



## Low-level nitrogen deposition significantly inhibits methane uptake from an alpine meadow soil on the Qinghai–Tibetan Plateau



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### ABSTRACT

It is crucial to understand the effects of enhanced nitrogen (N) deposition on soil methane (CH<sub>4</sub>) uptake to develop a better comprehension of carbon (C) dynamics in terrestrial ecosystems. A two-year field study was conducted to assess the effects of various forms of N (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) and associated N deposition rates (0, 10, 20 and 40 kg N ha<sup>-1</sup> yr<sup>-1</sup>) on alpine meadow soil CH<sub>4</sub> fluxes on the Qinghai–Tibetan Plateau, China. Soil CH<sub>4</sub> fluxes, soil temperature, and soil moisture were monitored weekly using the static chamber technique and gas chromatography. Soil inorganic N pools, soil pH and aboveground biomass were measured monthly to examine the key controlling factors of soil CH<sub>4</sub> flux. Our results showed that N addition significantly promoted plant growth and changed soil water-filled pore space (WFPS), but did not alter soil inorganic N storages over the short term. Low rates of N addition significantly decreased the seasonal amount of CH<sub>4</sub> uptake by 8.6% compared with the control. Soil CH<sub>4</sub> fluxes were mainly determined by soil WFPS, followed by inorganic N availability. N addition increased the contribution of soil WFPS, pH and soil NO<sub>3</sub><sup>-</sup> storage. The observed reduction in CH<sub>4</sub> uptake caused by N addition may be largely due to a decrease in physical diffusion, as the biochemical inhibition effects on methanotrophic bacteria are minor. These results suggest that soil inorganic N is a regulatory factor of soil CH<sub>4</sub> uptake, and its promotion or inhibition to soil CH<sub>4</sub> uptake depends on the N status in terrestrial ecosystems.

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### 1. Introduction

Methane (CH<sub>4</sub>) is a powerful greenhouse gas and contributes to approximately 17% of global warming, with a relative global warming potential 23 times that of CO<sub>2</sub> on a molar basis (Parry, 2007). Over the past 200 years, human activities such as fossil fuel exploration, rice production, large-scale animal husbandry of ruminants, biomass burning and landfill gas emission have resulted in an increase of atmospheric CH<sub>4</sub> concentration by 148%, which is currently increasing by about 0.9% yr<sup>-1</sup> (Parry, 2007). Meanwhile, atmospheric CH<sub>4</sub> reacts with hydroxyl radicals (OH) in the troposphere and transfers to the stratosphere, accounting for 84% and 7% of the global CH<sub>4</sub> sink, respectively (Dutaur and Verchot, 2007). Also, uptake of CH<sub>4</sub> by aerobic soils removes a significant amount from the atmosphere (10–44 Tg yr<sup>-1</sup>), and accounts for up to 10% of the global CH<sub>4</sub> sink (Lowe, 2006). However, there is evidence that increases of atmospheric nitrogen (N) deposition

can significantly decrease CH<sub>4</sub> uptake by soils (Liu and Greaver, 2009), which may also contribute to increasing atmospheric CH<sub>4</sub> concentrations.

N addition may increase (Saari et al., 2004; Veldkamp et al., 2001), decrease (Domingues et al., 2007; Liebig et al., 2008; Mosier et al., 1998) or have no effects on (Phillips and Podrebarac, 2009; Sawamoto et al., 2010; Van den Pol-van Dassel et al., 1999) CH<sub>4</sub> uptake from grassland ecosystems, appearing to depend on the form and the rate of N addition, as well as on soil properties (Rigler and Zechmeister-Boltenstern, 1999). Serious debates have focused on the role of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> on soil CH<sub>4</sub> consumption. Many studies have demonstrated that elevated soil NH<sub>4</sub><sup>+</sup> may significantly reduce CH<sub>4</sub> oxidation rates (Bodelier and Laanbroek, 2004). However, several studies reported that NO<sub>3</sub><sup>-</sup>, rather than NH<sub>4</sub><sup>+</sup>, had the greatest inhibitory effect on CH<sub>4</sub> oxidation in forest soils (Reay and Nedwell, 2004; Wang and Ineson, 2003; Xu and Inubushi, 2004; Xu and Inubushi, 2007). Unfortunately, most studies on the response of soil CH<sub>4</sub> uptake to the forms and rates of N addition have been conducted using soil core incubation in a laboratory, which differs from N addition experiments done in the field. Also, all of these studies were focused on temperate forest ecosystems (Reay and Nedwell, 2004; Wang and Ineson, 2003; Xu and Inubushi, 2004; Xu and Inubushi, 2007), with no data available

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from alpine meadow ecosystems. Overall, the patterns and intrinsic mechanisms of how  $\text{NO}_3^-$  and  $\text{NH}_4^+$  affect  $\text{CH}_4$  uptake in grassland ecosystems remain unclear.

As the largest grassland unit on the Eurasian continent, the Qinghai–Tibetan Plateau is averagely situated at 4000 m above sea level, and covers an area of approximately 2.5 million  $\text{km}^2$  (Yang et al., 2008). Soil carbon (C) storage for 1 m in depth is estimated to be 4.68 Pg C, accounting for about 1/10 of total soil C storage in China (Yang et al., 2008). Due to an increase in regional economic development as well as long-range transport of atmospheric reactive N, atmospheric N deposition is very obvious in the eastern Qinghai–Tibetan Plateau, ranging from 4 to 13.8  $\text{kg N ha}^{-1} \text{yr}^{-1}$  (Liu et al., 2013; Lü and Tian, 2007). Chronic N deposition can significantly change the soil  $\text{CH}_4$  uptake over the short term (C. Jiang et al., 2010), and can further offset C sequestration in the entire alpine meadow ecosystem (Fang et al., 2012). To clarify  $\text{CH}_4$  uptake in the alpine meadow system within the context of increased N deposition, it is necessary to explore the responses of soil  $\text{CH}_4$  uptake by the different forms and the rates of N addition.

We hypothesized that  $\text{NO}_3^-$  and  $\text{NH}_4^+$  played different roles to soil  $\text{CH}_4$  uptake. This hypothesis was tested by conducting a two-year N addition experiment in an alpine meadow on the Qinghai–Tibetan Plateau. The main purposes of this paper are: (1) to examine the effects of the forms and rates of N addition on soil variables (soil temperature, water-filled pore space (WFPS), inorganic N pools and pH) and above-ground biomass; (2) to quantify the effects of the forms and rates of N addition on  $\text{CH}_4$  uptake in the alpine meadow soil; and (3) to investigate which factors are responsible for changes in  $\text{CH}_4$  uptake from the alpine meadow soil induced by N addition.

## 2. Materials and methods

### 2.1. Study site

The experiment was conducted at the Haibei alpine meadow ecosystem research station, Chinese Academy of Sciences ( $37^\circ37' \text{N}$ ,  $101^\circ19' \text{E}$ ), located in the eastern of Qinghai–Tibetan Plateau. The site is subjected to a plateau monsoon climate. Weather data collected between 2008 and 2009 at the meteorological station showed a mean annual air temperature of  $-0.4^\circ\text{C}$ , with an overall minimum and maximum of  $-23.2^\circ\text{C}$  (January) and  $14.5^\circ\text{C}$  (July), respectively. The annual average precipitation was approximately 383.3 mm, of which 85.6% occurred in May and September (Fig. 1). The vegetation type is a typical *Kobresia humilis* meadow. Dominant species are *Kobresia humilis*, *Saussurea superba*, *Potentilla saundersiana*, *Leontopodium nanum*, *Lancea tibetica*, *Festuca ovina*, *Festuca rubra*, *Stipa aliena*, *Elymus nutans*, *Helictotrichon tibetica*, *Koeleria cristata* and *Poa crymophila* (Cao et al.,

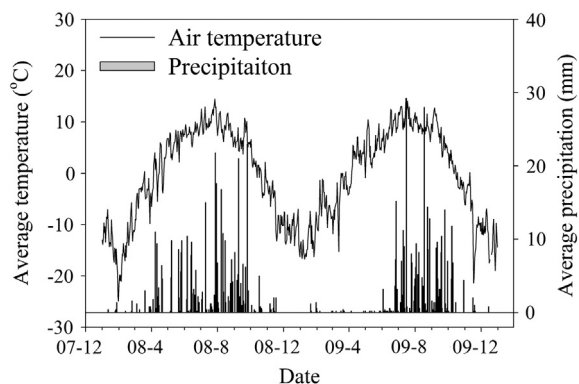


Fig. 1. The annual patterns of the daily average temperature and precipitation of the study site in 2008 and 2009.

2008). The soils developed in the Kobresia meadow are Mat-Gryic Cambisol (Cao et al., 2008).

### 2.2. Experimental design

The N addition experiment is a split plot design with N levels defining the main plots and N forms as subplots. Three N fertilizers ( $\text{NH}_4\text{Cl}$ ,  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{KNO}_3$ ) were applied as three levels (10, 20 and 40) of  $\text{kg N ha}^{-1} \text{yr}^{-1}$ . A control ( $0 \text{ kg N ha}^{-1} \text{yr}^{-1}$ ) was set at each plot and each N treatment had three replicates. Each plot had an area of  $9 \text{ m}^2$  ( $3 \text{ m} \times 3 \text{ m}$ ) and a 2 m isolation band was set between plots. The N addition experiment started on May 1, 2007. N fertilizer solutions were sprayed on the plots once a month, totally 12 equal applications over the year. Control plots received equivalent applications of water only. All plots are in the winter pastures, not grazed in the growing season, and grazed in non-growing season.

### 2.3. $\text{CH}_4$ flux measurement

Over the two growing seasons (from May to September) of 2008 and 2009, the soil  $\text{CH}_4$  fluxes were determined using the static chamber and gas chromatography method (Wang and Wang, 2003). At each plot, a stainless square box (length  $\times$  width  $\times$  height =  $50 \text{ cm} \times 50 \text{ cm} \times 50 \text{ cm}$ ), made up of one collar and one chamber, was installed. Gas samples were collected weekly between 9:00 AM and 11:00 AM (China Standard Time, CST) by fitting the chambers to the collars and removing headspace samples at ten-minute intervals over a 30-min period. Within 24 h following gas collection, the  $\text{CH}_4$  concentrations were analyzed on a gas chromatograph (HP Series 4890D, Hewlett Packard, USA), equipped with a flame ionization detector (FID). The  $\text{CH}_4$  fluxes were calculated, using chamber air temperature, barometric air pressure and the slope of the temporal change in  $\text{CH}_4$  concentration within the chamber headspace (Wang and Wang, 2003). The seasonal amount of  $\text{CH}_4$  uptake can be calculated using the following equation (J. Jiang et al., 2010):

$$E_{\text{CH}_4} = \frac{\sum_{i=1}^n 0.5 \times (F_i + F_{i+1}) \times (t_{i+1} - t_i) \times 24}{1000}$$

where,  $E_{\text{CH}_4}$  is the seasonal amount of  $\text{CH}_4$  uptake ( $\text{g CH}_4 \text{ m}^{-2}$ ),  $F_i$  and  $F_{i+1}$  are the  $\text{CH}_4$  fluxes of the  $i$ -th and  $i+1$ -th measurement ( $\text{mg CH}_4 \text{ m}^{-2} \text{h}^{-1}$ ), the term of  $(t_{i+1} - t_i)$  denotes the days between two adjacent days of the measurements,  $n$  is the total times of the measurements, and 24 is the number of hours per day.

Parallel to the flux measurements, soil temperature was recorded at the soil surface, 5 cm, and 10 cm belowground. Soil moisture was recorded at the 10 cm depth. Due to the proximity of the four subplots ( $<10 \text{ m}$  apart) and the simultaneous collection of air samples, soil temperature in the four subplots is considered to be the same. Volumetric soil moisture was transformed to water-filled pore space (WFPS):  $\text{WFPS} = \text{volumetric soil moisture} / (1 - \text{bulk density} / 2.65)$  (C. Jiang et al., 2010).

### 2.4. Field sampling and measurements

On the 15th day of each month during the two growing seasons, cores of mineral soil nearby the gas chambers were taken at 10 cm intervals to a depth of 50 cm using an auger (2.5 cm in diameter). Four core sets were collected at each plot and like depths were homogenized to obtain a sample. Soils were immediately passed through a 2 mm sieve to remove roots, gravel and stones. Sub-samples of each depth interval were extracted to determine  $\text{NH}_4^+ - \text{N}$  and  $\text{NO}_3^- - \text{N}$  concentrations using a continuous-flow autoanalyzer

(Bran Luebbe, Germany). Soil inorganic N storages (SIN,  $\text{g m}^{-2}$ ) at each plot were calculated using the following equation:

$$SIN = \sum_{i=1}^5 C_i \times BD_i \times 0.1$$

where, SIN is the storage of inorganic N at a plot ( $\text{mg m}^{-2}$ ),  $C_i$  is the concentration of inorganic N in the  $i$ -th horizon ( $\text{mg kg}^{-1}$ ),  $BD_i$  is soil bulk density of the  $i$ -th horizon ( $\text{g cm}^{-3}$ ), and 0.1 is the conversion factor.

Soil pH was determined in a 1:2 soil:water suspension using a standard pH meter (Mettler Toledo, Switzerland). In addition, aboveground biomass at each plot was measured by clipping vegetation at the ground level, drying plants at 60 °C to a constant mass, and weighing.

## 2.5. Statistical analysis

Repeated measurement analysis of variance was used to test the differences of soil temperature, WFPS, pH, inorganic N storages, aboveground biomass and  $\text{CH}_4$  fluxes among the different forms and rates of N addition. Comparisons of the means were conducted using the Tukey's HSD test. Simple and multiple stepwise regression analyses were used to examine the relationships between soil  $\text{CH}_4$  fluxes and related environmental variables. All statistical analyses were conducted using the SPSS software package (version 16.0). Statistical significant differences were set with  $p$  values < 0.05 unless stated otherwise.

## 3. Results

### 3.1. Soil $\text{CH}_4$ uptake

The alpine meadow ecosystem behaved as a net sink of atmospheric  $\text{CH}_4$  during the growing seasons in 2008 and 2009. Soil  $\text{CH}_4$  fluxes varied as a single-peak pattern, and the maximum occurred in early August 2008 (Fig. 2a–c). However, in 2009, the seasonality of soil  $\text{CH}_4$  fluxes weakened (Fig. 2a–c). In the control plots, the  $\text{CH}_4$  uptake fluxes from the alpine meadow ecosystem averaged  $31.1 \pm 3.5 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ , which was converted into the seasonal amount of  $104.69 \pm 5.60 \text{ g CH}_4 \text{ m}^{-2}$  (Table 1). N addition tended to

inhibit soil  $\text{CH}_4$  uptake, especially in medium N treatments (Table 1). N addition decreased the seasonal amount of  $\text{CH}_4$  uptake by  $-1.9\%$  to  $18.5\%$  without considering N addition types, with a mean of  $8.6\%$  (Table 1).

### 3.2. Soil temperature and WFPS

During the entire growing season, the soil surface temperature fluctuated greatly (Fig. 3a–c). Soil temperature at the 5 cm and 10 cm depths exhibited a mono-peak change, ranging from 3.2 to 15.9 °C (Fig. 3a–c). The maximum soil temperature occurred at the end of July (Fig. 3a–c). There was no significant difference in soil temperature from the surface, 5 cm or 10 cm depths among the different N addition rates (Table 1).

In contrast to soil temperature, soil WFPS exhibited an overall single-pit pattern in 2008. The high WFPS values were recorded in early May and at the end of September, and fluctuated violently in the other months (Fig. 4a–c). Among the different types of nitrogenous fertilizers, soil WFPS showed a similar trend, with low and high rates of N addition trending to decrease soil WFPS, while medium N significantly increased soil WFPS (Table 1). In addition, the effect of  $\text{NO}_3^-$ -N fertilizer on soil WFPS seemed to be stronger than those of  $\text{NH}_4^+$ -N fertilizers (Table 1).

### 3.3. Soil $\text{NH}_4^+$ -N and $\text{NO}_3^-$ -N pools

The pool of soil  $\text{NH}_4^+$ -N peaked in June, and either August or September, and obviously dropped in July (Fig. 5a–c). In the control plots, soil  $\text{NH}_4^+$ -N storage in the alpine meadow ranged from 1.83 to 7.86  $\text{g m}^{-2}$ , with an average of  $4.01 \pm 0.30 \text{ g m}^{-2}$  (Fig. 5a–c). N addition was seen to both deplete and accumulate soil  $\text{NH}_4^+$ -N pool, despite the fertilizer form, but the difference between N addition plots and control plots was not significant after three-year N addition (Table 1).

The monthly variation of soil  $\text{NO}_3^-$ -N pools was not consistent with that of soil  $\text{NH}_4^+$ -N pools. The peaks of soil  $\text{NO}_3^-$ -N pools occurred in June or July, and minimum values occurred in August or September (Fig. 6a–c). In the control plots, soil  $\text{NO}_3^-$ -N pools in the alpine meadow soils ranged from 0.59 to 3.14  $\text{g m}^{-2}$ , with an average of  $1.47 \pm 0.12 \text{ g m}^{-2}$ , which was significantly lower than that of soil  $\text{NH}_4^+$ -N pool (Table 1). In 2008 and 2009, soil  $\text{NO}_3^-$ -N pool accumulated in a positive trend with N addition; moreover, the cumulative effect of  $\text{NO}_3^-$ -N fertilizer was slightly higher than those of  $\text{NH}_4^+$ -N fertilizers (Table 1). However, the difference between N addition treatments and control was not significant (Table 1).

### 3.4. Aboveground biomass

Aboveground biomass was lowest in May, then gradually increased and reached its maximum in August, after which it began to decrease (Fig. 7a–c). In the control plots, aboveground biomass in the alpine meadow ranged from 147.6 to 581.3  $\text{g m}^{-2}$ , with an average of  $356.4 \pm 9.9 \text{ g m}^{-2}$  (Fig. 7a–c). Three levels of N addition consistently increased aboveground biomass during the growing seasons of 2008 and 2009; moreover, the positive effect of N addition on plant growth significantly increased with the N addition rate (Table 1).

### 3.5. Soil pH

Throughout the growing season, soil pH did not vary dramatically (Fig. 8a–c). In 2008, N addition tended to decrease soil pH, especially in the high N plots (Fig. 8a–c). In 2009, N addition significantly decreased soil pH values in the forms and levels of nitrogenous fertilizer addition (Fig. 8a–c). The decline of soil pH caused by N addition ranged from 0.07 to 0.21 units without considering N addition forms (Table 1). Moreover, the decrease of soil pH values caused by  $\text{NO}_3^-$ -N fertilizer was higher than that caused by  $\text{NH}_4^+$ -N fertilizers (Table 1).

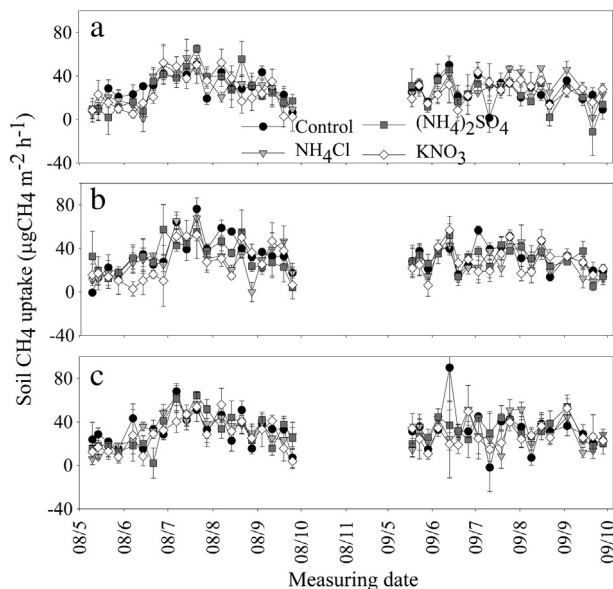


Fig. 2. Seasonal variations of soil  $\text{CH}_4$  fluxes in low (a), medium (b), and high (c) N treatments.

**Table 1**The seasonal amount of soil CH<sub>4</sub> uptake and relevant soil properties in different N treatments over the two growing seasons of 2008 and 2009.

N level	N form	Seasonal CH <sub>4</sub> uptake (g CH <sub>4</sub> m <sup>-2</sup> ) <sup>a</sup>	Soil temperature (°C) <sup>a</sup>			Soil WFPS (m <sup>3</sup> m <sup>-3</sup> ) <sup>a</sup>	Soil NH <sub>4</sub> <sup>+</sup> -N pool (g m <sup>-2</sup> ) <sup>a</sup>	Soil NO <sub>3</sub> <sup>-</sup> -N pool (g m <sup>-2</sup> ) <sup>a</sup>	Aboveground biomass (g m <sup>-2</sup> ) <sup>a</sup>	Soil pH <sup>a</sup>
			Surface	5 cm	10 cm					
Control	Control	104.69 ± 5.60 a	13.7 ± 0.7 a	9.2 ± 0.6 a	9.3 ± 0.7 a	0.37 ± 0.02 b	4.01 ± 0.30 ab	1.47 ± 0.12 a	356.4 ± 9.9 b	7.37 ± 0.02 a
Low N	NH <sub>4</sub> Cl	106.68 ± 1.56 a	13.0 ± 0.8 a	9.2 ± 0.6 a	9.2 ± 0.6 a	0.35 ± 0.03 b	3.81 ± 0.56 b	1.53 ± 0.16 a	376.0 ± 7.2 ab	7.29 ± 0.09 ab
Low N	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	103.83 ± 6.99 a				0.35 ± 0.03 c	4.30 ± 0.61 ab	1.58 ± 0.17 a	397.1 ± 7.6 a	7.30 ± 0.07 ab
Low N	KNO <sub>3</sub>	104.52 ± 3.19 a				0.34 ± 0.03 c	3.81 ± 0.56 b	1.78 ± 0.18 a	390.6 ± 7.3 ab	7.24 ± 0.17 ab
Medium N	NH <sub>4</sub> Cl	85.31 ± 9.76 b	13.5 ± 0.5 a	9.4 ± 0.6 a	9.3 ± 0.7 a	0.39 ± 0.02 ab	4.23 ± 0.60 ab	1.44 ± 0.18 a	378.3 ± 6.7 ab	7.30 ± 0.08 ab
Medium N	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	93.78 ± 6.91 ab				0.39 ± 0.03 ab	4.28 ± 0.62 ab	1.65 ± 0.19 a	398.8 ± 6.9a	7.32 ± 0.08 ab
Medium N	KNO <sub>3</sub>	87.37 ± 0.87 b				0.40 ± 0.02 a	4.20 ± 0.64 ab	1.68 ± 0.20 a	404.8 ± 8.1 a	7.15 ± 0.13 b
High N	NH <sub>4</sub> Cl	94.59 ± 8.22 ab	13.8 ± 0.9 a	9.6 ± 0.5 a	9.5 ± 0.7 a	0.36 ± 0.02 bc	4.54 ± 0.61 a	1.62 ± 0.18 a	390.7 ± 7.0 ab	7.28 ± 0.03 ab
High N	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	94.17 ± 1.18 ab				0.35 ± 0.03 c	4.15 ± 0.58 ab	1.58 ± 0.17 a	400.8 ± 7.7a	7.27 ± 0.05 ab
High N	KNO <sub>3</sub>	91.05 ± 4.22 ab				0.34 ± 0.02 c	3.85 ± 0.56 b	1.66 ± 0.16 a	409.8 ± 7.1a	7.19 ± 0.10 b

<sup>a</sup> Data are shown as means with standard errors; different lower case letters in a column indicate significant differences between treatments at  $p < 0.05$ .

### 3.6. Relationships between soil CH<sub>4</sub> fluxes and environmental variables

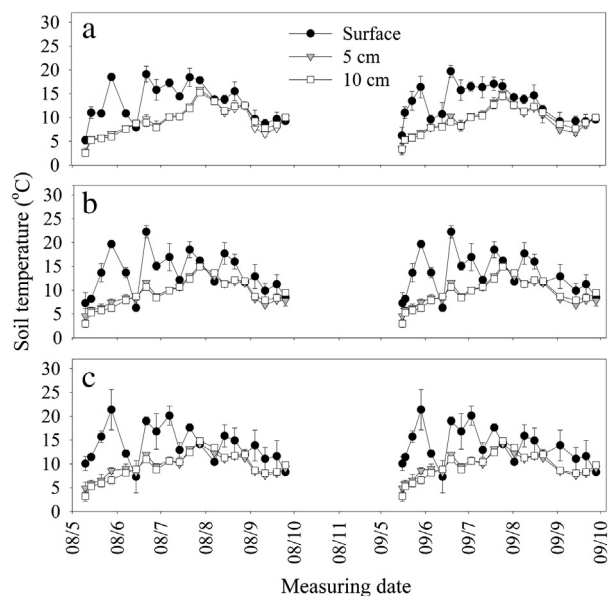
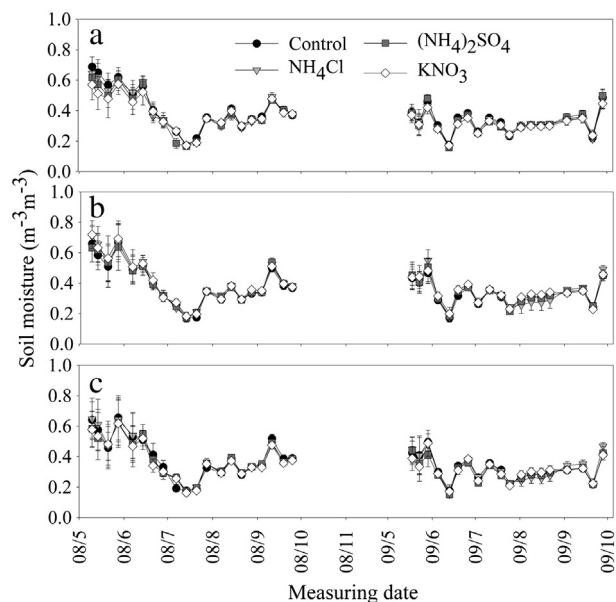
Soil CH<sub>4</sub> fluxes were positively correlated with soil temperature at the 5 cm depth, while negatively correlated with soil WFPS at the 10 cm depth (Fig. 9a–b). The relationships between soil CH<sub>4</sub> fluxes and soil WFPS could be well fitted with an exponential decay equation (Fig. 9b). Although soil CH<sub>4</sub> uptakes were positively correlated with soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N pools, only the relationships between CH<sub>4</sub> fluxes and soil NO<sub>3</sub><sup>-</sup>-N pools were significant (Fig. 9c–d and Table 2). These results showed that soil drought and NO<sub>3</sub><sup>-</sup>-N accumulation contributed to soil CH<sub>4</sub> consumption. Also, the relationships between soil CH<sub>4</sub> fluxes and aboveground biomass values were fitted by a quadratic equation (Fig. 9e–f), suggesting that vegetation has positive and negative effects on soil CH<sub>4</sub> uptake. Except for control plots, soil CH<sub>4</sub> fluxes at the N addition plots were positively correlated with soil pH, suggesting that soil acidification caused by N addition could inhibit soil CH<sub>4</sub> uptake. In the control plots, soil WFPS, temperature, aboveground biomass and NO<sub>3</sub><sup>-</sup>-N pool could explain 38.5%, 23.4%, 12.7% and 3.7% of the variation of soil CH<sub>4</sub> uptakes, respectively (Table 2). N addition tended to increase the contributions of soil WFPS, pH and NO<sub>3</sub><sup>-</sup> pool to CH<sub>4</sub> uptake, which could be reflected by regression coefficient ( $R^2$ ) values (Table 2). Multiple stepwise regression analysis indicated that soil CH<sub>4</sub> fluxes were mainly controlled by soil WFPS, followed by soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N pools at the control plots (Table 2). However, only soil WFPS and NO<sub>3</sub><sup>-</sup>-N pool dominated

soil CH<sub>4</sub> uptakes at the N addition treatment plots (Table 2). Therefore, our results suggest that the decreasing physical diffusion by soil WFPS rather than biochemical inhibition by other variables was responsible for the decrease in soil CH<sub>4</sub> uptake.

## 4. Discussion

### 4.1. Comparisons with other studies

Under natural conditions, the alpine meadow ecosystem is a sink of atmospheric CH<sub>4</sub>. The average CH<sub>4</sub> uptake flux during the study period ( $31.1 \pm 3.5 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ ) is close to the values ( $26\text{--}30 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ ) reported by other studies at the same site (Cao et al., 2008; Lin et al., 2009). However, the fluxes of CH<sub>4</sub> uptake are lower than fluxes reported in alpine steppe grasslands in the middle Tibetan Plateau, China ( $63.4\text{--}70.2 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ , Wei et al., 2012), the alpine grassland of the Tianshan Mountains, China ( $54.2 \pm 6.9 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ , Li et al., 2012) and the alpine dry meadow in the Eastern Alps ( $41.7\text{--}87.5 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ , Koch et al., 2007). CH<sub>4</sub> fluxes are significantly correlated with soil moisture (Fig. 9), and the difference in moisture availability is likely to be the most important driver of soil CH<sub>4</sub> fluxes in these alpine grasslands (Sjögersten et al., 2012). The soil moisture in the Haibei alpine meadow (37%) was higher than those in the above study sites (less than 30%), which is the main reason for the lower soil CH<sub>4</sub> uptake.

**Fig. 3.** Monthly variation of soil temperature in low (a), medium (b), and high (c) N treatments.**Fig. 4.** Monthly variations of soil WFPS in low (a), medium (b), and high (c) N treatments.

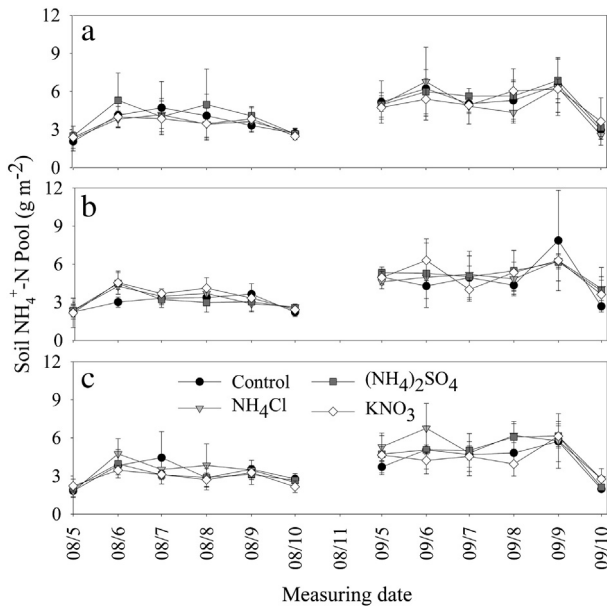


Fig. 5. Monthly variations of soil  $\text{NH}_4^+\text{-N}$  pool in low (a), medium (b), and high (c) N treatments.

Similar to many N addition experiments in grassland ecosystems (Domingues et al., 2007; Liebig et al., 2008; Mosier et al., 1998),  $\text{CH}_4$  uptake in the alpine meadow system is significantly inhibited by N addition over the short-term. This differs from some studies saying that N addition does not significantly affect or promote soil  $\text{CH}_4$  uptake (Phillips and Podrebarac, 2009; Sawamoto et al., 2010; Van den Pol-van Dasselaar et al., 1999). Moreover, the rates of N addition in our study were lower than those in the above studies, suggesting that the alpine meadow ecosystem is very sensitive to increased N deposition. The fact that N addition is inhibiting the  $\text{CH}_4$  uptake implies that the Tibetan Plateau may remove less  $\text{CH}_4$  under future increased nitrogen deposition conditions.

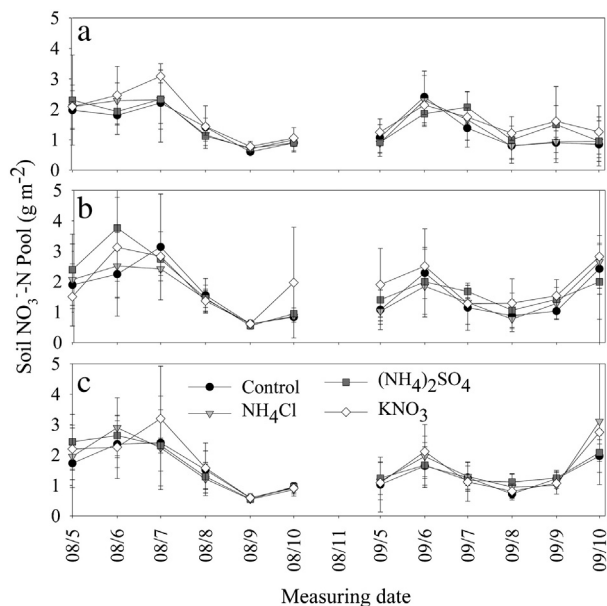


Fig. 6. Monthly variations of soil  $\text{NO}_3^-\text{-N}$  pool in low (a), medium (b), and high (c) N treatments.

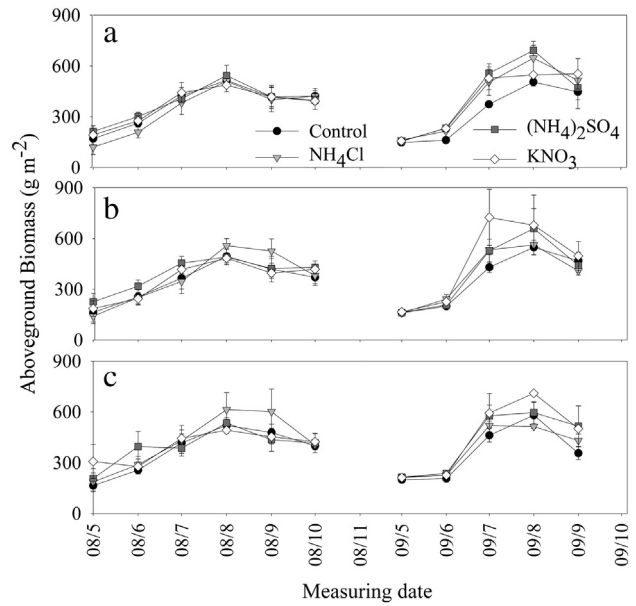


Fig. 7. Monthly variations of aboveground biomass in low (a), medium (b), and high (c) N treatments.

#### 4.2. Effects of soil WFPS on $\text{CH}_4$ uptake

Theoretically, the variation of soil moisture content depends on the dynamic balance between the input by precipitation and the loss by ecosystem evapotranspiration in semiarid alpine meadow ecosystems. In the study, precipitation was the same among the plots, and thus the differences in soil moisture content among the different N addition plots could be attributed to differences in ecosystem evapotranspiration among the plots. Ecosystem evapotranspiration is positively correlated with soil moisture (St Clair et al., 2009). N fertilizer application generally not only increases plant primary production and decreases species diversity, but also increases evapotranspiration (Sonnleitner et al., 2001). Our study showed that low and high N addition tended to decrease soil moisture, which could be related to the increase of ecosystem evapotranspiration. However, the responses of soil moisture

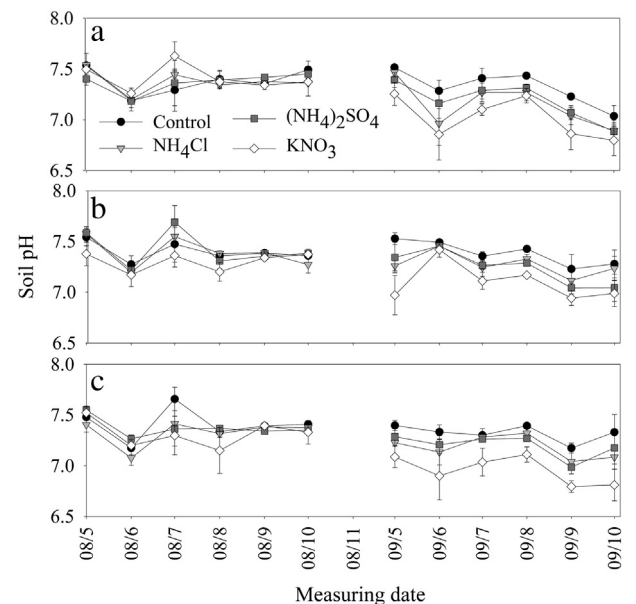


Fig. 8. Monthly variations of soil pH in low (a), medium (b), and high (c) N treatments.

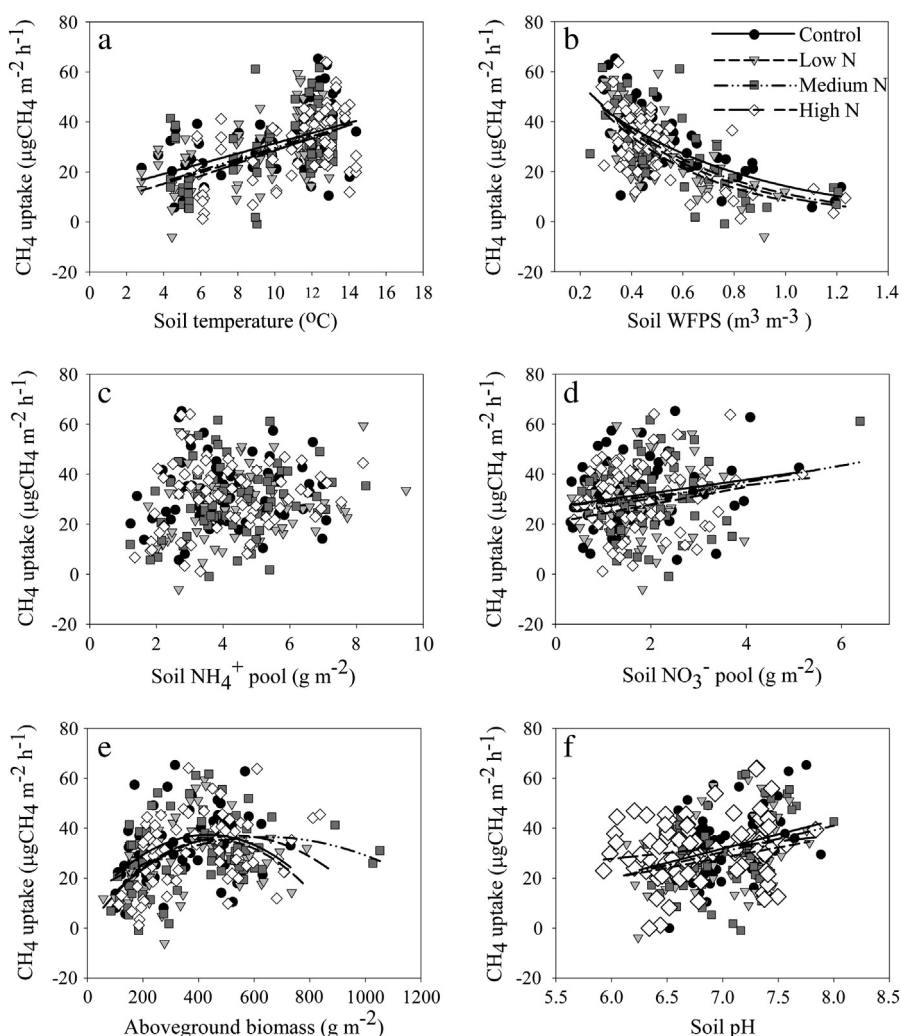


Fig. 9. Relationships between soil CH<sub>4</sub> fluxes and soil temperature, WFPS, inorganic N pools, pH and aboveground biomass.

to N addition are ambiguous—including positive (Fang et al., 2012), negative (Inouye, 2006), and neutral (Xia et al., 2009) results. Therefore, the driving mechanism behind this pattern needs to be examined further.

Because diffusion of CH<sub>4</sub> in water is 104 times slower than in air (Marrero and Mason, 1972), soil moisture controls the mass flow of air and diffusion of atmospheric CH<sub>4</sub> into the soil by altering the WFPS of soils (Domingues et al., 2007; Lin et al., 2009). The temporal variation in CH<sub>4</sub> uptake from each plot was consistent with that of soil WFPS (Figs. 2 and 4). Moreover, the fluxes of CH<sub>4</sub> uptake from N addition plots and control plots were significantly related to soil WFPS (Fig. 8b and Table 2). N addition usually stimulates vegetation growth in terrestrial ecosystems, and the subsequent changes in evapotranspiration enhance or lower soil WFPS, leading to higher or lower diffusion of CH<sub>4</sub> and O<sub>2</sub> into the soil (Veldkamp et al., 2001). In our study, medium N addition significantly increases soil WFPS, which corresponds to the significant decrease in soil CH<sub>4</sub> uptake (Table 1). Compared with other variables, soil WFPS plays a more important role in CH<sub>4</sub> uptake based on multiple regression results. This implies that physical diffusion dominates the direction and magnitude of the CH<sub>4</sub> uptake variation caused by N addition. Moreover, the determination coefficient ( $R^2$ ) between CH<sub>4</sub> uptake fluxes and soil WFPS values increases from 38.5% at control plots to 43.4–47.2% at N addition plots (Table 2), suggesting an increasing contribution of soil WFPS to the variation of CH<sub>4</sub> uptake induced by N addition.

#### 4.3. Effects of soil NH<sub>4</sub><sup>+</sup>-N variation on CH<sub>4</sub> uptake

Previous studies have shown that CH<sub>4</sub> oxidation in grassland soils can be inhibited by the addition of NH<sub>4</sub><sup>+</sup>-N fertilizers (Chan and Parkin, 2001; Dittert et al., 2005; Jacinthe and Lal, 2006), probably because (1) NH<sub>4</sub><sup>+</sup> competes with CH<sub>4</sub> for the same active site on the CH<sub>4</sub> monooxygenase of methanotrophs (Carlsen et al., 1991). (2) NH<sub>4</sub><sup>+</sup> oxidizes to the intermediate hydroxylamine (NH<sub>2</sub>OH) by CH<sub>4</sub> monooxygenase, or to its further oxidation by other enzyme systems of the methanotrophs to the end product nitrite (NO<sub>2</sub><sup>-</sup>). Hydroxylamine and nitrite in turn might lead to intoxication of CH<sub>4</sub> oxidizers (Schnell and King, 1994). (3) Indirect effects of N-treatments, such as high osmotic pressure, could have killed methanotrophs (Bodelier and Laanbroek, 2004). Generally, the oxidation of methanotrophic bacteria is optimally active at low osmotic stress (Saari et al., 2004). (4) N addition probably increases soil CH<sub>4</sub> emission to offset CH<sub>4</sub> uptake. Higher litter input under N enrichment alleviates C limitation to microbes (Bodelier and Laanbroek, 2004). As a result, the activities of methanogenic archaea are enhanced and more CH<sub>4</sub> is produced (Bodelier and Laanbroek, 2004).

However, in this study, it seems that decreased CH<sub>4</sub> uptake is likely to be unrelated to any competitive inhibition of monooxygenase by NH<sub>4</sub><sup>+</sup> and toxic inhibition by hydroxylamine or nitrite. The relatively insignificant or positive effect of NH<sub>4</sub><sup>+</sup> on CH<sub>4</sub> uptake observed here may be attributed to the following aspects. First, the alpine meadow

**Table 2**  
Regression model between soil CH<sub>4</sub> fluxes and environmental variables.

N addition rates <sup>a</sup>	a	b	c	d	p	R <sup>2</sup>
<i>(a) F<sub>CH4</sub> = a * T<sub>s</sub> + b</i>						
Control	2.04	11.03			<0.0001	0.234
Low N	2.27	6.20			<0.0001	0.266
Medium N	2.26	7.04			<0.0001	0.210
High N	2.54	3.12			<0.0001	0.253
<i>(b) F<sub>CH4</sub> = a * exp(-b * WFPS)</i>						
Control	72.27	1.62			<0.0001	0.385
Low N	86.51	2.33			<0.0001	0.434
Medium N	82.74	2.00			<0.0001	0.472
High N	85.36	2.15			<0.0001	0.449
<i>(c) F<sub>CH4</sub> = a * NH<sub>4</sub><sup>+</sup> + b</i>						
Control					0.4341	0.0006
Low N					0.1508	0.0154
Medium N					0.1436	0.0165
High N					0.3034	0.0011
<i>(d) F<sub>CH4</sub> = a * NO<sub>3</sub><sup>-</sup> + b</i>						
Control	2.71	26.92			0.039	0.037
Low N	3.54	20.77			0.029	0.042
Medium N	3.76	22.63			0.010	0.062
High N	2.20	26.77			0.014	0.057
<i>(e) F<sub>CH4</sub> = a * AB<sup>2</sup> + b * AB + c</i>						
Control	-0.0001	0.128	9.71		0.003	0.127
Low N	-0.0001	0.104	7.22		0.008	0.085
Medium N	-0.0001	0.073	13.31		0.005	0.118
High N	-0.0001	0.128	2.851		0.009	0.161
<i>(f) F<sub>CH4</sub> = a * pH + b</i>						
Control	4.67	-0.258			0.109	0.020
Low N	8.19	-29.08			0.011	0.066
Medium N	10.54	-43.32			0.004	0.087
High N	11.38	-47.83			0.002	0.103
<i>(g) F<sub>CH4</sub> = a * WFPS + b * NH<sub>4</sub><sup>+</sup> + c * NO<sub>3</sub><sup>-</sup> + d</i>						
Control	-44.39	-1.72	2.13	58.48	<0.001	0.433
Low N	-53.22		3.97	47.45	<0.001	0.458
Medium N	-44.61		2.03	49.44	<0.001	0.430
High N	-46.05		2.62	49.43	<0.001	0.428

<sup>a</sup> T<sub>s</sub> is soil temperature at 5 cm depth, WFPS is soil water-filled pore space at 10 cm depth, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> are soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N storages at 50 cm depth, and AB is aboveground biomass.

soil in our study site is N-limited and has a strong capacity to immobilize exogenous NH<sub>4</sub><sup>+</sup> (Song et al., 2007). The NH<sub>4</sub><sup>+</sup> immobilization by soil organic and mineral materials rapidly removes applied NH<sub>4</sub><sup>+</sup>, which would protect methanotrophs from exposure to NH<sub>4</sub><sup>+</sup> and accordingly mitigate the inhibition of methanotrophs. Second, the increasing nitrification by N addition would to some degree alleviate the inhibition of inorganic N to CH<sub>4</sub> oxidation (Chan et al., 2005). Although NH<sub>4</sub><sup>+</sup>-oxidizing bacteria are capable of oxidizing CH<sub>4</sub> at rates lower than methanotrophs, CH<sub>4</sub> oxidation is dominated by nitrifiers (Steudler et al., 1996). Thus, N addition enhances the availability of NH<sub>4</sub><sup>+</sup> to nitrifiers, which would accordingly decrease the extent to which CH<sub>4</sub> consumers are exposed to NH<sub>4</sub><sup>+</sup> (Chan et al., 2005). Finally, the ratio of NH<sub>3</sub>:NH<sub>4</sub><sup>+</sup> in soils is decreased by N addition. Gullede et al. (1997) suggested that NH<sub>3</sub>, rather than NH<sub>4</sub><sup>+</sup>, was the key determinant of this inhibition, because the decreased soil pH induced by N addition would decrease the ratio of NH<sub>3</sub>:NH<sub>4</sub><sup>+</sup> of soils. This is likely to decrease the degree of inhibition linked to N addition; however, no such clear regulation of NH<sub>4</sub><sup>+</sup> inhibition through pH has so far been identified (Gullede et al., 1997; Hütsch, 1996).

#### 4.4. Effects of soil NO<sub>3</sub><sup>-</sup>-N variations on CH<sub>4</sub> uptake

Similar with NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> also showed a very strong inhibitory effect in some forest ecosystems (Rigler and Zechmeister-Boltenstern, 1999). Moreover, some studies suggest that NO<sub>3</sub><sup>-</sup> may have a greater importance in the inhibition of CH<sub>4</sub> oxidation in forest soils than that attributed to

NH<sub>4</sub><sup>+</sup> (Wang and Ineson, 2003; Xu and Inubushi, 2004; Xu and Inubushi, 2007). Generally, NO<sub>3</sub><sup>-</sup> has been found inhibitory only in very high concentrations, which likely give rise to osmotic effects (Bodelier and Laanbroek, 2004). The other reason is that added NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> produced via NO<sub>3</sub><sup>-</sup> reduction in anaerobic 'microsites' are probably toxic to CH<sub>4</sub>-oxidizing bacteria (Schnell and King, 1994; Xu and Inubushi, 2004; Xu and Inubushi, 2007).

However, our study showed that NO<sub>3</sub><sup>-</sup> accumulation could significantly promote CH<sub>4</sub> uptake in soil (Fig. 9 and Table 2). Bodelier and Laanbroek (2004) proposed a schematic explanation about the stimulation of CH<sub>4</sub> oxidation by NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> addition to soils. For one thing, atmospheric methanotrophic bacteria are limited by N because they have a relatively high N requirement during C assimilation (Megm and Knowles, 1987). N addition enhances soil mineralization rates and C and N availability for soil microbes (Rigler and Zechmeister-Boltenstern, 1999). Besides utilizing CH<sub>4</sub> in the atmosphere and in soil pores, it has been demonstrated that atmospheric CH<sub>4</sub> consumers can profit from and even depend on some non-methane substrates such as methanol, formate, and acetate (Bodelier and Laanbroek, 2004; Jensen et al., 1998). The improved availability of these C sources would promote CH<sub>4</sub> consumption by aerobic soils. Also, during the rainy season, the alpine meadow soils may become partially anoxic following high precipitation events and begin producing CH<sub>4</sub>. The methanotrophic bacteria can profit from this enhanced CH<sub>4</sub> flux and grow when sufficient N is present (Bodelier and Laanbroek, 2004). However, in our study, N addition decreased overall soil CH<sub>4</sub> uptake. The promotion produced by NO<sub>3</sub><sup>-</sup> accumulation is almost masked by the inhibition associated with decreasing WFPS.

#### 4.5. Effects of other associated ions on CH<sub>4</sub> uptake

Besides NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup>, the associated ions of nitrogenous salts can also partly explain the different inhibition patterns. Some studies suggest that salt-effect may be a more reasonable explanation for reduced CH<sub>4</sub> oxidation than the specific NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> effect (Borken and Brumme, 2009; King and Schnell, 1998; Whalen, 2000). A laboratory study showed that high concentration of Cl<sup>-</sup> has a strong inhibitory effect on CH<sub>4</sub> oxidation by comparing the difference in ability to do so between N-salts (NH<sub>4</sub>Cl and NaNO<sub>3</sub>) and non-N-salt (NaCl) (Whalen, 2000). In the field, however, various N treatments ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>-acetate, urea, NH<sub>4</sub>Cl, NaNO<sub>3</sub>, NH<sub>4</sub>NO<sub>3</sub> and KNO<sub>3</sub>) added at a same rate of 30 kg N ha<sup>-1</sup> had little or no effect on CH<sub>4</sub> uptake within one year (Borken and Brumme, 2009). The rates of N addition (10–40 kg N ha<sup>-1</sup> yr<sup>-1</sup>) in our study are comparable to that of Borken and Brumme (2009), so added K<sup>+</sup>, SO<sub>4</sub><sup>2-</sup> and Cl<sup>-</sup> could have little contribution to the decrease in CH<sub>4</sub> uptake. Also, the K<sup>+</sup> can exchange NH<sub>4</sub><sup>+</sup> from the exchange sites in the soil and thus released NH<sub>4</sub><sup>+</sup> could then inhibit CH<sub>4</sub> oxidation. If K<sup>+</sup> was the responsible component of the added salt in the current study, ion exchange should result in a higher NH<sub>4</sub><sup>+</sup> concentration. However, the soil NH<sub>4</sub><sup>+</sup> pool did not accumulate significantly, and even declined in the low and high KNO<sub>3</sub> addition plots (Table 1). Thus, the salt effects could not be a main reason for the CH<sub>4</sub> uptake in our study.

#### 4.6. Effects of vegetation on CH<sub>4</sub> uptake

Plant communities affect soil CH<sub>4</sub> uptake through the following three ways: (1) increased N input to grasslands can increase soil moisture among the fine root biomass (Magill et al., 1997), which may decrease air-filled porosity and limit rates of diffusion and atmospheric CH<sub>4</sub> oxidation. Our data on soil WFPS and aboveground biomass partly supports this deduction (Figs. 4 and 7). (2) Plant-derived CH<sub>4</sub> emission can indirectly offset the CH<sub>4</sub> uptake of the entire ecosystem (Cao et al., 2008; Ferretti et al., 2007; Keppler et al., 2006; Keppler et al., 2008). However, serious debates and questions on CH<sub>4</sub> emission by living plants under aerobic conditions are raised. Dueck et al. (2007) reported

that there was no evidence for substantial aerobic CH<sub>4</sub> emission by plants. Moreover, aerobic CH<sub>4</sub> emission by plants is highly uncertain, and depends on species (Cao et al., 2008; Kirschbaum and Walcroft, 2008; Wang et al., 2007). Based on regression analysis results, CH<sub>4</sub> emission by alpine vegetation could have a small effect on ecosystem net CH<sub>4</sub> flux, although a significant relationship between CH<sub>4</sub> uptake and above-ground biomass is observed (Table 2 and Fig. 9e).

#### 4.7. Soil acidification and CH<sub>4</sub> uptake

N addition can, to some degree, result in soil acidification in the alpine meadow, and the NO<sub>3</sub><sup>-</sup>-N fertilizer seemed to have a stronger effect than the NH<sub>4</sub><sup>+</sup>-N fertilizer. Generally, soil H<sup>+</sup> mainly originates from acid deposition, microbial decomposition of organic matter, and soil nitrification (Arnold et al., 1994; Sun et al., 2006). The oxidation of soil NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> (NH<sub>4</sub><sup>+</sup> + 2O<sub>2</sub> → 2H<sup>+</sup> + NO<sub>3</sub><sup>-</sup> + H<sub>2</sub>O) can produce H<sup>+</sup>, so ammonium fertilizer application will directly result in soil acidification. Soil acidification caused by NO<sub>3</sub><sup>-</sup>-N fertilizer addition is attributed to the exchange between the accompanied cations, such as K<sup>+</sup> and H<sup>+</sup>, on soil particle exchange sites (Fang et al., 2012; Gao et al., 2013). Compared with NH<sub>4</sub>Cl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, the effects of KNO<sub>3</sub> fertilizer on soil acidification were stronger, indicating the different effects of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> on soil acidification. The added NH<sub>4</sub><sup>+</sup> is partly absorbed by plants, immobilized by soil microbes, fixed by clay minerals and lost through ammonia volatilization. Only the NH<sub>4</sub><sup>+</sup> involved in the N mineralization and nitrification can produce H<sup>+</sup>. On the contrary, most of the added NO<sub>3</sub><sup>-</sup> can be replaced by an H<sup>+</sup> due to negligible leaching and denitrification.

In some forest soils, negative effects of soil acidification on soil physical parameters and bioturbation have the potential to reduce CH<sub>4</sub> uptake (Borken and Brumme, 2009). However, stepwise regression analysis showed that pH was not a key factor in the control of CH<sub>4</sub> uptake, suggesting that: (1) soil pH plays a minor role in determining CH<sub>4</sub> oxidation compared with other variables, and (2) methanotrophs in the alpine meadow soils can tolerate low pH (Saari et al., 2004).

## 5. Conclusions

This study emphasizes the appropriateness of using realistic levels rather than saturating levels of N-inputs in attempts to simulate increased N deposition. Our study suggested that low rates of N addition had significantly reduced the CH<sub>4</sub> sink of alpine meadow on the Qinghai–Tibetan Plateau over the short term. This indicates that the alpine meadow has low potential to consume more CH<sub>4</sub> under future elevated N deposition conditions on the Tibetan Plateau. Soil CH<sub>4</sub> uptake was primarily driven by soil moisture. The observed reduction in CH<sub>4</sub> uptake induced by N addition may mainly result from the decrease in physical diffusion, which is different from those in N-rich ecosystems or in high-dose N addition experiments. Overall, N is a regulatory factor of CH<sub>4</sub> uptake in the alpine meadow ecosystems. To elucidate the complex mechanisms by which inorganic N affects soil CH<sub>4</sub> uptake, further investigation should be carried out to determine the biochemical inhibition effects of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> accumulation on soil methanotrophic bacteria.

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