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Temporal Variability in Soil Greenhouse Gas Fluxes and Influencing Factors of a Primary Forest on the Eastern Qinghai-Tibetan Plateau

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Abstract: Soil greenhouse gas (GHG) fluxes relate to soil carbon and nitrogen budgets and have a significant impact on climate change. Nevertheless, the temporal variation and magnitude of the fluxes of all three major GHGs (CO_2 , CH_4 and N_2O) and their influencing factors have not been elucidated clearly in primary forests on the eastern Qinghai-Tibetan Plateau. Herein, field chamber GHG fluxes from May to November, soil microbial community and enzyme activity were analyzed in a fir-dominated (Abies fargesii var. faxoniana) primary forest. The emission rates of CO₂ and N_2O ranged between 64.69–243.22 mg CO_2 m⁻² h⁻¹ and 1.69–5.46 ug N_2O m⁻² h⁻¹, exhibiting a temporally unimodal pattern with a peak in July. The soil acted as a CH_4 sink, and the uptake rate varied between 52.96 and 84.67 μ g CH₄ m⁻² h⁻¹ with the higher uptake rates in June and November. The temporal variation in the CO_2 flux was significantly correlated with the geometric mean of enzyme activities, suggesting that the soil CO₂ flux was determined by microbial activity rather than soil microbial biomass. The soil N2O flux was positively related to nitrate concentration with marginal significance, probably because N₂O was a byproduct of nitrification and denitrification processes. The soil CH_4 uptake was closely associated with methanotrophic biomass (18:1 ω 7c). The results highlight divergent temporal dynamics of GHG fluxes owing to different driving mechanisms and an important CH₄ sink in the primary forest soil, helping to evaluate the carbon and nitrogen budgets of primary forests on the eastern Qinghai-Tibetan Plateau.

Keywords: greenhouse gas; soil microbial biomass; enzyme activity; primary forest; eastern Qinghai-Tibetan Plateau

1. Introduction

Primary forests are an important component of the terrestrial biosphere and play an irreplaceable role in mitigating climate change; sustaining biodiversity, especially of imperiled and endemic species [1,2]; and providing economic benefits and biocultural value. Furthermore, primary forests are more resilient to climate change [3] that may enable them to better adapt to global changes. There is much evidence to highlight an indispensable role of primary forests in terms of climate warming mitigation because they harbor denser carbon (C) stocks [4] and continuing C accumulation to function as C sinks [5]. However, some case studies point out that primary forests could act as C sources [6,7], and the C sinks or sources are related to season [7].

Soil C is one of the most important C pools in primary forest ecosystems and constitutes a predominant component of the C cycle [8]. Soil carbon dioxide (CO₂) effluxes,



Citation: Liu, S.; Luo, D.; Xu, G.; Wu, J.; Feng, Q.; Shi, Z. Temporal Variability in Soil Greenhouse Gas Fluxes and Influencing Factors of a Primary Forest on the Eastern Qinghai-Tibetan Plateau. *Forests* **2023**, *14*, 2255. https://doi.org/10.3390/ f14112255

Academic Editor: Luca Belelli Marchesini

Received: 20 October 2023 Revised: 13 November 2023 Accepted: 14 November 2023 Published: 16 November 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). resulting from root and microbial respiration, serve as a primary pathway for soil carbon loss and contribute significantly to atmospheric CO_2 in forest ecosystems [9]. They have been observed to account for as much as 80% of the overall ecosystem respiration [10], making effluxes a key role in the ecosystem C budget. Generally, soil CO_2 effluxes vary with time, and quantifying temporal variations in soil CO_2 effluxes and identifying their major environmental and biotic drivers in primary forests are essential for comprehending the relative contribution of soil CO_2 effluxes to ecosystem C budgets. Furthermore, this knowledge is vital for predicting the C sink/source status in the context of climate change [11].

Apart from CO₂, methane (CH₄) and nitrous oxide (N₂O) are the two other important greenhouse gases (GHGs) that contribute to global warming [12]. Although atmospheric concentrations of CH₄ and N₂O are much lower, their respective warming potentials are 34- and 298-fold compared to CO₂ [13]. Therefore, an integrated assessment of these GHG fluxes in primary forests is key to understanding the C budget and to gauging their importance for climate mitigation [14]. Previous studies showed that forest soils are generally investigated as net sources of N₂O and net sinks of CH₄ for the atmosphere [15]. Nevertheless, N₂O uptakes and CH₄ emissions from forest soils have also been reported [16] at least temporarily [17,18]. This highlights that investigating the temporal dynamics of soil CH₄ and N₂O fluxes and their driving factors cannot be overlooked when assessing the role of primary forests in regulating climate change.

Climatic conditions, particularly soil temperature and moisture, are acknowledged as the primary factors controlling the seasonal patterns of soil GHG fluxes [19,20]. Higher soil temperatures could increase soil microbial biomass and enzyme activity, accelerate organic matter decomposition and promote root respiration, thus enhancing soil CO₂ effluxes [21]. An exponential relationship between soil CO₂ fluxes and soil temperature was observed in most studies; however, the temporal relationships between CH₄ and N₂O fluxes with soil temperature are inconsistent [14,17]. This may be due to the complex biological mechanisms of CH₄ and N₂O fluxes. N₂O is a by-product of nitrification and denitrification processes under aerobic and anaerobic conditions, respectively [22,23]. These processes determine soil mineral nitrogen (N) levels, affecting soil N availability and microbial methanogens and methanotrophs, which are related to CH₄ production and consumption, respectively [24]. Soil moisture might affect its aeration and soil microbial community, further altering the direction and magnitude of CH₄ and N₂O fluxes [23,25].

Primary dark coniferous forests are native forest types in the subalpine region of the eastern Qinghai-Tibetan Plateau. They play crucial ecological functions in maintaining the security of the upper reaches of the Yangtze River and in regulating regional climate change. Although there have been studies focusing on soil CO₂ fluxes in primary forests on the eastern Qinghai-Tibetan Plateau [21], to date, the research pertaining to CH₄ and N₂O fluxes is comparatively limited. The main factors affecting the temporal variability of GHG fluxes in primary forests on the eastern Qinghai-Tibetan on the eastern Qinghai-Tibetan Plateau are still unclear.

In this study, we investigated soil GHG fluxes in a primary forest on the eastern Qinghai-Tibetan Plateau from May to November and linked the GHG fluxes to climatic variables, soil microbial community composition and extracellular enzyme activity. The specific objectives of the present research were to quantify the magnitude of CO_2 , CH_4 and N_2O fluxes and to investigate their temporal patterns and key influencing factors. We hypothesized that (I) soil acts as a non-negligible CH_4 sink and N_2O source in the primary forest, and (II) soil CO_2 , CH_4 and N_2O fluxes showed different temporal patterns due to divergent responses to climatic factors and microbial attributes. The investigations of temporal variability and influencing factors of soil GHG fluxes simultaneously are important for exactly parameterizing GHG fluxes and estimating C and N budgets in subalpine primary forest ecosystems on the eastern Qinghai-Tibetan Plateau.

2. Materials and Methods

2.1. Study Site

The study was carried out in a subalpine dark coniferous forest, which is one of the typical forest types in western Sichuan, eastern Qinghai-Tibetan Plateau, China. The site of the study was located at Bipenggou Nature Reserve ($31^{\circ}14'$ N- $31^{\circ}19'$ N, $102^{\circ}53'$ E- $102^{\circ}57'$ E) in Lixian county, west of Sichuan Province, which is one of the key areas of the ecological barrier on the upper reaches of the Yangtze River. This region has large areas of wellpreserved primary forests, making it a representative site for the study of C and N cycling in primary forests. The site belongs to the alpine gorge region, with elevation between 2458 and 4691 m above sea level (a.s.l.), and has a typical Qinghai-Tibetan Plateau climate. The average annual temperature is 2.7 °C with the mean monthly temperature ranging from -18 °C in January to 23 °C in July, and the mean annual rainfall is approximately 850 mm. The land surface experiences seasonal snow cover, generally starting from early December and ending in April of the following year [26], which is the main reason for the sampling period from May to November in this study.

Three replicate plots (each 20×20 m in size) were randomly selected in the primary forest ($31^{\circ}14'31''$ E, $102^{\circ}53'5''$ E, 3500 m a.s.l.). The plots are on a southeast-facing slope with a gradient of 35° . The primary forest was dominated by *Abies fargesii* var. *faxoniana* with mean DBH (diameter at breast height) and stem density being 39.81 cm and 366.7 tree ha⁻¹, respectively. The understory was mainly composed of *Rhododendron delavayi*, *Cerasus duclouxii* and *Rosa sweginzowii* in the shrub layer and of ferns *Carex* spp. and *Cyperus* spp. in the herb layer. The soil of the studied forest is categorized as Cambisols in the FAO World Reference Base (WRB) for Soil Resources with basic characteristics from 0 to 10 cm being: BD (bulk density) of 0.778 g cm⁻³, soil organic C of 33.73 g kg⁻¹ and total N of 2.44 g kg⁻¹.

2.2. Soil Greenhouse Gas Flux Measurements

Fluxes of soil GHG were monitored monthly from May to November using the static chamber method and the gas chromatography technique. The results of this method could be affected by meteorological conditions, sampling time and chamber size and are limited by the inability to make continuous observations. Nonetheless, it has the advantages of simplicity, economy, convenience and relatively high accuracy and has been widely used for the determination of soil GHG fluxes. In each plot, three collars (25 cm diameter, 10 cm height) were inserted permanently into the soil in November of the year before the measurement. During sampling, a 30 cm high portable opaque chamber installed with a fan to mix the air was attached to the PVC ring [27]. Gas sampling procedures were usually conducted between 9:00 a.m. and 10:00 a.m. when the fluxes were close to the daily average values [28]. Gas samples were collected with a 100 mL gas-tight syringe at 0, 15 and 30 min after the chamber closure through a silicon tube equipped to the chamber headspace [29]. Meanwhile, air temperature and pressure were measured inside the chambers with a portable instrument during gas sampling. Simultaneously, we measured soil temperature at the depth of 5 cm with temperature probes and the soil water-filled pore space (WFPS) by drying soil samples collected with standard containers near each chamber.

The GHG concentrations were determined within one week using a gas chromatograph possessing a thermal conductivity detector, a flame ionization detector and an electron capture detector for CO₂, CH₄ and N₂O, respectively (Agilent GC-7890A, Agilent, Santa Clara, CA, USA). The GHG fluxes were estimated by the linear regression analysis model using the GHG concentrations of the three samples collected at an interval of 15 min from each chamber [29,30]. Meanwhile, we calculated the cumulative emission/uptake of GHG by integrating the area under the curve using daily fluxes and time intervals as dependent and independent variables, respectively [19].

2.3. Soil Microbial Community and Extracellular Enzyme Activity

Soil samples (0–10 cm) were collected from five selected locations in each plot in June, August and November following the collections of GHG samples. Five soil samples from

each plot were homogenized to one composited sample and then sieved through a 2 mm mesh. The composited samples were rapidly transported to the laboratory in an icebox and stored at 4 $^{\circ}$ C to test the concentrations of NH₄⁺-N and NO₃⁻-N and to analyze the soil microbial parameters.

The soil microbial community was estimated by phospholipid fatty acids (PLFAs) as described by Bossio et al. [31]. PLFAs were categorized into various functional groups based on fatty acid biomarkers to characterize the soil microbial community structure. Total bacterial biomass was calculated as the sum of Gram-positive (i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0), Gram-negative (16:1 ω 7c, 18:1 ω 5c, 18:1 ω 7c, cy17:0 and cy19:0) and other general bacterial biomarkers, including 15:0 and 17:0 [32,33]. The PLFAs 18:1 ω 9c and 18:2 ω 6,9c were selected as fungal signature markers, while the PLFA 16:1 ω 5c was addressed as a signature marker for arbuscular mycorrhizal fungi (AMF). The fatty acid of 18:1 ω 7c was chose as a biomarker for methanotrophs [34]. The abundances of each individual fatty acids and functional groups were expressed as nmol per gram dry weight of soil. The ratios of Gram-positive/Gram-negative bacteria and fungi/bacteria were calculated to investigate temporal variations in soil microbial community compositions. In addition, the ratios of cyclopropyl PLFAs to their monoenoic precursors (abbreviated cy/pre ratio) and saturated-to-monounsaturated PLFAs (abbreviated Sat/Mono ratio) were calculated to investigate the physiological stress of the soil microbial community [31,35].

The activities of five soil enzymes, α -glucosidase (AG), β -glucosidase (BG), β -Nacetylglucosaminidase (NAG), leucine aminopeptidase (LAP) and acid phosphatase (AP), were measured by a microplate reader (SpectraMax i3x, Molecular Devices, Santa Clara, CA, USA). These were involved in the cycling of C (AG and BG), N (NAG and LAP) and P compounds (AP) [36]. The assays were performed following the procedures described by German et al. [37]. Enzyme activities were expressed in nmol g⁻¹ dry soil h⁻¹.

2.4. Statistical Analysis

The geometric mean of soil enzyme activities (GMea) was used to integrate soil enzyme activities [38] and was calculated as:

$$GMea = \sqrt[5]{AG \times BG \times NAG \times LAP \times AP}$$
(1)

One-way analysis of variance (one-way ANOVA) and Tukey's HSD (honestly difference test) were used to identify differences among the sampling months for soil N availability and soil microbial attributes. The relationships between GHG fluxes and influencing variables were performed using regression modeling analysis. The data were analyzed with the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA), while the figures were generated using the SigmaPlot 12.5 software (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Soil Microclimate and Greenhouse Gas Fluxes

Soil temperature showed a distinctly unimodal temporal variation with higher values in July (9.6 °C) and August (9.5 °C) and lower values in November (2.5 °C). Soil water-filled pore space varied smoothly within the study period (54.8%–62.5%, Figure 1a). Soil CO₂ flux varied between 64.69 and 243.22 mg CO₂ m⁻² h⁻¹, showing a unimodal pattern with higher and lower emission rates in July (243.22 mg CO₂ m⁻² h⁻¹) and November (64.69 mg CO₂ m⁻² h⁻¹), respectively (Figure 1b). Soil CH₄ flux was negative within the study period, indicating net CH₄ uptake. Soil CH₄ flux ranged from -84.67 to -52.96 µg CH₄ m⁻² h⁻¹, with the relatively lower values being observed in June and November (Figure 1c). N₂O flux gradually increased from May to July, then sharply decreased from July to August, and then changed smoothly (Figure 1d).

The cumulative CO₂ emission over the study period was 778.48 g CO₂ m⁻², while the total N₂O efflux was 12.40 mg N₂O m⁻². The soil consumed CH₄, with the cumulative CH₄ uptake being 286.31 mg CH₄ m⁻² throughout the study period (Figure 2).



Figure 1. Temporal dynamics of soil temperature and soil water-filled pore space (**a**) and fluxes of CO₂ (**b**), CH₄ (**c**) and N₂O (**d**) investigated from May to November in the primary forest. Data are means \pm standard errors (vertical bars; *n* = 3 at each case). Positive and negative values of the fluxes indicate soil emission and uptake, respectively.



Figure 2. Cumulative emission/uptake of greenhouse gas during the study period.

3.2. Soil N Availability

Soil NH₄⁺-N concentration in November was 1.64 mg kg⁻¹ and 6.01 mg kg⁻¹ higher than that in June and August, respectively. Soil NO₃⁻-N concentration exhibited higher values in August and lower values in November. Nonetheless, there were no significant monthly differences in NH₄⁺-N or NO₃⁻-N concentrations (Figure 3).



Figure 3. The changes in soil N availability (NH_4^+ -N and NO_3^- -N concentrations) in Jun (June), Aug (August) and Nov (November). *ns*, not significant (*p* > 0.05).

Soil total PLFA and bacterial (including G–) and fungal (including AMF) PLFA concentrations were highest in June and lowest in August. However, only the sampling month had significant effects on G–, fungal, AMF and methanotrophic PLFAs (Figure 4a). The fungi/bacteria ratios in June and November were significantly greater than in August. In contrast, significantly higher values of G+/G– ratio and Sat/Mono ratio were measured in August (Figure 4b). The PLFA concentration ratio of cy/pre did not differ significantly among sampling months (Figure 4b).



Figure 4. Temporal patterns of PLFA biomarker amounts for microbial functional groups (**a**) and ratios of microbial functional groups (**b**). Total, total microbial PLFAs; G+, gram-positive bacteria; G-, gram-negative bacteria; AMF, arbuscular mycorrhizal fungi; F/B, ratio of fungi to bacteria; cy/pre, ratio of cyclopropyl PLFAs to their precursors; Sat/Mono, ratio of normal saturated to monounsaturated PLFAs. *ns*, not significant (*p* > 0.05); * and *** indicate significance at *p* < 0.05 and *p* < 0.001 levels, respectively. Bars with different letters denote significant differences between months (*p* < 0.05). Error bars are standard errors.

3.4. Soil Enzyme Activity

The LAP activity was significantly higher in June, and there was no significant difference between August and November. AG activity was relatively high in June, while BG, NAG and AP activities were relatively high in August and low in November with no significant month effects. The geometric mean of enzyme activities did not vary significantly among sampling months, though it was relatively high in June and August (Table 1).

Table 1. Soil enzyme activities (nmol $g^{-1} h^{-1}$) and the geometric mean of soil enzyme activities (GMea) in the three sampling months.

Sampling Month	AG	BG	NAG	LAP	AP	GMea
June	11.20 ± 1.04	244.34 ± 60.48	114.91 ± 12.59	$89.96\pm3.40a$	883.65 ± 87.63	118.84 ± 18.09
August	10.45 ± 1.46	269.12 ± 40.48	146.97 ± 33.15	$51.22\pm4.57\mathrm{b}$	1098.54 ± 157.62	116.24 ± 18.29
November	9.44 ± 1.68	201.49 ± 7.00	81.40 ± 6.57	$64.69\pm5.84b$	673.83 ± 53.14	91.76 ± 8.74
One-way ANOVA						
F-value	0.39 ns	0.66 <i>ns</i>	2.48 ns	17.44 **	3.83 ns	2.72 ns

AG, α-glucosidase; BG, β-glucosidase; NAG, β-N-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, acid phosphatase. Different letters indicate significant different between sampling months. ** p < 0.01. *ns*, not significant (p > 0.05).

3.5. Key Factors Affecting Soil GHG Fluxes

An exponential relationship between CO_2 flux and soil temperature was found, with 85.9% of the temporal variation in CO_2 flux explained by soil temperature (Figure 5a). Temporal variations in N₂O flux were linearly related to soil temperature, with the contribution being 31.5% (Figure 5a). Temporal changes in CH₄ flux were not significantly correlated with soil temperature (Figure 5a). Moreover, no significant relationships between GHG fluxes and soil WFPS were found across the study period (Figure 5b).



Figure 5. Dependency of soil greenhouse gas fluxes on (**a**) soil temperature and (**b**) soil waterfilled pore space (WFPS). Each datapoint in the figures is the mean per plot at each sampling time. Regression line is only shown when significant (p < 0.05). The equations were: CO₂ flux = 47.64 e^{0.169} ST; N₂O flux = 0.30 ST + 0.64. ST, soil temperature.

The linear regression analysis showed that GMea had a significantly positive correlation with CO₂ flux and explained 67.4% of the temporal variation in CO₂ flux (p = 0.007, Figure 6a). N₂O flux was positively correlated with NO₃⁻ concentration with a marginal significance (p = 0.063, Figure 6b). Methanotrophic biomass was significantly and positively related to CH₄ uptake rate, explaining 53.9% of the variation (p = 0.024, Figure 6c).



Figure 6. The relationships (**a**) between soil CO_2 flux and the geometric mean of enzyme activities, (**b**) between N_2O flux and NO_3^- concentration and (**c**) between CH_4 uptake rate and biomass of methanotrophs indicated by 18:1 ω 7c.

4. Discussion

4.1. The Direction and Magnitude of GHG Fluxes

Soil CO₂ efflux rates of the primary forest ranged between 64.69 and 243.22 mg CO₂ m⁻² h⁻¹ from May to November (Figure 1b), and the average CO₂ efflux rate was 163.03 mg CO₂ m⁻² h⁻¹ (equivalent to 39.13 kg CO₂ hm⁻² d⁻¹), which was similar to the level of a Chinese fir (*Cunninghamia lanceolata*) forest in the subtropical zone [39] and a pine (*Pinus tabulaeformis*) forest in the temperate zone [40]. The cumulative CO₂ emission during the study period was compared to a global dataset presented by Wei et al. [41] and fell at the lower end of the range of annual soil CO₂ effluxes from global forests and was lower than the annual soil respiration of 16 primary subtropical forests in China [42]. The

lower soil CO_2 flux in this study might have resulted from two specific reasons. First, it was likely a result of a lower temperature in the high-altitude subalpine site [43], as a close relationship between mean annual temperature and soil respiration has been observed at the global as well as regional scales [41,42]. Indeed, our result was comparable to the values recorded for a shrubland at a similar altitude but was lower than a coniferous forest at a lower altitude from a nearby study [21]. Second, the soil CO_2 efflux was measured within the growing season in our study, while the respired CO_2 from soil beneath the winter snow during the dormant season constituted a proportion of the annual CO_2 efflux [44].

The low soil temperature in this site may have a certain effect on other biogeochemical processes, such as CH₄ and N₂O fluxes. However, a previous study found a limited contribution of climatic variables for explaining the variability of forest soil CH₄ fluxes [45]. That might be due to the fact that soil CH₄ flux is a balanced result of production carried out by methanogens and consumption oxidized by methanotrophs [24], mainly occurring in anaerobic and aerobic conditions, respectively [46]. The CH₄ fluxes were between -52.96 and $-84.67 \ \mu\text{g} \text{ CH}_4 \ \text{m}^{-2} \ \text{h}^{-1}$ (i.e., $-12.71 \ \text{to} -20.32 \ \text{g} \ \text{CH}_4 \ \text{hm}^{-2} \ \text{d}^{-1}$, Figure 1c), indicating that the primary forest soil acted as a sink for atmospheric CH₄, as was demonstrated for 90% of the forest sites worldwide from a global synthesis [47]. The uptake rate was comparable with the mean CH₄ uptake rate from a temperate ecoregion in China [48], and the total CH₄ uptake was 286.31 mg CH₄ m⁻² (Figure 2), falling within the range of CH₄ fluxes from global forests [25,47]. Nonetheless, the uptake capacity was near the mean values for boreal and tropical forests [47] with distinct climatic conditions. These results partly support the first hypothesis and might indicate a complex mechanism controlling soil CH₄ fluxes and different driving factors across biomes [49].

Likewise, soil N₂O flux is determined by its consumption and production processes [18]. The N₂O fluxes in this study were positive, with the rate being 1.69–5.46 ug N₂O m⁻² h⁻¹ (Figure 1d), suggesting a net source. The emission rate was comparable in magnitude to those determined in a Sitka spruce forest in Scotland [50], Douglas fir forests in coastal Oregon [51], cypress and hardwood forests in Japan [52] and an old-growth lowland forest in Indonesia [53]. Nonetheless, the total efflux throughout the study period in our case was lower compared to most forests worldwide [54], which might be possibly ascribed to the lower soil temperature, similar to the soil CO₂ flux.

Ecosystem type/land cover change has an important effect on soil GHG fluxes. The primary forest in this study had a lower soil CO_2 flux but similar CH_4 uptake rate and N_2O emission rate compared to secondary and plantation forests at nearby sites [55], possibly resulting from differences in soil climatic conditions induced by elevation and/or soil C and N pools induced by vegetation [21]. This emphasizes that climate change and/or the conversion of primary forests might have a stronger impact on the soil C budget through soil respiration. In addition to forests, meadows, scrublands and village lands form important landscape components in the study area, but knowledge of their soil GHG fluxes is still lacking. Therefore, it is necessary to carry out the estimation of soil GHG fluxes for different land use types, which can help to understand the overall C and N budgets at the regional scale.

4.2. Environmental Controls on the Temporal Variability of GHG Fluxes

Temporal variations in GHG fluxes have been reported in various forest ecosystems [19], despite some studies finding no obvious pattern, for example N₂O fluxes in an old-growth temperate rainforest [14] and CH₄ fluxes in a humid tropical forest [17]. In this study, soil CO₂ and N₂O fluxes generally displayed a unimodal pattern, reaching a peak in the middle of the period (Figure 1). This trend was consistent with many findings reported by previous studies [56,57]. Soil CH₄ fluxes decreased, increased and then decreased over the entire period (Figure 1c), indicating higher CH₄ uptakes at the start and middle of the period.

Soil CO₂ and N₂O fluxes of the primary forest in our study were significantly and positively correlated with soil temperature, exhibiting an exponential relationship and a linear relationship, respectively (Figure 5a). This reflects an inhibitory effect of soil temperature and resulted in lower soil CO₂ and N₂O emissions at the start and end of the study period. However, CH₄ fluxes were nonsignificantly correlated with soil temperature (Figure 5a), similar to previous studies that found that soil temperature exerted a minor effect on CH₄ fluxes through temporal data [58] and a warming experiment [59]. Consistently, soil moisture could not significantly explain the temporal variations in GHG fluxes (Figure 5b). The possible reason might be due to the smooth temporal fluctuation in soil moisture (Figure 1a), as soil moisture is not a limiting factor in the temporal dynamics of GHG fluxes. These results demonstrate that soil temperature was the dominant climatic variable regulating the temporal dynamics of GHG fluxes. Therefore, it might be unfavorable for C and N accumulations from the perspective of increased soil respiration and N₂O emission under future warming scenarios.

4.3. Temporal Dependence of Soil GHG Fluxes on Soil Microbial Attributes

Alteration of climatic conditions with month may individually or interactively affect soil CO₂, N₂O and CH₄ fluxes, which involve different biological processes; the main biotic drivers of these fluxes therefore may be inconsistent [59,60]. We found CO_2 fluxes were not significantly related to either the biomass of total microbes or of specific microbial groups, suggesting microbial biomass was not the determining factor of CO_2 fluxes. Moreover, CO_2 flux was higher in the middle of the study period, accompanying a lower but nonsignificant total microbial biomass (Figure 4a). The relatively lower biomass in the middle of the study period might be due to the stronger microbial metabolic stress, for example a relatively lower soil NH_4^+ concentration, as observed by higher Sat/Mono and cy/pre ratios [35]. Our results contradicted those reported significant relationships between soil respiration and microbial biomass [61]. The result in our study that high soil respiration occurred with a relatively low microbial biomass confirmed the finding of Ali et al. [62]. This seems to indicate a shift in the microbial strategy toward catabolic processes at high soil temperatures [62] in the middle of the study period, whereas microbes allocated more nutrients to biosynthesis to maintain their populations at low temperatures [63]. Indeed, the geometric mean of soil enzyme activities explained most temporal variations in CO₂ fluxes (Figure 6a), supporting the finding that CO₂ fluxes were predominantly regulated by microbial activity instead of microbial biomass [61,63].

In the case of N₂O and CH₄ fluxes, soil aeration plays a pivotal role in their budgets, since it affects O₂ diffusion [25]. Although the small fluctuation in soil moisture failed to explain the temporal variation in N₂O and CH₄ fluxes, the high WFPS in the site might, to some extent, have affected these fluxes. A marginally significant correlation between N₂O and NO₃⁻ concentration was observed in our study (Figure 6b). This, on the one hand, might be attributed to the stimulation of nitrifier activities as temperatures increase, thus enhancing N₂O emissions derived from the intermediates of nitrification, which nitrated NH₄⁺ to NO₂⁻ and NO₃⁻ under an aerobic environment [22,28]. On the other hand, previous studies have demonstrated that anaerobic microbial processes in water-filled pores could occur simultaneously with aerobic microbial processes in aerobic pores [64,65]. Therefore, denitrification, involving the transformation of NO₃⁻ to N₂O and NO₃⁻ concentration. Nonetheless, it seems possible that nitrification was the main process producing N₂O in our study according to the positive relationship between the N₂O flux and NO₃⁻ concentration. Overall, these results supported our second hypothesis.

Previous studies have demonstrated inconsistent findings regarding the temporal variation in CH_4 fluxes, such as a unimodal pattern due to temperature rises, a bimodal pattern or no pronounced pattern as the joint effect of multiple factors [52,67]. We found soil CH_4 uptake showed a bimodal pattern during the study period (Figure 1c). Although the result was similar to the finding documented by Xu et al. [68], we preferred to deem that this bimodal pattern was ascribed to a decline in CH_4 uptake in the middle of the study period. This reduction was likely due to the decrease in methanotrophs, as there

is a positive correlation between CH_4 uptake and methanotrophs (Figure 6c). Several underlying mechanisms, potentially derived from the coupling of GHG fluxes, could lead to this result. First, oxygen is a common substrate for soil respiration, nitrification and CH_4 oxidation. The high CO_2 and N_2O emissions caused by high soil temperatures in the middle of the study period might increase competitive inhibition of oxygen availability and/or decrease diffusion of CH_4 under the condition of high soil moisture, thus reducing the methanotrophs and CH_4 uptake. Second, the potential increased nitrification might enhance the competition for methane monooxygenase, which could catalyze both CH_4 oxidation and NH_4^+ oxidation [19].

We investigated the temporal variability of soil GHG fluxes and the influencing factors and quantified the soil GHG budgets within a growing season, similar to many other studies [67,69]. Notably, the roles of GHG fluxes during the dormant season are not negligible in their annual budget estimates according to previous evidence [70]. For example, the CO_2 emission [71] and the uncertainty regarding CH_4 emission or uptake [70] beneath snow cover and the N_2O emission pulse during the freeze-thaw period [72] are important aspects determining the magnitude of annual GHG budgets in seasonally snowcovered regions. Therefore, soil GHG fluxes during winter and their response to climate change warrants further study. In addition, plants, as well as microbes, play an important role in soil GHG fluxes, which may exhibit diurnal variability. Although using the values measured at mid-morning, as in our study, has the smallest average bias to characterize daily average fluxes, it may still be over- or under-valued [73]. A study of the diurnal patterns of soil GHG fluxes is needed for accurate estimation of C and N budgets in primary forests. Moreover, aboveground litter property and decomposition rate may have important effects on the soil-atmosphere exchange of GHG [74], and the contributions of litter to soil GHG fluxes need to be strengthened in primary forests. Overall, our results highlighted the different underlying mechanisms regulating the seasonality of soil GHG fluxes and provided available data to estimate GHG fluxes from primary forests on the eastern Qinghai-Tibetan Plateau.

5. Conclusions

Soil GHG fluxes showed clear temporal patterns from the primary forest on the eastern Qinghai-Tibetan Plateau. Soil CO₂ and N₂O emission rates presented unimodal trends within the period, generally peaking in July. Soil CH₄ fluxes were negative and showed a bimodal pattern during the study period. The distinct temporal patterns are attributed to different drivers of GHG fluxes. The temporal variations in CO₂ fluxes were attributed to the microbial activity rather than soil microbial biomass. The N₂O flux was positively related to NO₃⁻ concentration with a marginal significance. A positive relationship between CH₄ uptake and methanotrophs indicated that the lowered methanotrophs in the middle of the study period was attributed to the reduced CH₄ oxidation. Moreover, soil temperature significantly explained the temporal variations in the fluxes of CO₂ and N₂O, while the contribution of soil moisture was small. The results provide insights into the underlying mechanisms regulating the temporal variability of soil GHG fluxes and are important for predicting C and N budgets of primary forests on the eastern Qinghai-Tibetan Plateau and for evaluating their potential role in climate change mitigation.

Author Contributions: Conceptualization, methodology, investigation, writing—original draft, S.L.; methodology, investigation, D.L. and Q.F.; investigation, G.X. and J.W.; Conceptualization, writing—review and editing, Z.S.; funding acquisition, S.L., Z.S. and G.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fundamental Research Funds of CAF (CAFYBB2022SY021, CAFYBB2021ZA002-2 and CAFYBB2022QC002).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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