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Greenhouse Gas Emissions from the Tibetan Alpine Grassland: Effects of Nitrogen and Phosphorus Addition

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Abstract: The cycle of key nutrient elements nitrogen (N) and phosphorus (P) has been massively altered by anthropogenic activities. Little is known about the impacts on greenhouse gas (GHG) emission of the large nutrient additions occurring in the alpine grasslands of the Tibetan Plateau. We investigated soil surface emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) under control, N, P and combined nitrogen and phosphorus (NP) additions from July 2011 to September 2012. Compared to the control, CO_2 flux significantly increased by 14.6% and 27.4% following P and NP addition, respectively. The interaction of NP addition had a significant influence on CO_2 flux during the non-growing season and the spring thaw period. Compared to the control, CH_4 flux decreased by 9.9%, 23.2% and 26.7% following N, P and NP additions, respectively, and no interactive effect of NP addition was found in any period. Soil N_2O flux was significantly increased 2.6 fold and 3.3 fold, following N and NP addition treatments, respectively, and there was no interaction effect of NP addition together. The contribution of cumulative CO_2 emission during the non-growing season was less than 20% of the annual budget, but cumulative CH_4 and N_2O emissions during the same period can account for 37.3–48.9% and 44.7–59.5% of the annual budget, respectively. Methane and N_2O emissions did not increase greatly during the spring thawing period, with contributions of only 0.4–3.6% and 10.3–12.3% of the annual budget, respectively. Our results suggest that N and P addition could increase CO_2 and N_2O emissions and reduce CH_4 emission. Furthermore, although the non-growing season is very cold and long, cumulative CH_4 and N_2O emissions are considerable during this period and cannot be neglected by future studies evaluating the greenhouse gas emission budget in the Tibetan plateau.

Keywords: carbon dioxide (CO_2); methane (CH_4); nitrous oxide (N_2O); growing season; non-growing season; Tibetan plateau

1. Introduction

Global warming is a major issue of common concern for the international community and can be attributed to anthropogenic emissions of greenhouse gases (GHG). Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are the three main GHG emissions that naturally occur in the atmosphere, but are largely added by human activities and contribute to global warming [1]. With the intensification of global warming, it is necessary to accurately estimate the regional budgets of GHG emissions in the world.

As "the third pole" of Earth, the Tibetan Plateau is one of the largest high elevation grasslands in the world, covering nearly one-quarter of the area of China [2]. Among all types of land cover, alpine grassland is the dominant ecosystem over the Tibetan plateau, covering 62% of the whole plateau area [3]. However, with rapid economic and social development, the deterioration of the grassland and the expansion of forage production have increased the need for herdsman to improve plant productivity, which translates to the addition of nutrients to the alpine grassland [4]. As the key nutrient elements for plant growth, nitrogen (N) and phosphorus (P) have been extensively added to the soil as synthetic fertilizers. Therefore, the cycle of soil N and P have been drastically altered by anthropogenic activities [5]. Nitrogen and P addition has proved to increase yield and quality of forage and improve the soil availability of farming and animal husbandry [6] but has also caused vegetation succession and pasture degradation [7] and extensively influenced the GHG budget [8–10]. Numerous studies have investigated the effects of N addition on GHG emission in widely distributed regions [11–14]. In the naturally occurring soils of the Tibetan plateau, P availability is limited [7,15]. The activity and productivity of microorganisms and their related functions in these soils are likely to be limited by P. However, the influence of P addition on GHG emission has rarely been considered. Therefore, it remains unclear how soil N and P enrichment due to anthropogenic activities affect the Plateau's GHG budget and its components in alpine grasslands and whether the response of GHG to N is distinct from the response of GHG to P addition.

The plateau has a unique climate condition that has strong solar radiation and a long cold non-growing season. Wang et al. [16] reported that the cumulative soil respiration during the long non-growing season is only 11.8–13.2% of the annual total respiration in the Tibetan alpine grassland. Song et al. [17] reported that CH_4 emissions during the non-growing season in an alpine wetland accounted for almost half of the annual emission. However, to our knowledge, few studies have explored the influence of nutrients (N, P and NP interaction) addition on GHG emissions under non-growing season in alpine grasslands. Moreover, soil freeze-thaw action frequently occurs on the Tibetan Plateau due to its high elevation and cold temperature. Freezing and thawing has been shown to cause significant soil physical and biological changes and impacts on GHG emissions [18,19]. For example, Wolf et al. [19] showed that a short-lived pulse of N_2O emission during spring thaw dominate the annual N_2O budget. Therefore, studying the GHG emissions during the non-growing season (including freeze-thaw periods) in the alpine grassland of the Tibetan Plateau should help in accurately assessing the GHG emissions budget of the plateau under nutrient addition.

In order to improve our predictions of the GHG emissions in response to different nutrient additions in the Tibetan alpine grassland and to evaluate the contribution of GHG emissions during the non-growing season, we conducted an *in-situ* experiment from June 2011 to September 2012 to: (1) evaluate the effects of nutrient addition on CO_2, CH_4 and N_2O emissions; (2) explore the dynamics of GHG emission and the contributions of growing/non-growing seasons; and (3) find the relationship between environmental factors and GHG emissions.

2. Materials and Methods

2.1. Site Description

This study was conducted in an alpine grassland at the Haibei Alpine Meadow Ecosystem Research Station, Northwest Plateau Institute of Biology, Chinese Academy of Sciences (37°37′ N,

101°12′ E, 3200 m a.s.l.), located in the northeast of the Tibetan Plateau, China. The site has a typical plateau continental climate that has strong solar radiation, long cold non-growing season and short and cool growing season. During the experimental period, the daily mean air temperature was −1.7 °C. The highest daily mean temperature was 15.6 °C in July, while the lowest was −22.1 °C in January. In this region, the annual mean precipitation ranged from 426 to 860 mm, more than 90% of which falls during the short summer growing season from May to September [20]. Although the non-growing season is lengthy, it receives only 6–8% of the annual precipitation; this period is usually characterized as dry, cold with no persistent snowpack [16]. Wang et al. [16] reported a more precise definition of the non-growing season by the phenological method but here we defined that the growing season is from May to September, non-growing season is from October to April and the spring thawing period occurs in April. The soils, sampled in August 2009, are classified as Mat-Gryic Cambisol. The characteristics of the studied soils are listed in Table 1.

Table 1. The soil physicochemical characteristics at this study site in the Tibetan alpine grassland before the start of nutrient addition sample in July 2009.

Soil Layer (cm)	pH (H_2O)	TC (g kg^{-1})	TOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})
0–5	7.5 ± 0.04	89.0 ± 1.33	72.4 ± 0.16	8.7 ± 0.12	0.84 ± 0.006	15.2 ± 0.09	6.2 ± 0.009	244.1 ± 0.28
5–10	7.6 ± 0.03	66.9 ± 0.72	62.4 ± 0.25	6.9 ± 0.08	0.69 ± 0.006	13.8 ± 0.08	5.2 ± 0.008	225.2 ± 0.23
10–20	8.0 ± 0.02	54.8 ± 0.46	42.8 ± 0.16	5.9 ± 0.07	0.56 ± 0.018	9.6 ± 0.08	3.2 ± 0.008	100.7 ± 0.17
20–40	8.4 ± 0.01	40.4 ± 0.28	33.0 ± 0.19	3.7 ± 0.07	0.44 ± 0.006	7.4 ± 0.08	2.2 ± 0.011	84.7 ± 0.16
40–60	8.6 ± 0.02	30.6 ± 0.29	23.6 ± 0.11	1.9 ± 0.06	0.30 ± 0.011	3.7 ± 0.08	0.2 ± 0.006	61.5 ± 0.09
Average	8.2 ± 0.02	55.6 ± 0.64	46.4 ± 0.51	5.3 ± 0.07	0.56 ± 0.007	9.9 ± 0.12	3.4 ± 0.06	141.6 ± 2.13

TC: total carbon, TOC: total organic carbon, TN: total nitrogen, TP: total phosphorus, TK: total potassium, AP: available phosphorus, AK: available potassium. The value is means ± SE (n = 3).

2.2. Experimental Design

A small area of alpine grassland (75 m × 110 m) with flat topography and a relatively uniform vegetation distribution was fenced in May 2009, to prevent grazing disturbance. The field experiment was conducted using a randomized complete block design with four treatments and six replications. Twenty-four plots (3 m × 3 m) were set up for the four treatments and divided into six blocks in a complete randomized block design. Some of them (block 1, 2 and 4) were used to study the effects of nutrient addition on GHG emissions from July 2011 to September 2012. The blocks were separated by a 2 m-wide buffer zone and the plots were separated by a 1 m-wide buffer zone in order to minimize the disturbance from neighboring treatments. The four treatments were: (1) Control: no fertilizer was added; (2) N: N addition in the form of urea with a rate of 100 kg N ha^{-1} $year^{-1}$; (3) P: P addition in the form of triple superphosphate with a rate of 50 kg ha^{-1} $year^{-1}$; and (4) NP: combined N and P addition in the same amounts as the solo treatments. All nutrients were evenly spread on 15 July, 5 July, 27 July and 22 June, respectively from 2009 to 2012 by experienced farmers after sunset in order to ensure higher moisture.

2.3. Sampling and Measurement

Fluxes of CO_2, CH_4 and N_2O from soil were measured using an opaque, static, stainless steel chamber and gas chromatography techniques [21]. The static chamber consisted of two parts, a stainless steel collar (0.4 m (length) × 0.4 m (width) × 0.1 m (height)) topped by a water groove to ensure that the chamber was airtight and a removable lid (0.4 m (length) × 0.4 m (width) × 0.4 m (height)) covered with heat-insulating material. An open-bottom square box and a fan on the top wall of each lid were installed to make turbulence when the chamber was closed, as described by Zhang et al. [4]. In June 2011, two weeks before samples were collected, the stainless steel collar was placed 20 cm away from the plot edge and inserted into the soil at 10 cm depth at each plot. During sample collection, the lid was sealed on the square collar and gas inside the chamber was sampled at 0, 10, 20 and 30 min using a 60 mL plastic syringe. Greenhouse gas emission was measured three times a month during the growing season and once a month during the non-growing season. During the

spring thawing period, sampling frequency was increased to every 3 days from 2 April to 28 April of 2012. Samples were collected at the same time each day (between 9:00 a.m. to 11:00 a.m.), representing one-day average emission as described in previous studies [21]. Gas chromatography (Agilent 6890A GC System, Agilent Technologies, Wilmington, DE, USA) was used to analyze the GHG concentrations of the gas samples within 72 h following gas sampling. Standard CO_2, CH_4 and N_2O gases were provided by the National Standard Material Research Center, China. The gas emission ratios were calculated from the slope of the linear regression between concentration and time using the equation described by Song et al. [22].

$$J = \frac{dc}{dt} \times \frac{M}{V_o} \times \frac{P}{P_o} \times \frac{T_o}{T} \times H \quad (1)$$

where J is emission flux (mg m^{-2} h^{-1}), dc/dt is the slope of the linear regression of gas concentration at time approaching zero, M is the mole mass of the measured gas (g mol^{-1}), P is the atmospheric pressure (P_a), T is the absolute temperature inside the chamber (K); V_o, P_o, T_o are volume (mL) and absolute temperature (K) and pressure (P_a) at standard condition and H is chamber height above the water surface (cm).

For all measurements, the mean values of gas emission and the standard errors were calculated from three replicates. The linear interpolation technique was used to estimate the cumulative emissions of CO_2, CH_4 and N_2O amount: the mean emission fluxes of two subsequent sampling dates were multiplied by the time interval between two sampling duration and then added up over the season [23].

2.4. Environmental Conditions

Air temperature (1.5 m above ground level), surface soil temperature (sensor installed on the top of the soil covered lightly with earth), soil temperature and soil moisture at a 10 cm depth were measured once an hour using sensors connected to a data logger (EM50, Decagon Devices Inc., Pullman, WA, USA) at the first block of the experimental site. Precipitation was measured once an hour near the experimental site with a rain gauge also connected to a data logger (EM50, Decagon Devices Inc., Pullman, WA, USA). The daily air temperature, soil temperature and mean moisture at a depth of 10 cm were used to constrain the correlation coefficient between GHG emissions and temperature and moisture.

2.5. Statistical Analysis

The repeated measures ANOVA was performed with nitrogen addition and phosphorus addition as the main factors (between-subject) and with sampling date as the within-subject factor including their interactions to test the effects of the main factors on CH_4, CO_2 and N_2O fluxes. The effects of nutrient addition on CH_4, CO_2 and N_2O emissions, individually and in combination, were evaluated using one-way analysis of variance (ANOVA), followed by a least significant difference (LSD) calculation. Correlation and stepwise regression analyses were used to identify the relationship between environmental variables and GHG fluxes. All significances mentioned in the text were at the 0.05 level. All statistical analyses were completed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Environmental Variables

Details of the environmental variables at the study site can be found in Figure 1. In the alpine grassland, air temperature (AT), soil temperature (ST_0 cm and ST_10 cm), soil moisture (SM_10 cm) and precipitation showed seasonal patterns. The daily mean AT of the growing season, the non-growing season, the spring thawing period and the whole experimental period were 7.8 °C, −8.0 °C, −0.9 °C and 0.5 °C, respectively (Figure 1a). Surface soil was readily influenced by strong solar radiation, which explained the strong daily fluctuations of ST_0 cm. The mean value of the daily maximum ST_0 cm was over 25.0 °C and the mean value of the daily minimum ST_0 cm was

−5.1 °C, for the whole experimental period. Although the bulk soil was frozen, the daily maximum ST_0 cm was frequently higher than 0 °C during the non-growing season, with a mean value at 19.9 °C (Figure 1b). Compared with AT and ST_0 cm, the ST_10 cm was relatively stable throughout the experimental period with a daily mean value of 4.6 °C. The daily mean values of ST_10 cm were 9.1 °C, −0.5 °C and 0.0 °C for the growing season, the non-growing season and the spring thawing period, respectively (Figure 1c). The daily mean SM_10 cm of the whole experimental period was 22.7%, 15.4% during the non-growing season, 13.6% during the spring thawing period, reaching 29.1% during the growing season (Figure 1d). During the whole experimental period, the total precipitation was 669.0 mm. During the non-growing season, the total precipitation, over nine months, was 56.3 mm, contributing to 13.7% of the annual total precipitation (from July 2011 to June 2012) (Figure 1e).

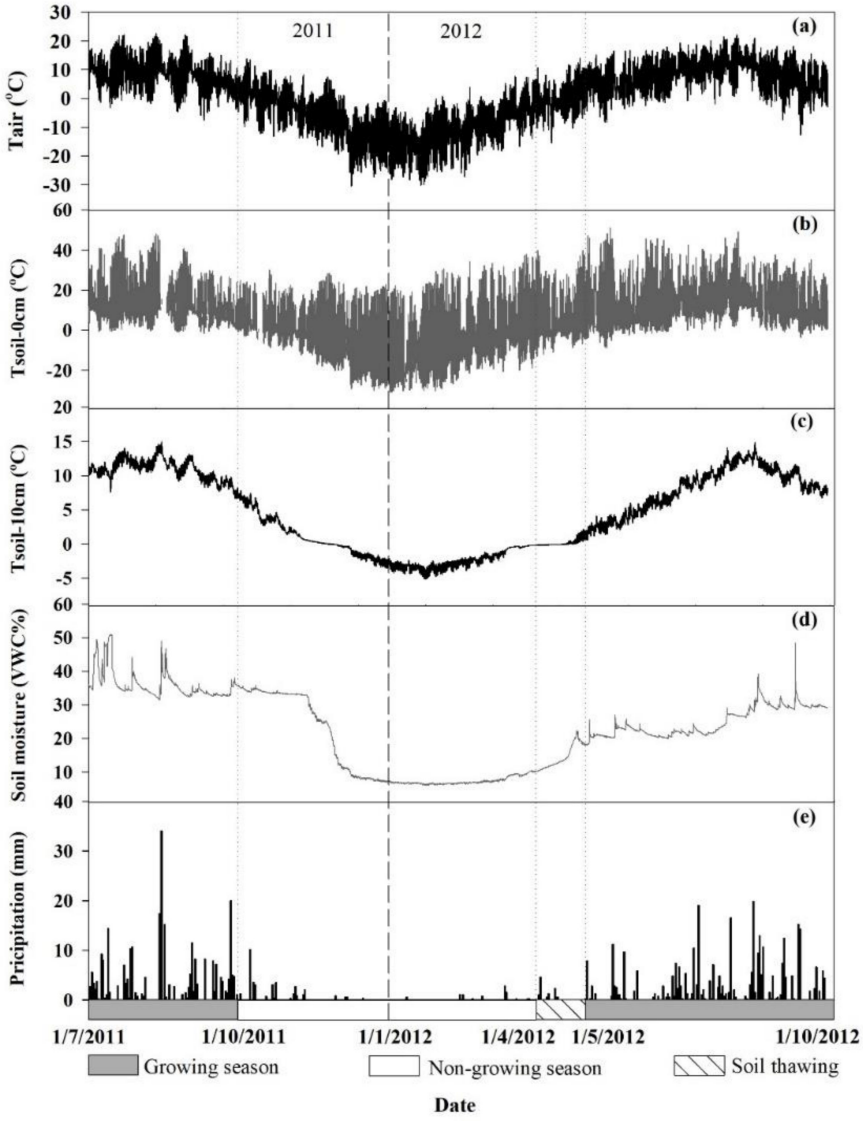

Figure 1. Mean of (**a**) daily air temperature (AT), (**b**) daily surface soil temperature (ST_0 cm), (**c**) daily soil temperature at 10 cm depth (ST_10 cm), (**d**) soil moisture at 10 cm depth (SM_10 cm) and (**e**) annual precipitation during different seasons.

3.2. CO_2 Emission

The CO_2 flux resulting from nutrient addition treatments had a trend similar to that of the control (Figure 2a). For all treatments, CO_2 flux peaks were recorded during late July and ranged from 998.1 mg m^{-2} h^{-1} to 1220.1 mg m^{-2} h^{-1}. Minimums occurred from early January to the middle of

February of the non-growing season, with values less than 100 mg m^{-2} h^{-1}. The CO_2 flux resulting from NP addition was greater than that resulting from N addition, P addition and control for all seasons (all $p < 0.01$). However, compared to the control for all seasons, there was no indication that N addition significantly increased the flux of CO_2 but P addition did. Repeated measures ANOVA analysis indicated that nutrient addition significantly affected the flux of CO_2 in different seasons but no significant influence of NP addition was observed in this alpine grassland, neither during the whole experimental period, nor during the growing season (Table 2).

Table 2. The mean fluxes of CO_2, CH_4 and N_2O under the nutrient addition (N and P) during different experimental periods in the Tibetan alpine grassland (Mean ± SE, n = 3).

	Treatments	Different Time-Scale			
		GS	NSG	STP	TEP
CO_2 flux ($mg\ m^{-2}\ h^{-1}$)	Control	645.8 ± 33.1 ^{c}	89.3 ± 8.2 ^{c}	100.3 ± 6.4 ^{c}	431.8 ± 32.5 ^{c}
	N	699.9 ± 33.3 ^{bc}	91.5 ± 8.0 ^{c}	98.4 ± 4.4 ^{c}	465.9 ± 34.4 ^{bc}
	P	740.6 ± 35.1 ^{b}	101.6 ± 8.2 ^{b}	114.8 ± 6.2 ^{b}	494.9 ± 36.2 ^{b}
	NP	818.2 ± 34.0 ^{a}	121.6 ±10.8 ^{a}	130.3 ± 6.6 ^{a}	550.3 ± 37.4 ^{a}
CH_4 flux ($\mu g\ m^{-2}\ h^{-1}$)	Control	−33.6 ± 1.7 ^{a}	−14.6 ± 4.1 ^{a}	−11.6 ± 6.4 ^{a}	−26.3 ± 2.1 ^{b}
	N	−30.0 ± 1.6 ^{a}	−13.6 ± 5.1 ^{a}	−9.2 ± 8.1 ^{a}	−23.7 ± 2.3 ^{ab}
	P	−29.8 ± 1.6 ^{a}	−4.8 ± 4.9 ^{a}	0.2 ± 7.8 ^{a}	−20.2 ± 2.4 ^{ab}
	NP	−28.2 ± 1.6 ^{a}	−5.0 ± 6.1 ^{a}	3.7 ± 9.6 ^{a}	−19.3 ± 2.7 ^{a}
N_2O flux ($\mu g\ m^{-2}\ h^{-1}$)	Control	7.5 ± 1.0 ^{c}	8.0 ± 1.7 ^{b}	9.5 ± 2.7 ^{b}	7.7 ± 1.4 ^{b}
	N	27.0 ± 2.9 ^{b}	28.7 ± 3.0 ^{a}	31.8 ± 3.7 ^{a}	27.6 ± 3.1 ^{a}
	P	10.3 ± 0.9 ^{c}	10.8 ± 1.9 ^{b}	12.3 ± 2.8 ^{b}	10.5 ± 1.4 ^{b}
	NP	37.4 ± 4.0 ^{a}	26.2 ± 3.0 ^{a}	34.3 ± 3.5 ^{a}	33.1 ± 3.8 ^{a}
***p*-Values of RM-ANOVA**					
CO_2 flux	Nitrogen (N)	**0.012**	**0.005**	**0.042**	**0.008**
	Phosphorus (P)	**0.001**	**<0.001**	**<0.001**	**<0.001**
	Date (D)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	N × D	**0.003**	**<0.001**	**0.023**	**<0.001**
	P × D	**0.044**	0.19	0.14	**<0.001**
	N × P	0.58	**0.016**	**0.015**	0.43
	N × P × D	**<0.001**	**0.01**	**0.019**	**<0.001**
CH_4 flux	Nitrogen (N)	0.19	0.92	0.69	0.38
	Phosphorus (P)	0.16	0.06	0.084	0.024
	Date (D)	**<0.001**	**0.018**	0.37	**<0.001**
	N × D	**0.005**	0.69	0.66	0.52
	P × D	0.057	**<0.001**	**0.001**	**<0.001**
	N × P	0.59	0.89	0.9	0.67
	N × P × D	0.051	**0.001**	**0.004**	**<0.001**
N_2O flux	Nitrogen (N)	**<0.001**	**<0.001**	**0.001**	**<0.001**
	Phosphorus (P)	**0.001**	0.95	0.57	**0.007**
	Date (D)	**<0.001**	**<0.001**	**0.006**	**<0.001**
	N × D	**<0.001**	**<0.001**	**0.010**	**<0.001**
	P × D	**<0.001**	**<0.001**	**0.001**	**<0.001**
	N × P	0.068	0.37	0.97	0.28
	N × P × D	**0.01**	**0.011**	0.13	**<0.001**

Date: sampling date; Nitrogen: nitrogen addition treatment; Phosphorus: phosphorus addition treatment; N × D: the interaction of nitrogen and date; P × D: the interaction of phosphorus and date; N × P: the interaction of NP co-addition treatment; N × P × D: the interaction of nitrogen, phosphorus and date. GS: growing season; NGS: non-growing season; STP: spring thawing period; TEP: the whole experimental period. Different letters denote significant different ($p < 0.05$) between treatments by LSD; Boldface *p*-value: the *p* value < 0.05.

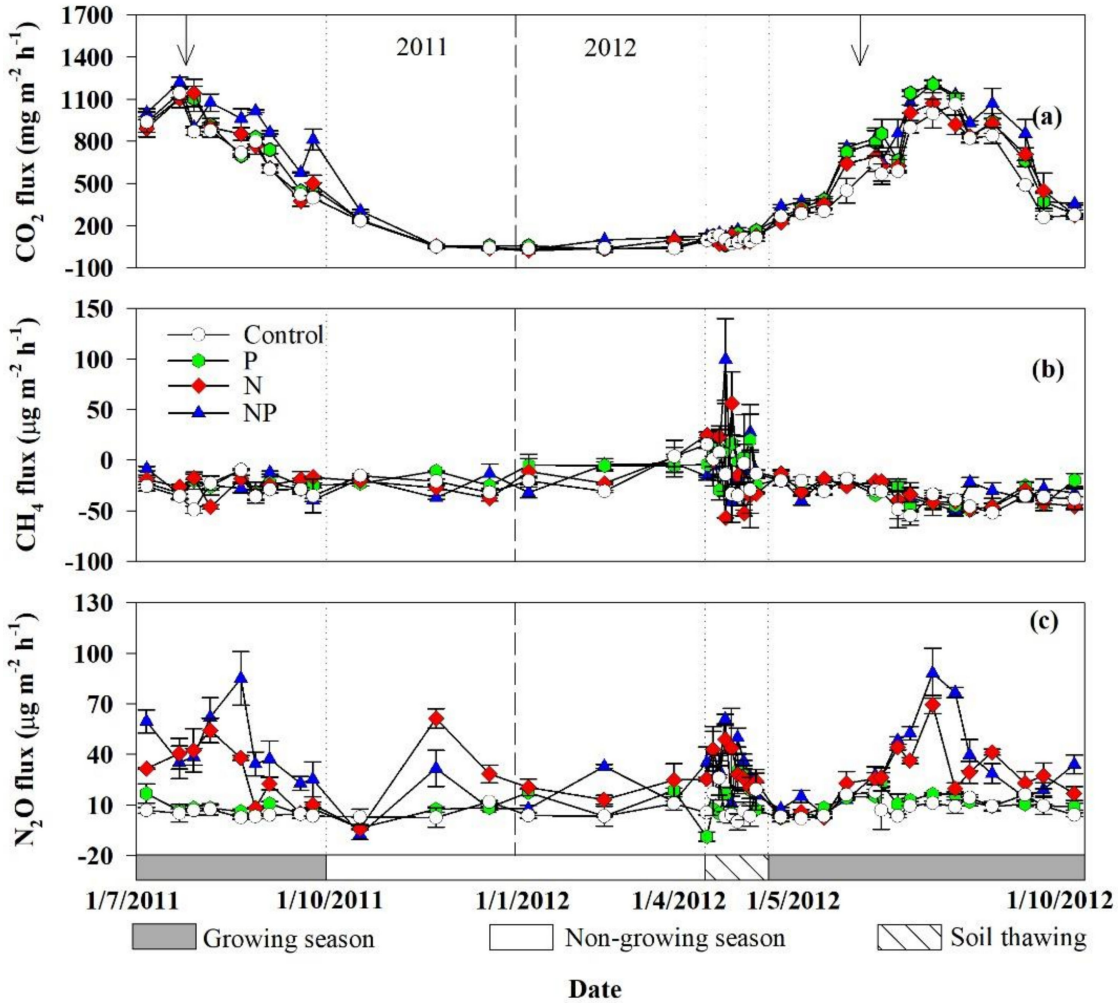

Figure 2. Trends of (**a**) CO_2, (**b**) CH_4 and (**c**) N_2O fluxes in Tibetan alpine grassland under nutrient addition (nitrogen and phosphorus) from 6 July 2011 to 30 September 2012. Bars indicate mean ± 1SE, arrows in the figures show the fertilizer date.

During the growing season, the cumulative CO_2 emission accounted for 82.8%, 82.4%, 82.9% and 80.0% of the annual emission under control, N, P and NP addition treatments, respectively (Figure 3). The spring thawing period only contributed to the cumulative CO_2 emission of the non-growing season by 10%, without any significant variation with time. The one-way ANOVA showed that there was no significant difference in the cumulative CO_2 emission between N addition and control, while P addition and NP addition significantly increased the cumulative CO_2 emission for all seasons compared with the control (Table 3), reaching 13.3%, 12.2%, 14.5% and 13.1% and by the addition of NP, reaching 28.2%, 54.1%, 31.4% and 32.7%, for the growing season, the non-growing season, the spring thawing period and the whole experimental period, respectively.

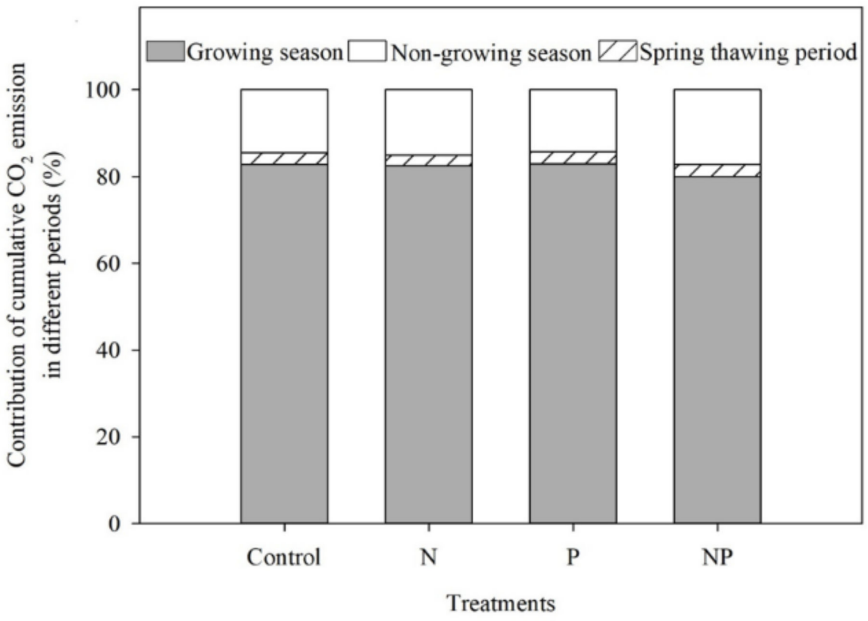

Figure 3. The contribution of growing season, non-growing season and spring thawing period on annual cumulative CO_2 emission (the annual means from 6 July 2011 to 6 July 2012), CK: control; N: nitrogen addition treatment; P: phosphorus addition treatment; NP: NP co-addition treatment.

Table 3. The mean cumulative CO_2, CH_4 and N_2O emissions under nutrient addition (N and P) treatments during different experimental periods in the Tibetan alpine grassland (Mean ± SE, n = 3).

	Treatments	Different Time-Scale			
		GS	NGS	STP	TWY
Cumulative CO_2 emission (kg ha^{-1})	Control	21,845.2 ± 850.5 ^{c}	4537.4 ± 33.6 ^{b}	720.5 ± 7.1 ^{c}	26,382.5 ± 884.0 ^{c}
	N	23,495.0 ± 968.4 ^{bc}	5016.3 ± 227.3 ^{b}	717.9 ± 17.4 ^{c}	28,511.3 ± 1172.2 ^{bc}
	P	24,740.5 ± 239.5 ^{b}	5092.1 ± 37.8 ^{b}	824.8 ± 19.1 ^{b}	29,832.6 ± 256.8 ^{b}
	NP	28,008.7 ± 843.8 ^{a}	6992.6 ± 292.7 ^{a}	946.5 ± 30.6 ^{a}	35,001.4 ± 925.7 ^{a}
Cumulative CH_4 emission (g ha^{-1})	Control	−1001.2 ± 68.5 ^{a}	−926.1 ± 63.9 ^{a}	−68.8 ± 19.9 ^{a}	−1927.3 ± 101.2 ^{a}
	N	−899.5 ± 44.7 ^{a}	−861.5 ± 169.1 ^{a}	−54.2 ± 23.3 ^{a}	−1761.0 ± 208.5 ^{a}
	P	−905.1 ± 43.5 ^{a}	−539.4 ± 71.6 ^{a}	−3.9 ± 75.2 ^{a}	−1444.5 ± 71.0 ^{a}
	NP	−892.9 ± 89.6 ^{a}	−822.3 ± 167.9 ^{a}	14.4 ± 33.6 ^{a}	−1715.2 ± 180.4 ^{a}
Cumulative N_2O emission (g ha^{-1})	Control	239.5 ± 35.1 ^{c}	301.1 ± 34.5 ^{c}	66.7 ± 7.2 ^{b}	540.7 ± 69.6 ^{b}
	N	854.1 ± 36.6 ^{b}	1252.5 ± 101.3 ^{a}	223.1 ± 55.2 ^{a}	2106.6 ± 118.5 ^{a}
	P	325.5 ± 26.1 ^{c}	415.3 ± 48.1 ^{c}	78.2 ± 9.3 ^{b}	740.8 ± 70.9 ^{b}
	NP	1199.4 ± 62.5 ^{a}	956.9 ± 126.7 ^{b}	242.1 ± 20.6 ^{a}	2156.3 ± 92.8 ^{a}

N: nitrogen addition treatment; P: phosphorus addition treatment; NP: NP co-addition treatment. GS: growing season; NGS: non-growing season; STP: spring thawing period; TWY: the whole year (6 July 2011 to 5 July 2012). Different small letters in a column denote significant different ($p < 0.05$) between treatments by LSD.

Carbon dioxide emission was significantly positively correlated with AT and ST_10 cm during all monitored seasons, with the exception of the spring thawing period. No significant correlation between CO_2 emission and SM_10 cm was observed during the growing season (Table 4).

Table 4. The relationship between air temperature (AT), soil temperature/moisture (ST_10 cm and SM_10 cm) at 10 cm depth with GHG (CO_2, CH_4 and N_2O) emissions at different nutrient addition (N and P) treatments of different experimental periods in the Tibetan alpine grassland.

	Treatments	GS			NGS			STP			TEP		
		AT	ST	SM	AT	ST	SM	AT	ST	SM	AT	ST	SM
CO_2 flux ($mg\ m^{-2}\ h^{-1}$)	Control	**0.72** ***	**0.85** ***	0.009	**0.58** *	**0.71** **	na	−0.30	−0.18	na	**0.76** ***	**0.89** ***	na
	N	**0.74** ***	**0.86** ***	0.037	**0.67** **	**0.78** **	na	0.22	0.35	na	**0.78** ***	**0.90** ***	na
	P	**0.76** ***	**0.78** ***	−0.071	**0.66** **	**0.77** **	na	0.60	0.66	na	**0.78** ***	**0.89** ***	na
	NP	**0.70** ***	**0.84** ***	−0.035	**0.66** **	**0.73** **	na	0.32	0.22	na	**0.78** ***	**0.91** ***	na
CH_4 flux ($\mu g\ m^{-2}\ h^{-1}$)	Control	−0.068	−0.14	−0.32	0.19	0.15	na	−0.55	−0.55	na	**−0.36** *	**−0.51** ***	na
	N	0.041	−0.16	−0.23	−0.005	−0.041	na	−0.49	−0.46	na	−0.26	**−0.36** *	na
	P	−0.081	−0.15	−0.078	0.17	0.049	na	−0.087	−0.081	na	**−0.47** **	**−0.64** ***	na
	NP	−0.038	−0.12	0.009	0.25	0.14	na	−0.058	−0.099	na	−0.22	**−0.39** *	na
N_2O flux ($\mu g\ m^{-2}\ h^{-1}$)	Control	0.14	0.17	−0.12	0.075	0.068	na	−0.30	−0.084	na	0.036	0.011	na
	N	**0.70** **	**0.60** **	−0.059	−0.063	−0.041	na	−0.64	−0.55	na	0.15	0.098	na
	P	**0.59** **	0.26	−0.37	0.12	−0.022	na	0.52	0.40	na	0.15	0.004	na
	NP	**0.77** ***	**0.71** ***	−0.17	0.23	0.054	na	−0.17	−0.41	na	**0.48** **	**0.41** *	na

N: nitrogen addition treatment; P: phosphorus addition treatment; NP: NP co-addition treatment. GS: growing season; NGS: non-growing season; STP: spring thawing period; TEP: the whole experimental period. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, the significance levels are shown in bold and no asterisk denotes no significant effect was found, na mean missing value.

3.3. CH_4 Uptake

With the exception of the spring thawing period, all treatments were revealed to be significant sinks for CH_4 throughout the experiment (Figure 2b). Indeed, Figure 2b showed large variability in patterns of CH_4 dynamics in the spring thawing period and no significant peak was observed. Nutrient addition significantly influenced the CH_4 accumulation in soil throughout the experimental period, but there was no influence of combined NP addition (Table 2). Methane accumulation capacities corresponding to NP addition was not only less than that of N addition and P addition treatments, but also significantly less than that of the control during the whole observation period. During the growing season, the non-growing season and the spring thawing period, the CH_4 flux decreased under nutrients addition treatments. However, regarding the CH_4 flux no significant difference is observed between nutrient addition treatments and the control for the same periods.

Compared with the control, nutrient addition did not significantly affect the cumulative CH_4 sink in any of the investigated periods (Table 3). The contributions of cumulative CH_4 flux from the non-growing season to the annual cumulative CH_4 budget were 48.5%, 48.9%, 37.3% and 47.9% for control, N, P and NP addition treatments in the whole year, respectively (Figure 4). About 50% of the cumulative CH_4 flux corresponds to the non-growing season, although the spring thawing period contributed to a smaller extent. Combined NP addition treatment resulted in a negative contribution regarding the cumulative CH_4 uptake during the non-growing season.

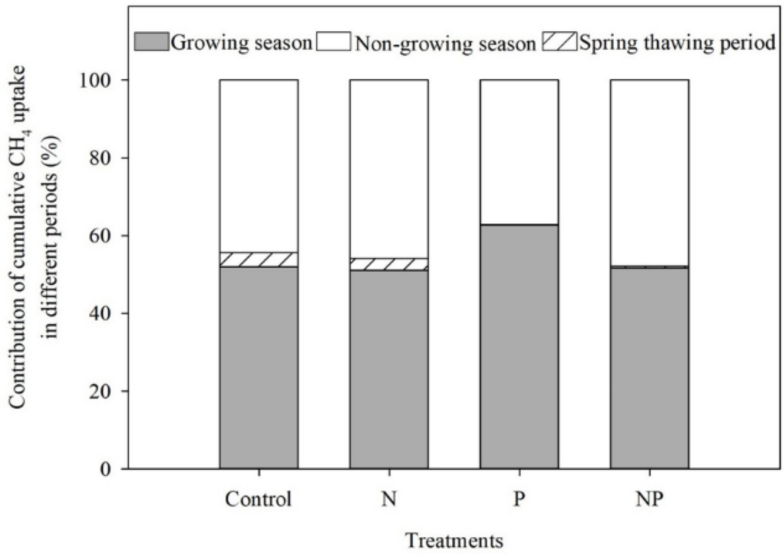

Figure 4. The contribution of growing season, non-growing season and spring thawing period on annual cumulative CH_4 emission (the annual means from 6 July 2011 to 6 July 2012), CK: control; N: nitrogen addition treatment; P: phosphorus addition treatment; NP: NP co-addition treatment.

Air temperature was negatively correlated to the CH_4 uptake with P addition and control treatments. Throughout the year, changes observed along the ST_10 cm profile significantly influenced the CH_4 uptake, regardless of which nutrients were added (Table 4).

3.4. N_2O Emission

Positive N_2O fluxes were observed for all treatments over the whole experimental period, except for a couple of occasions under NP addition treatment on 18 October 2011 and under P addition treatment on 4 April 2012 (Figure 2c). Indeed, the flux of N_2O showed large variations over time under N addition and NP addition treatments and several N_2O peaks were observed in the whole experimental period. Compared to N addition and NP addition treatments, N_2O flux corresponding to the P addition treatment had almost the same pattern as the control. Compared with the control, the

emissions of N_2O under N addition and NP addition were significantly greater for the whole study period. Nitrous oxide flux under NP addition was, however, significantly greater than that recorded under N addition in the growing season only. Repeated measures ANOVA analysis indicated that the N_2O flux was significantly influenced by the addition of N for all seasons, but also by P addition during the whole experimental period and especially during the growing season (Table 2).

Compared with the control, the addition of N and NP significantly stimulated the cumulative N_2O emission by more than 3 orders of magnitude, while an increasing trend was observed under P addition during the whole experimental period (Table 3). The contribution of the non-growing season to cumulative N_2O emissions over the whole season budget reached very high levels (55.7% for the control, 59.5% for N addition, 56.1% for P addition and 44.4% for NP addition), mostly resulting from the spring thawing period (Figure 5).

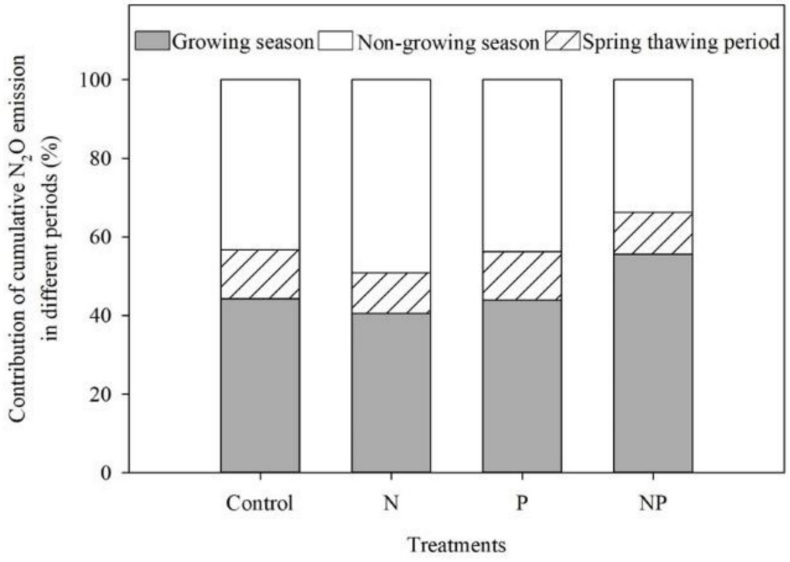

Figure 5. The contribution of growing season, non-growing season and spring thawing period on annual cumulative N_2O emission (the annual means from 6 July 2011 to 6 July 2012), CK: control; N: nitrogen addition treatment; P: phosphorus addition treatment; NP: NP co-addition treatment.

We found that N_2O emission and AT showed a positive correlation ($p < 0.05$) under nutrient addition treatments and a positive correlation with ST_10 cm under N addition and NP addition during the growing season. There was no correlation between N_2O emission and SM_10 cm in the growing season (Table 4).

4. Discussion

4.1. CO_2 Emission

During the growing season, the daily mean CO_2 flux reached about 700 mg m^{-2} h^{-1} in our study, which is consistent with the results of Cao et al. [24]. During the non-growing season, the CO_2 emission decreased and reached a minimum during the peak winter period (January/February 2012), while it started to increase with a rise in soil temperature. Although the seasonal pattern of CO_2 emission is similar to the observations made by Merbold et al. [25], the climate conditions of the alpine grassland in Tibet and of the subalpine grassland in the Swiss Alps that were studied by this group are entirely different. Merbold et al. [25] proposed that the soil temperature and snow cover were environmental factors that control the CO_2 exchange with the atmosphere. However, the Tibetan alpine grassland studied herein only has a low soil temperature without persistent snow cover during the non-growing season. Soil respiration represented the main CO_2 exchange between soil and the atmosphere in the non-growing season, due to the death of grass aboveground biomass. Soil

respiration consists of both autotrophic respiration and heterotrophic respiration. As the temperature decreases in the non-growing season, the autotrophic respiration (root respiration and rhizospheric respiration) would decline along with it. The CO_2 produced by soil microorganism metabolism was the heterotrophic respiration, which had positive feedback to soil temperature. Therefore, it is possible that soil temperature is the major environmental factor influencing the actives of grass roots and microorganism metabolism to control the CO_2 exchange in this region during the non-growing season. This is consistent with the fact that the CO_2 flux of Tibetan alpine grasslands are more sensitive to global warming [7,26].

It has been reported that N deposition decreases the emission of CO_2 [7,27]. However, this present study showed that the addition of N increased CO_2 emission, which is consistent with the reports of Li et al. [28] who found significant increasing CO_2 emission in response to N additions during the growing season in an alpine grassland. Two mechanisms can explain the observed increase of CO_2 emission after N addition at our experimental site: (1) more N was added during our experiment (100 kg ha^{-1} yr^{-1}) than in those of Jiang et al. (2010, 20 kg ha^{-1} yr^{-1}) and Burton et al. (2004, 30 kg ha^{-1} yr^{-1}), so that the concentration of N can meet the requirements of soil microbes; (2) more N addition led to shifts in plant species composition, favoring greater productivity and greater GHG emissions [29]. Elser et al. [5] found that N and P played important roles in plant growth since they are both essential nutrients for plants. Indeed, some studies have shown that P addition can increase the microbial (fungal and bacterial) biomass and induce changes in the soil microbial communities [30]. In this experiment, the data showed that the addition of P led to a rapid increase of CO_2 emission because P addition significantly increased autotrophic respiration and the ratio of autotrophic respiration to soil respiration and improved root nitrogen content and aboveground biomass [29]. Elser et al. [5] also reported that combined NP addition strongly induced positive synergistic responses in all ecosystems. However, we could not find a significant influence of combined NP addition on CO_2 emission in growing season with the present data. which may be because this is a P limited system and the added P has a larger impact on grassland aboveground biomass. The interaction of NP addition was significant only in non-growing season and thawing period (Table 2). It could be highlighted that N and P addition affected the heterotrophic respiration/autotrophic respiration components of soil respiration directly without aboveground respiration in the non-growing season as shown in previous research in the same experimental site [19].

The CO_2 emission recorded during the non-growing season significantly contributed to the annual CO_2 emission budgets [31]. For example, Grogan and Chapin Iii [32] reported an extremely large value for the non-growing season, contributing up to 52% of the annual total CO_2 emission. In heath tundra ecosystems, the non-growing season cumulative soil CO_2 emission accounts for 14–56% of the annual CO_2 emission [33,34]. Due to the inhibition of microbial activity by low temperature and aboveground-biomass death, the CO_2 emission of the ecosystem in the Tibetan alpine grassland could be very small [35]. Combined NP addition affected CO_2 flux (soil respiration) via different processes: N mainly affected heterotrophic respiration, whereas P largely influenced autotrophic respiration [19]. In addition, the interaction of NP addition was significant in the non-growing season (Table 2), which led to more CO_2 emission in the non-growing season with NP addition. Therefore, NP addition significantly increased the contribution ratio of the non-growing season to the annual cumulative CO_2 emission. However, the contribution of the non-growing season to the annual total CO_2 emission of the Tibetan alpine grassland is less than that of the annual contributions reported for the heath tundra ecosystems [16,34]. More specifically, in the long non-growing season, the Tibetan alpine grassland receives extremely low rates of precipitation and rarely has persistent snowpack.

4.2. CH_4 Uptake

During the spring thawing period, CH_4 flux did show large variation, which could be due to variations of surface soil temperature and soil moisture since these parameters are critical in CH_4 emission or uptake [36]. Also, this emission pattern is significantly different from that reported by

Li et al. [28] in an alpine grassland of the Tianshan Mountains, China. However, compared to the site studied by Li et al. [28], our experimental site has a higher elevation and unique hydrothermal conditions that are described above. These studies report a significant CH_4 sink and values comparable to the range of CH_4 flux in a typical semi-arid grassland in Inner Mongolia, China [37] and in a subalpine meadow in the Rocky Mountains, USA [38]. We found that nutrient addition could significantly inhibit the sink of CH_4 in this site over the whole season and that the observed differences may be due to a greater sensitivity of CH_4 oxidizing microbes to enhanced nutrient availability [39]. The possible mechanism of inhibition in CH_4 oxidation by N addition suggests that there may be multiple inhibition mechanisms: (1) NH_4^+ (or rather NH_3) can act as a competitive inhibitor of CH_4 oxidation due to the lack of specificity of methane monooxygenase (MMO) in methanotrophs; (2) the toxicity of hydroxylamine and nitrite produced by fortuitous NH_4^+ oxidation has been suggested to suppress methanotrophic activity; (3) occasionally, inhibition by NH_4^+ still occurs even though NH_4^+ concentration has decreased to background levels. In addition to the above mentioned inhibition mechanisms, the addition of P also had an inhibitory effect on CH_4 oxidation and this could be due to: (1) phosphate fertilization stimulating methanogenic activity in P-deficient soils, which supports our results for the spring thawing period [40]; (2) phosphate concentration in the rhizosphere is another factor which possibly regulates CH_4 production or oxidation [41]. Saari et al. [39] also reported that the inhibition of CH_4 oxidation by nitrogenous and non-nitrogenous salts mainly resulted from a general salt effect (osmotic stress) though NH_4^+ did have some additional inhibitory properties. Therefore, the interaction of NP addition may indeed cause the inhibition of CH_4 oxidation. We found no evidence supporting the fact that nutrient addition had a significant influence on CH_4 uptake in the growing season, the non-growing season and the spring thawing period.

Song et al. [18] demonstrated that the greater CH_4 flux in summer was mainly induced by the ice and snow thaw in spring. and Mastepanov et al. [42] reported that the integral CH_4 flux during the freezing period was approximately equal to the amount of CH_4 emitted during the entire summer season. We also detected greater cumulative CH_4 emission values during the non-growing season for all treatments at this site, but we found no CH_4 uptake or emission burst in the spring thawing season. This may be attributed to the fact that there was no snow cover at this site and we saw large diurnal fluctuation in soil temperature during the non-growing season, which could have led to a consistent CH_4 emission throughout the winter. The proportion of the non-growing season on the cumulative CH_4 emission accounted for nearly half of the annual emissions, emphasizing the important contribution of the non-growing season. This is consistent with the results of our previous studies in an alpine wetland [17]. Future research should concentrate on the mechanisms of methane oxidation in this region of low temperature conditions.

The sink of CH_4 measured in this study correlated with AT and ST_10 cm over the year but showed no strong relationship with soil moisture during the growing season. This indicates that CH_4 consumption in the soils of the alpine grassland might be more sensitive to changes in global temperature as well.

4.3. N_2O Emission

Mean nitrous oxide flux rates from this region were higher than those from bare patches on alpine steppe soils (0.8 $\mu g\ m^{-2}\ h^{-1}$) and on temperate steppe soils (5.9 $\mu g\ m^{-2}\ h^{-1}$) [43,44]. The great difference in N_2O flux between these grasslands may be caused by the greater moisture in alpine grassland soils than in alpine steppe soils or temperate steppe soils. However, the daily mean N_2O flux was smaller than that in the agricultural soils of the Northern China Plain (43.1 $\mu g\ m^{-2}\ h^{-1}$) [45], indicating that in the latter, large N fertilizer inputs may have enhanced the emissions of N_2O from agricultural soils by stimulating microbial growth and activity and promoting nitrification and incomplete denitrification. We found that P addition increased N_2O emission, albeit moderately. In this P limited system, it is possible that P-enrichment stimulates the activity of denitrifier and nitrifier colonies, along with other microbes within the soil, enhancing the N_2O emission from

soils by stimulating conversion of ammonium to nitrate and providing substrate for nitrous oxide production [46].

In temperate ecosystems, particular attention has focused on the N_2O emission of the non-growing season, because much of the annual cumulative emission appears to occur during this season and during the transition from winter to spring, when freeze-thaw events are common [47]. Our results suggest that in the non-growing season, N_2O emission from the alpine grassland on the plateau contributes >40% (expect NP addition treatment) to the annual budget and a greater contribution of N_2O emissions during the spring thawing period accounts for more than 10% of the annual budget. It could be because that N_2O emission affected by the variations of surface soil temperature and soil moisture. The mean of daily maximum surface soil temperature was over 25 °C in non-growing season, caused thin surface soil freezing and thawing frequently and enhanced soil microbial activity, led to more N_2O produced [19,30]. So we observed the higher contribution of the non-growing season to the annual budget, compared with in the alpine grassland of the Tianshan Mountains, China [28]. However, we saw no evidence of N_2O emission bursts in the spring thawing season. Seasonal patterns of the N_2O emission ratio in Tibetan alpine grassland were shown to be entirely different from continental steppes [19], marshes [48] and agricultural systems [49,50]. Moreover, it was shown to be different in another alpine grassland in the southern Tianshan Mountains, China [51]. Again, this confirms the uniqueness of the Tibetan plateau. In addition, nutrient addition shows decreasing trends for the cumulative N_2O emission proportion ratio of the spring thawing season. Compared with the control, while N addition significantly decreases the cumulative N_2O emission proportion ratio of the growing season, NP addition significantly increases the ratio. These results also confirmed that N_2O production is a very complex process mainly taking place through nitrification-denitrification processes, which depend on freezing-thawing processes [52], soil temperature/moisture [53], N-inputs [7,54], soil types [23,55] and microbial processes [56].

In this study, we found that in the growing season, temperature variations (AT and ST_10 cm) significantly correlate with N_2O emission under nutrient addition, contrarily to soil moisture (SM_10 cm). We inferred that nutrient addition can improve the response of N_2O emission to temperature variations (AT and ST_10 cm).

5. Conclusions

Nutrient addition significantly influenced the fluxes and emissions of CO_2, CH_4 and N_2O from soil. The cumulative CO_2 emission of the growing season dominated the total annual budget, however there were significant contributions from the non-growing season also. The nutrient addition tended to inhibit the flux of CH_4 for all periods and P addition significantly altered the sink or source patterns of CH_4 during the spring thawing period, meaning that the fertilized alpine grassland was a limited source of CH_4. Only Nitrogen addition and NP addition significantly increased the emission of N_2O during all periods. Focus should be made on the emission of CH_4 and N_2O outside the growing season because the cumulative emission of the non-growing season was nearly half of and a critical contributor to the annual budget. The contribution of the spring thawing period to the total annual CH_4 budget was very limited. While, the contribution of the cumulative N_2O emission during the spring thawing period to the annual budget was moderate. It is suggested that the lack of snow cover in the non-growing season and consequent large variation in diurnal soil temperature is a bigger factor in annual budgets of GHG emissions in this system. While we see no influence of soil moisture on the emissions of the three greenhouse gases reported here, the air and soil temperatures seem to be of great significance on the emission of these GHG. These results highlight the fact that temperature is a determinant environmental factor controlling the emission of GHG in the Tibetan alpine grassland and therefore climate change is likely to have a large impact on this biome as a source of further GHG emissions.

Author Contributions: Z.Z. and G.W. conceived and designed the experiments; G.W., F.R. and X.Y. performed the experiments; Z.M. and Y.G. analyzed the data; G.W. and Y.L. wrote the paper; T.G. revised the manuscript.

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