



1 Effects of water table level and nitrogen deposition on methane and 2 nitrous oxide emissions in an alpine peatland

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11 Abstract

12 Alpine peatlands are recognized as a major natural contributor to the budgets of atmospheric methane (CH₄) but as a weak
13 nitrous oxide (N₂O) source. Anthropogenic activities and climate change have put these fragile nitrogen (N)-limited peatlands
14 under pressure by altering water table (WT) levels and enhancing N deposition. The response of greenhouse gas (GHG)
15 emissions from these peatlands to these twin changes is uncertain. To address this knowledge gap, we conducted a mesocosm
16 experiment in 2018 and 2019 investigating individual and interactive effects of three WT levels (WT₋₃₀, 30 cm below soil
17 surface; WT₀, soil-water interface; WT₁₀, 10 cm above soil surface) and multiple levels of N deposition (0, 20, 40, 80 and 160
18 kg N ha⁻¹ yr⁻¹) on growing season CH₄ and N₂O emissions in the Zoige alpine peatland, Qinghai-Tibetan Plateau. We found
19 that the elevated WT levels increased CH₄ emission, while the N deposition had non-linear effects (stimulation at moderate
20 levels and inhibition at higher). In contrast no clear pattern of the effect of WT levels on the cumulative N₂O emission was
21 evident, while N deposition led to a consistent and linear increase (emission factor: 2.3%-2.8% and 1% in IPCC), and this was
22 dependent on the WT levels. Across the two years, the scenario with the greatest GWP (from CH₄ and N₂O) was an N deposition
23 of ca. 20 kg N ha⁻¹ yr⁻¹ and high WT levels (at soil surface or above). Given the current N deposition in the Zoige alpine
24 peatland (1.08-17.81 kg N·ha⁻¹), our results suggested that the CH₄ and N₂O emissions from the alpine peatlands could greatly
25 increase in response to the possible doubling N deposition in the future. We believe that our results provide insights into how
26 interactions between climate change and human disturbance will alter GHG emissions from this globally important habitat.

27 **Keywords** Cumulative GHG emissions; multi-level N enrichment; critical threshold; non-linear effect; Qinghai-Tibetan
28 Plateau

29 1. Introduction

30 Peatlands only cover ca. 3% of the land surface of the Earth but store one third of the global carbon pool (Yu et al. 2010).



2

31 In pristine peatlands, the shallow water table (WT) and waterlogged conditions allow accumulation of organic matter and
32 favour anaerobic production of methane (CH_4) and nitrous oxide (N_2O). Traditionally, the large carbon pool is nitrogen
33 deficient and is recognized as a moderate CH_4 source and a weak N_2O source (Frolking et al. 2011, Han et al. 2019).
34 Nevertheless, these conditions could be markedly changed by anthropogenic disturbance and climate change, and growing
35 evidence shows that peatlands are experiencing drainage and increasing nitrogen deposition (Chen et al. 2013, Evans et al.
36 2021). Consequently, the magnitude of CH_4 and N_2O emissions from peatlands is potentially changing from low to high. The
37 high-altitude or alpine peatlands are of particular interest because this carbon-rich ecosystem is vulnerable and highly sensitive
38 to the climate change and anthropogenic activities (Squeo et al. 2006).

39 Large-scale artificial drainage of peatlands was initiated hundreds of years ago and escalated in the 20th century (Evans
40 et al. 2021). As a result, about 10-20% of the global peatlands were primarily drained for the purposes of agriculture, peat
41 extraction and forestry (Frolking et al. 2011). The resulting lower WT altered the anaerobic conditions of the peat soil and led
42 to oxidative loss of peat (Laine et al. 2019, Wilson et al. 2016). Generally, the drainage decreased the CH_4 efflux and increased
43 N_2O emissions (Cao et al. 2017). The increase in N_2O emissions from drained peatlands is small, but may potentially reach a
44 high level at sufficient nutrient input, especially when the soil is fertilized (Laine et al. 2019). Ecological restoration has been
45 proposed as a measure to conserve the drained or degraded peatlands, particularly in order to meet the demand for mitigation
46 of GHG emissions outlined in the Paris Agreement (Evans et al. 2021). Numerous studies have reported a remarkably decreased
47 CO_2 efflux in rewetted or restored peatlands, but the rising WT levels have also augmented the emissions of CH_4 and N_2O
48 (Audet et al. 2013, Järveoja et al. 2016).

49 Atmospheric N deposition, primarily caused by anthropogenic activities (i.e. fossil fuels combustion, fertilizer application),
50 has increased consistently during the past decades (Gomez-Casanovas et al. 2016, IPCC 2013), and it is predicted to increase
51 two- or three-fold in terrestrial ecosystems by the end of the century (Lamarque 2005). The increasing N deposition could
52 alleviate the N stress on peatlands, but the N effects on CH_4 and N_2O emissions are controversial (Deng et al. 2019). Thus,
53 positive (Juutinen et al. 2018), negative (Gao et al. 2014) or neutral (Wang et al. 2017) effects of N deposition on CH_4 emissions
54 in peatlands have been observed. We speculate that the contrasting results probably are a result of the prevailing environmental
55 conditions and the N addition rate. Besides CH_4 emission, N deposition generally stimulates N_2O emissions from peatlands due
56 to the increasing supply of N substrate (Wang et al. 2017). However, previous studies have also shown that a higher N input
57 leads to a transition of the grassland into a state of declining N saturation as well as a reduction of the sensitivity of the GHG
58 exchange to the continuously increasing N deposition (Gomez-Casanovas et al. 2016). To eliminate the possible gap resulting
59 from the N addition rate, multiple levels of N deposition are required to study the possible linear or non-linear effects of
60 deposition on GHG emissions.



3

61 Numerous studies have reported on the individual effects of WT and N deposition on GHG emissions in peatlands (Evans
62 et al. 2021, Saiz et al. 2021). To our knowledge, only a few studies (Gao et al. 2014, Wang et al. 2017) exist that focus on their
63 interactive effects on peatland GHG emissions. Gao et al. (2014) found that N addition in peatlands decreased CH₄ emissions
64 but increased N₂O emissions without any significant interaction with WT levels. Wang et al. (2017) observed no interactive
65 effects of a lower WT and increasing N deposition on GHG emissions in an alpine wetland. The above-mentioned studies were,
66 however, limited to a single level of N addition and associated water addition. The response of GHG emissions in peatlands to
67 the gradients of N deposition and WT levels remains to be elucidated, in particular at the N saturation stage, even though it
68 may be a key factor in shaping GHG emissions. The large uncertainties regarding the interactive effects of N deposition and
69 WT levels on GHG emissions severely hamper obtaining a reliable estimation of the response of peatlands to climate change
70 and anthropogenic activities.

71 To address this knowledge gap we conducted a mesocosm investigation to study the influence of three WT levels (from
72 drained to inundated) and multi-level N deposition (from non-addition to 160 kg N ha⁻¹ yr⁻¹) on the soil CH₄ and N₂O emissions
73 in the Zoige alpine peatland, located on the eastern edge of the Qinghai-Tibetan Plateau. Being the largest and highest swamp
74 wetland area in China, its sensitivity to the global climate change and human activities is high (Chen et al. 2013). Exposure to
75 a potential influence of drainage, restoration or increasing N deposition (Yang et al. 2017, Zhang et al. 2011) may increase the
76 risk of high GHG emissions from this area. In this study, we hypothesised that i) a slight increase in N deposition might
77 stimulate both CH₄ and N₂O emissions, but a high level would inhibit GHG emissions. ii) The effects of N deposition on CH₄
78 and N₂O emissions would be associated with WT levels due to the influence of WT-induced aerobic conditions on the efficiency
79 of utilising nutrients for CH₄ and N₂O production.

80 2. Methods and Materials

81 2.1 Study site

82 This study was conducted in the Zoige alpine wetland, situated on the eastern edge of the Qinghai-Tibetan Plateau,
83 Southeast China, during the 2018 and 2019 growing seasons. This alpine wetland covers an area of 6180 km², which is 31.5%
84 of the whole Zoige plateau. The mean annual temperature is 1.4°C, with a maximum of 9.1 to 11.4°C in July and a minimum
85 of -8.2 to -10.6°C in January, while the average annual precipitation is approximately 650 mm (Chen et al. 2013, Yang et al.
86 2014). Over the past four decades, the mean annual air temperature has increased by 0.4°C per decade, while the total annual
87 precipitation has decreased by 22 mm per decade (Chen et al. 2013, Yang et al. 2014). Data on precipitation and air temperature
88 in this study were obtained from the closest meteorological station belonging to the Chinese National Meteorological
89 Information Center (www.nmic.gov.cn) and are shown in Figure S1. The depth of peat in the vertical profile is around 1.2 m,



90 soil pH is 6.8-7.2 and soil bulk density around 0.78 g m^{-3} (Zhang et al. 2020). The plant growing season ranges from July to
91 September, and the dominant plants are *Carex muliensis*, *Lancea tibetica*, *Potentilla anserina* L. and *Trollius farreri* Stapf.

92 2.2 Experimental design

93 Our experiment was carried out at the Sichuan Zoige Wetland Ecosystem Research Station, Tibetan Autonomous
94 Prefecture of Aba ($33^{\circ}57'N$, $102^{\circ}52'E$, 3500 m a.s.l.). A homogeneous swamp wetland was selected for collection of soil and
95 plants to be used in the mesocosm. Forty-five tanks ($0.6 \text{ m length} \times 0.6 \text{ m width} \times 0.6 \text{ m height}$) were kept above ground and
96 filled with intact soil cores and vegetation. The bottom of the tanks was welded, and the outside of the tanks was wrapped with
97 polystyrene foam to avoid heat exchange with the surroundings.

98 The experimental treatments consisted of five levels of added N and three water table levels and applied in a factorial
99 design (5 N addition \times 3 water table). The treatments were replicated three times, giving a total of 45 experimental plots. Based
100 on previous studies indicating water levels effects on GHG emission in the Zoige peatland (Cui et al. 2017, Yang et al. 2017),
101 three water table levels (WT₋₃₀, -30 cm below the soil surface; WT₀, 0 cm at the soil-water interface; and WT₁₀, 10 cm above
102 the soil surface) were selected. To maintain the water table at the selected three levels, we developed a water table control
103 system composed of three water table detectors, a manostat, a relay and micropumps. Three water table detectors were placed
104 in the PVC pipe (diameter 3 cm) of each tank at the exact water table level and at +2 cm and -2 cm water table. When the
105 water table was below the -2 cm detector, the pump switched on, supplying the tanks with local tap water until the water table
106 reached the +2 cm detector. To prevent the water table from becoming too high due to pump water or precipitation, four small
107 holes (diameter 1 cm, and two holes for two sides) were drilled at the precise position of the water table in each tank to allow
108 water overflow.

109 The current N deposition in the Zoige area is $1.08\text{-}17.81 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$, NH_4^+ and NO_3^- being the main component, and N
110 deposition is expected to increase further in the future (Han et al. 2019). NH_4NO_3 was adopted as N source to simulate the
111 different stages of the response of alpine peatlands to multi-level N deposition, and five N addition levels were established for
112 each water table level, namely 0 (N_0), 20 (N_{20}), 40 (N_{40}), 80 (N_{80}), and 160 (N_{160}) $\text{kg N ha}^{-1} \text{ yr}^{-1}$. The annual added N doses
113 were further divided into four portions and applied at the beginning of every month from June to September in 2018 and 2019.
114 25% of the added N was dissolved into 1 L water and sprayed evenly upon the surface of each plot. while the control plot only
115 received 1 L water (Wang et al. 2017).

116 2.3 GHG sampling and measurements

117 We measured CH_4 and N_2O fluxes once a month from June to October in 2018 and two or three times a month from June
118 to September in 2019. In each tank, CH_4 and N_2O fluxes were measured using static opaque chambers and gas chromatography



119 (GC) (Zhang et al. 2017). The cubic chamber was made of stainless steel (0.5 m length × 0.5 m width × 0.5 m height; without
120 bottom). At the top surface of the chamber, there were two ports for headspace gas sampling and enclosed air temperature
121 measurements, respectively. A dry battery-powered fan was placed in the chamber to avoid stratification of the gases during
122 sampling. Meanwhile, 45 square collars (0.5 m length × 0.5 m width × 0.2 m height) were produced and buried into the middle
123 part of the soil core in each tank at about 0.4 m depth. Before placing the chambers on top of the collars to collect gas samples,
124 the collars were sealed with water to ensure minimum gas exchange between chamber and atmosphere.

125 Gas samples (20 mL) were collected from each chamber using a rubber tube connected to the valve of the chamber and
126 a syringe at 10 min intervals over a 20-minute period (0, 10 and 20 min). The samples were then injected into a pre-evacuated
127 10 ml vacuum vial (Aladdin, Shanghai, China). The samples were kept cold and dark until the CH₄ and N₂O fluxes were
128 determined via GC (Agilent 7890A, Agilent Co., Santa Clara, CA, USA) within 72 hours. The GC was equipped with a flame
129 ionization detector (FID) to analyse the CH₄ concentration and an electron capture detector (ECD) to analyse the N₂O
130 concentration. The carrier gas was N₂, and the operation temperature for the FID was set at 250 °C and ECD at 300 °C.

131 The CH₄ and N₂O fluxes were calculated by the slopes of linear regression between gas concentration and sampling time
132 (0, 10, 20 min after chamber closure). Each linear regression was assessed individually, and the estimates of the data quality
133 of the fluxes were uniformly dependent on R-squared values. However, the coefficients of determinations (R²) of the linear
134 regression for CH₄ and N₂O were sometimes low (<0.4), particularly when the fluxes were low. We did not want to create bias
135 against these low fluxes and therefore kept them if the CO₂ concentration (data not shown) showed a good linear trend with
136 time (Laine et al. 2019). Apart from these fluxes, values were generally accepted only if the R-squared values of the linear
137 regressions were equal to or greater than 0.8 and 0.7 for CH₄ and N₂O (Lafuente et al. 2020, Laine et al. 2019), respectively.
138 The CH₄ flux had 5.29% discarded values, while the N₂O flux had 3.70% discarded values.

139 **2.4 Analysis of soil properties**

140 To determine soil properties, soil samples were collected in late September, considered as the end of the growing seasons
141 in 2018 and 2019. Three sub-samples of soil were collected from each tank at the top 5 cm depth and then bulked into a
142 composite sample representing a reliable replicate. The collected soil samples were stored under cold and dark conditions and
143 then forwarded to the laboratory within three days. The soil samples were passed through a 2 mm sieve and air dried for the
144 determination of soil pH, soil organic carbon (SOC) and total nitrogen (TN). Soil pH was measured at a soil:water ratio of
145 1:2.5 with a pH electrode (PHS 29, China). SOC and TN were determined via dry combustion using an Elementar Vario Max
146 CN analyzer (Hanau, Germany). Soil water content (SWC) was determined by using a TDR300 moisture meter (Spectrum
147 Technologies Inc., Plainfield, Illinois, USA).



148 2.5 Statistical analysis

149 Generalized least square (GLS) ANOVA was used to assess the effect of WT and N (fixed factor) on the cumulative CH₄
150 and N₂O emissions in 2018 and in 2019 (respectively), and the soil properties were determined via the R package *nlme* (Pekár
151 et al. 2016, Tiemeyer et al. 2016). We also used the GLS method to compare the effects of N deposition on CH₄ and N₂O
152 emissions at each WT level in each year, followed by a Tukey HSD test. The GLS model included an autoregressive structure,
153 accommodated for unequal time of sampling, and a variance function allowing for unequal variance in the fixed factors
154 (Wanyama et al. 2019).

155 A Generalized Additive Model (GAM) was used to fit the relationship between the cumulative CH₄ emissions and N
156 deposition dosages at different water table levels as well as the relationship between the global warming potential (GWP) of
157 growing season GHG emissions and N deposition dosages. Compared to the linear models, GAM directly and smoothly fitted
158 the non-linear relationship between the response variable and the multiple explanatory variables, despite the data distribution
159 (Chen et al. 2021).

160 The cumulative GHG emissions in the growing seasons in each year were calculated by the following equation:

161 Cumulative CH₄ (or N₂O) emission =

$$162 F_1 \times (t_1 - t_{start}) \times 24 + \sum_{i=1}^n (F_i + F_{i+1}) / 2 \times (t_{i+1} - t_i) \times 24 + F_n \times (t_{end} - t_n) \times 24$$

163 where F is the CH₄ (g C m⁻² h⁻¹) and N₂O flux (g N m⁻² h⁻¹), n is the total number of measurements each year, F_1 and F_n stand
164 for the GHG flux from the first and last sampling each year, and t_1 and t_n represent the time of the first and last sampling each
165 year. i is the i th measurement, $(t_{i+1} - t_i)$ is the days between two adjacent measurements, t_{start} and t_{end} are 1 June and 30 September
166 each year. To reduce the heterogeneity of the first and last sampling occasion each year, the GHG emission from 1 June to the
167 first sampling and from the last sampling to 30 September was taken into consideration and compared to the results of previous
168 studies (Goldberg et al. 2010).

169 GWP was used to define the cumulative impacts of the growing season GHG budgets (CH₄ and N₂O) as a time-integrated
170 radiative force over a period of 100 years. The GWP of cumulative CH₄ and N₂O emissions was calculated using the formula:

$$171 \text{GWP (g CO}_2\text{-eq m}^{-2}\text{)} = 28 \times F_{\text{CH}_4\text{-C}} \times 16/12 + 265 \times F_{\text{N}_2\text{O-N}} \times 44/28$$

172 where $F_{\text{CH}_4\text{-C}}$ and $F_{\text{N}_2\text{O-N}}$ are the growing season cumulative emissions of CH₄ (g CH₄-C m⁻² yr⁻¹) and N₂O (g N₂O-N m⁻² yr⁻¹)
173 based on the mass of C and N, respectively. The radiative forcings of CH₄ and N₂O are 28 and 265 in terms of a CO₂-eq unit
174 at a 100-year time horizon.

175 Statistical analysis was carried out by applying the statistic R software (version 3.4.3) (R Development Core Team, 2011),
176 and graphs were drawn using OriginPro 9.8.0.200. Final p values were Bonferroni adjusted to mitigate the risk of type I error.
177 A significance level of $\alpha = 0.05$ was used for all statistical tests.



178 **3. Results**

179 **3.1 Soil properties**

180 During the two years of the growing season mesocosm experiment, the soil water content (SWC) varied from 63.5% to
 181 81.1% and was only significantly affected by the water table levels (Table 1 and 2). The higher WT levels significantly
 182 increased the SWC in both years. The soil pH varied within the range 7.3 to 7.8 and was only significantly affected by N
 183 deposition. Large variability of SOC was observed within the range 215.9 g kg⁻¹ to 296.1 g kg⁻¹, and both the WT and N
 184 treatments showed significant effects on SOC, without any interactive effects. Compared with the control treatment without N
 185 deposition, N deposition increased SOC by 1.4% to 31.1% (except WT₀N₁₆₀ in 2019). Soil TN varied between 12.9 g kg⁻¹ and
 186 19.1 g kg⁻¹ and was elevated by N deposition, whereas no significant response to the WT treatments was observed. N deposition
 187 enhanced soil TN by 1.3% to 110.5% compared to the N₀ treatment at each WT level.

188

189 Table 1. Soil properties (mean ± SE) (n=3) in the different water table (WT) treatments and nitrogen deposition (N) levels in
 190 2018 and 2019.

		2018			2019		
		WT ₋₃₀	WT ₀	WT ₁₀	WT ₋₃₀	WT ₀	WT ₁₀
SWC (%)	N ₀	65.8±2.5	67.4±2.6	69.8±1.5	62.8±1.7	70.4±1.0	81.1±1.6
	N ₂₀	66.8±1.8	74.2±1.6	74.2±1.9	63.5±2.0	71.3±0.4	79.0±2.0
	N ₄₀	67.3±1.5	73.2±3.0	71.0±1.7	64.4±1.9	71.6±1.9	79.8±1.6
	N ₈₀	67.4±2.2	72.0±0.8	73.1±1.1	67.8±0.8	69.6±1.4	77.4±2.1
	N ₁₆₀	64.4±1.3	68.0±2.2	72.8±1.8	68.1±0.7	72.2±1.8	81.1±1
pH	N ₀	7.6±0	7.7±0.1	7.7±0.1	7.7±0	7.8±0.1	7.8±0.1
	N ₂₀	7.5±0	7.7±0	7.4±0.1	7.7±0.1	7.5±0.2	7.6±0.1
	N ₄₀	7.3±0	7.6±0.1	7.6±0.1	7.5±0.1	7.6±0.2	7.7±0.2
	N ₈₀	7.6±0	7.4±0.1	7.5±0.1	7.4±0.1	7.6±0.1	7.4±0
	N ₁₆₀	7.5±0	7.6±0	7.3±0.1	7.5±0.1	7.4±0.1	7.5±0
SOC (g kg ⁻¹)	N ₀	231.3±5.4	237±24.3	246.6±16.0	215.9±3.6	227.3±14.4	218.3±14.0
	N ₂₀	296.1±5.7	285.8±8.4	279.2±23.4	228.7±9.6	249.9±12.0	273.3±11.8
	N ₄₀	292.3±14.1	281.2±18.7	262.8±20.9	241.8±6.7	281.0±17.8	253.3±17.5
	N ₈₀	265.4±17.7	294.3±7.7	291.4±9.3	240.9±12	230.6±10.8	286.2±9.1
	N ₁₆₀	275.6±7.0	276.8±10.1	266.8±32.4	254.6±18.2	226.8±13.7	251.5±19.1
TN (g kg ⁻¹)	N ₀	17.6±0.8	16.1±1.1	18.7±0.8	12.9±0.9	14.4±0.3	14.7±2.1
	N ₂₀	18.7±0.7	19.1±0.6	19.3±0.8	21.9±2.2	21.2±3.2	23.3±5.0
	N ₄₀	18.4±1.1	18.8±0.9	19.2±0.4	19.1±1.5	21.4±4.0	15.0±2.7



N ₈₀	18.3±0.8	19.4±0.2	19.7±0.2	18.6±1.1	16.2±1.0	31.0±2.6
N ₁₆₀	18.1±0.8	18.2±0.4	19.0±0.4	19.5±2.0	21.7±6.6	21.9±4.6

191 SWC, soil water content; SOC, soil organic carbon; TN, total nitrogen.

192

193 Table 2. Effects of water table (WT) and nitrogen (N) deposition levels and their interactions on soil properties using
 194 generalized least square (GLS) models.

	SWC		pH		SOC		TN	
	F	P	F	P	F	P	F	P
WT	19.4	<0.001***	0.34	0.7103	9.92	<0.001***	2.08	0.1319
N	0.64	0.6352	6.78	<0.001***	5.18	0.001**	4.49	0.002**
WT×N	0.25	0.9807	0.35	0.944	0.91	0.5147	0.74	0.6526

195 Bold font denotes significant values. The statistical significance levels used were: *: 0.01 < P < 0.05; **: 0.001 < P < 0.01; ***:

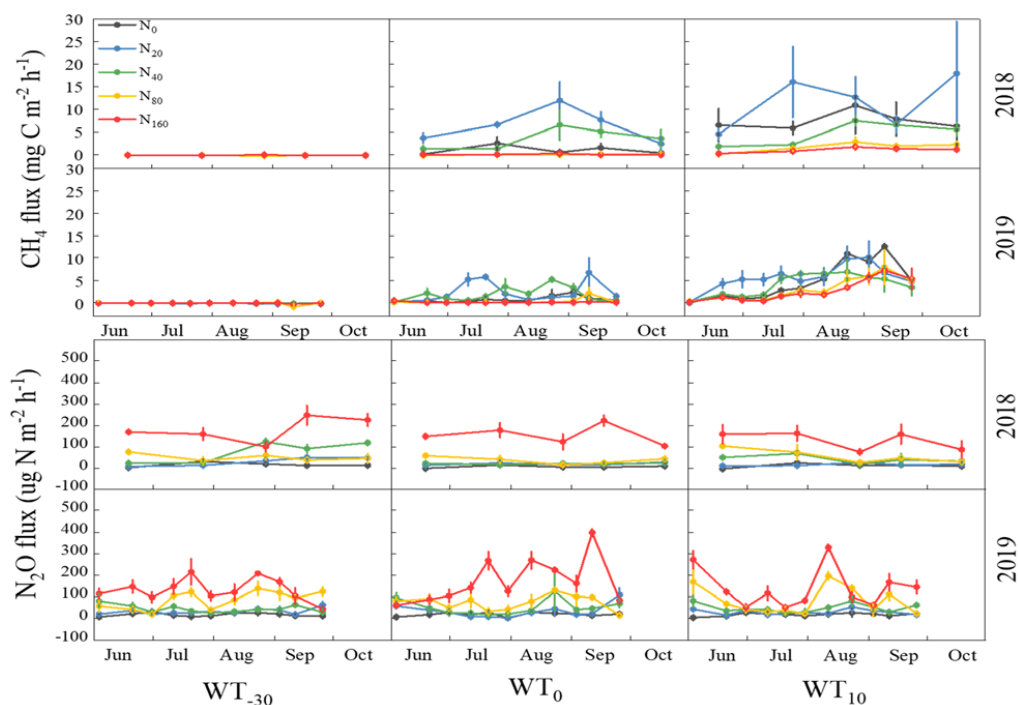
196 P < 0.001. SWC: soil water content; SOC: soil organic carbon; TN: total nitrogen.

197

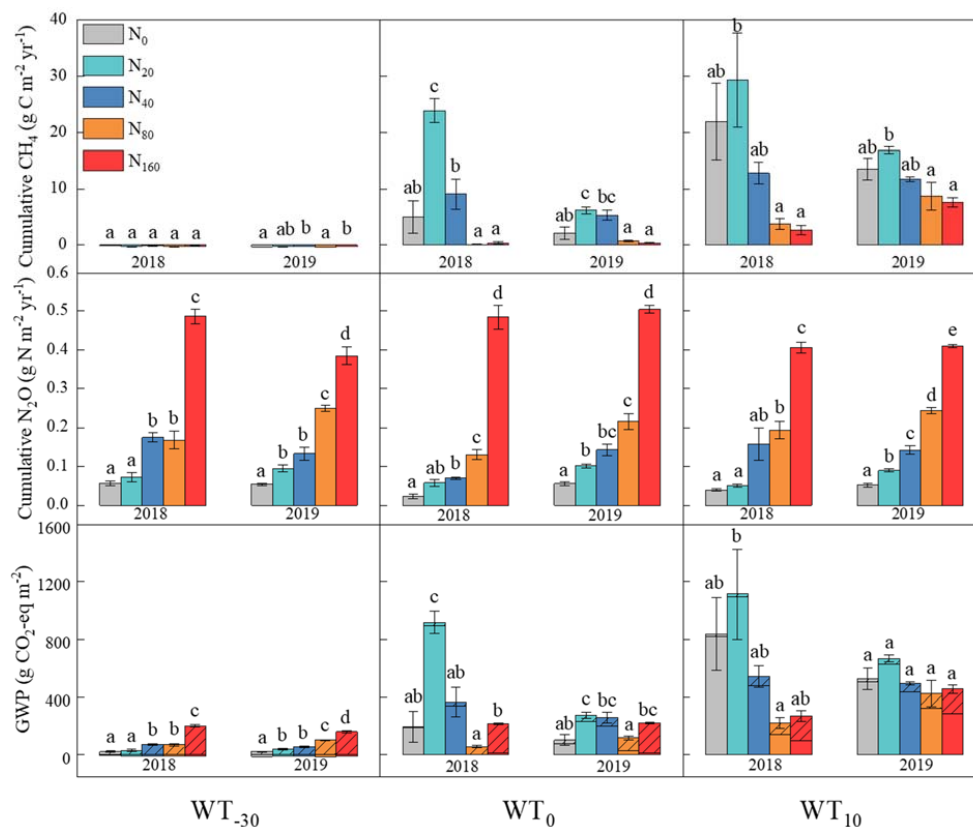
198 3.2 Methane

199 The Zoige alpine peatland acted as a net source of CH₄ in the WT₀ and WT₁₀ treatments throughout the two growing
 200 seasons, although the CH₄ flux was almost 0 in the WT₃₀ treatment. Seasonal variability of the CH₄ flux was observed (Figure
 201 1). The cumulative CH₄ emissions of the growing season ranged from -0.26 to 29.26 g CH₄-C m⁻² yr⁻¹ in 2018 and from -0.35
 202 to 16.36 g CH₄-C m⁻² yr⁻¹ in 2019 (Figure 2). During the two years, the WT treatments and their interaction with the N
 203 treatments had significant effects on the cumulative CH₄ emissions, while N deposition only had significant effects in 2019
 204 (Table 3). Along the WT level gradient from WT₃₀ to WT₁₀, the cumulative CH₄ emissions increased markedly. The response
 205 of the cumulative CH₄ emissions to N deposition was non-linear under WT₀ and WT₁₀ conditions (Figure 3), the highest value
 206 occurring in the N₂₀ treatment. Compared to the N₀ treatment, the N₈₀ and N₁₆₀ treatments remarkably decreased the cumulative
 207 CH₄ emissions by 36.5% to 97.4%, while N₄₀ was in the same order of magnitude as in the N₀ treatment. The GAM results
 208 showed that the cumulative CH₄ emissions could be explained by N deposition for 55.9% under WT₀ conditions and for 45.4%
 209 under WT₁₀ conditions. The modelling results also indicated that the critical thresholds for the highest cumulative CH₄
 210 emissions were 14.41 g C m⁻² yr⁻¹ with 20.9 kg ha⁻¹ yr⁻¹ N deposition under WT₀ conditions and 21.60 g C m⁻² yr⁻¹ with 16.2
 211 kg ha⁻¹ yr⁻¹ N deposition under WT₁₀ conditions.

212



213 Figure 1. Temporal variation of the response of CH₄ and N₂O fluxes to nitrogen deposition at three water table levels during
214 the growing seasons in 2018 and 2019. Error bars represent the SE (n=3).
215



216 Figure 2. Effects of nitrogen deposition levels on cumulative CH₄, N₂O emissions and GWP (from CH₄ and N₂O) at three
 217 water table levels during the rowing seasons in 2018 and 2019. For the GWP figures, the shaded area indicates GWP generated
 218 from cumulative N₂O, while the unshaded area from CH₄. Error bars represent the SE (n=3). Different letters above the bars
 219 indicate statistically significant differences ($P < 0.05$).

220

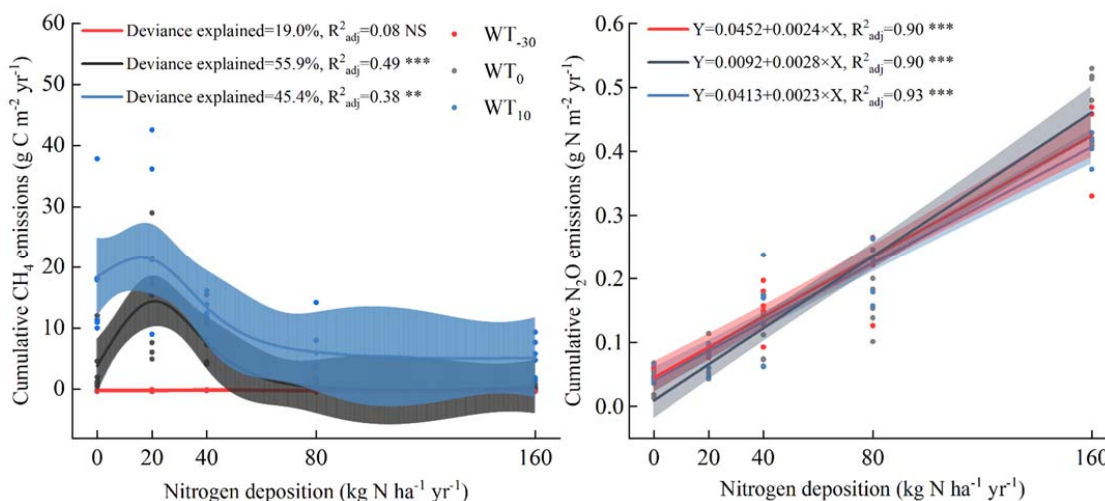
221 Table 3. The individual and interactive effects of water table (WT) and nitrogen (N) deposition levels on GWP (global warming
 222 potential), cumulative CH₄ and N₂O emissions in 2018 and 2019 using generalized least square (GLS) models.

	CH ₄		N ₂ O		GWP	
	F	P	F	P	F	P
2018						
WT	24.88	<0.001***	36.68	<0.001***	177.63	<0.001***
N	1.37	0.27	239.38	<0.001***	103.82	<0.001***
WT×N	15.15	<0.001***	4.28	0.002**	13.59	<0.001***
2019						
WT	615.89	<0.001***	351.26	<0.001***	702.04	<0.001***
N	5.99	0.001**	989.75	<0.001***	66.67	<0.001***



WT×N	18.01	<0.001***	5.23	<0.001***	7.61	<0.001***
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223 Bold font denotes significant values. The statistical significance levels used were: *:0.01<P<0.05; **: 0.001<P<0.01; ***:
 224 P<0.001.
 225



226 Figure 3. The relationship between cumulative greenhouse gas (CH₄ and N₂O) emissions and nitrogen deposition levels at
 227 three water table levels. A linear model was used to estimate cumulative N₂O emission at five nitrogen deposition levels,
 228 while a generalized additive model (GAM) was used to assess the response of cumulative CH₄ emission to nitrogen
 229 deposition levels. The statistical significance levels used were: * = P < 0.05 and > 0.01; ** = P < 0.01 and > 0.001; ***
 230 P < 0.001; NS = not significant (P > 0.05). The shaded areas indicate 95% confidence intervals.

231

232 3.3 Nitrous oxide

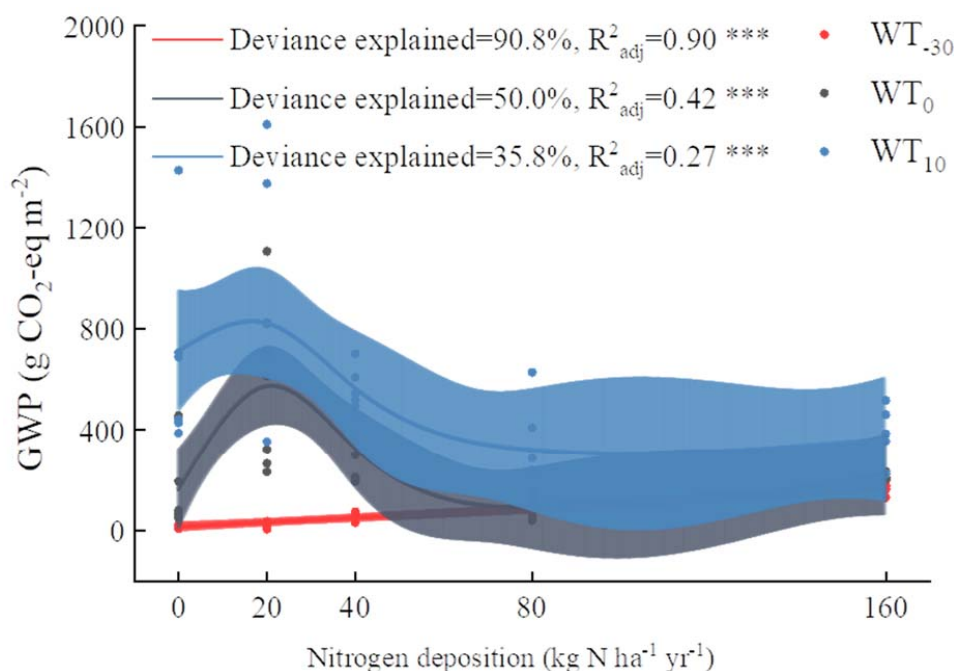
233 The Zoige alpine peatland acted as a net N₂O source during the growing seasons, the N₂O fluxes showing clear seasonal
 234 variability in 2018 and 2019. The N₂O flux tended to be highest in early September 2018 and in mid-August 2019, while the
 235 lowest flux was observed at the start or the end of the growing seasons (Figure 1). The cumulative N₂O emissions ranged from
 236 0.02 to 0.49 g N₂O-N m⁻² yr⁻¹ in 2018 and from 0.05 to 0.50 g N₂O-N m⁻² yr⁻¹ in 2019. The cumulative N₂O emissions were
 237 significantly affected by the WT levels, N deposition and their combination (Table 3). N deposition significantly increased the
 238 cumulative N₂O emission by 28.9% to 1974.6%, most significantly in the N₁₆₀ treatment. However, there was no clear effect
 239 of WT levels on N₂O emissions. We observed a significantly positive and linear relationship between the cumulative N₂O
 240 emissions and N application doses, and the slope and intercept of the linear correlation depended on the WT levels (Figure 3).
 241 The linear results also showed that the 1 kg N ha⁻¹ addition increased the cumulative N₂O emission by 0.0028, 0.0024 and



242 0.0023 g N₂O-N m⁻² yr⁻¹ under WT₋₃₀, WT₀ and WT₁₀ conditions, respectively.

243 3.4 Global warming potential

244 During the two study years, the combined GWP from the cumulative CH₄ and N₂O emissions varied within the range
245 11.17 g CO₂-eq m⁻² to 1113.86 g CO₂-eq m⁻² (Figure 2 and Table S1). Both the WT and N treatments and their combination
246 showed significant effects on GWP (Table 3). According to the GAM results (Figure 4), the higher WT level increased GWP,
247 the predicted curve following the order: WT₁₀ > WT₀ > WT₋₃₀. Similar to the cumulative CH₄ emissions, the GWP emission
248 showed a non-linear relationship with N deposition levels under WT₀ and WT₁₀ conditions (Figure 4), and the highest value
249 occurred in the N₂₀ treatment. Nevertheless, under WT₋₃₀ conditions, the response of GWP to N deposition was positive and
250 linear and thus similar to the pattern of N₂O, with the highest value being recorded in the N₁₆₀ treatment. Meanwhile, the
251 contribution of cumulative CH₄ emissions to GWP was negative (-112% to -4%) in the WT₋₃₀ treatment but much higher in
252 the WT₀ (3% to 97%) and WT₁₀ (35% to 97%) treatments, especially for the N₂₀ treatment (84% to 97%) (Table S1). The
253 contribution of cumulative N₂O emissions to total GWP was highest in the low WT level (WT₋₃₀) and high N deposition
254 treatments (N₈₀ and N₁₆₀).



255 Figure 4. Effects of nitrogen deposition levels on the global warming potential (GWP) of greenhouse gas (CH₄ and N₂O)
256 budgets at three water table levels using generalized additive models. The statistical significance levels used were: * = $P <$



257 0.05 and > 0.01 ; ** = $P < 0.01$ and > 0.001 ; *** $P < 0.001$; NS = not significant ($P > 0.05$). The shaded areas indicate 95%
258 confidence intervals.

259

260 4. Discussion

261 To better understand the sensitivity of alpine peatlands to climate change and anthropogenic disturbance, our mesocosm
262 experiment focused on the CH_4 and N_2O emissions in response to multiple levels of N deposition at three WT levels in an
263 alpine peatland. Ours is one of few studies (Gao et al. 2014, Wang et al. 2017) exploring the interactive effects of WT levels
264 and N deposition on GHG emissions in alpine peatlands. Our main finding was that N deposition non-linearly affected the
265 cumulative growing season CH_4 emissions and linearly enhanced the N_2O emission, the effect being highly dependent on the
266 WT levels. Our results partially supported hypothesis I – that N deposition would consistently enhance the N_2O emissions and
267 that moderate N deposition would increase and high N deposition decrease the emissions (hypothesis I). This provides clear
268 evidence of the interactive effects of WT levels and N deposition on GHG emissions, supporting hypothesis II.

269 4.1 Effects of WT and N deposition on CH_4 emission

270 In the two-year mesocosm experiment, corresponding with the results of previous studies (Hoyos-Santillan et al. 2019,
271 Wang et al. 2017), WT levels had a significant positive effects on CH_4 emissions, and the higher WT levels generally increased
272 the SWC and CH_4 emissions. The extent of the CH_4 release from the Zoige peatland to the atmosphere depended on the balance
273 between the microbial processes of CH_4 production and oxidation. At the higher WT levels, the oxygen content in the surface
274 peat declined and the exposure of CH_4 production process to anaerobic conditions increased, elevating CH_4 emissions (Evans
275 et al. 2021, Hoyos-Santillan et al. 2019, Zhang et al. 2020).

276 The effects of N deposition on peatland CH_4 emissions reported in previous studies varied considerably with positive
277 (Juutinen et al. 2018), negative (Gao et al. 2014) or neutral (Wang et al. 2017) relationships reported. In the current study, a
278 moderate level of N deposition positively stimulated the CH_4 emissions, but subsequently the positive effect declined with
279 further N addition, despite the extremely low uptake or emission of CH_4 at low WT levels. This result is not unique as those
280 of Li et al. (2012), Lafuente et al. (2020) and Qu et al. (2021) all showed a similar non-linear pattern for grasslands, drylands
281 and steppe, respectively, although the aforementioned three regions acted as CH_4 sinks. The response of CH_4 emissions to N
282 deposition was depending on a threshold value of added N doses. Below this threshold, we observed positive effect – the
283 increasing N input alleviated of N constraints on microbial metabolism and, finally, increased CH_4 production (Currey et al.
284 2009, Deng et al. 2019). Above the threshold, the large amounts of available NO_3^- led to negative and inhibitory effects on the
285 methanogenic activity due to the competition of NO_3^- -reducing bacteria with methanogens (Liu et al. 2020). In addition, NO_3^-



286 might be used as an alternative electron acceptor to O₂ in order to support the microbial CH₄ oxidation process, and thus the
287 produced CH₄ was more consumed by the methanotrophic microbes (Qu et al. 2021, Wang et al. 2017).

288 The interactive effects of WT levels and N deposition on the cumulative CH₄ emissions were distinct in our study (Table
289 3 and Figure 3). Thus, we found that the WT levels were more likely to determine the direction and magnitude of CH₄ emissions
290 from alpine peatlands than N deposition. The N deposition non-linearly affected the CH₄ emissions, but the optimal scenario
291 for CH₄ emissions was roughly ca. 20 kg N ha⁻¹ yr⁻¹, which could be slightly changed by the WT levels. This was not consistent
292 with the findings in previous studies showing no interactive effects of WT and N treatments on the CH₄ uptake or emissions
293 in the Qinghai-Tibetan Plateau (Wang et al. 2017, Wu et al. 2020). A possible explanation of this may be that WT levels may
294 significantly influence the positive or negative effects of N deposition on the microbial process of CH₄ production due to its
295 limitation on peat anaerobic conditions. It is likely that the manipulated N supply and aerobic conditions simultaneously
296 affected the microbial processes of CH₄ production and oxidation and thereby determined the level of CH₄ emissions. We also
297 speculate that the WT levels were associated with nutrient exploitation by microorganisms and, accordingly, that the higher
298 WT levels promoted diffusion of the added N in the water-filled soil pore, N thus becoming readily accessible in the microbial
299 process (Wang et al. 2017).

300 4.2 Effects of WT and N deposition on N₂O emission

301 The mesocosms in the Zoige alpine peatland acted consistently as a N₂O source in the two years, and we observed
302 significant positive and linear effects of N deposition on N₂O emissions but no clear pattern of WT effects. This is not unique,
303 and Wang et al. (2017) reported that elevated WT levels under drained to inundated conditions had no effects on the N₂O flux
304 in the alpine peatlands of the Tibetan Plateau. The present results show that the N₂O emissions from alpine peatland were
305 likely primarily determined by N deposition rather than by WT levels. This is partially consistent with the study of (Gao et al.
306 2014), which also indicated the N addition (5.0 g N m⁻² yr⁻¹) significantly increased and the higher WT level slightly decreased
307 N₂O emissions in the alpine peatlands of the Qinghai-Tibetan Plateau. The alpine peatlands are generally under pressure by N
308 deficits and highly sensitive to the climate change (Squeo et al. 2006). The N input via deposition could supply more N
309 substrate and activate the microbial process of N₂O production. Our study showed that the N deposition did increase soil TN
310 (F = 4.49, P = 0.002); however, this did not exhibit a linear relationship similar to N₂O emissions with N deposition. This is
311 common, and some studies have even reported no effects of N addition on TN on the alpine steppe and grasslands of the
312 Tibetan Plateau, respectively (Qu et al. 2021, Zhao et al. 2017). The possible reason is WT- or N deposition-induced availability
313 of N substrate in soil peat, which is favourable for microbial N₂O production (Cui et al. 2016, Yue et al. 2021, Zhu et al. 2020).
314 Both the WT levels and N deposition may affect the N availability in top layer soil and consequently regulate the denitrification
315 process (Han et al. 2019, Wang et al. 2017).



316 Studies on the interactive effects of WT levels and N deposition on N₂O emissions in peatlands are rare and have
317 contradictory findings. For example, Wang et al. (2017) revealed no interactive effects of WT and N addition on N₂O emissions
318 in the alpine peatlands of the Tibetan Plateau, while Gao et al. (2014) showed the N addition increased the N₂O emissions;
319 albeit this was slightly inhibited by water addition. Our results confirmed the occurrence of an interactive effect of WT and N
320 deposition on N₂O emissions, but it was neither synergistic nor antagonistic. N deposition had linear positive effects on N₂O
321 emission, and WT did not alter this linear relationship but slightly changed the slope and intercept. This finding is quite novel,
322 and we have not identified any similar results in the previous studies. Because of this, we speculate that the N supply was the
323 primary factor determining the N₂O emissions in the N-limited Zoige alpine peatland, but this process was associated with the
324 WT levels via impacts on the efficiency of utilising N substrate for microbial N₂O production.

325 The growing season (June to September) N₂O emission responded to the 1 kg N ha⁻¹ yr⁻¹ deposition and increased by
326 0.0023-0.0028 g N m⁻² yr⁻¹ in the current study. This is slightly lower than the levels of previous studies, for instance that of
327 (Gong et al. 2019), who found that 1 kg annual N ha⁻¹ addition led to an increase of ca. 0.0076 g N₂O-N m⁻² yr⁻¹ during the
328 growing season (May to October) in a boreal peatland. This could be attributed to the relatively low air temperature at this
329 particular alpine peatland, which hampered the microbial N₂O production. Furthermore, IPCC (2013) suggested that the
330 emission factor (the fraction of nitrogen added that is released as N₂O) is 1%, indicating that 1 kg annual N ha⁻¹ may increase
331 N₂O emissions by 0.001 g N m⁻² yr⁻¹. The relatively higher emission factor for N₂O-N in our study was probably due to the
332 high dose of N addition.

333 4.3 Effects of WT and N deposition on GWP

334 The 100-year GWP was used as a tool to quantify the combined effect of CH₄ and N₂O emissions and its response to N
335 deposition at different WT levels in the investigated Zoige alpine peatland. The response of GWP to the N deposition was
336 complex, and the largest increase in the GWP occurred at high WT levels (i.e. WT at soil surface or above) and ca. 20 kg N
337 ha⁻¹ yr⁻¹ deposition. This is partially consistent with the results of a previous study, which indicated that the threshold value of
338 N addition rates for the highest 100-year GWP (CO₂, CH₄ and N₂O) in the Qinghai-Tibetan Plateau was 20 kg N ha⁻¹ yr⁻¹ in
339 2013 (Qu et al. 2021). We also observed that the elevated WT levels increased the magnitude of total GWP in the region due
340 to the contribution of largely enhanced CH₄ emissions, this being supported by an earlier study in Qinghai-Tibetan Plateau by
341 Wang et al. (2017) indicating that the strength of the CH₄ source was the primary reason for the changed GWP in response to
342 the changed WT levels. Meanwhile, N deposition showed linear and non-linear effects on GWP depending on the WT levels.
343 The reason behind this was the main contributor to the total GWP changed from CH₄ to N₂O along the elevated WT levels,
344 which led to a change in the response of total GWP to N addition from linear to non-linear. This result is quite novel, and
345 further studies should be conducted with focus on the long-term interactive effects of WT and N deposition on GWP from CO₂,



346 CH₄ and N₂O combined.

347 **4.4 Implications for future GHG emissions in alpine peatlands**

348 The undisturbed peatlands are currently a weak carbon sink (~0.1 Pg C yr⁻¹) with a moderate source of methane (~0.03
349 Pg CH₄ yr⁻¹) and a very weak source of nitrous oxide (~0.00002 Pg N₂O-N yr⁻¹), but artificial drainage transitioned it into a
350 net C source (~0.1 Pg C yr⁻¹), with a 10% decrease in CH₄ emissions and 20-fold increase in N₂O emissions (Frolking et al.
351 2011). In addition, a further study indicated that if we do not rehabilitate or restore the drained peatlands, they might constitute
352 12-41% of the GHG emissions budget by 2100 (Leifeld et al. 2019). Restoration of the drained peatlands is already regarded
353 as crucial to safeguard natural ecosystems and decrease GHG emissions (Laine et al. 2019), particularly to meet the
354 requirement of mitigation of GHG emissions in the Paris Agreements. Numerous studies have confirmed the relief of GHG
355 emissions (primarily mitigation of CO₂ but acceleration of CH₄) from peatlands due to the artificial restoration (Evans et al.
356 2021, Laine et al. 2019). However, accurate estimates of CH₄ and N₂O emissions are still unavailable, in particularly associated
357 with the climate change.

358 Alpine or high-altitude peatlands constitute an exceptional group among the global peatlands (Le et al. 2020) and
359 traditionally, they are more fragile water bodies and sensitive to climate changes and human disturbances (Squeo et al. 2006).
360 The Zoige alpine peatland, the largest and highest wetland area in Qinghai-Tibetan Plateau, accounts for 6.2% of the SOC
361 storage in China and 1% in the world (Cao et al. 2017). Previous studies have already shown the Qinghai-Tibetan Plateau is
362 experiencing increasing N deposition (Zhu et al. 2020), drainage (Zhang et al. 2011) or ecological restoration (Xu et al. 2021).
363 However, the response of this vulnerable but large carbon pool to the increasing N deposition and fluctuating WT levels as
364 well as its contribution (particularly CH₄ and N₂O emissions) to the future global GHG budgets is still uncertain.

365 Our study estimated the CH₄ and N₂O emissions from an alpine peatland in response to the multi-level increasing N
366 deposition and fluctuating WT levels, and our results do not argue against full restoration or drainage of peatlands or the
367 reliability of the maybe too high level of N deposition. Our scenario of water table level (at soil surface or above) and N
368 deposition (ca. 20 kg N ha⁻¹ y⁻¹) effectively increased the CH₄ and N₂O emissions from the studied Zoige alpine peatland.
369 Furthermore, additional N deposition enhanced the N₂O emissions and slightly increased or even inhibited CH₄ emissions.
370 Considering the current N deposition of 1.08-17.81 kg N·ha⁻¹ in the Qinghai-Tibetan Plateau (Han et al. 2019) and of 21.1 kg
371 N ha⁻¹ in China (Liu et al. 2013), the future trend predicting a possible doubling or tripling of N deposition by the end of the
372 century (Lamarque 2005) as well as the restoration of drained peatlands to safeguard their function and to mitigate GHG
373 emissions, we need to pay attention to the probably increasing CH₄ emissions and the shift in the presently underestimated
374 N₂O emissions from low to high levels. We believe that our results are strongly useful for predicting the GHG emissions from
375 alpine peatlands in response to the climate change and anthropogenic activities in the future.



376 **5. Conclusion**

377 The N-limited fragile alpine peatlands are highly sensitive to climate change and anthropogenic activities. Hence, we
378 need to improve our understanding of the response of GHG emissions from alpine peatlands to the increasing nitrogen
379 deposition and changing water table levels. Our results showed that CH₄ emissions were determined by N deposition, WT
380 levels and their interactive effects. A modest input of N deposition and high WT levels both stimulated CH₄ emissions. N₂O
381 emissions were remarkably sensitive to N deposition, which consistently and linearly increased the N₂O emissions, irrespective
382 of WT levels. The N deposition supplied more nutrients and substrate for the GHG-related microbes, while the WT levels
383 determined the soil aerobic conditions. Moreover, we speculated that the WT levels influenced the exploitation of nutrients for
384 CH₄ and N₂O production. The highest GWP was observed at high WT levels and ca. 20 kg N ha⁻¹ yr⁻¹ deposition. The current
385 annual N deposition in the Qinghai-Tibetan Plateau (1.08-17.81 kg N·ha⁻¹) is not as high as in China as a whole (21.1 kg N·ha⁻¹).
386 However, the projected increasing N deposition suggests that the GHG emissions from alpine peatlands have not yet peaked,
387 and there is therefore a risk for higher CH₄ and N₂O emissions in the future.

388 **Data availability**

389 All data are available from the corresponding author by request.

390 **Author contributions**

391 **Wantong Zhang:** Conceptualization, Data curation, Software, Writing - original draft, Writing - review & editing,
392 Validation, Formal analysis. **Zhengyi Hu:** Conceptualization, Supervision. **Joachim Audet:** Data curation, Supervision,
393 Writing – review & editing. **Thomas A. Davidson:** Supervision, Data curation, Writing – review & editing. **Enze Kang:**
394 Investigation. **Xiaoming Kang:** Investigation, Project administration. **Yong Li:** Investigation. **Xiaodong Zhang:**
395 Investigation, Project administration. **Jinzhi Wang:** Investigation, Conceptualization, Project administration, Writing -
396 review & editing..

397 **Competing interests**

398 The contact author has declared that neither they nor their co-author has any competing interests.

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