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

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Abstract

Alpine peatlands are recognized as a major natural contributor to the budgets of atmospheric methane (CH_4) but as a weak nitrous oxide (N_2O) source. Anthropogenic activities and climate change have put these fragile nitrogen (N)-limited peatlands under pressure by altering water table (WT) levels and enhancing N deposition. The response of greenhouse gas (GHG) emissions from these peatlands to these changes is uncertain. To address this knowledge gap, we conducted a mesocosm experiment in 2018 and 2019 investigating individual and interactive effects of three WT levels (WT_{-30}, 30 cm below soil surface; WT_0, 0 cm at the soil surface; WT_{10}, 10 cm above soil surface) and multiple levels of N deposition (0, 20, 40, 80 and 160 kg N ha^{-1} yr^{-1}) on growing season CH_4 and N_2O emissions in the Zoige alpine peatland, Qinghai-Tibetan Plateau. We found that the elevated WT levels increased CH_4 emission, while N deposition had non-linear effects (with stimulation at moderate levels, but inhibition at higher levels). In contrast no clear pattern of the effect of WT levels on the cumulative N_2O emission was evident, while N deposition led to a consistent and linear increase (emission factor: 2.3%-2.8%), and this was dependent on the WT levels. Given the current N deposition in the Zoige alpine peatland (1.08-17.81 kg N·ha^{-1}), our results suggested that the CH_4 and N_2O emissions from the alpine peatlands could greatly increase in response to the possible doubling N deposition in the future. We believe that our results provide insights into how interactions between climate change and human disturbance will alter GHG emissions from this globally important habitat.

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

1. Introduction

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). In pristine peatlands, the shallow water table (WT) and waterlogged conditions allow accumulation of organic matter and favour anaerobic production of methane (CH_4) and nitrous oxide (N_2O). Traditionally, this nitrogen-limited ecosystem is recognized as major CH_4 sources and weak N_2O sources (Frolking et al., 2011). Nevertheless, these conditions could be markedly changed by

anthropogenic disturbance and climate change, and growing evidence shows that peatlands are experiencing drainage and increasing nitrogen deposition (Chen et al., 2013; Evans et al., 2021). Consequently, the magnitude of CH_4 and N_2O emissions from peatlands may be severely altered, particularly the high-altitude or alpine peatlands that are especially vulnerable and highly sensitive to the climate change and anthropogenic activities (Squeo et al., 2006).

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

Atmospheric N deposition, primarily caused by anthopogenic activities (i.e. fossil fuels combustion, fertilizer application), has increased consistently during the past decades (Gomez-Casanovas et al., 2016, IPCC, 2013), and it is predicted to increase two- or three-fold in terrestial ecosystems by the end of the century (Lamarque, 2005). The increasing N deposition could alleviate the N stress on peatlands, but the N effects on CH_4 and N_2O emissions are unclear (Deng et al., 2019). Thus, positive (Juutinen et al., 2018), negative (Gao et al., 2014) or neutral (Wang et al., 2017) effects of N deposition on CH_4 emissions in peatlands have been observed. We speculate that the contrasting results probably are a result of the prevailing environmental conditons and the N addition rate. Besides CH_4 emisison, N deposition generally stimulates N_2O emissions from peatlands due to the increasing supply of N substrate (Wang et al., 2017). However, previous studies have also shown that a higher N input 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 exchange to the continuously increasing N deposition (Gomez-Casanovas et al., 2016). To eliminate the possible gap resulting from the N addition rate, multiple levels of N deposition are required to study the possible linear or non-linear effects of deposition on GHG emissions.

Numerous studies have reported on the individual effects of WT and N deposition on GHG emissions in peatlands (Evans 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 interactive effects on peatland GHG emissions. Gao et al. (2014) found that N addition in peatlands decreased CH_4 emissions but increased N_2O emissions without any siginificant interaction with WT levels. Wang et al. (2017) observed no interactive effects of a lower WT and increasing N deposition on GHG emissions in an alpine wetland. The above-mentioned studies were, however, limited to a single level of N addition and associated water addition. The response of GHG emissions in peatlands to the gradients of N deposition and WT levels remains to be elucidated, in particular at the N saturation stage, even though it may be a key factor

in shaping GHG emissions. The large uncertainties regarding the interactive effects of N deposition and WT levels on GHG emissions severely hamper obtaining a reliable estimation of the reponse of peatlands to climate change and anthropogenic activities.

To address this knowledge gap we conducted a mesocosm investigation to study the influence of three WT levels (from drained to inundated) and multi-level N deposition (from non-addition to 160 kg N ha^{-1} yr^{-1}) on the soil CH_4 and N_2O emissions in the Zoige alpine peatland, located on the eastern edge of the Qinghai-Tibetan Plateau. Being the largest and highest swamp wetland area in China, its sensitivity to the global climate change and human activities is high (Chen et al., 2013). Exposure to a potential influence of drainage, restoration or increasing N deposition (Yang et al., 2017; Zhang et al., 2011) may increase the risk of high GHG emissions from this area. In this study, we aim to address the following two questions: i) with the N deposition consistently increasing, do the positive effects of N deposition on CH_4 and N_2O emissions persist? ii) if there is interaction between N depostion and WT level, how do they combine to influence CH_4 and N_2O emissions in the alpine peatland?

2. Methods and Materials

2.1 Study site

This study was conducted in the Zoige alpine wetland, situated on the eastern edge of the Qinghai–Tibetan Plateau, Southeast China, during the 2018 and 2019 growing seasons. This alpine wetland covers an area of 6180 km^2, which is 31.5% 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 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., 2014). Over the past four decades, the mean annual air temperature has increased by 0.4°C per decade, while the total annual precipitation has decreased by 22 mm per decade (Chen et al., 2013; Yang et al., 2014). Data on precipitation and air temperature in this study were obtained from the closest meteorological station belonging to the Chinese National Meteorological 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, 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 June to September, and the dominant plants are *Carex mulienses*, *Lancea tibetica*, *Potentilla anserina* L. and *Trollius farreri Stapf.*

2.2 Experimental design

Our experiment was carried out at the Sichuan Zoige Wetland Ecosystem Research Station, Tibetan Autonomous Prefecture of Aba (33°57′N, 102°52′E, 3500 m a.s.l.). A homogeneous swamp wetland was selected for collection of soil and plants to be used in the mesocosm. Forty-five tanks (0.6 m length × 0.6 m width × 0.6 m height) were kept aboveground and filled with intact soil cores and vegetation (Figure S2). The bottom of the tanks was welded, and the outside of the tanks was wrapped with polystyrene foam to avoid heat exchange with the surroundings.

The experimental treatments consisted of five levels of added N and three water table levels and applied in a factorial design (5 N addition × 3 water table). The treatments were replicated three times, giving a total of 45 experimental plots. Based on previous

studies indicating water levels effects on GHG emission in the Zoige peatland (Cui et al., 2017; Yang et al., 2017), three water table levels (WT_{-30}, 30 cm below the soil surface; WT_0, 0 cm at the soil surface; and WT_{10}, 10 cm above the soil surface) were selected. To maintain the water table at the selected three levels, we developed a water table control system composed of three water table detectors, a manostat, a relay and micropumps. Three water table detectors were placed 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 water table was below the -2 cm detector, the pump switched on, supplying the tanks with local tap water until the water table reached the +2 cm detector. To prevent the water table from becoming too high due to pump water or precipitation, four small 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 water overflow.

The current N deposition in the Zoige area is 1.08-17.81 kg $N \cdot ha^{-1} \cdot yr^{-1}$, NH_4^+ and NO_3^-being the main component, and N deposition is expected to increase further in the future (Han et al., 2019). NH_4NO_3 was adopted as N source to simulate the different stages of the response of alpine peatlands to multi-level N deposition, and five N addition levels were established for each water table level, namely 0 (N_0), 20 (N_{20}), 40 (N_{40}), 80 (N_{80}), and 160 (N_{160}) kg N ha^{-1} yr^{-1}. The three lowest levels (N_0, N_{20} and N_{40}) cover the gradient of current and near-future deposition levels while the two highest levels (N_{80} and N_{160}) represent levels of N-enrichment resulting from extreme deposition levels possibly combined with N input from fertilization or livestock excreta. The annual added N doses were further divided into four portions and applied at the beginning of every month from June to September in 2018 and 2019. 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 received 1 L water (Wang et al., 2017).

2.3 GHG sampling and measurements

We measured the CH_4 and N_2O fluxes with the sampling events of 1-3 times per month during the growing seasons in 2018 and 2019 in our study. In total, 16 sampling occasions of individual fluxes were recorded for CH_4 and N_2O. In each tank, CH_4 and N_2O fluxes were measured using static opaque chambers and gas chromatography (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 bottom). At the top surface of the chamber, there were two ports for headspace gas sampling and enclosed air temperature measurements, respectively. A dry battery-powered fan was placed in the chamber to avoid stratification of the gases during sampling. Meanwhile, 45 square collars (0.5 m length × 0.5 m width × 0.2 m height) were produced and buried into the middle part of the soil core in each tank at about 0.2 m depth. Before placing the chambers on top of the collars to collect gas samples, the collars were sealed with water to ensure minimum gas exchange between chamber and atmosphere.

Gas samples (20 mL) were collected from each chamber using a rubber tube connected to the valve of the chamber and 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 10 mL vacuum vial (Aladdin, Shanghai, China). The samples were kept cold and dark until the CH_4 and N_2O fluxes were determined via GC (Agilent 7890A, Agilent Co., Santa Clara, CA, USA) within 72 hours. The GC was equipped with a flame ionization detector

(FID) to analyse the CH_4 concentration and an electron capture detector (ECD) to analyse the N_2O concentration. The carrier gas was N_2, and the operation temperature for the FID was set at 250 °C and ECD at 300 °C. The CH_4 and N_2O fluxes were calculated as follows.

$$F = \frac{M}{V_0}\frac{P}{P_0}\frac{T}{T_0}\frac{dc}{dt}H$$

where dc/dt is the slope of the linear regression for the gas concentration gradient through time; M is the molecular mass of CH_4 or N_2O; P is the atmospheric pressure at the sampling site; T is the absolute temperature during sampling; V_0, P_0, and T_0 are the gas mole volume, atmospheric pressure, and absolute temperature under standard conditions, respectively; and H is the chamber height.

Each linear regression was assessed individually, and the estimates of the data quality of the fluxes were uniformly dependent on R-squared values. However, the coefficients of determinations (R^2) of the linear regression for CH_4 and N_2O were sometimes low (<0.4), particularly when the fluxes were low. We did not want to create bias against these low fluxes and therefore kept them if the CO_2 concentration (data not shown) showed a good linear trend with time (Laine et al., 2019). Apart from these fluxes, values were generally accepted only if the R-squared values of the linear regressions were equal to or greater than 0.8 and 0.7 for CH_4 and N_2O (Lafuente et al., 2020; Laine et al., 2019), respectively. The CH_4 flux had 5.29% discarded values, while the N_2O flux had 3.70% discarded values.

2.4 Analysis of soil properties

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

2.5 Statistical analysis

Generalized least square (GLS) ANOVA was used to assess the effect of WT and N (fixed factor) on the cumulative CH_4 and N_2O emissions in 2018 and in 2019 (respectively), and the soil properties were determined via the R package *nlme* (Pekár et al., 2016, Tiemeyer et al., 2016). We also used the GLS method to compare the effects of N deposition on CH_4 and N_2O emissions at each WT level in each year, followed by a Tukey HSD test. The GLS model included an autoregressive structure, accommodated for unequal time of sampling, and a variance function allowing for unequal variance in the fixed factors (Wanyama et al., 2019).

A Generalized Additive Model (GAM) was used to fit the relationship between the cumulative CH_4 emissions and N deposition

dosages at different water table levels. Via the R package "mgcv" (Wood, 2017), we used method "gam" to perform the GAM analysis and method "predict.gam" to see the response value of GHG emissions along the N deposition gradient from 0 to 160 kg N ha^{-1} yr^{-1}. Compared to the linear models, GAM directly and smoothly fitted the non-linear relationship between the response variable and the multiple explanatory variables, regardless of the data distribution (Chen et al., 2021).

The cumulative GHG emissions in the growing seasons of each year were calculated by linear interpolation between sampling events using the trapezoidal rule (Goldberg et al., 2010). In addition to the cumulative GHG emissions between the first and the last sampling event, the GHG emissions from 1st June to the first sampling and from the last sampling to 30th September were taken into consideration.

Statistical analysis was carried out using R (version 3.4.3) (R Core Team, 2017), and graphs were drawn using OriginPro 9.8.0.200. Final *p* values were Bonferroni adjusted to mitigate the risk of type I error. A significance level of $\alpha = 0.05$ was used for all statistical tests.

3. Results

3.1 Soil properties

During the two years of the growing season mesocosm experiment, the SWC varied from 63.5% to 81.1% and was only significantly affected by the water table levels (Table 1 and 2). The higher WT levels significantly 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 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 treatments showed significant effects on SOC, without any interactive effects. Compared with the control treatment without N deposition, N deposition increased SOC by 1.4% to 31.1% (except WT_0 N_{160} in 2019). Soil TN varied between 12.9 g kg^{-1} and 19.1 g kg^{-1} and was elevated by N deposition, whereas no significant response to the WT treatments was observed. N deposition enhanced soil TN by 1.3% to 110.5% compared to the N_0 treatment at each WT level.

Table 1. Soil properties in the different water table (WT) treatments and nitrogen deposition (N) levels in 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

Each value represents mean ± SE (n=3). SWC, soil water content; SOC, soil organic carbon; TN, total nitrogen.

Table 2. Effects of water table (WT) and nitrogen (N) deposition levels and their interactions on soil properties using 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

Bold font denotes significant values. The statistical significance levels used were: *: $0.01<P<0.05$; **: $0.001<P<0.01$; ***: $P<0.001$. SWC: soil water content; SOC: soil organic carbon; TN: total nitrogen.

3.2 Methane

The Zoige alpine peatland acted as a net source of CH_4 in the WT_0 and WT_{10} treatments throughout the two growing seasons, although the CH_4 flux was almost 0 in the WT_{-30} treatment. Temporal variability of the CH_4 flux was observed (Figure 1). The cumulative CH_4 emissions of the growing season ranged from -0.26 to 29.26 g CH_4-C m^{-2} in 2018 and from -0.35 to 16.36 g CH_4-C m^{-2} in 2019 (Figure 2). During the two years, the WT treatments and their interaction with the N treatments had significant effects on the cumulative CH_4 emissions, while N deposition only had significant effects in 2019 (Table 3). Along the WT level gradient from WT_{-30} to WT_{10}, the cumulative CH_4 emissions increased markedly. The response of the cumulative CH_4 emissions to N deposition was non-linear under WT_0 and WT_{10} conditions (Figure 3), with the highest value observed in the N_{20} treatment.

Compared to the N_0 treatment, the N_{80} and N_{160} treatments remarkably decreased the cumulative CH_4 emissions by 36.5% to 97.4%, while N_{40} was in the same order of magnitude as in the N_0 treatment. The GAM results showed that the cumulative CH_4 emissions could be explained by N deposition for 55.9% under WT_0 conditions and for 45.4% under WT_{10} conditions. The modelling results also indicated that the critical thresholds for the highest cumulative CH_4 emissions were 14.41 g C m^{-2} with 20.9 kg ha^{-1} yr^{-1} N deposition under WT_0 conditions and 21.60 g C m^{-2} with 16.2 kg ha^{-1} yr^{-1} N deposition under WT_{10} conditions.

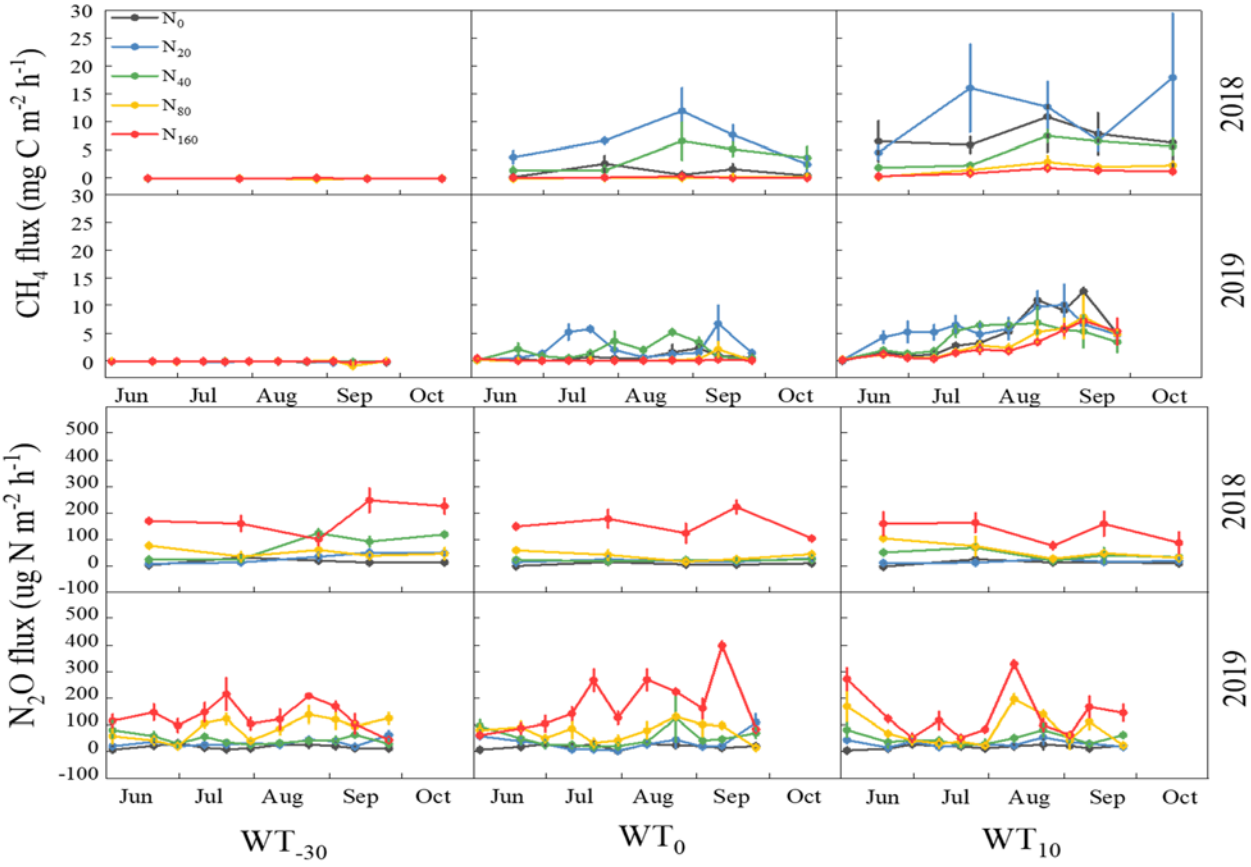

Figure 1. Temporal variation of growing-season CH_4 and N_2O fluxes under five levels of nitrogen deposition (0, 20, 40, 80 and 160 kg N ha^{-1} yr^{-1}) and three water table levels in 2018 and 2019. Error bars represent the SE (n=3).

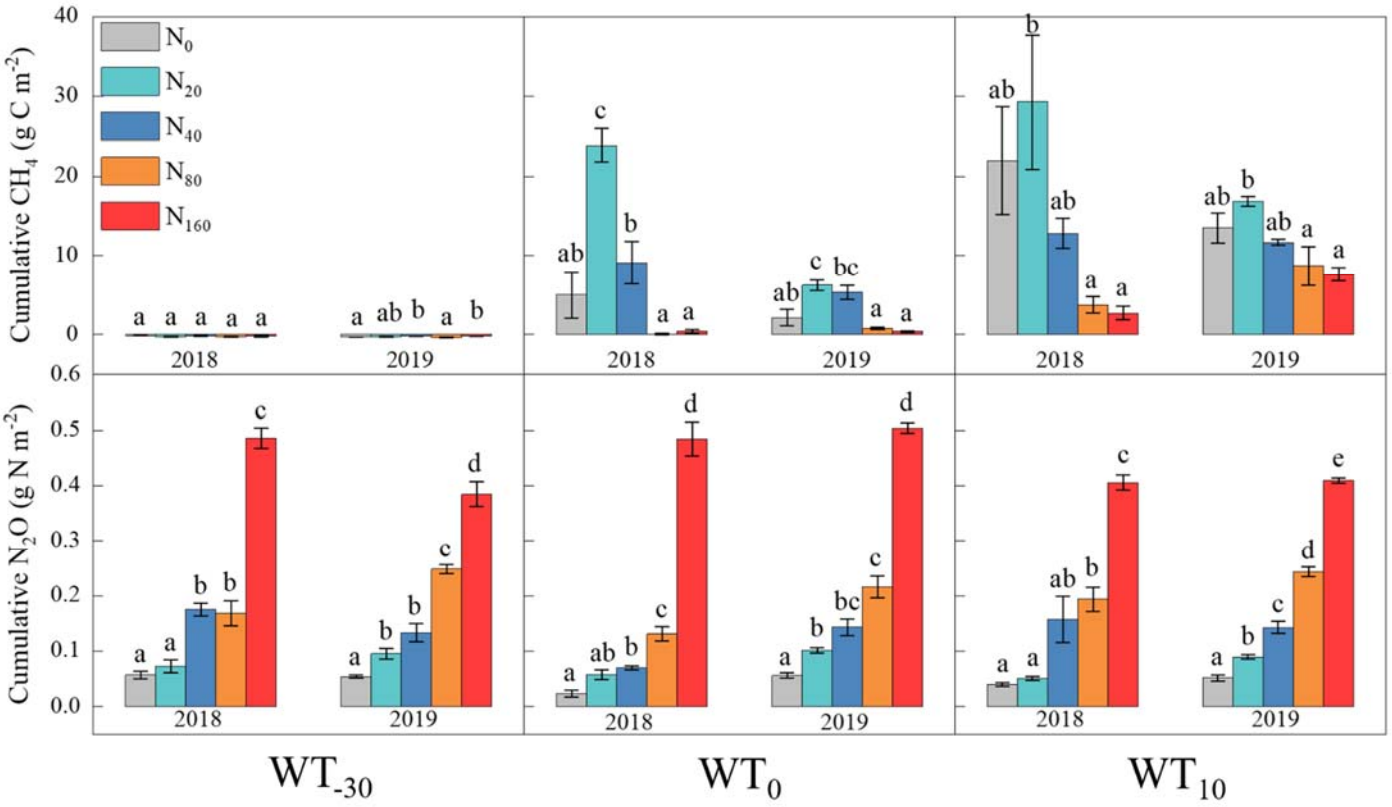

Figure 2. Effects of nitrogen deposition levels on cumulative CH_4 and N_2O emissions at three water table levels during the growing seasons in 2018 and 2019. Error bars represent the SE (n=3). Different letters above the bars indicate statistically significant differences ($P < 0.05$).

Table 3. The individual and interactive effects of water table (WT) and nitrogen (N) deposition levels on cumulative CH_4 and N_2O emissions in 2018 and 2019 using generalized least square (GLS) models.

	CH_4		N_2O	
	F	*P*	F	*P*
2018				
WT	24.88	**<0.001*****	36.68	**<0.001*****
N	1.37	0.27	239.38	**<0.001*****
WT×N	15.15	**<0.001*****	4.28	**0.002****
2019				
WT	615.89	**<0.001*****	351.26	**<0.001*****
N	5.99	**0.001****	989.75	**<0.001*****
WT×N	18.01	**<0.001*****	5.23	**<0.001*****

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

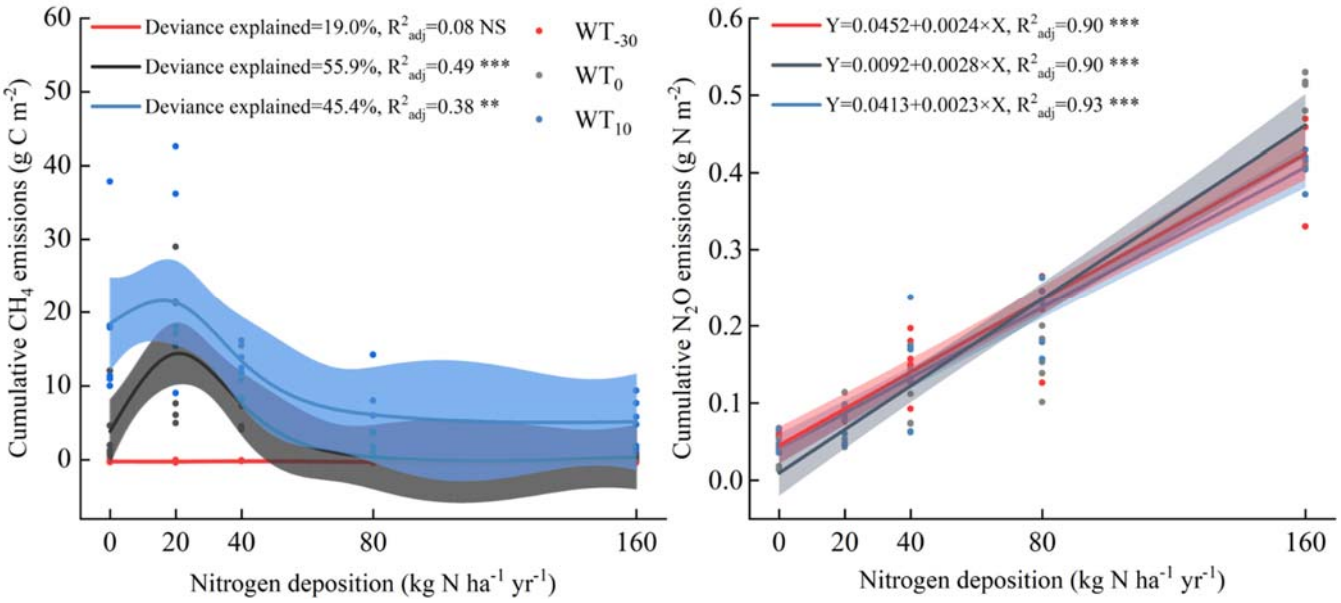

Figure 3. The relationship between cumulative greenhouse gas (CH_4 and N_2O) emissions and nitrogen deposition levels at three water table levels. A linear model was used to estimate cumulative N_2O emission at five nitrogen deposition levels, while a generalized additive model (GAM) was used to assess the response of cumulative CH_4 emission to nitrogen deposition levels. The statistical significance levels used were: * = $P < 0.05$ and > 0.01; ** = $P < 0.01$ and > 0.001; *** $P<0.001$; NS = not significant ($P > 0.05$). The shaded areas indicate 95% confidence intervals.

3.3 Nitrous oxide

The Zoige alpine peatland acted as a net N_2O source during the growing seasons, the N_2O fluxes showing clear temporal variability in 2018 and 2019. The N_2O flux tended to be highest in early September 2018 and in mid-August 2019, while the lowest flux was observed at the start or the end of the growing seasons (Figure 1). The cumulative N_2O emissions ranged from 0.02 to 0.49 g N_2O-N m^{-2} in 2018 and from 0.05 to 0.50 g N_2O-N m^{-2} in 2019. The cumulative N_2O emissions were significantly affected by the WT levels, N deposition and their interaction (Table 3). N deposition significantly increased the cumulative N_2O emission by 28.9% to 1974.6%, most significantly in the N_{160} treatment. However, there was no clear effect of WT levels on N_2O emissions. We observed a significantly positive and linear relationship between the cumulative N_2O emissions and N application doses, and the slope and intercept of the linear correlation depended on the WT levels (Figure 3). The linear results also showed that the 1 kg N ha^{-1} addition increased the cumulative N_2O emission by 0.0024, 0.0028 and 0.0023 g N_2O-N m^{-2} under WT_{-30}, WT_0 and WT_{10} conditions, respectively.

4. Discussion

4.1 Effects of WT and N deposition on CH_4 emission

The cumulative CH_4 emissions from the Zoige alpine peatland in our study ranged from -0.35 to 29.26 g CH_4-C m^{-2} across the two years, which is in the same order of magnitude as the cumulative CH_4 emissions (25.4-29.6 g CH_4-C m^{-2}) from an alpine wetland of the Qinghai-Tibetan Plateau in a previous study (Wang et al., 2017). As expected, WT levels had a significant positive effect on CH_4 emissions, with higher WT levels increased CH_4 emissions. This corresponds with the results of previous studies, due to the manipulative effects of WT levels on the soil redox conditions (Hoyos-Santillan et al., 2019; Wang et al., 2017). With higher WT levels, SWC increased and likely formed more anaerobic conditions conducive to CH_4 production, leading to elevated CH_4 emissions (Evans et al., 2021; Hoyos-Santillan et al., 2019; Zhang et al., 2020).

In the current study, a moderate level of N deposition positively stimulated the CH_4 emissions, but subsequently the positive effect declined with further N addition. However, this non-linear effect of N deposition was only observed at high WT levels, and the close to zero emissions of CH_4 at low WT levels were not affected by N deposition. To our knowledge, the non-linear effects of N deposition on CH_4 fluxes in the upland ecosystems has been reported in numerous studies (Li et al., 2012; Lafuente et al., 2020; Qu et al., 2021), however, there have been very few attempts in alpine peatlands. Song et al. (2013) reported that the N addition (0-240 kg N ha^{-1} yr^{-1}) showed non-linear positive effects on CH_4 fluxes in a wetland with the highest CH_4 flux at 60 kg ha^{-1} yr^{-1} N addition, but not reaching a significant level. Wu et al. (2022) conducted a global meta-analysis and found the N addition (0-300 kg N ha^{-1} yr^{-1}) could consistently increase the CH_4 emissions in the global wetlands, but the highest value occurred at 50-100 kg N ha^{-1} yr^{-1} N addition. The results of the aforementioned studies were not in agreement with the present study, due to the differences between the threshold values and the inhibitory or promotive effects of further N addition on CH_4 emission. However, in accordance with the previous studies, the relatively low level of N addition probably alleviated N constraints on microbial metabolism in soils and increased CH_4 production (Currey et al., 2009; Deng et al., 2019). With the further N addition, large amounts of available NO_3^- likely led to negative and inhibitory effects on the methanogenic activity due to the competition of NO_3^--reducing bacteria with methanogens (Liu et al., 2020).

The interactive effects of WT levels and N deposition on the cumulative CH_4 emissions were distinct in our study (Table 3 and Figure 3). We found that the WT levels were more likely to determine the direction and magnitude of CH_4 emissions from alpine peatlands than N deposition. This is in line with the previous study (Evans et al., 2020), which indicated that WT was the overriding factor to control GHG emissions from the peatlands at a global scale. The N deposition non-linearly affected the CH_4 emissions, and the scenario for maximum CH_4 emissions was roughly ca. 20 kg N ha^{-1} yr^{-1}, which could be slightly changed by the WT levels. As far as we know, this is the first mesocosm experiments in an alpine peatland comparing the interactive effects of multi-level N deposition and WT levels on CH_4 emissions, which makes it challenging to put in context the scope of these results, and we have not found any theory in the previous studies which could best explain the underlying mechanism. We speculate that the N deposition

supplied N substrate and WT levels were associated with N utilization by microorganisms. Precisely, the higher WT levels promoted diffusion of the added N in the water-filled soil pore, and N thus becoming readily accessible in the microbial process to support CH_4 production (Wang et al., 2017).

4.2 Effects of WT and N deposition on N_2O emission

The mesocosms in the Zoige alpine peatland were consistently acting as N_2O sources, and the cumulative N_2O emissions (0.02-0.49 g m^{-2} in 2018 and 0.05-0.50 g m^{-2} in 2019) did not show significant differences between the two years. The cumulative N_2O emission from the Zoige peatland in our study was relatively higher than that in a previous report (0.08-0.2 g m^{-2}), which focused on the drainage or lower water table level (Cao et al., 2018). Unlike the CH_4 emissions, we did not observe a clear pattern for the effects of WT levels on N_2O emissions in our study. This is different from the numerous previous studies, which all confirmed the positive effects of WT on N_2O emissions in peatlands, due to its positive effects on anaerobic denitrification and N_2O production (Gao et al. 2014; Regina et al., 1999; Regina et al., 1996). However, Wang et al. (2017) reported that elevated WT levels from drained to inundated conditions had no effects on N_2O fluxes in an alpine wetland of the Qinghai-Tibetan Plateau, which is in line with our study. The possible reason for this could be due to the large differences of habitat types, soil properties or precipitations among the regional heterogeneous peatlands.

N deposition stimulated N_2O emissions across the two years in our study, and same results were also found in the previous reports (Gao et al., 2014; Deng et al., 2019). The N deposition increased soil TN ($F = 4.49$, $P = 0.002$) in our study and is likely to supply more N substrate (NH_4^+ and NO_3^-) in soil (Zhu et al., 2020). The consequently increased N substrate could potentially activate the microbial process of N_2O production and increase N_2O emissions (Yue et al., 2021). Additionally, contrary to the CH_4 emissions, the N deposition showed consistently linear effects on N_2O emissions in the peatland, regardless of the WT levels. This is not in line with a previous study (Song et al., 2013), which indicated that N addition ranging from 0-120 kg N ha^{-1} yr^{-1} did not show significant effects on N_2O emissions in peatlands, while high level (240 kg N ha^{-1} yr^{-1}) largely increased the N_2O emissions. However, a global meta-analysis showed that N_2O emissions from wetlands demonstrated significant positive response to N enrichment (Deng et al., 2019), which agreed with our study. The possible explanation could be that the current level of N deposition in the Zoige peatland did not meet the N-saturation stage, after which the sensitivity of N_2O emission from the ecosystem to further N addition would decline (Gomez-casanovas et al., 2016).

The N_2O emission from the alpine peatland was likely primarily determined by N deposition rather than by WT levels. We confirmed the occurrence of an interactive effect of WT and N deposition on N_2O emissions, but it was neither synergistic nor antagonistic. N deposition had linear positive effects on N_2O emission, and WT level did not alter this linear relationship but slightly changed the slope and intercept. The N_2O emissions could be generated from both the aerobic nitrification and anaerobic denitrification processes. This is confirmed by the previous study (Bateman et al., 2005), which indicate that the denitrification was the major contributor to N_2O emission at water-filled pore space (WFPS) > 60% while nitrification was the major one at WFPS <

60%. The overall production of N_2O could not be uniquely manipulated by the WT levels, though the WT levels could influence the redox conditions and the microbial processes. Additionally, the Zoige peatland being a N-limited ecosystem (Squeo et al., 2006), we observed the overwhelming effects of N deposition on N_2O emission in the peatland, rather than WT levels.

The growing-season N_2O emission in the current study increased by 0.0023-0.0028 g N_2O-N m^{-2} in response to the additional 1 kg NH_4NO_3-N ha^{-1} yr^{-1} deposition. This is slightly lower than the levels from previous studies (Gong et al., 2019), which showed that 1 kg annual N ha^{-1} addition led to an increase of ca. 0.0076 g N_2O-N m^{-2} during the growing season in a boreal peatland. This could be attributed to the relatively low air temperature at this particular alpine peatland, which hampered the microbial N_2O production. Furthermore, IPCC (2013) suggested that the default emission factor (the fraction of nitrogen added that is released as N_2O) is 1%, indicating that 1 kg annual N ha^{-1} addition may increase N_2O emissions by 0.01 kg N_2O-N ha^{-1} (or 0.001 g N_2O-N m^{-2}). The relatively higher emission factor for N_2O-N in our study was probably due to the high dose of N addition.

4.3 Implications and limitations

To what extent the magnitude of non-CO_2 emissions from alpine peatlands could be upscaled in response to the increasing N deposition, and how this effect interacts with the WT levels is still high uncertain. Our study demonstrated that the CH_4 and N_2O emisions from the alpine peatland system could reach a new state if the projected increases in N deposition continued, in particular the CH_4 emission could be disproportionally increased. In the Zoige peatland, the current level of N deposition was 1.08-17.81 kg N·ha^{-1} yr^{-1} (Han et al., 2019), and the N deposition was predicted to be a possible doubling or tripling by the end of the century (Lamarque, 2005). Given the highest CH_4 emissions occurred at the level of ca. 20 kg N ha^{-1} yr^{-1} deposition and the consistent linear correlation between N_2O emissions and N deposition levels in our study, we infer that the CH_4 and N_2O emissions had not yet peaked, and the increasing N deposition could still result in the alpine peatland becoming a potential hotspot of GHG emissions in the future. Moreover, the fluctuation of WT levels resulting from anthropogenic drainage or rewetting could affect the GHG emissions but not suppress the N deposition-induced trend. However, our study is limited to a specific habitat, and thus the upscaling of this conclusion contains large uncertainty. To the best of our knowledge, very few advances heretofore have been achieved to elaborate this general conclusion. We believe that our results are useful for predicting the GHG emissions from alpine peatlands in response to the climate change and anthropogenic activities in the future.

It should be noted that some levels of N deposition (80 or 160 kg N ha^{-1} yr^{-1}) in our study were much higher than the local N deposition (1.08-17.81 kg N ha^{-1} yr^{-1}). This should not affect our general conclusion, because the non-linear and linear effects of N deposition on CH_4 and N_2O emissions (respectively) were primarily dependent on the low levels of N deposition (0-40 kg N ha^{-1} yr^{-1}), and the higher levels did not alter the relationship pattern. Meanwhile, note must be made that we did not measure the net ecosystem CO_2 exchange and wintertime GHG fluxes, which might hamper estimating the annual carbon budget from GHG emissions and SOC change. However, our study focused on the growing-season non-CO_2 emissions from the peatland at different WT levels under the future scenarios of increasing N deposition, and also the non-growing-season GHG emissions had only a minor

contribution to the yearly budget due to the low temperature and microbial activities (Peng et al., 2019). In addition, the low frequency of GHG sampling in 2018 could cause uncertainties in the temporal variation and cumulative emissions of CH_4 and N_2O, and this might result in bias in the present result. The monthly measurements of GHG fluxes from peatlands have already been found in the previous study (Cao et al., 2018), and we also increased the sampling frequency in 2019 to better support the current conclusion. However, further monitoring of GHG fluxes from the peatland would still be required to eliminate the uncertainties.

5. Conclusion

This study explored the response of non-CO_2 GHG emissions in the alpine peatland to the increasing N deposition at different WT levels. We found that CH_4 emissions were determined by N deposition, WT levels and their interactive effects. A modest input of N deposition and high WT levels both stimulated CH_4 emissions. N_2O emissions were remarkably sensitive to N deposition, which consistently and linearly increased the N_2O emissions, irrespective of WT levels. The projected increasing N deposition suggested an inevitable risk for higher CH_4 and N_2O emissions from the alpine peatland in the future.

Data availability

All data are available from the corresponding author by request.

Author contributions

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

Funding

This study was financially supported by the National Natural Science Foundation of China (41877421 and 31770511).

Competing interests

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

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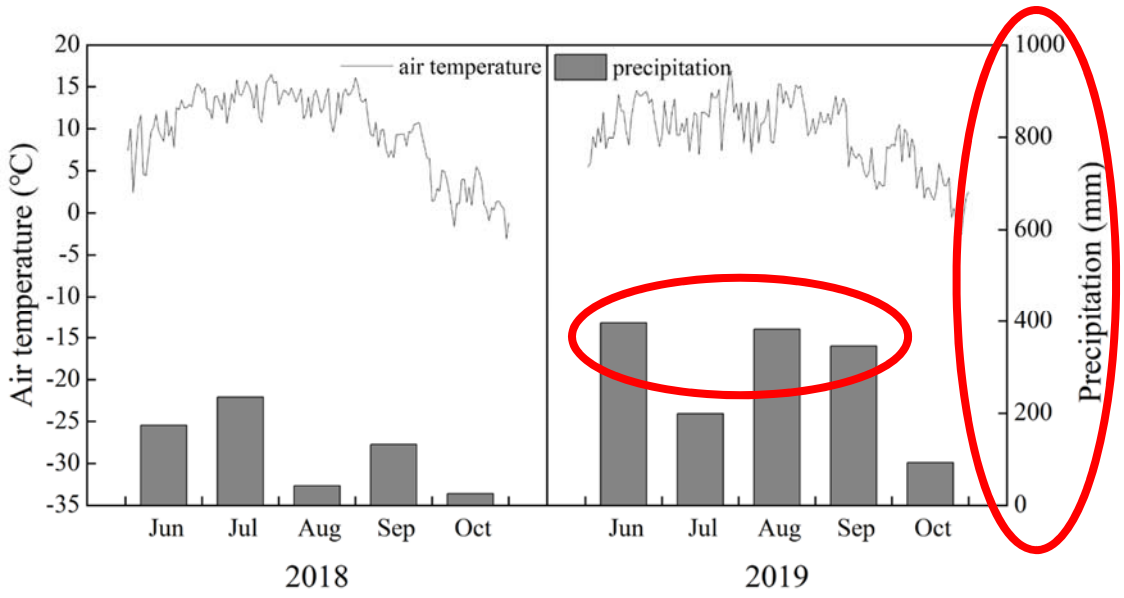

Figure S1. Variations of daily air temperature and monthly precipitation during the growing seasons in 2018 and 2019.

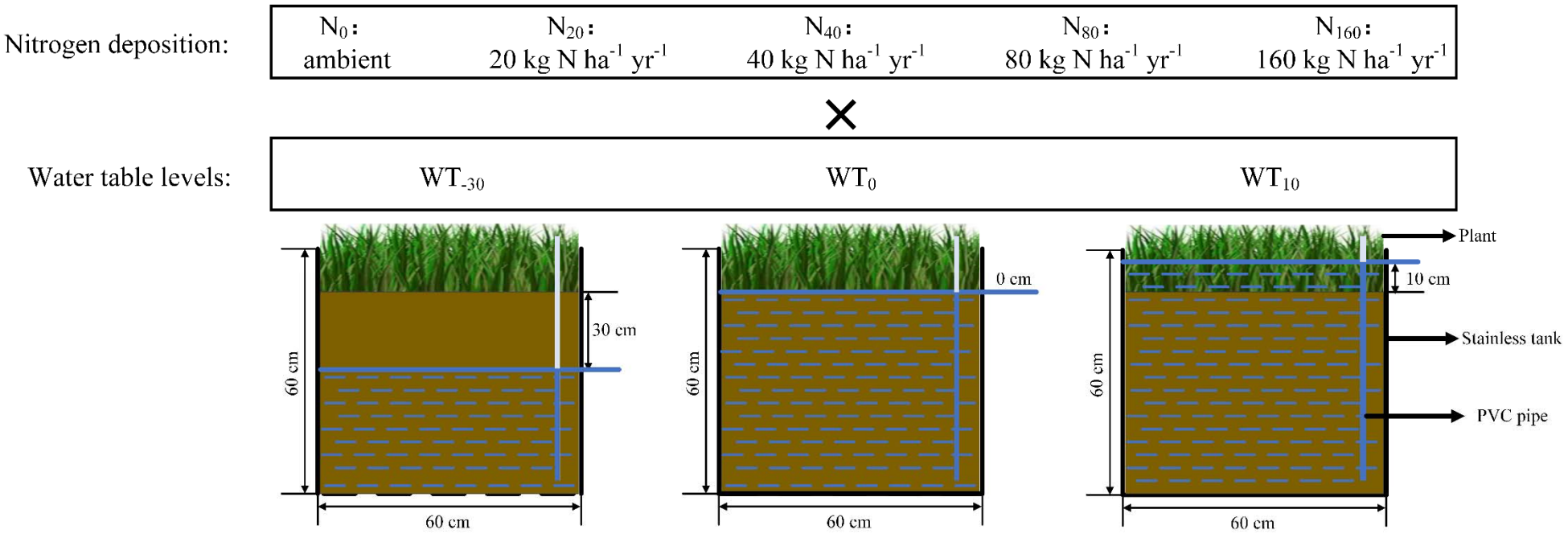

Figure S2. The sketch map of the experiment system in the present study