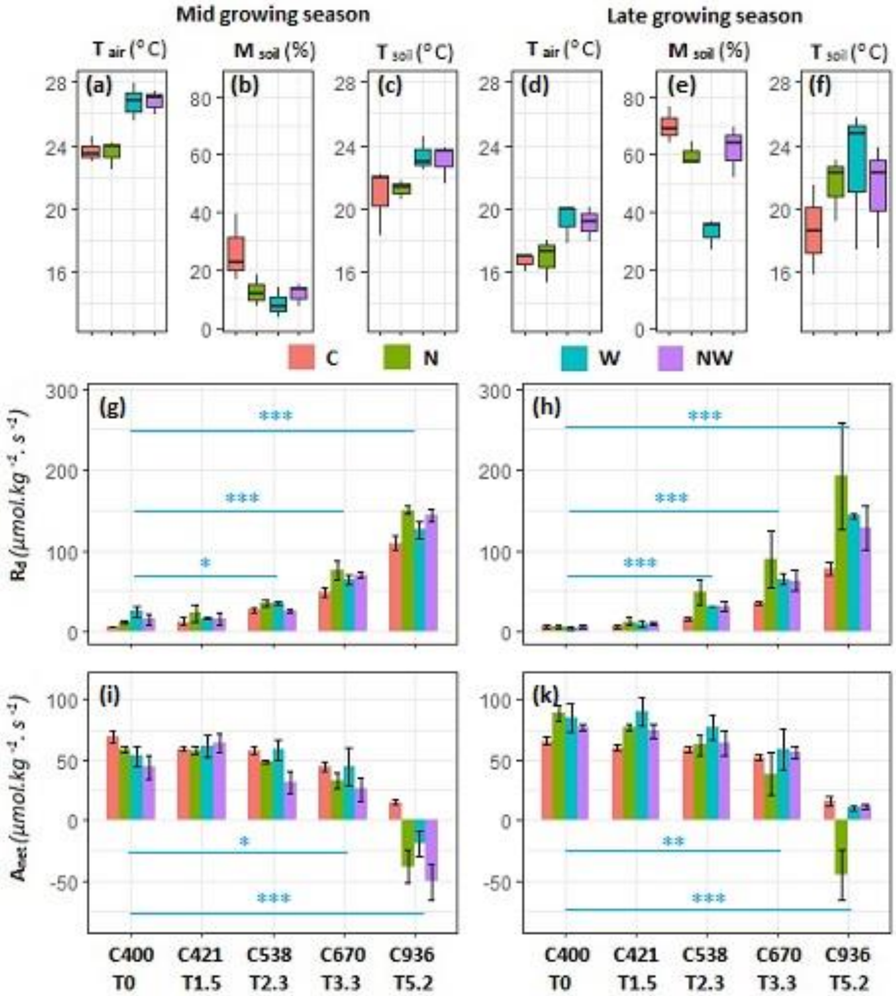

Figure 5-4. The dark respiration (R_d) (g, h) and net photosynthesis (A_{net}) (i, k) of T. cespitosum in the elevated CO_2 and air temperature conditions (eCT) under simulated future scenarios for the year 2100 in the Control (C-red bars), N addition (N-green bars), warming (W-blue bars) and both N addition and warming (NW-purple bars) plots. Error bars represent the standard error of the mean (n = 3). Stars indicate the p-value of the Tukey test for significant differences of R_d and A_{net} between levels of eCT (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$). Box plots show environmental variables, including air temperature (T_{air}), soil moisture (M_{soil}), and soil temperature (T_{soil}) during the mid (a, b, c) and late (d, e, f) growing season.

5.4.3. Effects of warming and nitrogen addition treatment on environments

W significantly affected T_{air}, M_{soil}, T_{soil} during both the mid-growing season and late-growing seasons ($P<0.001$), while the warming did not influence NH_4^+ and NO_3^- in soil water samples (Table S 5-1). Specifically, mean T_{air} was higher at the warming treatment plots than at the control plots by approximately 3.2 ^{o}C during the mid-growing season and by 2.5 ^{o}C during the late-growing season (Table 5-3). Mean T_{soil} was higher at the warming treatment plots than at the control plots by approximately 3.6 ^{o}C during the mid-growing season and by 3.3 ^{o}C during the late-growing season (Table 5-3). Mean M_{soil} was 11% during the mid-growing season and 33% during the late-growing season at the warming treatment plots, which were dramatically lower than in the control plots with 36% and 74% during the mid and late-growing seasons, respectively (Table 5-3).

Table 5-3. Air temperature (T air), soil moisture (T air) and soil temperature (T air) in Control (C), nitrogen addition (N), warming (W), both warming and N addition (WN) treatments in the mid- growing season (Mid-season) and late-growing season (Late-season).

Treatment	**T_{air}** (^{o} C)		**M_{soil}** (%)		**T_{soil}** (^{o} C)	
	Mid-season	Late-season	Mid-season	Late-season	Mid-season	Late-season
C	21.63±0.46	17.33±0.43	36.33±2.90	73.60±2.26	21.01±0.36	17.92±0.61
N	21.60±0.45	17.40±0.44	16.01±1.20	64.93±1.99	21.43±0.23	18.68±0.68
W	24.08±0.68	19.83±0.61	11.18±1.34	32.85±1.47	24.58±0.43	21.23±0.93
WN	24.09±0.66	19.77±0.64	14.11±0.81	61.68±1.90	23.69±0.33	19.95±0.68

Data are presented as mean ± standard error*($n=48$)*.

Nitrogen addition strongly influenced concentrations of NH_4^+ ($p<0.05$) and M_{soil} ($p<0.001$), while the treatment had no effects on NO_3^-, $T_{air,}$ or T_{soil} ($p>0.05$) (Table S 5-1). Mean concentration of NH_4^+ increased 3-times at 10cm depth and 2-times at 40 cm depth during both mid-and late-growing seasons (Table 5-4). M_{soil} in the N plots was significantly lower than in the control plots, especially during the mid-growing season with only 16% (Table 5-3).

Table 5-4. Ammonium (NH_4^+) and nitrate (NO_3^-) concentration in soil water at 10 cm and 40 cm depths in control (C), N addition (N), warming (W), and warming and N addition (WN) treatments in the mid- growing season (Mid-season) and late-growing season (Late-season).

Treatment	**NH_4^+** *(mg/L)*		**NO_3^-** *(mg/L)*	
	Mid-season	Late-season	Mid-season	Late-season
10cm depth				
C	0.366±0.043	0.384±0.080	0.025±0.008	0.190±0.130
N	0.982±0.156	1.054±0.355	0.036±0.006	0.511±0.470
W	0.454±0.125	0.220±0.063	0.031±0.014	0.056±0.032
WN	0.910±0.178	1.001±0.297	0.090±0.041	0.504±0.222
40cm depth				
C	0.637±0.134	0.642±0.161	0.038±0.011	0.028±0.010
N	1.250±0.156	1.087±0.133	0.020±0.007	0.018±0.003
W	0.579±0.064	0.680±0.203	0.056±0.019	0.021±0.006
WN	1.127±0.154	0.980±0.142	0.035±0.013	0.020±0.005

Data are presented as mean ± standard error *(n=24)*.

Table S 5-1. Statistical analysis for effects of N addition (N) and warming (W) on environmental variables during the mid-growing season (Mid-season) and late-growing season (Late-season).

Source of variation	df	Mid-season		Late-season	
		F	P	F	P
Air Temperature (°C) (n=48)					
W	1	20.5	<.001	30.3	<.001
N	1	0.0	0.994	0.0	1.000
W x N	1	0.0	0.970	0.0	0.881
Soil moisture (%) (n=48)					
W	1	78.4	<.001	140.7	<.001
N	1	32.4	<.001	29.5	<.001
W x N	1	57.9	<.001	102.2	<.001
Soil temperature (°C) (n=48)					
W	1	129.4	<.001	17.9	<.001
N	1	0.8	0.368	0.2	0.640
W x N	1	6.6	<.05	3.6	0.066
NH_4^+ at 10 cm depth (mg/L) (n=24)					
W	1	0.0	0.878	0.3	0.613
N	1	10.1	<.01	11.0	<.01
W x N	1	0.2	0.699	0.1	0.810
NO_3^- at 10 cm depth (mg/L) (n=24)					
W	1	1.5	0.249	0.1	0.801
N	1	1.5	0.249	2.1	0.167
W x N	1	0.5	0.507	0.1	0.815
NH_4^+ at 40 cm depth (mg/L) (n=24					
W	1	1.0	0.330	0.0	0.827
N	1	8.9	<.01	5.7	<.05
W x N	1	0.0	0.797	0.2	0.649
NO_3^- at 40 cm depth (mg/L) (n=24)					
W	1	1.3	0.265	0.2	0.692
N	1	2.0	0.179	0.9	0.358
W x N	1	0.0	0.922	0.9	0.359

We found the combined effects of W and N on M_{soil} during both mid and late-growing seasons ($p<0.001$), as well as on T_{soil} during the mid-growing season ($p<0.05$) (Table S 5-1). W enhanced the negative effects of N on soil moisture by 8% and 37% during the mid and late-growing seasons, respectively (Table 5-3). N reduced the increase of T_{soil} due to warming by 25% during the mid-growing season (Table 5-3).

5.5. Discussion

Boreal bogs have long been recognized as crucial ecosystems with a large carbon budget (Loisel et al. 2014) due to unique features such as low temperature and low available nutrients (Gorham 1991, Joosten and Clarke 2002, Page and Baird 2016). However, there is increasing evidence that boreal bogs are subject to elevated CO_2, warmer and nitrogen-enriched conditions due to climate changes (Collins et al. 2013, Stocker et al. 2014) and/or increasing nitrogen deposition (Matson et al. 2002, Galloway et al. 2008). These changes in CO_2, temperature, and nutrients may significantly affect these bogs (Bobbink et al. 1998a, Walker et al. 2015, Hedwall et al. 2017, Gong et al. 2021, Le et al. 2021). Here, we provide the evidence that short-term combined increases in CO_2 and temperatures increased R_d and decreased A_{net} in boreal bog plants, which were regulated by long-term warmer, nitrogen-enriched treatments.

5.5.1. Responses of dark respiration

Most previous studies have focused on the individual effects of temperature or eCO_2 on R_d, while the combined effects of these environmental changes on R_d are understudied (Dusenge et al. 2019). Regarding responses of R_d to eCO_2, previous studies have shown idiosyncratic results on the response of foliar R_d to eCO_2, including increases (Gonzàlez-Meler et al. 1996, Li et al. 2013a, Markelz et al. 2014); no effects (Amthor 2000, Tjoelker et al. 2001, Dusenge et al. 2020); and, decreases (Curtis 1996, Tjoelker et al. 2001, Ayub et al. 2014). Meanwhile, significant increases in R_d due to eT have been consistently observed in several previous experiments (Tjoelker et al. 1999, Slot and Kitajima 2015, Heskel et al. 2016, Aspinwall et al. 2017, Dusenge et al. 2020).

In this study, we did not examine the individual effects of eCO_2 and eT on R_d; instead, we tested the effects of the concomitant increase in CO_2 and temperature projected under future climate scenarios (Meinshausen et al. 2011, Zhang et al. 2019) in our study area. Results indicated that eCT significantly increases R_d in our dominant boreal bog plant species (Figure 5-1g, 5-1h, 5-2g, 5-2h, 5-3g, 5-3h, 5-4g, 5-4h, table 5-1). The findings are in line with previous studies that suggest eT increases R_d (Tjoelker et al. 1999, Slot and Kitajima 2015, Heskel et al. 2016, Aspinwall et al. 2017, Dusenge et al. 2020). These results indicate the possibility of temperature playing a dominant role in stimulating R_d under eCO_2, high-temperature conditions (Zha et al. 2003, Dusenge et al. 2019, Dusenge et al. 2020).

This study revealed that the increase of R_d due to eCT was regulated by long-term warming in two boreal bog shrubs, including *A. glaucophylla* during the mid-growing season and *G. bigeloviana* during the late-growing season (Table 5-1). Exposure to the 7-year warming treatment reduced the positive effects of eCT on R_d by approximately 23-30% in these boreal bog shrubs (Table 5-1) compared to the decrease by 80% at boreal and temperate species of Reich et al. (2016). Thermal acclimation of R_d to temperature increase (Tjoelker et al. 1999, Atkin and Tjoelker 2003, Smith and Dukes 2013, Heskel et al. 2014, Way and Yamori 2014, Slot and Kitajima 2015, Reich et al. 2016, Dusenge et al. 2020) might be a primary interpretation for this finding.

We also found that responses of R_d to eCT were modified by long-term N addition (Table 5-1). N addition accelerated the increases in R_d due to high levels of eCT in *T. cespitosum* by approximately 25-30% during the mid-growing season (Figure 5-4g). There is evidence to indicate the positive effects of leaf nitrogen concentration on R_d (Reich et al. 1998, Heskel et al. 2012, van de Weg et al. 2013) and on the response of R_d to eCO_2 (Shapiro et al. 2004, Markelz et al. 2014). The increases in mitochondrial density and size due to N addition (Heskel et al. 2012) may accelerate R_d under high eCT conditions.

5.5.2. Responses of net photosynthesis

The individual effects of eCO_2 and/or eT on A_{net} are well documented. The increases in A_{net} due to eCO_2 have been reported in previous studies (Curtis 1996, Gunderson et al. 2002, Ainsworth and Rogers 2007, Ellsworth et al. 2012, Li et al. 2013b, Ayub et al. 2014). Also, many recent studies have indicated that temperature increases lead to an improvement of A_{net} when increases are less than optimal (T_{opt}), while the increase of temperature reduces A_{net} if the increases are more than T_{opt} (Tjoelker et al. 1999, Medlyn et al. 2002, Kirschbaum 2004, Niu et al. 2008, Dusenge et al. 2020). However, the combined effects of eCO2 and eT on Anet are still unclear, with contradictory results reported from recent experiments (Dusenge et al. 2019). The increases (Teskey 1997, Lewis et al. 2001, Ghannoum et al. 2010, Wang et al. 2012), no effects (Wang et al. 1995, Ruiz-Vera et al. 2013, Lewis et al. 2015, Ward et al. 2019) or even decrease (Ward et al. 2019) of A_{net} due to combined effects of eCO_2 and high temperature have been observed.

Our results indicated that eCT decreased A_{net} in boreal bog vascular plants and *Sphagnum* moss (Table 5-2). This finding did not support the hypothesis that eCT would increase A_{net} in vascular plants and decrease A_{net} in *Sphagnum*. In this study, substantial increases of R_d in the boreal bog plants due to high levels of eCT (C670T3.3 and C936T5.2) may partly explain the decrease in A_{net}. The decreases in A_{net} due to eCT in a boreal bog plant was also found by Ward et al. (2019), which indicated that eCT reduced A_{net} of C. *calyculata* by 50% when they examined the combined effects of eCO2 (+ 500 ppm) and eT (+2-8 ^{o}C) on A_{net} of the species. These findings indicate that high levels of eCT scenarios (RCP6.0 and RCP8.5) may reduce photosynthesis efficiency in existing boreal bog plants, at least in the short term.

We also showed that decreasing levels of A_{net} due to eCT are species-specific (Table 5-1). The substantial decrease of A_{net} in S. *fuscum* was found at the moderate level of eCT (C538T2.3) (Figure 5-3k). Meanwhile, decreases in A_{net} were only detected at higher eCT (C670T3.3, C936T5.2) in G. *bigeloviana*, T. *cespitosum* (Figure 5-2i, 5-2k, 5-4i, 5-4k), and only at C936T5.2 in A. *glaucophylla* (Figure 5-1i, 5-1k). These results suggest that S. *fuscum* was more sensitive to eCT than vascular plants. Being keystone species controlling the growth of other plants as well as vital biochemical processes in boreal bogs (Limpens and Berendse 2003, Turetsky et al. 2007, Turetsky et al. 2012a, Sheppard et al. 2013), the low efficient photosynthesis in *Sphagnum* under eCT conditions, even in scenarios of moderate CO_2 emission (RCP4.5 or C528T2.3 condition), may lead to significant changes in vegetation composition and vital ecosystem services in future.

This research also revealed that long-term N addition regulated the effect of eCT on A_{net}, wherein the decreases in A_{net} due to eCT in T. *cespitosum* was stimulated by N addition during the mid-growing season (Figure 5-4i, table 5-2). The intense stimulation of R_d due to N addition in *T. cespitosum* (Figure 5-4g) may partly explain the decrease of A_{net} in the plant species in the nitrogen addition plots (N and NW). In addition, the substantial decrease in soil moisture (Table 5-3, S 5-1) could be another reason for accelerating the negative effects of eCT on A_{net} in *T. cespitosum* in the nitrogen addition plots.

We also found that long-term warming reduced the negative effects of eCT on A_{net}, wherein the decreases in A_{net} due to high eCT (C936T5.2) in *G. bigeloviana* in long-term warming plots was 93% lower than in control plots during the late-growing season (Table 5-2, figure 5-2k). The thermal acclimation of A_{net} had been observed in several previous studies (Tjoelker et al. 1999, Niu et al. 2008, Chi et al. 2013, Way and Yamori 2014, Lombardozzi et al. 2015, Smith et al. 2016). In this study, the higher A_{net} in *G. bigeloviana* at warming plots was only detected during the late-growing season (Figure 5-3k) and not during the mid-growing season (Figure 5-3i). This finding may indicate that the thermal acclimation of the plant was only effective during the late-growing season. Dry condition (Figure 5-2b, Table 5-3) may be a major limiting factor of the thermal acclimation capacity of *G. bigeloviana* during the mid-growing season. Decreases in stomatal and mesophyll conductance photosynthesis due to low soil moisture conditions may explain the decrease of A_{net} (Cornic and Fresneau 2002, Flexas et al. 2006, Lawlor and Tezara 2009, Albert et al. 2011). Therefore, dry conditions may erase or limit the thermal acclimation capacity of boreal bog plants (Reich et al. 2018). The long-term warmer condition also mitigated the negative effect of long-term N addition on A_{net} in *T. cespitosum* during the late-growing season (Table 5-2, Figure 5-4k). The thermal acclimation after exposure to a 7 year-warming treatment may be a primary reason for this fiding.

These combined effects of eCT and long-term warming and N addition indicate that the long-term environmental treatments play crucial roles in regulating responses of R_d and

A_{net} in boreal bog plants to short-term combined increases in CO_2 and temperatures. However, the intensity and direction effects of long-term warming and N addition on boreal bog plant R_d and A_{net} might depend on levels of these treatments, that have not been shown in this study where the experiment only composited 2 levels of warming (Control vs. ~2 ^{o} C) and 2 levels of N addition (Control vs. 6.4 gN.m^{-2}. yr^{-1}). Thus, multiple levels of warming and N addition experiments are urgently required to determine the potential responses of R_d and A_{net} in boreal bog plants to the predicted scenarios of atmospheric CO_2, temperatures, and N deposition.

In this study, we did not examine the specific long-term effects of eCO_2 on R_d and A_{net} responses to eCT. However, there is evidence that long-term exposure to eCO_2 reduces the positive effects of short-term eCO_2 on A_{net} (Ainsworth et al. 2004, Erice et al. 2006, Ainsworth and Rogers 2007, Leakey et al. 2009, Albert et al. 2011). Therefore, the decreases in A_{net} due to eCT may be more considerable under the long-term increases in CO_2, temperature, and nitrogen-enriched conditions, leading to more severe impacts on boreal bog plants in the future. These factors indicate the need and direction for continued research in these and related areas.

5.6. Conclusion

This study provides evidence that combined increases in CO_2 and temperature can increase R_d and reduce A_{net} in four dominant boreal bog plants, at least in the short term. The short-term responses of boreal bog plants to combined increases in CO_2 and temperature are regulated by long-term N addition and warming treatments. Long-term N addition accelerated the increases in R_d and the decreases in A_{net} in one boreal bog graminoid;

meanwhile, long-term warming mitigated the increases in R_d in two bog shrubs and the decreases in A_{net} in one bog shrub. Although mechanisms that underlie our findings need further studies, we highlight the importance of long-term warming and nitrogen addition in regulating responses of boreal bog plants to combined increases in CO_2 and temperature. Thus, the long-term experiments composited multiple levels of CO_2, warming, and N addition are required to project the responses of boreal bog plants in the future.

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Chapter 6 Conclusions

This thesis provides a deeper understanding of functions and potential changes in boreal bog vegetation under global changing conditions (Figure 6-1).

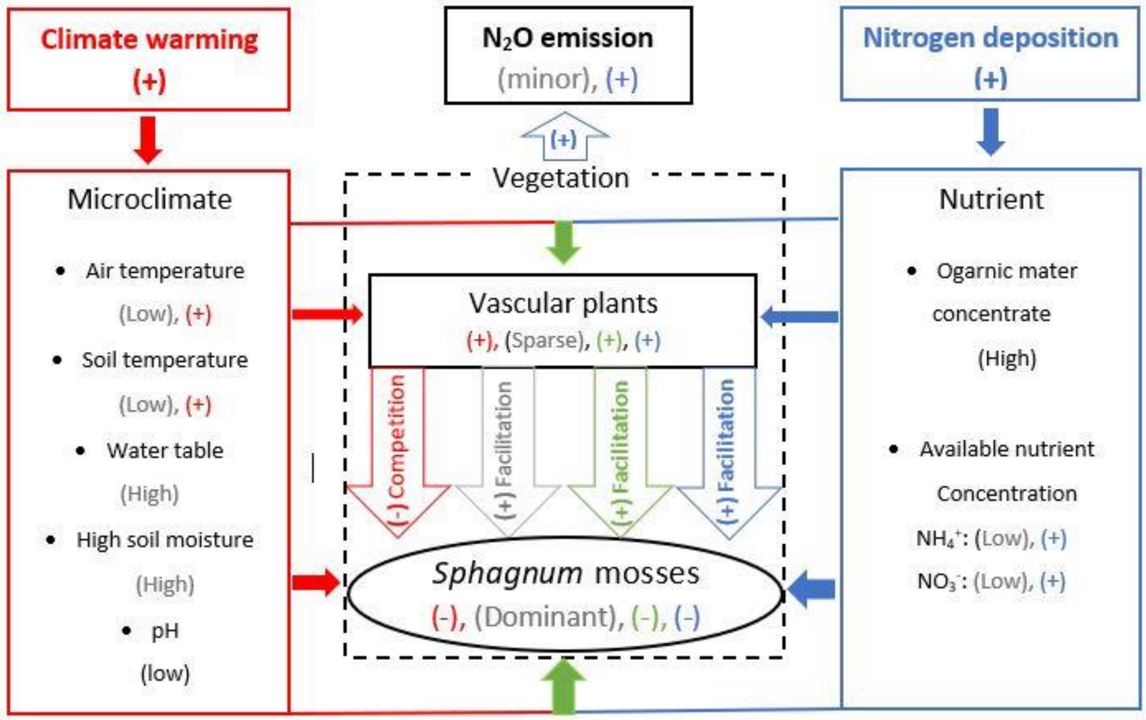

Figure 6-1. Dynamics of boreal bog vegetation due to warming (red), enriched nitrogen availability (blue), and their combination (green) and the function of boreal bog vegetation in N_2O emission. The grey color indicates the boreal bog characteristics without disturbances.

This study indicates that vascular plants, especially graminoids, grow better under warmer and nitrogen-enriched conditions. By contrast, these environmental changes resulted in rapid losses of *Sphagnum* in boreal bogs. The responses of *Sphagnum* to warmer, nitrogen-enriched conditions are regulated by vascular plants. In the warmer and nitrogen-enriched conditions, vascular plants postponed the *Sphagnum* losses; however, the facilitation from vascular plants could not eliminate the harmful effects of these environmental changes on the existing boreal bog mosses. This study also highlights the crucial importance of long-term warming and N addition on regulating responses of boreal bog plants in dark respiration and net photosynthesis to short-term increases in both CO_2 and temperature.

This study also reveals that graminoids accelerate increases in N_2O emission due to N addition. This finding suggests that boreal bogs can become hotpots of N_2O emissions under elevated nitrogen availability if boreal bog vegetation switches from the dominance of *Sphagnum* to more abundance of vascular plants, especially graminoids.

These findings provide evidence that vegetation and services of boreal bogs are sensitive to warmer and nitrogen-enriched conditions. Therefore, mitigations of these environmental changes need to be considered key countermeasures to manage and maintain the vital services of boreal bogs sustainably.

This study also provides evidence for the interactive effects of abiotic changes (i.e., CO_2, temperature, and nitrogen availability) and biotic changes (plant-plant interaction) on the dynamics of vegetation and ecosystem services in boreal bogs. It is therefore necessary to consider the combined effects of these changes when projecting responses of boreal bogs vegetation and services in the future. For example, the interaction with vascular plants should be accounted when projecting responses of *Sphagnum* to warmer, N-enriched conditions. The ignorance of interaction with vascular plants may lead to overestimating the losses of *Sphagnum* due to warmer, N- enriched treatments.

These significant dynamics of boreal bog vegetation due to warming and N addition are highlighted in a 7-year experiment. However, the boreal bog vegetation might continuously change and achieve a new equilibrium in the coming years. Therefore, it is necessary to investigate the dynamics of vegetation and services of boreal bogs over a more extended period. Moreover, this study did not investigate the effects of long-term exposure to elevated CO_2 concentration that might alter the responses of photosynthesis and respiration of boreal bog plants to concurrent increases in short-term CO_2 concentration, temperature, and nitrogen availability by acclimation mechanism. The responses of boreal bog plants to increases in CO_2, temperature, and nitrogen availability may also depend on levels of these increases that have not been examined in this study. These limitations of this study recommend investigating responses of boreal bog plants to the long-term treatments composited multiple levels of CO_2, warming, and N addition in future research.

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