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Characterizing nitric oxide emissions from two typical alpine ecosystems

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ABSTRACT

A portion of alpine meadows has been and will continue to be cultivated due to the concurrent increasing demands for animal- and crop-oriented foods and global warming. However, it remains unclear how these long-term changes in land use will affect nitric oxide (NO) emission. At a field site with a calcareous soil on the Qinghai-Tibetan Plateau, the authors measured the year-round NO fluxes and related variables in a typically winter-grazed natural alpine meadow (NAM) and its adjacent forage oat field (FOF). The results showed that long-term plow tillage, fertilization and growing forage oats significantly yielded ca. 2.7 times more ($p < 0.01$) NO emissions from the FOF than the NAM (conservatively 208 vs. 56 g N/(ha·year) on average). The spring freeze–thaw period and non-growing season accounted for 17%-35% of the annual emissions, respectively. The Q_{10} of surface soil temperature (T_s) was 8.9 in the NAM (vs. 3.8 in the FOF), indicating increases of 24%–93% in NO emissions per 1–3 °C increase. However, the warming-induced increases could be smaller than those due to land use change and management practices. The T_s and concentrations of ammonium, nitrate and water-extractable organic carbon jointly explained 69% of the variance in daily NO fluxes from both fields during the annual period ($p < 0.001$). This result indicates that temporally and/or spatially distributed NO fluxes from landscapes with calcareous soils across native alpine meadows and/or fields cultivated with forage oats can be predicted by simultaneous observations of these four soil variables.

Introduction

Nitric oxide (NO) is an important nitrogen gas. It is a precursor for generating secondary aerosol particles in the formation of haze and for nitric acid in the formation of acid rain. Nitric oxide is also involved in a number of atmospheric photochemical reactions that produce tropospheric ozone, which is not only a very important atmospheric pollutant but also a short-lifetime greenhouse gas. NO clearly contributes to both air pollution and climate change (IPCC, 2007). Atmospheric NO mainly originates from the combustion of both fossil fuels and biomass and from emissions from cultivated

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soils. Soils are usually the predominant atmospheric NO sources in remote regions (e.g., Bouwman et al., 2002). Global soils amended with nitrogen fertilizers are estimated to release NO at approximately 1.4 Tg N/year (1 Tg = 10^{12} g), which is nearly 42% of the nitrous oxide (N_2O) emission originating from the same sources (Stehfest and Bouwman, 2006).

Grasslands account for approximately 40% of the land area of China (www.fao.org/faostat/). Since 1950, approximately 19.3 million ha grasslands have been converted to cultivated lands, which is approximately 5% of the current national grassland area and approximately 20% of the total cultivated land area in China (Su et al., 2005). Alpine meadows (approx. 377 million ha) account for approximately 22% of the national grassland area (Piao et al., 2007), of which approximately 77% is located on the Qinghai-Tibetan Plateau (QTP) (Zhang et al., 2010). The landscapes of the grasslands in the QTP have been modified greatly since the 1960s, mainly due to intensified anthropogenic activities such as heavy grazing and cultivation of alpine crops (Chen et al., 2013). In particular, the area of cultivated alpine meadows has greatly increased (QPBS, 1997, 1993). In the regions surrounding Qinghai Lake, for instance, the total area of cultivated alpine meadows in 1957 to 1998 sometimes even increased at a very high rate of up to 7.03%/year (Du, 2002). In fact, long-term cultivation of some alpine meadows to grow forage crops has conventionally occurred widely on the QTP to solve the problem of forage shortage for grazing yaks and Tibetan sheep during the long and cold winters. Oat (*Avena sativa*) is the most important crop in the region and accounts for up to 70% of the total forage crop area on the QTP (Zhao et al., 2004).

In China, alpine meadows account for approximately 26% and 11% of the total soil organic carbon (SOC) and total nitrogen (TN) stocks in grassland soils at the 0–1 m depth (Ni, 2002; Tian et al., 2006). Compared to cultivated soils, native grassland soils usually have relatively higher SOC and TN concentrations (e.g., Ni, 2002; Tian et al., 2006). Therefore, soil aeration by tillage can promote the mineralization of SOC and organic nitrogen in the soils of cultivated grasslands, and thus stimulate NO production and emissions (e.g., Skiba et al., 1997). In addition, NO emissions in cultivated grasslands can be intensified by more nitrogen substrates directly provided by fertilization (e.g., Bouwman et al., 2002). For meadows particularly in alpine or mid- to high-latitude regions, these effects can be even more interesting because of the long periods with frequent freeze–thaw alternations (hereinafter referred to as FTP) that usually occur in early spring or late autumn (Katayanagi and Hatano, 2012; Mukumbuta et al., 2017). Thus far, however, studies to address the effects of alpine meadow cultivation on NO emission are scarce. This study was an attempt to improve the understanding of this knowledge gap.

In this study, the authors performed a case study involving an experimental year-round field campaign in the northeastern region of the QTP. This campaign aimed at (i) establishing a field experiment, which involved a typically winter-grazed natural alpine meadow (NAM) mainly dominated by *Kobresia humilis* and an adjacent forage oat field (FOF) that was converted in 1998 from a piece of NAM and has since then been consecutively cultivated with forage oats; (ii) simultaneously observing the NO emissions and other related variables in both NAM and FOF; and (iii) identifying the differences in the NO emissions between the two treatments and revealing their regulatory mechanisms. These efforts were to test the hypothesis that the conventional land use practices in the QTP region, i.e., cultivation of alpine meadows for long-term consecutive forage crop production, could amplify NO emissions from the ecosystems more intensively than climate warming.

1. Materials and methods

1.1. Site description and field experimental layout

The selected field site (37°36′45″N, 101°18′48″N, 3203 m above sea level) was situated aside the Haibei Alpine Meadow Ecosystem Research Station (HAMERS), which is located in Qinghai Province, China. The site is subject to a continental plateau climate in the temperate zone, with an annual average air temperature of −0.3°C and an annual average precipitation of 484 mm in 2012–2014. The site has a calcareous Matti-Cryic Cambisol soil (Cao et al., 2008), with a silty clay loam texture and pH above neutral (Table 1).

As one of the two ecosystems involved in this field case study, the NAM was a native *Kobresia humilis* meadow commonly in the region. The plant species *Kobresia humilis*, *Elymus nutans*, *Gentiana farreri*, *Poa annua* and *Saussurea pulchra* commonly dominate the community (e.g., Zhang et al., 2014). Since the 1960s, the NAM area has been conventionally winter-grazed with yaks and Tibetan sheep exclusively during the period from September to May of the following year. The

Table 1 – Selected soil properties and other features at the experimental sites in the natural alpine meadow (NAM) and the forage oat fields (FOF).

Treatment	NAM	FOF
Latitude	37°36′45″N	
Longitude	101°18′48″E	
Altitude (m)	3203	
Soil type	Mattic-Cryic Cambisol	
Soil (0–20 cm) texture	Silty clay loam	
Sand (0.05–2 mm) (%)	34 (1)	32 (1)
Silt (0.002–0.05 mm) (%)	50 (1)	51 (1)
Clay (< 0.002 mm) (%)	16 (1)	17 (1)
Soil (0–20 cm) organic carbon (g C/kg d.s.)	46 (1)	46 (2)
Soil (0–20 cm) total nitrogen (g N/kg d.s.)	4.8 (0.1)	4.4 (0.1)
pH (H_2O)	8.0 (0.1)	8.3 (0.3)
Soil (0–6 cm) bulk density (g/cm^3)	0.69 (0.03)	0.88 (0.04) **
Soil (0–6 cm) gas permeability (cm/s)	0.7 (0.4)	2.3 (0.6) **
Soil (0–10 cm) microbial carbon (mg C/kg d.s.)	505 (77)	181(9) **
Aboveground net primary productivity (g/(m^2·year))	322 (7)	1068 (38) **
Aboveground plant nitrogen content (g/kg)	14.5 (0.6)	8.6 (0.3) **

The given data are means of 3–5 spatial replicates, with standard errors showing within the parentheses. ** indicates significant differences between the NAM and FOF treatments at p < 0.01. d.s.: dry soil.

stocking rate approximated 6.8 sheep units (SU)/(ha·year). No fertilizer amendment and hay-making occurred in the NAM.

As another ecosystem involved in this study, the FOF was a forage oat field with an area of 0.93 ha. It was surrounded by vast NAM lands. The FOF area was a piece of NAM before 1998 and has since then been consecutively cultivated with the forage crop. The FOF had been amended with both synthetic fertilizers and yak manure during the first 10 years since its conversion but then with only synthetic fertilizers thereafter. During this experimental campaign (from April 22, 2013 to April 21, 2014), the regionally common practices for forage oat cultivation were adopted in the FOF. The soil was plowed to a depth of 20 cm on June 2, when basal synthetic fertilizers (diammonium hydrogen phosphate and urea, equal to 41 kg N/ha and 20 kg P/ha) were basally applied together when oat sowing at 410 kg/ha (dry seed matter, containing 8 kg N/ha). The fertilizers and seeds were incorporated mechanically into and mixed with the topsoil shortly after broadcasting. Herbicide (2,4-D butyl ester) was sprayed at 488 g/ha prior to oat germination. Top-dressing urea was broadcasted on July 24. There was no irrigation. The nitrogen inputs to the FOF, in the forms of organic nitrogen in the oat seeds and synthetic compounds, totaled 77 kg N ha/year. The aboveground biomass was harvested on September 16 and then sun-dried and stored for feeding during winter.

Four days before the campaign period, four replicated field plots (each with a size of 5 × 5 m^2) were established in both NAM and FOF. The FOF plots were distributed randomly in the forage oat area; each was situated at least 2 m from the closest field boundary between the NAM and FOF. The NAM plots were established randomly within the areas and each was situated at least 5 m from the closest field boundary between the NAM and FOF but as close as possible to the FOF land in the northern, western and southern directions. The area in the eastern direction was excluded due to a passing road. Such layouts of the field plots were chosen to minimize the effects of land boundaries. The exact location and area of each field plot were fixed during the entire campaign period. All the field plots were situated on a largely flat landform to ensure least heterogeneities in soil properties (Table 1), hydrological features and meadow vegetation communities among the eight plots prior to the conversion of the NAM area to the FOF. The least heterogeneities were required to facilitate statistical comparisons of experimental results between the two field treatments.

For the convenience of arranging temporary intensified observations, a FTP was defined in this study as a period of at least 5 d during which the daily mean air temperature consecutively fell within the range from −10 to 0°C. Accordingly, the spring FTP occurred during the period from February 21 to April 21, 2014 (Fig. 1).

1.2. Measurement of nitric oxide fluxes

Nitric oxide fluxes were measured during the entire campaign using a technique of combining the chemiluminescence analysis of NO concentrations with gas sampling by opaque, static chambers (Liu et al., 2009, 2015; Mei et al., 2009). The flux

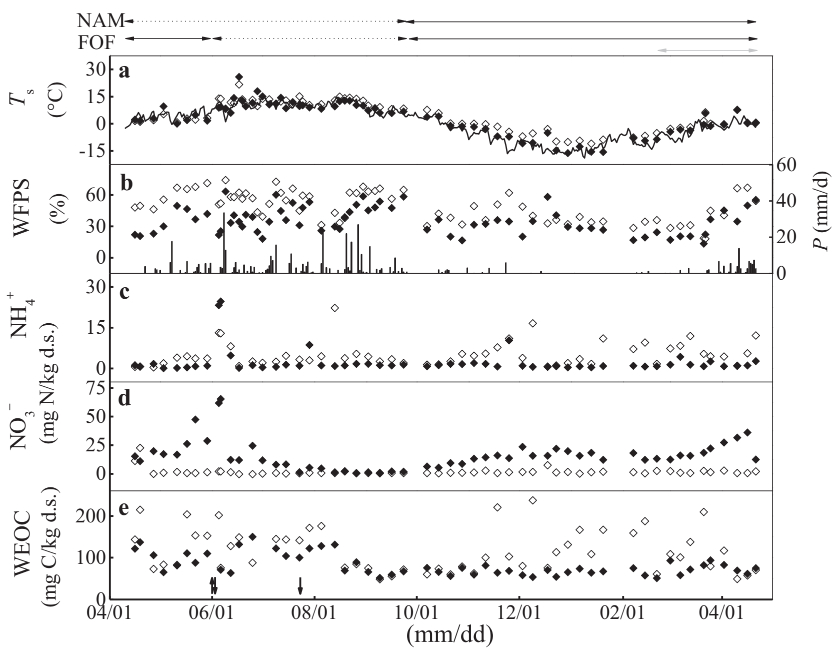

Fig. 1 – Year-round dynamics of soil and other environmental variables. For the definitions of FTP, NGS and GS as well as details on presented soil variables, refer to Tables 1–2. (a) Air temperature (T_a, line) and T_s. (b) WFPS and precipitation (P, bar). (c–e) Soil NH_4^+, NO_3^- (mg N/kg d.s.) and WEOC concentrations (mg C/kg d.s.), respectively. The empty and filled diamonds represent the means of the NAM and FOF treatments, respectively (Table 1), with the standard errors of four spatial replicates not shown for plot clarity. The upward and downward arrows indicate the tillage and fertilization dates of the FOF, respectively. The gray solid, black solid and dashed horizontal arrows indicate the FTP, NGS and GS, respectively. d.s.: dry soil.

measurements for all field plots were manually performed daily or every other day during FTP and the periods of 10–14 day following plowing and fertilizing; otherwise, the measurements were performed once every 3–4 day.

Four days before the first observation, two chamber base frames were installed in the center of each replicated field plot in the NAM and FOF. To minimize the effects of sampling operations on plant growth, the frames were situated approximately 2 m from each other for weekly alternative use in gas sampling thereafter. Both base frames remained permanently in each field plot during the entire campaign period, except in the FOF plots. The frames in the FOF were temporarily removed and reinstalled to allow for soil plowing and follow-up mechanical operations. Each frame was made of stainless steel (each 50 × 50 × 15 cm in length, width, height; each wall was 3.0 mm in thickness) and was inserted fully into the soil; only the upper-edge collar extended out of the soil surface. A rubber band (6 mm thick) was applied to the upper-edge collar of each base frame for gas-tight sealing of the joint with the chamber. Each chamber was made of stainless steel (50 × 50 × 40 cm in length, width, height; each wall was 1.0 mm thick; no bottom). The walls were coated with polystyrene foam boards that were covered with tinfoil to minimize the temperature change within the headspace enclosure during gas sampling. When the vegetation was taller than 40 cm, an alternative chamber with an 80 cm height was adopted to avoid physical damage to the plants within each base frame. There was a tube (7.4 mm inner diameter and 12 cm length) on the top wall of each chamber to allow for an air connection between the headspace and the atmosphere to minimize the pressure difference during sampling. To measure the flux from each plot, a chamber was temporarily mounted onto one of the two base frames to establish a headspace enclosure for gas sampling.

The methods of gas sampling, instrument calibration, and analyses of both NO and nitrogen dioxide (NO_2) concentrations in the gas samples as well as the flux calculations were in accordance with those detailed by Mei et al. (2009) and Zhang et al. (2018a). The sample NO that was collected into a gas bag until analysis could be converted partly into NO_2 in the presence of ozone. In this regard, the sum of the simultaneously measured NO and NO_2 fluxes was regarded as the measured NO flux. A single NO flux measured by gas sampling during the local time 08:00–10:00 a.m. was used to represent the daily average value (e.g., Liu et al., 2010). According to the instrument precision for NO or NO_2 analysis (0.3 nmol/mol) and the enclosure time (10 min), the detection limits of NO fluxes for the adopted chamber heights (40 and 80 cm) were 0.4 and 0.8 μg N/(m^2 hr), respectively.

It should be noted that the NO fluxes measured in this study represented only conservative magnitudes for the investigated ecosystems. The reason is that the applied method with a linear-change assumption regarding the gas concentrations in individual static, opaque chamber enclosures might significantly underestimate NO fluxes, e.g., by approximately 31% (ranging from 3% to 59% at the 95% confidence interval), in comparison with a nonlinear approach (Mei et al., 2009; Yao et al., 2015b). Similarly, underestimations also significantly occur to some extent for N_2O fluxes measured with a linear-change assumption in a static chamber enclosure, which have been commonly neglected by the overwhelming majority of researchers (e.g., Wang et al., 2013). In fact, the underestimations due to a linear-change assumption indeed do not allow accurate quantification of gas emissions. Nevertheless, the high sensitivity of the applied method to measure the NO fluxes allowed investigations of the differences between field treatments and of the regulatory effects of soil conditions and other factors.

1.3. *Auxiliary measurements*

The authors measured the aboveground biomass and its nitrogen content in the NAM late in the growing season (GS) and in the FOF at harvest. The harvested plant material was incubated for 30 min at 105°C and then dried for 48 hr at 60°C to obtain the mass weight. The measured biomass was considered approximate representation of the aboveground net primary productivity (ANPP). The nitrogen contents in the dried plant samples (Plant-N) were analyzed using the Kjeldahl method (Bao, 2000).

The soil (0–10 cm) microbial biomass carbon (SMBC) concentrations were measured seasonally five times in total using the chloroform fumigation method (Joergensen, 1996). The topsoil (0–6 cm) bulk density (BD) and gas permeability (GP) were seasonally measured three and five times, respectively. An air permeability test system (PL-300, Eijkelkamp Agrisearch Equipment, The Netherlands) was employed to measure GP. For each of these items with multiple observations, the mean was reported.

The other soil properties of the 0–20 cm soil depth, including SOC, TN, pH and soil texture, were measured once in mid-autumn. The SOC and TN concentrations were analyzed using the potassium dichromate oxidation and the Kjeldahl methods, respectively (Bao, 2000). A water-to-soil ratio of 2.5 was used to determine the pH values. The fractions of clay (<0.002 mm), silt (0.002–0.05 mm), and sand (0.05–2 mm) were measured using Malvern laser particle analysis (Yang et al., 2009).

In addition to the above items, the authors also simultaneously measured topsoil (5 cm) temperature (T_s).

The daily precipitations and air temperatures (T_a) were measured at the HAMERS, which provided the measurements. The headspace air temperature and T_s were manually measured during gas sampling using digital thermal couples (JM624, Jin Ming Instrument Co. Ltd., China).

On each day of flux measurement, the topsoil (0–6 cm) volumetric water content (θ_v, cm^3/cm^3) was manually measured during the unfrozen periods using a portable frequency-domain reflector moisture meter (ML2x ThetaKit, Delta-T Devices, UK). During the frozen periods or FTPs, the mass ratios (θ_w, g/g) of the soil water in ice and/or liquid phases were measured by oven-drying the soil sample and then converted the values to θ_v units by multiplying with BD (g/cm^3). Finally, each soil moisture content within the water-filled pore space (WFPS) was calculated as WFPS = $100\theta_v/(1–BD/2.65)$.

In addition, the concentrations of ammonium (NH_4^+), nitrate (NO_3^-), and water-extractable organic carbon (WEOC) in the 0–10 cm depth were measured weekly on one of the days when the NO fluxes were measured. At each time, four samples were collected (each was a mixture of soil samples from five random points within the corresponding field plot), well mixed, sieved with a 2-mm mesh, and ultimately subsampled via three replicates. The subsamples were extracted on the same day as the NH_4^+, NO_3^- (1 mol/L potassium chloride, solution-to-soil ratio = 5; shaking for 1 hr, and filtering by filter paper) and WEOC (distilled water, water-to-soil ratio = 5; shaking for 1 hrh, centrifuging for 5 min at 6000 r/min, and filtering by polyethersulfone membrane with <0.45 μm pores) contents were analyzed. Each extract was saved in a 50-mL polyethylene-terephthalate bottle at approximately −18°C for later assay. The concentrations of NH_4^+, NO_3^- and WEOC in the extracts were analyzed shortly after thawing for 24 hrh at 4°C using a continuous flow analyzer (San^{++}, Skalar Analytical B.V., The Netherlands).

1.4. Data analysis and statistics

Correlation analysis was adopted to test the correlations between the NO fluxes during different periods and each of the simultaneously observed soil variables as well as the correlations among the soil variables (T_s, WFPS, and the concentrations of NH_4^+, NO_3^- and WEOC). For this purpose, the data of NO fluxes and those of each soil variables within a specific period were normalized by their variances using Eq. (1).

$$Z_{ki} = (x_{ki} \cdot -\overline{x}_k)/\sigma_k \quad (1)$$

where, z_{ki} and x_{ki} denote the ith values of the kth soil variable or NO fluxes after and before normalization, respectively, and $\overline{x_k}$ and σ_k are the mean and the standard deviation of the measurement array.

The correlation coefficients between all variable pair were then calculated, and their significances were tested. The results were reported in triangle matrixes attached as the supplementary materials to this paper.

The general linear model for repeated measurements was used to test the significance of the NO fluxes or the soil variables between the two treatments. In addition, the general linear model analysis for univariate measures was applied for testing the significance between the two ecosystems with respect to ANPP, Plant-N, SMBC and soil properties or between different seasons with respect to NO emissions or soil variables. Univariate linear and nonlinear regressions were also used to investigate the dependences of NO fluxes on the soil variables.

The SPSS 19.0 software package (SPSS Inc., Chicago, USA) was used for the above statistical analysis. The Origin 8.0 software package (OriginLab Ltd., Guangzhou, China) was used for plotting the data. The raw experimental data were calculated and organized using the Excel software package of the Microsoft Office Standard 2010 (© 2010 Microsoft Corporation).

The standard errors of means for three to five spatial replicates were given to report the results in this paper if not otherwise specified.

2. Results

2.1. Soil properties and related variables

Table 1 lists the measured values of soil properties and other related variables for both ecosystems. Compared to the NAM, no significant differences occurred for the pH, SOC and TN contents and texture in the FOF; however, in the FOF, the BD and GP were 0.28 and 2.3 times greater on average ($p < 0.01$), respectively, and the SMBC concentrations were approximately 64% lower ($p < 0.01$). Although the Plant-N concentrations were approximately 41% lower ($p < 0.01$), the total nitrogen uptake by the aboveground plants in the FOF (92 ± 7 vs. 47 ± 2 kg N/(ha·year) in the NAM) was approximately 95% higher ($p < 0.05$) due to the approximately 2.3 times greater ANPP ($p < 0.05$).

During the entire experimental campaign, as Fig. 1a illustrates, the annual T_a averaged −0.1°C, with the maximum of the monthly averages occurring in August (11.6°C) and the minimum in January (−13.5°C). The T_s in the NAM and FOF averaged 4.8 and 3.3°C, respectively, during the annual period (AP); −3.3 and − 2.3°C, respectively, during the non-growing season (NGS); and 10.1 and 10.8°C, respectively, during the GS (Table 2). The monthly T_s averages in the NAM and FOF showed maximums of 13.3 and 10.9°C, respectively, in August and minimums of −9.7 and − 14.6°C, respectively, in January (Fig. 1b).

The total precipitation amounted to 486 mm during the AP, which was very close to the average (484 mm) in 2012–2014. As 66%–80% of the annual precipitation fell in the GS, the WFPSs during the season were significantly higher ($p < 0.05$) than those during NGS (Fig. 1b). Meanwhile, as Table 2 shows,

Table 2 – Precipitation (P); soil (5 cm) temperature (T_s); soil (0–6 cm) moisture in water-filled pore space (WFPS); and concentrations of soil (0–10 cm) ammonium (NH_4^+), nitrate (NO_3^-) and water-extractable organic carbon (WEOC) during the spring freeze–thaw period (FTP), the non-growing season (NGS), the growing season (GS) and the annual period (AP).

	P (mm)		T_s (°C)		WFPS (%)		[NH_4^+] (mg N/kg d.s.)		[NO_3^-] (mg N/kg d.s.)		[WEOC] (mg C/kg d.s.)	
	NAM	FOF	NAM	FOF	NAM	FOF	NAM	FOF	NAM	FOF	NAM	FOF
FTP	76	76	0.4 (1.1)	−0.7 (1.4)	40 (5)	31 (4) **	6.2 (1.2)	1.7 (0.4) **	1.6 (0.3)	20.5 (2.7) **	99 (15)	74 (5) **
NGS	99	166	−2.3 (1.0)^{b}	−3.3 (1.2)^{B}	39 (2) ^{B}	31 (3) $^{B\,**}$	5.7 (0.8)	1.4 (0.3) **	1.5 (0.3)	18.0 (1.4) **	114 (10)	75 (5)$^{B\,**}$
GS	387	320	10.1 (0.7)^{a}	10.8 (0.7)^{A}	56 (2) ^{A}	41 (2) $^{A\,**}$	4.4 (0.9)	4.0 (1.7)	1.9 (0.9)	12.2 (4.4) **	120 (10)	96 (8)$^{A\,**}$
AP	486	486	4.8 (1.0)	3.3 (1.1)	49 (2)	36 (2) **	5.1 (0.6)	2.3 (0.6) **	1.7 (0.5)	16.1 (1.8) **	118 (7)	82 (4) **

For full treatment names, refer to Table 1. The FTP occurred between February 21 and April 21, 2013, and the GSs for the NAM and FOF were from April 14 to October 1 and from June 2 to September 26, respectively. The given data are means of 3–5 spatial replicates, with standard errors showing within the parentheses, The different superscript letters indicate significant differences between the GS and NGS within a treatment at $p < 0.05$, and ** indicates significant differences between the two treatments at $p < 0.01$. d.s.: dry soil.

the WFPSs in the NAM were typically higher than those in the FOF ($p < 0.01$).

In comparison with the concentrations in the NAM, as Table 2 lists, those in the FOF for NH_4^+ were significantly lower (−55%, $p < 0.01$), but NO_3^- concentrations were greatly higher (+8.5 times, $p < 0.01$). The NH_4^+ concentrations in the NAM were lower than those in the FOF only during the short periods immediately following nitrogen fertilization events (Fig. 1c). After the basal fertilization in the FOF, the NH_4^+ and NO_3^- concentrations were higher than their annual averages (1.8 vs. 16.1 mg N/kg dry soil); this phenomenon occurred consecutively for 12 and 23 d, respectively, with 24.6 versus 65.5 mg N/kg dry soil as maximum values (Fig. 1c–d).

The WEOC concentrations in the NAM were typically higher ($p < 0.01$) than those in the FOF (Fig. 1e). Compared to the NGS, the WEOC concentrations in either ecosystem during the GS were significantly higher (Table 2). Significant correlations (p <0.05 or 0.01) occurred among these soil variables (Table S1).

2.2. Nitric oxide fluxes

The conservative NO fluxes varied between 0 and 5.7 μg N/(m^2 hr) in the NAM and between −0.2 and 28.5 μg N/(m^2 hr) in the FOF, occasionally falling within the negative and positive detection limits (Fig. 2a). The gas emissions from the NAM and FOF conservatively accumulated to 56 ± 4 and 208 ± 13 g N/(ha year), respectively (Table 3), with the latter being 2.7 times higher ($p < 0.01$). The conservative emissions during the spring FTP, NGS and GS accumulated to 30 ± 2, 27 ± 4 and 29 ± 1 g N/(ha year), respectively, in the NAM and to 36 ± 5, 106 ± 7 and 103 ± 16 g N/(ha year), in the FOF, respectively (Fig. 2b). The emissions in the NAM during any of the periods were clearly significantly lower than those in the FOF (Table 3). In addition, the seasonal variation pattern in the NO fluxes also differed between the two ecosystems (Fig. 2a). The fluxes in the NAM were more definitive during the spring FTP and GS, relative to those during the other periods. The spring FTP and NGS contributed 35% and 47% of the annual cumulative emission from the NAM, respectively (Table 3). The most intense fluxes in the FOF occurred following the basal fertilization event (Fig. 2a). The greater fluxes over the annual mean in the FOF (3.2 μg N/(m^2 hr)) lasted for 17 d and accounted for up to 29% of the annual emissions. Smaller fluxes occurred following the nitrogen top-dressing or during the spring FTP, compared to those following the basal

Table 3 – Cumulative nitric oxide (NO) emissions of each treatment during different periods and their contributions (R_{NO}) to the annual cumulative emissions.

	NAM		FOF	
	NO (g N/ha)	R_{NO} (%)	NO (g N/ha)	R_{NO} (%)
FTP	20 (2)	35	36 (5) *	17
NGS	27 (4)	47	106 (7) **	51
GS	29 (1)	53	103 (16) **	49
AP	56 (4)	100	208 (13) **	100

For the full treatment and period names, as well as the meanings of superscript letters, refer to Table 1–2. * and ** indicate significant differences between the treatments at $p < 0.05$ and 0.01, respectively.

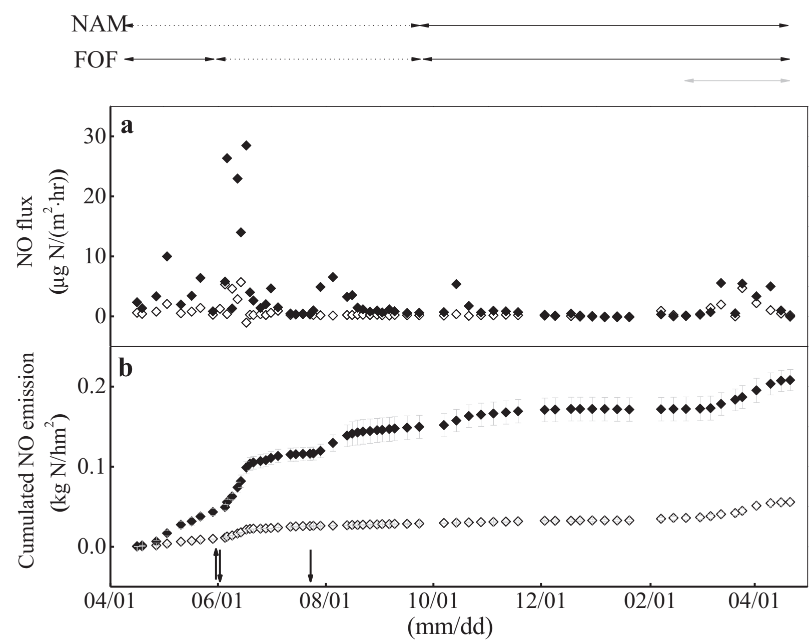

Fig. 2 – Year-round dynamics of nitric oxide (NO) fluxes (a) and cumulative emissions (b). The empty and filled diamonds indicate the means of four spatial replicates for the NAM and FOF treatments, respectively (Table 1). The errors in panel (a) are not shown for plot clarity, and the vertical bars in panel (b) show standard errors. Other details are shown in the footnotes of Fig. 1.

fertilization. In the FOF, the spring FTP and NGS accounted for 17% and 51% of the annual emission, respectively (Table 3). Of the cumulative emissions during the NGS, the spring FTP contributed 77% ± 5% in the NAM but only 35% ± 2% in the FOF, which showed a significant difference ($p < 0.001$).

2.3. *Effects of soil variables on nitric oxide fluxes*

The significant correlations between NO fluxes and soil variables presented in the online supplementary materials (Table S1) could be described with linear or nonlinear regressions (Table S2). According to the coefficient of determination (r^2) values, the variances of the NO fluxes in the NAM could be attributed to the following: (i) the T_s alone by 8%, 35% and 15% during the NGS, GS and AP, respectively; (ii) the NO_3^- concentrations alone by 32% during the GS; (iii) the NH_4^+ concentrations and T_s jointly by 46% during the GS; (iv) the concentrations of NH_4^+, NO_3^- and WEOC jointly by 63% during the GS; and (v) the T_s and concentrations of NH_4^+ and WEOC jointly by 29% during the AP. Regarding the NO fluxes in the FOF, the variances could be explained by the following: (i) the T_s alone by 67%, 27% and 31% during the NGS, GS and AP, respectively; (ii) the T_s and NO_3^- concentrations jointly by 52% and 56% during the GS and AP, respectively; and (iii) the five simultaneously measured soil variables jointly by up to 75% or more during the GS or AP (Table S2). Furthermore, the variances of all the NO fluxes in both treatments could be attributed to the following: (i) the T_s alone by 40%, 26% and 24% during the NGS, GS and AP; (ii) the T_s and NH_4^+ concentrations jointly by 54%-58% during the NGS, GS and AP (Table S2); and (iii) the T_s and concentrations of NH_4^+, NO_3^- and WEOC concentrations jointly by up to 69% during AP (Eq. (2)).

$$F = \left(0.068\left[NH_4{}^+\right] + 0.092\left[NO_3{}^-\right] – 0.00155[WEOC]\right)e^{0.132Ts} \tag{2}$$

($n = 94$, $r^2 = 0.69$, $p < 0.01$, $Q_{10} = 3.7$)

As the significant regressions show, the effects of the T_s on the NO fluxes from either the NAM or FOF during any period could be described by an exponential function of this factor. Accordingly, these empirical exponential coefficients resulted in Q_{10} values (i.e., the fold changes in NO fluxes due to a 10-degree change in T_s) of 8.9 for the NAM during the AP, 3.7 for the FOF during the GS and AP, and 5.1 for the FOF during the NGS (Table S2). The Q_{10} values indicate that a future warming of the surface soils by 1–3°C would amplify the NO emissions from the NAM during the AP by 24%-93% and those from the FOF during the GS and NGS by 14%-48% and 18%-63%, respectively.

3. Discussion

3.1. *Nitric oxide emission during the freeze–thaw period*

Previous studies on NO emissions from temperate terrestrial ecosystems reported significant contributions during the winter, FTP or NGS (Martin et al., 1998; Katayanagi and Hatano, 2012; Mukumbuta et al., 2017), although other studies did not report this (Koponen et al., 2006; Filippa et al., 2009; Wu et al., 2010; Cui et al., 2012; Zhang et al., 2018a). As Martin et al. (1998) reported, the winter period accounted for approximately 25% of the annual NO emission from a temperate grassland in North America. In a temperate grassland in Japan and a cultivated cropland originating from it, the FTPs contributed 1%–32% of the annual NO emissions (Mukumbuta et al., 2017), with significant NO fluxes especially occurring late during the FTP (Katayanagi and Hatano, 2012). Zhang et al. (2018a) reported marginally low NO fluxes during the NGS at two replicated typical alpine meadows on the eastern QTP and showed that the FTPs accounted for approximately 3% (ranging between 1%–14%) of the annual emissions. The FTP contributions in the cases of Zhang and coauthors were clearly much smaller than those reported in this study (Table 3).

Whether the FTPs contributed to the annual NO emissions significantly or not might have been due to the soil water contents and/or soil acidity.

The frequent freezing and thawing alternations of the surface soil water during a FTP usually disrupt soil aggregates and microbe cells, thus releasing NH_4^+ or organic nitrogen such that both rich substrates are provided for living microbes and organic nitrogen mineralization is further stimulated (e.g., Teepe et al., 2001; Wolf et al., 2010). This explanation might be supported by the 13%± 7% higher (but not statistically significant) NH_4^+ and NO_3^- concentrations, on average, during the FTP than during the NGS (Table 2). More available substrates would be more favorable for the activities of microbes (Matzner and Borken, 2008), especially those that are more tolerable to extreme environments, such as ammonia-oxidizing archaea (AOA), heterotrophic nitrifiers and denitrifiers. During the FTP, these microbial organisms might have catalyzed nitrification and/or denitrification, whereby NO was produced as a by-product or an intermediate product (e.g., Papen et al., 1989). A lower soil moisture during a FTP would be more favorable for nitrification and NO diffusion through the more developed air-filled pores to reach the atmosphere. In contrast, a higher soil moisture during a FTP would be more favorable for denitrification but not for NO diffusion through the more developed water-filled pores. Before the NO from denitrification diffused out of the soil, it could be captured by a denitrifier and further reduced to N_2O or even dinitrogen. Therefore, a FTP with a higher soil moisture content would not be favorable for NO emissions and vice versa. This inference can be supported by experimental evidence not only from this study but also from that of Zhang et al. (2018a). As Tables 2 and 3 list, the FTP in the NAM showed significantly higher WFPS but lower contributions to the annual NO emissions compared to the FTP in the FOF. The data presented by Zhang et al. (2018a) showed that the WFPS (%) and fluxes of N_2O and NO (μg N/(m^2 hr)) during the FTP were 66, 0.18 and 4.06, respectively, at one site but were 35, 0.32 and 1.42, respectively, at another site (with significantly different WFPS and N_2O fluxes between the two sites at $p < 0.01$).

Although the climate, vegetation community, ANPP, winter-grazing practices and soil moisture (40% vs. 35% WFPS during the FTP) were nearly comparable to those in one of the Zoige alpine meadows studied by Zhang et al. (2018a), the cumulative NO emission in the NAM during the

FTP was approximately 5.7 times higher (on average 20 vs. 3 g N/ha, respectively). This difference might have been attributed to the higher soil acidity in the NAM (pH 8.0 vs. 6.7) (Table 1; Zhang et al., 2018a). As the calcareous soil in the NAM enabled quick NH_4^+ depletion by nitrification (e.g., Hu et al., 2014), the NH_4^+ concentrations decreased to a level dominated by the equilibrium of the microbial biomass turnover. During the FTP, the NH_4^+ concentrations in the NAM were 75% lower than those in the Zoige meadow (approximately 6 vs. 24 mg N/kg dry soil, respectively). In the NAM case, AOA could survive in the soil with low NH_4^+ concentrations even during the FTP under low temperature, but ammonia-oxidizing bacteria (AOB) could not (e.g., Hu et al., 2014). The much higher NH_4^+ levels in the Zoige case could be favorable for AOB that may be inhibited by the low temperature (e.g., Hu et al., 2014) during the FTP. Governed by this mechanism, the first step of the nitrification (i.e., ammonia oxidation) during the FTP was inhibited in the Zoige case but still proceeded by AOA in the NAM. As a result, the FTP substantially contributed to the annual NO emission in the NAM (Table 3), but did not substantially contribute in the Zoige case (Zhang et al., 2018a).

The intensified NO emission from temperate or alpine ecosystems with calcareous soils during the FTP is important because, due to ongoing global warming, longer FTPs would gradually replace the long freezing period that currently occurs and would thus stimulate more NO emissions from the ecosystems in alpine or high-latitude regions. However, the frequencies of FTPs would decrease due to the ongoing global warming, thus reducing NO emissions (Yao et al., 2010). Additional studies are needed to elucidate the importance of these contrasting effects for the NO emissions from terrestrial ecosystems in cool or cold regions.

3.2. Differences in nitric oxide emissions between the NAM and FOF

3.2.1. Different nitric oxide emissions

The conservative annual NO emissions in the NAM (approximately 0.056 kg N/(ha year)) fell within the range of those (0.021, 0.29 and 0.094 kg N/(ha year) from the three Zoige alpine meadows (Gao et al., 2016; Zhang et al., 2018a). They were slightly lower than the emissions (0.11–0.14 kg N/(ha year) from a typical semiarid temperate steppe in Inner Mongolia (Holst et al., 2007; Zhang et al., 2018a). They were even much lower than those (1.3–1.5 kg N/(ha year)) from the temperate grasslands in North America and southern Germany (Martin et al., 1998; Tilsner et al., 2003). This finding indicates that alpine meadows are weak sources of atmospheric NO.

As for the FOF, which was converted from the alpine meadow of the NAM in 1998, its annual NO emissions (0.208 ± 0.013 kg N/(ha year)) were at the level of the lower bound of the range (0.2–23 (mean: 1.1) kg N/(ha year)) reported in previous studies on cultivated uplands (Davidson and Kingerlee, 1997; Stehfest and Bouwman, 2006). They were lower than those (0.7–5.7 (mean ± SD: 1.7 ± 1.2) kg N/(ha year)) from fertilized uplands with calcareous soils (Mei et al., 2009; Liu et al., 2010, 2015; Yan et al., 2015; Yao et al., 2015a, 2017a, b; Zhang et al., 2018b). They were even much lower than those (6.6–47.1 (mean ± SD: 15.2 ± 12.6) kg N/(ha year)) from vegetable fields or tea gardens with non-calcareous soils (Deng et al., 2012; Huang and Li, 2014; Yao et al., 2015b; Yao et al., 2018). This finding show that the NO emission intensities of fertilized alpine uplands, which originate from alpine meadows and have been long-term cultivated with forage oats, are clearly much lower than those from fertilized uplands in non-alpine regions.

Nevertheless, the difference in the annual NO fluxes between the NAM and FOF (Table 3) indicate that NO emission intensities of fertilized alpine ecosystems, which originate from alpine meadows and have long-term been cultivated with forage oats, are much higher than those from original alpine meadows. This stimulatory effect would be even larger than that of climate warming, e.g., with amplification factors of 3.7 (Table 3) versus 1.9 times per 3-degree increase in T_s (estimated by an equation in Table S2), which proved the hypothesis stated above.

3.2.2. Primary drivers and mechanisms governing different nitric oxide emissions

The much lower NO emissions in the NAM than in the FOF (Table 3) were attributed to two major primary drivers.

One of the major primary drivers was the addition of synthetic nitrogen fertilizer in the FOF. The exogenous nitrogen inputs improved the availability of nitrogen substrates for microbes, and thus enhanced NO production by nitrification. As stated above, the higher NO_3^- concentrations in the FOF indicate that the complex microbial transformations of amended nitrogen mainly ended with nitrification, whereby NO_3^- and NO were formed as a final product and a by-product, respectively (e.g., Butterbach-Bahl et al., 2013). In the NAM without exogenous nitrogen input except for atmospheric deposition, the nitrification process rates were lower due to the limits of insufficient nitrogen substrate and thus were proportionated to the smaller NO production rates and fluxes. The reduced nitrification might have been reflected by the much smaller ratios of NH_4^+ to NO_3^- concentrations (Table 2) recorded in the NAM than in the FOF (approximately 0.3 vs. 8.7, respectively). In addition, the two addition events of synthetic nitrogen per year might have resulted in different compositions of microbial communities between the NAM and FOF, particularly with respect to the species directly involved in NO production. As Fig. 1c shows, for instance, the NH_4^+ concentrations immediately following the addition of the basal fertilizers became very high. Following the urea addition, the increased pH of the soil solutions surrounding the fertilizer particles might especially have inevitably elevated the ammonia availability, and thus the activity of the AOB, which have a much lower ammonia affinity than do AOA (Hu et al., 2014). This effect might have significantly stimulated the NO_3^- and NO productions in the AOB-mediated nitrification, thus enhancing the NO fluxes in the FOF (Fig. 1d and 2a).

The other major primary driver involved both the plow tillage and forage oat cultivation in the FOF. Similar to this study, other researchers (e.g., Civerolo and Dickerson, 1998; Fang and Mu, 2007; Yao et al., 2009) also reported significantly increased NO emissions due to tillage in croplands. As mentioned above, the plow tillage that occurred once per year created a favorable soil structure for nitrification, such as a much higher GP (Table 1), and consequently stimulated NO production and emissions. Moreover, the forage oat cultivation enhanced the ecosystem evapotranspiration due to an amplified ANPP (Table 1) and a longer NGS with bare soil surface (Fig. 1), therefore reducing the soil moisture (Table 2). Thus phenomenon in turn was more favorable for nitrification and NO emissions from the FOF. In the NAM, however, the soil had never been disturbed by tillage while it had been repeatedly compressed by winter-grazing yaks and sheep. In addition, the soil in the NAM was covered for a longer period per year by vegetation with a lower ANPP (Table 1). These features resulted in significantly lower GP and soil moisture, both of which were more favorable for denitrification to consume not only NO_3^- (Table 1–2) but also NO; ultimately, these conditions were less favorable for NO emissions (Table 3). Furthermore, the different long-term management practices between the two ecosystems led to not only different microbial biomass magnitudes by a factor of 2.8 (Table 1) but also likely different microbial functions. For instance, the soil in the NAM was likely more favorable for heterotrophic nitrification to transform both NH_4^+ and organic nitrogen into NO_3^- and NO (e.g., Papen et al., 1989; Butterbach-Bahl et al., 2013; Hu et al., 2014), which could be particularly supported by an equation given in Table S2.

The above mechanical interpretations could be supported by the significant regression results for both ecosystems during the GS (Table S2).

Although the positively linear dependences of NO fluxes upon NO_3^- concentrations alone were significant for both ecosystems, the coefficients of determination were smaller in the FOF than in the NAM (r^2 = 0.18 vs. 0.32, respectively, with the former not shown in Table S2 or elsewhere). This finding suggests that more nitrogen transformation processes jointly dominated the NO emission in the NAM than in the FOF.

As Table S2 shows, the multivariate regressions with maximum coefficients of determination, which involved two soil variables, were obtained jointly for NH_4^+ and T_s in the NAM (r^2 = 0.46) but for NO_3^- and T_s in the FOF (r^2 = 0.52). This finding implicates that the most important processes dominating the NO emissions might have been the autotrophic nitrification in the FOF and the heterotrophic nitrification of NH_4^+ in the NAM.

As for the multivariate regressions with maximum coefficients of determination (r^2 = 0.63 in the NAM and 0.75 in the FOF), which involved three or more soil variables, the NH_4^+ and NO_3^- concentrations showed positive effects while the WEOC concentrations showed negative effects on the NO fluxes from both ecosystems (Table S2). In the FOF, however, the positive effects of T_s and the negative effects of WFPS also occurred at the same time (Table S2). This finding implies that not only the autotrophic and heterotrophic nitrification of NH_4^+ mediated by AOB and AOA but also the heterotrophic nitrification of organic nitrogen dominated by fungi positively accounted for the NO emissions that were both positively regulated by soil temperature and inhibited by denitrification.

3.3. Predictability of soil variables on nitric oxide fluxes

With respect to the variance of the NO fluxes from both the NAM and FOF during the AP, four soil variables, including the T_s and concentrations of NH_4^+, NO_3^- and WEOC, could jointly account for up to 69% (Eq. (2)). This finding suggests that temporally and/or spatially distributed NO fluxes across alpine meadow landscapes with native and/or long-term cultivated calcareous soils may be easily predicted, providing the temporal and spatial data of these four soil variables are available. Before Eq. (2) is applied for accurate predictions, however, its current estimated parameters relying on conservative measurements of NO fluxes still need to be corrected. The parameter corrections require experimental comparisons between the observational methods used in this study and other approach(es) that can more accurately measure NO fluxes (e.g., dynamic chambers).

4. Conclusions

The winter-grazed ecosystem of natural alpine meadow (NAM) with a calcareous soil and a *Kobresia humilis* community, which appears widely on the Qinghai-Tibetan Plateau, is a marginally weak source of atmospheric NO. The relatively high soil pH (approx. 8.0) results in a relatively high contribution (i.e., about one-third) from the spring freeze–thaw period (FTP) to the annual NO emissions. In comparison with the NAM, the forage oat field (FOF), which originated from the NAM and have been consecutively cultivated with forage oats since 1998, has significantly larger aboveground net primary productivity, higher soil bulk density, largely improved soil gas permeability, lower soil moisture, much smaller microbial biomass, lower water-extractable soil organic carbon contents, and greatly enhanced ratios of nitrate to ammonium concentrations. These significant differences are closely associated with the management practices of long-term fertilization, plow tillage and forage oat cultivation in the FOF. These management practices result approximately threefold greater annual NO emissions from the FOF, wherein the FTP contributions are reduced by approximately a half. Nevertheless, the GS and NGS contribute almost equally to the annual NO emissions in either ecosystem. The sensitivities of NO emissions to climate warming differ in the order of the NAM during the full year > the FOF during the NGS > the FOF during the GS. However, the stimulatory effect of climate warming on NO emissions from the NAM is smaller as compared to that due to the land use change converting the NAM to cultivated lands for long-term growing forage oats. This study also indicates the possibility to reliably predict temporally and spatially distributed NO fluxes across alpine meadow landscapes with native and cultivated ecosystems by simultaneous observations of topsoil temperature and concentrations of ammonium, nitrate and water-extractable organic carbon.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.08.011.

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