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Nitrous oxide emissions from different land uses affected by managements on the Qinghai-Tibetan Plateau

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ABSTRACT

We evaluated the N_2O emissions from four land use types (a native alpine meadow with winter grazing (NAM), an abandoned pasture (APL), a perennial *Elymus nutans Griseb.* pasture (PEN) and an annual *Avena sativa* L. pasture (AAS)) with and without three management practices (nitrogen (N) fertilizer, sheep manure and no tillage (NT)) in a Gelic Cambisol soil underlying an alpine meadow on the Qinghai-Tibetan Plateau in 2009 and 2010. Our results show that, compared with NAM, APL had significantly higher cumulative-average seasonal N_2O emissions. Converting unmanaged APL to PEN or AAS significantly increased cumulative-average seasonal N_2O emissions by 35% and 75%, respectively. Sheep manure and N fertilizer application significantly increased N_2O emissions due to increased soil inorganic N concentration. The effect of sheep manure addition on N_2O emissions was lower than that of N fertilizer. For AAS, tillage significantly decreased the effect of sheep manure application on N_2O emissions. Compared with tillage, NT significantly decreased N_2O emissions from AAS. Therefore, our results suggest that cultivating natural grassland would increase N_2O emissions, and fertilizer application would amplify the magnitude of emissions, whereas NT could mitigate the fertilizer impact on N_2O emission. Furthermore, the structural equation analysis revealed that land use change affected N_2O emissions directly by influencing the number of plant species and soil characteristics. There were two different underlying mechanisms regulating N_2O emissions in response to N fertilizer and sheep manure addition.

1. Introduction

The atmospheric concentration of nitrous oxide (N_2O), which has a global warming potential approximately 300 times that of carbon dioxide (CO_2), has increased from 271 ppbv before the Industrial Revolution to 324 ppbv in 2011, and has been responsible for 6% of the enhanced greenhouse effect (IPCC, 2013). Global emissions of N_2O from managed grasslands are estimated at ~0.81 Tg N_2O-N yr^{-1}, accounting for 32% of total N_2O emissions from grassland sources (Ussiri and Lal, 2013).

Nitrous oxide emissions depend on the balance between the production and consumption of N_2O and its diffusion from the soil to the atmosphere (Qin et al., 2014). Nitrous oxide is mainly produced in soils by denitrifying microorganisms which convert NO_3^- or NO_2^- to N_2O and N_2 under anaerobic conditions, and by nitrifying bacteria, which create N_2O as an intermediate product of the oxidation of NH_4^+ to NO_2^- or NO_3^- under aerobic conditions (Khalil et al., 2004; Hamonts et al., 2013; Denk et al., 2017). Denitrification by reduction to N_2 is the major microbial process of N_2O consumption under low oxygen (Flechard et al., 2007; Liu and Graver, 2009). Land use change alters N_2O emissions by altering plant community composition and soil characteristics that are indirectly and directly associated with N_2O production, consumption and diffusion processes (Merbold et al., 2014; Liu and Greaver, 2009; Fig. 1). Management (such as N fertilizer

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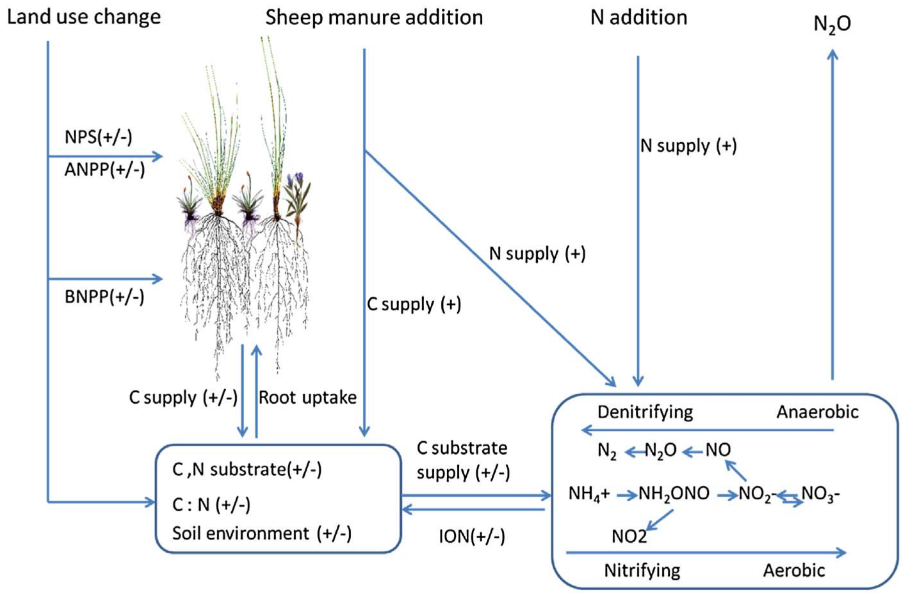

Fig 1. A conceptual model of how land use change and management practices regulate N_2O production and consumption processes (Liu and Greaver, 2009). NPS: the number of plant species; ANPP: aboveground net primary productivity; BNPP: belowground net primary productivity; C:N, soil C/N ratio; ION, soil inorganic N.

application and no-tillage (NT)) has been found to amplify or sometimes modify the effects of land use change through regulation of plant and microbial activities (van Groenigen et al., 2005; Hamonts et al., 2013; Du et al., 2016; Molina-Herrera et al., 2017). Nitrous oxide emissions from grasslands are strongly dependent on soil texture and the availability of mineral N and organic C (Khalil et al., 2004; van Kessel et al., 2013; Abalos et al., 2014; Merbold et al., 2014). The conversion of native grasslands to cultivated pasture is expected to increase the availability of soil C and N by altering plant community composition and soil characteristics (such as soil temperature, moisture, bulk density and N availability), and to result in increased N_2O emissions (Simona et al., 2004; Merbold et al., 2014). However, the potential mechanisms that regulate N_2O emissions under different management practices are still not well understood. The NT practice is widely used to conserve water and reduce soil organic matter losses in cultivated croplands. Some studies have shown an increase in N_2O emissions from NT soils, because of compaction, reduced porosity and increased denitrification in humid climate conditions (Rochette et al., 2008). However, others have reported lower emissions under NT than tillage (T) due to improvements in soil structure and lower soil temperature in dry climate conditions (Ruan and Robertson, 2013). The effect of tillage on N_2O emissions is probably highly dependent on the local environmental conditions (van Kessel et al., 2013). Results from manipulative field experiments indicate that chemical fertilizer or livestock manure increases N_2O emissions, but the magnitude of increase varies widely across grassland ecosystems, environmental conditions, fertilizer rates (0–300 kg^{-1} N ha^{-1} y^{-1}), type of fertilizer (manure organic fertilizer, chemical fertilizer), and application time (preplanting, sidedressing) (Meng et al., 2005; Jones et al., 2005; Flechard et al., 2007; Lu et al., 2011). Livestock manure, which has a high concentration of easily mineralizable organic C, could reduce C constraints on denitrification while at the same time increasing supply of labile N, which could stimulate microbial activity and emit more N_2O than chemical fertilizer (Del Grosso, 2010). Above all, there is high uncertainty in N_2O emission rates from individual fields and regions with different management. Even though an increasing number of models have been used to assess the characteristics of N_2O emissions and develop efficient mitigation strategies for reducing N_2O emissions (Du et al., 2008), the prediction of N_2O emissions in grassland under different land use types and management is difficult without an improved understanding of the potential mechanisms.

As one of the most important dominant vegetation types on the Qinghai-Tibetan Plateau, alpine meadows cover an area of about 2.5 million km^2 and emit an average of 0.3 Tg N_2O annually (Du et al., 2008). Because livestock numbers have increased by more than 200% since 1978, actual livestock numbers have greatly exceeded the theoretical carrying capacity (Cui et al., 2006). Large areas of native grassland have been converted into pasture and cultivated cropland to improve herbage production to meet the forage demand of the livestock population. With economic and social development, fertilizer application has now become the main management practice adopted by herders in this area to increase the productivity of cultivated pasture. Since the early 2000's, a number of government programs have been implemented, aiming to restore grassland vegetation on formerly cultivated land. Therefore, to reduce uncertainty in the estimation of N_2O emissions from the Qinghai-Tibetan Plateau, field quantification of N_2O emissions and the determination of model parameters for accurate model simulations are urgently needed for alpine grasslands under different land uses and managements. Previous studies reported that plowing could accelerate soil N transformation rates and decrease soil organic C in an alpine meadow on the Qinghai-Tibetan Plateau (Sun et al., 2005; Li et al., 2006). Given that N_2O emissions are mainly dependent on soil texture and the availability of soil N, organic C, we hypothesized that the conversion of abandoned pasture to cultivated pasture would increase N_2O emissions due to increased soil N and C transformation rates and a decreased number of plant species, and that natural restoration following the abandonment of cropland would decrease N_2O emissions. In addition, we hypothesized that N_2O emissions would increase in response to N fertilizer, sheep manure addition and tillage due to the increased mineral N, and we expected that sheep manure addition would cause more N_2O emissions than chemical fertilizer application. To test these hypotheses and study the potential mechanisms that regulate the N_2O emissions under different land uses and with different management practices, we set up experiments to measure N_2O emissions from four types of land use with three management practices in an alpine meadow on the Qinghai-Tibetan Plateau in 2009 and 2010.

2. Materials and methods

2.1. Site description and experimental design

Details about the experimental site were previously reported (Zhang et al., 2012). In brief, the experiment was conducted at the Haibei Alpine Grassland Ecosystem Research Station, Northwest Plateau Institute of Biology, Chinese Academy of Sciences, in Qinghai province (37°36′N, 101°12′E), which is located at a mean elevation 3250 m above sea level. The mean annual precipitation is 580 mm, of which 80% is concentrated in the growing season (from May to September), and the mean annual air temperature and soil temperature at 10 cm depth are −1.7 °C and 1.8 °C, respectively (Li et al., 2004). The soil is clay-loam textured and is classified as Mat-Gryic Cambisol (WRB, 1998).

A detailed description of the experimental design is shown in Table S1 (Zhang et al., 2012). In May 2007 an experimental site of 100 m × 100 m was fenced. The soil properties of the experimental site at 10 cm depth were: total organic C, 55.8 g kg^{-1}; phosphorus (P), 0.70 g kg^{-1}; N, 5.37 g kg^{-1} with about 1% inorganic N; $pH_{H2O,}$ 8.2; and bulk density 1.05 g cm^{-3}. Four types of land use (a native alpine meadow with winter grazing (NAM), an abandoned pasture (APL), a perennial *Elymus nutans Griseb.* pasture (PEN), and an annual *Avena sativa* L. pasture (AAS)) were examined in 2009 and 2010. Nitrogen fertilizer (69 kg N ha^{-1} $year^{-1}$) was applied to treatment plots within the APL, PEN, and AAS, and sheep manure (93 kg N ha^{-1}) was applied in the PEN and AAS treatment plots. Meanwhile, tillage (T) management was only performed (operating at 20 cm depth with a spade) in the AAS treatment with a split plot design. There were three replicates for each treatment. Thus, in total, there were 27 plots of 18 m^2 (4 m × 4.5 m) fully randomized throughout the study site. The sheep manure was collected from a sheepfold. Each plot was separated by a 2 m-wide buffer zone.

2.2. Soil sampling and moisture measurements

Fresh soil samples were collected monthly (from June to September) from 0 to 10 cm depth at each plot in 2010, brought to the lab immediately to pass through a 2-mm sieve, and then extracted with 2 mol L^{-1} KCl to measure the nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations. The extracts were analyzed using a Skalar Flow Analyzer (Skalar Analytical, Breda, The Netherlands). Soil samples were collected in each plot in late September 2010 to measure bulk density and total organic C and N. These samples were air dried and passed through a 2-mm sieve before analysis. Soil total organic C and N were measured using an elementalanalyzer (Vario ELIII, Germany). Soil bulk density was determined with core cutters (100 cm^3, diameter 5 cm). In 2009 and 2010 the volumetric water contents and temperature at 5 cm below the soil surface were measured simultaneously to the N_2O flux measurements near the chamber in each plot, using a Time Domain Reflectometer (JS-TDR300, Meridian Measurement, USA) and a digital temperature sensor (JM 624 digital thermometer, Living–Jinming Ltd., China).

2.3. Aboveground and belowground biomass and number of plant species

The aboveground biomass and number of plant species were estimated by clipping a 1 m × 1 m quadrat 20 cm away from the plot edge in each plot in late August each year. Belowground biomass was estimated using the soil cores method. The aboveground and belowground biomass sampling method has been described by Zhang et al. (2012).

2.4. N_2O measurement and cumulative emissions calculation

Similarly to previous studies (Du et al., 2008; Hu et al., 2010), N_2O emissions were measured weekly during the growing seasons (from May 1 to September 30) in 2009 and 2010 using a static opaque chamber and gas chromatography. The static closed opaque chamber (without a bottom, 0.4 m (length) × 0.4 m (width) × 0.4 m (height)) was made of 1 mm-thick stainless steel with a white adiabatic cover attached to the external surface to reduce the impact of direct radiative heating. The stainless-steel collar was inserted 10 cm into the soil at the beginning of the growing season. A battery-operated fan was installed on the top wall of each chamber to ensure good air mixing when the chamber is closed (Hu et al., 2010). At each measurement date, the gas samples were collected between 09:00 a.m. and 11:00 a.m. Gas samples were collected using 100 ml plastic syringes at 10 min intervals (0, 10, 20 and 30 min) and analyzed within 24 h using a gas chromatograph (HP Series 6820 plus, Hewlett Packard, USA). The chromatograph was equipped with an electron capture detector (ECD) for N_2O concentration. The carrier gas was N_2, and the operating temperature for the ECD was set at 300 °C. The calculations for N_2O emissions were based on the slope of the linear regressions of the concentrations over time in the chamber (Zhang et al., 2012). The cumulative emissions of N_2O produced over the growing season were estimated by multiplying the average emissions measured on two consecutive dates by the time interval, and by summing the cumulative fluxes calculated for each time interval in the growing season.

2.5. Statistical analysis

General Linear Model (GLM)–Repeated Measures Analyses of Variance (SPSS 20.0, SPSS Inc., Chicago, IL, USA), with land use as the main factor (between- subject) and sampling date as the within-subject factor, including interactions, was used to test the effects of land use change on N_2O emissions, soil water content, and soil inorganic N concentration when no additional management practices were applied. Two-way ANOVA was performed with management practices (fertilization or tillage) and sampling date to assess the effects of these practices on N_2O emissions, soil water content and soil inorganic N concentration under the same land use. The effects of land use change or fertilizer addition in APL and PEN on cumulative N_2O emissions during the growing season, soil bulk density, water content of soil and the ratio of total soil organic C and N (C/N) were investigated using a one-way ANOVA and least standard difference (LSD) test. Three-way ANOVA was performed with different land uses (PEN or AAS), fertilization and year to assess the effects of species on N_2O emissions induced by fertilizer addition. The effect of fertilizer addition and tillage systems on cumulative N_2O fluxes during the growing season, soil bulk density, water content of soil, and the ratio of total soil organic C and N (C/N) in AAS were investigated using a two-way ANOVA and LSD test. Nonlinear regressions were analyzed between daily N_2O emissions and soil water content, or cumulative precipitation during the one week before flux measurement. All statistical analyses were performed with SPSS (SPSS 20.0, SPSS Inc., Chicago, IL, USA) using the GLM procedure and type III sum of squares during the growing season. Significant differences are reported at the $p < 0.05$ level unless otherwise noted. We further fitted a piecewise structural equation model (SEM) to estimate the direct and indirect effects of land use change and management on seasonal cumulative N_2O emissions using the *piecewiseSEM* package in R software(R 3.1.3, R Development Core Team, 2014). The details of piecewise SEM analyses have been described by Jing et al. (2015).

3. Results

3.1. Soil physical and chemical properties

The seasonal average air temperature and total precipitation from May 1 to September 30 were 8 °C (daily range from −1 to 15 °C) and 9 °C (daily ranging from −3 to 18 °C), and 317 mm and 418 mm in 2009 and 2010, respectively. The seasonal range of daily precipitation was between 0 and 29 mm in 2009 and 0 and 25 mm in 2010 (Fig. S1a).

The seasonal variability in water content of soil under all land uses was very large, as affected by precipitation (Fig. S1b, Table 1). Unmanaged APL, PEN and AAS had significantly lower seasonal average soil water content ($p < 0.001$) and soil C/N ratios ($p < 0.001$), but had significantly higher soil bulk density ($p < 0.001$) compared with NAM. AAS had the lowest soil water content among the four land uses. There was no significant difference in soil inorganic N concentration ($p = 0.753$) among the four unmanaged land uses (Table 2). For AAS, tillage (T) significantly decreased soil water content by 16%

Table 1
Cumulative N_2O fluxes (mg m^{-2}) ± standard error and seasonal average soil water content (%) ± standard error from different land uses as influenced by different management practices in 2009–2010.

Year	Land uses	Soil water content			Cumulative N_2O emissions		
		0	M	N	0	M	N
2009	NAM	28.93 ± 1.51A			5.09 ± 0.11C		
	APL	24.48 ± 1.37B		25.05 ± 1.37	17.3 ± 0.98Bb		46.59 ± 1.02a
	PEN	20.94 ± 1.14BC	22.05 ± 1.08	21.77 ± 1.14	22.35 ± 1.94Ac	51.76 ± 1.33a	41.33 ± 2.22b
	AAS-T	19.37 ± 1.08C	19.37 ± 1.05	19.49 ± 1.13	18.64 ± 0.85ABb	11.21 ± 0.26 cd	24.84 ± 0.94a
	AAS-NT	20.20 ± 1.15	20.72 ± 1.11	20.89 ± 1.12	7.20 ± 1.48e	15.42 ± 0.97c	14.32 ± 1.29c
2010	NAM	47.22 ± 2.23A			5.81 ± 0.37C		
	APL	43.11 ± 2.03B		45.56 ± 2.10	7.65 ± 1.10Cb		35.74 ± 7.71a
	PEN	41.15 ± 1.96B	42.0 5 ± 1.84	43.51 ± 1.72	10.79 ± 0.41Bb	10.37 ± 1.38b	30.23 ± 0.74a
	AAS-T	29.32 ± 1.53C	29.15 ± 1.57	31.54 ± 1.64	18.57 ± 0.90Ac	19.50 ± 1.71c	78.97 ± 0.6a
	AAS-NT	35.47 ± 1.92	36.31 ± 1.88	31.54 ± 1.64	13.59 ± 0.47d	17.44 ± 1.64c	45.08 ± 1.12b

NAM: native alpine meadow with winter grazing; APL: abandoned cropland/pasture; PEN: perennial *Elymus nutans Griseb.* pasture; AAS: annual *Avena sativa* L. pasture; T: tillage; NT: no tillage; 0: without any fertilizer; M: with sheep manure; N: with urea. Values are means ± 1SE (n = 3). Different capital letters indicate significant differences among land uses in the same year at the $p < 0.05$ level. Different lowercase letters indicate significant differences among management practices in the same land use and year at the $p < 0.05$ level.

(p = 0.006), soil C/N by 6% (p = 0.027), and soil bulk density by 10.5% compared with no-tillage (NT) in 2010 (Table 2). However, there was no significant difference between T and NT in soil inorganic N concentration (p = 0.258) in 2010 for AAS. Sheep manure addition did not significantly alter the soil water content or soil C/N ratio, but N fertilizer significantly decreased the C/N ratio for all land uses ($p < 0.001$), and neither fertilization treatment significantly altered soil bulk density (Tables 2 and S2). There was a trend that N fertilizer increased soil inorganic N concentration in APL but it was not significant (Tables 2 and S2). Both sheep manure and N fertilizer addition significantly increased soil inorganic N concentrations in PEN and AAS, and the effects varied with sampling date (Fig. 3, Tables 2 and S2).

3.2. Daily and seasonal pattern of N_2O emissions

Daily N_2O fluxes varied over a large range during the growing seasons, but did not show clear patterns for all unmanaged land uses (Fig. 2). The maximum value of daily N_2O fluxes from APL with N fertilizer were 106.6 and 75.6 μg N_2O m^{-2} h^{-1}, whereas the maximum value of daily N_2O fluxes from APL without N fertilizer were 26.3 and 10.2 μg N_2O m^{-2} h^{-1} in 2009 and 2010, respectively. The daily N_2O fluxes from PEN with N fertilizer showed one peak with a short duration in both 2009 and 2010. N_2O fluxes from PEN with sheep manure resulted in two peaks that were both longer lasting and larger in magnitude compared to PEN with N fertilizer in 2009. In general, daily N_2O flux was significantly affected by land use change, sampling date and their interaction ($p < 0.001$, Table 2). Nitrogen fertilizer significantly increased N_2O fluxes for APL ($p < 0.001$), and this effect varied with sampling date ($p < 0.001$) (Table S2). Nitrogen fertilizer and sheep manure significantly increased N_2O fluxes for PEN (p = 0.004), and this effect varied with sampling date ($p < 0.001$) (Table S2).

For the AAS treatment fertilizer, NT and the interactions between fertilizer and/or NT and sampling date significantly affected N_2O fluxes, and their effects varied with year (Table S2). Nitrogen fertilizer significantly increased N_2O emissions for AAS, and there was one peak in the seasonal pattern of N_2O fluxes both in T (27.5 and 212.2 μg N_2O m^{-2} h^{-1} in 2009 and 2010, respectively) and NT treatments (15.3 and 75.5 μg N_2O m^{-2} h^{-1} in 2009 and 2010, respectively). Sheep manure application did not significantly affect N_2O emissions (p = 0.284). Compared with T, NT significantly decreased N_2O emissions by 43% for AAS without fertilizer ($p < 0.001$). In addition, there were significant interactive effects between T and sheep manure application ($p < 0.001$). In NT plots, sheep manure addition significantly increased seasonal average N_2O emissions for AAS (p = 0.009), whereas it did not significantly affect N_2O emissions under T treatment for AAS (p = 0.080). In addition, the effect of fertilizer on N_2O emissions was significantly different for AAS and PEN ($p < 0.001$), and the difference varied with sampling date and year.

3.3. Cumulative N_2O emissions

Land use ($p < 0.001$), sampling year ($p < 0.001$) and their interaction ($p < 0.001$) significantly affected the cumulative-average seasonal N_2O emissions during the growing season. Over the growing seasons, compared with NAM (5.09 ± 0.11 mg N_2O m^{-2} in 2009 and 5.81 ± 0.37 mg N_2O m^{-2} in 2010), APL had significantly higher cumulative-average seasonal N_2O emission by 240% in 2009 and 32% in 2010 (Table 1). Compared with APL, PEN and AAS with tillage had significantly higher cumulative-average seasonal N_2O emission by 29% and 8% in 2009, and by 41% and 143% in 2010

Table 2
Soil bulk densities (g m^{-2}), soil C/N ratio and seasonal average soil inorganic N (NO_3^--N + NH_4^+-N (mg kg^{-1} soil) in 10 cm depth from different land uses as influenced by different management practices in 2010.

Land uses	Soil bulk density			Soil C/N ratio			Soil inorganic N		
	0	M	N	0	M	N	0	M	N
NAM	0.96 ± 0.02C			19.15 ± 0.15			33.83 ± 1.46A		
APL	1.81 ± 0.11B		1.84 ± 0.04	10.48 ± 0.08		9.80 ± 0.05	33.72 ± 1.45Aa		36.73 ± 2.14a
PEN	1.86 ± 0.07A	1.88 ± 0.11	1.95 ± 0.06	10.13 ± 0.02	10.09 ± 0.06	9.88 ± 0.07	33.29 ± 0.95Ac	35.45 ± 1.19b	36.86 ± 1.18a
AAS-T	1.86 ± 0.02Aa	1.78 ± 0.05b	1.83 ± 0.04a	10.19 ± 0.07	10.23 ± 0.10	10.12 ± 0.06	32.32 ± 1.23Ab	32.51 ± 1.27b	34.04 ± 1.31a
AAS-NT	1.92 ± 0.06a	1.80 ± 0.01b	1.84 ± 0.02a	10.13 ± 0.02	9.88 ± 0.11	10.26 ± 0.12	31.63 ± 1.18Ab	32.82 ± 1.06ab	32.95 ± 1.35ab

NAM: native alpine meadow with winter grazing; APL: abandoned cropland/pasture; PEN: perennial *Elymus nutans Griseb*. pasture; AAS: annual *Avena sativa* L. pasture; T: tillage; NT: no tillage; 0: without any fertilizer; M: with sheep manure; N: with urea. Values are means ± 1SE (n = 3). Different capital letters indicate significant differences among land uses in the same year at the $p < 0.05$ level. Different lowercase letters indicate significant differences among management practices in the same land use and year at the $p < 0.05$ level.

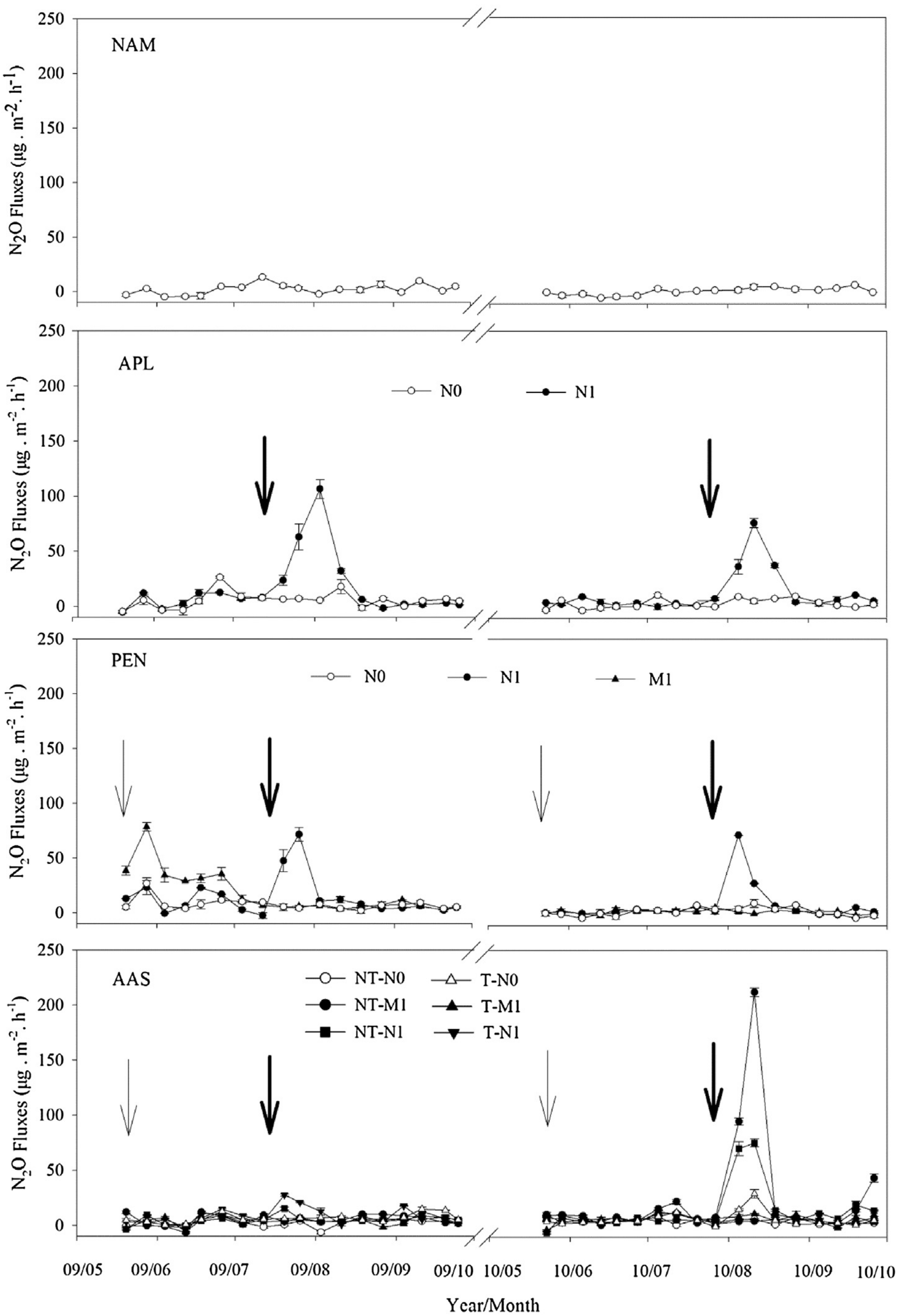

Fig. 2. Daily N_2O emissions from different land uses as affected by different management practices during the study period. NAM: native alpine meadow with winter grazing; APL: abandoned pasture; PEN: perennial *Elymus nutans Griseb.* pasture; AAS: annual *Avena sativa* L. pasture. Arrows show fertilizer application dates. The thick arrow indicates sheep manure application and the thinner arrow indicates urea application. Bars indicate mean ± 1SE (n = 3).

(17.30 ± 0.98 mg N_2O m^{-2} in 2009 and 7.65 ± 1.10 mg N_2O m^{-2} in 2010, Table 1).

Nitrogen fertilizer significantly increased cumulative-average seasonal N_2O emission by 270% for APL, by 110% for PEN, by 240% and 170% for AAS under T and NT treatments, respectively (Table 1). Sheep manure addition significantly increased cumulative-average seasonal N_2O emissions by 132% for PEN, but only in 2009 (Table 1). In AAS plots under NT treatment, sheep manure addition significantly increased cumulative-average seasonal N_2O emissions by 71% (28–114%), whereas it significantly decreased cumulative-average seasonal N_2O emissions by 40% emission under T treatment in 2009. Moreover, for AAS plots, NT significantly decreased cumulative-average seasonal N_2O emissions by 44% compared with T (Table 1).

3.4. Factors affecting N_2O emissions

Precipitation was the key factor affecting N_2O emissions for different land uses in the alpine meadow (Fig. 4). When data from all treatments are pooled, a positive relationship ($r^2 = 0.64$, $p < 0.001$) was found between the average cumulative precipitation in a one week period before the sampling date and daily N_2O emissions, especially during the period after fertilizer application in 2010. However, we did not find a significant relationship between soil water content and daily N_2O fluxes during the growing season ($p > 0.05$).

Considering the strong collinearity among affecting factors, we fitted a piecewise structural equation model (SEM) to evaluate the causal relationships among these factors and then to estimate the direct and indirect effects of land use change and different types of fertilizer on cumulative seasonal N_2O emissions (Fig. 5a–c). The influences of

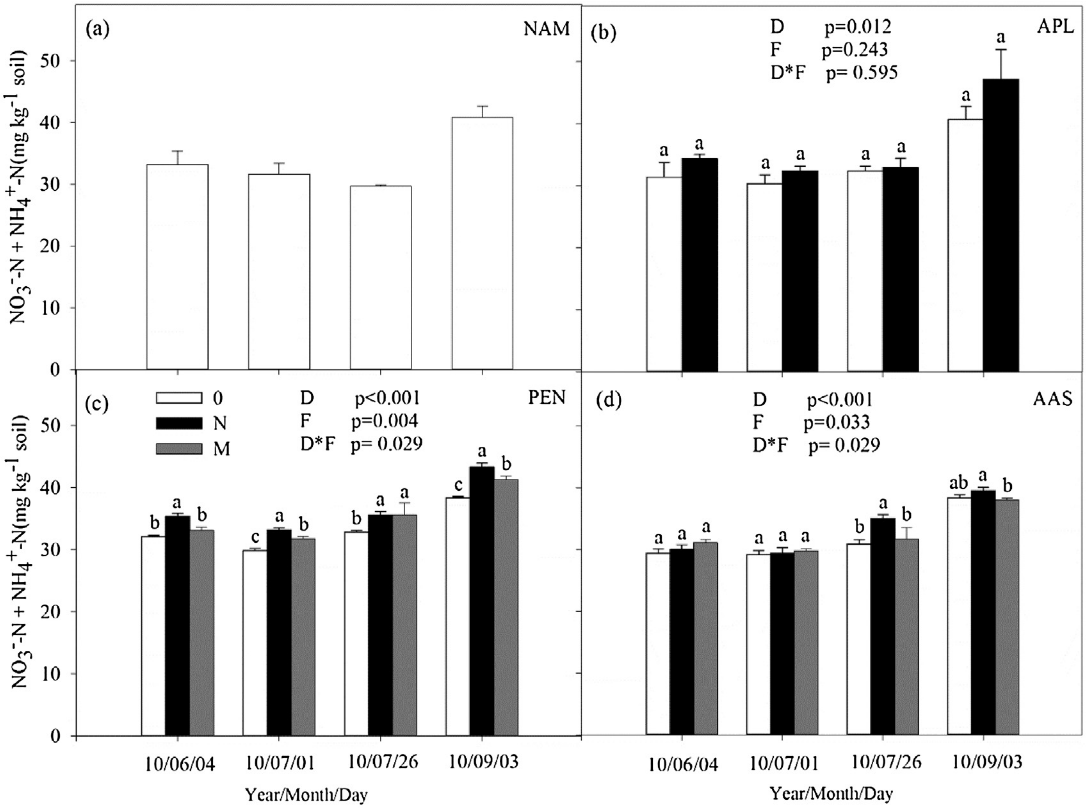

Fig. 3. (a): Seasonal variability of inorganic N (NO_3^--N + NH_4^+-N(mg kg^{-1} soil)) at a depth of 10 cm in NAM; (b): Seasonal variability of inorganic N (NO_3^--N + NH_4^+-N(mg kg^{-1} soil)) at a depth of 10 cm in APL with different managements; (c): Seasonal variability of inorganic N (NO_3^--N + NH_4^+-N(mg kg^{-1} soil)) at a depth of 10 cm in PEN with different management; (d): Seasonal variability of inorganic N (NO_3^--N + NH_4^+-N(mg kg^{-1} soil)) at a depth of 10 cm in AAS under different management. NAM: native alpine meadow with winter grazing; APL: abandoned pastureland; PEN: perennial *Elymus nutans Griseb.* pasture; AAS: annual *Avena sativa* L. pasture; D: sampling date; F: Fertilization; 0: without fertilizer; M: with sheep manure; N: with urea. Bars indicate mean ± 1SE. Different letters above bars indicate significant differences among treatments at the $p < 0.05$ level.

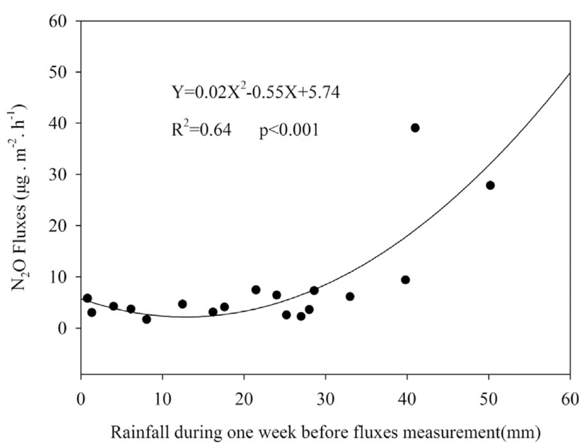

Fig. 4. The relationship between cumulative N_2O emissions and precipitation during the one week before emissions measurement across all treatments.

land use change on cumulative seasonal N_2O emissions were mainly mediated directly through soil water content, soil bulk density and the number of plant species. Land use change indirectly influenced soil C/N ratio and soil inorganic N concentration through soil water content and bulk density (Fig. 5a). The strongest relationship was between soil bulk density and C/N ratio ($\beta = -0.92$, standardized coefficient), however, there was a weaker relationship between soil C/N ratio and cumulative N_2O emissions. N fertilizer directly impacted on cumulative N_2O emissions through soil C/N ratio and soil bulk density (Fig. 5b). In addition, N fertilizer and soil water content directly influenced soil in organic N, which affected cumulative N_2O emissions. Unlike N fertilizer, sheep manure addition mainly indirectly influenced soil total organic C, soil in organic N, and soil water content, affecting cumulative N_2O emissions (Fig. 5c).

4. Discussion

4.1. Effect of land use change on N_2O emissions

Our results generally confirmed our hypothesis that converting APL to cultivated pasture (i.e. PEN and AAS) significantly increased N_2O emissions, and that natural restoration following the abandonment of cultivated pasture decreased N_2O emissions. The SEM results suggested that there were several reasons that account for this phenomenon. First, it is likely that following conversion from APL to cultivated pasturelands, a decline in soil aggregation and belowground biomass significantly increased soil bulk density (Table 2, Zhang et al., 2012), which could easily leads to a reduction in soil oxygen supply and therefore beneficial to N_2O production (Khalil et al., 2004; van Kessel et al., 2013; Merbold et al., 2014). Second, the cultivated pasture may have more available C for N_2O production due to accelerating mineralization of soil organic C induced by plowing (Ruan and Robertson, 2013). This can be seen from the soil C/N ratios (Table 2). Third, the cultivated pasture, with higher soil bulk density, may have more available soil inorganic N for N_2O production. Plant species have been reported to control N_2O emissions indirectly by influencing the physicochemical properties of the soil, especially mineral N availability, as differences in species composition can be associated with significant differences in the uptake of N (Xu et al., 2004; van Kessel et al., 2013; Abalos et al., 2014). Plant growth was limited by N availability in this area (Xu et al., 2004). Therefore, increased plant inorganic N uptake ability may increase N constraints on microbial processes to produce N_2O in the alpine region. The SEM analyses showed that all the factors

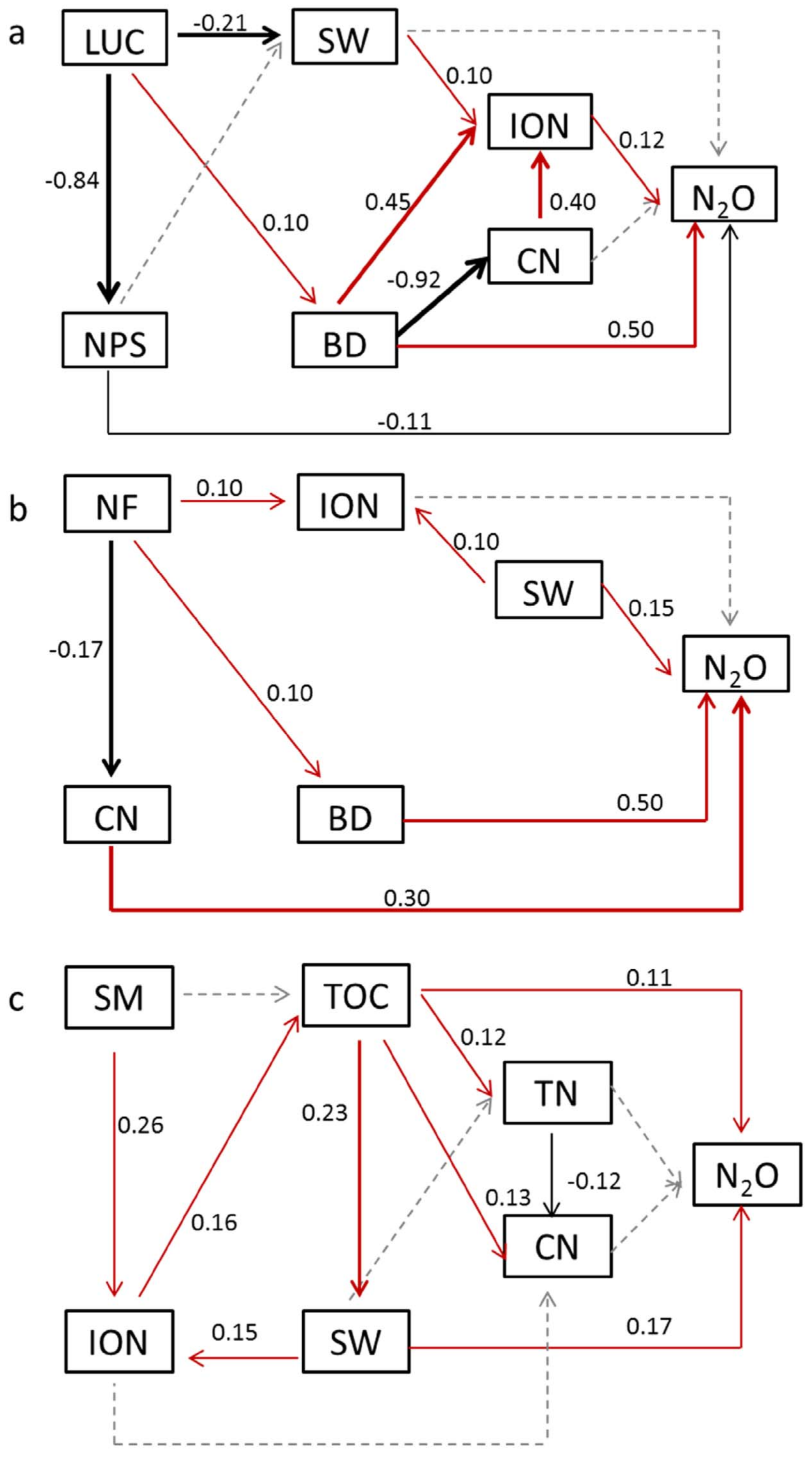

Fig. 5. The final SEM for cumulative N_2O emissions affected by land use change (a, Fisher C = 3.99, p = 0.99, AIC = 55.99), N fertilizer addition (b, Fisher C = 5.1, p = 0.75, AIC = 33.1) and sheep manure addition (c, Fisher C = 4.17, p = 0.94, AIC = 60.17). Width of arrows indicates the strength of the relationships. Solid red arrows represent positive paths ($p < 0.05$), solid black arrows represent negative paths ($p < 0.05$) and dotted gray arrows represent non-significant paths ($p < 0.05$). LUC, land use change; SW, soil water content; BD, bulk density; TOC, soil total organic carbon; TN, soil total nitrogen, CN, soil C/N ratio; ION, soil inorganic N; NPS, number of plant species; NF, N fertilizer; SM, sheep manure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

we observed have limited explanatory power to N_2O emissions variation (Fig. 5a). Further studies should investigate additional microbial mediated mechanisms for the influence of land use change on N_2O emissions.

In contrast to other studies (Flechard et al., 2007; Luo et al., 2013), in this study we did not find significant relationships between soil water content and daily N_2O fluxes under different land uses during the growing season. The SEM results showed that there was a significant but weaker relationship between soil water content and cumulative N_2O emissions. However, there was a significant exponential relationship between the temporal N_2O emissions and soil temperature from unmanaged PEN and APL (Fig. 4a, b). Given the low temperatures of the environment studied, it is possible that soil temperature plays a key role when soil water is not limiting (Dobbie and Smith, 2003). In addition, there was a significant and positive correlation between daily N_2O emissions and cumulative precipitation within a one-week period before the sampling date (Fig. 4c). It is possible that precipitation events can limit O_2 diffusion into the soil and create anaerobic conditions, which can stimulate N_2O emission through the promotion of denitrification (Dobbie and Smith, 2003; Luo et al., 2013; Kanter et al., 2016). In addition, the SEM analyses showed that there was a significant and positive correlation between soil water content and N_2O emissions both in N fertilizer and sheep manure treatment.

4.2. Effects of fertilizer or NT on N_2O emissions under different land uses

Previous studies have shown that the application of organic fertilizers could contribute to longer lasting and higher N_2O emissions compared with chemical fertilizers (Jones et al., 2005; Wei et al., 2014). For example, Jones et al. (2005) found that plots receiving inorganic fertilizers (urea, 300 kg N ha^{-1}) had short term peaks of up to 93 μg N_2O m^{-2} h^{-1}, whereas losses from plots receiving organic manures (cattle slurry, 300 kg N ha^{-1}) were both longer lasting and greater in magnitude, with emissions of up to 837 μg N_2O m^{-2} h^{-1}. In our study, although both N fertilizer and sheep manure addition significantly increased N_2O emissions, contrary to our hypothesis, the effect of sheep manure on N_2O emissions was lower than that of N fertilizer. In particular, this result was obtained even though there was adequate precipitation after application in 2010, and despite a higher N application rate from sheep manure addition compared to urea addition. Moreover, for AAS, there were interactive effects between sheep manure and tillage on N_2O emissions. This may be due to the different underlying mechanisms that regulate the N_2O emissions' response to N fertilizer and sheep manure addition (Fig. 5b and c). The different underlying mechanisms may not be the result of the rate of fertilizer application, but the type, timing and method of N fertilizer application (Ma et al., 2010; Zhang et al., 2013). On one hand, N fertilizer as urea was applied in the middle of July under warm and wet climate conditions, and it had a greater effect on soil C/N ratio and bulk density than sheep manure, which was applied at the end of May in 2010. On the other hand, a longer time is required for mineralization to transform part of the N and C from sheep manure into the forms that plants and microorganisms can utilize, especially if the broadcast method of application is used. Even though we analyzed so many driving factors of N_2O emissions, the SEM analyses showed that all the factors have limited explanatory power to N_2O emissions variation within different management (Fig. 5b and c). Further studies should investigate additional microbialmediated mechanisms for the influence of different management on N_2O emissions.

N_2O emissions from NT were significantly lower than T in both unfertilized plots and N fertilized plots in our study period. In addition, there was a trend for N_2O emissions from NT to be lower than T with sheep manure application (Table 1). Similar results have been reported by Ruan and Robertson (2013), who showed 64.8% decreased N_2O emissions from NT compared with T plots. Low N_2O emissions could be induced by the decrease in available N and C from soil organic matter mineralization after NT (Fig. 1, Ruan and Robertson, 2013). Available N could be one of the most important driving factors for N_2O emissions (van Kessel et al., 2013). However, in this study, there was no significant difference in soil inorganic N concentration between T and NT (Table 2). This is likely because the KCl extraction method only measures the soil-available N pool, which can be rapidly utilized by microorganisms or leached out of the soil, so the difference cannot be detected (Ruan and Robertson, 2013). However, in this study, the soil C/N ratios from NT were significantly lower than from T in unfertilized plots and N fertilized plots. There was a trend for soil C/N ratios from NT to be lower than T under sheep manure application. Conversely, Almaraz et al. (2009) found that NT enhanced cumulative N_2O emissions by 62% compared with T without N fertilizer application and by 35% with N fertilizer during two years in humid, rainy weather due to the increased soil water content, which enhanced denitrification activity (Rochette et al., 2008). This explanation is consistent with our result, in which daily N_2O emissions were significantly correlated with average cumulative precipitation in a one week period before the sampling date (Fig. 3C). However, unlike Almaraz et al. (2009), we found that for AAS plots, T could mitigate the magnitude of increased N_2O emissions caused by fertilizer by decreasing soil water content (Table 1). van Kessel et al. (2013) conducted a meta-analysis of 239 direct comparisons globally from sites with both dry and humid conditions, and found that there was no difference in N_2O emissions between T and NT/reduced tillage, and that the interaction between T/NT and N fertilizer depends on the fertilizer application method. However, our results suggest that converting T to NT may decrease N_2O emissions in alpine meadows on the Qinghai-Tibetan Plateau.

5. Conclusions

Our results show that cultivating pasture significantly increases N_2O emissions, and that natural restoration from abandoned cropland significantly decreases N_2O emission directly, through its influence on soil characteristics and the number of plant species. Even though both sheep manure and N fertilizer significantly increase N_2O emissions, there were two different underlying mechanisms regulating the N_2O emissions in response to N fertilizer and sheep manure addition. For AAS, no-tillage decreases N_2O emissions under both sheep manure and N fertilizer treatments, but tillage could mitigate the magnitude of increased N_2O emissions caused by fertilizer for AAS. These findings suggest that we can reduce the increased N_2O emissions induced by land use change by establishing mixed artificial perennial grasslands. For the existing cultivated pasture, we can improve the management (such as applying sheep manure instead of urea, especially in wet years, and adopting no-tillage or red uced tillage instead of tillage) to reduce N_2O emission in alpine meadow with high soil total N and low inorganic N.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agrformet.2017.06.013.

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