Research Article

Response of Soil Respiration to Grazing in an Alpine Meadow at Three Elevations in Tibet

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Alpine meadows are one major type of pastureland on the Tibetan Plateau. However, few studies have evaluated the response of soil respiration (R_s) to grazing along an elevation gradient in an alpine meadow on the Tibetan Plateau. Here three fenced enclosures were established in an alpine meadow at three elevations (i.e., 4313 m, 4513 m, and 4693 m) in July 2008. We measured R_s inside and outside the three fenced enclosures in July–September, 2010-2011. Topsoil (0–20 cm) samples were gathered in July, August, and September, 2011. There were no significant differences for R_s, dissolved organic C (DOC), and belowground root biomass (BGB) between the grazed and ungrazed soils. Soil respiration was positively correlated with soil organic C (SOC), microbial biomass (MBC), DOC, and BGB. In addition, both R_s and BGB increased with total N (TN), the ratio of SOC to TN, ammonium N (NH_4^+-N), and the ratio of NH_4^+-N to nitrate N. Our findings suggested that the negligible response of R_s to grazing could be directly attributed to that of respiration substrate and that soil N may indirectly affect R_s by its effect on BGB.

1. Introduction

Soil respiration (R_s) is an important flux in the C cycle [1–3]. Raich and Schlesinger [1] indicated that R_s is the second in magnitude to gross primary production but equivalent to or even greater than net primary production. Root and microbial respiration are the two most important components of R_s; thus the factors which affect root growth and microbial activity could all influence R_s [4–6]. Water availability and temperature are the two most important abiotic factors controlling R_s at various spatial and temporal scales [7, 8]. The positive relationship between R_s and temperature could be weakened or masked by other factors (e.g., respiration substrate) [9, 10]. Previous studies have shown that R_s increases with respiration substrate, including labile C (e.g., microbial biomass C, MBC; dissolved organic C, DOC) and belowground root biomass (BGB) [5, 10–12]. Soil N also affect R_s by influencing plant growth and microbial activity [6, 13].

Grazing is a major type of land use in grasslands and previous studies have shown inconsistent results on the response of R_s to grazing [14, 15]. Many studies have indicated that grazing significantly decreased R_s [14, 16, 17], whereas other studies have shown quite the contrary result [18, 19]. The responses of soil C and N (e.g., MBC, DOC, microbial biomass N, and dissolved organic N) to grazing differ among previous studies [20–23]. There are also inconsistent results on the response of BGB to grazing [15, 24]. The response of R_s to grazing is complex and may be dependent on the responses of respiration substrate [25] and soil N.

Alpine meadows are a major type of pastureland on the Tibetan Plateau [14, 22] and store 4.68 Pg soil organic C (SOC) with density of 9.05 kg m^{-2} at depth of 0–100 cm [26]. Few studies have evaluated the response of R_s to grazing in an alpine meadow along an elevation gradient, although pasture for domestic sheep and yak is a common land use type on the Tibetan Plateau [22]. Here, we investigated the grazing effect

on R_s in an alpine meadow at three elevations (i.e., 4313 m, 4513 m, and 4693 m) on the Northern Tibetan Plateau.

The main objectives of this study were to examine (1) the effect of grazing on R_s, soil C, and N and (2) the relationships between R_s and respiration substrate (soil organic C, MBC, DOC, and BGB) and soil N (total N, microbial biomass N, dissolved organic N, ammonium N, and nitrate N) along an elevation gradient in an alpine meadow in Tibet.

2. Materials and Methods

2.1. Study Area. The study area (30°30′–30°32′N and 91°03′–91°04′E) was located at the Damxung Grassland Observation Station, Tibetan Autonomous Region in China. Annual mean solar radiation was 7527.6 MJ m^{-2} and sunlight was 2880.9 h [12]. Annual average precipitation was around 476.8 mm and annual potential evapotranspiration was about 1725.7 mm [27]. Annual mean air temperature was 1.3°C [12]. The soil was classified as a shallow sandy loam (∼ 0.5–0.7 m), with organic matter of 0.3–11.2%, total N of 0.03–0.49%, and pH of 6.0–6.7 [22]. The vegetation surrounding the study site was *Kobresia*-dominated alpine meadow [28]. Roots are mainly concentrated in the topsoil layer (0–20 cm) [29].

Based on meteorological observations from 1963 to 2012 at the Damxung Station (4288 m, approximately 4 km from our study site), there was no significant change for annual precipitation, while annual mean air temperature increased at a rate of 0.04°C a^{-1} [12].

2.2. Experimental Design. Three sites (about 20 m × 20 m for each) were fenced in an alpine meadow on a south-facing slope on the Nyainqentanglha Mountains along an elevation gradient (i.e., 4313 m, 4513 m, and 4693 m) in July 2008. Before enclosure, the site at elevation 4313 m was winter pasture, while the other two sites were summer pasture [22]. A more detailed description of the experimental design can be found in Fu et al. [22].

Soil temperature (T_s) at a depth of 5 cm, soil water content (SWC) at a depth of 10 cm, and air temperature and relative humidity at a height of 15 cm were continuously monitored using data loggers (HOBO weather station, Onset Computer Corporation, USA) at each elevation [22]. Both air temperature and T_s increased with decreasing elevation [22].

2.3. Measurement of R_s. Soil respiration was measured using a soil CO_2 flux system (LI-8100, LI-COR Biosciences, Lincoln, NE, USA) [6, 30] during the period from July to September in 2010 and 2011 (Figure 1). Soil respirationat 9:00–11:00 am was close to daily average R_s [6, 31]; thus R_s was measured between 9:00 and 11:00 (local time) in this study. Four polyvinyl chloride (PVC) collars (20 cm in diameter and 5 cm in height) were inserted into the soil to depths of about 2-3 cm on each measuring date. All the PVC collars were installed and the aboveground biomass was removed at least 12 h before R_s measurement in order to reduce disturbance [6, 12]. The opaque survey chamber was manually mounted

TABLE 1: Repeated-measures analysis of variance for the main and interactive effects of grazing (G) and measuring date (D) on soil respiration (R_s, μmol CO_2 m^{-2} s^{-1}) in an alpine meadow at three elevations (i.e., 4313 m, 4513 m, and 4693 m) on the Tibetan Plateau ($n = 4$).

Model	4313 m		4513 m		4693 m	
	F	P	F	P	F	P
G	5.94	0.051	2.39	0.173	1.22	0.311
D	**34.12**	<0.001	**29.08**	<0.001	**10.02**	<0.001
G × D	1.60	0.249	1.39	0.277	0.31	0.837

on PVC collars for R_s measurements [30]. One cycle was performed on each measuring date.

2.4. Soil Sampling and Analysis. Topsoil samples (0–20 cm depth) inside and outside the three fenced enclosures were collected (using a soil auger of 3.0 cm in diameter) on July 7, August 9, and September 10, 2011 [22]. Five soil subsamples were randomly sampled and composited into one soil sample for each of the four replicates. The composited soil samples were stored in an icebox and transferred to laboratory. We sieved soil samples (with a sieve of 1 mm diameter) and picked up any visible roots from the sieved soil. Subsamples of the sieved soil were used to measure NO_3^--N, NH_4^+-N, DOC, and DON. All the roots in the soil samples were washed, dried at 65°C for 48 h, and weighed.

We extracted 20 g fresh soil samples using 100 mL K_2SO_4. The K_2SO_4 extracts were filtered through 0.45 μm filter membrane and then soil available N (SAN, NO_3^--N, and NH_4^+-N) in the extracts were analyzed on a LACHAT Quickchem Automated Ion Analyzer.

The methods of Jones and Willett [32] were used to determine DOC and dissolved total N (DTN). Briefly, we extracted 20 g fresh soil samples using 100 mL ultrapure water and filtered the extracts through 0.45 μm filter membrane. We analyzed the extractable soil organic C and total N in the ultrapure water extracts using a Liqui TOC II elementar analyzer (Elementar Liqui TOC, Elementar Co., Hanau, Germany) and a UV-1700 PharmaSpec visible spectrophotometer (220 nm and 275 nm), respectively. Dissolved inorganic N (DIN) concentrations in the ultrapure water extracts were also determined on a LACHAT Quickchem Automated Ion Analyzer. Then, DON was calculated as the difference between DTN and DIN. Soil organic C, TN, MBC, and MBN data were obtained from Fu et al. [22].

2.5. Statistical Analysis. Repeated-measures analysis of variance (ANOVA) was used to estimate the main and interactive effects of measuring date and grazing on R_s for each site (Table 1). Repeated-measures ANOVA was used to estimate the main and interactive effects of sampling date and grazing on DOC, DON, DOC/DON ratio, NO_3^--N, NH_4^+-N, NH_4^+-N/NO_3^--N ratio, SAN, and BGB (Table 2). Student-Newman-Keuls multiple comparisons were performed among the three sites. Linear relationships of R_s with SOC, TN, SOC/TN ratio, MBC, MBN, DOC, DON, NH_4^+-N, SAN, NH_4^+-N/NO_3^--N ratio, and BGB were conducted,

TABLE 2: Repeated-measures analysis of variance for the main and interactive effects of grazing (G) and sampling date (D) on dissolved organic C (DOC, mg kg^{-1}) and N (DON, mg kg^{-1}), the ratio of DOC and DON (DOC/DON ratio), nitrate N (NO$_3^-$-N, mg kg^{-1}), ammonium N (NH$_4^+$-N, mg kg^{-1}), the ratio of NH$_4^+$-N and NO$_3^-$-N (NH$_4^+$-N/NO$_3^-$-N ratio), soil available N (SAN, mg kg^{-1}), and belowground root biomass (BGB, kg m^{-2}) in an alpine meadow at three elevations (i.e., 4313 m, 4513 m, and 4693 m) on the Tibetan Plateau ($n = 4$).

Elevation	Model	DOC	DON	DOC/DON ratio	NO$_3^-$-N	NH$_4^+$-N	NH$_4^+$-N/NO$_3^-$-N ratio	SAN	BGB
	G	0.77	1.65	0.46	27.10**	26.05**	1.05	95.61***	0.00
4313 m	D	26.25***	4.80*	5.00*	57.55***	10.47**	8.95**	29.95***	2.79
	G × D	2.64	1.46	0.29	11.02**	3.00	2.16	7.13*	1.45
	G	0.00	2.23	5.51	7.29*	13.99**	0.04	16.50**	1.52
4513 m	D	35.53***	4.38*	0.40	51.70***	29.87***	12.29***	57.31***	2.74
	G × D	3.15	3.81	3.49	1.85	2.05	0.51	3.07	0.98
	G	0.00	0.16	0.12	0.82	0.27	0.08	0.65	0.64
4693 m	D	1.33	3.67	3.03	9.24**	15.27***	0.94	15.78***	3.85
	G × D	3.41	1.24	0.04	1.79	6.95**	0.30	5.67*	0.95

*, **, and ***means $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

respectively. All the statistical tests were performed using the SPSS software (version 16.0; SPSS Inc., Chicago, IL).

3. Results

At elevation 4313 m, NO$_3^-$-N, NH$_4^+$-N, and SAN under grazing were 31.78% (1.95 mg kg^{-1}), 39.14% (2.34 mg kg^{-1}), and 35.41% (4.29 mg kg^{-1}) lower compared with ungrazed soils across all the three sampling dates (Figure 2 and Table 2). Similarly, at elevation 4513 m, NO$_3^-$-N, NH$_4^+$-N, and SAN under grazing were 22.00% (1.41 mg kg^{-1}), 23.60% (1.33 mg kg^{-1}), and 22.75% (2.75 mg kg^{-1}) lower than that of ungrazed soils (Figure 2 and Table 2). In contrast, there were no significant differences of NO$_3^-$-N, NH$_4^+$-N, and SAN between ungrazed and grazed soils at elevation 4693 m (Figure 2 and Table 2).

In addition, grazing had no significant effects on NH$_4^+$-N/NO$_3^-$-N ratio, DOC, DON, DOC/DON ratio, and BGB for the three alpine meadow sites (Figure 2 and Table 2). Regardless of grazing, NO$_3^-$-N, NH$_4^+$-N, NH$_4^+$-N/NO$_3^-$-N ratio, SAN, DOC, DON, DOC/DON ratio, and BGB all showed similar seasonal dynamics among the three elevations (Figure 2).

No significant differences of DOC, NH$_4^+$-N, and NH$_4^+$-N/NO$_3^-$-N ratio were found between elevations 4313 m and 4513 m, whereas they were significantly lower compared with elevation 4693 m whether or not grazing was present. Average DOC at elevations 4313 m and 4513 m across all the three sampling dates was 33.01% and 29.31% lower than that of elevation 4693 m, respectively, irrespective of grazing ($P < 0.05$). Average NH$_4^+$-N at elevations 4313 m and 4513 m was 68.40% and 67.23% lower compared with elevation 4693 m, respectively ($P < 0.05$). Average NH$_4^+$-N/NO$_3^-$-N ratio at elevations 4313 m and 4513 m was 66.76% and 70.52% lower compared with elevation 4693 m, respectively ($P < 0.05$).

Average DON at elevations 4313 m and 4513 m across all the three sampling dates was 50.78% and 33.84% lower than that of elevation 4693 m under grazing, respectively ($P < 0.05$), whereas there was no significant difference between elevations 4313 m and 4513 m. There were no significant

differences of average DON among the three sites when grazing was absent.

Average SAN at elevations 4313 m and 4513 m across all the three sampling dates was 38.69% and 38.88% lower than that of elevation 4693 m when grazing was absent, respectively ($P < 0.05$), while no significant difference between the two lower elevations was found. By contrast, average SAN increased with increasing elevation under grazing ($F = 375.30$, $P < 0.001$).

No significant differences of DOC/DON ratio and NO$_3^-$-N were found among the three sites whether or not grazing was present.

The main effect of grazing and its interactive effect with measuring date on R_s were not significant for each alpine meadow site (Figure 1 and Table 1). Grazing only tended to decrease the average R_s across all the measuring dates by 14.02% (0.25 μmol CO$_2$ m^{-2} s^{-1}), 4.70% (0.11 μmol CO$_2$ m^{-2} s^{-1}), and −4.07% (−0.15 μmol CO$_2$ m^{-2} s^{-1}) at elevations 4313 m, 4513 m, and 4693 m, respectively. In contrast, there was significant seasonal variation for R_s (Figure 1 and Table 1). Regardless of grazing, R_s showed similar seasonal dynamics among the three elevations (Figure 1).

There were significant elevation effects on R_s ($F = 147.94$, $P < 0.001$ for ungrazed condition; $F = 227.25$, $P < 0.001$ for grazed condition) and BGB ($F = 315.20$, $P < 0.001$ for ungrazed condition; $F = 58.81$, $P < 0.001$ for grazed condition) across all the measuring dates.

Belowground biomass was positively related to TN, NH$_4^+$-N, SAN, SOC/TN ratio, and NH$_4^+$-N/NO$_3^-$-N ratio, respectively (Figure 3), but not to NO$_3^-$-N (data not shown).

R_s was positively correlated with SOC, TN, SOC/TN ratio, MBC, MBN, DOC, DON, NH$_4^+$-N, SAN, NH$_4^+$-N/NO$_3^-$-N ratio, and BGB, respectively (Figure 4). However, R_s was not linearly correlated with DOC/DON ratio, MBC/MBN ratio, and NO$_3^-$-N (data not shown).

4. Discussion

Previous studies indicated that grazer urine and dung stimulated soil microbial activity and accelerated nutrient cycling
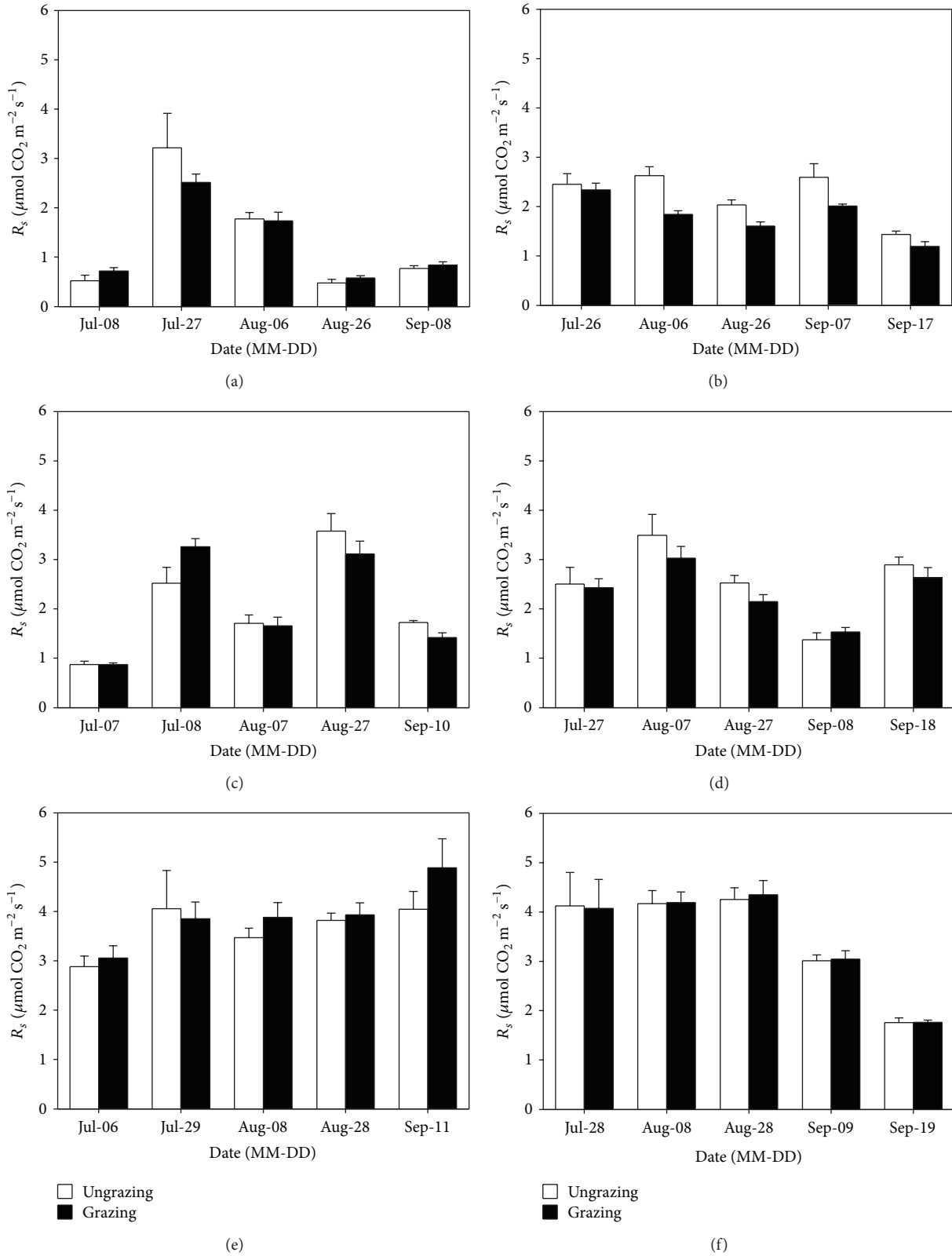
FIGURE 1: Effects of grazing on soil respiration (R_s, μmol CO_2 m^{-2} s^{-1}) in an alpine meadow located at elevations of 4313 m (a, b), 4513 m (c, d), and 4693 m (e, f) on the Tibetan Plateau in 2010 (a, c, and e) and 2011 (b, d, and f), respectively. Error bars represent standard error ($n = 4$).
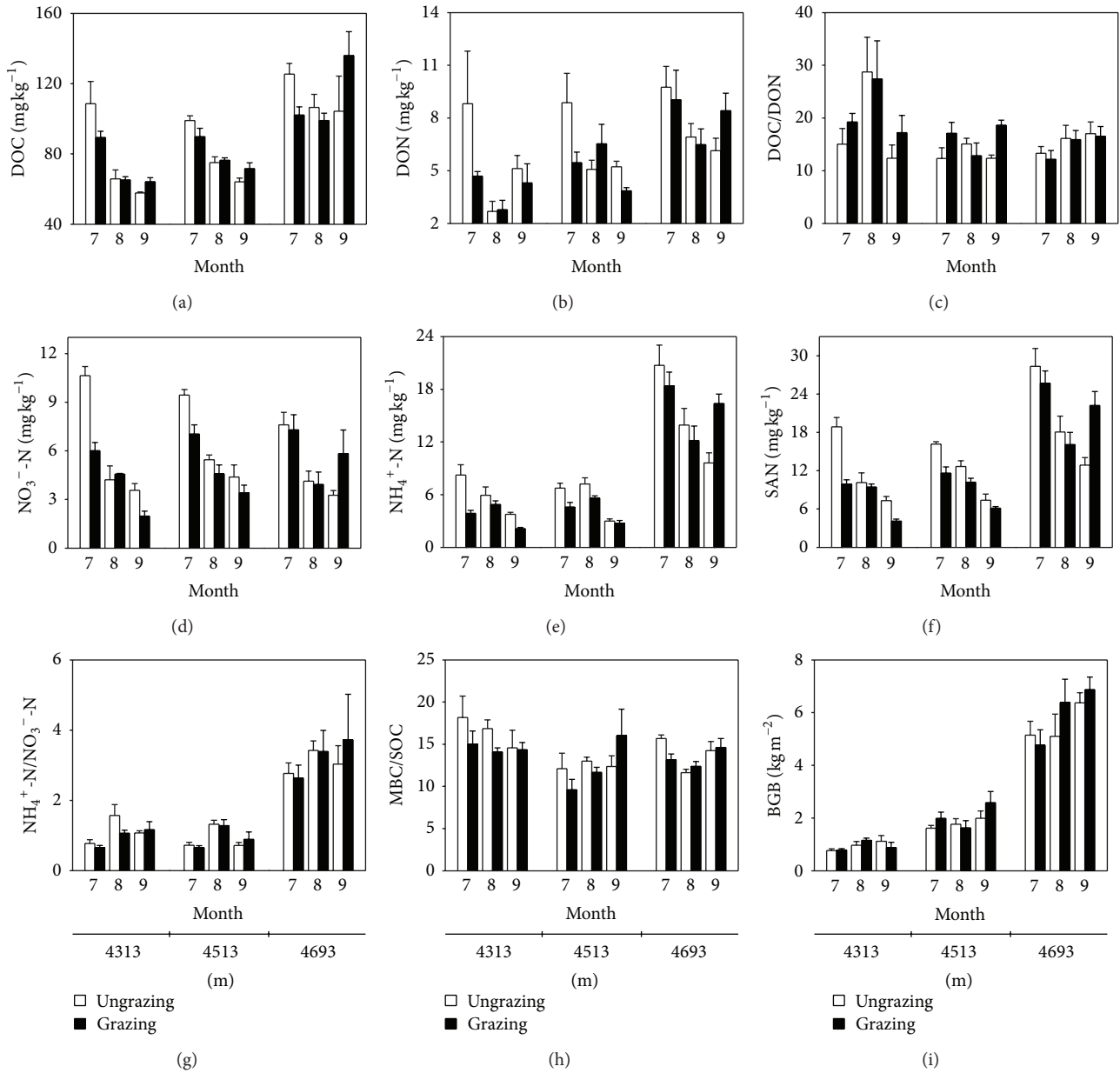
FIGURE 2: Effects of grazing on dissolved organic C (DOC, mg kg^{-1}), dissolved organic N (DON, mg kg^{-1}), the ratio of DOC and DON (DOC/DON ratio), nitrate N (NO$_3^-$-N, mg kg^{-1}), ammonium N (NH$_4^+$-N, mg kg^{-1}), soil available N (SAN, mg kg^{-1}), the ratio of NH$_4^+$-N and NO$_3^-$-N (NH$_4^+$-N/NO$_3^-$-N ratio), and belowground root biomass (BGB, kg m^{-2}) in an alpine meadow at three elevations (i.e., 4313 m, 4513 m, and 4693 m) on the Tibetan Plateau. Error bars represent standard error ($n = 4$). MBC and SOC data were obtained from Fu et al. [22].

in grasslands [33, 34]. However, this effect may be often weakened at the three alpine meadow sites because the dung of yak and goat was removed by local residents.

Generally, grazing did not alter the distributions of R_s, DOC, DON, DOC/DON ratio, NO$_3^-$-N, NH$_4^+$-N, and BGB along the elevation gradient, which was in line with previous studies [22, 35, 36].

Soil microbial biomass N at elevations 4313 m and 4693 m and SAN (including NO$_3^-$-N and NH$_4^+$-N) at elevations 4313 m and 4513 m under grazing were significantly lower

compared with ungrazed soils, while there were no significant differences of SOC, TN, DOC, DON, BGB, and R_s between grazed and ungrazed soils (Tables 1 and 2, [22]). This suggests that soil microbial biomass and available N may respond more rapidly to grazing than SOC, TN, DOC, DON, BGB, and R_s. The negligible response of R_s to grazing was consistent with some previous studies conducted on the Tibetan Plateau (e.g., [15]).

Previous studies showed that BGB increased with increasing TN in alpine grasslands on the Tibetan Plateau
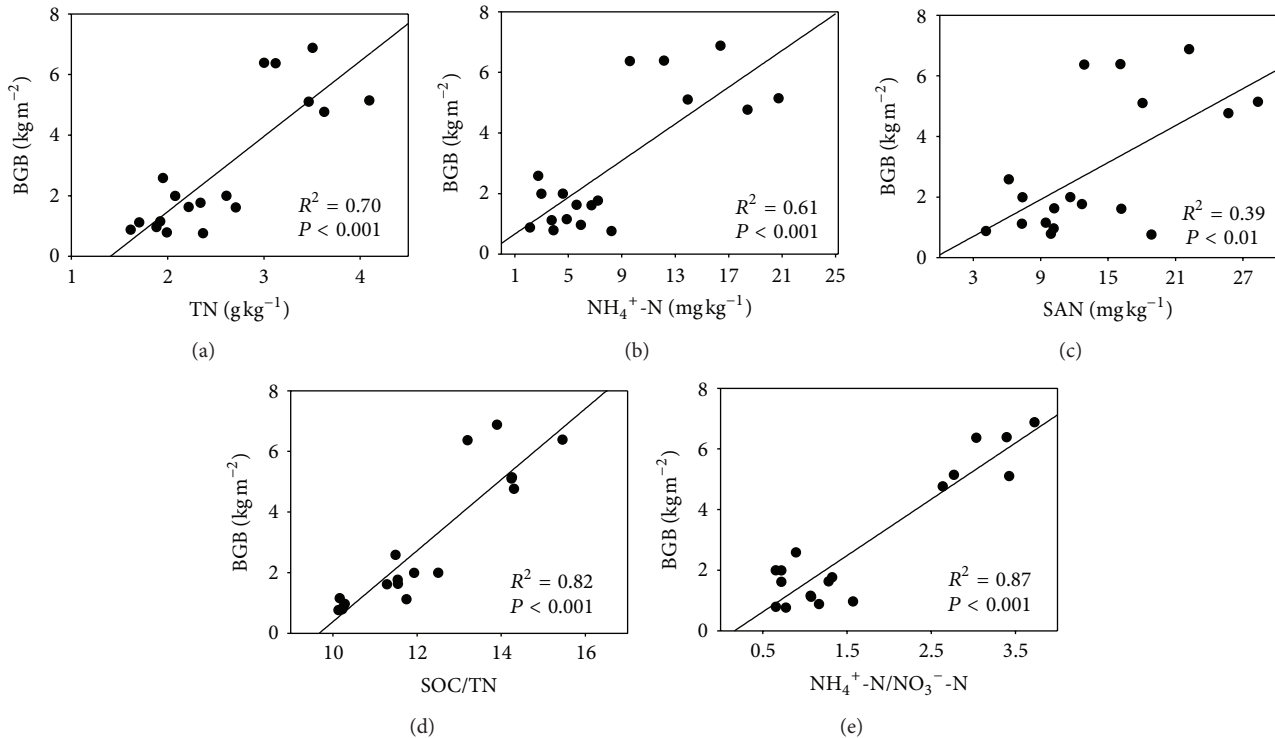
FIGURE 3: Relationships between belowground root biomass (BGB, $kg\,m^{-2}$) and total N (TN, $g\,kg^{-1}$), ammonium N (NH_4^+-N, $mg\,kg^{-1}$), soil available N (SAN, $mg\,kg^{-1}$), the ratio of SOC and TN (SOC/TN ratio), and the ratio of NH_4^+-N and nitrate N (NH_4^+-N/NO_3^--N ratio), respectively. SOC and TN data were obtained from Fu et al. [22].

[36, 37]. Our study confirmed this finding (Figure 3). Besides, BGB increased with increasing NH_4^+-N, SAN, and NH_4^+-N/NO_3^--N ratio, but not with NO_3^--N. Therefore, the positive relationship between BGB and SAN may be mainly attributed to that between BGB and NH_4^+-N. In addition, the ratio of different soil available N forms could affect BGB.

Similar to BGB, R_s increased with TN, SOC/TN ratio, NH_4^+-N, SAN, and NH_4^+-N/NO_3^--N ratio, respectively (Figure 4). Meanwhile, R_s was positively correlated with BGB (Figure 4). Therefore, the effect of soil N availability and form on R_s was probably associated with the effect of soil N availability and form on BGB. In addition, the negligible response of R_s to grazing may be directly attributed to that of SOC, MBC, DOC, and BGB.

The positive relationships between DOC and SOC, DON and TN, DOC and MBC, DON and MBN, and SAN and MBN (data not shown) were in accordance with previous studies which were made in alpine meadows on the Tibetan Plateau [22, 38] and an upland grassland of northern England [39]. Previous studies found that DOC was a good index in reflecting C availability of soil microorganisms [40, 41]. Therefore, the variation of soil microbial biomass along the elevation gradient may be not only associated with that of SOC and TN [22] but also with that of DOC and DON [6, 11, 42]. In other words, soil microbial activity may regulate

the balances of soil inorganic and organic C and N pools in this alpine meadow.

Many studies have found the positive relationship between R_s and T_s in various ecosystems [3, 15]. In contrast, R_s increased significantly with increasing elevation, while both soil and air temperatures declined in the current study. In other words, the relationship between R_s and T_s was negative along the elevation gradient. This implied that other factors (e.g., respiration substrate) probably regulated or confounded the positive relationship between respiration and temperature [10, 25]. This viewpoint was as confirmed by the positive relationships between R_s and BGB, SOC, MBC, and DOC (Figure 4).

5. Conclusions

In this study, we measured soil respiration under grazed and ungrazed conditions in an alpine meadow along an elevation gradient (4313–4693 m with approximate 200 m interval) in Tibet during the period from July to September in 2010-2011. We found that grazing did not significantly affect soil respiration, which was probably attributed to the insignificant response of respiration substrate (e.g., soil organic C and belowground root biomass) to grazing. Soil N availability and the ratio of ammonium to nitrate N might also influence soil respiration by affecting belowground root growth.
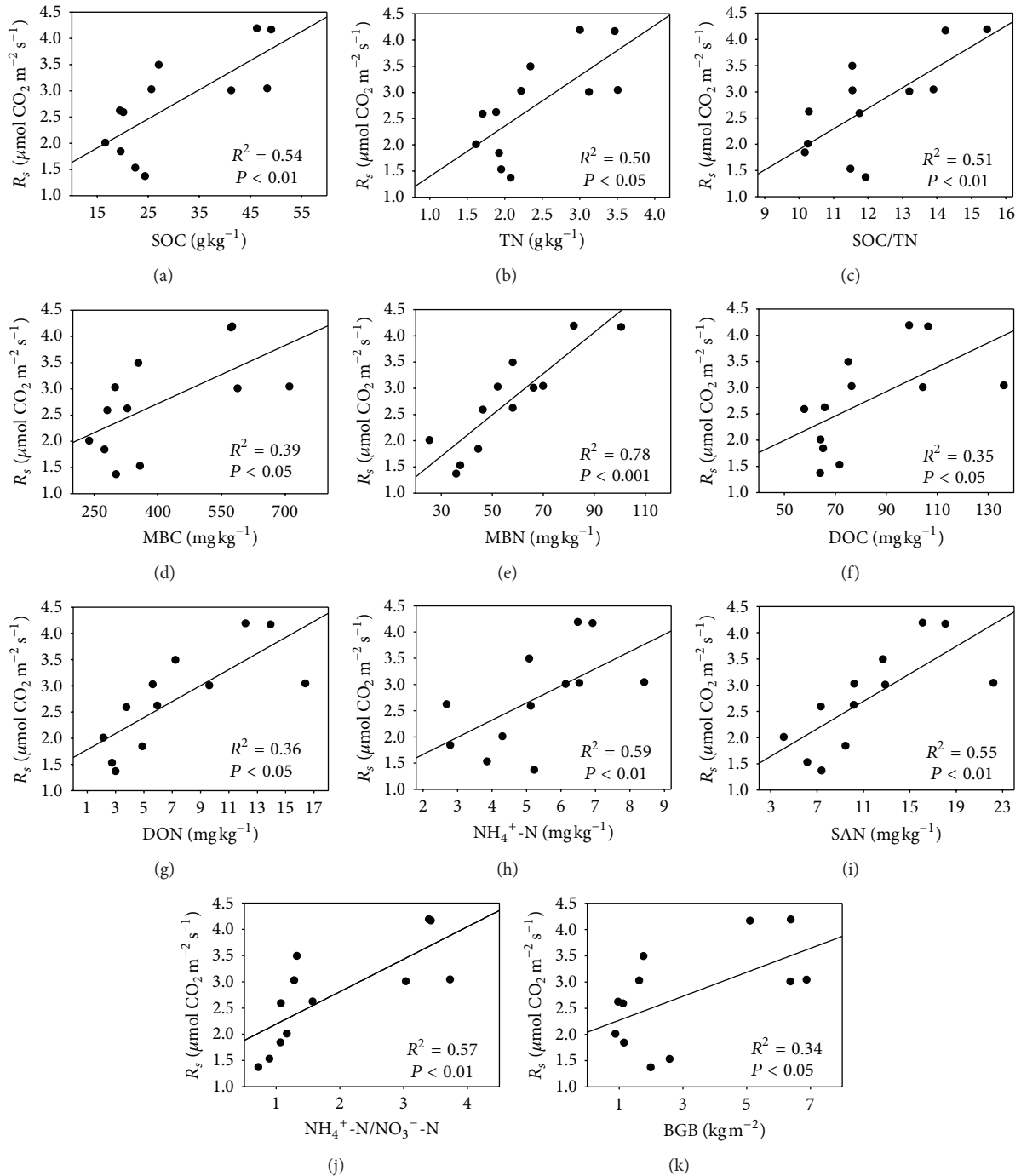
FIGURE 4: Relationships between soil respiration (R_s, μmol CO_2 m^{-2} s^{-1}) measured early in August and September, 2011, and soil organic C (SOC, g kg^{-1}), total N (TN, g kg^{-1}), the ratio of SOC and TN (SOC/TN ratio), microbial biomass C (MBC, mg kg^{-1}), microbial biomass N (MBN, mg kg^{-1}), dissolved organic C (DOC, mg kg^{-1}), dissolved organic N (DON, mg kg^{-1}), ammonium N (NH$_4^+$-N, mg kg^{-1}), soil available N (SAN, mg kg^{-1}), the ratio of NH$_4^+$-N and nitrate N (NH$_4^+$-N/NO$_3^-$-N ratio), and belowground root biomass (BGB, kg m^{-2}), respectively. SOC, TN, MBC, and MBN data were obtained from Fu et al. [22].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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