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Effects of warming on N₂O fluxes in a boreal peatland of Permafrost region, Northeast China



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HIGHLIGHT

GRAPHICAL ABSTRACT

- Different vegetation surfaces showed different response on warming.
- Warming increased N₂O fluxes by 147% in boreal peatlands.
- N₂O fluxes were strongly correlated with soil temperature at 5, 10 and 15 cm depth and active layer depth.



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ABSTRACT

Climate warming is expected to increasingly influence boreal peatlands and alter their greenhouse gases emissions. However, the effects of warming on N₂O fluxes and the N₂O budgets were ignored in boreal peatlands. Therefore, in a boreal peatland of permafrost zone in Northeast China, a simulated warming experiment was conducted to investigate the effects of warming on N₂O fluxes in *Betula. Fruticosa* community (*B. Fruticosa*) and *Ledum. palustre* community (*L. palustre*) during the growing seasons from 2013 to 2015. Results showed that warming treatment increased air temperature at 1.5 m aboveground and soil temperature at 5 cm depth by 0.6 °C and 2 °C, respectively. The average seasonal N₂O fluxes ranged from 6.62 to 9.34 µg m⁻² h⁻¹ in the warming plot and ranged from 0.41 to 4.55 µg m⁻² h⁻¹ in the control plots. Warming treatment increased N₂O fluxes by 147% and transformed the boreal peatlands from a N₂O fluxes by increasing soil temperature and active layer depth, whereas soil moisture showed a weak correlation with N₂O fluxes. The results indicated that warming promoted N₂O fluxes by increasing soil temperature and active layer depth in a boreal peatland of permafrost zone in Northeast China. Moreover, elevated N₂O fluxes persisted in this region will potentially drive a noncarbon feedback to ongoing climate change.

1. Introduction

The permafrost zone contains approximately 50% of global soil organic matter (SOM), storing 1400–1800 Pg of carbon (C) (Tarnocai et al., 2009; Hugelius et al., 2014; Schuur et al., 2015) and 40–60 Pg of nitrogen (N) (Jonasson et al., 1999; Weintraub and Schimel, 2003; Harden

* Correspondence author. *E-mail address:* songcc@neigae.ac.cn (C. Song). et al., 2012). Rapid warming in northern latitudes would result in permafrost degradation and thaw (Romanovsky et al., 2010), accelerating decomposition of permafrost SOM and stimulating greenhouse gas emissions (Yang et al., 2010; Sierra et al., 2015). While past studies focused on gaseous C dynamics (Schuur et al., 2015), growing evidence suggest that Arctic soils are large N reservoirs and emit high amounts of Nitrous oxide (N₂O) (Repo et al., 2009; Elberling et al., 2010; Marushchak et al., 2011; Voigt et al., 2017a, 2017b). N₂O is a potent greenhouse gas (GHG) that has 310 times radiative forcing of CO_2 over a 100-year time horizon, and also takes part in the depletion of stratospheric ozone (Ravishankara et al., 2009). N₂O generation is regulated by availability of mineral N and is subject to microbial processes of nitrification and denitrification (Bouwman, 1990). Warming may accelerate N mineralization from SOM and leaching of mineral N forms (ammonium and nitrate) may occur. Thus, increased ammonium (NH₄⁺) pool could boost nitrification, and hence enhanced (NO_3^-) concentrations through amplified nitrification could accelerate denitrification, and elevate the concentrations of N₂O (Butterbach-Bahl et al., 2013).

Most recently, soils may generate N₂O as a result of high potential nitrification and denitrification rates in boreal ecosystem (Buckeridge and Grogan, 2010; Harms and Jones, 2012; Stewart et al., 2014) and release high N₂O in permafrost peatlands (Repo et al., 2009; Marushchak et al., 2011). Moreover, high N₂O potential has also been found in subarctic peatlands after permafrost thawing (Abbott and Jones, 2015; Voigt et al., 2017b), highlighting the importance of warming to permafrost N₂O fluxes. Previous studies have found that warming promoted N₂O release in a subarctic tundra and an alpine meadow of permafrost region (Voigt et al., 2017a; Chen et al., 2017a), reduced N₂O emission in an alpine meadow (Hu et al., 2010; Shi et al., 2012), however, warming also had no effect on N_2O fluxes in a high Arctic tundra and mountain tundra (Lamb et al., 2011; Zhou et al., 2016). These inconsistent results are mainly due to the various effects of warming on soil moisture, soil SOM content, C/N ratio and plant growth in Arctic soils (Marushchak et al., 2011; Voigt et al., 2017a, 2017b).

The Great Hing'an Mountains of China is characterised by low temperature, a short growing season, partial water-saturation as well as permafrost, leading to an accumulation of organic matter (Wang et al., 2010). Consequently, the permafrost is susceptible to climate change, which permafrost boundary has moved northward by a deepening of the active layer in mountain area (Jin et al., 2007). Generally, permafrost peatlands with large N stocks have negligible N₂O fluxes because of low N minerlization rates (Regina et al., 1996). However, the permafrost degradation will make vast stocks of previously frozen SOM may become available and enhance decomposition of SOM (Hobbie et al., 2000; Schuur et al., 2015). Additionally, experimental warming of both boreal and arctic soils accelerate soil nitrogen pools and cycling (Schimel et al., 2004; Natali et al., 2011; Weedon et al., 2012), likely caused by enhanced rates of nitrogen mineralization or nitrification (Rustad et al., 2001; Keuper et al., 2012). Thus, permafrost thaw and climate warming in this area will in turn enhance nitrogen release from the permafrost and potentially drive a positive feedback to ongoing climate warming (Abbott and Jones, 2015; Macdougall et al., 2016).

To verify the effects of warming on N_2O fluxes in boreal peatlands, we established a warming experiment using opaque chambers in a boreal peatland of permafrost zone in the Great Hing'an Mountains, Northeast China. In this study, we reported the changes in N_2O fluxes and environmental factors in two habitat types following three years of warming during the growing seasons of 2013–2015. The main objectives of this study are to (i) observe temporal variation in N_2O fluxes at different time scales (i.e., daily, monthly, seasonally); (ii) assess the effects of warming on N_2O fluxes; and (iii) investigate the factors that influence N_2O fluxes from permafrost peatlands.

2. Materials and methods

2.1. Study site

The study was conducted in a minerotrophic peatland located in the continuous permafrost zone of the Great Hing'an Mountains, Northeast China (52°94′ N, 122°86′ E, 477 m a.s.l). The study area experiences a cool continental climate with a mean annual temperature of -3.9 °C, a monthly mean temperature ranging from 19.8 °C in July to -31.9 °C in January, and a mean annual precipitation of 452 mm (with 45% of all precipitation occurring between July and August; Miao et al., 2012). The permafrost of this region belongs to an undivided portion of Eurasian continuous permafrost (Jin et al., 2007). The growing season normally starts in early May and lasts until late September. The dominant plant community mainly consists mostly of Betula fruticosa, Ledum palustre, Vaccinium uliginosum, Sphagnum spp., Rhododendron parvifolium, Chamaedaphne calyculata, Eriophorum vaginatum, and Larix gmelini Rupr. The main microtopographies in the peatland surface are distinguished by hummocks, tussocks and hollows. About 50% of the surface is hummocks covered by moss and some shrubs.

Our study was conducted in two habitat types, a higher shrub community dominated by the deciduous shrub *Betula fruticosa* and *Sphagnum* mosses, and a dwarf shrub community dominated by evergreen shrub *Ledum palustre* and *Sphagnum* mosses. For simplicity's sake, we will refer to these two habitat types as *B. fruticosa* and *L. palustre* hereafter. The height, coverage and above-ground biomass of *B. fruticosa* were 135 ± 14.4 cm, $84 \pm 7.1\%$ and 467.1 ± 73.1 g m⁻², respectively. The height, coverage and above-ground biomass of *L. palustre* were 50.3 ± 7.1 cm, $44.7 \pm 22.8\%$ and 269.6 ± 133.6 g m⁻², respectively. The distance between these two habitat types was 200 m and the other focal species were all abundant in both habitat types. These two species were selected because they were abundant at our study site and were widespread in the peatland of the Great Hing'an Mountains (Miao et al., 2012).

2.2. Experimental design

The OTCs were passive warming chambers designed according to the established ITEX protocol in order to obtain guasi-natural transmittance of visible wavelengths and to minimize the transmittance of re-radiated infrared wavelengths (Marion et al., 1997; Aronson and McNulty, 2009). The OTCs had a diameter, height, and open-top diameter of 2.6, 2.3, and 1.3 m, respectively. They consisted of an octagonal galvanized steel structure made with steel tubes, and the warming material was organic glass with a thickness of 6 mm. At 1.8 m height, the vertical beams of the structure were bent inwards to have a truncated pyramid pen-top of 1.3 m diameter and a 60° tilt. A door of 1 m length and 1.8 m height was also installed on one side of each OTC to allow access to the inside, but was kept closed during the experiment. In June 2012, we installed 3 OTCs at each habitat type paired with 3 control plots. At each habitat type, the distance between two adjacent OTCs was around 4 to 5 m, and the distance between an OTC and the adjacent control plot was approximately 3 m.

2.3. N₂O flux measurements

Three replicates of each treatment were randomly selected for N_2O flux measurements. The N_2O fluxes were measured every 7–10 days using the static chamber and gas chromatography during the growing seasons of 2013 to 2015. The static chamber was made by stainless steel and consisted of two parts: a top removable aluminum chamber (50 cm \times 50 cm \times 50 cm) and a square base collar (50 cm \times 50 cm). The chambers were put on the collar during gas sampling and immediately removed after gas samples collected. The chambers were coated with Styrofoam to prevent an increase in the inner air temperature during sampling. The collars

were pre-installed into the peat layer of a depth of 15 cm, and kept in the soil throughout the experiment. Pressure equilibration tube was fixed on each chamber to keep the air mixed in the chamber closure during sampling. Boardwalks were built to minimize disturbance on the plant and soil microenvironments around collars after the collars were installed. Gas samples were collected in the morning (09:00 and 11:00 a.m) because the flux during this period is almost equal to the daily mean flux (Tang et al., 2006). Before gas sampling, we filled the grooves (2 cm wide) of the base collar with water to ensure a gas-tight enclosure. Within the overall 30 min period of gas measurement, four air samples were taken from the same chamber at intervals of 10 min (including zero time). The samples were taken with 60 mL plastic syringes with three-way stopcocks, and put into Tedlar^R air sample bags (100 mL, Delin Ltd., Liaoniang, China). The N₂O concentrations in the gas samples were analyzed within a week after sampling with modified gas chromatography (Agilent HP-7820A, USA), which was remodeled by adding an independent sample injector by the Institute of Atmospheric Physics. Chinese Academy of Sciences, and equipped with a flame ionization detector. The air sample bags with known standard concentration of N₂O were delivered with the collected samples to the laboratory to evaluate the leakage of trace gases during transport and analysis. No significant changes in the concentration of the standards were found during one week of store. The N₂O fluxes generally rose or fell linearly with time and the detection limit of N_2O fluxes was 2.0 µg N_2O m⁻²⁻ h^{-1} (Li et al., 2016). In this study, the zero or near zero fluxes were still retained to avoid biasing the results when N₂O concentrations had no evident increase or decrease in the chamber headspace over the 30 min period. Details on the N₂O concentration analyses and the associated methods for calculating the flux can be found in Song et al. (2009).

2.4. Abiotic variables

Daily precipitation was measured with a rain gauge located near the sampling plot. In both the OTC and the adjacent outside control, air temperature at 1.5 m aboveground was measured every 30 min using an automatic recording system (UTBI-001, Onset, USA). Soil temperature, active layer depth, and soil moisture were measured at the same time as gas sampling was performed. Air temperature inside the chamber and soil temperature at 5, 10, and 15 cm belowground were measured next to the chambers using a portable digital thermometer (JM 624, Jinming Instrument CO., Ltd., Tianjin, China). The depth of the active layer was simultaneously measured with a steel rod. Soil moisture at 5 and 10 cm depths were measured in each plot using a portable time-domain reflectometry instrument (Field Scout TDR-100, Spectrum Technologies Inc., Plainfield, IL, USA).

2.5. Field sampling and analyses

We selected four replicates of each treatment for surveys of vegetation and soil properties. The plant cover and height of each species, aboveground biomass were investigated on August 2014. Soil samples were collected and air-dried, sieved through a 2-mm sieve and sent to the laboratory to measure soil pH, total nitrogen (TN), bulk and organic carbon (SOC) on August 2012. The soil samples were analyzed following the methods reported by Cui et al., 2016. The properties of soil layer (0– 10 cm, 10-20cm, 20-30cm) in *B. fruticosa* and *L. palustre* were shown in Table 1.

2.6. Statistical analysis

An Independent Samples Test was used to test the differences in abiotic factors and average yearly N_2O fluxes between the warming and control plots. Repeated-measures analyses of variance (ANOVA), with warming and community as the main factors (between-subject factors) and with sample date as a within-subject factor including interactions, was performed to test the effects of the main factors on soil N₂O fluxes. In all analyses, differences with a P < 0.05 were considered statistically significant. Linear regression analysis was used to examine the relationship between N₂O flux and abiotic factors. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, USA) and all graphs were prepared using the Origin 8.0 package (Origin Lab Corporation, USA) and SigmaPlot 12.5 (Systat Software, Inc. USA) for Windows.

3. Results

3.1. Responses of abiotic factors to warming

Warming treatment significantly increased air temperature at 1.5 m above-ground by 0.6 °C during the two growing seasons in 2014 and 2015 (P < 0.05, Fig. 1). During the growing seasons of 2013–2015, the soil temperature significantly increased by 2 °C, 2.1 °C, and 1.95 °C at 5, 10, and 15 cm depth, respectively (P < 0.05, Fig. 2a, b, c, d, e, f). Warming treatment significantly increased soil moisture at 10 cm depth by 8.25% but had no significant on soil moisture at 5 cm depth (P < 0.05, Fig. 2g, h). Warming treatment significantly increased active layer depth by 6.6 cm (P < 0.05, Fig. 2i, j).

3.2. Responses of N₂O fluxes to warming

The N₂O fluxes exhibited seasonal variations, with ranges of -14.21 $\mu g~m^{-2}~h^{-1}$ to 13.09 $\mu g~m^{-2}~h^{-1}$ in the control plots and -1.82 $\mu g~m^{-2}~h^{-1}$ to 37.62 $\mu g~m^{-2}~h^{-1}$ in the warming plots during three growing seasons (Fig. 2k,l). The N₂O fluxes peaked in August of 2014 and September of 2015, whereas the peak of N₂O fluxes occurs in May of 2013. More N₂O uptake occurred in middle growing season in the control plots (Fig. 2k, l).

Warming treatment exerted significant effects on the N₂O fluxes from both vegetation communities, and the effects varied with the sampling date and the vegetation types (P < 0.05, Fig. 2k, l; Table 2). In the warming plots, the average seasonal N₂O fluxes from *B*,*fruticosa* and *L*. *palustre* were 7.89 \pm 0.79 µg m⁻² h⁻¹ and 7.69 \pm 0.45 µg m⁻² h⁻¹, respectively (Table 3). Warming treatment significantly increased N₂O fluxes in *B*. *fruticosa* and *L*. *palustre* by 156% and 137%, respectively (P < 0.05, Fig. 3). Moreover, warming treatment significantly enhanced cumulative N₂O fluxes, which cumulative N₂O fluxes in the warming plots from *B*. *fruticosa* and *L*. *palustre* were 26.11 mg m⁻² and 23.55 mg m⁻², respectively (P < 0.05, Fig. 4).

3.3. Relationships between N₂O flux and abiotic factors

Across three growing seasons, N_2O fluxes in *B. fruticosa* exhibited a positive linear relationship with soil temperature at 5, 10 and 15 cm depth, and active layer depth (Table 4). However, N_2O fluxes in *L. palustre* only showed a significant linear relationship with soil temperature at 5, 10 and 15 cm depth. N_2O fluxes in *B. fruticosa* also linearly correlated with soil temperature at 5, 10 and 15 cm depth in 2014 and 2015, whereas the correlation between N_2O fluxes and active layer depth was only found in 2015. In addition, N_2O fluxes in *L. palustre* only positively correlated with soil temperature at 5, 10 and 15 cm depth was only found in 2015. In addition, N_2O fluxes in *L. palustre* only positively correlated with soil temperature at 5, 10 and 15 cm depth, soil moisture at 10 cm depth and active layer depth in 2015.

4. Discussion

4.1. Effects of warming on abiotic factors

In our study, the open top chambers (OTCs) were used for simulated warming in the field, which were especially appropriate for ecosystems located in high-latitude and high-altitude due to low cost, easy to operation and fewer technological maintenance requirements (Oechel et al., 2000; Walker et al., 2006). The OTCs increased air temperature at 1.5 m

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Table I	
Soil properties of the soil	in <i>B.fruticosa</i> and <i>L.palustre</i> .

Community	Layer	SOC	TN	C/N ratio	рН	Bulk density
B.fruticosa	0–10 cm	479.53 ± 7.91	19.28 ± 4.92	24.88 ± 0.41	4.20 ± 0.03	11.97 ± 0.19
	10-20 cm	462.03 ± 8.73	25.69 ± 3.35	17.99 ± 0.34	4.58 ± 0.15	15.65 ± 0.48
	20-30 cm	451.23 ± 5.95	26.07 ± 3.55	17.31 ± 0.23	4.97 ± 0.15	19.29 ± 1.85
L.palustre	0–10 cm	439.63 ± 7.26	18.60 ± 5.62	23.64 ± 0.39	4.48 ± 0.07	7.14 ± 0.96
	10-20 cm	429.33 ± 9.62	24.02 ± 6.43	17.87 ± 0.40	4.84 ± 0.14	14.88 ± 0.96
	20-30 cm	451.23 ± 5.95	26.76 ± 5.62	15.22 ± 0.09	4.63 ± 0.04	14.88 ± 0.81

SOC, soil organic carbon; TN, total nitrogen; The units of SOC and TN: g kg⁻¹; The units of Bulk density: g cm⁻³. Values are means \pm SE (n = 3).

aboveground and soil temperature at 5 cm depth by 0.6 °C and 2 °C, respectively, as compared to the control plots. Such an air temperature rise was in accordance with other warming studies with OTCs in the Arctic (Dorrepaal et al., 2009; Weedon et al., 2012), whereas was much less than that other warming studies in a subarctic tundra and an alpine swamp meadow of a permafrost region (e.g., 0.3–2.1 °C for Voigt et al., 2017a, 2017b, 6.2 °C for Chen et al. (2017a); 4.5 °C for Chen et al. (2017b)). The magnitude of elevated soil temperature was more than warming studies in a subarctic tundra and an alpine meadow (e.g., 0.5-0.7 °C for Voigt et al., 2017a, 2017b, 0.62 °C for Qin et al. (2015), 1.7 °C for Zhu et al. (2017)). In contrast with many previous studies (Oin et al., 2015; Voigt et al., 2017a, 2017b; Chen et al., 2017a; Chen et al., 2017b), the OTCs had a stronger effect on soil temperature than air temperature, which was consistent with previous results (Yin et al., 2012). The reason was mainly that there was sphagnum layer in this peatlands, which could prevent the heat to loss when there was no sunshine.

Surprisingly, the OTCs increased soil moisture at 10 cm depth by 8.25%, since many warming experiment reported a decrease or no effect on soil moisture (Dorrepaal et al., 2009; Bokhorst et al., 2011; Jassey et al., 2013; Zhu et al., 2015). Two possible underlying mechanisms may result in this phenomenon in a wetland ecosystem of permafrost region. Firstly, warming enhances the release of frozen soil moisture and promotes its upward diffusion. Secondly, local warming diffuses the heat to the surrounding environment, leading to increased water content and

the thawing of surrounding permafrost (Chen et al., 2017a). Thus, warming increased soil moisture due to frozen soil thawing and soil moisture diffusion.

4.2. Temporal variation in N₂O fluxes

The values of N₂O fluxes in the control plots ranged from negative to positive, indicating that there was no consistent uptake or emission in boreal peatlnds of permafrost region. The results were in accordance with those from a boreal peatland (Regina et al., 1996), a high arctic lowland (Ma et al., 2007), a northern boreal fen (Lohila et al., 2010) and an alpine swamp meadow (Chen et al., 2017a). N₂O production in soil is the result of nitrification and denitrification, and N₂O flux depends on the balance between production and consumption processes (Conrad, 1996). N₂O uptake has been thought to be caused by anaerobic denitrification processes and be favored by low mineral N and high water contents (Schmidt et al., 2004; Chapuis-Lardy et al., 2007), leading to the consumption of N₂O in denitrification due to the reduction of most of the nitrate into N_2 and the reduced NO_2^- supplied to denitrifiers by nitrification (Jungkunst and Fiedler, 2007). In the case of shortage in nitrate supply, denitrifying bacteria might use atmospheric N₂O as an alternative electron acceptor to nitrate, leading to N₂O uptake (Rosenkranz et al., 2006). In addition, incubation experiment at this site showed that N₂O fluxes in control treatment were limited by low nitrate content (<5 mg kg⁻¹ soil depth 0-15 cm) and low denitrification



Fig. 1. The seasonal variation of air temperature for warming plots (solid line) and control plots (dotted line) during the growing seasons of 2014 and 2015.



N2O flux (µg m2 h1) Active layer depth (cm) Soil -10 .20 Jul Aug 2015 Jul Aug Sep 2013 Jul Aug 2015 May May Aug Set May Jun Sep Oct May Jun May Jun Aug Ser MayJun Sep Oct ^{Jun} Ju 2014 Date (Month) 2014 Fig. 2. The seasonal variation of soil temperature at 5 cm depth (a,b), soil temperature at 10 cm depth (c,d), soil temperature at 15 cm depth (e,f), soil moisture at 10 cm depth (g,h), active

layer depth (i,j), N₂O flux (k,l) for warming plots and control plots during the growing seasons of 2013–2015. Error bars represent \pm SE (n = 3).

activity (Cui et al., 2016). Thus, N₂O uptake and low N₂O fluxes in our study may be caused by low availability of mineral N and labile SOM, efficient N absorption by plants and microorganisms, low temperature and high water content (Davidson et al., 2000; Chapuis-Lardy et al., 2007; Stewart et al., 2014).

Our results showed that N₂O fluxes peaked at the end of May during the 2013 growing season, while N₂O fluxes peaked during August-September in 2014 and 2015. The peak of N₂O at the end of May has been

Table 2

Soil temperature (°C)

(%)

moisture 60 41

21

15

30 20

Result (F-values) of repeated measures ANOVA indicating effects of Warming (W), Community (C), sampling times and the their interactions on N₂O fluxes ($\mu g m^{-2} h^{-1}$).

	N ₂ O flux		
	2013	2014	2015
С	0.36	2.19	1.27
W	23.21***	42.67***	43.06***
Time	5.66**	5.39***	8***
C imes W	0.23	0.25	0.01
$Time \times C$	3.16*	2.32	2.39**
$Time \times W$	3.09*	0.89	0.993
$Time \times C \times W$	0.48	1.03	0.36

*,**, and *** represents significant at P < 0.05, 0.01, and 0.001, respectively.

observed by other researches in subalpine meadow of Colorado (Filippa et al., 2009) and Qinghai-Tibetan Plateau (Jiang et al., 2010). The pulse of N₂O in 2013 could be due to the thawing process of peatlands, resulting in a quick release of N₂O that was accumulated in an unfrozen water film (Teepe et al., 2001; Koponen et al., 2006), or because of the release of available carbon and nitrogen substrates (Møkved et al., 2006). In 2014 and 2015, N₂O peaks occurred in early September and the middle of August were as a result of the discontinued vegetation growth, which caused an increase in nitrogen availability. During three growing seasons, the uptake of N₂O in control plots from June to July was probably because the mineral nitrogen was almost entirely absorbed by plants (Jiang et al., 2010). Although N₂O fluxes during the growing seasons were different in the two community species, the influence of the community species was not significant. Hence, the differences of substrate availability and environmental factors in the two microhabitats were probably not the crucial factors influencing N₂O fluxes.

4.3. Warming effects on N₂O fluxes

We found that warming promoted N₂O fluxes during three years of experimental warming in our study of a boreal peatland at permafrost

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Table 3

Average seasonal flu	ixes of N ₂ O from	B. fruticosa and	L. palustre.
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Community	2013		2014	2014 2015		2015		Total	
	Warm	Control	Warm	Control	Warm	Control	Warm	Control	
B.fruticosa L.palustre	$\begin{array}{c} 7.71 \pm 0.41 \\ 7.89 \pm 0.26 \end{array}$	$\begin{array}{c} 0.41 \pm 0.03 \\ 2.07 \pm 0.03 \end{array}$	$\begin{array}{c} 9.34 \pm 1.26 \\ 6.83 \pm 1.86 \end{array}$	$\begin{array}{c} 4.55 \pm 0.26 \\ 3.32 \pm 0.26 \end{array}$	$\begin{array}{c} 6.62 \pm 2.26 \\ 8.34 \pm 1.35 \end{array}$	$\begin{array}{c} 0.64 \pm 0.16 \\ 2.58 \pm 0.27 \end{array}$	$\begin{array}{c} 7.89 \pm 0.79 \\ 7.69 \pm 0.45 \end{array}$	$\begin{array}{c} 1.87 \pm 1.34 \\ 2.66 \pm 0.36 \end{array}$	

The units of N₂O fluxes: μ g m-² h⁻¹, Values are means \pm SE (n = 3).

site. Our results were consistent with those warming experiments from a High Arctic tundra (Lamb et al., 2011), a subarctic tundra (Voigt et al., 2017a) and an alpine swamp meadow (Chen et al., 2017a). The observed change in fluxes was thus the result of three overlapping environmental manipulations: 1) warming could speed N mineralization, nitrification and denitrification (Rustad et al., 2001; Bai et al., 2013); 2) increased soil temperature in combination with elevated soil moisture promoted N₂O fluxes via denitrification (Marushchak et al., 2011; Butterbach-Bahl et al., 2013; Voigt et al., 2017b); 3) deeper thaw increased substrate availability and release of trapped N₂O in frozen soil (Voigt et al., 2017a, 2017b).

In this study, N₂O fluxes were positively correlated with soil temperature and active layer depth. Experimental warming could stimulate mineralization of organic nitrogen and decomposition of organic matter in systems that were not water limited (Rustad et al., 2001; Pendall et al., 2004). Moreover, increased temperatures may result in lower plant N uptake and offer better N availability for soil microbes in the Arctic (Voigt et al., 2017a). Thus, higher soil temperature stimulated rates of N mineralization and enhanced the availability of mineral N (Rustad et al., 2001; Schimel et al., 2004), and thereby provided nutrient supply for nitrification and denitrification. Although N₂O emissions showed weak relationship with soil moisture, enhanced soil moisture in combination with elevated soil temperature also created ideal conditions for N₂O production (Marushchak et al., 2011; Butterbach-Bahl et al., 2013; Voigt et al., 2017b). Elevated soil temperature and soil moisture also deepen thaw depth. A recent study in subarctic peatlands found that N₂O emissions from subarctic peatlands increased as the permafrost thaws and a gradual deepening of the active layer will create a strong noncarbon climate change feedback (Voigt et al., 2017b). Probably, permafrost thaw would liberate labile dissolved organic carbon and mineral N from the organic layer (Cory et al., 2013; Abbott et al., 2014), which provide substrate for nitrification and serve as electron donors during denitrification (Abbott and Jones, 2015). Because of the altered environmental factors, the increased N₂O emissions



Fig. 3. Changes in N_2O flux induced by warming from 2013 to 2015. Error bars represent \pm SE (n = 3).



Fig. 4. Effects of warming on the N₂O cumulative fluxes in *B*_f*tuticosa* and *L* palustre during the growing seasons of 2013–2015. Error bars represent \pm SE (n = 3). Different symbols represent significant treatments differences. * *P* < 0.05, ** *P* < 0.01.

induced by warming in a boreal peatland of permafrost zone would further drive positive feedback to undergoing climate change.

5. Conclusions

By conducting a warming experiment in a boreal peatland of permafrost zone over three growing seasons, we showed that warming treatment by means of OTCs caused an increase in air and soil temperature, and deepened the active layer. Warming treatment promoted N₂O fluxes and transformed N₂O sink to source, indicating that warming might stimulate soil N₂O production in boreal peatlands. Moreover, increased soil temperature and deepen active layer depth induced by warming would stimulate N₂O release during the growing seasons, thus further intensify mechanisms and microbial activity responsible for enhanced N₂O from soils in a boreal peatland of permafrost zone.

Table 4

Coefficient matrix table between N_2O fluxes and soil temperature and soil moisture at different soil depths, and active layer depth during the growing season.

Community		Soil temperature		Soil moisture	Active layer depth		
			5 cm	10 cm	15 cm	10 cm	
	B.fruticosa	All	0.43	0.59	0.69	0.01	0.08
		2013	0.13	0.05	-0.10	0.03	-0.02
		2014	0.91	0.68	0.91	0.00	0.074
		2015	1.03	0.88	1.03	0.10	0.15
	L.palustre	All	0.45	0.66	0.86	0.05	0.04
		2013	0.33	0.07	-0.43	-0.10	-0.16
		2014	0.72	0.16	0.72	0.13	0.14
		2015	0 92	1 34	1 52	0 19	0.18

The bond fonts represents significant at P < 0.05.

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References

- Abbott, B.W., Jones, J.B., 2015. Permafrost collapse alters soil carbon stocks, respiration, CH_4 , and N_2O in upland tundra. Glob. Chang. Biol. 21 (12), 4570–4587.
- Abbott, B.W., Larouche, J.R., Jones, J.B., Bowden, W.B., Balser, A.W., 2014. Elevated dissolved organic carbon biodegradability from thawing and collapsing permafrost. J. Geophys. Res. Biogeosci. 119, 2049–2063.
- Aronson, E.L., McNulty, S.G., 2009. Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. Agric. For. Meteorol. 149, 1791–1799.
- Bai, E., Li, S., Xu, W., Li, W., Dai, W., Jiang, P., 2013. A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. New Phytol. 199, 441–451.
- Bokhorst, S., Huiskes, A., Convey, P., Sinclair, B.J., Lebouvier, M., Van de Vijver, B., et al., 2011. Microclimate impacts of passive warming methods in Antarctica: implications for climate change studies. Polar Biol. 34, 1421–1435.
- Bouwman, A.F., 1990. Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere. In: Bouwman, A.F. (Ed.), Soils and the Greenhouse Effect. Wiley, Chichester, pp. 61–127.
- Buckeridge, K.M., Grogan, P., 2010. Deepened snow increases late thaw biogeochemical pulses in mesic low arctic tundra. Biogeochemistry 101, 105–121.
- Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S., 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos. Trans. R. Soc.: Biol. Sci. 368, 20130122.
- Chapuis-Lardy, L., Wrage, N., Metay, A., Chotte, J.L., Bernoux, M., 2007. Soils, a sink for N₂O? A review. Glob. Chang. Biol. 13, 1–17.
- Chen, X.P., Wang, G.X., Zhang, T., Mao, T.X., Wei, D., Song, C., Hu, Z.Y., Huang, K.W., 2017a. Effects of warming and nitrogen fertilization on greenhouse flux in an alpine swamp meadow of a permafrost region. Sci. Total Environ. 601–602, 1389–1399.
- Chen, X.P., Wang, G.X., Zhang, T., Mao, T.X., Wei, D., Hu, Z.Y., Song, C.I., 2017b. Effects of warming and nitrogen fertilization on GHG flux in the permafrost region of an alpine meadow. Atmos. Environ. 157, 111–124.
- Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H₂, CO, CH₄, OCS, N₂O, and NO). Microbiol. Rev. 60, 609–640.
- Cory, R.M., Crump, B.C., Dobkowski, J.A., Kling, G.W., 2013. Surface exposure to sunlight stimulates CO₂ release from permafrost soil carbon in the Arctic. Proc. Natl. Acad. Sci. U. S. A. 110, 3429–3434.
- Cui, Q., Song, C.C., Wang, X.W., Shi, F.X., Wang, L.L., Guo, Y.D., 2016. Rapid N₂O fluxes at high level of nitrate nitrogen addition during freeze-thaw events in boreal peatlands of Northeast China. Atmos. Environ. 135, 1–8.
- Davidson, E.A., Keller, M., Erickson, H.E., Verchot, L.V., Veldkamp, E., 2000. Testing a conceptual model of soil emissions of nitrous and nitric oxide. Bioscience 8, 667–680.
- Dorrepaal, E., Toet, S., van Logtestijn, R.S., Swart, E., van de Weg, M.J., Callaghan, T.V., Aerts, R., 2009. Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. Nature 460 (7255), 616–619.
- Elberling, B., Christiansen, H.H., Hansen, B.U., 2010. High nitrous oxide production from thawing permafrost. Nat. Geosci. 3, 332–335.
- Filippa, G., Freppaz, M., Williams, M.W., Helmig, D., Liptzin, D., Seok, B., Hall, B., Chowanski, K., 2009. Winter and summer nitrous oxide and nitrogen oxides fluxes from a seasonally snow-covered subalpine meadow at Niwot Ridge, Colorado. Biogeochemistry 95, 131–149.
- Harden, J.W., Koven, C.D., Ping, C.L., Hugelius, G., David, McGuire.A., Camill, P., Schuur, E.A., 2012. Field information links permafrost carbon to physical vulnerabilities of thawing. Geophys. Res. Lett. 39 (15).
- Harms, T.K., Jones, J.B., 2012. Thaw depth determines reaction and transport of inorganic nitrogen in valley bottom permafrost soils. Glob. Chang. Biol. 18, 2958–2968.
 Hobbie, S.E., Schimel, J.P., Trumbore, S.E., 2000. Controls over carbon storage and turnover
- in high-latitude soils. Glob. Chang. Biol. 6, 196–210. Hu, Y., Chang, X., Lin, X., Wang, Y., Wang, S., Duan, J., Zhang, Z., Yang, X., Luo, C., Xu, G.,
- Fu, F., Chang, A., Lin, A., Wang, F., Wang, S., Dudai, J., Zhang, Z., Tang, A., Luo, C., Au, G., Zhao, X., 2010. Effects of warming and grazing on N₂O fluxes in an alpine meadow ecosystem on the Tibetan plateau. Soil Biol. Biochem. 42, 944–952.
- Hugelius, G., Strauss, J., Zubrzycki, S., Harden, J.W., Schuur, E.A.G., Ping, C.L., et al., 2014. Estimated stocks of circumpolar permafrost carbon with quantified uncertainty ranges and identified data gaps. Biogeosciences 11 (23), 6573–6593.
- Jassey, V.E.J., Chiapusio, G., Binet, P., Buttler, A., Laggoun-Defarge, F., Delarue, F., et al., 2013. Above- and belowground linkages in Sphagnum peatland: climate warming affects plant-microbial interactions. Glob. Chang. Biol. 19, 811–823.
- Jiang, C., Yu, G., Fang, H., Cao, G., Li, Y., 2010. Short-term effect of increasing nitrogen deposition on CO₂, CH₄ and N₂O fluxes in an alpine meadow on the Qinghai-Tibetan Plateau, China. Atmos. Environ. 44 (24), 2920–2926.
- Jin, H.J., Yu, Q.H., Guo, D.X., He, R.X., Yu, S.P., Sun, G.Y., Li, Y.W., 2007. Degradation of permafrost in the Xing'an Mountains, Northeast China. Permafr. Periglac. Process. 18 (3), 245–258.

- Jonasson, S., Michelsen, A., Schmidt, I.K., 1999. Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. Appl. Soil Ecol. 11, 135–146.
- Jungkunst, H.F., Fiedler, S., 2007. Latitudinal differentiated water table control of CO₂, CH₄ and N₂O fluxes from hydromorphic soils: feedbacks to climate change. Glob. Chang. Biol. 13, 2668–2683.
- Keuper, F., van Bodegom, P.M., Dorrepaal, E., Weedon, J.T., van Hal, J., van Logtestijn, R.S.P., Aerts, R., 2012. A frozen feast: thawing permafrost increases plant-available nitrogen in subarctic peatlands. Glob. Chang. Biol. 18, 1998–2007.
- Koponen, H.T., CE, Duran, Maljanen, M., Hytönen, J., Martikainen, P.J., 2006. Temperature responses of NO and N₂O emissions from boreal organic soil. Soil Biol. Biochem. 38, 1779–1787.
- Lamb, E.G., Han, S., Lanoil, B.D., Henry, G.H.R., Brummell, M.E., Banerjee, S., Siciliano, S.D., 2011. A high arctic soil ecosystem resists long-term environmental manipulations. Glob. Chang. Biol. 17 (10), 3187–3194.
- Li, F., Zhu, R., Tao, B., Wang, Q., Hua, X., 2016. Sunlight stimulates methane uptake and nitrous oxide emission from the high arctic tundra. Sci. Total Environ. 572, 1150–1160.
- Lohila, A., Aurela, M., Hatakka, J., Pihlatie, M., Minkkinen, K., Penttilä, T., Laurila, T., 2010. Responses of N_2O fluxes to temperature, water table and N deposition in a northern boreal fen. Eur. J. Soil Sci. 61 (5), 651–661.
- Ma, W.K., Schautz, A., Fishback, L.E., Bedard-Haughn, A., Farrell, R.E., Siciliano, S.D., 2007. Assessing the potential of ammonia oxidizing bacteria to produce nitrous oxide in soils of a high arctic lowland ecosystem on Devon Island, Canada. Soil Biol. Biochem. 39, 2001–2013.
- Macdougall, H.A., Avis, A.C., Weaver, J.A., 2016. Significant contribution to climate warming from the permafrost carbon feedback. Nat. Geosci. 5, 719–721.
- Marion, G.M., Henry, G.H.R., Freckman, D.W., Johnstone, J., Jones, G., Jones, M.H., Levesque, E., Molau, U., Møgaard, P., Parsons, A.N., Svoboda, J., Virginia, R.A., 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. Glob. Chang. Biol. 3, 20–32.
- Marushchak, M.E., Pitkämäki, A., Koponen, H., Biasi, C., Seppälä, M., Martikainen, P.J., 2011. Hot spots for nitrous oxide emissions found in different types of permafrost peatlands. Glob. Chang. Biol. 17, 2601–2614.
- Miao, Y., Song, C., Sun, L., Wang, X., Meng, H., Mao, R., 2012. Growing season methane emission from a boreal peatland in the continuous permafrost zone of Northeast China: effects of active layer depth and vegetation. Biogeosciences 9, 4455–4464.
- Møkved, P.T., Dösch, P., Henriksen, T.M., Bakken, L.R., 2006. N₂O emissions and product ratios of nitrification and denitrification as affected by freezing and thawing. Soil Biol. Biochem. 38, 3411–3420.
- Natali, S.M., Schuur, E.A.G., Trucco, C., Hicks Pries, C.E., Crummer, K.G., Baron Lopez, A.F., 2011. Effects of experimental warming of air, soil and permafrost on carbon balance in Alaskan tundra. Glob. Chang. Biol. 17, 1394–1407.
- Oechel, W.C., Vourlitis, G.L., Hastings, S.J., Zulueta, R.C., Hinzman, L., Kane, D., 2000. Acclimation of ecosystem CO₂ exchange in the Alaskan Arctic in response to decadal climate warming. Nature 406, 978–981.
- Pendall, E., Bridgham, S., Hanson, P.J., Hungate, B., Kicklighter, D.W., Johnson, D.W., Law, B.E., Luo, Y., Megonigal, J.P., Olsrud, M., Ryan, M.G., Wan, S., 2004. Below-ground process responses to elevated CO₂ and temperature: a discussion of observations, measurement methods, and models. New Phytol. 162, 311–322.
- Qin, Y., Yi, S., Chen, J., Ren, S., Wang, X., 2015. Responses of ecosystem respiration to shortterm experimental warming in the alpine meadow ecosystem of a permafrost site on the qinghai–tibetan plateau. Cold Reg. Sci. Technol. 115, 77–84.
- Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N₂O): the dominant ozone-depleting substance emitted in the 21st century. Science 326, 123–125.
- Regina, K., Nykanen, H., Silvola, J., Martikainen, P.J., 1996. Fluxes of nitrous oxide from boreal peatlands as affected by peatland type, water table level and nitrification capacity. Biogeochemistry 35, 401–418.
- Repo, M.E., Susiluoto, S., Lind, S.E., Jokinen, S., Elsakov, V., Biasi, C., Martikainen, P.J., 2009. Large N₂O emissions from cryoturbated peat soil in tundra. Nat. Geosci. 2 (3), 189–192.
- Romanovsky, V.E., Smith, S.L., Christiansen, H.H., 2010. Permafrost thermal state in the polar northern hemisphere during the international polar year 2007–2009: a synthesis. Permafr. Periglac. Process. 21 (2), 106–116.
- Rosenkranz, P., Bruggemann, N., Papen, H., Xu, Z., Seufert, G., Butterbach-Bahl, K., 2006. N₂O, NO and CH₄ exchange, and microbial N turnover over a Mediterranean pine forest soil. Biogeosciences 3, 121–133.
- Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126, 543–562.
- Schimel, J.P., Bilbrough, C., Welker, J.A., 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. Soil Biol. Biochem. 36, 217–227.
- Schmidt, I., van Spanning, R.J.M., Jetten, M.S.M., 2004. Denitrification and ammonia oxidation by Nitrosomonas Europaea wild-type, and NirK- and NorB-deficient mutants. Microbiology 150, 4107–4114.
- Schuur, E.A.G., McGuire, A.D., Schädel, C., Grosse, G., Harden, J.W., Hayes, D.J., Natali, S.M., 2015. Climate change and the permafrost carbon feedback. Nature 520 (7546), 171–179.
- Shi, F., Chen, H., Chen, H., Wu, Y., Wu, N., 2012. The combined effects of warming and drying suppress co2, and n2 o emission rates in an alpine meadow of the eastern Tibetan plateau. Ecol. Res. 27 (4), 725–733.
- Sierra, C.A., Trumbore, S.E., Davidson, E.A., Vicca, S., Janssens, I., 2015. Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture. J. Adv. Model. Earth Syst. 7 (1), 335–356.

- Song, C.C., Xu, X.F., Tian, H.Q., Wang, Y.Y., 2009. Ecosystem-atmosphere exchange of CH₄ and N₂O and ecosystem respiration in wetlands in the Sanjiang Plain, Northeastern China. Glob. Chang. Biol. 15, 692–705.
- Stewart, K.J., Grogan, P., Coxson, D.S., Siciliano, S.D., 2014. Topography as a key factor driving atmospheric nitrogen exchanges in arctic terrestrial ecosystems. Soil Biol. Biochem. 70, 96–112.
- Tang, X., Liu, S., Zhou, G., Zhang, D., Zhou, C., 2006. Soil atmospheric exchange of CO₂, CH₄, and N₂O in three subtropical forest ecosystems in southern China. Glob. Chang. Biol. 12, 546–560.
- Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G., Zimov, S., 2009. Soil organic carbon pools in the northern circumpolar permafrost region. Glob. Biogeochem. Cycles 23, 1–11.
- Teepe, R., Brumme, R., Besse, F., 2001. Nitrous oxide emissions from soil during freezing and thawing periods. Soil Biol. Biochem. 33, 1269–1275.
- Voigt, C., Lamprecht, R.E., Marushchak, M.E., Lind, S.E., Novakovskiy, A., Aurela, M., Martikainen, P., Biasi, C., 2017a. Warming of subarctic tundra increases emissions of all three important greenhouse gases - carbon dioxide, methane, and nitrous oxide. Glob. Chang. Biol. 23, 3121–3138.
- Voigt, C., Marushchak, M.E., Lamprecht, R.E., Jackowicz-Korczyński, M., Lindgren, A., Mastepanov, M., Granlund, L., Christensen, T.R., Tahvanainen, T., Martikainen, P.J., Biasia, C., 2017b. Increased nitrous oxide emissions from arctic peatlands after permafrost thaw. Proc. Natl. Acad. Sci. U. S. A. 114 (24), 6238.
- Walker, M.D., Wahren, C.H., Hollister, R.D., Henry, G.H., Ahlquist, L.E., Alatalo, J.M., Epstein, H.E., 2006. Plant community responses to experimental warming across the tundra biome. Proc. Natl. Acad. Sci. 103 (5), 1342–1346.
- Wang, X.W., Li, X.L., Hu, Y.M., Lv, J.J., Sun, J., Li, Z.M., Wu, Z.F., 2010. Effect of temperature and moisture on soil organic carbon mineralization of predominantly permafrost

peatland in the Great Hing'an Mountains, Northeastern China. J. Environ. Sci. 22, 1057–1066.

- Weedon, J.T., Kowalchuk, G.A., Aerts, R., van Hal, J., van Logtestijn, R., Tas, N., Röling, W.F., van Bodegom, P.M., 2012. Summer warming accelerates sub-arctic peatland nitrogen cycling without changing enzyme pools or microbial community structure. Glob. Chang. Biol. 18, 138–150.
- Weintraub, M.N., Schimel, J.P., 2003. Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in arctic tundra soils. Ecosystems 6, 129–143.
- Yang, Z.P., Ou, Y.H., Xu, X.L., Zhao, L., Song, M.H., Zhou, C.P., 2010. Effects of permafrost degradation on ecosystems. Acta Ecol. Sin. 30 (1), 33–39.
- Yin, H., Chen, Z., Liu, Q., 2012. Effects of experimental warming on soil n transformations of two coniferous species, eastern tibetan plateau, china. Soil Biol. Biochem. 50 (50), 77–84.
- Zhou, Y., Hagedorn, F., Zhou, C., Jiang, X., Wang, X., Li, M.H., 2016. Experimental warming of a mountain tundra increases soil CO₂ effluxes and enhances CH₄ and N₂O uptake at Changbai Mountain, China. Sci Rep 6, 21108.
- Zhu, X., Luo, C., Wang, S., Zhang, Z., Cui, S., Bao, X., Jiang, L., Li, Y.M., Li, X., Wang, Q., Zhou, Y., 2015. Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the tibetan plateau. Agric. For. Meteorol. 214-215, 506–514.
- Zhu, J., Zhang, Y., Jiang, L., 2017. Experimental warming drives a seasonal shift of ecosystem carbon exchange in Tibetan alpine meadow. Agric. For. Meteorol. 233, 242–249.