# Nitrogen addition turns a temperate peatland from a near-zero source into a strong sink of nitrous oxide

Boli Yi<sup>1,2,3</sup>, Fan Lu<sup>1,2,3</sup>, Zhao-Jun Bu<sup>1,2,3</sup>\*

<sup>1</sup>Key Laboratory of Geographical Processes and Ecological Security in Changbai Mountains, Ministry of Education, School of Geographical Sciences, Northeast Normal University, Changchun, P.R. China

<sup>2</sup>State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, Institute for Peat and Mire Research, Northeast Normal University, Changchun, P.R. China <sup>3</sup>Jilin Provincial Key Laboratory for Wetland Ecological Processes and Environmental Change in the Changbai Mountains, Changchun, P.R. China

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**Abstract:** Peatlands, as important global nitrogen (N) pools, are potential sources of nitrous oxide ( $N_2O$ ) emissions. We measured  $N_2O$  flux dynamics in Hani peatland in a growing season with simulating warming and N addition for 12 years in the Changbai Mountains, Northeastern China, by using static chamber-gas chromatography. We hypothesised that warming and N addition would accelerate  $N_2O$  emissions from the peatland. In a growing season, the peatland under natural conditions showed near-zero  $N_2O$  fluxes and warming increased  $N_2O$  emissions but N addition greatly increased  $N_2O$  absorption compared with control. There was no interaction between warming and N addition on  $N_2O$  fluxes. Pearson correlation analysis showed that water table depth was one of the main environmental factors affecting  $N_2O$  fluxes and a positive relationship between them was observed. Our study suggests that the  $N_2O$  source function in natural temperate peatlands maybe not be so significant as we expected before; warming can increase  $N_2O$  emissions, but a high dose of N input may turn temperate peatlands to be strong sinks of  $N_2O$ , and global change including warming and nitrogen deposition can alter  $N_2O$  fluxes via its indirect effect on hydrology and vegetation in peatlands.

Keywords: climate change; greenhouse gas; denitrification; terrestrial ecosystem; Sphagnum

Peatlands cover only 3% of the global land area (Gorham 1991), but sequestrate approximate 16% of the global nitrogen storage (Limpens et al. 2006), and hence play an important role in the terrestrial ecosystem nitrogen budget. As an important greenhouse gas and the major ozone-depleting compound in the atmosphere, nitrous oxide ( $N_2O$ ) holds a warming potential about 298 times higher than carbon dioxide ( $CO_2$ ) at a 100-year scale (Ravishankara et al. 2009). The concentration of  $N_2O$  in the atmosphere has increased by more than 20% over the past 250 years,

from 270 ppb to 331 ppb, and the increase rate has been accelerating in the past 50 years (Hall et al. 2007). Peatlands storing ca. 8–15 Gt nitrogen (N), are the potential source of  $N_2O$  emission (Regina et al. 1996, Leppelt et al. 2014, Liimatainen et al. 2018, Minkkinen et al. 2020).

Peatlands in the middle and high latitudes of the northern hemisphere are suffering from relatively strong climate change. These effects, in turn, influence the labile N pools of peatlands (Alm et al. 1999). Updegraff et al. (1995) found that the labile

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<sup>\*</sup>Corresponding author: buzhaojun@nenu.edu.cn

N pools subjected to warming would greatly affect the N mineralisation rate in peatlands. In peatlands, especially the *Sphagnum*-dominated ombrotrophic ones, decomposition was very slow due to the acidic, cold and anaerobic environment (Clymo and Hayward 1982). Warming may accelerate the decomposition of organic matter in peatlands and turn, provide more organic substrates for the nitrification and denitrification processes by nitrifying and denitrifying microorganisms. These effects, then, promote the production and emission of  $\rm N_2O$  (Voigt et al. 2017, Cui et al. 2018).

Anaerobic environment is beneficial to denitrification in peatlands. It is generally believed that denitrification is the main way for producing N<sub>2</sub>O in peatlands (Rückauf et al. 2004). However, the NO<sub>3</sub> supply was generally low in peatlands, especially bogs and poor fens where the NO<sub>3</sub> can be limited (Wassen et al. 1995). Along with atmospheric nitrogen deposition, N input from near farmlands (Vitousek et al. 1997, Frolking et al. 2011) would greatly increase the substrate of denitrification and intensify the N<sub>2</sub>O production and N<sub>2</sub>O emissions. However, there was uncertainty in N<sub>2</sub>O emission response to high levels of N addition in peatlands. For example, 1 year of 40 kg N/ha/a addition had no effect on N<sub>2</sub>O emissions in two Swedish bogs (Lund et al. 2009), and 100 kg N/ha/a addition for 6 years did not increase N<sub>2</sub>O emissions in a Finnish pine bog (Nykänen et al. 2002). A study in tropical artificial peatlands showed that N2O fluxes under 130 kg N/ha/a addition were significantly higher than other levels of N addition (with a mean below 100 kg N/ha/a) (Chaddy et al. 2019).

The current global change is altering peatland vegetation composition, which has a great impact on  $N_2$ O emissions (Bubier et al. 2007, Gong et al. 2020). In the nutrient-limited peatland ecosystem, increased nutrient availability may promote the growth of vascular plants, whereas inhibiting Sphagnum (Bubier et al. 2007, Larmola et al. 2013). Sphagnum was one of the main users of N in peatlands, and it was also a filter for atmospheric N deposition (Lamers et al. 2000). Nitrogen deposition may negatively affect the N absorption efficiency of Sphagnum (Aerts et al. 1992). Vitt et al. (2003) found that the N absorption of Sphagnum was greatly reduced when N deposition was greater than 100 kg N/ha/a, resulting in more N entering into the soil, stimulated the nitrification and denitrification process to produce more N2O, and even promoted the growth of nitrophilic plants like dwarf shrubs (Woodin and Lee 1987, Bragazza et al. 2004, Wieder et al. 2020). Nitrogen addition may increase the content of easily degradable components in *Sphagnum* and vascular plants, and accelerate the decomposition of organic matter (Rudolph and Voigt 2010), which can increase the available substrates to promote the production and emission of  $\rm N_2O$  in peatlands.

Besides warming, N addition, vegetation succession, and other environmental changes may also affect N<sub>2</sub>O emissions from peatlands (Amha and Bohne 2011, Maljanen et al. 2014). The water table is a key factor affecting oxygen availability along with the peat depth and then nitrification and denitrification in peatlands (Eickenscheidt et al. 2014). Previous studies have shown that water table drawdown will affect the N mineralisation rate, leading to a substantial increase in N<sub>2</sub>O emissions (Regina et al. 2010). The increase in pH and soil water content increased the denitrification of peats greatly, no matter ombrotrophic or minerotrophic (Amha and Bohne 2011). The freeze-thaw cycle (Teepe et al. 2001, Yu et al. 2010), soil oxidation-reduction potential, peatland type and the available C concentration also affect N<sub>2</sub>O fluxes (Frasier et al. 2010, Buchen et al. 2019, Hatano 2019, Minkkinen et al. 2020).

So far, the global change and the interaction between high N levels and warming on N<sub>2</sub>O emissions from peatlands in the mid-temperate zone have been rarely studied. We used static chamber-gas chromatography to measure the N<sub>2</sub>O emission characteristics of a peatland in the Changbai Mountains during the 2019 growing season. We hypothesised: (1) the control plots would clearly show N<sub>2</sub>O emissions since natural peatlands usually are the N<sub>2</sub>O source during growing seasons; (2) warming would increase N<sub>2</sub>O emissions due to increased N availability by facilitating decomposition; (3) N addition would promote N2O emissions because it increases the substrate of nitrification and denitrification, and (4) warming + N addition would greatly increase N<sub>2</sub>O emissions and hence strengthen N<sub>2</sub>O source function of the ecosystem.

## MATERIAL AND METHODS

**Study site.** The study site, Hani peatland (126°31'05"E, 42°12'50"N), is located in the west foot of the Changbai Mountains in Northeast China, with an altitude of 900 m a.s.l. It is a large peatland with an area of *ca.* 16.8 km<sup>2</sup> and a peat depth of *ca.* 3–10 m

(Zhang et al. 2019). The peatland is a transitional mire from eutrophic to oligotrophic. The temperature is low throughout the year, with an average annual temperature of 2.5-3.6 °C, and an annual active accumulated temperature of  $\geq 10$  °C is about  $2\,600$  °C. The annual precipitation is 757-930 mm. The tree *Larix gmelinii* var. *olgensis* (A. Henry) Ostenf. & Syrach, the dwarf shrub *Betula ovalifolia* Rupr., the graminoids *Carex lasiocarpa* Ehrh., *Eriophorum polystachion* L. and *Phragmites australis* (Clav.) Trin., and the bryophytes *Sphagnum magellanicum* Brid., *S. fuscum* (Schimp.) Klinggr., *S. imbricatum* Hornsch. ex Russow and *S. subsecundum* Nees. are common in the peatland (Bu et al. 2011).

Experimental design. A field experiment was conducted in the long-term global change simulation plots (initiated from 2007) in Hani peatland (Bu et al. 2011). Of the 72 plots (0.8 m  $\times$  0.8 m), 16 plots, including 4 treatments (control (CK), N addition (N), warming (W), and warming + N addition (WN)), each with 4 replicates, were chosen for the experiment. Warming was achieved through passive temperature increase with open-top chambers (OTC). In May 2018, OTCs (1.2 m  $\times$  1.2 m at the bottom and  $0.8 \text{ m} \times 0.8 \text{ m}$  at the top) were placed to surround the plots. Nitrogen addition was achieved by applying NH<sub>4</sub>NO<sub>3</sub> solution with a dose of 100 kg N/ha/a which is 4 times of average nitrogen deposition level in the area of the Changbai Mountains (Zhou et al. 2015). Three hundred mL of NH<sub>4</sub>NO<sub>3</sub> solution with an N concentration of 4.26 g/L was prepared and sprayed in each plot monthly during the growing season (from May to September). The same amount of pure water was added in the plots without N addition. Boardwalk was paved in the previous autumn to reduce the possible disturbance to gas sampling.

Gas sampling and analysis. The static chamber-gas chromatography method was used to measure  $\rm N_2O$  fluxes. In each sampling plot, a PVC soil respiration collar (25 cm in diameter, 14 cm in height) with a groove for chamber placement was fitted in the soil at 5 cm depth in October 2018, to allow recovery of any damaged roots and disturbance caused by collar installation. From May to September in 2019, gas samples were collected twice in May, July and September and once in June and August. During gas sampling, an opaque acrylic plexiglass static chamber with a diameter of 25 cm and a height of 50 cm was placed on the collar. The collar was supplied with water sealing at the top groove to ensure an airtight connection with the chamber, and the top of the

chamber is equipped with a gas extraction tube and a balanced air pressure tube, as well as a mini fan for evening the air and reducing the temperature in the chamber. Gas samples were taken from the chamber headspace and then stored using 60 mL gas syringes at 0, 10, 20 and 30 min, respectively, after closure. After each sampling, the static chamber was lifted from the soil respiration collar to restore the temperature and N<sub>2</sub>O concentration in the chamber to the surrounding environment. The sampling time was from 9:00 a.m. to 2:00 p.m. Gas chromatograph (Agilent 7980B, Santa Clara, USA) was employed to analyse the gas in the Biogeochemistry Laboratory of School of Geographical Science, Northeast Normal University within two weeks after sampling. Nitrous oxide concentration was detected by an electron capture detector. The concentration of the 4 gas samples collected within 30 min was linearly related to the sampling interval, and the slope of the linear equation was used to calculate the N2O flux of the sample. Fluxes were accepted when the determination coefficient between fluxes and sampling time intervals was greater than 0.75.

Measurement of environmental factors and estimation of vegetation cover. Meteorological data including rainfall, temperature, humidity, air pressure, and photosynthetic radiation was obtained from the weather station in the study site. A button thermometer (Lascar Electronics Ltd., Whiteparish, UK) was kept 10 cm above the moss surface by being tied to a brush and inserted into the soil to monitor the air temperature of the four typical treatment samples continuously and automatically throughout the whole growing season. The soil temperature of 5 cm and 20 cm underground was measured around the collar with a soil thermometer while measuring the N<sub>2</sub>O emission. Water table depth (WTD, distance from moss surface to water table) was measured by inserting a PVC observation well next to each sample square. Peat water pH was measured by a multi-parameter analyser (HQ30D, Shanghai Reunion Science Instrument Co. Ltd, Shanghai, China). Soil temperature, pH and WTD were measured twice a month. At the end of July 2019, plant cover in each soil respiration collar of each plot was estimated by the visual inspection method (Liu et al. 2015).

**Peat water/soil sampling and analysis.** In early August 2019, peat porewater 10 cm below the moss surface in the PVC well and soil 5–10 cm below the moss surface in the soil respiration collar were col-

lected from each plot. In the laboratory, peat water was filtered by an oil-free diaphragm vacuum pump with 0.45 µm microfiltration membrane and then measured dissolved organic carbon (DOC) concentration with a TOC analyser (Aurora 1030, OI Analytical, College Station, USA). Peat soil samples were dried at 60 °C to a constant weight, then put into a ball mill (GT200, Grinder, Beijing, China) for grinding and homogenising. After being weighted, the samples were then employed TC and TN analysis with an element analyser (Euro Vector 3000, Pavia, Italy).

**Data analysis.** Repeated measures analysis of variance (ANOVA) was used to analyse the effects of warming, N addition, and their interaction on  $N_2O$  emissions. Normality test of the data was analysed before, and the data of TN and TC were logarithmic transformation. Two-way ANOVA was used to analyse the effects of different treatments on vegetation cover, TC, TN, DOC, soil temperature (5 cm and 20 cm), WTD and other non-biological environmental factors. Pearson correlation analysis was used to analyse the correlation between  $N_2O$  fluxes and environmental factors. Statistical analysis was performed in R 3.5.3 (Development Core Team, 2019) and SPSS 19 statistical software package (SPSS, Inc., Chicago, USA).

#### RESULTS

**Environmental factors.** During the growing season, the warming treatment significantly increased the average air temperature by 0.51 °C (Figure 1). The differences in environmental factors among treatments are shown in Table 1. Although there was no significant difference in the soil temperature at 5 cm and 20 cm depth among the four treatments, they were lower in warming treatment than control by

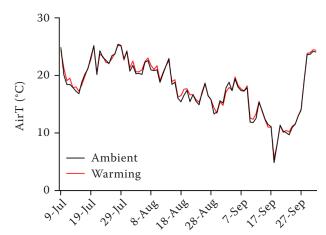


Figure 1. Daily mean air temperature in the warming plots (red line) and control plots (black line) in the growing season of 2019

1.1 °C and 0.5 °C, respectively. WTD in N addition and WN plots was lower than control. From May to July 2019, WTD of all the four treatments gradually decreased over time (Figure 2), being lowest in August, but tended to increase in September, nearly the same as that in early May. DOC concentration of control was lower than the other three treatments, among which no significant difference in DOC was observed. TN concentration of the two treatments with N addition was higher than that without N addition treatments. TC concentration of the WN treatment was higher than that without N addition treatments. The C/N ratio among the four treatments was different, the C/N ratio in control plots was higher than in the other three treatments, and in N addition plots were higher than without N addition treatment plots. No significant difference in pH among the four treatments was found.

N<sub>2</sub>O fluxes. Repeated measurement ANOVA showed that both warming and N addition had

Table 1. Environmental parameters of peat soil in different treatments during the growing season of 2019 (mean ± standard error of the mean)

Treatment	T <sub>soil</sub> , 5 cm	T <sub>soil</sub> , 20 cm	WTD	DOC	ъU	TN	TC	C/N
	(°C)		(cm)	$(\mu g/mL)$	pН	(%)		C/N
CK	$18.25 \pm 0.5^{a}$	11.82 ± 1.0 <sup>a</sup>	$29.94 \pm 3.8^{\circ}$	$4.29 \pm 0.7^{a}$	$6.19 \pm 0.1^{a}$	$1.11 \pm 0.1^{a}$	$37.79 \pm 0.3^{b}$	$34.04 \pm 0.9^{c}$
W	$17.32 \pm 0.9^{a}$	$11.36 \pm 0.8^{a}$	$23.91 \pm 3.0^{bc}$	$6.68\pm0.6^{\rm b}$	$5.97 \pm 0.1^{a}$	$1.32\pm0.1^{\rm b}$	$34.13 \pm 2.1^{b}$	$26.03 \pm 2.1^{b}$
N	$16.53 \pm 1.2^{a}$	$10.67 \pm 0.5^{a}$	$15.65 \pm 1.8^{a}$	$7.29\pm0.8^{\rm b}$	$6.00 \pm 0.2^{a}$	$1.50 \pm 0.1^{bc}$	32.96 ± 1.8ab	22.15 ± 1.8 <sup>a</sup>
WN	$15.35 \pm 1.4^{a}$	$10.07 \pm 0.4^{a}$	13.58 ± 1.1 <sup>a</sup>	$6.69 \pm 0.6^{\rm b}$	$6.05 \pm 0.2^{a}$	$1.71 \pm 0.1^{c}$	33.93 ± 1.1 <sup>a</sup>	$20.06 \pm 1.4^{a}$

CK – control; W – warming; N – N addition; WN – warming + N addition; WTD – water table depth; DOC – dissolved organic carbon; TN – total nitrogen; TC – total carbon. Different lowercase letters represent significant differences (P < 0.05) between the treatments

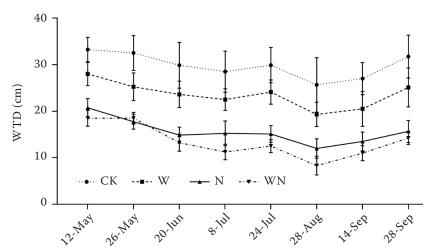


Figure 2. Temporal variation of water table depth (WTD) in Hani peatland in the growing season of 2019 (mean  $\pm$  standard error of the mean, n = 4). CK – control; W – warming; N – N addition; WN – warming + N addition

a significant effect on  $\rm N_2O$  fluxes, but no interaction between the two factors was observed (Table 2). Under natural conditions (control), a  $\rm N_2O$  flux  $-37.8\pm61.6\,\rm g/m^2/a$  was monitored but it was not significantly different from zero (Figure 3, P=0.562). Nitrogen addition enhanced  $\rm N_2O$  absorption and warming enhanced  $\rm N_2O$  emissions. However,  $\rm N_2O$  fluxes in the combined treatment of warming and N addition had no difference from that in the control treatment, and it was also in a state of absorption. Compared with the control treatment, N addition increased  $\rm N_2O$  absorption by 126.45 g/m²/a, while warming increased  $\rm N_2O$  emissions by 92.07 g/m²/a.

Temporal variation of  $N_2O$  fluxes. The temporal variation of  $N_2O$  fluxes was consistent with the water table depth. During the growing season (May to July), all the treatments showed similar  $N_2O$  flux dynamics with a relatively steady zero flux from early May to early August, a strong absorption peak in late August, and a clear emission peak in late September. The first peak may be related to emission obstacle due to rich rainfall and high water table in late August; while the second peak may be subject to the delayed emission obstacle release due to sudden decrease of the water table (Figures 2 and 4). This inference can be supported experimentally by Martikainen et al. (1993) and Kachenchart et al. (2012), both of whom

Table 2. Repeated measures ANOVA of  $\mathrm{N}_2\mathrm{O}$  fluxes under different treatments

Treatment	df	F	<i>P</i>
Warming	1	4.503	0.036*
N addition (N)	1	4.225	0.042*
N × warming	1	0.334	0.853

found increased  $\rm N_2O$  emissions when water table depth increased.

Vegetation cover change and the relationship between  $N_2O$  fluxes and environmental factors. Both N addition and warming + N addition decreased *Sphagnum* cover compared with control (P < 0.05 for both), and there was no significant effect of experimental treatment on vascular plant cover (Figure 5).

Pearson correlation analysis showed that soil temperatures at 5 cm and 20 cm, WTD, DOC in peat porewater and TN and TC in peat soil had no relation with  $\rm N_2O$  fluxes, while WTD showed a positive relation with  $\rm N_2O$  fluxes (Table 3, Figure 6).

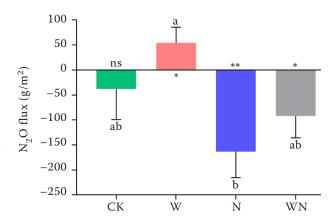


Figure 3. Cumulative  $N_2O$  fluxes (mean  $\pm$  standard error of the mean, n=4) in Hani peatland in the growing season of 2019 (153 days in total). CK – control; W – warming; N – N addition; WN – warming + N addition. Different lowercase letters represent significant differences (P < 0.05). Asterisks denote  $N_2O$  flux significantly different from zero. \*P < 0.05; \*\*P < 0.01; ns – no significant difference

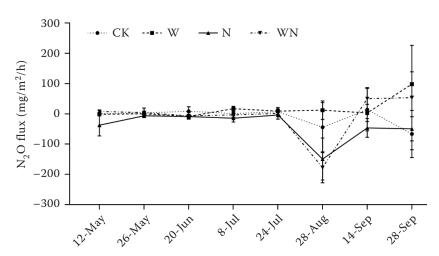


Figure 4. Temporal variation of  $N_2O$  fluxes in Hani peatland in 2019 (mean  $\pm$  standard error of the mean, n = 4). CK – control; W – warming; N – N addition; WN – warming + N addition

#### **DISCUSSION**

Source and sink function of N<sub>2</sub>O in the natural peatland. It is well believed that natural peatlands are an important potential source of  $N_2O$  emissions (Dinsmore et al. 2009, Maljanen et al. 2010, Burgin and Groffman 2012, Cui et al. 2018). However, in contrast to our first hypothesis, our experiment found that Hani peatland in the growing season was not a source of but even tended to be a sink of  $N_2O$ . Recent studies in a boreal peatland of Canada, similarly, reported a net-zero N<sub>2</sub>O flux during the growing season although yearly variation was observed (Gong et al. 2018, 2019). In seasonal dynamics, the peatland even performed as a clear N2O sink in July 2015 and August 2016, rather similar to our observations in late August and late September 2019. The contrasted result for N2O fluxes is probably related

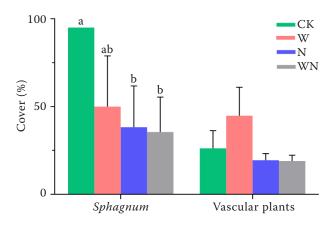


Figure 5. Vegetation cover in the plots in different treatments (mean  $\pm$  standard error of the mean, n=4). CK – control; W – warming; N – N addition; WN – warming + N addition. Different lowercase letters represent significant differences (P < 0.05)

eco-hydrological type of the peatland (Li et al. 2019, Chen et al. 2020). As a transitional mire, long-lasting high water table and anaerobic environment of Hani peatland are not in favour of  $\rm N_2O$  emission under natural state since the intermediate product  $\rm N_2O$  of denitrification would be reduced to  $\rm N_2$  and emitted into the atmosphere. This might be the reason why a waterlogging peatland was a sink of  $\rm N_2O$  (Bowden 1986, Rückauf et al. 2004). Liimatainen et al. (2014) found that denitrification was the main reaction to produce  $\rm N_2O$  under anaerobic conditions, but more  $\rm N_2O$  was produced after acetylene was used to inhibit the reduction of  $\rm N_2O$  to  $\rm N_2$ , which indirectly indicated that more  $\rm N_2O$  was reduced to  $\rm N_2$  in peatlands with poor aeration conditions.

Source and sink function of  $N_2O$  and warming. Inconsistent with the second hypothesis, warming plots were detected to perform as a source of  $N_2O$  fluxes. The cover of vascular plants in warming treatment was the highest among all the treatments, indicating that warming promoted the growth of

Table 3. Pearson correlation between N<sub>2</sub>O fluxes and environmental parameters

Environmental parameter	r	P
T <sub>soil</sub> 5 cm	0.216	0.421
T <sub>soil</sub> 20 cm	0.121	0.654
WTD	0.538	0.042*
DOC	0.034	0.901
pH	0.082	0.762
TC	0.281	0.292
TN	-0.152	0.575

T – temparature; WTD – water table depth; DOC – dissolved organic carbon; TC – total carbon; TN – total nitrogen

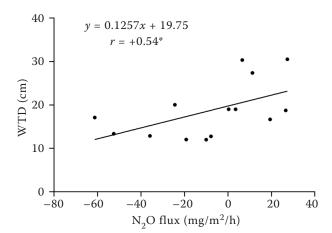


Figure 6. Correlation between  $N_2O$  fluxes and water table depth (WTD)

vascular plants. Given no significant difference in water table depth between warming and control plots (Table 1), the increase of N<sub>2</sub>O emissions relative to control plots may be attributed to the cover increase of vascular plants. The aerenchyma of vascular plants could promote N2O emissions by providing pathways for N2O produced in deep peat to escape to the atmosphere in the warming treatment (Le et al. 2021). Low C/N ratio and high DOC in warming plots due to accelerated decomposition of organic matter may provide more energy sources for denitrifying microorganisms to increase N<sub>2</sub>O production and fluxes (Hu et al. 2016). It is well known that the most suitable temperature for denitrification was 30-65 °C (Malhi et al. 1990). In our study, the soil temperature of all four treatments was relatively low in the whole growing season. This may explain why the peatland in natural or even warming conditions showed low N<sub>2</sub>O emissions.

Source and sink function of  $N_2O$  and N addition. In contrast to the third hypothesis, N addition did not increase  $N_2O$  emissions but enhanced absorption, which was rarely observed in previous studies (Couwenberg et al. 2010, Oktarita et al. 2017). Similar to our study, Leeson et al. (2017) found that 13 years of N input in the level of 64 kg/ha/a which was lower than the level in our study could reduce  $N_2O$  emissions, and they inferred that vegetation composition might be related to the  $N_2O$  flux. We recognise that the N addition level in our study is rather higher than most of the previous studies (e.g. Lund et al. 2009, Leeson et al. 2017, Gong et al. 2018). The unusual  $N_2O$  absorption may be related to surface subsidence because of continuous high-level

of N addition killing Sphagnum mosses (Figure 6) and increase surficial peat decomposition (Bubier et al. 2007, Moore et al. 2019), which could be indicated by decreased C/N ratio in peat and elevated DOC in porewater. This result could be explained as that soil moisture increased with water table depth decrease, and the N<sub>2</sub>O produced by denitrification was further reduced to N2, which eventually led to a decrease in N2O fluxes. Another possibility is that our N availability in the long-term N addition plots with TN 35% greater than control was so high that it inhibited denitrification microbes to produce N<sub>2</sub>O (Tedeschi et al. 2021). In further studies, NO<sub>3</sub> concentration measurement may answer whether NO<sub>3</sub> is rich enough to inhibit denitrification like in mineral soils (Sosulski et al. 2020).

Source and sink function of  $N_2O$  after N addition and warming. In the study, we found no interaction between warming and N addition. And contrary to the fourth hypothesis, warming + N addition did not greatly increase  $N_2O$  emissions. The  $N_2O$  fluxes in warming + N addition treatment were negative during the whole growing season.

However, the absorption intensity was lower than that in N addition plots, probably due to the effect of warming on N<sub>2</sub>O emissions partly offsetting the N<sub>2</sub>O absorption effect caused by N addition. In a similar N addition and warming experiment, Gong and Wu (2021) found no significant emissions under warming  $(1.2-2.6 \,^{\circ}\text{C}) + \text{N}$  treatment  $(64 \, \text{kg N/ha/a})$ . They believed that warming promoted the growth of vascular plants which would compete with denitrifiers for nitrogen to reduce the positive effect of N addition on N<sub>2</sub>O emissions. This mechanism may also work in our experiment since compared with N treatment, warming + N treatment indeed decreased N<sub>2</sub>O emissions. In seasonal emission dynamics, slightly stronger N<sub>2</sub>O emissions in warming + N addition treatment was detected in September which should be mainly attributed to the warming effect.

**Vegetation and N\_2O fluxes.** There were obvious differences in vegetation cover among the four treatments, especially in N addition treatment compared with control. As mentioned above, the effect of each treatment on N $_2$ O fluxes might be indirectly caused by the change of vegetation composition and other environmental factors, such as WTD. Long-term N addition and warming treatment, resulting in significant changes in vegetation composition among treatments, and the impact of this change on N $_2$ O fluxes has exceeded the direct impact of our treat-

ment on  $N_2O$  fluxes. In other words, the long-term cumulative effect caused by global change is greater than the short-term instantaneous effect (Cheng et al. 2016, Moore et al. 2019).

In summary, the study monitored N<sub>2</sub>O emission characteristics of Hani peatland under simulated environmental changes including warming and N addition. We found a near-zero N<sub>2</sub>O emission in the peatland during the whole growing season, and the peatland became a strong N<sub>2</sub>O sink when there is a continuous 100 kg N/ha/a input for 12 years. Warming of about 0.5 °C in the growing season promoted N<sub>2</sub>O emissions, resulting in net N<sub>2</sub>O emissions while the emissions were weakened by N addition, leading to a near-zero N<sub>2</sub>O flux. N<sub>2</sub>O fluxes in all the treatments showed similar seasonal dynamics, with near-zero emissions from May to July, and an absorption peak in late August, which accounted for approximately 45% of the whole growing season flux, and then minor emissions in September. Pearson correlation analysis showed that the water table was the key environmental factor leading to seasonal variation in N<sub>2</sub>O emissions. Furthermore, a high level of N addition exacerbated the death of *Sphagnum*, while warming promoted the growth of vascular plants. Our study suggests that a long-term global change, especially N deposition, may strongly affect N2O fluxes in temperate peatlands, and even change the source or sink function of peatlands, which may be achieved indirectly via affecting hydrology or vegetation.

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