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1	Contribution of the nongrowing season to annual N_2O emissions from the
2	continuous permafrost region in Northeast China
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23	Abstract. Permafrost regions store large amounts of soil organic carbon and nitrogen,
24	which are major sources of greenhouse gas. With climate warming, permafrost
25	regions are thawing, releasing an abundance of greenhouse gases to the atmosphere
26	and contributing to climate warming. Numerous studies have shown the mechanism
27	of nitrous oxide (N ₂ O) emissions from the permafrost region during the growing
28	season. However, little is known about the temporal pattern and drivers of
29	nongrowing season N_2O emissions from the permafrost region. In this study, N_2O
30	emissions from the permafrost region were investigated from June 2016 to June 2018
31	using the static opaque chamber method. Our aims were to quantify the seasonal
32	dynamics of nongrowing season N_2O emissions and its contribution to the annual
33	budget. The results showed that the N_2O emissions ranged from -35.75 to 74.16
34	$\mu g {\cdot} m^{-2} {\cdot} h^{-1}$ during the nongrowing season in the permafrost region. The mean N_2O
35	emission from the growing season were $1.75-2.86$ times greater than that of winter
36	and 1.31-1.53 times greater than that of spring thaw period due to the mean soil
37	temperature of the different specified periods. The nongrowing season N ₂ O emissions
38	ranged from 0.89 to 1.44 kg ha ⁻¹ , which contributed to 41.96–53.73% of the annual
39	budget, accounting for almost half of the annual emissions in the permafrost region.
40	The driving factors of N ₂ O emissions were different among during the study period,
41	growing season, and nongrowing season. The N_2O emissions from total two-year
42	observation period and nongrowing season were mainly affected by soil temperature,
43	while the N ₂ O emissions from growing season were controlled by soil temperature,
44	water table level, and their interactions. In conclusion, nongrowing season N ₂ O





- 45 emissions is an important component of annual emissions and cannot be ignored in
- 46 the permafrost region.
- 47

48 1 Introduction

49 Permafrost regions cover approximately 25% of terrestrial land and store large amounts of nitrogen stocks (31-102 Pg) in soils (Harden et al., 2012). With climate 50 51 warming, permafrost regions are thawing and degrading globally (IPCC, 2013). Large 52 amounts of soil nitrogen have been released to the atmosphere from the permafrost 53 region. Nitrous oxide (N2O) is a major component of N exchanged between terrestrial ecosystems and atmosphere in the permafrost region and feedback to climate warming. 54 N₂O is the third most important greenhouse gas with 265 times the global warming 55 potential of CO₂ and 9 times that of CH₄, which contributes 6% to global climate 56 57 warming (IPCC, 2013). Soil biological processes, which release approximately 60% of total natural N2O emissions, are the largest source of N2O emissions to the 58 atmosphere (IPCC, 2013). Permafrost regions were considered to release negligible 59 60 amounts of N2O emission because of the limited mineral N content. Recently, "hot spots" for N₂O emissions from permafrost regions were found in the subarctic 61 (Marushchak et al., 2011;Repo et al., 2009). The rates of N₂O emissions from bare 62 peatland could reach 31-31.4 mg m⁻² day⁻¹, which are as high as N₂O emissions from 63 64 tropical soil (Marushchak et al., 2011; Repo et al., 2009; Castaldi et al., 2013). The cumulative N₂O emissions range from 0.9 to 1.4 g m^{-2} during the growing season, 65 indicating that the permafrost region is also an important source of N2O emissions 66





- (Repo et al., 2009). In the past, research on N₂O emissions from permafrost regions
 were mainly focused on the growing season (Repo et al., 2009;Gao et al., 2019b;Chen
 et al., 2017). However, in the permafrost region, N₂O emissions from the nongrowing
 season are unclear.
- 71

72 N₂O emissions have been widely researched during the nongrowing season in 73 different ecosystems (Maljanen et al., 2010;Merbold et al., 2013;Furon et al., 2008). A 74 significant release of N₂O emissions have been observed during the nongrowing 75 season, particularly during the spring thaw period. During the nongrowing season, the rates of N₂O emission could be more than 230 g N ha⁻¹ d⁻¹ (Glenn et al., 2012;Flesch 76 et al., 2018; Chantigny et al., 2017) and the cumulative N2O emissions released can be 77 78 as high as 40 kg ha⁻¹ in agricultural soil (Dunmola et al., 2010). The importance of 79 nongrowing season N₂O emissions to the annual budget, which contributed more than 50% of the annual values, have been shown in different ecosystems (Fu et al., 80 2018; Virkajärvi et al., 2010; Yanai et al., 2011). Scientists have focused primarily on 81 82 N₂O emissions during the nongrowing season in the agricultural (Furon et al., 2008; Dietzel et al., 2011), grassland (Virkajärvi et al., 2010; Merbold et al., 2013), 83 forest (Maljanen et al., 2010), wetland (Hao et al., 2006), and tundra ecosystems 84 (Brooks et al., 1997). Nongrowing season N2O emissions are an essential component 85 86 of global N cycling. Permafrost regions, which are characterized by cold temperatures, are mainly distributed in high-latitude and high-altitude areas, and are extremely 87 sensitive to climatic warming. The nongrowing season lasts for more than half of the 88





- year in the permafrost region. Determining nongrowing season N₂O emissions is
 important for accurately evaluating annual N₂O emission from permafrost regions.
 However, N₂O emissions from permafrost regions still remain uncertain during the
 nongrowing season.
- 93

Daxing'an Mountains, located in Heilongjiang province of Northeast China, are 94 95 a unique high latitude and the second largest permafrost region in China. Under the 96 threat of global warming, the permafrost region in the Daxing'an Mountains has been 97 significantly degrading and thawing (Jin et al., 2007). The area of the permafrost region has decreased by 35%, leading to the deepening of the active layer, thinning of 98 the permafrost layer, and increasing ground temperatures, which changes the N cycle 99 100 (Jin et al., 2007). The previous in-situ N_2O measurements from permafrost region of 101 the Daxing'an Mountains have primarily been reported during the growing season or the spring thaw period (Gao et al., 2019b;Cui et al., 2018;Gao et al., 2019a). In the 102 context of global climate warming, N2O emission during the nongrowing season are 103 104 unclear in the permafrost region of the Daxing'an Mountains.

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106 The typical vegetation in the permafrost region of the Daxing'an Mountains is 107 cool-temperate coniferous forest dominated by *Larix gmelinii*, forming the southern 108 boundary of the boreal forest. In this study, in-situ N_2O emission were measured from 109 the permafrost region at three forest sites in the Daxing'an Mountains for two full 110 years. The objectives of this study were to: (i) characterize the nongrowing season





- 111 N₂O emissions from continuous permafrost regions; (ii) evaluate the contributions 112 from the nongrowing season, particularly the spring thaw period, to annual N₂O 113 emissions; and (iii) investigations the key regulatory factors on N₂O emissions. 114 Observation of the nongrowing season N₂O emissions from permafrost regions 115 provides insight into regional climate warming and the impact of the permafrost 116 region on global climate change.
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118 2 Materials and Methods

119 2.1 Site description

The experimental site was located on the continuous permafrost region in the 120 Heilongjiang Mohe Forest Ecosystem Research Station at the Daxing'an Mountains, 121 122 Northeast China (122°06'-122°27'E, 53°17'-53°30'N; 290-740 m elevation). The 123 study region has a typical cold temperate continental climate with a long cold winter and short hot summer. Air temperature ranges from -52.3 to 36.6 °C with a mean 124 annual temperature of -4.9 °C. Mean annual precipitation is 430-550 mm, 60% of 125 126 which falls as rain primarily in the summer. Snow accumulation is 20-40 cm and covers the land for more than half of the year (from October to April). The soil at the 127 study site is primarily brown forest soil, interspersed with meadow soil and marsh 128 129 soil.

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131 The typical vegetation in this permafrost region is a temperate coniferous forest 132 with *L. gmelinii* as the dominant species. Other overstory species include *Betula*





133	platyphylla, Pinus sylvestris var. mongolica. Shrub species include Ledum palustre
134	var. dilatatum, B. fruticose, Vaccinium uliginosum, V. vitis-idaea, Rhododendron
135	dauricum, and Alnus sibirica. Herbaceous species include Carex appendiculata, C.
136	schmidtii, Eriophorum vaginatum, Rubus clivicola, and Sanguisorba officinalis.
137	According the water table level from low to high, three types of typical swamp forests
138	located in the permafrost region were studied: L. gmelinii - Ledum palustre var.
139	dilatatum swamp forest (LL), L. gmelinii - Carex appendiculata swamp forest (LC),
140	and Betula fruticose swamp forest (B). The soil physicochemical properties at the
141	three swamp forests are shown in table 1.
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155 Table 1.

- Soil properties at the three swamp forest sites in the permafrost region of Daxing'an 156
- Mountains, Northeast China (mean \pm SD). 157

Environmental factor	<i>LL</i> site	<i>LC</i> site	<i>B</i> site
WTL	$-13.19 \pm 9.73b$	$-4.51 \pm .51a$	$0.26 \pm 5.67a$
SM ₀₋₁₀	$117.30 \pm 14.92c$	$174.20 \pm 14.58a$	$162.31 \pm 16.14b$
SM ₁₀₋₂₀	49.54 ± 8.28b	$115.86 \pm 10.98a$	115.91 ± 9.13a
pH ₀₋₁₀	$4.77 \pm 0.16c$	$4.99 \pm 0.08a$	$4.89 \pm 0.11b$
pH ₁₀₋₂₀	$4.93 \pm 0.18c$	$5.09 \pm 0.07a$	$4.99 \pm 0.11b$
NH4 ⁺ -N0-10	$5.49 \pm 2.15a$	$5.98 \pm 3.03a$	$4.92 \pm 2.65a$
NH4 ⁺ -N10-20	$3.05 \pm 1.57a$	$3.87 \pm 1.94a$	$3.43 \pm 1.88a$
NO ₃ ⁻ -N ₀₋₁₀	$1.71 \pm 0.73a$	$1.81 \pm 1.02a$	$1.58 \pm 0.63a$
NO ₃ ⁻ -N ₁₀₋₂₀	$1.29 \pm 0.57 ab$	$1.44 \pm 1.02a$	$1.02 \pm 0.42b$
TOC ₀₋₁₀	<mark>39.95</mark> ± 6.91a	$42.01 \pm 4.43a$	$35.57 \pm 5.22 b$
TOC ₁₀₋₂₀	$15.62\pm3.95b$	$18.25\pm2.71a$	16.62 ± 2.1ab
TN_{0-10}	$2.19\pm0.37\text{b}$	3.78 ± 0.51a	$1.97\pm0.69b$
TN ₁₀₋₂₀	$0.83 \pm 0.15 b$	$1.03 \pm 0.21a$	$0.91 \pm 0.13 b$
C/N ₀₋₁₀	$17.94 \pm 4.17a$	$11.27 \pm 144b$	$17.08\pm3.55a$
C/N ₁₀₋₂₀	$19.26\pm5.65a$	$18.29 \pm 4.24a$	$18.57\pm3.82a$

158

WTL, water table level; SM₀₋₁₀, soil moisture at 0-10 cm; SM₁₀₋₂₀, soil moisture 159 at 10-20 cm; pH₀₋₁₀, pH at 0-10 cm; pH₁₀₋₂₀, pH at 10-20 cm; NH₄⁺-N₀₋₁₀, ammonium nitrogen at 0-10 cm; NH4+-N10-20, ammonium nitrogen at 10-20 cm; 160





161	NO_3^- - N_{0-10} , nitrate nitrogen at 0–10 cm; NO_3^- - N_{10-20} , nitrate nitrogen at 10–20 cm;
162	TOC_{0-10} , total organic carbon at $0-10$ cm; TOC_{10-20} , total organic carbon at $10-20$ cm;
163	$TN_{0-10},$ total nitrogen at 0–10 cm; $TN_{10-20},$ total nitrogen at 10–20 cm; $C/N_{0-10},$
164	carbon-to-nitrogen ratio at 0–10 cm; C/N $_{10-20}$, carbon-to-nitrogen ratio at 10–20 cm.
165	
166	2.2 N ₂ O emission measurements
167	The field experiment was conducted from June 2016 to June 2018. Three 20×20
168	m plots were permanently established at each LL, LC, and B site, respectively. N ₂ O
169	emissions were measured with the static opaque chamber technique (Hutchinson et al.,
170	2000). The polypropylene chamber collar with a water-filled channel (50 cm \times 50 cm
171	\times 20 cm height) was randomly inserted 20 cm into the soil. Gas samples were
172	measured from 9:00 am to 11:00 am, the hours that were most representative of the
173	daily mean N ₂ O emissions (Alves et al., 2012). During each N ₂ O measurement period,
174	chambers (50 cm \times 50 cm \times 50 cm height) were sealed by filling the collars with
175	water and used to collect N_2O from the soil. Four-chamber headspace air samples
176	were taken using a 50-mL plastic syringe at 0, 15, 30, and 45 min after chamber
177	closure (Liu et al., 2019). Samples were injected into pre-evacuated 100 mL gas
178	sampling bags (Delin Gas Packing Co., Dalian, China) for subsequent laboratory
179	analysis. The air temperature inside the chamber was recorded when gas samples were
180	being retrieved. Gas samples were taken twice per month during the growing season
181	from June to September, monthly during the winter from October to December, and

182 every three to ten days during the spring thaw period from March to May (45





- 183 sampling events in total).
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185	The gas N_2O concentration was analyzed with a gas chromatograph coupled with
186	an electron capture detector (ECD) (Shimadzu GC2010, Shimadzu Analytical and
187	Measuring Instruments Division, Kyoto, Japan). Compressed air containing 0.378
188	ppm N_2O was used for calibration. N_2 was used as the carrier gas with a flow rate of
189	20 mL min ⁻¹ . The N_2O was separated using a 1-m stainless steel column with an inner
190	diameter of 2 mm from Porapak Q (80/100 mesh), and was detected by an ECD. The
191	temperature for gas separation was maintained at 70 $^\circ C$ and the detector was set at
192	250 °C.

193

194 2.3 Measurements of meteorological and soil physiochemical properties

Soil temperature (ST) at 5, 10, and 15 cm deep was monitored at each collar using a portable digital thermometer with a thermocouple probe (JM-624, Jinming Corp., Tianjin, China). During the growing season, the water table level (WTL) was measured near the chamber in each plot using a ruler (Dobbie and Smith, 2006). In

199 the nongrowing season, soil moisture was determined by the oven-drying mothed.

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Using a 3.8 cm diameter stainless-steel sampling probe, soil samples were taken from the upper 0–10 cm and lower 10–20 cm soil layers close to each collar. Fine roots and visible organic debris were removed by passing the soil samples through a 2-mm sieve. Then, samples were stored in an insulated box (Esky) and stored at 4 °C





- 205 for subsequent chemical analysis.
- 206

Soil moisture contents were determined by drying at 105 °C for 48 h followed by 207 calculating the weight loss. Soil samples were air dried and sieved to <2 mm 208 209 aggregate size and used to measure the soil pH. Soil pH was measured in a 2:5 air-dried soil: deionized water mixture using an InoLab pH meter (WTW InoLab pH 210 211 730, Weilheim, Germany). Soil mineral N, ammonium (NH_4^+ -N), and nitrate (NO_3^- -N) 212 content were determined on 10 g samples of fresh soil based on a 1 mol/L KCl 213 solution extraction procedure. The extracts were filtered through a 0.45 µm pore-diameter syringe filter, and then soil mineral N was analyzed using a Lachat 214 flow-injection auto-analyzer (Seal Analytical AA3, Norderstedt, Germany). For the 215 216 analyses of total N and C, sub-samples were further ground to a fine powder (<0.15217 mm). Total N concentration was measured on aliquots of 1.0 g of soil using semi-micro-Kjeldahl method. The TOC content was determined by oxidation at 218 170 °C, with potassium dichromate in the presence of sulfuric acid. The excess 219 220 potassium dichromate was titrated with a solution of Mohr's salt.

221

222 2.4 Statistical analysis

The N₂O emissions were calculated as reported by (Hou et al., 2012). A positive regression indicates the emission from soil to the atmosphere. A negative regression indicates a net uptake by the soil from the atmosphere. Cumulative N₂O emissions were linearly and sequentially accumulated from the emissions between every two





227 adjacent intervals of the measurements following the procedure described by Ding et

228	al. (2007).
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The calendar year was divided into three seasons: winter, spring thaw period, and growing season. The winter was defined as the period during which the daily mean air temperature remained below 0 °C for at least five consecutive days. The spring thaw period was defined as the period when the daily maximum air temperature exceeded 0 °C and ended at soil thawing to a depth of 20 cm. The growing season was defined as the period that lasts from the end of the spring thaw period to the beginning of winter. We designated the winter and spring thaw period as the nongrowing season.

237

238 A one-way analysis of variance was used to test the difference of N₂O emissions 239 and environment factors in the three swamp forest sites. A T-test was used to identify the differences in N_2O emissions between 2016/2017 and 2017/2018. The correlations 240 between the N₂O emissions and environmental factors were tested using Pearson's 241 242 correlation analysis. A linear correlation analysis and multivariate regression analysis were conducted to create explanatory models using the same variables as those used 243 for describing the temporal variation of N₂O emissions. R software (Version 3.4.1, 244 https://www.r-project.org/) was used for the statistical analyses. Significance was 245 246 analyzed using Fisher's least significant difference (LSD) test at a probability level of 95% (P < 0.05). All figures were drawn using OriginPro 2018 software (OriginLab 247 Corp., Northampton, MA, U.S.A.). 248





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250	2	Doculto
250	Э	results

251 3.1 Temporal variation of N₂O emissions

During the two-year observation period, there was significant temporal variation 252 253 in N₂O emissions in the permafrost region of the Daxing'an Mountains (Fig. 1a). However, the temporal pattern of N₂O emissions were different in the three swamp 254 255 forests. The N₂O emissions ranged from -17.40 to 74.16, -35.75 to 64.73, and -26.47to 79.25 μ g·m⁻²·h⁻¹ in the *LL*, *LC*, and *B* sites, respectively (Fig. 1a). The highest N₂O 256 257 emissions occurred in different periods in three swamp forests, namely, they occurred 258 in the beginning of the spring that period in the LL site and at the end of growing season in the LC and B sites. The lowest N₂O emissions from the three swamp forest 259 260 sites were all observed in the spring thaw period. Negative emissions mainly occurred during the winter and spring thaw period. The N₂O emissions during the nongrowing 261 season mainly ranged from -17.40 to 74.16, -35.75 to 60.76, and -26.47 to 71.82 262 263 $\mu g \cdot m^{-2} \cdot h^{-1}$ in the *LL*, *LC*, and *B* sites, respectively.



265 Figure 1. N₂O emissions (A) and cumulative N₂O emissions (B) from three types of





swamp forests in the permafrost region of the Daxing'an Mountains, Northeast China

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268	The annual mean N_2O emissions ranged from 27.80 to 32.51, 26.10 to 37.89, and
269	25.86 to 33.16 μ g·m ⁻² ·h ⁻¹ in the <i>LL</i> , <i>LC</i> , and <i>B</i> sites, respectively (Table 2). The mean
270	N_2O emissions from the growing season typically higher than that of winter and the
271	spring thaw period in the three swamp forest sites. In 2016/2017, the mean N_2O
272	emissions were all highest in the growing season and lowest in the winter. In contrast,
273	during 2017/2018, the mean N_2O emissions were lowest during the spring that
274	period in the LC site and highest during the spring that period in the B site. For the
275	different types of swamp forests, the mean N2O emissions from the LC site were
276	significantly higher than the mean N_2O emissions in the <i>B</i> site in the 2017/2018
277	growing season. There was no significant difference in N_2O emissions during the
278	winter, spring thaw period, and annually in the three swamp forests. The $N_2 O$
279	emissions from different periods were generally not significantly different between
280	the two years. Differences in N_2O emissions were found during the growing season
281	and spring thaw period. The mean N_2O emissions during the growing season from the
282	LC site and the N ₂ O emissions during the spring that period from the B site were
283	both significantly higher in 2017/2018 than 2016/2017.



Table 2.	
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Summary of N2O emissions during specified periods from the three swamp forest sites in the permafrost region of the Daxing'an Mountains, 286

287 Northeast China

l		Duri	ation			Mean N ₂ O emissi	ons $(\mu g \cdot m^{-2} \cdot h^{-1})$		
	Specified period	(D	ays)		2016/2017			2017/2018	
		2016/2017	2017/2018	LL site	LC site	B site	LL site	LC site	B site
De 17	Growing season	134	129	40.42 ± 15.97Aa	$29.54\pm7.30Ab$	38.35 ± 23.42Aa	$40.09 \pm 14.68 \mathrm{ABa}$	47.73 ± 12.27Aa	33.17 ± 12.48Ba
	Nongrowing season								
	Winter	155	161	$19.80\pm34.74\mathrm{Aa}$	$9.62\pm29.69\mathrm{Aa}$	17.41 ± 33.14Aa	$14.95\pm26.23\mathrm{Aa}$	33.31 ± 16.25Aa	$8.13\pm22.89\mathrm{Aa}$
	Spring thaw period	LL	75	22.49 ± 24.90Aa	27.56 ± 26.06Aa	$20.89 \pm 21.73 Ab$	32.74 ± 16.69 Aa	31.04 ± 31.31 Aa	$41.66\pm18.78\mathrm{Aa}$
	Annual	366	365	$27.80\pm24.36\mathrm{Aa}$	$26.10 \pm 22.44 Aa$	25.86 ± 24.06 Aa	$32.51\pm18.31\mathrm{Aa}$	37.89 ± 22.26Aa	$33.16\pm19.55\mathrm{Aa}$
286	3 The different	capital letter	rs indicate th	hat the N ₂ O emist	sions were signif.	icantly different amon	ig the types of swan	np forest; differen	it lowercase

letters indicate that the N2O emissions were significantly different between the two years. 289







290 3.2 Seasonal contribution of N2O emissions to the annual budget

291	Cumulative N ₂ O emissions primarily increased during the study period (Fig. 1b).
292	The annual N_2O emissions ranged from 2.27 to 2.68, 1.92 to 2.90, and 2.00 to 2.24 kg
293	ha^{-1} yr ⁻¹ in the <i>LL</i> , <i>LC</i> , and <i>B</i> sites, respectively (Table 3). The cumulative N ₂ O
294	emissions during the growing season ranged from 1.02 to 1.46 kg ha $^{-1}$, which
295	contributed to 46.27 to 58.04% to the annual emissions. The cumulative $N_2 O$
296	emissions from the growing season were higher than the cumulative $N_2 O$ emissions
297	during the winter and spring thaw periods, and were 1.2 to 3.2 times greater that
298	of the winter and 1.5 to 3.7 times greater than that of the spring thaw period. The
299	cumulative N_2O emissions during the nongrowing season were mainly lower than
300	during the growing season, which contributed to 41.96–53.73% to annual emissions.
301	The cumulative N_2O emissions during the spring thaw period ranged from 0.35 to
302	0.66 kg ha ^{-1} , contributing to 15.63 to 33.00% to the annual emissions.
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Table 3.	
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The cumulative N₂O emissions and its contribution to annual N₂O emissions from the three swamp forest sites in the permafrost region of 305

306 Daxing'an Mountains, Northeast China.

	Voor		Cumulativ	e N2O emissio	ns (kg ha ⁻¹)		Contri	bution to annus	al N2O emissioi	IS (%)
rotest types	ICAI	Annual	GS	NGS	Winter	STP	GS	NGS	Winter	STP
LL site	2016/2017	2.68 ± 0.15	1.24 ± 0.06	1.44 ± 0.21	1.00 ± 0.20	0.44 ± 0.04	46.27	53.73	37.31	16.42
	2017/2018	2.27 ± 0.16	1.21 ± 0.07	1.06 ± 0.18	0.56 ± 0.16	0.50 ± 0.03	53.30	46.70	24.67	22.03
LC site	2016/2017	1.92 ± 0.14	1.03 ± 0.13	0.89 ± 0.13	0.48 ± 0.11	0.41 ± 0.02	53.65	46.35	25.00	21.35
	2017/2018	2.90 ± 0.11	1.46 ± 0.03	1.44 ± 0.10	0.93 ± 0.12	0.51 ± 0.03	50.34	49.66	32.07	17.59
B site	2016/2017	2.24 ± 0.21	1.30 ± 0.15	0.94 ± 0.08	0.58 ± 0.07	0.35 ± 0.01	58.04	41.96	25.89	15.63
	2017/2018	2.00 ± 0.22	1.02 ± 0.11	0.98 ± 0.22	0.32 ± 0.20	0.66 ± 0.08	51.00	49.00	16.00	33.00



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GS: growing season; NGS: nongrowing season; STP: spring thaw period.





309 3.3 Temporal control of soil N₂O emissions

310	The relationship between N ₂ O emissions and environmental factors during the
311	different specified periods are shown in Table 4. During the entire two-year
312	observation period, the N_2O emission were all significantly positively correlated with
313	soil temperature at 5, 10, and 15 cm in the three swamp forest sites. Except for the soil
314	temperature, the N_2O emissions from the LL site were also positively correlated with
315	air temperature (P<0.05) and C/N ₀₋₁₀ (P<0.05) and negatively correlated with pH_{0-10}
316	(P<0.01), pH_{10-20} (P<0.05), $NO_3^-N_{0-10}$ (P<0.05), $NO_3^-N_{10-20}$ (P<0.05), and TN_{0-10}
317	(P<0.05). The N ₂ O emissions from the LC site were significantly positively correlated
318	with TOC ₀₋₁₀ (P<0.01) and TN ₁₀₋₂₀ (P<0.01). The N ₂ O emissions from the <i>B</i> site were
319	significantly positively correlated with air temperature (P<0.001), NH_4^+ - N_{0-10}
320	(P<0.05), and NH ₄ ⁺ -N ₁₀₋₂₀ (P<0.05). Similar to the entire period, the N ₂ O emissions
321	from the nongrowing season were mainly significantly positively correlated with soil
322	temperature in the LC and B sites and weakly positively correlated with soil
323	temperature in the LL sites. The N ₂ O emissions from the LL site were also
324	significantly negatively correlated with $pH_{0\mathchar`-10}$ (P<0.05) and TN_{0\mathchar`-10} (P<0.001); the
325	N_2O emissions from the <i>LC</i> site were significantly positively correlated with TN_{0-10}
326	(P<0.05). The N ₂ O emissions from the <i>B</i> site were significantly positively correlated
327	with air temperature (P<0.05), NH_4^+ - N_{0-10} (P<0.01), NH_4^+ - N_{10-20} (P<0.01), and
328	$NO_3^N_{0-10}$ (P<0.05). For the growing season, the impact of environmental factors on
329	N_2O emissions were complicated. The N_2O emissions were significantly positively
330	correlated with T_{15} (P<0.05) and negatively correlated with NO ₃ ⁻ -N ₀₋₁₀ (P<0.05) and





- 331 $NO_3^--N_{10-20}$ (P<0.01) in the LL site. The N₂O emissions from LC site were
- 332 significantly influenced by air temperature, water table level, NO₃⁻-N, TOC, TN, and
- 333 C/N ratio. The N₂O emissions from the B site were only significantly positively
- 334 correlated with water table level (P < 0.05).





The relationship between N₂O emissions and environmental factors from three swamp forest sites in the permafrost region of the Daxing'an 336

Table 4.

335





·· * *	it $P < 0.01$	nt effects a	ndicates significa	0.05; **: ii	sets at $P < 0$	gnificant effe	indicates sig	P < 0.1; *:	nt effects at	: indicates significa
	0.05	-0.13	-0.13	0.04	-0.54***	-0.18	0.04	-0.14	0.06	C/N ₁₀₋₂₀
	-0.14	-0.17	0.10	-0.05	0.53***	0.13	-0.08	0.15	0.21*	C/N_{0-10}
	-0.15	0.26*	-0.06	-0.16	0.65***	0.19	-0.16^+	0.26^{**}	0.01	TN_{10-20}
	0.03	0.14	-0.43***	-0.04	-0.35*	0.08	-0.03	0.06	-0.21*	TN_{0-10}
	-0.09	0.11	-0.14	-0.01	-0.02	0.03	-0.06	0.10	0.08	TOC 10-20
	-0.05	0.01	-0.16	-0.15	0.48^{***}	0.23	-0.14	0.26^{**}	0.13	TOC_{0-10}
	0.03	0.11	0.01	-0.02	0.36*	-0.36**	-0.11	0.0	-0.22*	$NO_{3}^{-}-N_{10-20}$
	0.29*	0.09	-0.08	-0.16	0.28*	-0.32*	0.01	0.05	-0.24*	NO3 N ₀₋₁₀
	0.34**	0.15	0.21^{+}	0.14	-0.23	-0.08	0.24*	0.01	0.05	$\rm NH_4^{+-}N_{10-20}$
	0.32**	-0.11	0.07	-0.06	0.19	0.21	0.20*	0.05	0.08	$\rm NH_{4}^{+-}N_{0-10}$
	-0.19	0.22^{+}	-0.17	0.05	0.14	-0.05	-0.12	0.15	-0.21*	pH_{10-20}



338

indicates significant effects at P < 0.001.





340	The Pearson correlation analysis showed that the soil temperature was the key
341	environmental factor controlling the N_2O emissions during the entire observation
342	period and the nongrowing season. During the entire observation period, soil
343	temperature at 5, 10, and 15 cm could explain 10.39 to 14.48, 6.07 to 8.34, and 10.66
344	to 12.02% of the temporal variation of N ₂ O emissions in the LL, LC, and B sites,
345	respectively. During the nongrowing season, N_2O emissions from the LL site were
346	weakly positively correlated with soil temperature, explaining 1.73 to 3.27% of N_2O
347	emissions. The soil temperature could explain 5.02 to 9.54% and 7.51 to 9.36% of the
348	N_2O fluctuation in the <i>LC</i> and <i>BC</i> sites, which were lower than the entire observation
349	period.







351

Figure 2. The linear models between N_2O emissions and soil temperature during the entire observation period and nongrowing season in the three swamp forest sites in the permafrost region of the Daxing'an Mountains, Northeast China.

355

During the growing season, multivariate regression analyses showed that the N₂O emissions were affected by soil temperature, water table level, and their interactions (Table 5). Soil temperature, water table level, and their interactions could explain 26.35, 19.46, and 12.36% of the temporal variation of N₂O emissions in the three swamp forest sites, respectively.





361

362 Table 5.

- 363 Models of N₂O emissions during the growing season against soil temperature and
- 364 water table level for the three swamp forest sites in the permafrost region of the

Forest type	а	b	с	d	R ²	Р
LL site	-11.55	4.03	-3.19	0.25	0.2635	< 0.001
LC site	22.44	0.94	-3.99	0.28	0.1946	< 0.01
<i>B</i> site	27.57	0.77	-1.50	0.21	0.1236	< 0.05

365 Daxing'an Mountains, northeast China.

366 The regression models are: $y = a + b \times T_5 + c \times WTL + d \times T_5 \times WTL$, where a, b, c, and d

367 are the regression coefficients.

368

369 4 Discussion

370 **4.1 Soil temperature controls the mean N₂O emissions from the different periods**

In previous studies, the N₂O emissions from the permafrost region primarily 371 focused on emissions during the growing season (Gil et al., 2017;Lamb et al., 372 373 2011; Voigt et al., 2017). Permafrost regions are mainly distributed in high-altitude and high-latitude zones. It was difficult to measure N2O emissions during the nongrowing 374 season in the cold climate conditions of the permafrost region. Publications on 375 nongrowing season N₂O emissions are scarce and the difference of mean N₂O 376 377 emissions among the winter, spring thaw period, and growing season are unknown in 378 the permafrost region.





379

380	The nongrowing season N_2O emission ranged from –35.75 to 74.16 $\mu g \cdot m^{-2} \cdot h^{-1}$
381	in the permafrost region of the Daxing'an Mountains, northeast China. The results
382	were similar to the rate of annual N_2O emission (–35.75 to 79.25 $\mu g {\cdot} m^{-2} {\cdot} h^{-1})$ in the
383	permafrost region of the Daxing'an Mountains and within the range of N_2O emissions
384	reported in permafrost ecosystems (–35.75 to 2662 $\mu g \cdot m^{-2} \cdot h^{-1}$) (Gao et al., 2019a;Mu
385	et al., 2017). The N_2O emissions confirmed our previous findings that the N_2O
386	emissions from the Daxing'an Mountains ranged within the intermediate range for
387	permafrost ecosystems (Gao et al., 2019b).

388

The annual N₂O emissions showed significant temporal variations in grasslands 389 390 (Du et al., 2006). There were significant differences in the mean N₂O emissions in the 391 spring, summer, autumn, and winter in grasslands, whereas the temporal pattern over the course of the five-year study (Du et al., 2006). The N₂O was taken up during the 392 freezing period and emitted during the thawing period and growing season in marshes, 393 394 indicating that the emissions were different among the three specified periods (Hao et 395 al., 2006). These trends were also observed in the permafrost region. In the "hot spots" of N2O emission from permafrost region, high N2O emissions were observed during 396 the nongrowing season in the bare peatland, which contributed 20-69% to the annual 397 398 emissions from the bare peatland (Marushchak et al., 2011). In the vegetated permafrost ecosystem, the N₂O emissions during the nongrowing season, growing 399 season, and annually were mainly negligible (Marushchak et al., 2011). The N₂O 400





401 emissions from the spring thaw period and nongrowing season were lower than that of 402 growing season in the permafrost region of the Daxing'an Mountains (Chen et al., 2017;Gao et al., 2019a;Wu et al., 2019). However, the drivers of N₂O emissions 403 between the nongrowing season and growing season were not clear in the permafrost 404 405 region. Our results showed that the N2O emissions were the highest during the growing season and lowest during the nongrowing season. The mean N₂O emissions 406 407 from the growing season were significantly higher than the emissions from the winter 408 in the LL and B sites, whereas the N₂O emissions during the spring thaw period was 409 not significantly different from growing season and winter in the three swamp forests 410 (Fig. 3). The N_2O emissions from the growing season were 1.75–2.86 times greater than the winter emissions and 1.31-1.53 times greater than during the spring thaw 411 412 period in the three forest types.



413

414 Figure 3. The difference of N₂O emissions among the growing season, winter, and

415 spring thaw period in the permafrost region of the Daxing'an Mountains, Northeast





- 416 China.
- 417

We found that the mean N₂O emissions of the three specified periods were 418 significantly positively correlated with soil temperature at 5, 10, and 15 cm, which 419 420 could explain 91.36–94.07, 91.97–95.92, and 81.71–92.85% of the temporal variation of mean N_2O emissions in the *LL*, *LC*, and *B* sites, respectively (Fig. 4). In a 421 422 laboratory experiment, the N₂O emissions were strongly dependent on soil 423 temperatures above zero (Oquist et al., 2004). The net N₂O production rates at -4 °C 424 equaled those observed at +10 to +15 °C in the boreal forest soil (Oquist et al., 2004). However, the field soil temperature in the winter in the Daxing'an Mountains was 425 significantly lower than the simulated cold temperature in the laboratory, meaning that 426 427 the winter N₂O emissions may be lower than N₂O emissions during the growing 428 season (Oquist et al., 2004). In the nongrowing season, soil moisture was consistently saturated in the 0-10 cm soil layer of three swamp forests, which implies that N₂O 429 production occurred predominantly due to denitrification. The denitrification rates 430 431 showed similar temporal variations of N2O emissions in agricultural soil in the winter (Tatti et al., 2014). The copy numbers of denitrifier genes (nirS and nirK) remained 432 stable from November to January and increased in March and April, indicating that 433 N₂O emissions during the spring thaw period were higher than during the winter (Tatti 434 435 et al., 2014). During the growing season, N₂O emissions were significantly positively correlated with soil temperature in the permafrost region (Marushchak et al., 2011;Cui 436 et al., 2018; Chen et al., 2017). Thus, the soil temperature controlled the mean N₂O 437





- 438 emissions during the winter, spring thaw period, and growing season. Soil
- 439 temperature was the key environmental factor determining the temporal variation of
- 440 N₂O emissions in the permafrost region of the Daxing'an Mountains.



Figure 4. The relationship between mean N₂O emission from different specified
periods and soil temperature in the permafrost region of the Daxing'an Mountains,
Northeast China.

446

447 **4.2 Contribution of nongrowing season to annual N₂O budget**

The cumulative N₂O emissions from the permafrost region was mainly evaluated
during the growing season (Repo et al., 2009;Takakai et al., 2008;Gao et al., 2019b).





450	The N ₂ O emissions from the permafrost region were as high as 14 kg ha^{-1} and
451	released approximately 0.1 Tg yr^{-1} N ₂ O emissions to the atmosphere in the bare peat
452	region of the Arctic, accounting for up to 0.6% of the global annual N ₂ O emissions
453	(Repo et al., 2009). A few studies have indicated that the nongrowing season
454	contributed greatly to the annual N ₂ O emissions from the frigid terrestrial ecosystems
455	(Li et al., 2012;Zhang et al., 2018;Fu et al., 2018). However, the contribution of the
456	nongrowing season to the annual N ₂ O budget is uncertain in the permafrost region.
457	
458	The cumulative N ₂ O emissions during the nongrowing season ranged from 0.89
459	to 1.44 kg ha ⁻¹ , which contributed to 41.96–53.73% of the annual budget in the
460	permafrost region of the Daxing'an Mountains. In frigid terrestrial ecosystems, the
461	N_2O emissions of the nongrowing season contributed to 20–74% of the annual
462	emissions; therefore, our results were within the range of previous studies (Li et al.,
463	2012;Zhang et al., 2018;Fu et al., 2018;Marushchak et al., 2011). During the spring
464	thaw period, the soil temperature and soil moisture dramatically changed, which
465	significantly affected the release of N_2O emissions. A pulse or burst of N_2O emissions
466	have been observed in agriculture (Flesch et al., 2018), grassland (Virkajärvi et al.,
467	2010), forest (Wu et al., 2010), marsh (Song et al., 2008), and peat ecosystems (Flesch
468	et al., 2018). The pulse N_2O emissions during the spring thaw period had enormous
469	influence on the contribution of the nongrowing season to the annual budget in the

470 frigid terrestrial ecosystems (Li et al., 2012;Fu et al., 2018). When the pulse of N_2O

471 emissions occurred during the spring thaw period, emissions from the non-growing





472	seasons dominated (67–74%) the annual total emissions (Fu et al., 2018). When no
473	pulse of N_2O emissions were found during the spring thaw period, the contribution of
474	the spring thaw period to the total annual N ₂ O budget was very small and accounted
475	for only 6.6% of the annual emissions (Li et al., 2012). In the present study, there was
476	no significant large burst of N2O emissions during the spring thaw period. The
477	cumulative N_2O emissions during the spring thaw period ranged from 0.35 to 0.66 kg
478	ha ⁻¹ and contributed 15.61 to 33.00% of the annual budget in the permafrost region of
479	the Daxing'an Mountains, and these ranges were generally lower than the emissions
480	during the winter and growing season.

481

The mean N₂O emissions were the lowest in the winter and highest in the 482 483 growing season, which were not the same as the cumulative N₂O emissions in the three swamp forests. The permafrost region of the Daxing'an Mountains was located 484 at a high latitude, which had a longer nongrowing season than growing season. In the 485 permafrost region of the subarctic, the nongrowing season lasts for more than 9 486 mouths (283 days) (Marushchak et al., 2011). The length of winter was more than 487 twice of spring thaw spring in the Daxing'an mountains. Although the mean N2O 488 emissions in winter were lower than during spring thaw period, the cumulative N2O 489 emissions in winter were higher than the emissions from the spring thaw period. The 490 491 cumulative N₂O emissions during winter were as important as the spring thaw period. Only one study reported the cumulative N₂O emissions during nongrowing seasons in 492 the permafrost region. Marushchak et al. (2011) found that the N₂O emissions from 493

515





494	the nongrowing season contributed 20–69% to the annual emissions in the bare peat
495	zone of the permafrost region. Our results confirmed that half of the N_2O emissions
496	were released during the nongrowing season, indicating that the N2O emissions during
497	the nongrowing season cannot be ignored in the permafrost regions. In the future, the
498	N_2O emissions of the nongrowing season should be emphasized in the permafrost
499	region, especially in the context of global climate change.
500	
501	4.3 Drivers of N2O emissions on different temporal scales
502	Most previous studies on the permafrost region have focused exclusively on the
503	growing season (Gao et al., 2019b; Repo et al., 2009; Cui et al., 2018). The N_2O
504	emissions during the growing season were mainly influencing by air temperature, soil
505	temperature, water table level, soil moisture, precipitation, pH, NH4+-N, NO3-N,
506	TOC, gross N mineralization, N content, and C/N ratio in the permafrost region (Ma
507	et al., 2007;Gil et al., 2017;Marushchak et al., 2011;Chen et al., 2017;Cui et al.,
508	2018;Paré and Bedard-Haughn, 2012). However, the drivers of nongrowing season
509	N ₂ O emission remain unknown in the permafrost region.
510	
511	N ₂ O production processes are very complex and can be produced by nitrification,
512	nitrifier denitrification, and denitrification in the permafrost region (Ma et al.,
513	2007;Gil et al., 2017;Siljanen et al., 2019). Soil water content controls the redox
514	conditions in the soil, which determines the pathway of N_2O emissions. The soil water

content showed significant temporal variation in the permafrost region of the





516	Daxing'an Mountains. Thus, the pathway of N_2O emissions may be different in the
517	nongrowing season and growing season. During the growing season, the water table
518	level ranged from -31.6 to 10.27 cm in the three swamp forests. N ₂ O emission may
519	have come from simultaneous nitrification and denitrification in the LL site and
520	denitrification in the LC and BC sites (Gao et al., 2019b). In the 2016 growing season,
521	the N ₂ O emissions mainly controlled multiple environmental factors (Gao et al.,
522	2019b). Our results show that the N_2O emissions were driven by soil temperature and
523	water table level and their interactions, explaining 12.36-26.35% of temporal
524	variation of N_2O emissions during the two growing seasons. As the permafrost
525	ecosystem is strongly temperature limited, the N2O emissions were mainly
526	significantly positively correlated with soil temperature in the permafrost region
527	(Marushchak et al., 2011;Cui et al., 2018;Chen et al., 2017). Soil temperature could
528	increase nitrification and denitrification, thus promoting the release of N_2O flux. Wu
529	et al. (2019) found that the N_2O emissions were significantly negatively correlated
530	with soil moisture in the three forests of the Daxing'an Mountains. Our results
531	confirm that the decrease of the water table level was beneficial to the release of $N_2 O$
532	emissions. Soil temperature and water table level were key factors controlling the
533	emission of N ₂ O during the growing season. In contrast, the nongrowing season N ₂ O
534	emissions were mainly controlled by soil temperature in the permafrost region of the
535	Daxing'an Mountains.

536

537 During the nongrowing season, the soil moisture was consistently exceeded 60%





538	in the three swamp forests. The N_2O emissions were major produced by
539	denitrification in the permafrost region. The nongrowing season N ₂ O emissions were
540	positively correlated with soil temperature in the three swamp forests. The denitrifier
541	genes were stable during the winter and increased during the spring thaw period (Tatti
542	et al., 2014). Wertz et al. (2013) found that the community structures of denitrifiers
543	were different between below-zero and above-zero temperatures. During the
544	nongrowing season, the soil temperature affected the abundance and community
545	structures of denitrifiers and thus the release of N_2O emissions in the permafrost
546	region of the Daxing'an Mountains. In the field, environmental factors are always
547	changing; therefore, any factor can be a limiting factor for a long period. The $N_2 O$
548	emissions from the two-year study period were controlled by soil temperature in the
549	permafrost region of the Daxing'an Mountains. Soil temperature was the major
550	limiting factor related to annual N2O emissions in the permafrost region. Except for
551	soil temperature, the N ₂ O emissions from the permafrost region were also affected by
552	pH, NO ₃ -N, TN, and C/N ratio.

553

554 5 Conclusions

The N₂O emissions from the nongrowing season were quantified in the permafrost region. The N₂O emissions ranged from -35.75 to $74.16 \ \mu g \cdot m^{-2} \cdot h^{-1}$ during the nongrowing season. The mean N₂O emissions were lowest in the winter and highest in the growing season, and were controlled by soil temperature. The cumulative N₂O emissions during the nongrowing season greatly contributed to the

560





561	emissions had different key limiting factors in the permafrost region. The nongrowing
562	season and the annual N ₂ O emissions were driven by soil temperature, whereas the
563	growing season N ₂ O emissions were affected by soil temperature, water table level,
564	and their interaction.
565	
566	Data availability. The data used in the present study are available in the Supplement.
567	
568	Supplement. The supplement related to this article is available online at:
569	https://doi.org/
570	
571	Author contributions. Dawen Gao and Hong Liang designed and guided the
572	experiment, provided supervision, and contributed to revise of the manuscript. Tijiu
573	Cai and Houcai Sheng provided and set up field experimental sites. Liquan Song
574	conducted laboratory analysis. Weifeng Gao conducted the field sampling, analyzed
575	the data and wrote the manuscript.
576	
577	Competing interests. The authors declare that they have no conflict of interest.
578	

annual budget, which cannot be ignored. In the different periods studied, N2O

- 579 Acknowledgements. We are sincerely grateful to Heilongjiang Mohe Forest Ecosystem
- 580 Research Station.
- 581





- 582 Financial support. This work was supported by the National Natural Science
- 583 Foundation of China (No. 31870471, 31971468).
- 584
- 585 *Review statement*. This paper was edited by and reviewed by.
- 586
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