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CONTRIBUTION OF THE VEGETATION LAYERS IN THE NITROUS OXIDE EMISSION FROM ALPINE *KOBRESIA HUMILIS* SERG. MEADOW ECOSYSTEM ON THE TIBETAN PLATEAU

ABSTRACT: Nitrous oxide (N_2O) was one of the major atmospheric greenhouse gases. Its budget was poorly understood in alpine meadow, a dominant vegetation type on the Tibetan Plateau. To characterize a *Kobresia humilis* meadow on the plateau, N_2O emission rates were monitored from June 2003 to June 2006 in the study area located at 3280 m a.s.l. Nine plots with 1 m × 1 m each were divided into three treatments, i.e. intact herbaceous community (HCK), removal of aboveground plant biomass (CBK), and removal of both above and belowground plant biomass (BSK), to estimate contribution of plants, rhizosphere and bulk soil to the total N_2O emission. N_2O emission from plant aboveground biomass was calculated by flux difference between HCK and CBK, denoted as $F_{(HCK-CBK)}$, from rhizosphere by $F_{(CBK-BSK)}$, and from bulk soil was the flux in BSK treatment. Static chambers (height 50 cm, area 0.5 × 0.5 m^2)were used for gas collection. N_2O emission rate was significantly correlated with soil temperature at 5 cm depth in both HCK and BSK (P <0.001). Both treatments demonstrated a seasonal peak rate in growing season and minimum rate in dormancy period. The mean emission rates in the three years were 39.7±2.9 and 30.6±2.5 μg m^{-2} h^{-1} in HCK and BSK, respectively, with the former significantly higher than the latter (P <0.05). In CBK, however, the emission rate did not show consistent correlation with soil temperature, especially in growing season. Its three-year mean emission rate was 36.2±3.3 μg m^{-2} h^{-1}. In the *K. humilis* meadow, bulk soil contributed much more than plants and rhizosphere. The mean emission rate was 3.5±2.9, 5.7±3.8, and 30.6±2.5 μg m^{-2} h^{-1} (P <0.001) from plants, rhizosphere and bulk soil, and these accounted for 9, 14 and 77%, separately. Our results implied that N_2O emission rate decreased little with grazing as indicated by the difference between HCK and CBK in *K. humilis* meadow (P <0.05). N_2O emission from alpine meadow could not be ignored in addressing regional greenhouse gases budget on the Tibetan Plateau, considering the vast area and much higher radiative forcing of N_2O.

KEY WORDS: nitrous oxide, grazing impact, bulk soil, sink, alpine meadow

1. INTRODUCTION

N_2O is a greenhouse gas with high radiative forcing, approximate 298 times more than carbon dioxide (CO_2) and an annual increment of about 0.25% was reported for atmospheric N_2O concentration (IPCC 2007). The present contribution was estimated to be almost 6% to the total observed global warming (WMO 2006). Therefore there has been a growing interest in quantifying N_2O sources and sinks, and finding available practice to decrease its production in different ecosystems (Flessa *et al.* 2002, Oquist *et al.* 2007).

Soil was one of the main sources of atmospheric N_2O (Fluckiger *et al*, 1999). Grassland soil represented a significant source of N_2O emission, next to cultivated soils (Bremmen and Feijtal 1990, Papen and Butterbach-Bahl 1999, Watanabe *et al.* 2000, Skiba *et al.* 2004). N_2O was predominantly produced in nitrification and denitrification processes by soil microbes (Firestone *et al.* 1980, Wrage *et al.* 2001, Cycoń and Piotrowska-Seget 2007). They accounted for 65% of the total emission (Prather *et al.* 1994). Other biotic (plant biomass) and abiotic processes accounted for only a small portion (Smart and Bloom 2001, Zou *et al.* 2005). These processes were sensitive to rhizospheric carbon deposition as well as plant, soil and rhizospheric properties (Brumme 1995).

Animal grazing may change belowground carbon and nitrogen processes. For instance, CO_2 efflux rate under high grazing intensity was about twice of that under light grazing intensity in a study (Cao *et al.* 2004), while N_2O emission and CH_4 uptake rates decreased (Du *et al.* 2001, Liu *et al.* 2007, Cao *et al.* 2008, Du *et al.* 2008, Holst *et al.* 2008).

Greenhouse gases emissions could be separated into several components in order to clarify the underlying mechanisms. Gas exchange between air and bulk soil was difficult to measure *in situ* directly. A treatment of plant aboveground biomass removal was adopted, instead, to approximate that of bulk soil (Sanhueza and Donoso 2006, Hu *et al.* 2008). N_2O emission from plants could be estimated by comparing the above treatment with undisturbed community. Plant N_2O emission was evidently observed in cole and barley (Chang *et al.* 1998, Zou *et al.* 2005). And N_2O emission from crop and vegetable production was potential source of national N_2O inventory (Mosier *et al.* 1998, Xiong *et al.* 2006).

Due to the large area and high fragileness, alpine ecosystems on the Tibetan Plateau are supposed to be not only sensitive, but also have important feedback to human activities and climate change. Alpine meadow is one of the dominant vegetation types on the Tibetan Plateau. It covers more than 2.5×10^6 km^2, approximately 35% of the whole plateau area (Zheng and Zhu 2000). With the rapid growth of local population and economic scale, the alpine meadow has been suffering from overgrazing and degradation. Vegetation coverage decreased to various extents. Bare soil patches appeared and expanded under serious conditions (Klein *et al.* 2007). Intensive studies have been conducted on greenhouse gases budgets of alpine ecosystems on the plateau in recent years. For example, over 5 sets of eddy covariance equipments and more chambers have been monitoring CO_2 fluxes of several types of alpine meadow vegetation since 2001. However, most of these studies were about carbon absorption and release. There have been few researches about N_2O emission from ecosystems on the Tibetan Plateau till now (Pei *et al.* 2004, Du *et al.* 2008). Therefore, we still know little about the characterization and quantity of N_2O emission from the vast plateau, even less on the influence of human activities or climate change.

We therefore conducted a long-term N_2O monitoring experiment in a typical alpine meadow on the Tibetan Plateau. Our objectives were: (1) to characterize seasonal N_2O emission pattern; (2) to quantify N_2O emission; and (3) to clarify relative contribution of plant biomass, rhizosphere and bulk soil to total N_2O emission on the ecosystem *in situ*. Effect of serious degradation was estimated by partial or entire removal of plant biomass.

2. SITE DESCRIPTION

The study site was located at Haibei Alpine Meadow Research Station (37°32`N, 101°15`E, 3280 m altitude), the Chinese Academy of Sciences. Annual precipitation averaged 560 mm in the past 20 years, of which 85% was concentrated in growing season from May to September (Li *et al.* 2004). Annual rainfall was 546, 536, 450, and 540 mm, respectively, in the experimental period from 2003 to 2006. Mean annual air temperature was –0.9, –1.1, –0.5 and –0.7°C in these years.

N_2O emission was measured in a *Kobresia humilis* Serg. meadow, which was one of the dominant alpine meadow types in the plateau. The community has more than 40 species per square meter. Vertically it has two layers. The upper layer is dominated by *Festuca ovina* Linn., *F. rubra* Linn., *Stipa aliena*

Table 1. Basic soil properties of the alpine *K. humilis* meadow.

Soil depth (cm)	pH	Organic C (%)	Bulk density (g cm^{-3})
0–10	7.3±0.4	5.5	0.75±0.05
10–20	7.4±0.5	3.3	1.11±0.09
20–30	–	2.7	1.13±0.04
30–40	–	1.9	1.15±0.03

Keng., *Elymus nutans* Griseb., *Helictotrichon tibetica* Henr., *Koeleria cristata* Linn., and *Poa crymophila* Keng., and the lower layer by *K. humilis* Serg., *Saussurea superba* Anth., *Potentilla saundersiana* Royle., *Leontopodium nanum* Hand.-Mazz. and *Lancea tibetica* Hook. f. et Thoms. The experimental site was on a flat plain, where yaks and goats grazed from late September to the end of April since 1982. Vegetation coverage ranges from 75 to 80%. Bare soil resulted from serious degradation occupied about 2% of the total community area (Zhou and Wu 2006).

The soil is Mat-Gryic Cambisol (Chinese Soil Taxonomy Research Group. 1995). It has high organic C content and belongs to udic soil moisture regime (Bao *et al.* 1995, Cao *et al.* 1998). Soil basic properties are presented in Table 1.

3. MATERIAL AND METHODS

3.1. Experimental treatment

In May 2003, three treatments were setup in the *K. humilis* meadow. One was intact herbaceous community treatment (HCK), one was bare soil treatment (BSK), and the third was removal of aboveground biomass (CBK). There were three plots in each treatment. All the nine plots were arranged totally randomly in the field. The bare soil plots were prepared by digging three pits, 1 m ×1 m×1 m for each. Soils were refilled to the pits according to their original soil layers after removing roots by sieving. Soil preparation was completed 6 weeks before N_2O measurements.

3.2. Samples collection and measurements

Nitrous oxide emission was measured by a static chamber method (Cao *et al.* 2008). In each plot a stainless steel pedestal was installed permanently. The lower edge of the pedestal reached 10 cm soil layer. The ground area in the pedestal was 0.5 × 0.5 m^2. Opaque plexiglas chamber with height of 50 cm was used for gas sampling. Each chamber was equipped with two electric fans to mix the air and a thermo-probe to monitor temperature in the chamber during measurements. To avoid too much increase of air temperature inside, the chambers were covered by foam and white waterproof cloth.

Just before gas sampling, the chambers were mounted on the pedestal and sealed by sticky clay soil. Pre-experiment of sampling at 2 h interval showed that flux rate in 9:00 to 10:00 local time was close to that of diurnal average. Therefore, samples were taken within this hour during the study. Gas samples were collected from the chambers every 10 min using 100 ml plastic syringes. Thus, it took about 30 minutes for each chamber. Samples were gathered and immediately measured every 4–5 days during growing seasons and twice per month in the rest of time. Moreover, diurnal sampling was performed once per month, at 2 h interval during daytime and 3 h interval during nighttime. If snow covered the plots, it was not cleared in the pedestals to avoid disturbance during measurement.

The samples were analyzed by an improved gas chromatograph (HP4890D, Agilent Co.) system with electron capture detector (ECD). Injection/detection and column (stainless steel 3 m × 2 mm Porapak Q, Agilent Co.) oven temperature was 55°C and 330°C, respectively. Ultra pure N_2 was used as carrier gas with a flow rate of 30 ml min^{-1} (Wang *et al.* 2003, Wang and Wang 2003). A certified N_2O standard with a concentration of 355×10^{-9} L L^{-1} (China National Research Center for Certified Reference Materials, Beijing) was used for calibration. Analysis accuracy of samples is ±5 10^{-9} L L^{-1} for N_2O measurements. Three replicates were measured for each treatment.

During gas samples collection, soil temperature at 5 cm depth was measured using

JM624 thermometer and volumetric soil moisture at 10 cm soil depth was measured by a TDR (Time-domain reflectometer, Campbell Scientific, Inc., North Logan, UT, USA).

3.3. Calculation, data analysis and statistics

Hourly N_2O emissions (μg N_2O m^{-2} h^{-1}) were calculated based on the slope of the linear increase in N_2O concentration over the sampling period, as follows:

$$Flux_{N_2O} = \rho \times \frac{V}{A} \times \frac{P}{P_0} \times \frac{T_0}{T} \times \frac{dC_t}{dt}$$

where $Flux_{N2O}$ is hourly N_2O emission rate (μg N_2O m^{-2} h^{-1}). ρ is air density inside the chamber. C_t denotes N_2O concentration in the chamber at time *t*. *V* is the volume of the chamber. A is the ground area covered by the chamber. P_0 and T_0 are air pressure and temperature at standard state (1.103×10^4 Pa and 273 K, respectively). *P* and *T* are local air pressure and air temperature in the chambers.

Soil temperature and moisture were recorded in each plot from 9:00 to 10:00 during gas sampling. Arithmetical average values were calculated and used with corresponding N_2O emission rate to establish their correlations in the study period.

N_2O emission from plant aboveground biomass, rhizospheric soil, and bulk soil was separated by biomass removal method (Hanson *et al.* 2000, Hu *et al.* 2004, 2008). N_2O emission from plant aboveground biomass was calculated during three years records by flux difference between HCK and CBK, denoted as $F_{(HCK-CBK)}$, emission from rhizosphere by $F_{(CBK-BSK)}$, and bulk soil emission was supposed to be the flux in BSK treatment (F_{BSK}).

The effects of treatments and environmental factors (soil temperature, moisture) were analyzed by one-way ANOVA. Critical LSD values for 5% error probability were calculated for post hoc test. The difference of flux between growing and dormancy period, as well as that between rhizospheric and bulk soil were examined by independent-samples T-test. Correlation between N_2O emission and environmental factors was calculated by bivariate process correlations in SPSS® 11.5 (System Software Inc.).

4. RESULTS

4.1. Seasonal dynamics of N_2O emission

Both the HCK and BSK treatments showed remarkable seasonal dynamics of N_2O emissions (Fig. 1), and the maximal emission rates appeared in growth season from May to September, while they reached the lowest in dormancy period from October

Table 2. N_2O emission rate of different components in alpine *K. humilis* meadow. N_2O flux rates for aboveground biomass and rhizospheric soil were calculated as the difference between that of HCK and CBK, and that of CBK and BSK. Means ± standard errors were presented.

Component	N_2O emission rate (μg m^{-2} h^{-1})			
	Entire experimental period [1]	Season		
		Growth season [2]	Dormancy period [3]	T-test
Total (HCK)	39.7±2.9 n=125 a	48.2± 4.4 (n=77)	25.8±1.9(n=48)	0.000
Belowground (CBK)	36.2±3.3 n=125 a	34.9± 2.9 (n=77)	38.5±7.3(n=48)	0.596
Bulk soil (BSK)	30.6±2.5 n=125 b	36.4± 3.5 (n=77)	21.4±2.8(n=48)	0.001
Rhizospheric soil	5.7±3.8 n=125	–1.6± 3.2 (n=77)	17.1±7.9 (n=48)	0.000
Aboveground	3.5±2.9 n=125	13.3±4.0 (n=77)	–12.7±3.5(n=48)	0.000

Different letters mean significant difference of the same item (P <0.05).

[1] Entire experiment, from June 2003 to June 2006.

[2] Growth season, from June to September in 2003, May to September in 2004 and 2005, and May to June in 2006.

[3] Dormancy period, from October to the next April in 2003–2006.

to the next April. As to CBK, three brief and rapid increases were observed, i. e. from February to April in 2004, from April to May in both 2005 and 2006, and from August to October in 2005 (Fig. 1).

N_2O emission rate averaged 39.7±2.9, 36.2±3.3, and 30.6±2.5 μg m^{-2} h^{-1} in the HCK, CBK, and BSK treatment during the entire experimental period from June 2003 to June 2006 The former two treatments had significantly higher rate than the last one (P <0.05, Table 2). Mean N_2O emission rate in growing seasons was also significantly higher than that in dormancy period for HCK and BSK treatments (P <0.01, Table 2), but there was no significant difference for CBK (P> 0. 05, Table 2).

4.2. Contribution of aboveground biomass, rhizospheric and bulk soil

Average N_2O emission rate from aboveground biomass, rhizospheric and bulk soil was 3.5±2.9, 5.7±3.8, and 30.6±2.5 μg m^{-2} h^{-1}, respectively, in the entire experiment period. These three components accounted for 9, 14, and 77% of N_2O flux in this alpine meadow (represented as the mean flux rate of HCK). Relative contribution was significantly different between growing season and dormant period for all these components (P <0.01, Table 2). Aboveground biomass consumed N_2O at an average rate of 12.7±3.5 μg m^{-2} h^{-1} in the dormancy periods, and rhizospheric soil absorbed at 1.6 ±3.2 μg m^{-2} h^{-1} during growing seasons.

4.3. Relationship between N_2O emission and soil temperature and moisture

Seasonal variation of soil temperature synchronized with that of N_2O emission rates in all treatments, both peaked in growth seasons and reached the lowest in dormant seasons (Fig. 2). Mean soil temperature at 5 cm soil depth was 5.8, 6.3 and 6.5°C in HCK, CBK, and BSK, respectively in the whole period. The difference among them was not statistically significant (P> 0.05, Table 3, Fig. 2). Soil moisture in growth seasons were 40.1, 36.3 and 31.3 % in HCK, CBK and BSK, and the difference was significant (P <0.05, Fig. 2).

N_2O emission rate was significantly correlated with soil temperature in both HCK and BSK (P <0.001, Table 3), but not in CBK, or in bulk data including all plots. N_2O emission rate in neither treatments nor bulk data was significantly correlated with soil moisture (Table 3).

5. DISCUSSION

5.1. Plant removal method to separate N_2O emission from vegetation components

Apparent N_2O emission from aboveground plant biomass may be mixture of a phytogenic N_2O and N_2O produced by soil microorganisms but transported by plant. Plants produce N_2O during their N assimilation (Dean and Harper 1986). They can also transport soil-generated N_2O with water flow transpiration, and subsequently release through stomata (Ferch and Römheld 2001, Ma *et al.* 2008). Quite a few researches had been carried out to quantify N_2O emission from living plants by removing aboveground biomass or by applying ^{15}N-labeled nitrate or nitrite to plants (Goshima *et al.* 1999, Muller 2003, Zou *et al.* 2005, Ding *et al.* 2007). The former method calculated N_2O emitted from aboveground biomass as the

Table 3. The correlations and P values between N_2O emission and 5 cm soil temperature, and 10 cm soil moisture. HCK means herbaceous community; CBK was removal of aboveground biomass, and BSK was bare soil treatment in the *K. humilis* meadow.
Different letters mean significant difference of the same item (P<0.05). R denotes correlation coefficient.

Treatment	Temperature			Moisture		
	Mean (°C)	R	P	Mean (%)	R	P
HCK	5.8 a	0.393	0.000**	40.1 a	–0.046	0.671
CBK	6.3 a	0.071	0.436	36.3 b	0.029	0.791
BSK	6.5 a	0.311	0.000**	31.3 c	0.028	0.795

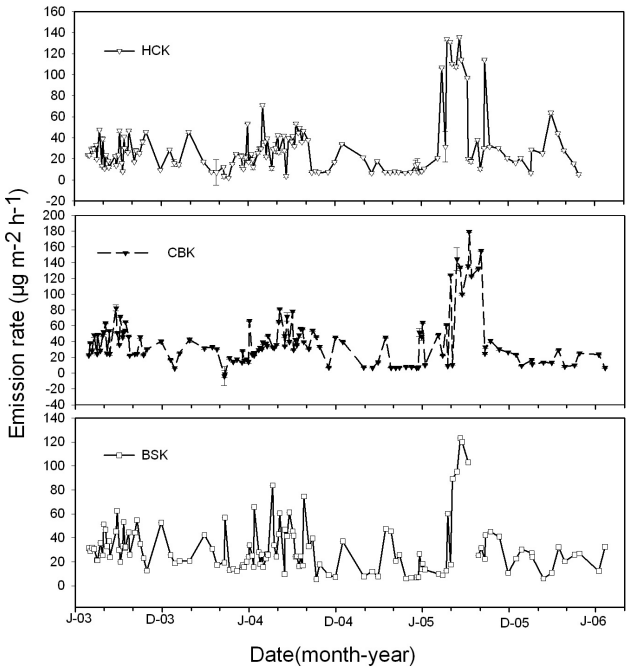

Fig. 1. Seasonal dynamics of N_2O emission in the *K. humilis* meadow. Mean ± standard errors of three replicates in each treatment were presented. HCK denoted intact herbaceous community treatment; CBK was aboveground biomass removal treatment, and BSK was bare soil treatment. J and D were abbreviations of June and December.

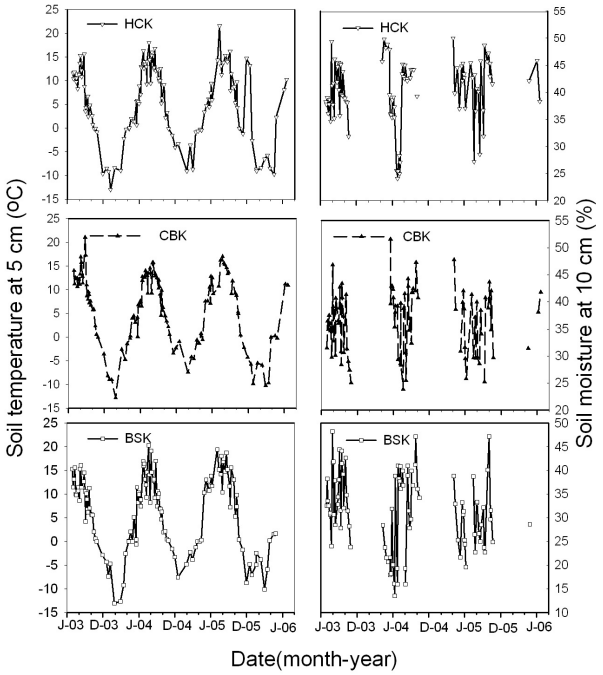

Fig. 2. Seasonal dynamics of soil moisture at 10 cm depth and soil temperature at 5 cm in the *K. humilis* meadow. Soil moisture data were not collected in winter when soils were frozen. HCK denoted intact herbaceous community treatment; CBK was aboveground biomass removal treatment, and BSK was bare soil treatment. J and D were abbreviations of June and December.

difference in N_2O emission from intact community and that from aboveground removed treatment. It derived N_2O emission from rhizospheric soil by the difference between aboveground removal treatment and whole plant removal treatment.

Uncertainties were supposed to be derived from some sources in above method. Since aboveground plant biomass had fundamental influence on rhizospheric processes through rhizodeposition, which included exudation, secretion, and root cell slough, and provided carbon and energy sources for microorganisms, removal of aboveground biomass not only eliminated aboveground phytogenic N_2O, but also changed rhizospheric N_2O production and diffusion (Ding *et al.* 2007). Whole plant removal had further impact on soil physical properties. Therefore, the main uncertainty of biomass removal method was disturbance on soil microbial activities and composition, as well as soil environments. Zou *et al.* (2005) however did not detect obvious changes in soil N_2O fluxes after removing biomass. In this study, Formal gas sampling was started 6 weeks after plant removal and lasted for 3 years in the field. It may alleviate short term disturbance on soil, and also preclude root exudation in whole plant removal treatment (Cao *et al.* 2008, Du *et al.* 2008, Hu *et al.* 2008). Our experiment did not prove that N_2O was directly produced by aboveground biomass or by roots. Nevertheless, it did demonstrate that more than 23% of total N_2O emission was from aboveground biomass and rhizospheric soil. It also showed that these two components may release or absorb N_2O in different seasons.

5.2. Components of ecosystem N_2O emission

Plant emitted N_2O at an average rate of 3.3 μg m^{-2} h^{-1} in an Australian mangrove ecosystem with poor nutrient supply (Kreuzwieser *et al.* 2003). In a wheat cropland, N_2O emission from plant and the rhizospheric soil periods varied between 12.5 and 162.5 μg m^{-2} h^{-1} at different growning periods which contributed around 25% to ecosystem N_2O flux (Zou *et al.* 2005). The alpine meadow in this study emitted at an average rate of 9.2 μg m^{-2} h^{-1} through aboveground biomass and the rhizospheric soil, and it was about 23% of the ecosystem flux. This was lower than for the wheat, and the aboveground biomass emission rate was approached to that of the mangrove (Table 2). The study also demonstrated that plant biomass may also uptake N_2O from the air. Although the mean emission rate from plant biomass was 3.5±2.9 μg m^{-2} h^{-1}, aboveground biomass including litter consumed 12.7±3.5 μg m^{-2} h^{-1} during the dormancy period (Table 2). Litter cover could decreased water evaporation and maintain soil moisture (Zhang *et al.* 2008). It could also provide more nutrients for some functional groups of microbes. Consequently, denitrification process was stimulated and transformed more N_2O to N_2.

The alpine meadow ecosystem emitted N_2O dominantly from bulk soil. The rate from bulk soil was almost 5 times higher than that from rhizospheric soil in the entire experiment period. The alpine meadow had extremely high root/shoot ratio, high belowground primary production, and high soil organic carbon content (Klein *et al.* 2007, Du *et al.* 2008). These supported the high contribution of N_2O emission from bulk soil. In growing season the two soil components emitted N_2O at significantly higher rate than in dormancy period, suggesting an important control by temperature (Table 3). Slow uptake of N_2O by rhizosphere, about 1.6 ±3.2 μg m^{-2} h^{-1}, was detected in the growth season. This may be caused by stimulation of denitrification process due to increased carbon supply during root decomposition. Water loss decrease may also play a role. As a result, more N_2O was consumed and transformation to N_2 (Du *et al.* 2006, Ding *et al.* 2007).

5.3. Effect of grazing on N_2O emission rate in the alpine meadow

It was well established that N_2O was produced dominantly by microbial mediated nitrification and denitrification, which were influenced by soil oxygen, soil temperature, mineral N content, available soil carbon, and pH (Bolan *et al.* 2004, Saggar *et al.* 2004, 2007, Luo and Saggar 2008). Grazing by animal may have an impact on vegetation and soil properties above, thus, alters ecosystem N_2O flux. Therefore, the species of animals determined the degree of influence on N_2O emis-

sion through hoof trampling. Du *et al* (2001, 2006) found that grazing altered soil structure, increased denitrification bacterium quantity and decreased N_2O emission rate (from 5.9 to 3.7 μg m^{-2} h^{-1}) in Inner Mongolia grassland. Animal grazing created dung and urine patches, which may be strong N_2O emission source, especially after fertilization (Coby *et al.* 2008).

The alpine meadow ecosystems had been under degradation on the Tibetan Plateau. Aboveground biomass and it coverage decreased and bare soils appeared and enlarged. In this study, treatments of HCK, CBK and BSK simulated the succession of alpine meadow from original to most severe statuses. The biomass decreased largely from HCK (386.27±12.34 g m^{-2}) to CBK (no aboveground biomass) and bare soil, but N_2O emission rate fell much less from 39.7±2.9 to 36.2±3.3 and 30.6±2.5 μg m^{-2} h^{-1}. Especially, N_2O emission in CBK decreased little from HCK, and emission rate in dormancy period was higher than that in the whole experimental period and even growth period (Table 2). Hence, the total N_2O flux and rhizosphere emission rate increased greatly during the dormancy period. The insensitive response of N_2O emission to plant biomass removal was perhaps resulted from (a) prolonged high carbon supply to microorganisms due to large belowground root biomass, (b) likelihood of physiological stress for nitrous oxide consuming bacteria due to plenty rainfall in the alpine meadow on the plateau, (c) lack of animal trampling in the CBK and BSK treatments.

IPCC estimated that radiative forcing of N_2O was 298 times more than that of CO_2 (IPCC, 2007). Based on the average N_2O emission rate in this three-year study, the alpine meadow ecosystem was a non-negligible source of atmospheric N_2O. Grassland degradation does not seem to induce large drop in N_2O emission for a long time.

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