Title
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Permalink
https://escholarship.org/uc/item/5vh9n4cn

Journal
Global change biology, 23(2)

ISSN
1354-1013

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Publication Date
2017-02-01

DOI
10.1111/gcb.13467

Peer reviewed
Molecular mechanisms of water table lowering and nitrogen deposition in affecting greenhouse gas emissions from a Tibetan alpine wetland

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Abstract

Rapid climate change and intensified human activities have resulted in water table lowering (WTL) and enhanced nitrogen (N) deposition in Tibetan alpine wetlands. These changes may alter the magnitude and direction of greenhouse gas (GHG) emissions, affecting the climate impact of these fragile ecosystems. We conducted a mesocosm experiment combined with a metagenomics approach (GeoChip 5.0) to elucidate the effects of WTL (−20 cm relative to control) and N deposition (30 kg N ha⁻¹ yr⁻¹) on carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) fluxes as well as the underlying mechanisms. Our results showed that WTL reduced CH₄ emissions by 57.4% averaged over three growing seasons compared with no‐WTL plots, but had no significant effect on net CO₂ uptake or N₂O flux. N deposition increased net CO₂ uptake by 25.2% in comparison with no‐N deposition plots and turned the mesocosms from N₂O sinks to N₂O sources, but had little influence on CH₄ emissions. The interactions between WTL and N deposition were not detected in all GHG emissions. As a result, WTL and N deposition both reduced the global warming potential (GWP) of growing season GHG budgets on a 100-year time horizon, but via different mechanisms. WTL reduced GWP from 337.3 to −480.1 g CO₂-eq m⁻² mostly because of decreased CH₄ emissions, while N deposition reduced GWP from 21.0 to −163.8 g CO₂-eq m⁻², mainly owing to increased net CO₂ uptake. GeoChip analysis revealed that decreased CH₄ production potential, rather than increased CH₄ oxidation potential, may lead to the reduction in net CH₄ emissions, and decreased
Nitrification potential and increased denitrification potential affected N₂O fluxes under WTL conditions. Our study highlights the importance of microbial mechanisms in regulating ecosystem-scale GHG responses to environmental changes.

Keywords: carbon cycle, climate warming, methane, microbial functional gene, nitrous oxide, the Tibetan Plateau

Introduction

Carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are three major greenhouse gases (GHGs) that contribute to the anthropogenic greenhouse effect (IPCC, 2013). Wetlands play a dual role in affecting the atmospheric budgets of these GHGs. Waterlogged conditions lead to significant carbon (C) accumulation because of limited decomposition, which exerts a cooling effect on climate (Frolking et al., 2006; Yuan et al., 2015). Meanwhile, low redox potentials are conducive to CH₄ and N₂O emissions, which have a warming effect on climate (Smith et al., 2003; Frolking et al., 2006). Human activities and climate change have resulted in numerous environmental changes, including water table lowering (WTL) in wetlands (Dise, 2009). However, it still remains unclear how the GHG emissions and their net climate impact respond to these changes (Petrescu et al., 2015).

The water table is the key factor controlling the boundary between oxic and anoxic soils (Dinsmore et al., 2009) and has aroused considerable concern in peatlands and marshes in boreal (Aurela et al., 2007; Chivers et al., 2009), temperate (Webster et al., 2013; Yang et al., 2013) and subtropical zones (Malone et al., 2013). As the water table lowers, on the one hand, soil CO₂ emission increases because of accelerating organic matter decomposition (Aurela et al., 2007; Webster et al., 2013). On the other hand, water stress may reduce plant photosynthesis, leading to a decrease in CO₂ uptake (Chivers et al., 2009; Malone et al., 2013). Meanwhile, WTL also reduces CH₄ production and enhances CH₄ oxidation due to elevated soil oxygen content (Smith et al., 2003; Karbin et al., 2015), as well as stimulates N₂O emissions because of increased nitrogen (N) availability associated with N mineralization (Goldberg et al., 2010).

N deposition is another issue directly linked to wetland GHG emissions. N deposition affects CO₂ fluxes by increasing plant productivity, improving the chemical quality of litter (lower C/N ratio) and alleviating N constraints on microbial metabolism (Bragazza et al., 2006; Lebauer & Treseder, 2008). N also alters CH₄ emissions through impacts on microbes and plants, because N influences the activity of methanogens and methanotrophs (Liu & Greaver, 2009), and affects plant productivity and species composition involved in CH₄ production, oxidation and transport (Joabsson et al., 1999; Bubier et al., 2007; Lai et al., 2014). In addition, N input increases N₂O emissions by supplying available N for nitrifying and denitrifying bacteria (Dalal et al., 2003; Lohila et al., 2010). More importantly, nutrient status has been observed to modulate the effect of WTL on GHG emissions in northern
peatlands (Martikainen et al., 1993; Aerts & Ludwig, 1997); however, few studies have investigated the interactive effects of WTL and N deposition in alpine wetlands.

Currently, our understanding of GHG emissions is constrained by limited knowledge of microbial mediated mechanisms (McCalley et al., 2014). For CH$_4$ and N$_2$O, the linked processes of production and consumption involve methanogens and methanotrophs, as well as nitrifying and denitrifying bacteria (Le Mer & Roger, 2001; Dalal et al., 2003). However, determining the roles of these microbes in mediating GHG emissions under WTL and N deposition is difficult. The recently developed microarray-based metagenomics tool (GeoChip 5.0) enables the detection of over 144000 gene sequences from 393 gene families associated with biogeochemical processes (Wang et al., 2014a). For example, the CH$_4$ production potential of methanogens is detected by *mcrA* gene encoding methyl coenzyme M reductase A (Luton et al., 2002). The CH$_4$ oxidation potential of methanotrophs is detected by *pmoA* and *mmoX* genes. The nitrification potential is detected by *amoA* gene encoding ammonia monooxygenase and *hao* gene encoding hydroxylamine oxidoreductase. The denitrification potential is detected by *narG* gene encoding nitrate reductase, *nirS* and *nirK* genes encoding nitrite reductase, and *norB* gene encoding nitric oxide reductase (Yue et al., 2015). Therefore, GeoChip 5.0 offers an unprecedented opportunity to investigate the links between microbial functional potentials connected with these processes and net CH$_4$ or N$_2$O emissions (Yang et al., 2014b).

The aim of this study is to explore how and by what mechanisms GHG fluxes respond to WTL and N deposition in the alpine wetlands of the Tibetan Plateau, which play an important role in regulating regional GHG budget and are subjected to intensified human activities and rapid climate change (Chen et al., 2013). Over the past five decades, the wetlands in this plateau (with the exception of riverine and lacustrine wetlands) have undergone widespread degradation (Zhang et al., 2011b; Chen et al., 2013) that has largely been ascribed to artificial drainage for cultivation (An et al., 2007; Zhang et al., 2011a) or permafrost degradation associated with climate warming (Cheng & Wu, 2007; Piao et al., 2010; Zhang et al., 2011b). Meanwhile, this plateau has experienced increasing N deposition (Liu et al., 2015), particularly in the northeastern region, where N deposition rates (including dry and wet deposition) have ranged from 4 to 13.8 kg N ha$^{-1}$ yr$^{-1}$ (Lu & Tian, 2007; Fang et al., 2012). Although several studies have reported that CH$_4$ emissions are affected by water table (Chen et al., 2008, 2009; Yang et al., 2014a; Song et al., 2015), or N deposition (Gao et al., 2014) in this fragile ecosystem, no study to date has focused on the effects of these changes on net climate impact of GHG emissions and the molecular mechanisms underlying GHG responses.

We hypothesize that WTL decreases net CO$_2$ uptake and CH$_4$ emissions but increases N$_2$O emissions (hypothesis I), since aerobic conditions accelerate
organic matter decomposition, decrease CH₄ production, increase CH₄ oxidation, and provide more available N for nitrification and denitrification by stimulating N mineralization. We also hypothesize that N deposition increases net CO₂ uptake as well as CH₄ and N₂O emissions (hypothesis II), since increased N availability favors nitrifying and denitrifying bacteria and stimulates plant growth under N-limited conditions. Consequently, increased plant productivity could supply more substrate for CH₄ production and more conduits for CH₄ transport. Finally, we hypothesize that N deposition modulates the effects of WTL on GHG fluxes (hypothesis III). WTL could physiologically threaten the function (e.g. the production and consumption of GHGs) of soil microbes by imposing a lower environmental water potential (Schimel et al., 2007). N deposition would reduce the WTL-induced negative impact on the microbial function because more N can be used to synthesize chaperones and osmolytes (Csonka, 1989; Yerbury et al., 2005). In addition, we quantified the effect of WTL and N deposition on the global warming potential (GWP) of three GHGs on a 100-year time horizon. Using a metagenomics technique, this study is the first to investigate whether variations in CH₄ and N₂O emissions align with that of functional genes associated with CH₄ production and oxidation, nitrification and denitrification in a Tibetan wetland.

Materials and methods
Study site and mesocosm collection
This study was conducted at the Luanhaizi wetland (37°35′N latitude, 101°20′E longitude, 3250 m a.s.l.), located in the northeastern part of the Tibetan Plateau. This area is characterized by a continental monsoon climate, with short, cool summers and long, cold winters. From 1981 to 2010, the mean annual air temperature was −1.1 °C; the mean annual precipitation was 480 mm, and more than 80% of the precipitation was concentrated in the growing seasons (May to September). The wetland is dominated by the vascular plant *Carex pamirensis* and dotted with *Carex atrofusca*, *Hippuris vulgaris*, *Triglochin palustre* and *Heleocharis* spp (Fig. 1a). In the 0- to 10-cm soil layer of the wetland, the soil pH, total C and N content are 7.7, 16.2% and 1.1%, respectively. The local level of atmospheric N deposition ranges from 8.7 to 13.8 kg N ha⁻¹ yr⁻¹ (Fang et al., 2012).
A homogeneous area was selected for mesocosm collection. Twenty bottomless tanks (0.6 m length × 0.6 m width × 0.65 m height, n = 20) were inserted into the wetland soil in September 2010, which is close to the end of the growing season. After the soil froze at the end of October, the tanks were excavated (Fig. 1b), the bottom of the tanks was welded, and the outside of the tanks was wrapped with polystyrene foam to avoid heat exchange with their surroundings. To eliminate rain effects on the maintenance of the water table, the mesocosms were placed under a rainfall shelter that is approximately 1000 m away from the excavated site. The rainfall shelter was constructed with anti-ultraviolet plastic transparent plates (Fig. 1c). We let the mesocosms recover from collection and transportation until May 2011 by maintaining the water table at the same depth as that of the natural wetland.

Experimental design

Two water table levels and two N deposition levels were applied in a complete factorial design (2 water table × 2 N deposition). Each treatment had five replicates; twenty mesocosms were randomly arranged into five blocks. Two water table levels (WT₀, 3 cm above the soil surface, and WT⁻, 20 cm below the soil surface) simulated the present and future water table.
Two N deposition levels ($N^0$, 0 kg N ha$^{-1}$ yr$^{-1}$ addition; $N^+$, 30 kg N ha$^{-1}$ yr$^{-1}$ addition) mimicked the scenario of doubled N deposition.

The control water table was set to 3 cm above the soil surface, which was in line with the mean water table under natural growing season conditions. Because the lowest water table at this wetland dropped to approximately 20 cm below the soil surface in the 2010 growing season, the water table in the WTL plots was set to this water level. Wetland water was automatically supplemented by micropumps to control the water table accurately. Manostat systems connected to the micropumps (PULANDI 1205 Diaphragm Pump, Pulandi Machine Equipment Co., Shijiazhuang, China) were used to regulate the water table. When the water table was below the set points, the micropumps turned on and supplemented the tanks with wetland water transported from where the mesocosms were collected. Simulated N deposition was divided into four portions and applied monthly during the growing seasons from 2011 to 2013. The N deposition plots were sprayed with ammonium nitrate ($\text{NH}_4\text{NO}_3$) mixed with 1 L of water from the wetland, while the no-N deposition plots were sprayed with the same amount of water without $\text{NH}_4\text{NO}_3$.

GHG fluxes measurements

$\text{CO}_2$ fluxes

Net ecosystem $\text{CO}_2$ exchange (NEE) was measured with a transparent chamber (0.4 m length × 0.4 m width × 0.6 m height; without bottom) using an infrared gas analyzer (IRGA; LI-6400, LI-COR Inc., Lincoln, NE, USA). This method has been used and validated in a number of previous studies (e.g. Niu et al., 2008). Before the measurements, square collars (0.4 m length × 0.4 m width × 0.1 m height) were inserted 5 cm below the soil surface. During each measurement, the chamber was placed on the collar and sealed with water. A fan was fixed on the top of the sampling chamber to mix the air. Six consecutive values of $\text{CO}_2$ concentration were recorded at 10-s intervals during a measurement period of 1 min after steady-state conditions were reached. The $\text{CO}_2$ flux rate was calculated by the slope of linear regression of the six records in the time series of concentration. After the NEE measurements, we ventilated the chamber, replaced it on the same square collar, and finally covered it with an opaque cloth. These obtained values represent the ecosystem respiration (ER). Gross ecosystem productivity (GEP) was calculated by subtracting ER from NEE. Positive and negative values of $\text{CO}_2$ fluxes indicate $\text{CO}_2$ release and uptake, respectively. We measured $\text{CO}_2$ fluxes twice or thrice per month on sunny days between 9:00 and 12:00 local time for all treatments. We observed the diurnal patterns at 2-h intervals on 21 June, 14 July, 21 August and 6 September 2012.
CH₄ and N₂O fluxes

CH₄ and N₂O fluxes were measured according to Yu et al. (2013) using static opaque chamber and gas chromatography. The chambers were made of stainless steel and consisted of removable cover boxes (0.4 m length × 0.4 m width × 0.4 m height; without bottom) and square collars. The square collars were the same as those for the CO₂ measurements. A fan in each opaque chamber was used to mix the air during sampling. Gas samples were collected at 10-min intervals over 30 min using plastic syringes. Samples were stored in syringes following sampling and subsequently analyzed via gas chromatograph (Agilent 7890A, Agilent Co., Santa Clara, CA, USA) within 24 h. The chromatograph was equipped with a flame ionization detector (FID) to analyze the CH₄ concentration and an electron capture detector (ECD) to analyze the N₂O concentration (Wang & Wang, 2003). The carrier gas was N₂, and the operation temperature for the FID was set at 250 °C and ECD at 300 °C. CH₄ and N₂O fluxes were calculated by the slopes of linear regressions between gas concentrations and sampling time (0, 10, 20 and 30 min after chamber closure). The coefficients of determination (R²) of the linear regressions were sometimes low for N₂O (<0.4). To avoid a bias from omitting low fluxes, we kept those values when CH₄ concentrations showed a good linear trend with time as described by Dijkstra et al. (2013). Sampling was conducted twice or thrice per month between 9:00 and 12:00 local time for all treatments, and the diurnal patterns were measured on 26 June, 29 July, 21 August and 1 September 2012. CH₄ fluxes were measured from 2011 to 2013, and N₂O fluxes were measured in 2012 and 2013.

**GWP**

Daily fluxes were calibrated as the ratios of daily average values to daytime (9:00–12:00) average values. The calibration coefficients were 0.25 for NEE, 0.91 for ER, 0.86 for CH₄ and 0.98 for N₂O based on the diurnal patterns of CO₂, CH₄ and N₂O fluxes of the control plots in 2012 (Figs S4 and S5). Seasonal cumulative GHG fluxes were calculated by multiplying the average daily fluxes between two consecutive sampling dates by the time interval, and then by summing up the daily fluxes for all time intervals during the growing seasons. We further assessed the climate impact of growing season GHG budgets using the GWP, which is defined as time-integrated radiative forcing. The 100-year GWP (in g CO₂-eq m⁻²), which was adopted by the UNFCCC and the Kyoto Protocol, was calculated by adding the GWP from NEE, CH₄ (seasonal cumulative CH₄ fluxes in g CH₄ m⁻² multiplied by 28) and N₂O (seasonal cumulative N₂O fluxes in mg N₂O m⁻² multiplied by 265 × 10⁻³) (IPCC, 2013). It should be noted that the GWP in 2011 was calculated only by NEE and CH₄; however, the GWP showed no large differences because of the small contribution of N₂O (<5% in 2012–2013).

**Water table depth and soil temperature measurements**

While GHGs were sampled, water table depth and soil temperature at 10 cm depth were recorded. A slotted 2.5 cm diameter polyvinyl chloride pipe was
installed in each tank to measure water table depth (Fig. 1d). No significant changes in soil temperature at 10 cm depth were observed under WTL and N deposition conditions using portable temperature probes (JM 624 Digital Thermometer, Jinming Instrument Co., Ltd., Tianjin, China) (Fig. S1).

Estimation of aboveground net primary production and belowground biomass

The height and density of the plants were measured in the mesocosms during August in 2011, 2012 and 2013. A simulated method was used to estimate the aboveground net primary production (ANPP) nondestructively (Wang et al., 2012). To develop the simulation model, the height and density of the plants in 0.4 m length × 0.4 m width quadrats in the natural wetland were measured, and the aboveground biomass was harvested, dried and weighed in August 2012. Based on the measurements from the natural wetland, a linear equation was established to simulate aboveground plant biomass (APB): APB = −9.375 + 0.089 A + 0.972 H (P < 0.001, R² = 0.93, n = 41), where A is the total amount of plant stems and H is the mean plant height. The APB in each mesocosm was estimated using this equation to represent the annual ANPP because APB peaks in mid-August in this area.

Upon the completion of the experiment in September 2013, one soil core of 5.0 cm in diameter from each mesocosm was collected to a depth of 50 cm and washed with sieves. Live and dead roots were dried and weighed, and belowground biomass was estimated by the ratio of the live standing root crops to the total root biomass (0.56).

Soil sampling, DNA extraction and GeoChip 5.0 experiments

In mid-September 2013, three soil cores of 5.0 cm in diameter from a depth of 0−50 cm were randomly collected in each mesocosm and then well mixed as a composite soil sample by soil depth (0−10 cm, 10−20 cm, 20−30 cm and 30−50 cm). The soil samples were kept on ice before transportation to the laboratory. Soil samples were then sieved using 2-mm mesh to remove the plant roots and stones and preserved at −80 °C before DNA extraction. Soil samples of 0−10 cm were used for GeoChip 5.0 experiments because the topsoil was significantly affected by WTL and N deposition. Soil DNA was extracted using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA was further purified by precipitation with ethanol and then dissolved in nuclease-free water. Approximately 0.6 μg of community DNA was labeled with the fluorescent dye Cy-3 and hybridized with GeoChip 5.0 at 67 °C for 24 h in an Agilent hybridization oven. Then, GeoChip microarrays were scanned using a NimbleGen MS200 scanner (Roche, Madison, WI, USA). The detailed descriptions of DNA extraction, purification, labeling, hybridization to GeoChip 5.0, raw data processing and statistical analyses were reported in a previous study (Wang et al., 2014a).
Data analysis
Repeated-measures analysis of variance was used to test the effects of the main factors on CO$_2$, CH$_4$, N$_2$O and ANPP, with water table and N deposition as between-subject factors and year as a within-subject factor, including interactions. Repeated-measures analysis of variance was also used to examine the effects of the main factors on CO$_2$, CH$_4$ and N$_2$O fluxes within each year, with water table and N deposition as between-subject factors and sampling date as a within-subject factor, including interactions. We used post hoc tests (Tukey HSD) to test the differences among treatments. A paired test was used to analyze the effect of water table across different N deposition levels and the effect of N deposition across different water table levels. All statistical analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

Results
Aboveground net primary production and belowground biomass
Over the three years of the mesocosm experiment, WTL had no detectable effect on ANPP compared with the no-WTL plots (Fig. 2a). However, the effect of WTL varied with time, with a decrease (16.0%) in 2011, no changes in 2012 and an increase (13.7%) in 2013. Overall, N deposition enhanced ANPP by 11.5% across different water table levels (Fig. 2b). Additionally, this enhancement varied during the study period, with no effect on ANPP in 2011, an increase of 15.5% in 2012, and an increase of 12.4% in 2013.
At the end of the experiment, WTL significantly increased belowground biomass by 22.6% in comparison of the no-WTL plots, and N deposition elevated belowground biomass by 25.7% compared with the no-N deposition plots (Fig. 2c).

Carbon dioxide fluxes

GEP was higher than ER, leading to a net CO$_2$ absorption of 1019.1 g CO$_2$ m$^{-2}$ averaged by the three consecutive growing seasons (Fig. 3a–c). Seasonal
CO₂ flux dynamics ($P < 0.001$) were observed within each year (Table 1), and CO₂ fluxes peaked in July or August. Although WTL did not significantly affect net CO₂ uptake because of simultaneous increases in ER and GEP across the three years (Table 2), the effect varied with time and the trend changed from negative to positive in August 2012 (Fig. S2a). In comparison with the no-N deposition plots, N deposition elevated the net CO₂ uptake across the three years with an average increase of 25.2%, because of a smaller increase in ER than in GEP (Fig. S2a–c).

![Fig. 3 Seasonal dynamics of daytime (9:00–12:00) average net ecosystem carbon dioxide exchange (NEE) (a), ecosystem respiration (ER) (b) and gross ecosystem productivity (GEP) (c) under different treatments from 2011 to 2013. Bars show ± SE ($n = 5$). Negative values indicate sinks. The arrows indicate the dates of nitrogen application. CK, control; WT, water table lowering; N, nitrogen deposition; WT + N, combined water table lowering and nitrogen deposition.](image-url)
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WT, N and Date indicate water table, nitrogen deposition and sampling date, respectively. N₂O fluxes were not measured in 2011. *, ** and *** statistically significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$. 
Methane and nitrous oxide fluxes

CH₄ emissions ranged from 33.9 to 39.5 g CH₄ m⁻² across all treatments in the three years (Fig. 4a). Significant seasonal dynamics (P < 0.01) were observed (Table 1), and the highest value appeared in mid-August. WTL substantially reduced CH₄ emissions by 67.0% in 2011, 52.7% in 2012 and 52.7% in 2013 compared with the no-WTL plots, whereas N deposition had no significant effect on CH₄ emissions (Fig. S3a, Table 2).
The absorptions of N$_2$O were weak and varied from −3.4 to −12.9 mg N$_2$O m$^{-2}$ in the control plots (Fig. 4b). No significant seasonal dynamics were detected (Table 1), and the N$_2$O fluxes fluctuated around zero. WTL had no significant effect on N$_2$O fluxes, whereas N deposition significantly increased N$_2$O release ($P < 0.001$), resulting in net N$_2$O emissions (Fig. S3b, Table 2). Moreover, no significant WT × N interactions were observed.

**Global warming potential**

The GWP of growing season GHG budgets ranged from 14.6 to 876.3 g CO$_2$-eq m$^{-2}$ for the control plots (Fig. 5a). Overall, WTL turned the GWP of this wetland from positive (337.3 g CO$_2$-eq m$^{-2}$, warming) to negative (−480.1 g CO$_2$-eq m$^{-2}$, cooling) across different N deposition levels, and this change was mainly caused by the decrease in CH$_4$ emissions (Fig. 5b). Compared with the no-N deposition plots, N deposition reduced the GWP from 21.0 to −163.8 g CO$_2$-eq m$^{-2}$, because the increase in net CO$_2$ uptake exceeded the enhancement in CO$_2$ equivalents from CH$_4$ and N$_2$O emissions (Fig. 5c).
Abundance of genes associated with CH$_4$ and N$_2$O fluxes

The abundance of methane-producing gene (mcrA) markedly decreased ($P = 0.04$; Fig. 6a), whereas the abundance of methane-oxidizing genes (pmoA and mmoX) remained unchanged under WTL conditions (Fig. 6b). Similarly, N deposition considerably decreased the abundance of methane-producing genes ($P = 0.08$), but did not affect that of methane-oxidizing genes. These results imply that both WTL and N deposition mainly influence CH$_4$ production potential, rather than CH$_4$ oxidation potential.
WTL significantly decreased the abundance of genes (*amoA* and *hao*) involved in nitrification (*P* = 0.01; Fig. 6c), but marginally increased the abundance of genes (*nirS*, *nirK*, *narG* and *norB*) involved in denitrification (*P* = 0.06; Fig. 6d), suggesting that both nitrification and denitrification potentials were influenced by WTL. More specifically, WTL decreased the abundance of *amoA* or *nirS*, increased the abundance of *narG* and did not affect that of *hao*, *nirK* or *norB* (Fig. S6). N deposition did not affect the abundance of the nitrifying and denitrifying genes.

**Discussion**

Our results partially support hypotheses I and II; that is, WTL decreases CH$_4$ emissions and N deposition increases net CO$_2$ uptake and N$_2$O emissions. In contrast to our predictions, WTL did not affect net CO$_2$ uptake or N$_2$O fluxes,
and N deposition had no effect on CH$_4$ emissions. Hypothesis III, which states that the effects of WTL on GHG emissions are modulated by N deposition, is not supported. This study shows that both WTL and N deposition reduced the overall GWP although the mechanisms differed. That is, the WTL-induced reduction was mainly caused by decreased CH$_4$ emissions, whereas the N-induced reduction was largely caused by increased net CO$_2$ uptake. GeoChip analysis suggests that changes in CH$_4$ production potential, rather than CH$_4$ oxidation potential, provided explanation to the variations in net CH$_4$ emissions, and decreased nitrification potential and increased denitrification potential affected N$_2$O fluxes under WTL condition in this alpine wetland.

WTL decreased CH$_4$ emissions but did not affect NEE and N$_2$O fluxes

This three-year study provides direct evidence that simultaneous increases in ER and GEP led to little NEE response to WTL in this alpine wetland. Despite no detectable change across the three years, the response of NEE to WTL varied with time. In 2011, WTL decreased net CO$_2$ uptake, which is mainly attributed to increased soil CO$_2$ emission due to improved aeration (Wang et al., 2014b) and decreased water supply for plants (Malone et al., 2013). Interestingly, the negative influence of WTL on net CO$_2$ uptake became positive influence after August 2012. One possible explanation is plant acclimation to WTL. It is well documented that drought increases investment of carbon assimilation to roots (Farooq et al., 2009). More deep roots under WTL (Fig. 2c) would enable plants to access water in the subsoil. Another explanation is soil nutrient changes during the experiment. WTL-induced increase in N mineralization may stimulate photosynthetic CO$_2$ fixation in this low N availability ecosystem (Updegraff et al., 1995; Laiho, 2006). In short, this study shows that the effect of WTL on NEE varied with time, and demonstrates the necessity of long-term observations.

WTL substantially decreased CH$_4$ emissions over the three-year observation period, which is consistent with the finding of mesocosms experiment in Zoige peatlands (Yang et al., 2014a). This result is also supported by a study showing that seasonal CH$_4$ emissions are positively correlated with water table depth using eddy covariance methods in the same wetland (Song et al., 2015). Decreased CH$_4$ emissions can be caused either by inhibition of CH$_4$ production, or stimulation of CH$_4$ oxidation, or both. Using GeoChip approach, we further proved that decreased CH$_4$ production, rather than increased CH$_4$ oxidation, was responsible for the changes in CH$_4$ emissions under WTL conditions. The lack of change in CH$_4$ oxidation may be attributed to the following seasons. First, Carex pamirensis with well-developed aerenchyma has a higher capacity in transporting CH$_4$ to the atmosphere bypassing the oxidized zones of CH$_4$ consumption (Bridgham et al., 2013). Thus, CH$_4$ oxidation may play a minor role in net CH$_4$ emission, and improved aeration under WTL conditions led to a limited increase in CH$_4$ consumption. Second, the low oxygen density at high elevations (3200 m, approximately 70% of that at sea level) can to some extent limit CH$_4$ oxidation, because low
oxygen availability reduces the activities of methanotrophs (Le Mer & Roger, 2001). Further evidence is required to verify these speculations.

Generally, improved aerobic condition induced by WTL favors N mineralization and subsequent N$_2$O emissions (Updegraff et al., 1995). Nevertheless, our results show that WTL did not affect N$_2$O fluxes. This may be attributed to high C/N ratio (16.8) in the 0-30 cm soil layer in this alpine wetland (Song et al., 2015), since high C/N ratio limits the influence of WTL on N$_2$O emissions, as reported by Klemedtsson et al. (2005). It is well-known that nitrifying bacteria prefer aerobic conditions but denitrifying bacteria prefer anaerobic conditions. However, GeoChip analysis shows, on the one hand, that WTL decreased nitrification potential (amoA; Fig. S6). One explanation is that the alpine sedge negatively influences soil nitrification by competing for soil ammonium. In this present study, WTL increased ANPP and belowground biomass in 2013, thereby stimulating plant uptake of soil available N. Moreover, the alpine sedge prefers ammonium over nitrate (Raab et al., 1999), which may lead to less soil ammonium for nitrifiers. Other explanation is that the WTL-induced decrease in soil moisture reduces the diffusion of soil ammonium, resulting in low nitrification rate, as supported by a previous study (Osborne et al., 2016). On the other hand, GeoChip data show that WTL increased denitrification potential. This may result from the increased availability of soil nitrate because of the stimulating N mineralization under aerobic conditions and the relatively low uptake of the alpine sedge.

N deposition stimulated NEE and N$_2$O emissions but did not affect CH$_4$ emissions

N deposition stimulated net CO$_2$ uptake (Fig. 3a), suggesting that alpine wetlands may sequester more atmospheric CO$_2$ under N deposition during growing seasons. Notably, the observed changes in NEE may not be applied to future long-term projections, because shifts in plant community composition in the long term can influence the response of NEE to N treatment. For example, Bubier et al. (2007) and Larmola et al. (2013) reported that N addition increases vascular plants but decreases moss, leading to little impact after 5 years and a negative impact after 7 years on net CO$_2$ sink in an ombrotrophic bog. Hence, it remains unknown how long N-induced stimulation in NEE lasts in this wetland. However, at least in the short term, the increased CO$_2$ absorption partly compensated for the reduced CO$_2$ sink in ombrotrophic bogs.

CH$_4$ emissions were not affected by N deposition in this current study, which may result from the counteraction of direct and indirect influences. The direct influence was the reduced activity of methanogens, as indicated by decreased CH$_4$ production potential ($P = 0.08$; Fig. 6a). This is supported by the results of an experiment using soil columns in a peatland of the eastern Tibetan Plateau (Gao et al., 2014) showing that N addition inhibits CH$_4$ emissions. The indirect influence was caused by the increased plant ANPP,
which benefits CH$_4$ emissions (Hirota et al., 2004). Therefore, in the current study, the observed no effect of N deposition on CH$_4$ emissions is probably a result of a positive effect from plants being offset by a negative effect from microbes.

In the present study, N deposition generally shifted this alpine wetland from a N$_2$O sink to a N$_2$O source over the whole growing season, but variations existed in specific sampling period. For instance, no significant changes in N$_2$O emissions were detected under N deposition in mid-September 2013 (Fig. S3b). Our soil sampling for GeoChip experiment was also in this period. GeoChip data show no changes in nitrification and denitrification potentials, which is consistent with N$_2$O flux response to N deposition. We acknowledge that this low sampling frequency in GeoChip analysis due to the high cost is a limitation to the understanding of the mechanisms underlying the overall N$_2$O flux response.

No interactive effects of WTL and N deposition

Concurrent changes in water and N deposition in wetlands challenge the ability to extrapolate GHG responses based on the results of single-factor experiments. This study is among a few studies focusing on the interactions between water and N deposition at the ecosystem scale. For example, WTL stimulates N$_2$O emissions from nutrient-rich soils in peatlands, but has no impact on emissions from nutrient-poor soils (Martikainen et al., 1993; Aerts & Ludwig, 1997). In a California grassland, water addition alone increases net CO$_2$ uptake, but has no effect under N addition (Harpole et al., 2007). However, our finding suggests that N deposition did not alter the effects of WTL on GHG emissions in this alpine wetland. One possible explanation is that stimulated N mineralization increases N availability under WTL conditions in this wetland, thereby overlapping the effect of N deposition. Another explanation is that microbial community structure has changed except for physiological acclimation of microbes, when facing a larger soil water gradient. It has been reported that WTL increases fungi but decreases Gram-negative bacteria in the upper soil of a boreal fen (Jaatinen et al., 2007). We speculate that microbes reduce the demand for N because of shifting in community composition after three years of WTL in the current study.

WTL and N deposition both reduced the GWP of GHG emissions

Few studies have quantified the climate impact of alpine wetlands and its response to environmental changes. Using the widely used 100-year GWP, this study estimates that the pristine Tibetan alpine wetlands have a positive net GWP with growing season GHG budgets, that is exerting a net warming impact on global radiation balance. We further found that decreased CH$_4$ emissions led to reduction in GWP under WTL conditions, as supported by an earlier study in arctic wet tundra (Merbold et al., 2009). The weakened CH$_4$ source strength of alpine wetlands and the corresponding climate impact deserve more attention because the wetland of the Tibetan Plateau is one of
the two largest natural sources of CH$_4$ in China (Ding et al., 2004). By contrast, our results suggest that increased net CO$_2$ uptake was the dominant contributor to the reduction in GWP following N deposition. This finding is inconsistent with previous studies based on *in situ* experiments (Zhang et al., 2013), meta-analyses (Liu & Greaver, 2009) and coupled biogeochemical models (Lu & Tian, 2013), reporting that N-driven stimulation in CH$_4$ and N$_2$O emissions increases GWP. This discrepancy is possibly because of the larger responsive intensity of net CO$_2$ uptake than CH$_4$ and N$_2$O emissions in this alpine wetland.

On the Tibetan Plateau, continued climate warming has been projected by the IPCC global climate models, and it has been suggested that climate warming will lead to more permafrost degradation and WTL in typical alpine wetlands (Cheng & Wu, 2007; Trenberth *et al*., 2007). With intensified human activities, N deposition may increase in the near future. We estimated that the reduction in GWP ranged from 919.1 to 1132.5 g CO$_2$-eq m$^{-2}$ when WTL and N deposition were combined. If we simply extrapolate our results to the alpine wetlands (with the exception of riverine and lacustrine wetlands) of the Tibetan Plateau (6.3 $\times$ 10$^4$ km$^2$, Wei *et al*., 2015), approximately 57.9–71.3 Tg CO$_2$-eq per growing season will be taken up if all Tibetan wetlands are simultaneously subject to WTL and N deposition.

Our study was motivated by the alpine environments of the Tibetan Plateau, which are expected to strongly influence the GHG responses to environmental changes. The low oxygen density, among the unique environments, reduces the activities of methanotrophs. In addition, the alpine sedges acclimate to WTL by allocating more resources to deep roots. Furthermore, the sedge plants are efficient in CH$_4$ transportation and prefer ammonium over nitrate. Further studies should investigate these mechanisms for the influence of WTL and N deposition on GHG emissions.

It should be noted that wintertime GHG fluxes were not observed due to the harsh conditions of this wetland, which causes an underestimation of annual GHG emissions. However, this underestimation should not affect our general conclusions because the wetland is covered by ice during most of the non-growing season. In addition, we do not have inter-annual data on belowground biomass and soil properties to explain the inter-annual variations in ecosystem-scale GHG responses. These detailed ecosystem-level parameters, although need destructive sampling, warrant further investigation.

Here, for the first time, we examined the net climate impact of three GHG emissions and explored the possible molecular mechanisms underlying the changes in GHG emissions under WTL and N deposition in a Tibetan alpine wetland. Our results reveal that both WTL and N deposition decrease the GWP of GHG emissions by affecting different gas species. More importantly, the variances in microbial functional genes are aligned with the changes in ecosystem-scale GHG emissions. Our results suggest that microbial
mechanisms should be considered when predicting ecosystem-scale GHG responses to future environmental changes.

Acknowledgements

We thank Weimin Song and Biao Zhu for helpful suggestions on the manuscript, Li Li for assistance with the field measurements and Peter Schmid for language editing. This project was supported by the National Program on Key Basic Research Project (Grant No. 2014CB954004), National Natural Science Foundation of China (Grant No. 31270481 and 31321061) and the Programs of open ends funds from Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University. This collaboration is part of the ‘111 project’ of China.

References


